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Jones, M. A. & R. Burns. 1975. Title. Abbr. J. 00: 00-00.

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Jones, M. A., A. B. Skyler & H. H. Monroe. 1973. Title. Abbr. J. 00: 00-00.

Jones, M. A., R. Burns & L. O. Curtin. 1979. Title. Abbr. J. 00: 00-00.

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Books

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Mitchell, E. R. [Ed.]. 1981. Management of insect pests with semiochemicals: concepts and practice. Plenum, New York, 514 pp.

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Myler, A. 1985. Article or chapter title, pp. 00-00. *In* I. S. Burke, Jr. and L. B. Armstrong [Eds.], Book title. Publisher, city, state, 233 pp.

Reynolds, H. T., P. L. Adkisson & R. F. Smith. 1975. Cotton insect pest management, pp. 379-443. *In* R. L. Metcalf and W. H. Luckmann [eds.], Introduction to insect pest management. Wiley, New York, 587 pp.

Royer, T. A., J. V. Edelson & B. Cartwright. 1988. Onion thrips control, 1987, p. 129. *In* Insecticide and acaracide tests, vol. 13. Entomological Society of America, College Park, Maryland, 459 pp.

Proceedings

Reynolds, H. T. 1985. Pesticides: a dependable component of IPM, pp. 21-24. *In* Proceedings, Regional workshop on pesticide management, Nairobi, Kenya, 128 pp.

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Reports

Baker, W. H. 1972. Eastern forest insects. United States Department of Agriculture Forest Service Miscellaneous Publication 1175, Washington, D.C., 672 pp.

Colorado Agricultural Experiment Station. 1989. Annual report. Colorado State University, Ft. Collins, 62 pp.

Webster, J. A. & D. H. Smith, Jr. 1983. Developing small grains resistant to the cereal leaf beetle. United States Department of Agriculture Technical Bulletin 1673, Washington, D.C., 12 pp.

Young, D. A. 1986. Taxonomic study of the Cicadellinae (Homoptera: Cicadellidae). Part 3: Old World Cicadellinae. North Carolina Agricultural Experiment Station Technical Bulletin 281, Raleigh, 639 pp.

In Press

Rogers, L. E. & J. F. Grant. In press. Infestation levels of dogwood borer (Lepidoptera: Sesiidae) larvae on dogwood trees in selected habitats in Tennessee. J. Entomol. Sci.

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Department of Agriculture. 1985. Insects of eastern forests. United States Department of Agriculture Forest Service Miscellaneous Publication 1426, Washington, D.C., 608 pp.

International Rice Research Institute (IRRI). 1977. Title. International Rice Research Institute, Manila. Philippines, 336 pp.

Theses and Dissertations

Anway, C. L. 1982. Male-produced aggregation pheromone of the maize weevil and effect of diet on production and response. MS thesis, Univ. of Wisconsin, Madison, 66 pp.

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Abstracts and Translations

Barker, S. 1989. Toxicity of XXX. Chem. Abstr. 18: 193a.

Hooker, M. W. & E. M. Barrows. 1989. Clutch sizes and sex ratios in *Pediobius*. Ann. Entomol. Soc. Am. 82: 460 (abstr.)

Shenderovskaya, L. P. 1979. Introduced insect enemies and microorganisms. Zash. Rast. (Kiev) 3: 52-56 (in Russian).

Shenderovskaya, L. P. 1979. Introduced insect enemies and microorganisms. Zash. Rast. (Kiev) 3: 52-56. (translated in OTS 61: 31267), U. S. Department of Commerce, Washington, D.C.

Magazine Articles

Headley, J. C. 1979. Economics of pest control. Chem. Eng. News, Jan. 15, pp. 55-57.

Other

Code of Federal Regulations. 1986. Title. 7 CFR Chapter III, Section 318.13-46, pp. 128-129.

SAS Institute. 1985. SAS user's guide: statistics, version 5 ed. SAS Institute, Cary, North Carolina, 956 pp.

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New Device for Ethylene Release in Pest Management[1]

A. Peña,[2] M. Campos, and A. J. Sánchez-Raya

Estación Experimental del Zaidín, CSIC, c/Profesor Albareda, 1,
E-18008 Granada, Spain

J. Agric. Entomol. 15(1): 9–21 (January 1998)

ABSTRACT Ethylene, a plant phytohormone, has attracted a beetle pest of olive trees, *Phloeotribus scarabaeoides* (Bernard), both in laboratory bioassays and in the field. A device was optimized for the required release of ethylene to manage this pest under field conditions. The concentration and volume of ethrel, a formulation that releases ethylene, were investigated. Better release rates of ethylene were achieved with 1% and 0.1% dilutions, but the levels of ethylene reached were not maintained for the periods of time required for pest management. The best results were obtained using plastic dispensers with holes in their upper surface containing an aqueous solution of ethrel. The addition of different plant materials to these dispensers triggered the release of ethylene and, when a piece of olive log was added, ethylene was released in a continuous and durable way and at a sufficient rate to attract olive bark beetles.

KEY WORDS ethylene, dispenser, olive bark beetle, scolytids

Plants produce hundreds of volatile compounds, some of which can act as regulators of plant development and influence the growth and activity of adjacent plants and animals (Abeles et al. 1992). Volatile components have been involved in attracting insects to plants, and substances present on plant surfaces may potentially function in host recognition and feeding site selection (Tumlinson et al. 1993) or oviposition (Fletcher & Watson 1974). Other compounds in plants may serve as feeding stimulants or deterrents (Dillwith et al. 1991). The phytohormone ethylene has been involved in plant defense mechanisms (Kendall & Bjostad 1990, Popp & Johnson 1990) or in some insect-plant relationships as an insect pheromone regulator (Raina et al. 1992).

A field study of the chemicals involved in the colonization behavior of the olive bark beetle, *Phloeotribus scarabaeoides* (Bernard) (Coleoptera: Scolytidae), showed that ethylene released by the olive tree attracts olive bark beetles (González & Campos 1996), as shown first in laboratory assays (González et al. 1994, Campos & Peña 1995). Arambourg (1986) described the attraction of olive bark beetles to olive logs as due to nonspecific odors released by the plant.

[1]Accepted for publication 24 September 1997.

[2]To whom correspondence and reprint requests should be addressed.

Phloeotribus scarabaeoides is an olive pest that causes economic losses in olive groves throughout the Mediterranean coast (Russo 1938, Benazoun 1992, González & Campos 1994). These scolytids usually reproduce in logs left over from annual pruning and the emerging generations fly to the olive trees, first to feed on the young, potential fruit-bearing branches and later to overwinter, causing a reduction of crop and vegetative growth. At present, the recommended control of bark beetles consists of transporting and storing logs in well-closed and isolated rooms, once the insects have initiated reproduction (Civantos & Sánchez 1993), to prevent new generations from reaching olive trees and causing damage. But, for economic reasons, a high percentage of olive growers do not follow this cultural method, and the problems associated with the pest have been steadily increasing in the last years, necessitating the development of other control strategies.

It is well known that ethylene is produced by plants and that the application of ethrel (Etisa, Barcelona, Spain), also known as Ethephon (Maynard & Swan 1963), a formulation that contains an aqueous solution of 48% 2-chloro-ethyl-phosphonic acid, induces higher emission rates from plant tissues (Abeles et al. 1992, Campos et al. 1994). But, to our knowledge, there has been no implementation of the release of ethylene in the field for the control of insect pests. Therefore, the aim of this study was to develop a device capable of releasing ethylene, in a continuous and durable way, and at a sufficient concentration to manage these scolytids under field conditions.

Materials and Methods

Release devices. Different devices were tested as candidates for the release of ethylene: white polyethylene capsules of ca. 2-ml capacity (1 cm i.d. × 3 cm height) (Agrisense, BCS, Cardiff, United Kingdom), black polyethylene capsules of similar dimensions (a gift from Dr. Mazomenos, Aghia Paraskevi, Greece), and plastic containers (4.5 cm i.d. × 7 cm high) of ca. 100-ml capacity (Aulabor, Rubí, Barcelona, Spain). To determine ethylene release in each case, each device was introduced into a glass container of adequate capacity. For the white and black polyethylene capsules, glass tubes of 34-ml capacity, with screw caps with a central hole and a rubber seal on the top cover, were used. For the bigger devices, glass bottles of 0.5-liter capacity were adapted as follows (Fig. 1): a hole was drilled in the upper surface; a hollow glass tube was glued to the hole; and the glass tube was connected to a silicone tube that was sealed with a glass rod. The bottles were kept tightly closed for 2 h at 25°C, unless otherwise indicated. Then, the rubber seal or silicone tube was pierced with a gas-tight syringe and 3 ml of the inner bottle atmosphere was taken for the gas chromatographic analysis. The different devices were subsequently removed from the bottles, until the next analyses were performed. In all the experiments, ethrel was used for the production of ethylene. Each of the treatments described below was replicated three times.

Influence of temperature and ethrel concentration. Four different ethrel solutions were used (pure ethrel and aqueous dilutions at 1%, 0.1%, and 0.01%) at two different temperatures (25°C and 35°C). One milliliter of each solution was placed in white polyethylene capsules. After 168 h, an olive leaf

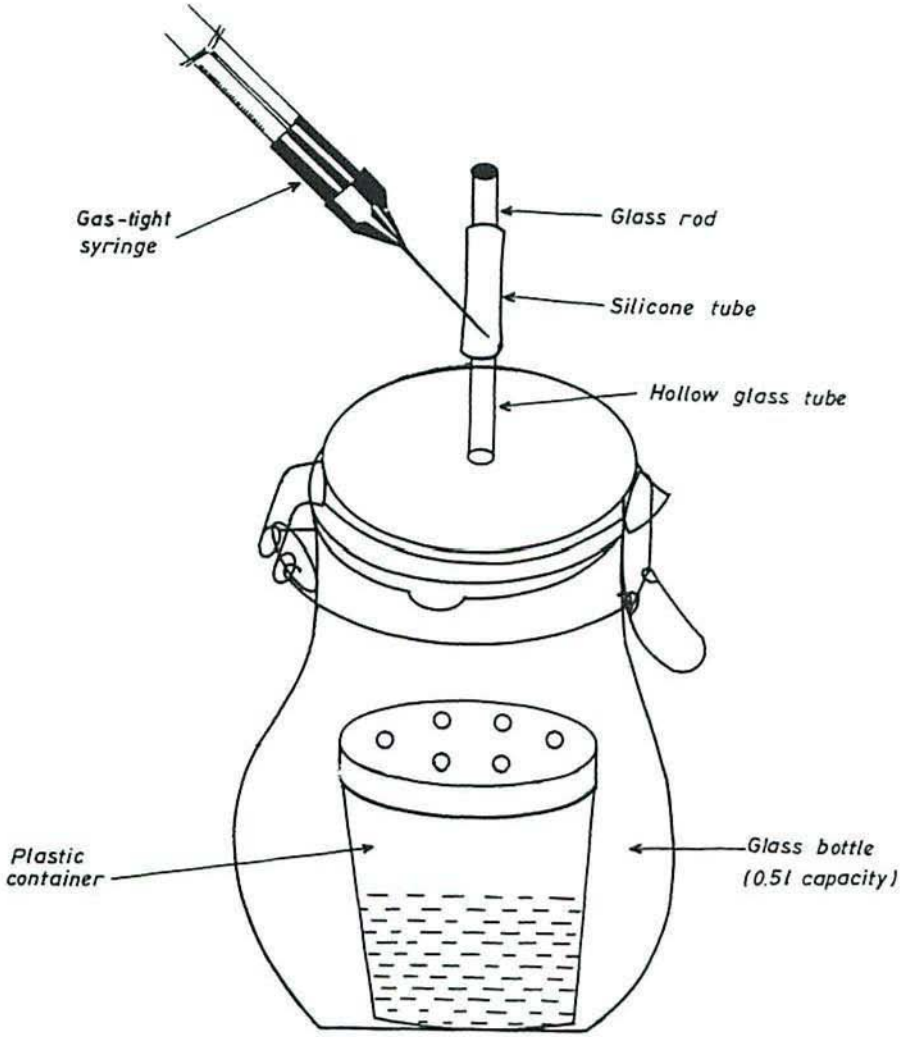

Fig. 1. System used for the determination of ethylene release. The container with six holes in the upper surface is placed inside a 0.5-liter glass bottle and tightly closed for 2 h at 25°C. Then, the silicone tube is pierced with a gas-tight syringe and 3 ml of the inner atmosphere sampled. Afterwards, the container is removed from the bottle until the next analysis is performed.

(*Olea europaea* L., cv. Picual) (mean length 3 cm and mean weight 0.18 g) was added to each capsule.

Influence of drilling a hole in the dispensers. Two types of polyethylene capsules of similar capacity, white and black, were used at two different temperatures (25°C and 35°C). Both types of capsules were tested with or without a hole in their top covers, pierced with a pin, to obtain different ethylene release rates. Olive leaves were cut with scissors into six pieces, three of which were placed in each capsule.

Influence of the volume of ethrel and of the number of leaves. Plastic containers of ca. 100-ml capacity were tested for their ability to release ethylene. In one experiment, 10 or 20 ml of a 1% ethrel solution was placed in a container, without holes, to which were added three or 15 olive leaves. In a second experiment, six holes were pierced in the upper surface of the containers, with 20 ml of the same ethrel dilution, to which 15 or 30 leaves were added.

Influence of the addition of sodium hydroxide (NaOH). Plastic containers with six holes pierced in their top covers were used. Twenty-five milliliters of a 1% ethrel solution was supplied to each container plus 1 ml of NaOH solution at 0.1, 0.01, or 0.001 M. One milliliter of water was added to the ethrel solution as the control.

Influence of the addition of different plant material. Twenty olive leaves, 20 small stems (ca. 5 cm high), or a small piece (ca. 3 cm diameter and 5 cm high) of a recently cut log or of one cut 3 d before the experiment, were put in the plastic containers with holes described above. In one experiment, each container contained 25 ml of a 1% ethrel solution. In a second experiment, 25 ml of water without ethrel was added and the same plant material was tested, except for logs cut 3 d previously. In all experiments, the stems and logs were placed with their basipetal side immersed in the solution. For the leaves, their stalks were placed in the same solution.

Gas chromatographic analysis. A Carlo Erba Fractovap 2350 gas chromatograph equipped with a flame ionization detector and a 2-m Porapak Q column was used. Nitrogen was the carrier gas. Injector and detector temperatures were held at 150°C. The chromatographic analysis was run isothermally at 60°C. Two milliliters of the total volume taken was injected for each sample. Ethylene standards were prepared from pure ethylene (99.3% purity) from a gas cylinder (SEO, Málaga, Spain) by serial dilution with air. A range of ethylene standards was prepared to calculate the actual concentration of ethylene in the samples by comparison with standards of similar concentration.

Results and Discussion

Influence of temperature and ethrel concentration. The influence of temperature on the release rate of ethylene from the dispenser was studied because these scolytids attack olive trees over a wide period of time (usually March-May) (Arambourg 1986), depending on the year and the climatic conditions, and because of the documented relationship between ethylene emission and temperature (Hansen 1945, Burg & Thimann 1960, Phan 1965).

The initial emission rate from ethrel solutions ranged from 0 to 2 nl/h at 25°C (Fig. 2A) and increased by a factor of 10 at 35°C (Fig. 2B). In the absence of plant material, ethylene release was similar for the three dilutions at 25°C and higher than undiluted ethrel for the dilutions at 35°C.

The addition of exogenous ethylene to plant material has been reported to enhance ethylene production (Goren et al. 1988). Therefore, an olive leaf was placed in the plastic capsules containing ethrel. The addition of an olive leaf triggered ethylene release at both temperatures tested (Figs. 2A, B), and the emission rates were higher and more prolonged for the dilutions assayed (1% to 0.01%) than for ethrel alone. According to Cooke & Randall (1968), the pH of the cytoplasm of plant cells facilitates ethylene emission from ethrel. However, the ethylene initially released by ethrel may act to promote the autocatalytic production of endogenous ethylene (Daniell & Wilkinson 1972, Ben-Tal & Lavee 1976). This latter mechanism would be responsible for the observed increase in ethylene release when plant material is added.

For the different concentrations tested, no conclusion can be drawn, as they behaved in different ways for the two temperatures studied. It seems that both the 1% and 0.01% dilutions provided the best results. Therefore, for the rest of the assays, ethrel at a 1% dilution in water was used.

Influence of drilling a hole in the dispensers. For the two temperatures studied, and independently of their color, plastic capsules with holes in their top cover showed higher ethylene release rates (Figs. 3A, B) compared with those without holes. This finding may be explained by an insufficient diffusion of ethylene through the plastic walls that may be compensated for by a rapid escape of the gaseous ethylene through the hole.

Ethylene emission rate from the white capsules without the hole was slightly lower than observed with the previous experiments (Figs. 2A, B). Because the only difference was that a smaller quantity of olive leaf had been added to the capsules, the influence of the quantity of plant material added has to be taken into account. The black polyethylene capsules, which are usually used to prevent photodegradation of UV-sensitive compounds, such as some insect pheromones, provided similar release rates (Figs. 3A, B). Nevertheless, due to the small size of both kind of capsules, the volume of ethrel and the quantity of leaves were limited. Therefore, new dispensers, with higher capacities were tested.

Influence of the volume of ethrel and number of leaves. When three olive leaves were added to ethrel solutions (Fig. 4A), ethylene release levels similar to those reached in the temperature experiments, which contained 1 ml of ethrel and an olive leaf (Fig. 2A), were obtained. The addition of 15 olive leaves produced more ethylene than when only three leaves were added, regardless of the volume of ethrel (Fig. 4A). This result confirms the previous findings of the effect of plant material.

Figure 4B shows the results with the same kind of dispensers at the same temperature (25°C), but with holes in their top covers. Similar trends in the release of ethylene for the different number of leaves were observed: release rate was higher with 30 than with 15 leaves. When the latter (15 leaves) was compared with data from Fig. 4A, the ethylene release rates reached clearly were higher.

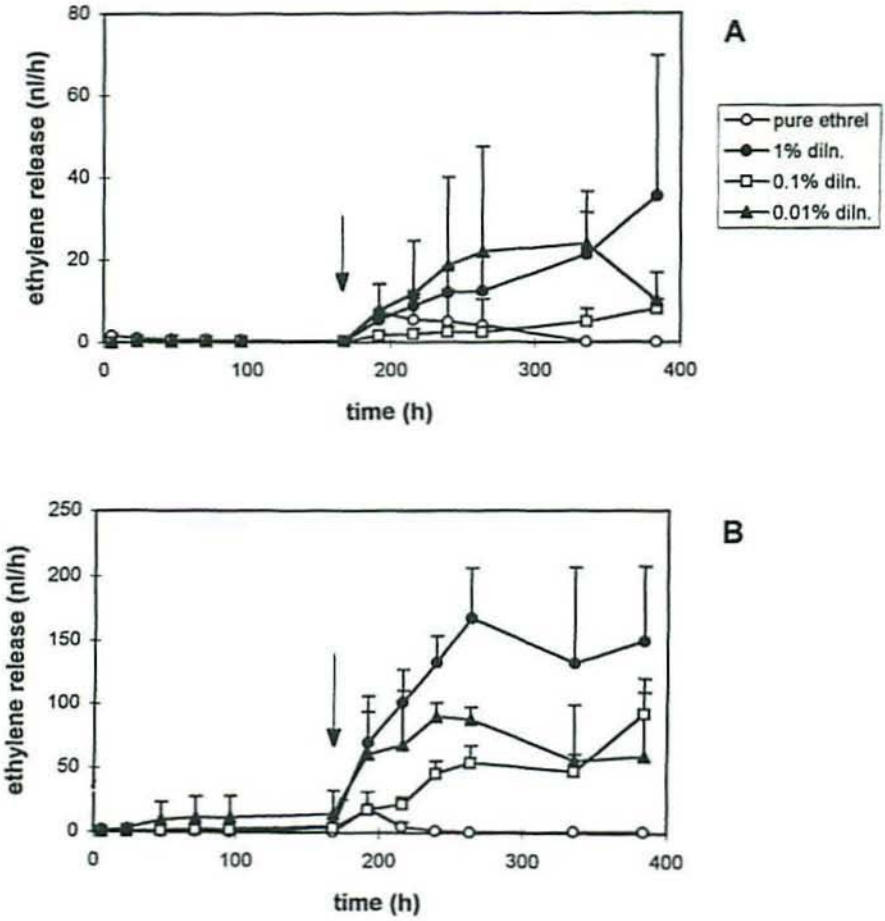

Fig. 2. Ethylene release (+ SD, n = 3) by different ethrel solutions (diln, dilutions) from white, plastic capsules. The arrow indicates the addition of an olive leaf. A. 25°C. B. 35°C.

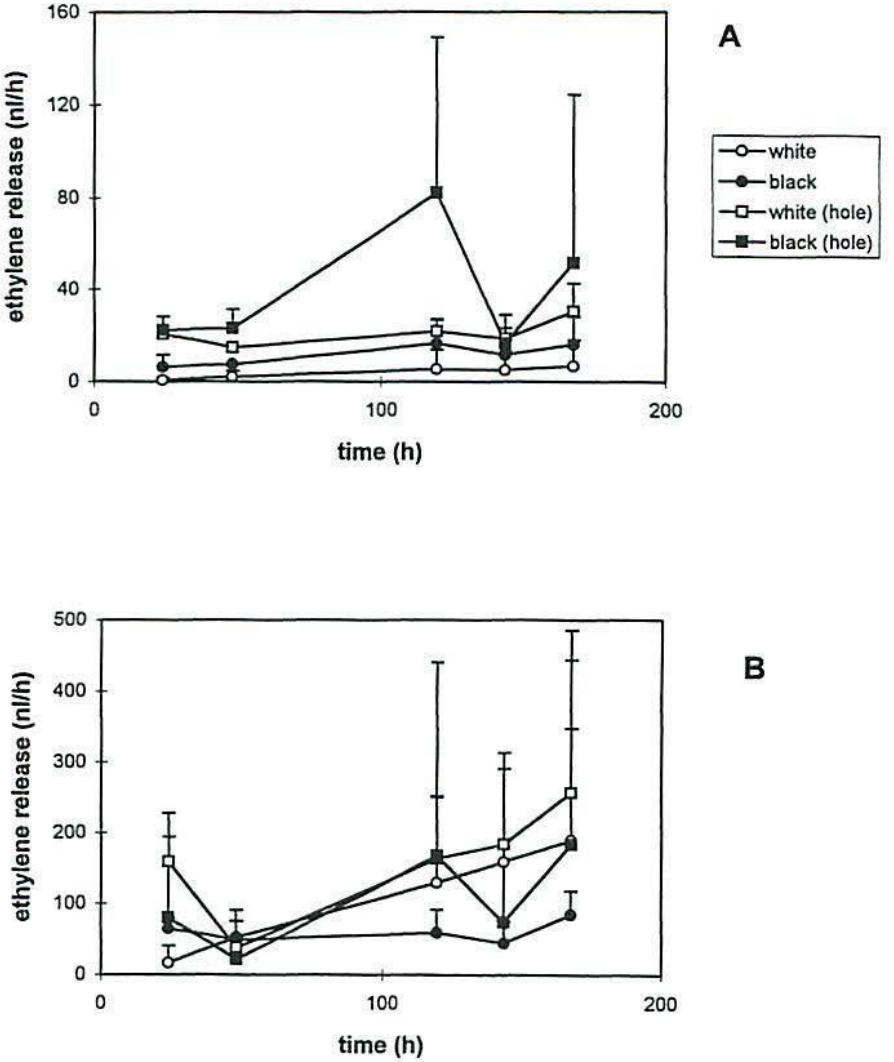

Fig. 3. Ethylene release (+ SD, n = 3) from white and black plastic capsules containing 1 ml of a 1% aqueous ethrel solution plus three pieces of an olive leaf. A. 25°C. B. 35°C.

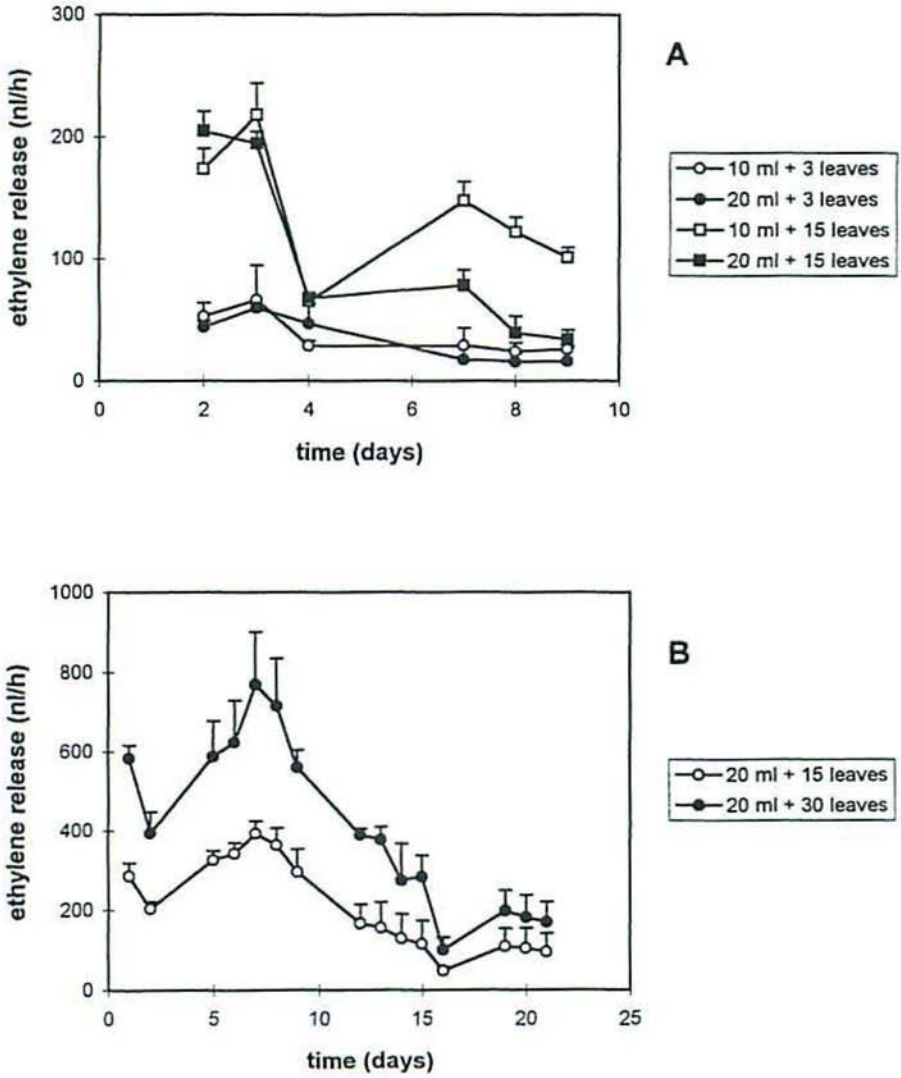

Fig. 4. Ethylene release (+ SD, n = 3) by plastic containers with different volumes of a 1% aqueous ethrel solution and different numbers of olive leaves at 25°C. A. Container without holes. B. Container with six holes.

The presence of holes in the dispenser not only facilitated ethylene liberation from the containers but also their gaseous interchange with the outer atmosphere, and allowed for better oxygenation of the plant material, a process of utmost importance for the production of ethylene from plant tissues (Abeles et al. 1992).

Influence of the addition of NaOH. The increase in the number of holes, together with the number of olive leaves, improved ethylene release rates but the duration was short, no more than 12–15 d, thus making the implementation of the device under field conditions laborious.

Yang (1969) reported that ethylene release from ethrel was improved in an alkaline solution. Thus, serial dilutions of sodium hydroxide were added to 20 ml of 1% ethrel. Figure 5 shows that, unlike what has been previously reported, ethylene emission when NaOH was present was not different from that of the control.

The ethylene levels reached were lower than those reported in the literature (Cooke & Randall 1968, Yang 1969), because the conditions in which those experiments were performed were more drastic, involving heating at 75 or 50°C for 2 h. As the aim of the present work was to design a system able to release ethylene under field conditions, this approach was considered inadequate.

Influence of the addition of different plant material. Different olive plant materials (leaves, stems, and logs) were tested for their ability to increase ethylene release. The absolute amounts of ethylene released per hour by the logs (Fig. 6A) were not only the highest found in all the experiments undertaken (values above 1,000 nl/h) but also their level of production was still high after more than a month. Olive logs, therefore, are more appropriate for the release of ethylene under field conditions than leaves or stems. Leaves and stems deteriorated relatively quickly in the ethrel solution, which may be explained by a higher capacity to lose water, due to a higher surface:mass ratio than in logs.

Recently cut logs and 3-d-old logs were both tested because, according to Campos et al. (1994), the release of ethylene is higher in recently cut olive logs and starts to decrease after 3 d. Hence, it was important to find out whether the age of the log, and its natural ability to release ethylene, had an influence on the enhancement of ethylene release from ethrel. No differences were observed (Fig. 6A).

The different plant materials also were placed in containers with water, to confirm that the presence of ethrel was essential for the liberation of high levels of ethylene (Fig. 6B). In general, plant material alone released very low levels of ethylene. The appearance of fungi after about a week did not substantially increase ethylene release. Therefore, to reach the high levels of ethylene needed for a pest management strategy, the combination of ethrel and plant material is required.

When the results obtained with the logs immersed in ethrel (Fig. 6A) are compared with those obtained by a previous method in which olive logs were directly sprayed with ethrel, similar initial values of ethylene released per log were obtained (Campos et al. 1994). But the proposed dispenser has some advantages: it lasts longer, is not affected by rain, and can be easily substituted. It is important to stress that, in general, the application of ethrel

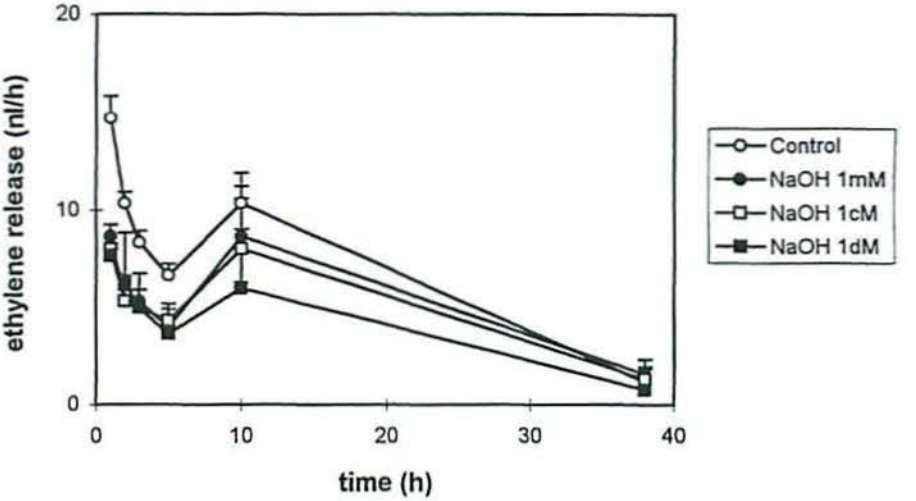

Fig. 5. Ethylene released (+ SD, n = 3) by plastic containers with six holes in their upper surface, each with 25 ml of a 1% aqueous ethrel solution and 1 ml of NaOH at different concentrations (25°C).

to plant tissues stimulates ethylene release for periods up to 10 d (Abeles et al. 1992), but the device presented here is able to maintain very high levels of ethylene for periods longer than a month.

The proposed system seems appropriate as a release device for ethylene, alone or in combination with other semiochemicals or insecticides for the integrated pest management of the olive bark beetle. This system could be adapted for the management of other pests that interfere in the ethylene cycle of a host plant. Thrips (Wien & Roesingh 1980, Kendall & Bjostad 1990) and cotton fleahoppers (Powell & Duffey 1978, Martin et al. 1988) have been reported as triggering ethylene release from host plants, causing higher releases than those produced by mechanical injury alone. It would be interesting to find out whether these pests orient to their plant hosts due to the unusually high levels of ethylene released, as in the case of the olive bark beetle (Campos & Peña 1995, González & Campos 1996). If so, an ethylene dispenser would be suitable for their management.

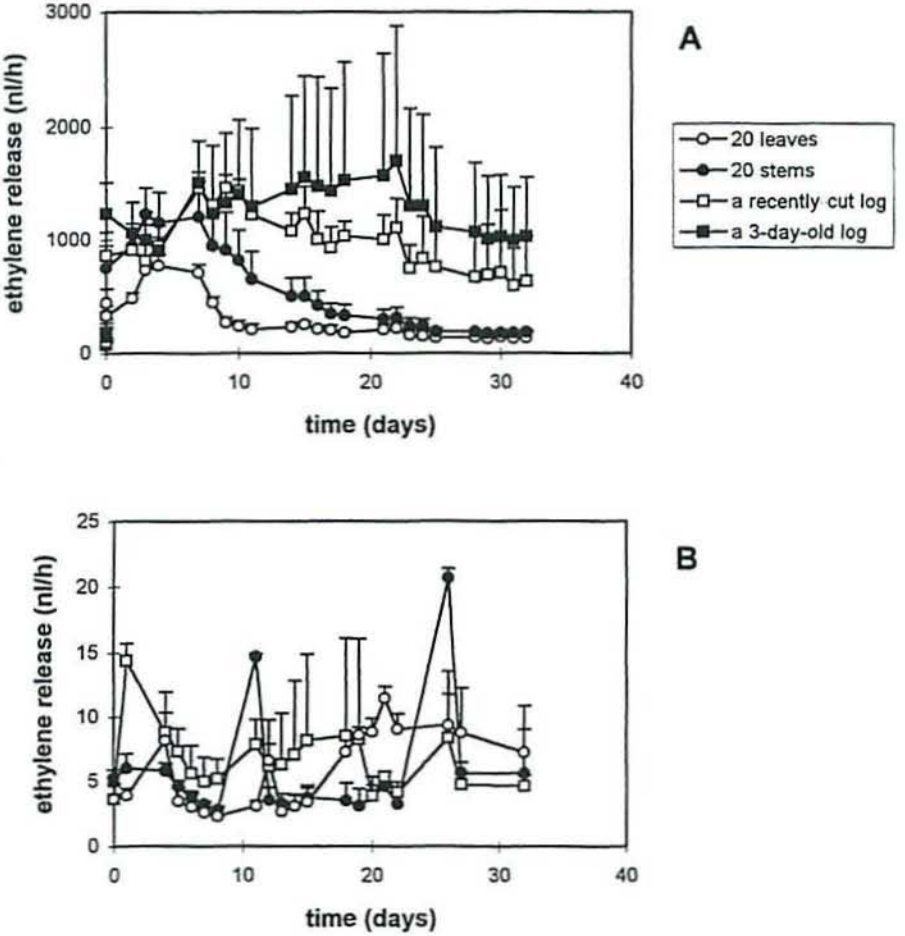

Fig. 6. Ethylene released (+ SD, $n = 3$) by plastic containers with six holes in their upper surface, each containing 25 ml of (A) a 1% aqueous ethrel solution or (B) water, to which different plant material was added (25°C).

Acknowledgement

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Ethylene Release under Field Conditions for the Management of the Olive Bark Beetle, *Phloeotribus scarabaeoides*[1]

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ABSTRACT The need to develop new methods for the management of *Phloeotribus scarabaeoides* (Bernard) (Coleoptera: Scolytidae), an important olive pest, has led to the optimization of an ethylene dispenser that can be used to attract the adult beetles to specific olive logs and trees in the field. The dispenser combines the use of ethrel®, a formulation that releases ethylene, and a piece of olive log, and has proven effective in laboratory experiments. Spraying olive logs with ethrel was the most effective treatment to attract beetles. When logs were sprayed with the insecticide cypermethrin, alone or in combination with ethrel, no colonization occurred. In olive trees, the efficacy of the dispensers for attracting adult beetles was similar to that of spraying trees with ethrel. Consequently, the dispensers can be used, avoiding the undesirable side effects observed when ethrel is directly applied to living trees.

KEY WORDS ethylene, dispenser, olive bark beetle, scolytids, IPM, cypermethrin

Ethylene is a plant growth regulator whose release depends on a plant's physiological stage (vegetative growth, flower and fruit formation) (Sánchez-Raya 1981) and on abiotic (drought, flooding, temperature) and biotic factors (viral, bacterial, and fungal diseases) (Abeles et al. 1992).

Field and laboratory observations have indicated that ethylene plays an important role in the primary attraction of the olive bark beetle, *Phloeotribus scarabaeoides* (Bernard) (Coleoptera: Scolytidae), to olive trees (*Olea europaea* L.) (Campos & Peña 1995, González & Campos 1996). In addition, *P. scarabaeoides* induces increased ethylene release from attacked olive logs (Campos et al. 1994), a response that also has been observed in several other plants after insect infestation (Popp & Johnson 1990, Rieske & Raffa 1995).

Phloeotribus scarabaeoides is one of the principal insect pests of olive trees throughout the Mediterranean coast. It causes harvest reductions (González & Campos 1994) and affects oil quality (Humanes & Civantos 1992). Adult beetles emerge from overwintering galleries and excavate galleries for egg laying in logs left

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over from the annual pruning, and the emerging generation disperses to the olive trees to feed on the young, potentially fruit-bearing branches in which they will overwinter (Arambourg 1986, Benazoun 1992).

Traditional cultural methods of management, such as burying infested logs (de la Torre 1975) and applying insecticide-based treatments to the affected crop, have been suggested to control this beetle (Civantos & Sánchez 1993). However, due to the ecological and health-related problems of insecticide application and to the reluctance of the farmers to apply insecticide, management systems based on the use of semiochemicals, frequently deployed in the control of other scolytids (Byers 1983, Miller & Borden 1992, Schlyter et al. 1992), have been promoted.

Ethylene could be used for the management of *P. scarabaeoides*, either through monitoring its population size, by using it in a mass trapping system, or by combining it with insecticides in a lure-and-kill strategy. The ethylene could be applied to the olive logs where beetles reproduce or to the olive trees (González & Campos 1995) where beetles feed and overwinter.

The direct spraying of ethrel, a formulation containing 2-chloroethyl phosphonic acid that releases ethylene, on olive trees to attract bark beetle adults has severe drawbacks. An excess of ethylene can cause undesirable side effects to the plant, such as leaf abscision and excessive fruit loss (Furuta et al. 1970). Avoidance of these problems would require the precise adjustment of application rates of ethylene to the plant (Abeles et al. 1992). Even on olive logs, where the problems of fruit loss or leaf abcission do not occur, the use of a dispenser is advantageous because ethylene release is longer than for sprayed logs (Campos et al. 1994), without needing substitution for at least 30 d and it is not washed off by rain.

Therefore, the aim of this research was to test the efficiency of a dispenser that combines ethrel and a piece of olive log, a combination that has proven effective in the laboratory (Peña et al. 1998), for releasing ethylene under field conditions for the management of the olive bark beetle. This dispenser could avoid the problems related to the direct application of ethylene to olive trees. In addition, the pyrethroid insecticide cypermethrin was tested on olive logs to determine whether it could be used in combination with ethrel for the management of the olive bark beetle in a lure-and-kill strategy.

Materials and Methods

Laboratory tests—ethrel spraying. Laboratory tests were carried out to confirm the reported effects of leaf abcission when trees are treated with high concentrations of ethrel and to verify the concentration of ethrel that could be safely applied to olive trees.

Three olive terminal branches were sprayed (ca. 1 ml) with each of five aqueous solutions of ethrel (Etisa, Barcelona, Spain) at 0.05%, 0.1%, 0.2%, 0.4%, and 2% and placed in airtight, 2-liter glass containers. Branches sprayed with water were used as a control. Water (60 ml) was added to each glass container to retard dessication of the branches. The open glass containers were maintained at 25°C, closed every day for 30 min, and 3 ml of the resulting atmosphere collected with a gas-tight syringe as previously reported (Campos

et al. 1994) for gas chromatographic analysis. At the end of the experiment the number of remaining leaves was noted. Each treatment was replicated three times.

Laboratory tests—ethrel in dispenser. To avoid the described undesirable effect of an autocatalytic production of ethylene by plant material, ethrel was not applied to the leaf surface, but rather branches were placed together with dispensers containing ethrel and a piece of olive log (Peña et al. 1998), to produce a sufficiently high ethylene level inside the container.

Three olive terminal branches were introduced into airtight, 2-liter glass containers together with a plastic dispenser containing each of five aqueous solutions of ethrel at 0.05%, 0.1%, 0.2%, 0.4%, and 2% plus a piece of an olive log (ca. 3 cm diameter by 5 cm long). Branches with a dispenser containing water were used as a control. Water (60 ml) was added to each glass container to retard dessication of the branches. Experimental conditions and sampling were as above. Each treatment was replicated three times.

Field tests. The field experiments were carried out in 1996 in an olive grove 20 km from Granada (Andalucia, south of Spain), between latitudes 37°18′04″ and 37°20′04″ and longitudes 3°38′10″ and 3°40′10″. The olive trees were 50-70 yr old and separated by a distance of ca. 10 m. Olive trees are pruned every year at the end of winter. The cut logs from pruning are stored for subsequent use as fuel by the farmers in the settlements close to the olive grove. These piled logs represent infestation sites for bark beetles.

Logs. Ethrel, in dispensers or sprayed, was tested in combination with the pyrethroid insecticide cypermethrin when bark beetles initiated the construction of their reproductive galleries. Cypermethrin was evaluated for its possible use in a lure-and-kill system.

Olive logs were set up in small piles because these scolytids are usually attracted to places in which logs are piled, and logs were subjected to the following treatments: (1) spraying the logs to runoff with a 1% aqueous solution of ethrel; (2) placing two dispensers, each containing 25 ml of the 1% ethrel solution plus a small (ca. 5 cm long) piece of an olive log on top of the piled logs; (3) spraying the logs to runoff with a 0.1% (AI) solution of cypermethrin (EC at 10%, Probelte, Murcia, Spain); (4) treatment 1 followed by treatment 3; (5) treatment 2 plus 3; and (6) a control sprayed with water. Each treatment was applied to a pile of 10 olive logs that was placed under an olive tree. The distance between the piles was ca. 30-40 m and a second replication was placed ca. 300 m apart. Olive logs of similar length (ca. 40 cm) and diameter (ca. 5 cm) that were pruned on the same day were used. The treated log piles were placed in the olive grove in April, when bark beetle attack usually takes place in the test area (González & Campos 1990), and remained in the field until the end of bark beetle attack (end of May to the beginning of June). Every week, logs were observed for new galleries that were noted by recording the number of penetration holes.

Additionally, the release of ethylene was followed in the laboratory for three dispensers as described in treatment 2, and for three logs each sprayed according to treatments 1, 4, and 6. Everyday, dispensers and logs were individually introduced for 2 h into airtight, 0.5- or 2-liter glass containers. After sampling 3 ml of the inner atmosphere for gas chromatographic analysis,

they were brought outdoors and sheltered under a roof to mimic the climatic conditions of the logs and dispensers in the field.

Trees. In olive trees, the efficacy of ethrel in dispensers for beetle attraction was compared with spraying. Two alternatives for enhancing ethylene release from the dispensers were considered: either increasing the concentration of ethrel in the dispensers or increasing the number of dispensers per tree.

Six olive trees per treatment were used. Each treatment was separated by 30–40 m and situated around the infestation center, at the edge of the olive crop. Treatments were carried out in June, before the start of bark beetle emergence. Treatments were: (1) spraying the trees with a 0.1% aqueous solution of ethrel; (2) placing six dispensers per tree, each containing 25 ml of 0.1% ethrel solution plus a small (ca. 5 cm long) piece of an olive log; and (3) a control sprayed with water. All the trees were sampled weekly during the period of insect emergence (July-August). In each tree, the terminal 30 cm of six twigs from the northeast orientation, where the attack level is the highest (González & Campos 1993), were selected randomly and inspected for the number of new feeding galleries.

Laboratory analyses of ethylene release. Ethylene release from the different samples was followed with a Carlo Erba Fractovap 2350 gas chromatograph equipped with a flame ionization detector and a 2-m Porapak Q column. Nitrogen was used as the carrier gas. The injector and detector temperature was 150°C. The chromatographic analysis was run isothermally at 60°C. Two milliliters of the total volume taken was injected for each sample. Results were compared with external standards of pure ethylene, expressed as nanoliters of ethylene released per hour and corrected for the different residence times and for the different capacities of the glass containers.

Data analysis. Data were subjected to non-parametric analysis of variance by using the Kruskal-Wallis test, with means separated by Tukey's honestly significant difference (HSD) test (Martín Andrés & Luna del Castillo 1990). All treatment effects and differences among means were tested at $\alpha \leq 0.05$.

Results and Discussion

Laboratory tests. Olive branches that were sprayed with aqueous solutions of ethrel showed an increase in ethylene release with increasing ethrel concentration (Fig. 1A). After 4 d, a degreening of the olive leaves was visible for the branches sprayed with ethrel at concentrations ≥0.2%, but only for the two highest concentrations of ethrel tested (0.4% and 2%) was leaf loss (58 and 49 leaves, respectively) higher than for the lower concentrations considered (between 2 and 10). Because ethylene release from ethrel is enhanced when temperature increases (Burg & Thiman 1960, Peña et al. 1998), and because an increase in the level of ethylene may produce plant damage (Furuta et al. 1970) in treatments in which temperature does not exceed 25°C, living branches can be sprayed with concentrations of ethrel up to 0.2% without increasing leaf abcission. Nevertheless, the treatments on living trees are usually applied in the warm summer months (July-August), when temperatures may reach or exceed 40°C. Due to the increase in ethylene release with increasing temperatures, caution must be exercised not to exceed the maximum bearable ethrel concentration.

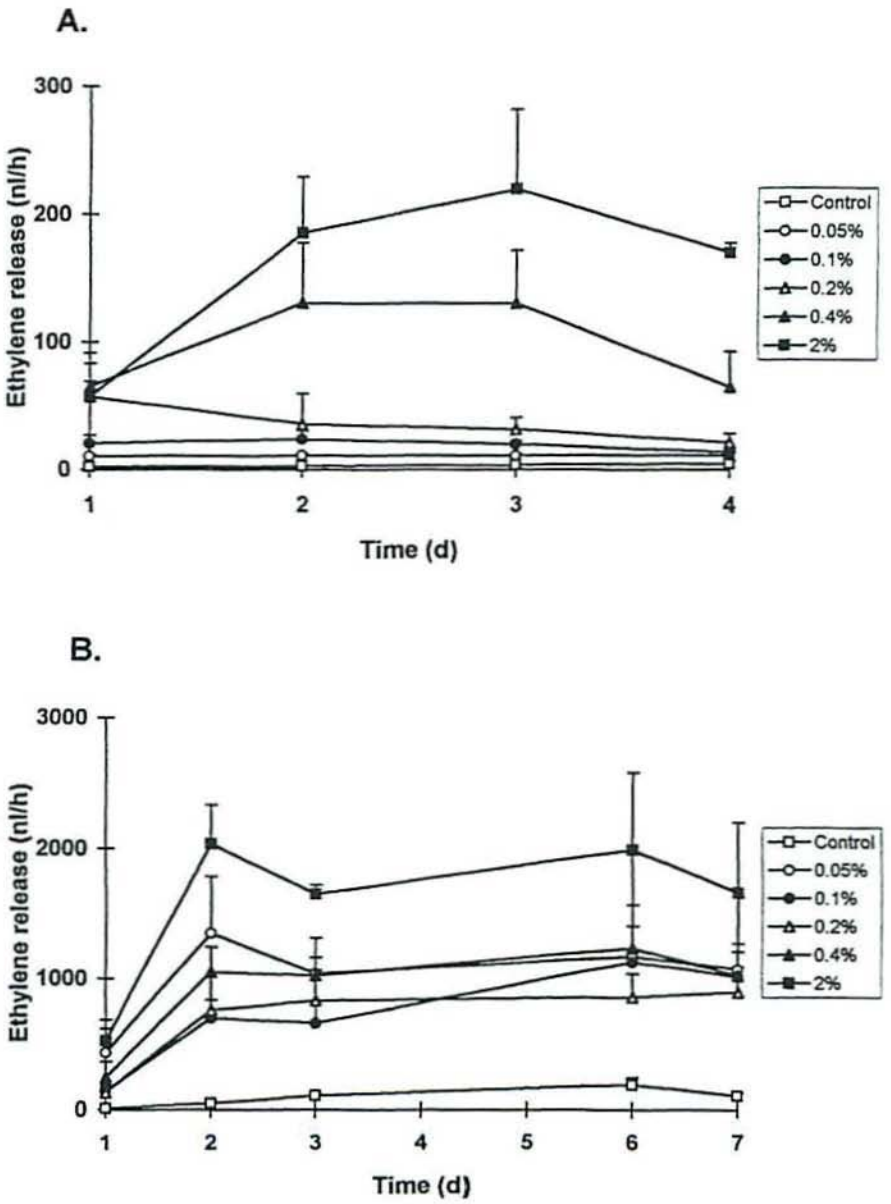

Fig. 1. Ethylene release (+ SD) from olive branches (A) sprayed with an ethrel solution and (B) placed with a dispenser containing an ethrel solution at different concentrations (25°C) ($n = 3$).

In the laboratory tests in which ethrel dispensers were placed together with the olive branches, ethylene release was high for all the treatments, as the dispensers were continuously releasing this gas. Similar ethylene release was reached in all treatments with ethrel except for the dispenser with the highest ethrel concentration (2%) (Fig. 1B). After 4 d, no degreening and negligible leaf abcission (between 0 and 6 leaves fell) was observed from any of the ethrel concentrations, including the control.

If ethylene levels reached in both laboratory experiments (spraying and dispensers) are considered (Figs. 1A, B), the dispenser releases 10 times higher levels of ethylene when compared with spraying at the same ethrel concentration. Thus, the use of the dispensers is advisable because the higher ethylene levels facilitate host finding by olive bark beetles and there is a lower or negligible effect on leaf loss by olive branches.

Field tests with logs. In all the treatments that contained cypermethrin, no reproductive galleries were initiated by beetles. Although the release of ethylene was not affected by the presence of the insecticide (Fig. 2), cypermethrin completely prohibited the colonization of the logs. Kohnle et al. (1992) described a short-range repellent effect by ash (*Fraxinus excelsior* L.) bolts sprayed with cypermethrin for *Hylesinus varius* (F.), a scolytid that also colonizes olive trees. Pyrethroid insecticides have been successfully applied for the control of several bark beetle species (Phillipsen et al. 1986, Pajares & Lanier 1989, Drumont et al. 1992), but in some instances, a lack of efficiency has been observed (Hall 1984, Shea et al. 1984).

Spraying an ethrel solution on olive logs resulted in significantly more penetrations of *P. scarabaeoides* per log (Fig. 3) than placing two ethrel dispensers among the pile of logs. At the end of the experiment the attack level of the logs with the dispensers did not significantly differ from that of the control logs. The release of ethylene from the dispensers was higher than from the sprayed logs (Fig. 2) during the whole experiment. The difference in attraction may lie in that 10 sprayed logs were used as a source of ethylene release in the spray treatments compared with two points of release from the dispensers.

During the field tests, in a 2-wk period between the end of April and the first 10 d of May, precipitation was above average and well in excess of a total of 100 liter/m^2. This may explain why the ethylene release from logs sprayed with ethrel was much longer and higher than in previous experiments (Campos et al. 1994). Laboratory observations have shown that when logs treated with ethrel are wetted, a peak of ethylene release is produced. This peak cannot be observed in Fig. 2 because the logs analyzed for ethylene release were sheltered from the rain.

Field tests with trees. The use of more dispensers of ethrel per tree was finally chosen, taking into consideration the results presented in Fig. 1B, where a 20-fold increase in ethrel concentration (from 0.1% to 2%) produces less than a two-fold increase in ethylene release. As the use of two dispensers per log pile had been insufficient to compete with the spraying with ethrel at the same concentration, the number of vials per tree was increased to six, and the vials were homogeneously distributed under the olive canopy. Figure 4 shows that both treatments were equally effective in attracting emerged bark beetles for

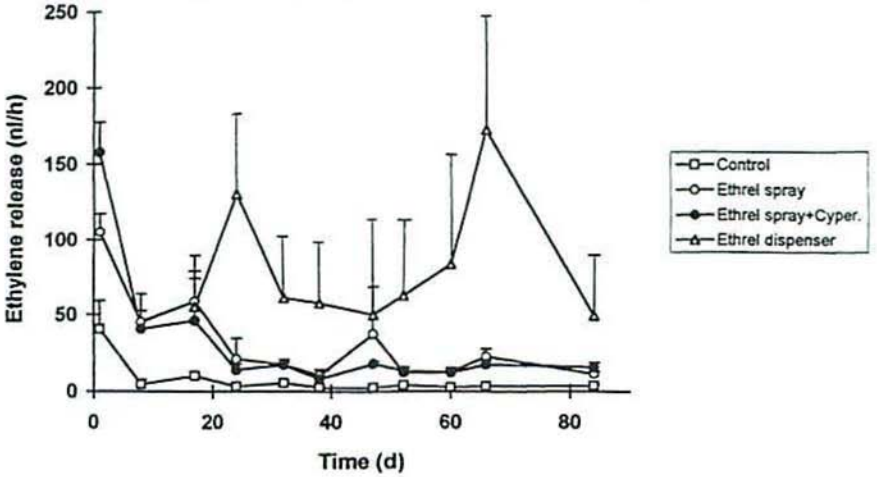

Fig. 2. Ethylene release (+ SD) from olive logs sprayed with ethrel alone or together with cypermethrin, as well as from the dispensers with an ethrel solution ($n = 3$).

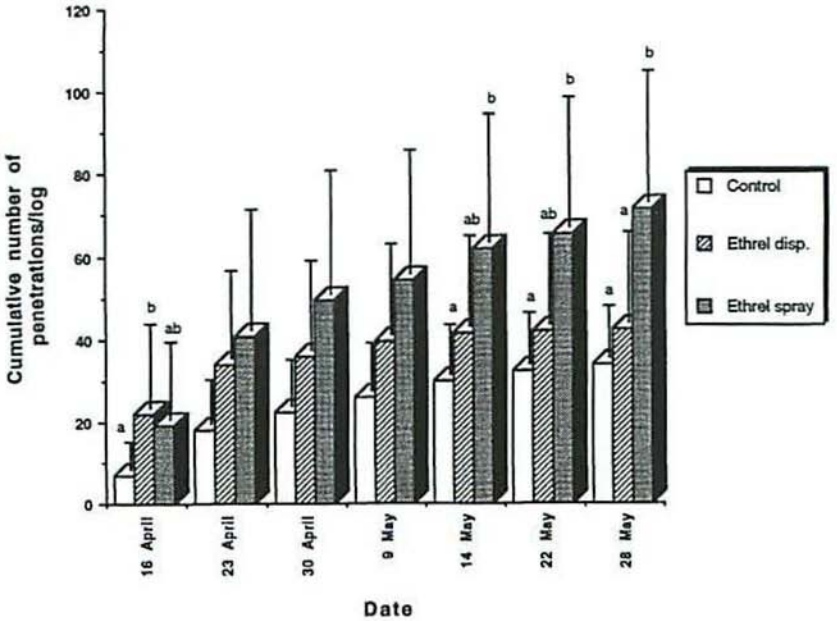

Fig. 3. Cumulative number of reproduction galleries (+ SD) of *P. scarabaeoides* in olive logs treated with ethrel (sprayed or in dispensers) ($n = 20$). Different letters above bars for the same date indicate that means are significantly different (Tukey test, $P < 0.05$)

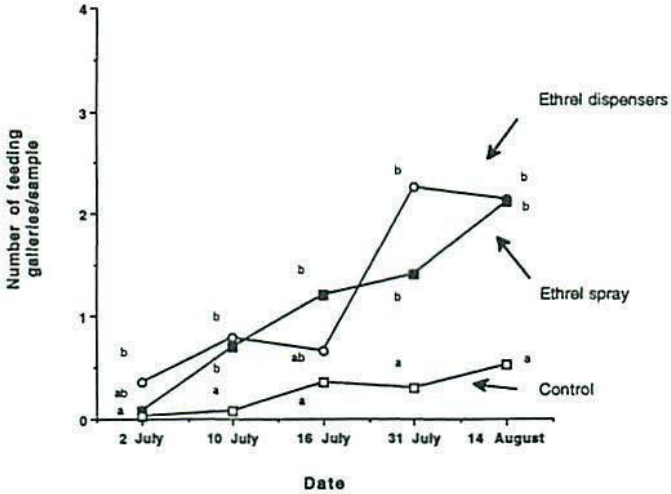

Fig. 4. Average number of feeding galleries of *P. scarabaeoides* found in olive trees (+ SD) treated with ethrel (sprayed or in dispensers) (n = 6). Different letters for the same date indicate that means are significantly different (Tukey test, $P < 0.05$)

feeding and that both were significantly more attractive than the untreated olive trees used as control, based on the average number of feeding galleries.

In summary, the proposed dispenser for ethylene release could be used instead of spraying trees with ethrel. Similar attraction has been achieved for olive bark beetles, and the possible undesirable effects on the plant are avoided. In addition, direct spraying of ethrel to olive trees has to be carefully controlled because an effect on leaf abcission has been observed when olive branches are sprayed with ethrel at concentrations ≥0.2%. For the olive logs, spraying was found to be more effective than using two dispensers per log pile. The lure-and-kill method in olive logs of combining ethrel with cypermethrin proved inefficient due to a repellent effect observed to the insecticide.

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NOTE

Development of Sticky Trap Sampling Technique for Potato Leafhopper Adults[1]

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KEY WORDS Homoptera, Cicadellidae, *Empoasca fabae*, sampling, sticky trap

The potato leafhopper, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), is a major pest of alfalfa in the north-central and northeastern United States. In many instances, potato leafhoppers are the only insects that cause significant economic loss (Smith & Ellis 1983). Potato leafhopper injury to alfalfa may result in reduced yield, quality, and stand longevity or in delayed phenological development (Smith & Ellis 1983, Hutchins & Pedigo 1989).

The potato leafhopper is a particularly difficult insect to manage because management tactics need to be implemented before the appearance of visual damage, specifically leaf chlorosis or "hopperburn" (Gessel et al. 1982). Various techniques have been used for sampling leafhoppers, including sweep net, pans, in situ counts, D-vac, and traps, but few techniques have been found adequate for management decision making (Lamp & Smith 1989). Sweep-net sampling is currently the main technique used for management programs (Gessel et al. 1982, Smith & Ellis 1982, Undersander et al. 1994).

Limited research has been conducted to develop other sampling techniques for use in potato leafhopper management. The use of sticky traps as a sampling technique may be acceptable to farmers. Sticky traps were first used for monitoring leafhopper flight activity (Pienkowski & Medler 1966) and later with limited success, for estimating leafhopper densities (Smith & Ellis 1982, Fleischer et al. 1983). But, the color, orientation, and height of the sticky traps (relative to the canopy) necessary for consistent potato leafhopper catches are not well understood. The objective of these studies was to develop a sticky trap sampling technique for potato leafhopper adults in alfalfa by using a series of studies to compare trap color, orientation, and height.

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Materials and Methods

Studies were conducted in 1994 at the Iowa State University Ross farm near Ames, Iowa. A 3-ha field was seeded with 'Apollo' alfalfa in the spring of 1993, and the studies were conducted in the second crop of alfalfa. When possible, trials within each study were initiated on consecutive days to minimize the influence of alfalfa plant height on trap catch. Three studies were implemented to compare number of potato leafhopper adults trapped according to trap color, orientation (horizontal versus vertical), and height. Trap color studies were repeated two times on consecutive days, whereas trap orientation and height studies were repeated three times. For each study, the number of adult leafhoppers adhering to the sticky traps was counted after 24 h in the field. Potato leafhopper numbers were typical for second crop alfalfa, ranging from one to 12 leafhoppers per sweep (38-cm-diameter sweep net) during the course of the studies. Leafhopper numbers exceeded the economic threshold used in Iowa of two leafhoppers per sweep in alfalfa 20 cm or taller. Alfalfa height ranged from 20 to 30 cm during the course of the studies. Insect counts for each study were analyzed by using analysis of variance (ANOVA) (SAS 1990).

Color preference study. Yellow non-baited Pherocon® AM sticky traps (23 × 28 cm [644 cm^2]) were compared with white sticky traps (20 × 32 cm [640 cm^2]) on 1 and 3 July 1994. Yellow- and white-colored traps were used because these are the main colors available for monitoring insect pests. Traps were folded and tied vertically on lath stakes. The bottoms of the traps were positioned even with the top of the canopy in the alfalfa. A yellow and a white sticky trap were placed in each quadrat (5 m × 5 m) according to a stratified random design. The stratified random sampling involved dividing the field into 10 quadrats and randomly placing each color of sticky trap within each quadrat.

Trap orientation study. Yellow sticky traps (described above) were placed either in a vertical or horizontal orientation in the alfalfa on 7, 9, and 10 July 1994. Traps were either folded, tied, and placed on lath stakes in a vertical orientation or placed flat and tied in a horizontal position on plywood platforms. Both trap orientations exposed the same amount of surface area. A horizontally and a vertically oriented sticky trap were placed in each of 10 quadrats (5 m × 5 m) in a stratified random design. The horizontally placed traps were positioned even with the top of the alfalfa canopy with sticky side facing up. The bottom edge of the vertical traps was placed even with the top of the canopy. The sticky surfaces of the vertical traps were oriented in north and south directions.

Trapping height study. Yellow sticky traps were placed either 25 cm above the canopy or even with the top of the alfalfa canopy on 12, 13, and 15 July 1994. Traps were placed flat and tied in a horizontal position on plywood platforms. The height of the trap was adjusted by using different-sized stakes. One sticky trap at each height (25 cm above canopy and even with top of canopy) was placed in each of 10 quadrats (5 m × 5 m) in a stratified random design.

Results and Discussion

Color preference study. In both trials (Table 1), significantly greater numbers ($P < 0.05$) of adult potato leafhoppers were collected on yellow sticky traps than on white traps. These data agree with Pienkowski & Medler (1966) who found the adult leafhoppers prefer yellow surfaces to white surfaces. Based on these findings, yellow sticky traps were used for the orientation study.

Table 1. Mean number ± SE of adult potato leafhoppers captured on white and yellow sticky traps placed vertically in alfalfa.

Trial	Yellow	White	*F* test
1	8.1 ± 1.2	3.0 ± 0.4	$F = 14.55$; df = 1, 9; $P = 0.004$
2	7.3 ± 1.0	1.6 ± 0.3	$F = 29.81$; df = 1, 9; $P = 0.029$

SE, standard error of mean.
α level = 0.05.

Trap orientation study. Significantly greater numbers ($P < 0.05$) of adult leafhoppers were captured on the horizontal sticky traps compared with the vertical traps in all three trials (Table 2). These findings conflict with Fleischer et al. (1983) who did not find any difference in numbers of leafhoppers captured per day when using horizontal and vertical trap orientations. But, in their study, 3-dimensional yellow cylinders, painted with Tac Trap®, were used instead of the 2-dimensional (flat) sticky traps used in these studies. The difference between vertical and horizontal spatial orientations, when using a 3-dimensional trap, was not as contrasting as when a 2-dimensional trap (90° difference in spatial plane) was used. Therefore, traps were placed horizontally in the alfalfa for the trapping height study.

Trapping height study. Significantly greater numbers ($P < 0.05$) of potato leafhoppers were collected on the traps placed even with the top of the alfalfa canopy compared with the traps placed 25 cm above the canopy in two out of three trials (Table 3). Alfalfa height ranged from 25 to 35 cm during the study. These findings are supported by Pienkowski & Medler (1966) who found that local flight patterns were those of low-level flight within the alfalfa. Fleischer et al. (1983) also captured more leafhoppers at lower sticky trap heights in alfalfa.

The results of our series of studies showed that yellow sticky traps, placed horizontally, even with the top of the alfalfa canopy, will capture the most potato leafhoppers. This sticky trap technique was developed for use in sampling programs to make relative estimates of potato leafhopper density (DeGooyer 1997).

Table 2. Mean number ± SE of adult potato leafhoppers captured on vertical and horizontal yellow sticky traps in alfalfa.

Trial	Horizontal	Vertical	*F* test
1	16.2 ± 2.1	3.0 ± 0.5	$F = 41.53$; df = 1, 9; $P = 0.0001$
2	28.9 ± 2.4	3.9 ± 0.5	$F = 126.12$; df = 1, 9; $P = 0.0001$
3	48.9 ± 4.8	0.6 ± 0.3	$F = 97.09$; df = 1, 9; $P = 0.0001$

SE, standard error of mean.
α level = 0.05.

Table 3. Mean number ± SE of adult potato leafhoppers captured on horizontal yellow sticky traps at two heights in alfalfa.

Trial	Even with top of canopy	25.4 cm above canopy level	*F* test
1	10.2 ± 2.4	7.2 ± 1.5	$F = 0.94$; df = 1, 4; $P = 0.3878$
2	31.0 ± 1.6	16.2 ± 9.0	$F = 15.27$; df = 1, 4; $P = 0.0174$
3	49.8 ± 4.2	24.0 ± 2.9	$F = 49.53$; df = 1, 4; $P = 0.0021$

SE, standard error of mean.
α level = 0.05.

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NOTE

A Freeze-Thaw Stable Diet for Lepidoptera[1]

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KEY WORDS Lepidoptera, diet

Artificial insect diets are an essential component in many insect rearing systems that produce insects for research purposes. Complex agar-gelled diets are generally made in large batches and used shortly after preparation because degradation of perishable diet ingredients such as vitamins and fatty acids can adversely affect insect quality (Brewer & Lindig 1984). However, the timing of diet preparation may be inconvenient and large batches wasteful if the unused, excess diet is discarded. Numerous, small batches of diet prepared on different days might reduce waste but they are likely to be more variable than a single, large quantity of diet prepared in 1 d. The quality control and preparation efficiency of finished diets could be increased by the development of diets with increased storage stability.

Hydrocolloids are a group of long-chain polymers that are widely used in the food industry to enhance the texture (viscosity), emulsification, and stability of products (Dziezak 1991). Xanthan gum, a high molecular weight polysaccharide produced by the microorganism *Xanthomonas campestris* strain NRRL B1459, is particularly useful for syneresis (exudate production) control and for increasing the freeze-thaw stability of processed foods (Ferrero et al. 1993, 1994). Gelatinized precooked waxy-maize starches also are widely used to increase the freeze-thaw stability and quality of processed food products (Kobs 1997). In the present study, freeze-thaw stable diets containing xanthan gum and xanthan gum combined with gelatinized starch were examined for suitability as lepidopteran diets.

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Materials and Methods

Diets. A modified version of the soybean flour-wheat germ diet originally described by Vanderzant (1974) and suitable for rearing many species of Lepidoptera (King & Hartley 1992) formed the basis for the diets. Premixed, dry diet, without agar, and antimicrobial solution (phosphoric acid + propionic acid) were purchased from Southland Products, Lake Village, Arkansas. The diet ingredients required to make approximately 1 L of freezable diet are listed in Table 1. Freeze-thaw stability was imparted to the diet by the addition of xanthan gum (Keltrol®, Kelco Co., San Diego, California) and precooked waxy-maize starch (Dura-Jel®, A. E. Staley Co., Decatur, Illinois).

Table 1. Soybean flour-wheat germ lepidopteran diet with freeze-thaw stability.

Ingredient	Amount (g/L)
Soybean flour (Nutrisoy Flour #40)	41
Wheat germ	35
Wesson salt	10
Sugar	41
Vitamin mix	9.5
Methyl paraben	1.0
Aureomycin (6% soluble powder)	1.0
Sorbic acid	1.0
Serva agar (Serva, Feinbiochemica GmbH & Co.)	13
Xanthan gum (Keltrol®)	2.35[a], 1.18[b]
Dura-Jel starch (A. E. Staley Co.)	30[b]
Water	930 ml
Mold inhibitor (phosphoric acid [3.8%] + propionic acid [42%])	3.0 ml

[a] Diet #1 (see text for details)

[b] Diet #2 (see text for details)

Three diet formulations were prepared in ca. 1-L batches. A magnetic hot plate was used to first dissolve 2.35 g of xanthan gum (diet #1), 1.18 g of xanthan gum and 3 g of Dura-Gel starch (diet #2), followed by 13 g of agar into 930 ml of distilled water. Control diet, diet #3, had only agar (no xanthan gum or starch). The ingredients were slowly added, with stir bar agitation, and heated to boiling. The hot liquid then was transferred to a blender, 165 g of dry diet mix and 3 ml of mold inhibitor were added, and the mixture was thoroughly blended for ca. 3 min. Diet was transferred to 500-ml wash bottles (with 1-cm spouts) and dispensed, in 1–1.5 ml amounts, into the wells (2-ml capacity) of 96-well trays (Jarold Co., St. Louis, Missouri) or in 7–9-ml amounts into wells (ca. 15-ml capacity) of 32-well trays (Oliver Products Co., Grand Rapids, Michigan). After 10–15 min of cooling/hardening under a BioHood, the diet was covered with heat-sealed Mylar plastic (Clear Lam Packaging, Elk Grove Village, Illinois) and then stored at ca. -20°C.

Feeding bioassay. The suitability of frozen-defrosted diet for supporting larval growth was determined using larvae of the tobacco budworm, *Heliothis virescens* (F.). To study diet #1, individual neonate larvae of *H. virescens* were placed into small wells of diet either freshly prepared or previously stored for 58 d at -20°C then thawed for ca. 30 min at 23°C. Four replicates of 20–24 larvae each were tested per treatment; total sample size was 86 larvae for the freshly prepared diet and 88 larvae for the frozen/thawed diet. Larvae were incubated for 6 d at 28°C then weighed to determine mean larval weight per replicate. One additional replicate of 24 larvae was tested on diet stored at ca. -20°C for 211 d. Diet #2 was tested in 32-well trays by comparing standard unfrozen diet (no xanthan gum or starch), freshly prepared, unfrozen diet #2, and diet #2 that was stored frozen for 7 d at -20°C and then thawed. Twenty-four neonate *H. virescens* larvae were initially placed on the test diets, allowed to develop at ca. 28°C until pupation was complete (15 d), and then pupae were individually weighed. Final sample sizes (surviving pupae) for the standard diet, unfrozen diet #2, and frozen diet #2 were 18, 17, and 18 pupae, respectively.

Data analysis. Weights of larvae and pupae, in diet #1 and diet #2 comparisons, were compared by analysis of variance using the general linear model (PROC GLM) of SAS (SAS Institute 1990).

Results and Discussion

Freezing and thawing the xanthan gum lepidopteran diet (diet #1) and long-term storage did not affect its suitability for supporting larval growth of *H. virescens*. The mean weights (± 1 SD) of *H. virescens* larvae after 6 d on diet that was freshly prepared (87.9 ± 7.0 mg) or stored for 58 d at -20°C (88.2 ± 8.8 mg) were not significantly different ($F = 0.002$; df = 1, 6; $P > 0.05$). Mean (87.9 ± 7.0 mg) weight of 24 larvae after 6 d growth on 211 d old frozen-thawed diet #1 was 108.6 ± 22.5 mg, suggesting that prolonged frozen storage did not have an adverse effect on diet quality. Similarly, mean weights (± 1 SD) of pupae reared on unfrozen control diet (343.0 ± 27.8 mg), unfrozen diet #2 (336.4 ± 32.5 mg), or frozen/thawed diet #2 (321.3 ± 29.7 mg) were not significantly different ($F = 2.47$; df = 2, 52; $P > 0.05$). Standard lepidopteran diet (diet #3) frozen and stored

at -20°C had greater ice recrystallization (large ice crystals outside of the diet) compared with diet #1 and diet #2. When thawed, diet #3 showed pronounced syneresis or "pooling" of liquid on the diet surface, making it unsuitable for *H. virescens* larval inoculation, and a spongy liquid texture similar to that of cottage cheese. A diet with the texture of thawed standard diet would likely be unsuitable for species such as European corn borer (*Ostrinia nubilalis* [Hübner]) or pink bollworm (*Pectinophora gossypiella* [Saunders]) with larvae that bore into diet.

The procedures used to prepare the diets described here could easily be applied to other insect diets, but the optimum concentrations of xanthan gum or modified starch may differ. A useful starting point would be 0.1% to 0.5% (total diet weight) xanthan gum combined with 1% to 3% starch (M. Bradford, A. E. Staley Co., personal communication). Higher percentages of xanthan gum and starch in diet might provide enhanced freeze-thaw stability, but these diets are more difficult to prepare due to greater viscosity. Furthermore, the potential of higher concentrations of gum and starch for reducing the rate of larval development would require investigation. The rate of freezing and the temperature used for frozen storage can affect deteriorative changes in starch-based foods (Ferrero et al. 1993, 1994). This suggests that, for best results, insect diet should be frozen rapidly and stored at the lowest possible temperature (-20°C or lower).

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Statewide Distribution of Parasitoids of the Alfalfa Weevil in Tennessee[1]

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ABSTRACT The alfalfa weevil, *Hypera postica* (Gyllenhal), is the most important insect pest of alfalfa, *Medicago sativa* L., in Tennessee. In 1994, a 2-yr statewide research project was initiated to monitor alfalfa weevil and its biological control agents in 27 alfalfa fields in nine counties. Alfalfa weevils overwintered both as eggs and as adults. Parasitism of all field-collected larvae (n = 6,215) was 11.39%, whereas parasitism of all field-collected adults (n = 460) was 0.43%. *Bathyplectes anurus* (Thomson) and *Bathyplectes curculionis* (Thomson), parasitoids of alfalfa weevil larvae, were recovered in all counties sampled. In eastern and middle Tennessee, *B. anurus* was the predominant larval parasitoid, suggesting *B. anurus* has displaced the previously predominant *B. curculionis* in these areas of Tennessee. Percent parasitism of alfalfa weevil larvae by *B. anurus* and *B. curculionis* was 7.12% (n = 6,215) and 4.12% (n = 6,215), respectively. Statewide, *Microctonus aethiopoides* (Loan) was recovered in low numbers, suggesting poor establishment of this parasitoid of adult alfalfa weevils in Tennessee. *Zoophthoora phytonomi* (Arthur) Batko, a fungal pathogen of alfalfa weevil larvae, was detected from early March to late April. Because few follow-up studies have been undertaken to determine the establishment and distribution of these introduced parasitoids in Tennessee, this research should provide baseline information to improve biological control of the alfalfa weevil in Tennessee.

KEY WORDS *Hypera postica*, alfalfa weevil, *Medicago sativa*, alfalfa, parasitoids, *Bathyplectes anurus*, *Bathyplectes curculionis*, *Microctonus aethiopoides*, biological control

The western strain of the alfalfa weevil, *Hypera postica* (Gyllenhal), was accidentally introduced into the United States from Europe at the beginning of the twentieth century (Anonymous 1991, Bryan et al. 1993). This insect pest was soon followed by the eastern strain, which was accidentally introduced into Maryland in 1951 and threatened alfalfa production, especially in the southeastern United States

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(Poos & Bissell 1953, Grant & Lambdin 1990). Because host-specific natural enemies were absent in the eastern United States, researchers directed emphasis towards implementing classical biological control to reduce populations of the eastern strain of the alfalfa weevil (Day 1981, Anonymous 1991, Bryan et al. 1993).

Since 1962, several exotic parasitoid species have been released against the alfalfa weevil in Tennessee (Bennett 1968, Brunson & Coles 1968, Dysart & Day 1976, Grant & Lambdin 1990, Bryan et al. 1993). Two of the most commonly released parasitoids, *Bathyplectes curculionis* (Thomson) and *Bathyplectes anurus* (Thomson), attack alfalfa weevil larvae (Dysart & Day 1976, Giles et al. 1994, Hogg 1994), and another parasitoid, *Microctonus aethiopoides* (Loan), attacks alfalfa weevil adults (Flessel & Niemczyk 1977, Grant & Lambdin 1990). This adult parasitoid is one of the most important biological control agents of the alfalfa weevil because it reduces egg production and has the greatest potential to reduce alfalfa weevil populations (Flessel & Niemczyk 1977, Van Driesche & Gyrisco 1979, Munir & Sailer 1984, Kingsley et al. 1993).

Although numerous releases of parasitoids have been made in Tennessee, little information is known about the success of these releases. A statewide assessment is necessary to determine the establishment, distribution, and role of parasitoids of the alfalfa weevil in Tennessee. The objective of this study was to determine parasitoid species composition and level of parasitism of the alfalfa weevil in Tennessee.

Materials and Methods

A 2-yr study was conducted during 1994 and 1995 to determine statewide establishment and distribution of introduced parasitoids. Nine counties were selected for evaluation, three each in eastern, middle, and western Tennessee (Fig. 1). In each county, three alfalfa fields were selected in cooperation with county agents, and each field represented a typical alfalfa field in production. Complete information on agronomic practices in each field was unavailable. All 27 alfalfa fields were sampled twice, ca. 4–8 wk apart, during early season (March to May), and all sampling was completed before the first cutting of alfalfa. Because of the design of this statewide study, it was not feasible to sample each of the 27 fields more than two times during early season. To minimize the chances of missing peak populations of alfalfa weevil, our first sample was made when larval populations were present and the second sample was made when alfalfa weevil adults were expected. This sampling methodology may have missed peak populations in some fields and would have not included sampling for adult weevils after the first cutting.

1994. On each sampling date, 100 sweep-net (15 cm diameter) samples were collected in four sets of 25 sweeps each. The contents of each set of sweeps were placed into a labeled paper bag (35 × 17 cm), placed into a cooler, taken to the laboratory, and processed.

In the laboratory, each labeled bag was emptied into a plastic pan (32 × 26 cm), and the contents were evaluated. Alfalfa weevil adults were counted, removed, and placed into petri dishes (100 × 15 mm) (n = 48) and provided

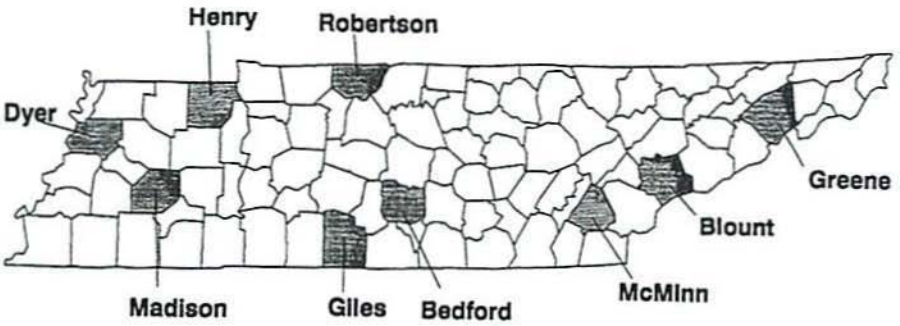

Fig. 1. Location of alfalfa weevil sampling sites in Tennessee, 1994–1995.

alfalfa foliage for food. The numbers of adult weevils placed into petri dishes were dependent upon the number collected; however, to avoid overcrowding, no more than 20 alfalfa weevil adults were placed into each petri dish. Larvae of the alfalfa weevil were placed in groups of 10 into petri dishes (100 × 15 mm) (n = 642) and provided alfalfa foliage. Alfalfa weevils were maintained in an insectary at room temperature (ca. 29°C) at the University of Tennessee Plant Science Farm. Adults and larvae were monitored every other day until death, adult emergence, or emergence of parasitoids. The numbers of parasitized larvae and adults, and percent parasitism, were determined. Actual parasitism percentages are probably larger than those presented because parasitism estimates were based on rearing of larvae and adults, which may underestimate parasitoid impact (Day 1994). In fact, the impact of a parasitoid species on an insect pest is difficult to accurately measure, especially when hosts that are collected from the field are used to evaluate parasitoid incidence and influence on pest populations (Simmonds 1948, Van Driesche 1983). However, studies of this type, conducted under field conditions, do provide a basic understanding of the possible influence of a parasitoid species on a pest species.

The larval parasitoids *B. curculionis* and *B. anurus* were identified by the color and shape of the cocoon. The adult parasitoid *M. aethiopoides* was identified by its cocoon and emergence from adult alfalfa weevils. The numbers of larvae infected by a fungal entomopathogen also were recorded. All data were compared among counties and geographical regions of the state.

1995. The same procedures as described for 1994 were used in 1995, with one modification. On each sampling date, an additional 100 sweep-net (15 cm diameter) sample in four sets of 25 sweeps each was collected in each field and taken to the laboratory. The contents of these four sets of sweeps were placed in a freezer (-17°C) and held until they could be sorted and processed. Each set was then emptied into a plastic pan (32 × 26 cm), and subsets of the samples were observed separately under a microscope. During processing, alfalfa weevil larvae and adults, as well as adult parasitoids, such as *B. curculionis*,

B. anurus, and *M. aethiopoides*, were separated from the samples, and parasitoids were labeled and pinned. All data were compared among counties and geographical regions of the state.

Data for all tests were analyzed using General Linear Models. Means were separated using Duncan's multiple range test ($P \leq 0.05$) (SAS 1989).

Results and Discussion

Alfalfa weevil larvae and adults were found in all nine counties. The mean number of larvae per 25 sweeps was highest in Robertson County and lowest in Bedford County (Table 1). In general, larval densities were higher in middle Tennessee and lower in western Tennessee, where less alfalfa is grown than in other areas of the state. Densities of larvae varied among counties, as significantly greater numbers of larvae were found in Robertson County than in all other counties except Giles County (Table 1). Environmental and agronomic conditions may have been more conducive for alfalfa weevil growth, development, and survival in Robertson County. Differences in larval densities occurred among counties in each region of the state (e.g., east, middle, and west).

Larval densities, averaged across all fields and both sampling times, were not significantly different between years (42.67 larvae per 25 sweeps and 41.37 larvae per 25 sweeps in 1994 and 1995, respectively). However, larval densities were significantly different between the first and second sampling dates for both years, as greater numbers of larvae were collected on the first sampling date (80.55 larvae per 25 sweeps [n = 204]) than on the second sampling date (3.22 larvae per 25 sweeps [n = 203]). These differences were expected and are attributed solely to the biology and seasonality of the alfalfa weevil.

Numbers of alfalfa weevil adults collected from sweeps were significantly different among counties (Table 1). The mean number of adults per 25 sweeps was highest in Blount County and lowest in Henry County. Densities of alfalfa weevil adults also differed between years, as significantly greater numbers of adult weevils were found in 1995 (2.58 weevils per 25 sweeps [n = 216]) than in 1994 (0.29 weevils per 25 sweeps [n = 191]). In 1995, environmental factors, such as mild winter conditions, may have been more favorable for survival of alfalfa weevil eggs, larvae, and adults.

The total numbers of *Bathyplectes* spp. reared from alfalfa weevil larvae were significantly different between years, as greater numbers of *Bathyplectes* spp. were reared from alfalfa weevil larvae in 1995 (493 *Bathyplectes* spp. [n = 3,776]) than in 1994 (200 *Bathyplectes* spp. [n = 2,439]). Differences also were noted between samping dates, as significantly more adult *Bathyplectes* spp. were found on the first sampling date (2.81 *Bathyplectes* spp. per 100 sweeps [n = 204]) than on the second sampling date (0.69 *Bathyplectes* spp. per 100 sweeps [n = 203]). This difference can be partially attributed to the expected presence of more alfalfa weevil larvae on the first sampling date.

Percent parasitism of alfalfa weevil larvae by *Bathyplectes* spp. was significantly affected by year, as parasitism of larvae by *Bathyplectes* spp. was greater in 1995 (11.29% parasitism [n = 3,776]) than in 1994 (7.23% parasitism [n = 2,439]). Numerical differences in percent parasitism among counties also

Table 1. Average densities of alfalfa weevil larvae and adults collected in nine counties throughout Tennessee during 1994 and 1995.

County	Alfalfa weevil		Sampling dates	
	Larvae	Adults	1994	1995
Bedford	20.19 ± 39.71c[a]	2.58 ± 4.82ab	12 May 20 May	24 March 6 May
Blount	39.36 ± 57.36bc	3.03 ± 5.17a	27 April 19 May	26 March 8 May
Dyer	36.71 ± 58.66bc	1.98 ± 5.35abcd	21 April 24 May	23 March 29 April
Giles	60.54 ± 96.66ab	1.08 ± 3.48bcd	22 April 20 May	24 March 6 May
Greene	23.71 ± 38.81c	0.96 ± 3.20bcd	20 April 18 May	26 March 7 May
Henry	31.79 ± 43.51bc	0.52 ± 0.95d	22 April 24 May	23 March 29 April
Madison	22.28 ± 41.55c	0.85 ± 1.99cd	21 April 29 May	23 March 29 April
McMinn	45.42 ± 66.25bc	0.77 ± 2.25cd	21 April 19 May	25 March 8 May
Robertson	91.31 ± 180.45a	2.42 ± 4.77abc	28 April 25 May	24 March 6 May

[a]Mean ± SD per 25 sweeps; means within a column followed by the same letter are not significantly different ($P > 0.05$).

were noted. In 1994 and 1995, parasitism was highest (26.23% [n = 568]) in Greene County and lowest in Madison County (4.67% [n = 515]) (Table 2). Percent parasitism in the other counties ranged from about 5.18% (Dyer County) to 18.75% (Blount County).

The numbers of *B. anurus* collected statewide were significantly affected by year, county, and sampling date. Significantly greater numbers of *B. anurus* were reared from alfalfa weevil larvae in 1995 (298 *B. anurus* [n = 3,776]) than in 1994 (122 *B. anurus* [n = 2,439]). Parasitism by *B. anurus* was higher in Blount, Giles, and Greene counties than in any other county (Table 2), and more *B. anurus* were found in the eastern region of the state. Numbers of *B. anurus* adults were significantly greater on the first sampling date (2.14 *B. anurus* per 100 sweeps) than on the second sampling date (0.01 *B. anurus* per 100 sweeps).

The numbers of *B. curculionis* collected statewide were significantly affected by year, as more *B. curculionis* were reared from larvae (195 *B. curculionis* [n = 3,776]) in 1995 than were reared in 1994 (78 *B. curculionis* [n = 2,439]). Parasitism by *B. curculionis* varied among counties (Table 2). Percent parasitism by *B. curculionis* was highest in Henry County and lowest in Blount County. In general, percent parasitism by *B. curculionis* was highest in western Tennessee and lowest in middle Tennessee.

Microctonus aethiopoides was not recovered in 1994, and only low numbers were recovered statewide in 1995. In 1995, 460 alfalfa weevil adults were collected and maintained in the laboratory to determine parasitism, and only two *M. aethiopoides* emerged from alfalfa weevil adults (0.43% parasitism). In Robertson County, one *M. aethiopoides* emerged from 69 adult weevils collected on the second sampling date (1.5% parasitism). In Dyer County, one *M. aethiopoides* emerged from 23 adult weevils collected on the second sampling date (4.3% parasitism). The greatest number of *M. aethiopoides* collected in sweep-net samples was found on the first sampling date, corresponding to the presence of overwintered alfalfa weevil adults. Seven *M. aethiopoides* were recovered in Henry, Madison, Bedford, Giles, Blount, and McMinn counties. One *M. aethiopoides* was recovered in Henry County on the second sampling date. These low numbers of *M. aethiopoides* may be attributed to its inability to adapt to the environmental and climatic conditions in Tennessee. However, as discussed in the methodology, these low numbers also may simply reflect our selective and restrictive sampling times, which may have missed population peaks of adults in some fields, especially after the first cutting. Additional sampling throughout the growing season and across the state may more accurately define the establishment level of *M. aethiopoides*.

Zoophthora phytonomi (Arthur) Batko, a fungal pathogen that infects and kills alfalfa weevil larvae, was detected in all nine sampled counties from March to late April. Alfalfa weevil larvae were generally infected by this pathogen in the spring after excessive rainfall and high humidity conditions. Atmospheric moisture levels have been shown to influence the impact of *Z. phytonomi* on alfalfa weevil (Harcourt et al. 1990). The major factor influencing seasonal infection levels of *Z. phytonomi* on *H. postica* is relative humidity, as determined partially by the amount and frequency of rainfall (Barney & Armbrust 1981). Because of the collection method used in this study, no effort

Table 2. Percent parasitism of alfalfa weevil larvae by *Bathyplectes* spp. during 1994 and 1995.

County	Total number of larvae collected[a]	Percent parasitized by		
		Bathyplectes spp.	*Bathyplectes anurus*	*Bathyplectes curculionis*
Bedford	354	5.93	5.08	0.85
Blount	544	18.75	18.01	0.74
Dyer	849	5.18	1.53	3.65
Giles	918	14.60	13.62	0.98
Greene	568	26.23	20.42	5.81
Henry	780	10.36	1.00	9.36
Madison	515	4.67	1.17	3.50
McMinn	718	9.75	3.20	6.55
Robertson	969	7.12	1.44	5.68
Total	6,215	$\bar{x}$=11.40	$\bar{x}$=7.27	$\bar{x}$=4.12

[a]Sampling dates for 1994 and 1995 are listed in Table 1.

was made to accurately define the level of infection by *Z. phytonomi* among larval samples. In previous studies, however, *Z. phytonomi* has been reported to be an important population regulating factor, occassionally causing mortality >50% and, in some cases, >90% (Harcourt et al. 1974, Harcourt et al. 1990, Giles et al. 1994). Further research is needed to better understand the role of *Z. phytonomi* in the population dynamics of the alfalfa weevil in Tennessee.

Alfalfa weevils were detected in each county, and alfalfa weevil parasitoids were active in each alfalfa field. In most of the sampled fields in eastern and middle Tennessee, *B. anurus* was the predominant larval parasitoid, suggesting *B. anurus* has displaced the previously predominant *B. curculionis* in these areas of Tennessee (Grant & Lambdin 1990). Percent parasitism of alfalfa weevil larvae by *B. anurus* and *B. curculionis* was 7.12% (n = 6,215) and 4.12% (n = 6,215), respectively. *Microctonus aethiopoides* is more numerous and appears to be more effective in alfalfa-producing areas north of Tennessee. In Ontario, parasitism rates of overwintered adult alfalfa weevils by *M. aethiopoides* were 76%–82%, whereas the parasitism rates of summer adult alfalfa weevils were 41%–60% (Abu & Ellis 1976). In New York, parasitism of alfalfa weevil adults by *M. aethiopoides* ranged from 20%–88%, and oviposition by parasitized alfalfa weevil populations was reduced an average of 47% (Van Driesche & Gyrisco 1979). In Missouri and Iowa, parasitism of alfalfa weevil adults by *M. aethiopoides* increased from 1.9% in 1981 to 36% in 1985 (Kingsley et al. 1993). The low numbers of *M. aethiopoides* collected in Tennessee indicate that further research should be conducted to better define the role of *M. aethiopoides* against alfalfa weevil in Tennessee. Although these three species of parasitoids are established in Tennessee, ca. 96% of the alfalfa fields in Tennessee were treated with chemical insecticides in 1994 (unpublished data). This high incidence of pesticide application is most likely suppressing biological control, which may be responsible for the low parasitism levels detected in this limited survey of typical alfalfa production fields. These data should provide baseline information to improve biological control of the alfalfa weevil in Tennessee.

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Population Dynamics and Diurnal Activity of Potato Leafhopper (Homoptera: Cicadellidae) in Central Iowa Alfalfa[1]

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ABSTRACT Studies were conducted in Iowa from 1994 through 1996 to assess the population dynamics and diurnal activity of potato leafhopper, *Empoasca fabae* (Harris), in alfalfa. The population dynamics study showed that potato leafhopper populations can reach economic levels in each of the three alfalfa crops during a growning season. However, economic thresholds were consistently exceeded only in the second alfalfa crop. In the second crop, current economic thresholds were exceeded approximately 3 weeks after first harvest. Potato leafhopper adults were collected in each of the three alfalfa crops, but nymphs were only collected in the second and third crops. The diurnal study showed that there were significant differences in the number of leafhoppers captured at different times of the day 66% of the time. However, there were no consistent trends in the relative number of leafhoppers captured at a particular time. For samples collected around 1000 h or at 1900 h or later, present economic thresholds may be too low. But there was not enough conclusive evidence from this study to justify development of different economic thresholds based on the time of day leafhoppers are sampled.

KEY WORDS potato leafhopper, population dynamics, diurnal activity, alfalfa

The potato leafhopper, *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), is a major insect pest of alfalfa in the north central and northeastern United States (Lamp 1991). In many instances, potato leafhoppers are the only insect causing economic loss (Smith & Ellis 1983). In addition to alfalfa, potato leafhoppers feed on over 200 other plant species (Poos & Wheeler 1949), some of which are important in maintaining populations before leafhoppers move into the alfalfa (Armburst 1989).

Because of its economic importance, the potato leafhopper is one of the most studied insect pests of alfalfa (Gyrisco et al. 1978, Armburst & Lamp 1989). In fact, the population dynamics of potato leafhopper have been well documented in most regions of the United States. However, the population dynamics of potato leafhopper

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in Iowa are not well understood. In Iowa, three to four crops (cuttings) of alfalfa are harvested per growing season. Because of the leafhopper's biology (e.g., migration from the southern United States in the spring and its polyphagous nature), each alfalfa crop during the season may have different factors that affect potato leafhopper densities. To more effectively manage potato leafhopper in Iowa, a better understanding of population dynamics, as it relates to current economic thresholds, is needed.

Potato leafhopper populations have been found to exhibit different levels of flight activity within a 24-h period. Dysart (1962) determined that over 50% of daily flight occurred within 30 min after sunset. Smith & Ellis (1982) sampled alfalfa every hour for a 38-h period and collected the most leafhoppers 1 h before sunrise and 2 h after sunset. Daytime leafhopper estimates fluctuated but always were less than nighttime estimates; however, sampling for potato leafhopper management is usually conducted during the daytime hours. Therefore, an understanding of the influence of diurnal activity on leafhopper capture could potentially improve management.

Field studies were conducted to evaluate potato leafhopper biology and ecology as it relates to alfalfa production in central Iowa. The objectives were to assess both the seasonal population dynamics and diurnal activity of the potato leafhopper as they relate to current economic thresholds in alfalfa.

Materials and Methods

Two fields, located at Iowa State University farms near Ames, Iowa, were selected for study from 1994 through 1996. A 3-ha field, located at the Ross farm 2 km north and 1 km west of Ames, was seeded with 'Apollo' alfalfa in the fall of 1993. A second 2-ha field, located at the Johnson farm 4 km south of Ames, was seeded with 'Defiant' alfalfa in the spring of 1994. Both alfalfa cultivars are commonly grown in the Midwest. Each field was divided into two sections. One section was used for the potato leafhopper population dynamics study, and the other section was used for the diurnal activity study. Alfalfa was cut and harvested when approximately 10% of the alfalfa stems were flowering.

Population dynamics study. This study was conducted at both locations in 1995 and 1996 throughout the growing season. Fields were divided into three quadrats (0.4 ha per quadrat). Insect sampling was conducted weekly between 1300 and 1400 h during the alfalfa growing season with a 38-cm-diameter sweep net to estimate potato leafhopper abundance. Thirty pendulum sweeps were taken at two sites (60 sweeps total) within each quadrat on each sampling date. Collecting two 30-sweep sampling units decreased the processing time for each treatment sample compared with processing one 60-sweep unit. Sampling units were collected, bagged, and frozen before counting. Adult and nymphal means were calculated to compare population trends at each site during both years. In addition, alfalfa stem height was estimated weekly by taking 25 alfalfa-stem samples from each quadrat.

Diurnal activity study. This study was conducted in the second alfalfa crops at the Ross farm location in 1994, at both locations in 1995, and at the Johnson farm location. Each alfalfa field was divided into 10 25 m × 25 m

quadrats. One 10 sweep-net sampling unit was collected per quadrat for each time of day by using a 38-diameter sweep net. Samples were collected between 1000 and 1030 h, 1500 and 1630 h, 1900 and 1930 h, and 2200 and 2230 h. These times were chosen based on two factors: (1) possible times a scout or grower could sample the alfalfa field and (2) times of day following dew evaporation in the morning and dew formation in the evening. These sampling times avoided wet sweep samples that might bias population estimates. Sweep samples for each time of day were collected in each quadrat according to a stratified random design. Data were analyzed by analysis of variance (ANOVA), and means were separated by using Fisher's protected least significant difference (LSD) (SAS 1990).

Results and Discussion

Population dynamics study: first crops. Immigrating potato leafhopper adults were first collected on 19 May in 1995 and on 21 May in 1996 at both locations (Figs. 1–4). In the first alfalfa crops, most adults were collected on the last two sampling dates. With the exception of the Johnson farm in 1996 (Fig. 3), population densities did not exceed current economic thresholds during the first alfalfa crop. Current economic thresholds are 0.1 leafhoppers per sweep for each 2.5 cm of plant height if alfalfa is less than 25 cm tall, and two or more leafhoppers per sweep if the alfalfa is taller than 25 cm (Rice 1996). Furthermore, the leafhopper density at the Johnson farm was only greater than the economic threshold on the last sampling date before first crop harvest (alfalfa was greater than 25 cm tall and leafhoppers averaged 2.7 per sweep) (Fig. 3). No nymphs were collected in the first alfalfa crops in either year of the study.

Second crops. Both adult and nymphal densities tended to increase on each subsequent sampling date in the second alfalfa crops (Figs. 1–4). No nymphs were collected 1 wk after the first harvest. The greatest number of all leafhopper stages collected during the alfalfa growing season always occurred on the last sampling date before the second harvest. Greater numbers of leafhoppers were collected at both locations during the second crop in 1995 than during the second crop in 1996. The number of nymphs was greater than adults on the last sampling date of the second crops in all but one instance. Leafhopper populations (adults and nymphs combined) exceeded economic thresholds 2 to 3 wk after the first alfalfa harvest at both locations in 1995 and 1996.

Third crops. Leafhopper populations did not reach economic levels in 1995 at either location (Figs. 1, 2). In 1996, populations increased to economic levels 2 (Ross) to 3 (Johnson) wk after the second alfalfa harvest; however, these populations declined to noneconomic levels by the next sampling date (Figs. 1–4). Potato leafhopper populations then remained noneconomic throughout the third alfalfa crops.

Both adult and nymphal numbers were reduced following each alfalfa cutting and harvest; however, because of their mobility, adult densities were affected less than those of nymphs in both years and locations. After cutting and harvest, many adult leafhoppers emigrate to adjacent fields or alternative

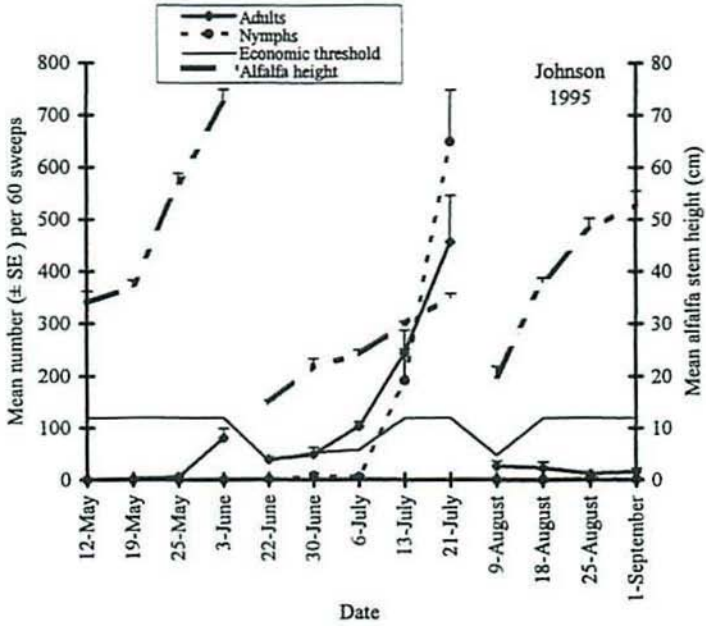

Fig. 1. Mean number of potato leafhoppers collected during the alfalfa growing season at the Johnson farm, 1995. Breaks in lines indicate when alfalfa was harvested. Error bars represent standard error of the associated mean.

hosts, whereas most nymphs are either removed with harvest or are dislodged from the alfalfa stems and starve to death (are unsuccessful at relocating a suitable host). Simonet & Pienkowski (1979) found that cutting alfalfa to a 2- to 5-cm stubble height resulted in nymphal and egg mortality near 95% and 100%, respectively.

The results of this study showed that potato leafhopper populations can reach economic levels in any of the three alfalfa crops in central Iowa; however, economic thresholds were consistently exceeded only in the second alfalfa crops. The dramatic reduction in potato leafhopper densities after alfalfa harvest suggests that early harvest may be an effective management tactic in alfalfa. Early harvest is an especially useful tactic for the first crop, in which leafhopper populations only build to economic levels late in the alfalfa growth cycle. But, adults can recolonize subsequent alfalfa crops and still reach economic populations, as happened in the second and third alfalfa crops. Early harvest is not as practical in the second crop because thresholds are exceeded when alfalfa is early in its growth cycle. At this time, the most probable management option for growers is an insecticide application to rapidly reduce leafhopper populations.

Diurnal activity study. The numbers of potato leafhoppers collected at three or four times during the day are shown in Figs. 5–8. Note that the scale

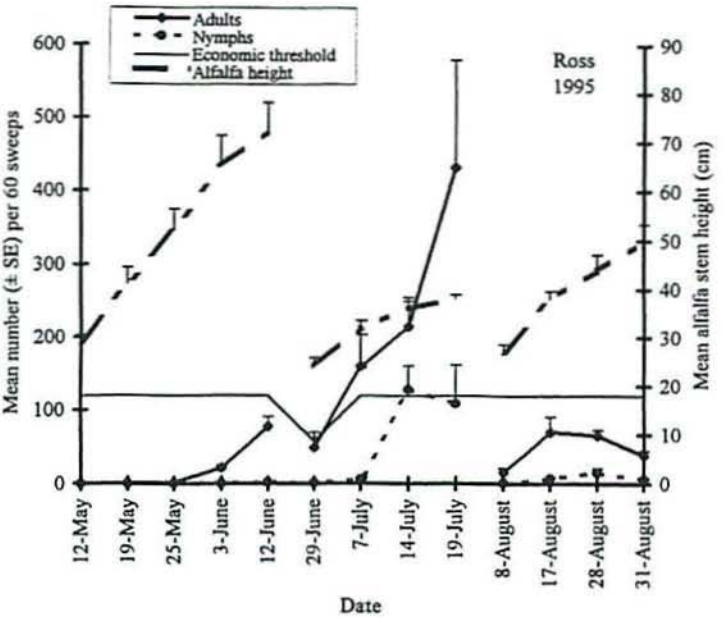

Fig. 2. Mean number of potato leafhoppers collected during the alfalfa growing season at the Ross farm, 1995. Breaks in lines indicate when alfalfa was harvested. Error bars represent standard error of the associated mean.

of the Y-axis was changed in these figures based on the population densities collected in each field in both years. With the exception of 28 June, significantly greater potato leafhopper numbers (F = 38.09; df = 6, 245; P < 0.001) were collected at 1900 and 2200 h (samples only collected on 28 and 30 June) compared with 1500 h at the Ross farm in 1994 (all sampling dates combined; Fig. 5). On the four sampling dates in 1994, 22% to 81% more leafhoppers were collected on the two later sampling times. Sampling, however, was discontinued at 2200 h after the first two sampling dates because of dew formation beginning on 5 July that interfered with sampling. In addition, consistently greater numbers of leafhoppers were collected at 1000 h compared with 1500 h on all four sampling dates in 1994. No consistent differences in the number of leafhoppers collected at different sampling times were found in 1995 (Figs. 6, 7). But there were a few instances in 1995 when significantly greater numbers (P < 0.05) were collected at 1900 h compared with the other sampling times. In 1996 when all sampling dates were combined, significantly greater densities of leafhoppers were collected at 1000 and 1900 h compared with 1500 h (F = 40.19; df = 4, 85; P < 0.001; Fig. 8).

When the data from all 3 yr of the study were combined, there were significant differences in the number of potato leafhoppers captured at different times of the day 66% of the time (Figs. 5–8). In 87% of the comparisons, greater

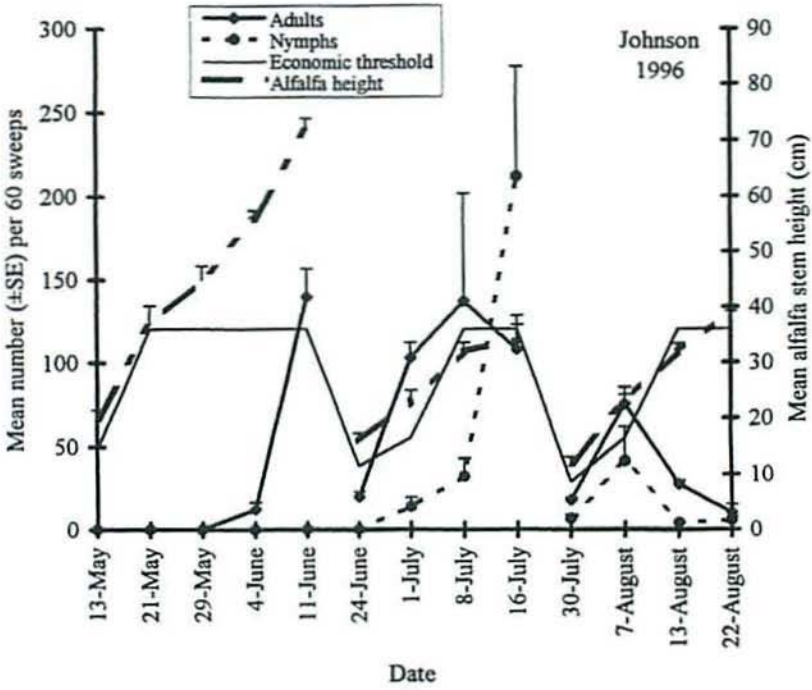

Fig. 3. Mean number of potato leafhoppers collected during the alfalfa growing season at the Johnson farm, 1996. Breaks in lines indicate when alfalfa was harvested. Error bars represent standard error of the associated mean.

numbers of leafhoppers were collected at either 1000 or 1900 h than at 1500 h. However, there were no consistent trends in the relative number of leafhoppers captured at a particular time.

The greater numbers collected at 1900 and 2200 h in 1994 (and to some degree in 1995) were likely the result of increased local flight activity amongst the vegetation (Pienkowski & Medler 1966). Adult leafhoppers are highly mobile and have increased flight activity near sunrise (between 0400 and 0600 h) and especially near sunset (between 1900 and 2200 h) (Dysart 1962, Smith & Ellis 1982). The greater numbers of leafhoppers collected at 1000 h compared with 1500 h may have been the residual of the increased activity of adults observed around sunrise.

For samples collected around 1000 h or at 1900 h or later, present economic thresholds may be too low; however, there is not enough conclusive evidence from this study to justify development of different economic thresholds based on the time of day leafhoppers are sampled. Field studies to develop management programs for potato leafhoppers were likely conducted during normal working hours (ca. 800 to 1800 h) (Saugstad et al. 1967, Simonet et al.1979), and growers (or scouts) are more likely to sample during this same time period, making current economic thresholds acceptable.

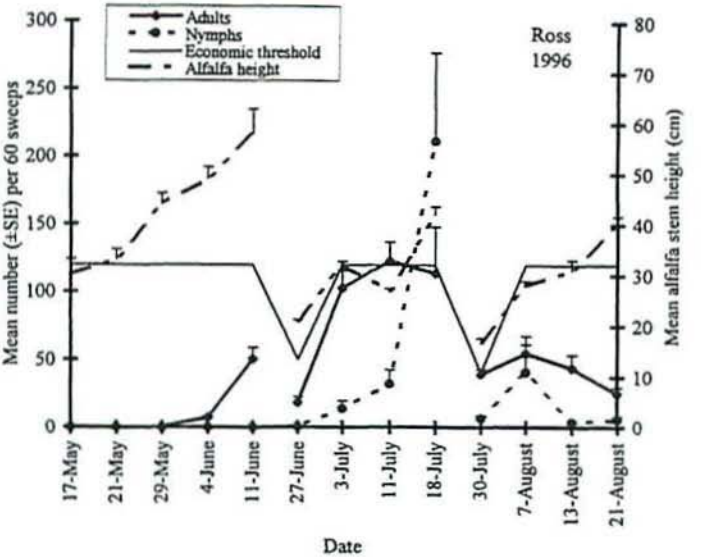

Fig. 4. Mean number of potato leafhoppers collected during the alfalfa growing season at the Ross farm, 1996. Breaks in lines indicate when alfalfa was harvested. Error bars represent standard error of the associated mean.

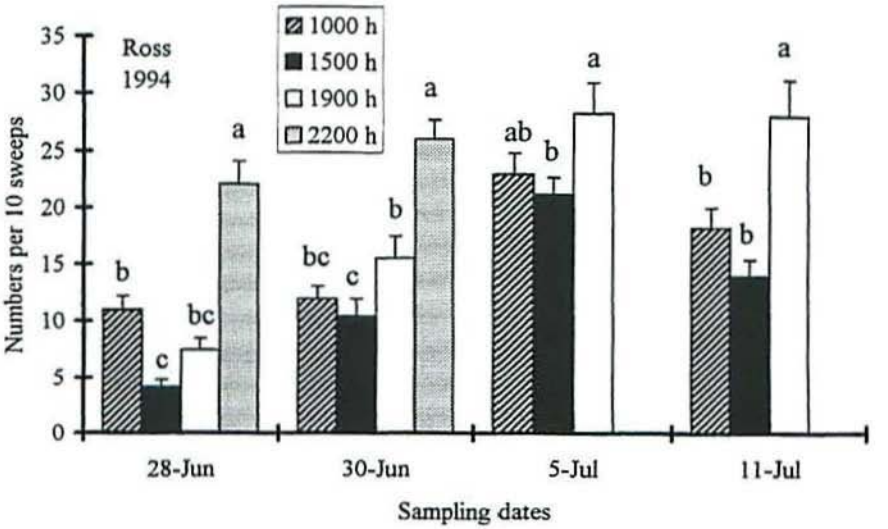

Fig. 5. Mean number of potato leafhoppers collected at four times of the day, Ross farm, 1994. Means (bars) on each sampling date with the same letter are not significantly different (ANOVA, LSD, $P > 0.05$).

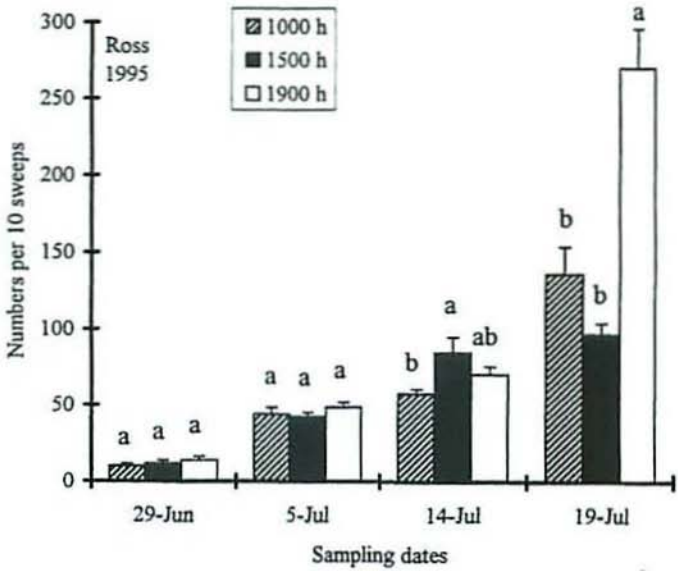

Fig. 6. Mean number of potato leafhoppers collected at three times of the day, Johnson farm, 1995. Means (bars) on each sampling date with the same letter are not significantly different (ANOVA, LSD, $P > 0.05$).

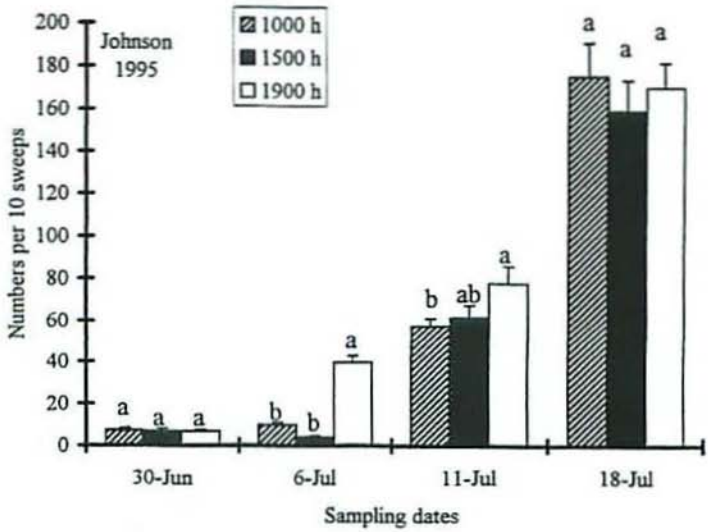

Fig. 7. Mean number of potato leafhoppers collected at three times of the day, Ross farm, 1995. Means (bars) on each sampling date with the same letter are not significantly different (ANOVA, LSD, $P > 0.05$).

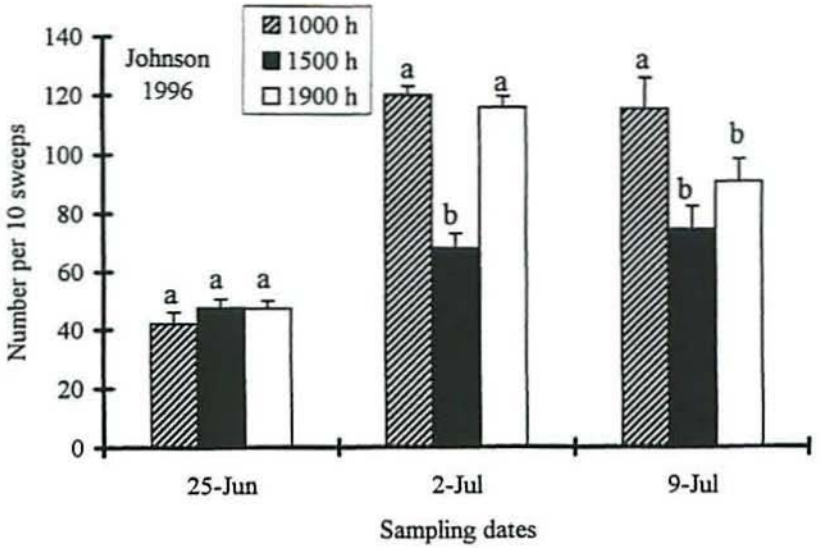

Fig. 8. Mean number of potato leafhoppers collected at three times of the day, Johnson farm, 1996. Means (bars) on each sampling date with the same letter are not significantly different (ANOVA, LSD, $P > 0.05$).

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Effect of Crop Habitat and Potato Management Practices on the Population Abundance of Adult *Harpalus rufipes* (Coleoptera: Carabidae) in Maine[1]

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ABSTRACT The effects of crop habitat and potato management practices on adult *Harpalus rufipes* (Coleoptera: Carabidae) were studied in Presque Isle and Stillwater, Maine, during 1990–1992. A relative index of population abundance was estimated using pitfall traps. Rotation crops may harbor a higher abundance of *H. rufipes* than potato fields. A higher beetle abundance late in the season was observed in barley rotation crops compared with potato. The type of the current year's rotation crop (oats vs. clover) significantly affected *H. rufipes* abundance; more beetles were found in the small grain than in clover. Factors such as crop planting density, weed biomass, or weed seed (barnyard grass) densities did not affect *H. rufipes* abundance in a wheat rotation crop. Within the potato crop, there was no significant effect of a previous year's rotation crop (oats vs. clover) on *H. rufipes* abundance. Organic soil amendments and insect pest management strategies in potato fields had no significant effect on adult abundance. The effects of two pesticides used in Maine potato production (a herbicide and a mycoinsecticide) on *H. rufipes* mortality were studied in the laboratory. No direct adult *H. rufipes* mortality resulted from the application of the herbicide metribuzin or the insect fungal pathogen *Beauveria bassiana* (Balsamo) Vuillemin. However, in the field, the combination of an application of metribuzin with chisel plowing significantly reduced *H. rufipes* abundance late in the season.

KEY WORDS Carabidae, *Harpalus rufipes*, habitat, weed seed predator, potato

Harpalus rufipes DeGeer (Coleoptera: Carabidae) is the dominant ground beetle species in Maine potato cropping systems (Zhang et al. 1997). The beetles will feed on soft-bodied immature insects, but primarily feed on seeds of various grains and weeds that they store in burrows or beneath plant residue (Chiverton 1987, Skuhravy 1959,

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Sunderland et al. 1987). Growing concern over the environmental impact of intensive herbicide use has led to increased interest in cultural and biological methods of weed management (Altieri & Liebman 1988). Adult *H. rufipes* appears to play a significant role as a weed seed predator in northern Maine (Zhang 1993, Hartke 1996), but little is known about its population abundance and ecology related to agricultural crop production methods, especially pest management strategies.

Because *H. rufipes* is endemic in Europe and was only recently introduced to the United States accidentally (Zhang et al. 1994), most of the ecological research on this carabid has been conducted in European cropping systems. *Harpalus rufipes* is associated with cultivated habitats and is most abundant in small crop fields compared with large fields (Wallin 1986). Another characteristic of agricultural fields that has been suggested to affect *H. rufipes* abundance is weed density. Speight & Lawton (1976) manipulated insect prey densities in areas of varying weed cover to test the effect of the weed *Poa annua* L. in cereal fields on the abundance of carabids, including *H. rufipes*. Within one field, areas of high weed density had more predatory ground beetles, and artificial prey suffered significantly greater 'mortality' than in areas with low weed density. Diversity of weed species has been suggested to be an important factor affecting ground beetle populations in general (Murdoch et al. 1972, Pimentel 1961, Speight & Lawton 1976). The relationship between beetle activity and the frequency and abundance of *P. annua* is probably complex; it is likely that weeds are important in protecting carabids from weather extremes, e.g., insulation from heat during the day and from desiccation, both during the day and at night (Speight & Lawton 1976). Rivard (1966) trapped more carabids in areas of high humidity, and Thiele (1964) considered humidity as a key factor in the abundance of most carabids, along with the microclimate in vegetation. Thus, *H. rufipes* abundance may be associated with high weed densities as a result of increased relative humidity. It is also possible that there is an indirect effect due to weed density on the abundance of insect prey, which may be more common in dense weed patches than in sparse patches (Speight & Lawton 1976). However, exception to the weediness hypothesis exists. Purvis & Curry (1984) found that none of the dominant carabids they studied (*H. rufipes* included) responded positively to increases in weed densities, although the activity of *Pterostichus melanarius* DeGeer appeared to persist longer in weedy plots in September. In Maine, *H. rufipes* was found to be equally active in potato plots with varying weed densities during peak abundance of the beetle in August (Zhang 1993).

Another characteristic of crop production that may affect *H. rufipes* abundance is cultivation. Soil disturbance may favor *H. rufipes* because of larval requirements for seeds as food. An open soil surface encourages weed growth, and *H. rufipes* larvae would be short of food in a field kept completely weed free (Luff 1980). After using pitfall traps to quantify the carabid fauna of arable land, Scherney (1960) concluded that certain Carabidae (*H. rufipes* included) were associated with cultivation. The numbers taken in pitfall traps in different habitats decreased in an order that reflects a lessening intensity of cultivation: wheat, barley, potato, clover, meadow, and densely weed covered fallow land. Although agricultural monocultures are considered unstable

habitats, they may provide stable conditions for species such as *H. rufipes* (Luff 1980) that are dependent on relatively bare, cultivated soil rich in weed seeds.

The objective of our study was to investigate the effects of selected potato production practices in Maine on the abundance of adult *H. rufipes*, a potentially important weed seed predator in Maine potato. The practices that we chose to investigate were those that represent: (1) the overall habitat, in this case, choice of rotation crop, tillage, and the use of organic soil amendments (compost and manure); (2) weed density; and (3) pest management tactics.

Materials and Methods

Field experiments were conducted in a 32.4-ha field at the University of Maine Aroostook Farm, Presque Isle, Maine. This site is dedicated to a long-term, 10-yr agroecosystem study to assess the affect of crop management practices on soil nutrient dynamics and pest population processes (Alford et al. 1996).

Tillage, rotation crop, and herbicide practice (TRH experiment). ***Field study.*** A randomized complete block split plot design was conducted to test the effects of tillage (moldboard vs. chisel plowing [main plots]), rotation crop type (oats, *Avena sativa* L. [seedling rate = 108 kg/ha], vs. berseem clover, *Trifolium alexandriunum* L. [seedling rate = 22 kg/ha]), and herbicides (none vs. preplant application of metribuzin [Sencor™] [0.56 kg AI/ha] in potato [*Solanum tuberosum* L] plots) on adult *H. rufipes* abundance (Liebman et al. 1996). The experiment consisted of four replicate 7.6 m × 4.6-m plots for a total of 32 plots (eight treatment combinations, 2 × 2 × 2). Plots were either moldboard plowed turning the top soil with weed seeds to a depth of ca. 25 cm or chisel plowed leaving the top soil and weed seeds at the surface. The rotation crops were planted in late May 1990 and 1992. Potatoes (cv. Atlantic) were planted in all plots on 22 May 1991. Metribuzin, a commonly used herbicide in Maine potato production, was applied to the potato plots in 1991. No herbicide was applied to the rotation crops in 1990 and 1992. A pitfall trap was dug into each of the 32 plots in 1991 and 1992, and a 1:1 water and antifreeze solution was added to the traps that had 20 × 20-cm^2 aluminum rain shelters. The traps were checked weekly and the numbers of adult *H. rufipes* were recorded. Mean adult density was analyzed by repeated measures analysis of variance with a randomized complete block, split plot design (Gagnon et al. 1989).

Laboratory study. A follow-up laboratory study designed to assess, more precisely, the direct effect of metribuzin on the survival of *H. rufipes* adults. It was conducted in July 1992. Metribuzin was applied to both soil and seeds at the rates of half (56.1 mg/m^2), standard (112.2 mg/m^2), and double (224.4 mg/m^2) the recommended field dosage. A water-only control also was included as a treatment. Petri dishes filled with a 5-mm depth of potato field soil mixed with barnyard grass seeds (*Echinochloa crusgalli* [L.] Beauvois) at a 2:1 ratio were sprayed with the Sencor solution. The number of seeds added to each dish was adequate to feed six beetles for a month. A completely randomized block design was set up on 28 July 1992. Each Petri dish contained three female and three male *H. rufipes* adults collected over a period of several days in mid-June, early July, mid-July, and late July. Experimental units (groups of six adults)

were partitioned into four cohorts (statistical blocks) according to collecting days, and each treatment was represented once within a block. Mortality was recorded at 1, 2, 7, and 14 d after Sencor application. Data were analyzed with a repeated measures analysis of variance, randomized complete block design (Gagnon et al. 1989).

Soil and pest management strategies (SPM experiment). ***Field study.*** This experiment was conducted in 96 plots (0.08 ha each) arranged in a randomized complete block, split plot design to test the effect of pest management (main plots, three levels), soil management (subplots, two levels), and potato variety (subplots, two levels) strategies on adult *H. rufipes* abundance. Treatments were randomly assigned into four statistical blocks with two entry points (2-yr potato–barley rotation). Potato (48 plots) and barley (rotation crop, 48 plots) plots were assigned next to each other and rotated every year.

Three pest management systems were conventional, reduced chemical input, and biological strategies. The conventional management system relied on agricultural production practices considered to be typical of most potato farms in northern Maine. Synthetic pesticides were used when appropriate, as recommended by the University of Maine Cooperative Extension Service. The biological management system relied on biological and mechanical control measures against insect pests and weeds when possible. In 1991, for the biological strategy the only insecticide used was the bacterium *Bacillus thuringiensis* subspecies *kurstaki* strain EG2424 , and plant disease (potato late blight) was monitored and controlled by applying copper sulfate. In 1992, changes were made in the biological management system, including heavy reliance on biocontrol of Colorado potato beetle, *Leptinotarsa decemlineata* Say, with application of the parasitic nematode *Steinernema carpocapsae* (Weiser); the fungus *Beauveria bassiana* (Balsamo) Vuillemin; the bacterium *B. thuringiensis* subspecies *kurstaki* strain EG2424; and releases of the coccinellid *Coleomegilla maculata* (DeGeer). Aggressive mechanical cultivation was used for weed control, copper sulfate was applied for disease control; and green manure (hairy vetch plus oats and field peas) was used in place of the barley rotation crop for improvements of soil physical, chemical, and biological characteristics. The reduced chemical input management system was similar to the conventional management system except that all economic thresholds were double that of the conventional system. In both these management systems standard pesticides for potato production recommended by Maine Cooperative Extension were used (see Alford et al. 1996 for more detail).

Two soil nutrient management regimes were used in both 1991 and 1992 as subplot treatments: organic soil amendments were (+) or were not (-) applied to appropriate plots. The organic amendments consisted of 13 ton/ha of waste potato compost and 44 ton/ha of beef-cattle manure. Chemical fertilizer rates at planting were 504 and 1,120 kg/ha (10-10-10) for the + and - organic amendment regimes, respectively, and an additional 56 kg/ha nitrogen was side dressed on 15 July 1991 (near tuber initiation) in both treatments.

The two potato varieties Superior and Atlantic were included in the design. Both varieties are early to mid-season maturing, round whites grown in significant acreage in the northeastern United States. Superior is susceptible

to water stress, early and late blight, and most other potato diseases. Atlantic is considered disease and stress tolerant. However, for this experiment we only estimated relative *H. rufipes* density in the Superior potato plots.

Adult *H. rufipes* abundance was estimated at two times during the growing season: 30 June–6 July and 12 August–18 August in 1991 and 26 June–1 July and 16 August–22 August in 1992. Five pitfall traps with 1:1 water and antifreeze solution were placed in each of 48 plots (24 barley and 24 potato) for a 7-d period. At the end of 7 d, the number of adult *H. rufipes* per trap was recorded. Trap catch data were log (x+1) transformed and analyzed with repeated measures analysis of variance by using a randomized complete block, split plot design (Gagman et al. 1989).

Laboratory study. Because *B. bassiana* was the major insecticide used in the biological pest management treatment, a laboratory study designed to assess the direct effect of this pathogen on *H. rufipes* adults was conducted in July 1992. Adult *H. rufipes* were inoculated individually with *B. bassiana* spores (RS252 strain) at a concentration of 1×10^6 conidia suspended in 0.01% Tween®. *Beauveria bassiana* was pipetted topically on the notal membrane between the thorax and abdomen of 30 adults with 5 µl of spore solution. Another 30 nontreated adults were held in soil (Caribou silt loam) sprayed with 5 µl of *B. bassiana* solution. A third group of 30 adults had only 0.01% Tween solution applied topically (topical control), and a fourth group of 30 adults was held in soil treated only with the 0.01% Tween solution as a control for the soil *B. bassiana* treatment. Adult *H. rufipes* were kept in a constant temperature environmental chamber at 25°C and 12:12 (L:D) h photoperiod for 10 d and checked daily for survival. Under similar conditions, mortality of Colorado potato beetle adults infected with *B. bassiana* occurs in 6–9 d. Mortality in the *B. bassiana*-treated groups was compared with mortality in the control groups with pairwise Bonferoni corrected *t*-tests for a completely randomized design (Gagnon et al. 1989).

Vegetation density. To test the effect of vegetation density on *H. rufipes* abundance, an experiment was conducted in a wheat field at Aroostook Research Farm in 1992. Wheat stands were manipulated by selective cutting of stems to create the treatments: no wheat (all stems removed), half wheat density, and standard wheat density (135 kg seed/ha). Each treatment had three replications laid out in a Latin square design with 3-m × 3-m plots. One pitfall trap was set out in the center of each plot on 28 July 1992. Pitfall traps were checked and *H. rufipes* densities were recorded weekly until the experiment was terminated on 17 August. Data were analyzed as a repeated measures analysis of variance with a one-way Latin square design (Gagnon et al. 1989).

Weed seed density. The response of adult *H. rufipes* to varying seed densities of the weeds barnyard grass (*E. crusgalli*) and wild mustard (*Brassica kaber* (D. C.) L. C. Wheeler, was tested. Seed densities were 0, 125, 500, and 2,000 seeds per square meter for each weed species. The experiment was conducted in a 2.5-ha potato field. The seed density treatments for each weed species were laid out in a Latin square design with four replicates. Plot size was 1.8 m × 1.8 m separated by a 1.8-m space between plots. One pitfall trap was set up in the center of each plot for 1 wk before the start of the experiment.

Seeds were sown on 24 July 1992. Pitfall trap catch was recorded once a week for 5 wk. Data were log (x+1) transformed and analyzed with a repeated measures analysis of variance, Latin square design. Polynomial contrasts were used to assess the regression relationship between adult beetle response to increasing weed seed density (Gagnon et al. 1989).

Results and Discussion

Effect of crop habitat. ***Previous year's crop.*** From the TRH experiment, we found no evidence to suggest that the previous year's (1990) rotation crop (oats vs. clover) affected *H. rufipes* capture in pitfall traps set out in potato (F = 1.495; df = 1, 154; P = 0.247). There were 0.57 ± 0.81 (mean ± SD) adults per trap per day throughout the season in plots following an oat rotation crop and 0.69 ± 0.66 adults per trap per day throughout the season in the plots following a clover rotation crop. Weed biomass (dominated mostly by lambsquarters, *Chenopodium album* L.) in these plots prior to planting to potato was significantly higher in the clover rotation compared with the oat rotation (Liebman et al. 1996). However, weed biomass in the following year's potato plots was not significantly different in potato plots preceded by clover compared with potato plots preceded by an oat rotation crop. Cultivation and hilling reduces any differences in initial weed densities between these two crop rotation treatments (Liebman et al. 1996). Therefore, any differences in the adult *H. rufipes* abundance in these potato plots would likely be due to the previous year's larval density or an adult numerical response due to any differences in nongerminated weed seeds comprising the seed bank in these potato plots. Hartke (1996) found that *H. rufipes* third instar densities were not significantly different in 10 barley rotation fields compared with 10 legume green manure rotation fields. Therefore, our finding of no differences in adult *H. rufipes* abundance due to the preceding year's rotation crop suggests that this is probably due to a lack of difference in the previous year's larval densities in these plots. In addition, Liebman et al. (1996) found that the potato plots preceded by clover had four times the weed seed bank compared with potato plots preceded by an oat rotation. Thus, our findings suggest that adults do not numerically respond to differences in weed seed abundance (see below for results of experiment designed to test numerical response to seed density).

Current year's crop. The two experiments designed to assess current crop habitat on *H. rufipes* abundance showed that crop habitat can have an effect on adult *H. rufipes* abundance. As far as comparisons between rotation crops, the TRH experiment showed that oats planted in 1992 led to a significantly (F = 4.485; df = 1, 38; P = 0.048) higher abundance of *H. rufipes* (1.78 ± 3.17) adults per trap per day averaged over growing season in 1992) compared with clover (0.98 ± 1.57) adults per trap per day averaged over the growing season). We have no ability from our experimental design to determine what factors caused the difference we observed. Differences in prey availability could be responsible, as could differences in the microclimate between oats and clover rotation crops. Both of these factors have been cited as affecting the abundance of *H. rufipes* in European studies (Rivard 1966, Speight & Lawton 1976). Rivard (1966) captured more *H. rufipes* in areas of high humidity. Although we

did not monitor the relative humidity in the two rotation crops, we captured more beetles in the less vegetatively dense plots of oats than we did in the more densely vegetated clover plots. In regards to prey availability, Skuhravy (1959) suggested that *H. rufipes* is predominantly herbivorous based upon morphology of the proventriculus and laboratory feeding studies. Zhang (1993) conducted laboratory prey preference studies that supported the idea of a predominant herbivorous life style. However, when both seeds and aphids were available to beetles, feeding was concentrated on seeds, aphids being consumed to a lesser extent. When aphids were offered in the absence of seeds, aphid consumption was high. Thus, differences in the ratio between insect and seed prey availability in these two rotation crops could result in differences in total prey available and explain differences in the adult abundance that we observed.

The SPM experiment showed that the barley rotation crop had a significantly higher trap catch of *H. rufipes* than potatoes (for 1991, $F = 101.00$; df = 1, 26; $P < 0.001$; for 1992, $F = 16.914$; df = 1, 26; $P = 0.003$ [Fig. 1]). Therefore, the type of ground cover or intensity of cultivation (tillage) can affect the abundance of *H. rufipes* under certain conditions. Scherney (1960) reported the same trend in that barley results in more beetles than potato. He attributed this trend to differences in soil disturbance due to cultivation. In Maine, potato production results in one to two weed cultivations in late June followed by a cultivation for hilling the potatoes in early July, whereas barley production usually has minimal if any cultivation and this occurs in May (Alford et al. 1996). We found a significant interaction for beetle trap captures between month and crop type in 1991. The difference in trap captures from June to August was much higher in barley plots than in potato plots ($F = 32.923$; df = 1, 26; $P = 0.001$, [Fig. 1]). August beetle populations are composed of both first and second generations (Zhang et al. 1997). The population abundance of second generation *H. rufipes* in 1992 was significantly lower than in 1991 ($F = 50.626$; df = 1, 92; $P < 0.0001$ [Fig. 1]). One difference between the 2 yr is that in 1992, there was greater precipitation than in 1991, leading to high soil moisture that may have detrimentally affected the development and emergence of the overwintered larvae, possibly leading to a low second generation abundance in 1992. Hamon et al. (1990) documented that wet spring weather can cause significant mortality of larval carabids.

Vegetation density. To test the hypothesis that vegetation density affects beetle abundance we manipulated the plant density of a wheat crop and sampled beetle abundance. Three weeks of pitfall trap catches in the wheat density plots suggested that no significant differences in *H. rufipes* abundance existed between plots with no wheat, half the standard wheat density, and the standard wheat density during 28 July–17 August 1992 ($F = 0.175$; df = 1, 14; $P = 0.688$). This finding does not support the hypothesis that increasing crop density and resultant relative humidity favor high populations of *H. rufipes*.

Weed seed density. There were no significant differences between *H. rufipes* abundance in experimental plots before the start of the experiment ($F = 0.035$; df = 1, 3; $P = 0.863$). Differentially added seed density and seed species did not change the resultant trap catch of *H. rufipes* adults for the 5-wk period after seed sowing ($F = 0.163$; df = 1, 12; $P = 0.713$). Thus, *H. rufipes* adults do not appear to rapidly respond to a change in the density of a seed prey resource.

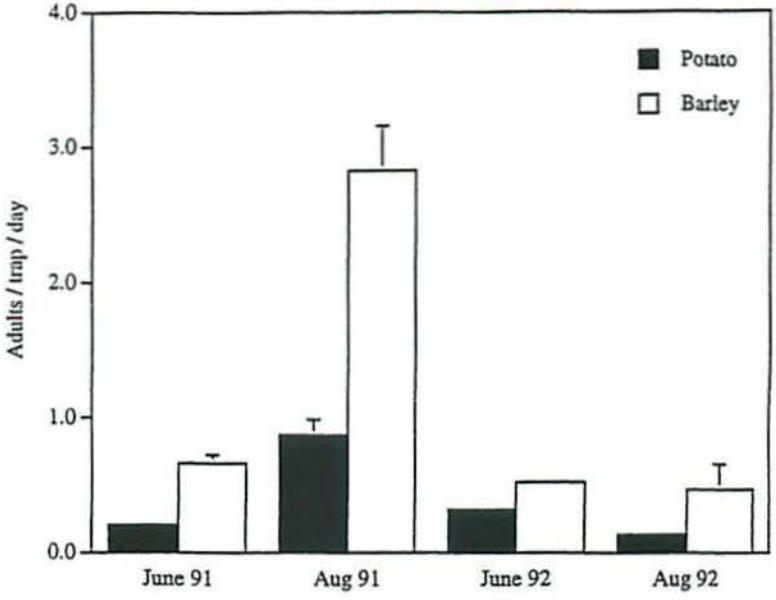

Fig. 1. Adult *H. rufipes* abundance in potato and barley plots in 1991 and 1992 (error bar = SE).

Effect of crop management. ***Soil amendments.*** The SPM experiment showed that there were no significant differences in beetle abundance between potato plots that received compost and manure applications compared with those that did (for 1991, $F = 2.009$; df = 1, 26; $P = 0.168$; for 1992, $F = 0.009$; df = 1, 26; $P = 0.926$). As far as we know there are no published reports on the effects of soil organic matter on the abundance or survival of harpaline carabids.

Tillage. The moldboard plow turns the top few inches of soil and weed seeds deep beneath the soil surface. We had hypothesized that spring or fall tillage might kill overwintering beetles (larvae and adults). However, we found no difference in abundance of adults caught in pitfall traps set out in chisel tillage plots (0.07 ± 0.08 adults per trap per day) compared with moldboard tillage plots (0.03 ± 0.06 adults per trap per day) during 10–15 May 1991, immediately after spring tillage operations prior to planting ($F = 0.351$; df = 1, 20; $P = 0.614$). There was also no significant reduction in abundance directly from moldboard plowing (0.56 ± 0.55 adults per trap per day) compared with the chisel plowing (0.71 ± 0.90 per trap per day) ($F = 0.152$; df = 1, 154; $P = 0.723$) after harvesting potatoes in the fall of 1991. In the third year of the experiment rotation (1992), oats and clover were planted following the potato crop of 1991. Repeated measures analysis showed that there was no significant difference in *H. rufipes* population abundance between moldboard plowed plots and chisel-plowed plots before planting ($F = 0.496$; df = 1, 38; $P = 0.532$). Therefore, in these trials, over a 3-yr period, tillage itself had no effect on *H. rufipes* abundance.

Comparison of pest management strategies. There were no significant differences in beetle abundance in potatoes managed by conventional, reduced chemical input, and biological pest management strategies (in 1991, $F = 0.502$; df = 2, 6; $P = 0.629$; in 1992, $F = 2.118$; df = 2, 6; $P = 0.201$). Conversely, these pest management strategies did effect abundances of Colorado potato beetle, the potato pest aphid species complex, potato flea beetle, lady beetle predators, and spider populations (Alford et al. 1996). This may not be surprising if prior to any pest management tactics being applied early season cultivation in potato fields (as speculated above) has a significant detrimental effect on beetle populations.

Mycoinsecticide. Application of *B. bassiana* also resulted in no significant increase in mortality of adults as compared with a 0.01% Tween® control. During the test period, 3.3% mortality was observed from a topical application of *B. bassiana* spores and 6.7% mortality was observed from the soil treatment with *B. bassiana* spores. The control treatments resulted in only 5.2% mortality. This indicates that *H. rufipes* is tolerant to *B. bassiana* when applied at rates that result in 69.7% mortality of the Colorado potato beetle (Groden 1989).

Herbicide. In 1991, the potato year of the TRH experiment, application of metribuzin was correlated with a reduction in adult population abundance ($F = 6.316$; df = 1, 154; $P = 0.029$ [Fig. 2]). There was also a significant interaction between tillage method and herbicide application in 1991 when potato was planted ($F = 4.556$; df = 1, 154; $P = 0.056$ [Fig. 2]). In the chisel-plowed plots, if a preplant herbicide was applied, a significantly low *H. rufipes* abundance occurred, whereas the plots that did not receive an application of herbicide had a higher beetle density ($P < 0.05$, Fisher's Protected LSD). There was also a significant interaction between tillage methods, application of herbicide, and sampling time ($F = 2.497$; df = 14, 154; $P = 0.003$). There appears to be no treatment interaction earlier in the season. Later in the season, chisel plowing and metribuzin reduced the population of second peak of *H. rufipes* indicating that metribuzin has an impact on the mortality of overwintered larvae that emerge producing a second generation (Fig. 3).

We also found that application of metribuzin in the laboratory had no significant effect on mortality of adults observed for 14 d as compared with a water control treatment ($F = 0.729$; df = 3, 159; $P = 0.540$). During the test period, mortality rates of 2.1% were observed from both standard and double dosage treatments, whereas, the water control resulted in 4.2% mortality. There was no mortality from the half dosage. These results suggest that metribuzin does not directly effect *H. rufipes* abundance through mortality. We did not assess repellency effects of metribuzin on the beetles or any effect that reduced weed density may have on altering the relative humidity of the microhabitat (Thiele 1964, Rivard 1966).

In summary, both in Europe and the United States (Maine), *H. rufipes* has been identified as a predominant weed seed predator in agroecosystems (Briggs 1965, Zhang et al. 1997). Attack rate on barnyard grass seeds and wild mustard seeds sown in potato and barley fields in Maine can range from 24% to 97% over a 1-wk period (Zhang 1993). Therefore, conservation of this exotic species may be desirable in northern Maine potato cropping systems (but see Zhang et al. 1994 for possible hybridization or competitive exclusion in regard to endemic *Harpalus* species). Our research suggests that rotation crops are an

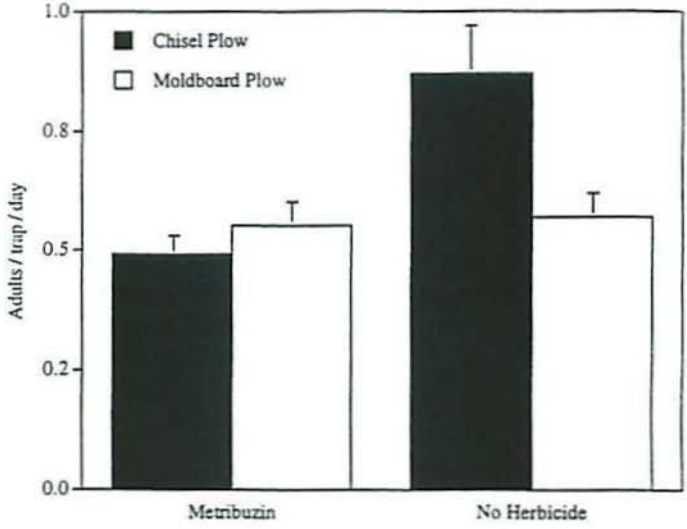

Fig. 2. Interaction between metribuzin application and tillage treatments on adult *H. rufipes* abundance at Aroostook Farm in 1991 (error bar = SE).

important refugia for these beetles with more *H. rufipes* found in barley than in adjacent potato fields. Of the rotation crops, grain may be a better refugia than green manure rotation crops because we found significantly higher abundance in oats compared with clover. Aspects of potato production that affect beetle numbers in potato crops are few. This lack of factors affecting beetle populations may be due to abundance being low overall in potato, possibly due to intensive early season cultivation. Therefore, we found that the previous year's rotation crop, insect pest management strategy, and soil management practice involving the use of amendments appear to have no effect on beetle abundance. Weed management in potatoes does affect beetle abundance with a metribuzin-based weed management program combined with spring chisel plowing being the most detrimental. Our laboratory study suggests that the effect of metribuzin does not appear to be a direct effect on beetle mortality. It may be mediated through the reduction of weed seeds and seedlings, the beetle's food resources (Zhang 1993, Murdoch et al. 1972, Pimentel 1961, Speight & Lawton 1976) or modification of the microhabitat as suggested by Rivard (1966) and Thiele (1964).

Given the moderate dispersal capability of *H. rufipes* both on the ground surface (Zhang et al. 1997) and through flight (Zhang et al. 1994, 1997), higher densities of this beetle might be maintained in potato cropping systems that are characterized by potato fields occurring adjacent to rotation crop grain fields. In addition, development of weed management programs for potato that rely less heavily on metribuzin and more on suppressive cover crops (Liebman et al. 1996) may result in better success in conservation of *H. rufipes*.

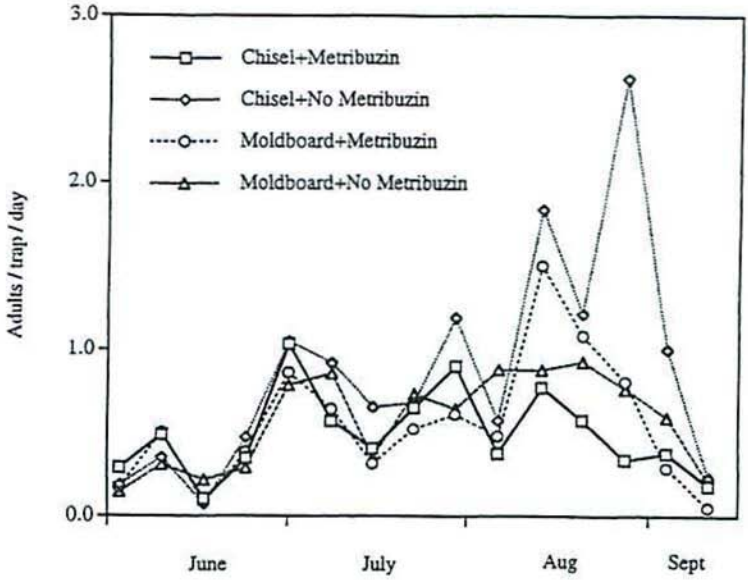

Fig. 3. Interaction between metribuzin application, tillage, and time of year on adult *H. rufipes* abundance at Aroostook Farm in 1991.

Acknowledgment

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Effect of Imidacloprid Seed Treatment on Infestations of Wheat Curl Mite (Acari: Eriophyidae) and the Incidence of Wheat Streak Mosaic Virus[1]

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ABSTRACT The survival of wheat curl mites (*Aceria tosichella* Keifer) collected from Kansas, Montana, Nebraska, South Dakota, and Texas was evaluated in the greenhouse on 'Larned', 'TAM 107', and 'Karl 92' wheat, with and without imidacloprid seed treatment. The effect of imidacloprid on the incidence of wheat streak mosaic virus (WSMV) transmitted by Kansas wheat curl mites was evaluated in the field and greenhouse. No significant differences occurred in wheat curl mite populations on any of the cultivars with and without imidacloprid. However, more wheat curl mites from each of the six collections tended to infest imidacloprid-treated Larned than untreated Larned. In one greenhouse test, the incidence of WSMV was significantly less for imidacloprid-treated than untreated Larned and TAM 107, but in two subsequent tests, no significant differences occurred. In the field at Garden City, Kansas, no significant differences occurred in wheat curl mite populations or incidence of WSMV between imidacloprid-treated and nontreated Karl 92. However, at Hays, imidacloprid-treated Karl 92 had significantly more wheat curl mites per spike than untreated Karl 92, but this did not affect the incidence of WSMV in the same plots. Results from both field and greenhouse tests indicated that imidacloprid seed treatment did not consistently control WSMV or its vector. However, imidacloprid may be useful in research with wheat curl mites and their transmission of WSMV to prevent aphids and other insects from contaminating experiments.

KEY WORDS wheat, wheat curl mite, Acari, Eriophyidae, *Aceria tosichella*, wheat streak mosaic virus, imidacloprid, resistance, vector

Wheat streak mosaic (WSM) is an important disease of wheat caused by wheat streak mosaic virus (WSMV), which is vectored by the wheat curl mite, *Aceria tosichella* Keifer (Slykhuis 1955). The recommended controls of destroying volunteer wheat and delayed planting have only limited success because WSMV caused an average annual loss in Kansas of 5.5 million hectoliters (15.5 million bushels) of grain between 1987 and 1991 (Harvey et al. 1994). Resistance to the wheat curl mite is

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effective in controlling WSM (Martin et al. 1984, Conner et al. 1991, Harvey et al. 1994) and is present in the cultivar 'TAM 107' (Wood et al. 1995). However, wheat curl mites have overcome the resistance of TAM 107 in Kansas (Harvey et al. 1997), and different collections or strains of wheat curl mites varied significantly in their responses to different sources of plant resistance (Harvey et al. 1995). Acaricides effective against wheat curl mite immediately after wheat emergence could control WSM because the most critical period for WSMV infection is in the early fall when wheat curl mites move from volunteer to planted wheat. Carbofuran applied at planting time controlled wheat curl mite and reduced the incidence of WSM (Harvey et al. 1979), and similar results were obtained with foliar-applied treatments (Hammon et al. 1993), but carbofuran is not recommended for use on wheat in Kansas. Imidacloprid seed treatment is registered for use on wheat and may reduce the incidence of aphid-borne virus diseases such as barley yellow dwarf and sugarcane mosaic (Dewar 1992, Gourmet et al. 1994, Harvey et al. 1996). Its effects on the wheat curl mite and WSMV have not been reported. The objective of this study was to evaluate the effects of imidacloprid seed treatment on wheat curl mite and its transmission of WSMV.

Materials and Methods

Different strains or sources of wheat curl mites and different cultivars of wheat may respond differently to imidacloprid, so we compared three cultivars of wheat with and without imidacloprid for its effect on survival of six collections of wheat curl mites. The identification of mite collections as *A. tosichella* was verified by J. W. Amrine (West Virginia University).

The wheat cultivars tested in the greenhouse were 'Larned', 'Karl 92', and TAM 107. These three cultivars accounted for 43% of the Kansas wheat hectarage in 1996. Seeds of each cultivar were treated with imidacloprid (Gaucho) at a rate of 1.2 g [AI]/kg (2 oz [AI]/hundred weight) by Gustafson, Inc., Dallas, Texas. Larned and Karl 92 are susceptible to wheat curl mites, and TAM 107 is susceptible to some Kansas wheat curl mites but resists wheat curl mites from other locations (Harvey et al. 1995).

The wheat curl mite collections were obtained originally from wheat in the field and then reared in the greenhouse at Hays, Kansas, on 'Tomahawk' wheat until used for testing. The following sources of wheat curl mites were tested for survival in the greenhouse: KS95 collected from Trego County, Kansas, in August 1995; KS96 collected from Ellis County, Kansas, in September 1996; TX collected at Amarillo, Texas, in May 1996 by Dr. C. Rush; NE collected at Scottsbluff, Nebraska, in May 1996 by Dr. T. Mohoomad; SD collected at Brookings, South Dakota, in February 1996 by Dr. R. Collins; and MT collected at Bozeman, Montana, in April 1996 by Dr. S. Blodgett.

Wheat curl mite tests. Seeds of each of the three cultivars described above were established at a rate of one plant per polycast tube (2.5 cm in diameter and 14 cm long) (Stuewe and Sons, Inc., Corvallis, Oregon), containing a standard greenhouse soil mixture. Five two-leaf-stage seedlings of each cultivar with and without imidacloprid were each infested with 10 wheat curl mites. The wheat curl mite transfers from the rearing host, Tomahawk wheat,

to the test plants were accomplished by placing infested leaf clippings from culture plants in 2-ml shell vials. After 1–3 h, wheat curl mites crawled from the leaf clippings to the lip of the vial, where they were picked up on the tip of a hair glued to a dowel and released in the whorl of the second leaf of each test plant. Only adult or second instars observed to have normal movement immediately after release were used. Transferring and counting wheat curl mites were accomplished by using a dissecting microscope with 15× magnification and a fiber optic system to provide cold light. The infested seedlings were covered with clear plastic cylinder cages and randomized into supportive holders, set in trays filled with water, and held in a greenhouse with natural light and variable temperatures (25 ± 10°C). Humidity (not measured) was probably high inside the cages, which were 25 mm × 305-mm vinyl-capped, nitrocellulose tubes (Sinclair and Rush Co., St. Louis, Missouri). The tubes were vented with eight 12-mm holes that were covered with nylon mesh having 16-µm openings (H.R. Williams Mill Supply Co., Kansas City, Missouri). The nylon screen provided a barrier against passage of wheat curl mite because the mesh size was less than the smallest reported size (33 µm × 80 µm) for first instars (Slykhuis 1955). Wheat curl mites may complete a generation in less than 10 d, so seedlings were excised, dissected, and examined to determine the numbers of mites per plant 7–8 d after infestation. Longer infestation periods allowed wheat curl mite numbers to become so large that accurate population estimates were difficult.

Five seedlings representing each of the six treatments (three wheat cultivars with and without imidacloprid) were infested at one time. One wheat curl mite strain constituted a test, and each test was repeated three times for each collection of mites.

Wheat WSMV transmission. Thirty, two-leaf-stage seedlings each of Larned, TAM 107, and Karl 92 with and without imidacloprid were placed in three blocks, each with 10 seedlings representing each of the six treatments (Larned, TAM 107, and Karl 92 with and without imidacloprid) in each block. The seedlings were established in polycast tubes and randomized in holding racks as previously described. Each seedling was infested manually with five wheat curl mites from the KS-96 collection. The wheat curl mites were obtained from cultures reared on Tomahawk wheat that had typical symptoms of WSM. Three weeks after wheat curl mites were transferred, the numbers of seedlings with and without visual symptoms of WSM were recorded.

Field test. Karl 92 wheat, treated with imidacloprid as previously described, was compared with untreated Karl 92 in field plots at Garden City and Hays, Kansas. At Garden City the plots were 2 m × 37 m and at Hays the plots were 1.2 m × 3 m with no buffers or borders between plots. Tests at both locations were planted with a 30-cm row spacing and four replications arranged in randomized complete blocks. Numbers of plants showing symptoms of WSMV in 30 m of row per plot were recorded on 5 May at Garden City and in 12 m of row per plot on 15 May 1997 at Hays. Five spikes were selected randomly from each plot at both locations on 4 June 1997. Numbers of wheat curl mites per spike were estimated by the sticky tape method (Harvey & Martin 1988) in which wheat curl mites are captured on tape as they abandon the drying spikes.

Values presented are means ± SD. Data were subjected to analysis of variance with treatment means separated by the Student-Newman-Keuls (Steel & Torrie 1960) multiple range test at $P < 0.05$.

Results and Discussion

Numbers of wheat curl mites on Larned, TAM 107, and Karl wheat with and without imidacloprid are shown in Table 1. Treatments did not result in significant differences in numbers of wheat curl mites on any of the cultivars. However, more wheat curl mites from each of the six collections tended to infest imidacloprid-treated Larned than untreated Larned. Further testing might determine that some wheat curl mite strains or collections, such as KS-95, MT, or NE, survive better on Larned treated with imidacloprid than on nontreated Larned, but such minor differences are unlikely to be of economic importance in the field. TAM 107 was resistant to wheat curl mite collections from Montana,

Table 1. Effect of imidacloprid seed treatment of three cultivars of wheat on populations of six collections of wheat curl mites (WCM).

Cultivar[a] Treatments	Number of WCM per plant by WCM source[b]					
	KS-95	KS-96	TX	MT	SD	NE
Larned						
Treated	67 ± 17a	50 ± 9a	74 ± 7a	70 ± 17a	47 ± 16a	62 ± 21a
Nontreated	52 ± 12a	47 ± 8a	66 ± 7a	56 ± 16a	45 ± 13a	46 ± 12a
TAM 107						
Treated	64 ± 11a	42 ± 7a	79 ± 14a	14 ± 5b	3 ± 1b	8 ± 2b
Nontreated	56 ± 10a	39 ± 16a	81 ± 10a	11 ± 6b	3 ± 1b	11 ± 4b
Karl						
Treated	63 ± 13a	43 ± 4a	82 ± 11a	69 ± 33a	42 ± 3a	49 ± 8a
Nontreated	65 ± 11a	45 ± 7a	79 ± 15a	64 ± 27a	49 ± 13a	55 ± 11a

[a]Seeds of each cultivar treated with imidacloprid (Gaucho) at rate of 1.2 g [AI]/kg (2 oz [AI]/CWT).
[b]Values are means ± SD. Means within a column followed by the same letter are not significantly different (Student-Newman-Keuls, $P < 0.05$). The WCM sources were KS, Kansas; TX, Texas; MT, Montana; SD, South Dakota; and NE, Nebraska.

Table 2. Effect of imidacloprid seed treatment of three cultivars of wheat on development of wheat streak mosaic virus (WSMV) transmitted by wheat curl mites in the greenhouse.

Cultivar[a] Treatments	WSM incidence (%) in three tests[b]			
	1	2	3	Mean
Larned				
Treated	37 ± 6a	90 ± 10a	44 ± 21a	58a
Nontreated	65 ± 13b	77 ± 32a	47 ± 21a	63a
TAM 107				
Treated	30 ± 10a	90 ± 10a	13 ± 15a	44a
Nontreated	61 ± 15b	80 ± 21a	31 ± 20a	57a
Karl				
Treated	47 ± 16a	97 ± 6a	40 ± 27a	61a
Nontreated	67 ± 6ab	90 ± 10a	40 ± 10a	68a

[a]Seeds of each cultivar treated with imidacloprid (Gaucho) at rate of 1.2 g [AI]/kg (2 oz [AI]/CWT).
[b]Values are means ± SD. Means within a column followed by the same letter are not significantly different (Student-Newman-Keuls, $P < 0.05$).

South Dakota, and Nebraska, and susceptible to wheat curl mite from Kansas and Texas. This is in agreement with previous wheat curl mite collections from Kansas, but TAM 107 was resistant to a 1994 collection from Texas (Harvey et al. 1995). Regardless of whether wheat curl mites were virulent or avirulent to plant resistance, imidacloprid did not significantly affect their survival on TAM 107.

Incidence of WSM in plants of Larned, TAM 107, and Karl 92 treated and nontreated with imidacloprid grown and infested with wheat curl mites in the greenhouse are shown in Table 2. In test 1, Larned and TAM 107 had lower percentages of symptomatic plants when treated with imidacloprid. However, in tests 2 and 3, no significant differences occurred between treated and untreated cultivars. The average percentage of symptomatic plants in test 1 (51%) was intermediate to those recorded in tests 2 and 3 (87% and 36%, respectively). Results of test 1 were not anticipated, because KS-96 wheat curl mites survived equally well on all three treated and nontreated cultivars. We have no explanation for the control of WSM by imidacloprid in test 1 and could

Table 3. Effect of imidacloprid seed treatment on numbers of wheat curl mites (WCM) per spike and numbers of wheat streak mosaic virus (WSMV)-infected plants.

	No. of WCM per spike[b]		No. WSMV-infected plants[b]	
Treatment[a]	GC[c]	HA[c]	GC	HA[c]
Treated	365 ± 152a	470 ± 186a	5.3 ± 4a	15.3 ± 5a
Nontreated	520 ± 405a	145 ± 155b	6.5 ± 3a	13.3 ± 2a

[a]Treated = Seeds of Karl-92 wheat treated with imidacloprid (Gaucho) at rate of 1.2 g AI/kg (2 oz AI/CWT). Nontreated = Karl-92 without imidacloprid.
[b]Mean no. of WCM from five spikes per plot. mean no. of WSMV-infected plants per 30 m of row at Garden City and 12 m of row at Hays. Values are means ± SD. means within a column followed by the same letter are not significantly different (Student-Newman-Keuls, $P < 0.05$).
[c]Tests located at Garden City (GC) and Hays (HA).

not repeat the results in subsequent tests. Based on these greenhouse results imidacloprid probably would not be an effective control method for WSM.

Average numbers of wheat curl mites per spike from Karl 92 with and without imidacloprid, at two locations are shown in Table 3. No significant differences occurred in wheat curl mite numbers or WSMV-infected plants at Garden City, but significantly more wheat curl mites per spike were recorded on imidacloprid-treated Karl 92 at Hays. However, differences in wheat curl mite populations at Hays did not result in a significant difference in the numbers of WSMV-infected plants (Table 3).

Although some inconsistencies were observed, the field and greenhouse results indicate that, regardless of wheat curl mite source or wheat cultivar, the use of imidacloprid seed treatment would have little or no effect on the incidence of WSM.

Because imidacloprid had no significant effect on wheat curl mites or their transmission of WSMV, it may be useful in research involving wheat curl mite and WSMV to maintain cultures and/or experiments free of aphids and other insects that it does control. We have reared wheat curl mites continuously for over a year on wheat grown from imidacloprid-treated seed in open flats in greenhouses that were simultaneously used for experiments involving greenbugs, *Schizaphis graminum* (Rondani), and Russian wheat aphids, *Diuraphis noxia* (Mordvilko), without any of the usual problems of wheat curl mite cultures becoming contaminated with aphids.

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Infestation Levels and Seasonal Incidence of the Meadow Spittlebug (Homoptera: Cercopidae) on Musk Thistle in Tennessee[1]

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ABSTRACT Infestation levels, seasonality, and distribution of the meadow spittlebug, *Philaenus spumarius* (L.), on musk thistle, *Carduus nutans* L., were monitored in eastern and middle Tennessee during a 2-yr study. Spittle masses were found on thistle from early to mid-April until late June, and most (ca. 60 to 80%) were found on the leaf internodes along the main stem. In eastern Tennessee, 50 to 90% of the plants were infested with spittle masses by early to mid-April; corresponding infestations were generally lower (0 to 10%) in middle Tennessee. Musk thistle development and populations of meadow spittlebug nymphs were well synchronized, as suitable host plant material was available for nymphs during mid-April to early June. Thus, musk thistle may be an important reservoir host for the meadow spittlebug in Tennessee. Spittlebug adults may subsequently move to grasses and economically important crops as musk thistle populations mature.

KEY WORDS *Philaenus spumarius, Carduus nutans,* meadow spittlebug, musk thistle, Homoptera, Cercopidae, insect–plant interactions

The meadow spittlebug, *Philaenus spumarius* (L.), is the most abundant, widespread, and economically important spittlebug in the United States (Johnson & Lyon 1991). This nearly cosmopolitan species feeds on almost 400 host plant species (Metcalf & Metcalf 1993), ranging from red spruce in the northeastern United States (Baker 1972) to thistle in several geographical regions (Batra et al. 1984). *Philaenus spumarius* also is one of the most widely distributed conifer-feeding spittlebugs in the United States (Johnson & Lyon 1991). This univoltine species occurs on a variety of agriculturally important plants, including grasses near wooded areas, pasture and meadow grasses, orchards, and row crops, such as strawberries and vegetables. Feeding by meadow spittlebug nymphs may cause wilting and stunting of host plants. The meadow spittlebug is an economic pest on several crops, such as alfalfa and strawberry. For example, nymphal feeding on strawberry reduced both the size and number of berries produced (Zajac & Wilson 1984).

[1]Accepted for publication 21 December 1997.

Musk thistle, *Carduus nutans* L., an adventive plant species introduced into North America during the middle of the 19th century, has continually spread over much of Tennessee, where it is now a major pest in most counties in the eastern and middle areas of the state (Grant & Lambdin 1993). Because it grows in abundance along highways, pastures, unused farmlands, and near suburban residences, it has become an economically and aesthetically unpleasant weed in these areas (Grant et al. 1990). During a study of arthropod diversity on musk thistle in Tennessee, Powell et al. (1996) found numerous species on this non-native plant species. However, only a few of these species, including the meadow spittlebug, fed extensively on musk thistle (Powell et al. 1992, 1996). In fact, Powell and et al. (1996) reported that cercopid nymphs and adults, primarily *P. spumarius*, were commonly encountered early in the growing season (rosette to bud stage), but they did not quantify its extent of feeding damage to thistle or assess the impact of musk thistle on population dynamics of the meadow spittlebug.

Little information is available on the development of the meadow spittlebug on musk thistle and its relationship to agricultural crops in Tennessee. Musk thistle may be used by the meadow spittlebug for population buildup before adults move into economically important agricultural crops (Powell et al. 1996). Thus, the objective of this study was to determine infestation levels, seasonality, and distribution of the meadow spittlebug on musk thistle in eastern and middle Tennessee.

Materials and Methods

Four thistle-infested study sites were selected for sampling in 1991 and 1992: two sites (mile markers 9 [Hamblen County] [site 1] and 18 [Greene County] [site 2] on I-81) in eastern and two (mile markers 269 [Putnam County] [site 3] and 221 [Davidson County] [site 4] on I-40) in middle Tennessee. At each site, one sampling area (3.1 m × 30.3 m) was randomly selected and delineated into 10 (3.1 m × 3.1 m) sampling blocks.

At each site, 20 plants (two per block) were randomly selected every 2 wk, depending upon environmental conditions, from 17 April to 17 June 1991, and each week from 8 April to 24 June 1992. The location (e.g., rosette, stem, first leaf, etc.) and height of each spittle mass were recorded. Five additional spittle masses were selected at each site and dissected to determine the number of nymphs per mass.

To determine infestation levels (i.e., the percentage of plants infested with spittle masses), 100 plants (10 per block) were visually inspected for spittlebug masses, which are secreted by the nymphs and easily distinguished by their frothlike or spittle appearance. The number of infested plants was recorded, and percent infestation levels were calculated. The number of spittlebug masses and adults on each plant were observed and recorded on each sampling date. Selected plant growth parameters (e.g., plant height, number of branches per plant, number of buds per plant, and number of flowers per plant) were measured on 100 plants biweekly during the study period. Plant density also was determined twice during the season (April-May and June-July) by counting the number of plants in each study plot.

Data were tabulated to determine infestation levels of spittlebugs and their seasonality at each site. Data were subjected to analysis of variance (ANOVA) to determine differences in the mean numbers of spittlebug masses per plant, nymphs per mass, and average plant height among sites in eastern and middle Tennessee (SAS Institute 1989).

Results and Discussion

Spittle masses were found on thistles throughout the sampling period, from early to mid-April until late June (Figs. 1, 2). As many as 10 spittle masses were found on rosettes, stems, and branches. Although their location on the plant varied, most (ca. 60 to 80%) of the spittle masses were found on the leaf internodes along the main stem of the plant (Table 1). Few (<4% in 1992 and none in 1991) spittle masses were found on rosettes, suggesting that spittlebug phenology is well synchronized with stem elongation and plant development.

In 1991, the average number of spittle masses peaked at ca. 4.0 per plant in mid-April in eastern Tennessee, while populations in middle Tennessee were lower, averaging <0.3 per plant (Fig. 1). In 1992, densities of spittle masses peaked at ca. 2.0 per plant during mid- to late May in eastern Tennessee and at ca. 0.8 per plant during early to mid-June in middle Tennessee (Fig. 2). Densities of spittle masses were generally higher (ca. 2–6×) in eastern Tennessee than in middle Tennessee (Figs. 1, 2). For example, significantly greater numbers of spittle masses were found in eastern than in middle Tennessee during both years of this study (1991: 1.4 and 0.3 masses per plant, respectively [F = 47.67; df = 1, 14; P = 0.0001]; 1992: 1.2 and 0.6 masses per plant, respectively [F = 20.23; df = 1, 14; P = 0.0005]) (Figs. 1, 2).

On most sampling dates, one to two nymphs were found in each spittle mass (Figs. 1, 2); however, as many as seven nymphs were found within a single spittle mass. Densities of nymphs per spittle mass were generally higher early in the season and somewhat consistent throughout the remainder of the sampling period. The seasonal average of nymphs per mass on each plant was similar among locations (1.3 and 1.1 nymphs per mass in eastern and middle Tennessee, respectively) and years (1.4 and 1.8 nymphs per mass in 1991 and 1992, respectively).

The average number of adults per plant was difficult to quantify because of their quick movement and behavior, but numerous adults were observed among the thistle plants at each site. Adult spittlebugs were first observed in late April to early/mid-May, and adult densities were greatest during May and June. Adults were present on musk thistle until the plant had fully matured, when they could have moved onto other host plants.

In eastern Tennessee, 50 to 90% of the plants were infested with spittle masses by early to mid-April (Fig. 3). However, infestations on thistle in middle Tennessee were low (<20%) until late May to early June when infestations peaked at about 50% in 1992. Infestation levels during 1991 and 1992 were greater (ca. 3–4×) in eastern than in middle Tennessee (45.4 and 11.1% [1991] and 34.7 and 12.2% [1992], respectively). Although our data do not fully explain these differences, environmental conditions and availability of alternative hosts may have been more favorable for spittlebug development in the eastern region

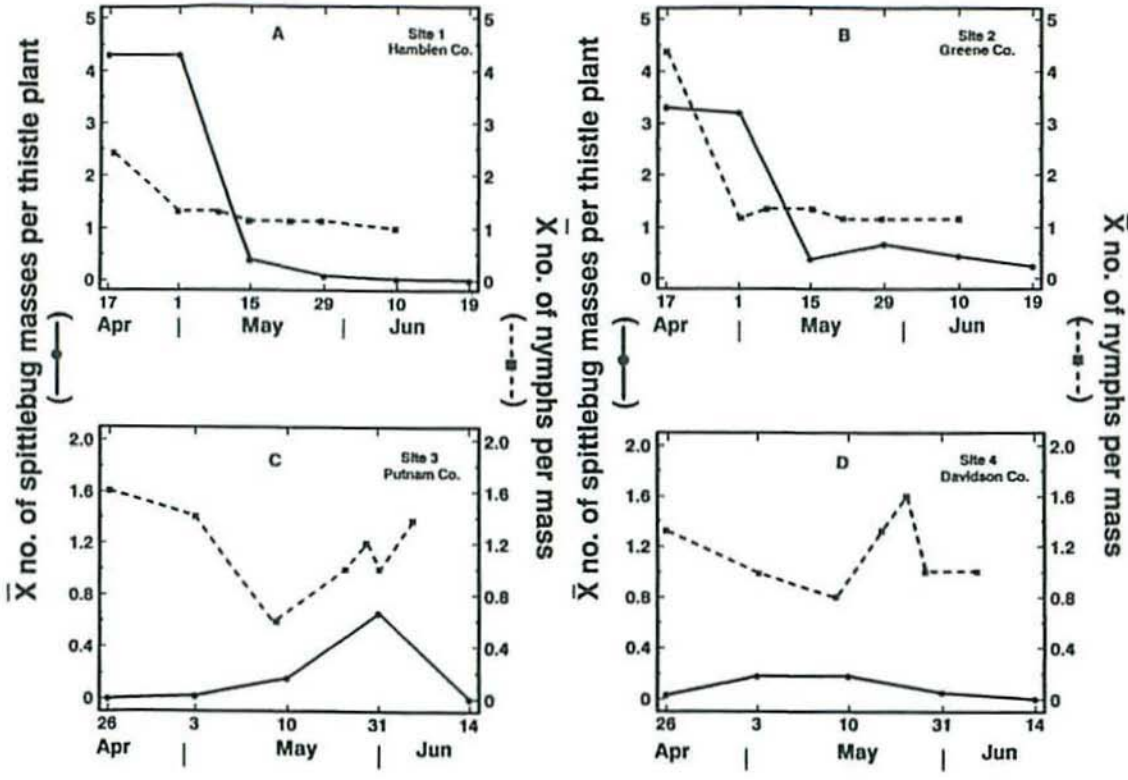

Fig. 1. Seasonal incidence of spittlebug immature masses and nymphs per mass on musk thistle at four sites in Tennessee, 1991: (A) Site 1, Hamblen County; (B) Site 2, Greene County; (C) Site 3, Putnam County; and (D) Site 4, Davidson County (sites 1 and 2 are in eastern Tennessee; sites 3 and 4 are in middle Tennessee).

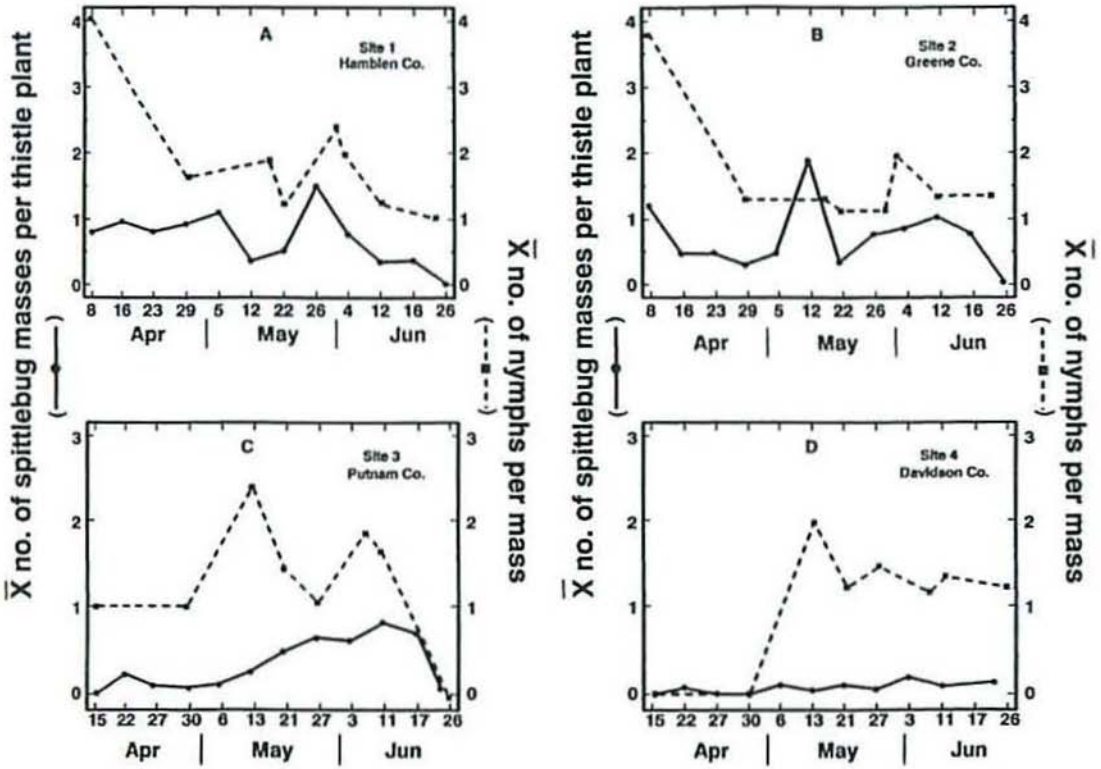

Fig. 2. Seasonal incidence of spittlebug immature masses and nymphs per mass on musk thistle at four sites in Tennessee, 1992: (A) Site 1, Hamblen County; (B) Site 2, Greene County; (C) Site 3, Putnam County; and (D) Site 4, Davidson County (sites 1 and 2 are in eastern Tennessee; sites 3 and 4 are in middle Tennessee).

Table 1. Distribution of nymphal spittle masses of *P. spumarius* on musk thistle (1991 and 1992).

Location of spittle mass	Site[a] 1	2	3	4	Mean per site
			1991		
rosette	0.0[b] (0)[c]	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
leaves	10.3 (10)	36.7 (79)	10.6 (5)	33.3 (10)	26.7 (104)
upper branches	22.6 (22)	7.0 (15)	10.6 (5)	0.3 (1)	11.1 (43)
middle branches	1.0 (1)	2.3 (5)	2.1 (1)	6.7 (2)	2.3 (9)
lower branches	0.0 (0)	1.4 (3)	0.0 (0)	0.0 (0)	0.8 (3)
upper main stem	19.6 (19)	20.9 (45)	—[d]	—[d]	—[e]
middle main stem	19.6 (19)	17.2 (37)	76.6 (36)[d]	56.7 (17)[d]	59.1 (230)[e]
lower main stem	26.8 (26)	14.4 (31)	—[d]	—[d]	—[e]
	(n = 97)	(n = 215)	(n = 47)	(n = 30)	(n = 389)
			1992		
rosette	6.5 (12)	5.1 (7)	1.1 (1)	0.0 (0)	4.3 (20)
leaves	12.0 (22)	19.9 (27)	12.8 (12)	26.8 (15)	16.2 (76)
upper branches	0.5 (1)	0.0 (0)	1.1 (1)	0.0 (0)	0.4 (2)
middle branches	0.0 (0)	2.9 (4)	0.0 (0)	0.0 (0)	0.9 (4)
lower branches	0.0 (0)	0.7 (1)	0.0 (0)	0.0 (0)	0.2 (1)
upper main stem	41.3 (76)	39.0 (53)	37.2 (35)	50.0 (28)	40.9 (192)
middle main stem	23.6 (43)	16.9 (23)	30.9 (29)	12.5 (7)	21.7 (102)
lower main stem	16.3 (30)	15.4 (21)	17.0 (16)	10.7 (6)	15.5 (73)
	(n = 184)	(n = 136)	(n = 94)	(n = 56)	(n = 470)

[a]Sites 1 and 2 are in eastern Tennessee; sites 3 and 4 are in middle Tennessee.
[b]Percentage of all spittle masses; due to rounding, combined values may not total 100%.
[c]Values in parentheses represent number of spittlebug masses.
[d]Values were grouped for the main stem (numbers were not separated for upper, middle, and lower main stem).
[e]Values for upper, middle, and lower main stems were grouped together to determine average percent infestation of main stems per site.

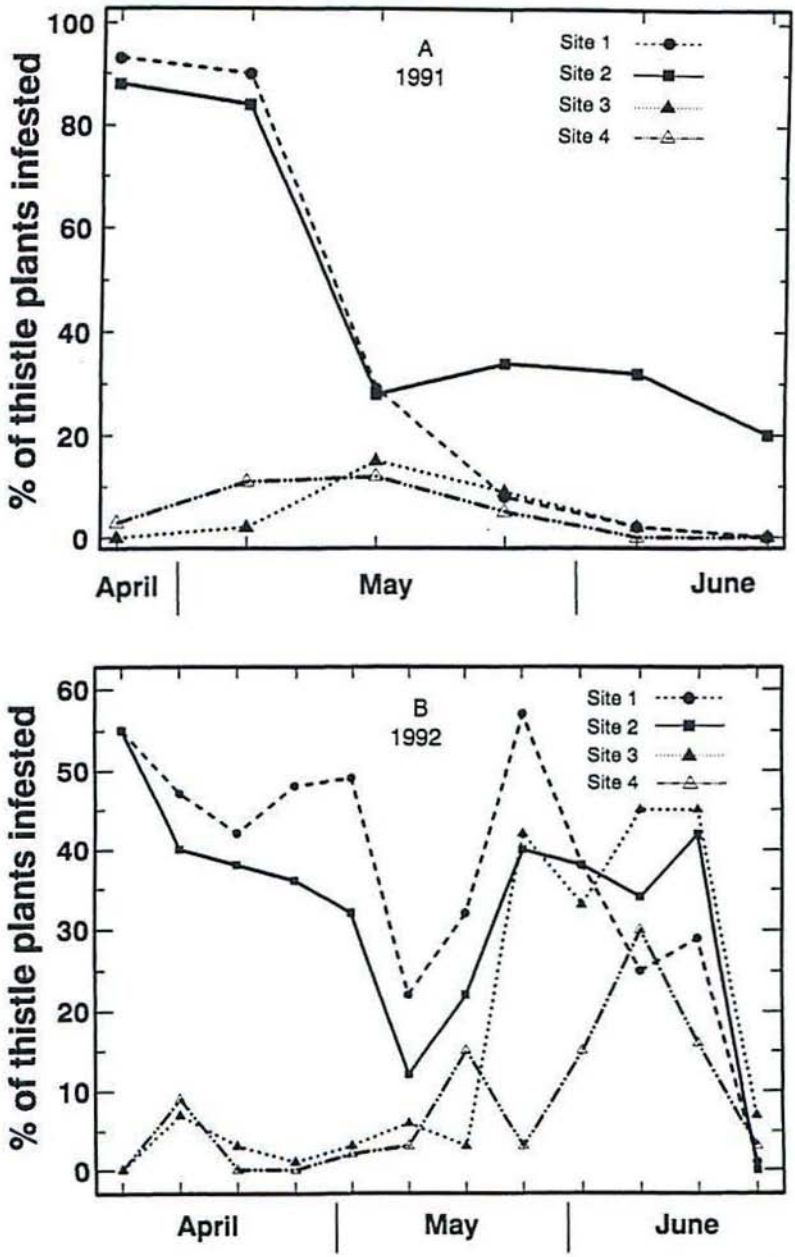

Fig. 3. Levels of infestation of spittlebug immature masses on musk thistle at four sites in Tennessee: (A) 1991 and (B) 1992. Site 1, Hamblen County; Site 2, Greene County; Site 3, Putnam County; and Site 4, Davidson County (sites 1 and 2 are in eastern Tennessee; sites 3 and 4 are in middle Tennessee).

of the state. In eastern Tennessee, infestation levels in 1991 declined steadily throughout the season (Fig. 3A); however, in 1992, infestation levels were sporadic during late season (Fig. 3B). Infestation levels, averaged throughout the season, were higher in 1992 (46.9%) than in 1991 (28.2%). The higher seasonal average and variability among infestation levels in 1992 may have been partially attributed to fewer plants available for infestation by spittlebugs.

Approximately 3.5× fewer plants were found in 1992 than in 1991. This reduction in densities of musk thistle was primarily attributed to an ongoing biological control program directed against musk thistle in Tennessee (Grant et al. 1990, Lambdin & Grant 1992). *Philaenus spumarius* had no apparent effect on plant densities. In 1991, plant densities were slightly greater in eastern Tennessee (9.2 plants per square meter) than in middle Tennessee (6.9 plants per square meter). In 1992, plant densities were similar among sites in eastern and middle Tennessee (averaging 2.8 and 2.3 plants per square meter, respectively).

In 1991, plants in eastern Tennessee were significantly taller than those in middle Tennessee ($F = 5.48$; df = 1, 14; $P = 0.0345$), averaging 66.8 and 51.8 cm, respectively. However, plant heights in 1992 were approximately 40% shorter than in 1991 and were not significantly different between eastern and middle Tennessee, averaging 38.2 and 30.7 cm, respectively. These differences in plant heights between years may be primarily attributed to seasonal variations in environmental conditions.

In areas where densities of musk thistle are high, such as in eastern Tennessee, it may serve as an important reservoir food source for spittlebug populations to increase and then move into, and colonize, agriculturally important crops, such as alfalfa. Adult meadow spittlebugs may feed during early summer on the host plant on which they develop; they may then move into legumes, corn, wheat, oats, and other suitable hosts, where they oviposit in late August and early September (Metcalf & Metcalf 1993). Because most musk thistle plants are fully mature by midsummer, some adult spittlebugs that develop on thistle may remain in the area and feed on other plants, such as grasses. However, other adult spittlebugs may move onto crop plants with more succulent tissue, where the overwintering eggs are deposited. These overwintering populations could adversely affect crop growth and yield, especially of alfalfa, during the next growing season.

Musk thistle development and nymphal populations of meadow spittlebug are well synchronized, as suitable host plant material is available for nymphs during mid-April to early June. After feeding and developing on this nonnative plant species during the spring and early summer, adults may move to grasses and economically important crops as thistle populations mature. Thus, the high infestation levels and numbers of spittlebugs on thistle early in the spring and summer suggest that musk thistle may be an important reservoir host for the meadow spittlebug throughout eastern and middle Tennessee. Additional studies are needed to clarify the role of musk thistle in population dynamics of meadow spittlebug and subsequent pest populations on economically important plants.

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Activity of Cry1 Endotoxins of *Bacillus thuringiensis* for Four Tree Fruit Leafroller Pest Species (Lepidoptera: Tortricidae)[1]

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ABSTRACT Nine formulated preparations of Cry1 endotoxins of *Bacillus thuringiensis* Berliner were bioassayed against four tree fruit pest species (Lepidoptera: Tortricidae): *Argyrotaenia citrana* (Fernald), *Choristoneura rosaceana* (Harris), *Pandemis pyrusana* Kearfott, and *Platynota stultana* (Walsingham). Larvae were fed surfaced-treated artificial diet in the laboratory and direct mortality was scored after 5 and 12 d. The LC_{50}s within species varied as much as 200-fold across preparations. Estimates of LC_{50}s were improved in the 12-d versus the 5-d assay for many of the endotoxins. The LC_{50}s to the Cry1A and Cry1E(a) endotoxins were significantly lower across all four species compared with Cry1C, Cry1D, Cry1E(b), and Cry1F endotoxins. Larval developmental time and pupal weights of surviving *P. pyrusana* were significantly affected in bioassays with two rates (0.01 and 0.001% stock solution) of each endotoxin. Larval development was delayed and pupal weight was significantly lower for both sexes at the higher rate compared with the lower rate of each endotoxin. The sex ratio of survivors for each endotoxin/rate tested was not correlated with larval survivorship.

KEY WORDS *Bacillus thuringiensis*, Cry1 endotoxin, leafrollers, orange tortrix, obliquebanded leafroller, omnivorous leafroller

Tortricid leafroller species that feed on leaves and fruits of horticultural crops have become an important suite of pests, especially as insecticide spray programs in these crops have been reduced and become more selective. For example, grower adoption of mating disruption for codling moth, *Cydia pomonella* (L.), in apple and pear has elevated leafrollers to major pest status (Beers et al. 1993, Knight 1995). Maintenance of integrated pest management programs in these crops requires that leafrollers be managed with selective interventions that will not disrupt the biological control of other secondary pests nor increase risks to environmental and farm worker safety.

[1]Accepted for publication 18 February 1998.

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Commercial formulations of the various subspecies of *Bacillus thuringiensis* Berliner (Bt) have been available for 40 yr (Beegle & Yamamoto 1992), and account for >90% of all microbial products used in pest management (Lacey & Goettel 1995). The most widely used Bt products against lepidopterous pests are derived from isolates of *B. thuringiensis* subsp. *kurstaki* and contain a blend of spores and CryIA(a,b,c) and CryII endotoxins (Höfte & Whiteley 1989). Efforts to improve the performance of products containing only *kurstaki* strains against *Spodoptera* spp., and the development of resistance to *kurstaki* strain toxins in *Plutella xylostella* (L.) (Tabashnik 1994), led to registration of new products based on the *B. thuringiensis* subsp. *aizawai* that contain genes encoding for other endotoxins (e.g., Cry1C, Cry1D, and Cry1E) (Höfte & Whiteley 1989). Developments in recombinant and nonrecombinant genetic engineering have allowed companies to tailor their Bt-based products against a wider range of pests by either increasing toxin production, expressing only specific toxins, or broadening the spectrum of activity by combining genes for several toxins (van Frankenhuyzen 1993). Incorporation of genes encoding for specific endotoxins has been used to develop several transgenic annual plants and a perennial plant, walnut (*Juglans regia* L.) (Vail et al. 1991, Dandekar et al. 1994). Recent use of transgenic plants expressing single Bt endotoxins in agriculture has intensified the need for more basic knowledge of pest species' responses to individual and groups of endotoxins. Adoption of successful resistance management strategies with Bt endotoxins requires knowledge of a given pest's levels of resistance, cross-resistance, and multiple-resistance among endotoxins (Tabashnik 1994).

The potential size of the market for Bt products to control leafroller pests in tree fruits is small relative to its use in other crops, and thus has had only a marginal effect on the development of these new products. However, from 1991 to 1995, the percentage of apple growers in Washington State who reported using Bt in their orchards increased from 0% to 23% (Washington Department of Agriculture 1995). The published literature of Bt against tortricid species in tree fruits in North America is limited to laboratory studies of commercial products (Rock & Monroe 1983; Brunner 1994; Li et al. 1995a, b) and their field efficacy (Pinnock & Milstead 1978, Vakenti et al. 1984, Westigard et al. 1986, Hardman & Gaul 1990, Brunner 1994). Screening of specific endotoxin activity for tortricid pests of tree fruits has not been reported. However, substantial literature exists on the efficacy of Bt products against the spruce budworm, *Choristoneura fumiferana* (Clemens) (van Frankenhuyzen 1993), including the activity of specific endotoxins (van Frankenhuyzen et al. 1991, Masson et al. 1992).

Our paper reports results from laboratory bioassays of nine formulated endotoxins against four tortricid species that are of importance in fruit crops in the western United States: orange tortrix, *Argyrotaenia citrana* (Fernald); oblique-banded leafroller, *Choristoneura rosaceana* (Harris); *Pandemis pyrusana* Kearfott; and omnivorous leafroller, *Platynota stultana* (Walsingham). *Argyrotaenia citrana* is a pest of small fruits in Oregon and Washington (Knight et al. 1988) and tree fruits in coastal areas of California (Zalom & Pickel 1988). *Choristoneura rosaceana* is a major pest in apple orchards in New York (Reissig 1978), across Canada (Madsen et al. 1984, Vincent & Bostanian 1989), and throughout

the western United States (University of California 1991, Beers et al. 1993). *Pandemis pyrusana* and its sibling species, *P. limitata* (Fernald) (threelined leafroller), are important pests of tree fruits in Washington (Knight 1995), British Columbia (Madsen et al. 1984), and California (Zalom & Pickel 1988). *Playtnota stultana* is primarily a pest of grapes, *Vitis* spp., in California but also can be a problem in stone fruit and kiwi (Powell 1983). The LC_{50}s from 5- and 12-d bioassays of neonates on surface-treated diet are reported. Sublethal effects of these endotoxins on larval development and pupal weight for *P. pyrusana* also are presented.

Materials and Methods

All formulated toxins were provided by Mycogen Corporation (San Diego, California). The Cry1D(a) endotoxin was expressed in a recombinant *B. thuringiensis* host, whereas all others were expressed in *Pseudomonas fluorescens* Migula by using the proprietary CellCap® encapsulation system. All toxins originated from *B. t.* subsp. *kurstaki* or *B. t.* subsp. *aizawai*. The *B. thuringiensis* sample contained live cells with spores, whereas the Cellcap® samples contained nonliving cells with crystals and no spores.

Samples were tested either as lyophilized cell culture powders or aqueous formulations. The powder samples, Cry1A(d), Cry1E(a), Cry1E(b), Cry1D(a), and Cry1Aa, were derived from shaker flask-grown *B. thuringiensis* or *P. fluorescens* cultures. Following culture, samples were centrifuged and the resulting cell pellet lyophilized. The liquid samples, Cry1A(c), Cry1C, Cry1F(a), and Cry1A(b), originated from *P. fluorescens* grown in fermentation tanks. Following cell concentration via removal of the supernatant, materials were formulated with dispersants, thickeners, and suspending agents to create aqueous flowable concentrates suitable for field application. The additives can be considered inert, having little or no insect toxicity at the levels applied in the bioassay (B. A. S., unpublished data). Endotoxin concentrations ranged from 0.8% to 3.6% in these preparations.

Insect colonies were reared on a synthetic bean diet (Shorey & Hale 1965). Colonies were maintained at 22°C, a photoperiod of 16:8 (L:D) h, and 55% RH. *Choristoneura rosaceana* and *P. pyrusana* were originally collected from apple, *Malus domestica* Borkham, and are supplemented with new wild-caught individuals every year. *Platynota stultana* was provide by V. Yokoyama (USDA, ARS Laboratory, Fresno, California). *Argyrotaenia citrana* was collected from caneberry, *Rubus* sp., in 1992 in Linn County, Oregon.

Laboratory bioassays were conducted using surface-treated artificial diet (9.0 g) within 25-ml plastic cups. Suspensions of each formulation were prepared from the stock material and serially diluted to 0.0001%. A micropipette was used to apply 200 µl of suspension to the surface of the diet in each cup (10 cm^2), and a glass rod was used to spread the material evenly over the diet surface. The diet was allowed to dry for 30 min to 1 h and a neonate larva was transferred to the middle of each cup. Twenty larvae were assayed per concentration per test. On each date, 3–4 concentrations were tested plus a water control. Tests were replicated on separate dates 3–5 times. Cups were held at 25 ± 1°C and a photoperiod of 16:8 (L:D) h. Larval mortality was assessed

after 5 and 12 d. Percentage of mortality was expressed as a function of endotoxin concentration per square centimeter of diet surface. The LC_{50}s and slopes of the dose-mortality regressions were estimated with the probit option of POLO (LeOra Software 1987). The LC_{50}s within species were considered significantly different if their 95% fiducial limits (FL) did not overlap. The LC_{50}s for each endotoxin were ranked for each species and compared across species with a Friedman test (SAS Institute 1985). Differences among toxins were separated by a nonparametric multiple range test (Conover 1980). Data were not transformed before analyses.

Additional bioassays were conducted to measure the sublethal effects of all endotoxins except Cry1A(d) and Cry1D on *P. pyrusana* larvae. Two rates of each endotoxin were used (0.01 and 0.001% stock suspension). Forty neonate larvae were placed individually on surface-treated diet. Larval survivorship, developmental time, pupal weight, and sex were recorded. Analysis of variance and correlation statistics were used to evaluate the relations among these factors (SAS Institute 1985).

Results and Discussion

Significant differences in the toxicity of endotoxins were found for larvae of each species in 5- and 12-d bioassays (Table 1). Mean ranks of the endotoxin's LC_{50}s across the four species varied significantly (Table 2). The LC_{50}s for the Cry1As and Cry1E(a) endotoxins ranked significantly higher (had lower LC_{50}s) than values for Cry1C, Cry1D, Cry1E(b), and Cry1F. These differences in activity of the Cry1 endotoxins may be an important consideration in the future development of transgenic fruit trees and effective resistance management strategies (Bauer 1995).

Estimates of LC_{50}s were substantially improved (tighter 95% FL) for many of the species/endotoxin bioassays by extending the bioassay from five to 12 d (Table 1). Tabashnik et al. (1993) examined data from 54 bioassays of Bt against the diamondback moth and found that shorter bioassays of 24–48 h provided similar information to longer tests up to 120 h. In contrast, Robertson et al. (1996) found that encapsulating Bt toxins inside *P. fluorescens* cells delayed the response time of *P. xylostella* larvae to the endotoxins. However, in their tests at 25°C there was no noticeable difference after 5 d. In our tests, the ranking of the LC_{50}s across all four species were highly correlated ($r_S = 0.89$, $P < 0.01$) between the 5- and 12-d bioassays (Table 2). One exception was the improved rank of the Cry1Ac endotoxin in the 12-d versus the 5-d bioassay. These results suggest that the standard bioassay (Robertson et al. 1996) for encapsulated Bt products may need to be extended beyond 7 d for leafroller species.

The general mode of action of Bt toxin is the disruption of osmotic balance through a change in membrane permeability in midgut epithelial cells leading to eventual rupture of the cell (Lüthy & Ebersold 1981, Ellar 1994). If the midgut is sufficiently damaged, death of the insect can be relatively rapid; or it may take several days. However, if feeding is interrupted before lethal amounts of endotoxin are ingested or if only sublethal amounts of the endotoxin are available, gut cells may sustain some damage but can be regenerated

Table 1. Concentration-mortality response of four leafroller species to surface-treated artificial diet with nine formulated Cry 1 *B. thuringiensis* endotoxins after 5 and 12 d.

			5 d		12 d	
			LC_{50} (95% FL)		LC_{50} (95% FL)	
Species	Endotoxin	*n*	Slope (SEM)	(ng/cm^2)	Slope (SEM)	(ng/cm^2)
A. citrana	Cry 1 A(a)	254	0.46 (0.08)	32.0 (NA)[a]	1.09 (0.14)	2.7 (0.8–5.3)ab
	Cry 1 A(b)	320	0.49 (0.10)	3.5 (NA)	0.64 (0.09)	5.0 (0.8–36.1)abc
	Cry 1 A(c)	339	0.85 (0.10)	32.3 (11.8–133.1)ab	1.33 (0.15)	4.7 (2.4–8.7)ab
	Cry 1 A(d)	220	0.83 (0.10)	13.9 (3.5–199.8)ab	1.58 (0.27)	1.8 (0.9–3.4)a
	Cry 1 C	377	0.47 (0.09)	195.7 (NA)	0.57 (0.08)	55.4 (NA)
	Cry 1 D	299	1.10 (0.12)	11.1 (4.2–25.2)a	1.38 (0.19)	5.5 (3.8–7.7)b
	Cry 1 E(a)	337	1.00 (0.15)	7.8 (1.9–54.5)ab	0.99 (0.13)	6.5 (2.6–14.7)abc
	Cry 1 E(b)	316	1.11 (0.22)	237.9 (42.9–1379.4)b	0.87 (0.12)	29.8 (11.9–72.0)c
	Cry 1 F	395	0.43 (0.07)	53.8 (NA)	0.36 (0.06)	66.2 (NA)
C. rosaceana	Cry 1 A(a)	219	0.94 (0.10)	1.8 (0.4–4.3)ab	2.2 (NA)	1.1 (0.4–1.8)a
	Cry 1 A(b)	279	1.17 (0.20)	1.7 (NA)	1.54 (0.28)	0.8 (NA)
	Cry 1 A(c)	277	1.44 (0.22)	2.4 (NA)	1.55 (0.29)	0.8 (NA)
	Cry 1 A(d)	217	1.26 (0.17)	1.7 (0.6–3.2)a	1.38 (0.19)	0.9 (0.6–1.2)a
	Cry 1 C	277	0.90 (0.11)	20.2 (4.6–74.3)b	0.90 (0.12)	12.6 (2.9–40.3)b
	Cry 1 D	297	1.51 (0.32)	122.4 (NA)	1.48 (0.28)	86.5 (38.9–173.3)c
	Cry 1 E(a)	252	0.91 (0.11)	1.6 (0.3–6.5)ab	1.42 (0.19)	0.8 (0.3–1.6)a
	Cry 1 E(b)	236	0.97 (0.12)	6.2 (3.5–10.5)b	0.77 (0.11)	2.2 (0.8–4.9)ab
	Cry 1 F	315	0.56 (0.08)	34.0 (NA)	0.68 (0.11)	15.1 (NA)
P. pyrusana	Cry 1 A(a)	291	0.91 (0.12)	1.1 (0.4–2.5)a	1.24 (0.16)	0.4 (0.02–0.4)a
	Cry 1 A(b)	368	0.92 (0.17)	12.6 (3.4–69.7)bcd	0.36 (0.24)	0.8 (0.02–1.7)ab
	Cry 1 A(c)	311	0.64 (0.09)	1.6 (0.8–3.9)ab	0.88 (0.09)	0.0 (0.02–0.8)ab
	Cry 1 A(d)	343	0.79 (0.09)	4.6 (1.5–12.2)ab	0.93 (0.11)	0.3 (0.03–0.6)a
	Cry 1 C	310	0.82 (0.15)	12.6 (4.6–65.9)cd	0.93 (0.14)	1.3 (0.8–2.5)b
	Cry 1 D	415	1.03 (0.16)	108.2 (60.7–171.6)d	0.91 (0.12)	28.8 (11.8–55.4)c
	Cry 1 E(a)	351	1.15 (0.23)	1.4 (0.5–3.0)a	0.72 (0.15)	0.3 (0.02–0.80)ab
	Cry 1 E(b)	354	1.20 (0.21)	27.3 (12.4–57.2)c	0.76 (0.12)	5.9 (2.7–12.7)c
	Cry 1 F	411	0.45 (0.07)	226.8 (37.8–1,480.4)cd	0.73 (0.08)	12.3 (4.7–26.5)c
P. sultana	Cry 1 A(a)	400	1.23 (0.15)	0.7 (0.4–1.1)a	1.42 (0.16)	0.4 (0.04–0.40)a
	Cry 1 A(b)	400	0.83 (0.11)	0.8 (0.01–0.9)a	0.98 (0.11)	0.02 (0.01–0.05)a
	Cry 1 A(c)	400	1.13 (0.12)	7.1 (3.9–11.0)bc	1.60 (0.18)	0.8 (0.7–1.6)b
	Cry 1 A(d)	400	1.14 (0.17)	1.2 (0.6–2.0)a	1.11 (0.12)	6.3 (0.3–0.6)a
	Cry 1 C	400	1.19 (0.14)	2.5 (1.3–4.2)b	1.65 (0.22)	1.3 (0.8–2.1)b
	Cry 1 D	400	1.60 (0.19)	102.1 (54.6–174.1)de	1.48 (0.17)	52.9 (26.4–93.7)c
	Cry 1 E(a)	400	1.44 (0.28)	27.5 (9.5–47.5)cd	1.21 (0.21)	14.0 (5.7–23.8)c
	Cry 1 E(b)	400	1.00 (0.16)	32.7 (8.9–82.4)cde	1.07 (0.15)	19.4 (4.9–48.3)c
	Cry 1 F	400	1.13 (0.19)	118.1 (60.5–195.6)e	1.26 (0.15)	17.0 (9.5–26.5)c

[a]NA, 95% fiducial limits could not be calculated due to the variability present in the data. The LC_{50}s were significantly different if 95% FL did not overlap.

Table 2. Average ranks of Bt endoxins LC_{50}s for four species of tortricid leafrollers in surface-treated bioassays after 5 and 12 d.

	Average rank of LC_{50} across species[a]	
Bt-endotoxin	5-d bioassay	12-d bioassay
Cry 1A(a)	2.8a	3.0a
Cry 1A(b)	2.5a	2.8a
Cry 1A(c)	4.8ab	2.0a
Cry 1A(d)	3.3a	3.3a
Cry 1C	6.0bc	6.3b
Cry 1D	7.0bc	8.0b
Cry 1E(a)	2.8a	3.8a
Cry 1E(b)	7.3bc	7.0b
Cry 1F	8.3c	8.3b

Mean ranks were compared with a Friedman test (SAS Institute 1985) followed by a nonparametric multiple range test (Conover 1980). The 5-d bioassay: $X^2 = 20.7$, df = 8, $P < 0.01$; 12-d bioassay: $X^2 = 22.6$, df = 8, $P < 0.005$.

[a]Spearman rank correlation between the 5-d and 12-d test, $r_s = 0.89$, $P < 0.005$.

(Lüthy & Ebersold 1981, Spies & Spence 1985). Recovery of insects receiving sublethal doses of endotoxin have been reported for a number of insect species, including the tortricids *C. fumiferana* (Clemans) (Fast & Régnière 1984) and *C. rosaceana* (Harris) (Li et al. 1995b).

Time to pupation for *P. pyrusana* larvae feeding on Bt-treated diet varied significantly among endotoxins and the check for both males ($F = 2.79$; df = 13, 151; $P = 0.001$) and females ($F = 5.53$; df = 13, 139; $P < 0.001$) (Table 3). Because of the differences in the initial concentrations of the stock solutions and subsequent dilutions, no mean separations among endotoxins in these analyses are reported. However, the mean (SEM) delay in larval development on endotoxin-treated diet compared with the check was 2.9 d (0.3) and 4.5 d (0.3) for males and females, respectively. The mean delay in larval development was negatively correlated with the mean larval survivorship at each dose across all endotoxins (male: $r = -0.68$, $P < 0.001$; female: $r = -0.83$, $P < 0.001$). Larval development was significantly affected by the concentration of endotoxin and was slower at the higher level for both males ($F = 9.5$; df = 1, 125; $P < 0.01$) and females ($F = 32.6$; df = 1, 122; $P < 0.0001$). The sex ratio of the survivors of each treatment was not correlated with mean larval survivorship ($P = 0.39$).

Differences in pupal weight among the check and the two rates of each endotoxin were found for both males ($F = 3.02$; df = 13, 151; $P < 0.001$) and females ($F = 2.31$; df = 13, 139; $P < 0.01$) (Table 3). There was no correlation of mean pupal weight with the mean larval development delay relative to the check with either sex (male, $P = 0.21$; female, $P = 0.23$) or with larval survivorship

(male, $P = 0.28$; female, $P = 0.18$). Pupal weight was significantly affected by the concentration of endotoxin, and was reduced at the higher concentration for both sexes (male: $F = 4.9$; df = 1, 125; $P < 0.05$; female: $F = 6.1$; df = 1, 122; $P < 0.05$). In contrast, Li et al. (1995b) found no significant effect on pupal weight with *C. rosaceana* feeding on Dipel-treated foliage (tested at the LC_{50}). Sublethal doses of Bt and *B. sphaericus* Neide that cause a reduction in pupal weight also can be responsible for small adult size, lower nutritional reserves, and reduced adult longevity and fecundity (Dulmage & Martinez 1973, Fast & Régnière 1984, Lacey et al. 1987). These effects have not been measured for any leafroller species.

The occurrence of sublethal exposure to Bt residues in treated leafroller populations has both positive and negative impacts on pest management. The timing of adult emergence and egg hatch for summer populations from spring Bt-treated orchards can de delayed by several weeks from populations in orchards not treated with Bt (A. L. K., unpublished data). Currently, models are used to time sampling activities and spray applications for the summer generation of leafrollers in Washington's apple orchards (Beers et al. 1993). The occurrence of sublethal effects from Bt exposure suggests that new phenology models are needed for these species within Bt-treated orchards. Delaying larval development in the spring also prolongs the window of exposure of larvae to predators and parasitoids. Field studies conducted with *P. pyrusana* and *C. rosaceana* in 1994–1995 found that 40–80% of individuals in weekly samples were parasitized in apple orchards treated with Bt after the time period when the majority of individuals in several untreated populations had pupated and adult emergence peaked (A. L. K., unpublished data). This parasitism was primarily by two species of tachinids, *Nemorilla pyste* (Walker) and *Pseudoperichaeta erecta* (Coquillet), that attacked large larvae late in the spring. The interactions of natural enemies and timings of Bt applications on leafroller management in orchards need to be explored more fully.

Cessation of feeding by leafrollers following sublethal exposure to these Bt endotoxins persists for 7–21 d depending on temperature (A. L. K., unpublished data), and also may contribute to reductions in fruit injury. However, eventual larval recovery and the short field persistence of residues (Nyouki & Fuxa 1994) limit the effectiveness of Bt insecticides in tree fruit pest management. Avoidance of Bt residues by some lepidopterous species, including *C. fumiferana* (Ramachandran et al. 1993), has been reported. In combination with rapid foliage growth by fruit trees in the spring and early summer, any avoidance behavior would likely increase the occurrence of sublethal effects in treated orchards (Knight 1997). Optimal use of Bt insecticides may require concentrated spray applications to ensure mortality of the target pest following consumption of one or a few droplets of insecticide (van Frankenhuyzen & Payne 1993). The addition of feeding stimulants to enhance the activity of Bt (Farrar & Ridgway 1995) also has been shown to be effective for leafrollers (Brunner 1994, Knight 1997). The use of supplemental chemicals to enhance Bt, such as caffeine, tannic acid, or inorganic salts (Morris et al. 1994, 1995; Gibson et al. 1995), has not been reported with leafrollers in tree fruits.

Table 3. Sublethal effects of Cry 1 endotoxins on larval development and pupal weight for *P. pyrusana* and *C. rosaceana*.

Species	Endotoxin	Conc. (ng/cm²)	Survivor-ship	Sex ratio (M/F)	No. days to pupation		Pupal weight (mg)	
					Male (SEM)	Female (SEM)	Male (SEM)	Female (SEM)
P. pyrusana								
	Check	-	0.70	1.3	32.3 (1.5)	35.4 (1.7)	52.6 (1.7)	81.2 (2.9)
	Cry 1A(a)	36.0	0.63	0.9	32.9 (1.7)	38.8 (2.3)	64.6 (2.5)	94.4 (3.1)
	Cry 1A(a)	3.6	0.50	1.2	30.0 (1.2)	35.0 (2.7)	68.3 (2.8)	94.7 (3.6)
	Cry 1A(b)	60.0	0.23	0.8	46.5 (6.4)	49.0 (4.1)	53.1 (0.8)	85.3 (6.0)
	Cry 1A(b)	6.0	0.63	1.1	36.5 (3.3)	34.2 (1.2)	59.7 (2.1)	104.7 (3.2)
	Cry 1 A(c)	127.0	0.00	-	-	-	-	-
	Cry 1 A(c)	12.7	0.48	1.7	38.6 (2.1)	40.7 (1.4)	56.1 (3.4)	92.7 (5.8)
	Cry 1 C	73.5	0.23	0.3	38.5 (8.5)	52.3 (4.1)	47.0 (4.4)	78.3 (5.6)
	Cry 1C	7.4	0.50	1.0	32.9 (2.1)	39.7 (3.0)	59.6 (2.3)	96.2 (5.3)
	Cry 1E(a)	48.4	0.65	1.9	38.8 (2.1)	39.8 (2.2)	48.4 (3.6)	96.9 (9.4)
	Cry 1E(a)	4.8	0.55	0.3	33.6 (2.3)	34.9 (1.9)	52.1 (1.3)	86.9 (1.8)
	Cry 1E(b)	28.3	0.85	1.3	32.8 (1.7)	34.0 (1.8)	60.5 (4.8)	88.3 (4.8)
	Cry 1E(b)	2.8	0.88	0.7	28.7 (1.0)	33.1 (1.1)	63.0 (2.1)	99.0 (4.8)
	Cry 1F	101.0	0.40	2.2	36.2 (2.7)	51.4 (8.6)	54.7 (3.1)	80.9 (7.3)
	Cry 1F	10.1	0.75	1.7	33.0 (1.8)	36.4 (1.8)	58.4 (1.2)	89.0 (4.7)
C. rosaceana								
	Check	-	1.00	1.1	22.6 (0.6)	24.3 (0.3)	67.1 (2.0)	111.9 (3.5)
	Cry 1 A(a)	36.0	0.85	1.4	26.5 (1.1)	28.9 (1.2)	67.8 (1.1)	110.4 (3.5)
	Cry 1 A(a)	3.6	0.90	1.0	24.7 (0.8)	28.0 (1.4)	69.5 (1.4)	113.6 (3.8)
	Cry 1 A(b)	60.0	0.85	1.6	31.5 (1.8)	32.2 (2.1)	71.7 (1.3)	125.2 (4.2)
	Cry 1 A(b)	6.0	0.93	1.6	27.3 (0.7)	29.9 (0.9)	87.2 (5.8)	121.7 (5.3)
	Cry 1 A(c)	127.0	0.50	0.7	30.1 (0.9)	34.8 (2.0)	71.6 (2.4)	112.2 (4.2)
	Cry 1 A(c)	12.7	0.85	1.6	23.8 (0.7)	29.8 (1.2)	72.8 (2.3)	116.6 (4.2)
	Cry 1 C	73.5	0.75	0.9	26.6 (1.3)	27.7 (1.3)	73.5 (5.1)	113.6 (3.0)
	Cry 1 C	7.4	0.83	0.7	26.5 (1.4)	30.0 (2.2)	70.3 (2.8)	120.0 (6.2)
	Cry 1 E(a)	48.4	0.95	0.9	23.4 (0.7)	27.1 (0.7)	72.9 (1.6)	119.5 (6.7)
	Cry 1 E(a)	4.8	0.88	1.3	25.3 (1.0)	25.3 (0.8)	70.3 (1.8)	122.3 (4.2)
	Cry 1 E(b)	28.3	0.85	1.1	23.6 (0.8)	26.9 (1.1)	70.2 (2.1)	118.8 (4.5)
	Cry 1 E(b)	2.8	0.93	0.5	22.0 (0.9)	23.5 (0.9)	70.4 (2.4)	131.7 (3.0)
	Cry 1 F	101.0	0.93	1.2	23.3 (0.6)	25.4 (0.6)	75.8 (2.1)	128.7 (4.0)
	Cry 1 F	10.1	0.98	1.6	22.0 (0.5)	24.0 (0.5)	70.0 (1.3)	138.5 (4.6)

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Evaluation of Transgenic Corn for Resistance to Corn Earworm (Lepidoptera: Noctuidae), Fall Armyworm (Lepidoptera: Noctuidae), and Southwestern Corn Borer (Lepidoptera: Crambidae) in a Laboratory Bioassay[1,2]

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ABSTRACT The development of crop plants expressing genes that encode insecticidal proteins isolated from the bacterium *Bacillus thuringiensis* Berliner (*Bt*) has provided a tremendous opportunity for improved control of insect pests. Although corn, *Zea mays* L., germplasm with resistance in the whorl stage to fall armyworm, *Spodoptera frugiperda* (J. E. Smith), and southwestern corn borer, *Diatraea grandiosella* Dyar, larval feeding has been identified and released, little progress has been made in identifying resistance to ear damage by these insects. Husks and silks were collected 3 to 4 d after anthesis from nontransgenic and transgenic corn hybrids and used in laboratory bioassays to determine the effects of the *Bt* insecticidal protein on larval survival and growth of fall armyworm, southwestern corn borer, and corn earworm, *Helicoverpa zea* (Boddie). Southwestern corn borer larvae that fed on diets containing husks of transgenic plants did not survive. Corn earworm larvae fed on diets containing husks or silks from transgenic plants died, and those that fed on diets containing silks harvested from transgenic plants exhibited reduced survival and growth. Fall armyworm larvae were least susceptible to the *Bt* protein, but larval survival and growth on diets containing husks of transgenic plants were reduced. Larval growth also was reduced on diets containing silks from transgenic plants. The bioassays indicated that expression in husks and silks of genes encoding *Bt* insecticidal proteins could be useful in reducing insect damage in ears of corn.

KEY WORDS Lepidoptera, Noctuidae, Pyralidae, *Spodoptera frugiperda, Diatraea grandiosella, Helicoverpa zea, Bacillus thuringiensis, Zea mays,* plant resistance, transgenic hybrids

Tremendous effort has been expended in the past few years on the development of crop plants expressing genes that encode insecticidal proteins

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having the property of those found in the bacterium *Bacillus thuringiensis* Berliner *(Bt)* (Boulder 1993). Corn, *Zea mays* L., plants expressing δ-endotoxin insecticidal proteins have been evaluated in field tests for resistance to European corn borer, *Ostrinia nubilalis* (Hübner), a major pest in the Corn Belt and a serious pest in parts of the southern United States. Resistance to both leaf feeding and stalk tunneling were observed (Armstrong et al. 1995, Koziel et al. 1993).

In the southern United States, fall armyworm, *Spodoptera frugipereda* (J. E. Smith), and southwestern corn borer, *Diatraea grandiosella* Dyar, are economically more important than European corn borer. Williams et al. (1997) observed high levels of resistance to leaf feeding by southwestern corn borer in whorl-stage plants of transgenic *Bt* corn hybrids and moderate resistance to leaf feeding by fall armyworm. They also found that when southwestern corn borer larvae were fed on laboratory diets containing lyophilized leaf or husk tissue of transgenic *Bt* corn hybrids, the larvae died. Fall armyworm larvae fed diets containing lyophilized leaf tissue with the *Bt* protein exhibited lower survival and reduced weight in comparison with those fed diets that did not contain the protein.

In this investigation, survival and growth of fall armyworm, southwestern corn borer, and corn earworm, *Helicoverpa zea* (Boddie), larvae were compared in a laboratory bioassay on diets containing lyophilized husk tissue of two transgenic corn hybrids with *Bt* insecticidal proteins and nontransgenic versions of the two hybrids lacking the protein. In addition, survival and growth of larvae were compared on diets consisting of lyophilized leaf tissue of either a leaf-feeding resistant or a susceptible corn hybrid and lyophilized silks of a transgenic or nontransgenic hybrid. The treatments constituted a 2×3 factorial set with leaf tissue being the primary factor and no silks, transgenic silks, or nontransgenic silks the secondary factor. Leaf tissue from both resistant and susceptible hybrids was used in the bioassays to determine whether the addition of the insecticidal proteins in transgenic silks affected larval survival and growth differently when used in combination with resistant leaf tissue than susceptible leaf tissue.

Materials and Methods

Plant materials. Two experimental transgenic *Bt* corn hybrids, N6800Bt and N7634Bt, and nontransgenic versions of these hybrids, N6800 and N7639, were provided by Novartis Seeds. The transgenic single-cross hybrids were produced by crossing *Bt* lines with elite inbred testers.

The gene used to confer insect resistance was *CryIA(b),* or *Bt* 11, from *B. thuringiensis* var. *kurstaki* (Höfte & Whiteley 1989). The coding sequence of the *CryIA(b)* gene was altered to increase its level of protein expression (Perlak et al. 1991). A 35S promoter from cauliflower mosaic virus (Gardner et al. 1981) was ligated to the *CryIA(b)* gene and then inserted into a pUC18 cloning vector (Yanisch-Perron et al. 1985). The phosphinothricin N-acetyltransferase (PAT) gene from *Streptomyces viridochromogenes* (Krainsky) (Wohlleben et al. 1988) was inserted into the same pUC18 cloning vector and used as a selectable marker. The *CryIA*-PAT construct was incorporated into corn and selected in

vitro with a glufosinate-ammonium herbicide. First-generation transformed plants were regenerated from calli. Reciprocal crosses were made with Northrup King Company elite, inbred lines. The progeny were subjected to selective herbicide sprays and enzyme-linked immunosorbent assay (ELISA) to ensure that only plants containing the *CryIA(b)* gene were advanced.

Approximately 100 plants of each hybrid were grown at Mississippi State, Mississippi, in 1996 following standard corn production practices to provide husks and silks for laboratory bioassays. Within 3 to 4 d after silk emergence, primary ear shoots were removed from the plant and placed on ice. Husks and silks were removed from the ear, separated, placed in plastic freezer bags, and frozen at -18°C. The husks and silks were later lyophilized and ground to a fine powder by using a laboratoy mill with a 1-mm screen.

Husk bioassay. On 26 February 1997, 20 cups of diet were prepared from the ground, lyophilized husk tissue of each hybrid. The transgenic and non-transgenic versions of the two hybrids formed a 2 × 2 factorial set of treatments. Following the procedures described by Williams & Buckley (1996), diets were prepared by combining 250 ml of distilled water, 2.5 g of agar, 528 mg of ascorbic acid, 132 mg of sorbic acid, and 12.5 mg of gentamicin sulfate and heating the mixture to 100°C while stirring. The mixture was then cooled to 82°C and 15 g of lyophilized husk tissue was added. The diet was dispensed into 30-ml plastic cups. Each cup was infested with a neonate southwestern corn borer and covered with a paperboard insert cap. Cups were arranged in a randomized complete block design with five replications of four cups per hybrid and placed in an environmental chamber maintained at 29°C and photoperiod of 12:12 (L:D) h. The larvae were counted and weighed 21 d later. Previous work indicated that southwestern corn borer larvae fed on husk tissue of highly susceptible corn genotypes reach the 5th instar, but do not pupate within 21 d (P.M.B., unpublished data).

On 4 March and 10 April, similar experiments were initiated for fall armyworm and corn earworm, respectively. All larvae were obtained from laboratory cultures maintained in the Corn Host Plant Resistance Research Unit. Field-collected larvae are infused into the cultures annually to avoid loss of vigor in the cultures. Because fall armyworm and corn earworm grow more rapidly than southwestern corn borer, the larvae were weighed after 14 d rather than 21 d so larvae would not pupate before weighing.

Silk bioassay. To ensure that the laboratory diets provided adequate nutrition for larval growth, the ground lyophilized silks were combined with lyophilized leaf tissue rather than being used alone in diets. Leaf tissue was harvested from two hybrids: Mp704 × Mp707, which is moderately resistant to fall armyworm and southwestern corn borer, and Ab24E × SC229, which is susceptible to both insects. In field evaluations Mp704 × Mp707 sustains less leaf damage that Ab24E × SC229 when infested with either fall armyworm or southwestern corn borer. Larvae recovered from Mp704 × Mp707 plants weigh less than those recovered from Ab24E × SC229 (Williams et al. 1997). Both a resistant and a susceptible hybrid were included to determine whether the effect of a combination of resistant leaf tissue and silks differed from a combination of susceptible leaf tissue and silks. The whorl tissue was harvested when plants were in the V7 stage of growth (Ritchie et al. 1986) and was processed following the procedures described for husks.

Diets were prepared following the same procedures described for husks except either 9 g of lyophilized leaf tissue or 9 g of lyophilized leaf tissue plus 2 g of either transgenic (N6800Bt) or nontransgenic (N6800) silks replaced the husks in the diets. With genotypes of leaf tissue as one factor and no silks, nontransgenic silks, or transgenic silks as the second factor, the six treatments constituted a 2 × 3 factorial set of treatments. Diet cups in the first experiment were infested with southwestern corn borer larvae on 12 March, and diet cups in the other experiments were infested with fall armyworm and corn earworm on 21 March. Experimental protocols were the same as for husks except the southwestern corn borer larvae were counted and weighed 14 d after infestation. Fall armyworm and corn earworm larvae were counted and weighed after 10 and 11 d, respectively. Larvae grow more rapidly on leaf tissue than on husk tissue so the larval feeding periods were reduced to avoid pupation on the susceptible hybrid.

Data on larval survival and weight from each experiment were subjected to analysis of variance. The significance of differences between means was determined by Fisher's Protected LSD ($P = 0.05$) (Steel & Torrie 1980).

Results and Discussion

The results of bioassays that used lyophilized husk tissue are presented in Table 1. All southwestern corn borer and corn earworm larvae that fed on husks of the transgenic hybrids were killed. Survival of fall armyworm larvae on the diets containing *Bt* protein was significantly (F=16.00; df=3, 12; P<0.0002) lower (35%) than on the diets lacking *Bt*. Weights of the surviving larvae also were greatly reduced. Interestingly, mean larval weights for all three species were consistently higher on the nontransgenic hybrid N6800 than on the hybrid N7639. This indicates that N7639 may possess at least some resistance to these insects in the early reproductive stages.

Survival and weights of larvae fed on diets containing mixtures of lyophilized leaves and silks are presented in Table 2. Larval survival was high (≥90%) for all species fed on diets containing lyophilized leaf tissue, but no silk tissue, whether the leaf tissue was from the leaf-feeding resistant hybrid Mp704 × Mp707 or from the susceptible hybrid Ab24E × SC229. The addition of silks from the nontransgenic hybrid N6800 significantly (F=107.63; df=5, 20; P<0.0001) reduced survival of southwestern corn borer larvae, but not fall armyworm or corn earworm survival. Adding silks of the transgenic hybrid N6800Bt to the diet resulted in death of all southwestern corn borer larvae with no visible sign of larval feeding, significantly (F=16.59; df=5, 20; P<0.0001) reduced corn earworm survival, but did not affect fall armyworm survival (F=0.55; df=5, 20; P>0.73).

Larvae reared on lyophilized leaf tissue of the resistant hybrid alone weighed about half as much as those reared on lyophilized leaf tissue of the susceptible hybrid. Additions of silks of the nontransgenic hybrid resulted in reduced size. Apparently, something detrimental to larvae of the three species is present in the silks that caused reduced weight of fall armyworm and corn earworm larvae and reduced survival of southwestern corn borer. Wiseman et al. (1986) reported that additions of leaf tissue to pinto bean diet resulted in

Table 1. Survival and weight of southwestern corn borer, fall armyworm, and corn earworm larvae fed on lyophilized husk tissue of transgenic and nontransgenic corn hybrids in a laboratory bioassay.

Hybrid	Southwestern corn borer[a]		Fall armyworm[b]		Corn earworm[b]	
	Survival (%)	Weight (mg)	Survival (%)	Weight (mg)	Survival (%)	Weight (mg)
N6800[c]	90 (22)	126 (12)	85 (22)	231 (11)	95 (11)	97 (14)
N7639[c]	95 (11)	78 (7)	90 (14)	100 (17)	95 (11)	39 (7)
N6800Bt[d]	0 (0)	—	55 (21)	16 (10)	0 (0)	—
N7639Bt[d]	0 (0)	—	50 (18)	15 (12)	0 (0)	—
LSD (0.05)	34	13	16	35	11	25

Means followed by standard deviations in parentheses.

[a]Larvae were counted and weighed after feeding 21 d.

[b]Larvae were counted and weighed after feeding 14 d.

[c]Nontransgenic hybrid.

[d]Transgenic (*Bt*) hybrid.

Table 2. Survival and weight of southwestern corn borer, fall armyworm, and corn earworm larvae fed in a laboratory bioassay on a diet containing lyophilized leaf tissue of genetically resistant (R) and susceptible (S) corn hybrids with additions of lyophilized silks of transgenic (T) and nontransgenic (NT) hybrids.

	Southwestern corn borer[a]		Fall armyworm[b]		Corn earworm[c]	
Diet	Survival (%)	Weight (mg)	Survival (%)	Weight (mg)	Survival (%)	Weight (mg)
S	100 (0)	120 (8)	95 (11)	466 (35)	95 (11)	250 (71)
S + NT silks	60 (22)	94 (11)	100 (0)	255 (33)	85 (22)	40 (12)
S + T silks	0 (0)	—	100 (0)	45 (5)	55 (21)	4 (0.8)
R	100 (0)	60 (8)	95 (11)	285 (75)	90 (14)	109 (13)
R + NT silks	85 (14)	59 (8)	95 (11)	67 (29)	100 (0)	20 (3)
R + T silks	0 (0)	—	100 (0)	8 (4)	40 (14)	1 (0.5)
LSD (0.05)	13	9	NS	50	18	40

Means followed by standard deviations in parentheses.

[a]Larvae were counted and weighed after 14 d.

[b]Larvae were counted and weighed after 10 d.

[c]Larvae were counted and weighed after 11 d.

increased fall armyworm larval weight, but additions of silk resulted in significant reductions in larval weight of fall armyworm and corn earworm larvae. The addition of transgenic silks to the diets resulted in marked reductions in larval weights whether the leaf tissue came from a resistant or susceptible hybrid. However, fall armyworm and corn earworm larvae fed on a combination of leaves from a resistant hybrid and silks of a transgenic hybrid were extremely small.

Of the three insects, the southwestern corn borer was most sensitive to the *Bt* insecticidal protein. All larvae were killed in any diet containing transgenic tissue, whether husk or silk. The corn earworm was the second most sensitive. The larvae were killed when fed on diets of husk tissue, and when silk and leaf tissue were combined, larval survival and weight were significantly (F=51.47; df=5, 20; P<0.0001) reduced. The fall armyworm was the least sensitive of the three insects to the *Bt* proteins. Survival was reduced on *Bt*-containing husk diets, but 50% of the larvae survived. On the silk–leaf tissue mixtures, survival was unaffected. The greatly reduced weights of fall armyworm larvae fed on any of the diets containing the *Bt* insecticidal proteins indicates the potential usefulness of this technology in controlling fall armyworm as well as the other two insects.

Combining native resistance with the *Bt* insecticidal protein in a hybrid could result in better control of less susceptible insects such as fall armyworm. Also, the enhancement from native resistance could hinder the development of resistance to *Bt* proteins in an insect population. Sachs et al. (1996) suggested pyramiding CryIA(b) insecticidal protein with conventional plant resistance in cotton to delay the development of resistant tobacco budworm, *Heliothis virescens* (F.), populations in cotton, *Gossypium hirsutum* L. Pyramiding could be especially useful with polyphagous insects such as fall armyworm and corn earworm that attack more than one crop in which transgenic hybrids and varieties have already been developed.

The effectiveness of the *Bt* protein in silks and husks against southwestern corn borer, fall armyworm, and corn earworm in these assays indicates the potential for reduced ear damage that would be important in eliminating the entrance of fungi into the ears and reducing contamination of grain with mycotoxins. This could be especially important in the southern United States where aflatoxin contaminated grain is a chronic problem in corn.

Although the bioassays described herein used husks and silks harvested soon after anthesis, other plant tissues or tissue collected at other growth stages also could be used. The bioassays also could be adapted for use with other insect pests and other crops.

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Effects of Summer Pruning and Hand Fruit Thinning on Obliquebanded Leafroller (Lepidoptera: Tortricidae) Fruit Damage in New York State Apple Orchards[1]

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ABSTRACT Two commercially used horticultural practices, summer pruning and fruit thinning by hand, reduced fruit damage caused by the obliquebanded leafroller, *Choristoneura rosaceana* (Harris), in apple orchards in New York. Results of a 2-yr study indicate that fruit thinning by hand reduced fruit damage by 2–3% in large and small trees, whereas the use of summer prunning reduced fruit damage by 4–6% in large, vegetative trees. Summer pruning had no effect on fruit damage in small trees. Pruning early in the growing season can increase fruit damage and decrease flower production the following year. Hand fruit thinning increased fruit size, and summer pruning increased fruit color. These findings indicate that horticultural techniques commonly used in commercial apple production can be slightly modified to aid in the control of obliquebanded leafrollers.

KEY WORDS *Choristoneura rosaceana*, Lepidoptera, cultural control, prunning, apple

Horticultural practices such as pruning and fruit thinning are used to improve fruit quality and maintain tree size and vigor in apple trees (Gardner 1920, Gaston 1942, Shaw 1935). Growers have adopted the use of summer pruning to maintain tree size in high-density apple plantings (Miller 1982, Morgan et al. 1984, Taylor & Ferree 1984). This technique removes unwanted vegetation during the growing season, usually after terminal bud set. It has proven effective at maintaining small tree size, and in general, increasing fruit quality. The use of fruit thinning with chemicals or by hand removes excess fruit, which reduces alternate bearing and increases the size and quality of the remaining fruits (Faust 1989).

The impact of these horticultural practices on arthropod pests of apples has not been examined. A previous study, which evaluated arthropod densities in apple orchards maintained under different management protocols, concluded

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that cultural practices such as pruning alter the resource base upon which a portion of the arthropod community develops. Therefore, the effects of cultural practices should be considered when examining an ecosystem approach to pest management (Brown & Welker 1992).

The obliquebanded leafroller, *Choristoneura rosaceana* (Harris), is a pest of apples throughout North America. In western New York, it damages as much as 20% of the fruit in some orchards (Lawson et al. 1996). Because of the development of insecticide resistance by this pest (Reissig et al. 1986, Lawson et al. 1997), alternative control strategies are being examined.

Obliquebanded leafroller adult populations are usually larger during the first generation than during the second. To explain this phenomenon, Chapman et al. (1968) developed the favorable food factor hypothesis that postulates that differences in moth densities between generations result from changes in larval food availability and quality. In June, young, succulent foliage is available and larvae develop rapidly, giving rise to a large adult population. However, in July and August, succulent foliage is not available, resulting in fewer larvae and subsequently fewer adults. Onstad et al. (1986) examined this hypothesis and found that larvae feeding on young, succulent foliage have greater fitness than those feeding on older foliage.

Most summer-generation, obliquebanded leafroller larvae feed on succulent tissue at the terminal ends of actively growing branches (Chapman & Lienk 1971, Onstad 1985). However, during this generation some larvae feed directly on developing fruits. It is unknown whether these larvae first feed on foliage and then move to fruit or feed exclusively on the fruit. However, larvae were shown to feed preferentially on foliage compared with fruit in a laboratory study (Onstad 1985). Factors shown to influence larval movement are changes in leaf texture and availability of refuge on host plants (Carriére 1992), both of which change during the growing season in apple trees. We have observed that apples that are clustered together are more severely damaged by obliquebanded leafroller larvae than are single fruits.

It appears that obliquebanded leafroller development and the location of feeding sites are closely associated with changes in the plant's development. It also appears that horticultural practices presently employed in apple production could be used to modify the host plant, making it less suitable for obliquebanded leafrollers and possibly influencing fruit damage levels. The goal of this research was to examine the effect of pruning and fruit thinning on obliquebanded leafroller fruit damage in commercial apple orchards. If these practices could be modified to reduce fruit damage, growers could improve leafroller control at no additional expense.

Material and Methods

Studies were conducted during a 2-yr period (1994–1995) in large and small apple trees to examine the effects of different summer pruning times with and without fruit thinning as a control tactic for the obliquebanded leafroller. We also were interested in examining the effects of these treatments on fruit quality and return bloom.

1994 Study. The effects of fruit thinning and summer pruning on fruit damage caused by obliquebanded leafrollers on small trees were examined in a 'Jersey Mac' apple orchard located in Orleans County, New York. Trees in this orchard were planted on M-9 rootstocks at a density of 988 trees per hectare with an average height of 2.5 m, trained on a 3 wire trellis. Trees were pruned on 30 June, 14 July, or 28 July. Growers applied normal chemical thinners in all of the plots and control treatments. On 29 June, fruit was hand thinned in half of the plots where trees were pruned. Control plots were unpruned trees with and without any additional hand thinning. Each of the plots contained five trees, and each treatment was replicated five times.

Strong upright branches, water sprouts, and hanging branches that shaded fruit were removed throughout the canopy in pruning treatments. Horizontal branches were removed if they shaded fruit or forked into two branches, in which case one of the branches was removed. Early in the season, strong upright growth was removed by pulling it from the tree by hand. This pruning removed the latent buds at the base of the branch and reduced regrowth. However, later in the season as branches hardened off, they could not be removed by pulling and were cut with pruning shears. An estimate of the percentage of shoots removed from the tree was made by counting all shoots from one tree per plot and then counting the number removed. Fruit was thinned so that individual fruits were separated by approximately 10 cm.

Approximately 150 fruits from each plot were sampled and rated for damage caused by summer generation obliquebanded leafrollers on 9 August. A fruit was considered to be damaged if any leafroller feeding could be detected, regardless of the area affected. Additionally, all sampled fruits were rated into two color categories by visually estimating the percentages of surface area: those with >50% red color and those with <50% red color. Twenty-five apples from each treatment were randomly chosen from the samples and measured for width and firmness. The average firmness of each apple was measured by using a Penetrometer (McCormick Fruit Tree, Inc, Yakima, Washington) to record the force required to push a 1.2-cm plunger 8.0 mm into the pared flesh on two sides of each apple. The soluble solids concentration (Brix) of each apple was estimated by placing an aliquot of juice remaining from the Penetrometer measurements (one reading per fruit) into a refractometer.

On 9 May 1995, when trees were at the tight cluster stage, two branches at least 2 cm in diameter were selected on opposite sides of two trees from each of the previous year's plots for bloom evaluation. All buds were rated as either producing vegetative growth or flowers along the entire length of the branch, except for 1-yr-old growth, in which case only the terminal bud was rated. The number of flower buds was divided by the total number of buds and expressed as a percentage.

1995 Study. During 1995, the effect of summer pruning and hand thinning of fruit on obliquebanded leafroller-damaged fruit was examined on trees with different heighths and canopy volumes. Smaller 'Paula Red' apple trees planted on M-9 rootstocks at a density of 988 trees per hectare with an average height of 2.5 m, trained on a 3 wire trellis, were compared with larger 'Red Delicious' trees planted on MM-106 rootstocks at a density of 287 trees per hectare with a tree height of 5.5 m. Both orchards were located in Orleans County, New York, and the same treatments were examined in each orchard.

In the small tree study, treatments were applied to 5-tree plots arranged into three blocks with 2 replicates per block. In the large-tree study, treatments were applied to single trees arranged in the same manner. Trees were pruned on 17 or 31 July, and fruit was thinned in half of the plots on 6 July, as previously described. However, the percentage of terminal growth removed was calculated only in the small-tree study. In the large-tree study, the total number of shoots removed was recorded.

Approximately 100 fruits from the small tree study were harvested on 15 August and rated for damage caused by summer generation obliquebanded leafrollers. Fruit firmness, soluble solids, and color were examined on 25 apples from each plot, as described previously.

Early season damage from the summer generation of obliquebanded leafrollers was estimated in treatments in the large-tree study on 14 August by sampling 200 fruits per tree from the midcanopy position. Only apples in this canopy position were sampled at this time to facilitate accurate detection of obliquebanded leafroller damage because fruit could not be removed from the tree prematurely before harvest. On 3 October, 100 fruits from the top and 100 from the bottom of each tree were harvested and rated for damage caused by summer generation obliquebanded leafrollers. A randomly collected sample of 25 apples from the top of the tree was examined for firmness and soluble solids, as previously described. Apples were sampled in the top portion of the trees because this area was where most of the vegetative shoots were removed and leafroller damage was most common. Color could not be rated because this apple variety was highly colored with >90% of the fruit surface red.

During both years of this study and in all orchards, growers continued to use their normal pesticide regime. In all plots, growers applied chemical fruit thinners; our hand thinning was performed in addition to these applications.

Statistical analyses. Fruit damage data were subjected to an analysis of variance (ANOVA) after transformation with the arcsine square-root transformation. Treatment effects were examined with contrasts between thinned and unthinned and between control and treatment plots, respectively (Abacus Concepts 1989). The 1995 large-tree fruit-damage data at harvest were analyzed with a repeated ANOVA, by using location within the tree (top and bottom) as the repeated factor. A significant interaction between location and treatment was found. Therefore, separate analyses were performed for each location. Differences in fruit width, firmness, and soluble solids data from thinned and unthinned treatments were examined with contrasts. Fruit color data were examined with a linear contrast in 1994, and in 1995 contrasts were constructed between thinned and unthinned treatments. Contrasts also were constructed to examine differences in return bloom between thinned and unthinned treatments.

Results

1994 Study. Fruit damage caused by obliquebanded leafrollers on the small 'Jersey Mac' trees ranged from 4 to 10% among the treatments, but damage was not significantly different than that in the control plots. Treatments had a significant effect on fruit quality, with fruit width significantly ($F = 27.06$; df =

1, 23; $P \leq 0.01$) greater in all treatments where fruit thinning occurred (Fig. 1). Fruit color was significantly ($F = 7.58$; df = 1, 32; $P \leq 0.01$) improved by pruning and thinning, and the highest percentages of apples with >50% red color were obtained when trees were pruned early in the growing season (Fig. 1). Pruning removed 59.4% of all shoots per tree. Soluble solids and fruit firmness were the same among all treatments. The effect of fruit thinning on the formation of blossoms the following year was significant ($F = 16.14$; df = 1, 32; $P \leq 0.01$) with the greatest percentage of flowers occurring in the fruit-thinned treatments (Fig. 1). Pruning on 30 June resulted in the lowest percentage of buds producing flowers.

1995 Study. Later pruning dates were chosen because of the deleterious effect of the 30 June pruning on flower production recorded in the 1994 study. In large trees, 483 ± 246 (mean ± SE) shoots per tree were removed during each pruning treatment. Early fruit damage ratings in these trees were significantly different ($F = 5.60$; df = 1, 28; $P \leq 0.02$) between thinned and unthinned treatments, with less damage in the thinned treatments (Fig. 2). However, at harvest, the only differences in fruit damage were recorded in the top of the tree canopy, where the control treatments had significantly more damaged fruit than all other treatments ($F = 9.24$; df = 1, 28; $P \leq 0.01$). As in the previous study, significant ($F = 21.09$; df = 1, 28; $P \leq 0.01$) differences in fruit width were observed, with fruit from thinned trees larger than fruit from unthinned trees (Fig. 2). Soluble solids and fruit firmness were the same among all treatments.

In the small-tree study, 53.3% of all actively growing shoots was removed during the pruning treatments. A significant ($F = 3.32$; df = 1, 28; $P \leq 0.08$) reduction in fruit damage was recorded in all treatments where fruit thinning occurred, but pruning had no effect (Fig. 3). Fruit width ($F = 13.3$; df = 1, 28; $P \leq 0.01$) and soluble solids ($F = 6.49$; df = 1, 28; $P \leq 0.01$) were significantly increased by fruit thinning (Fig. 3). However, fruit firmness was the same among all treatments. Fruit color was significantly ($F = 20.89$; df = 1, 28; $P \leq 0.01$) greater in treatments where pruning occurred (Fig. 3).

Discussion

The positive effects of summer pruning and fruit thinning on fruit quality and return bloom reported in our study have been documented and contribute to the use of these techniques in commercial apple production (Morgan et al. 1984, Talyor & Ferree 1984, Wesley & Greene 1990). Fruit thinning increases the leaf to fruit ratio, causing the remaining fruits to increase in size. This technique also reduces gibberellin levels in buds early in the season, which increases flower buds initiated and reduces alternate bearing (Russel & Pickering 1919, Faust 1989). Summer pruning increases fruit color because light penetration into the tree canopy is increased (Morgan et al. 1984, Talyor & Ferree 1984, Wesley & Greene 1990). This study also has shown that fruit thinning and summer pruning reduce fruit damage caused by obliquebanded leafrollers, although the effects may be variable on different sizes of trees.

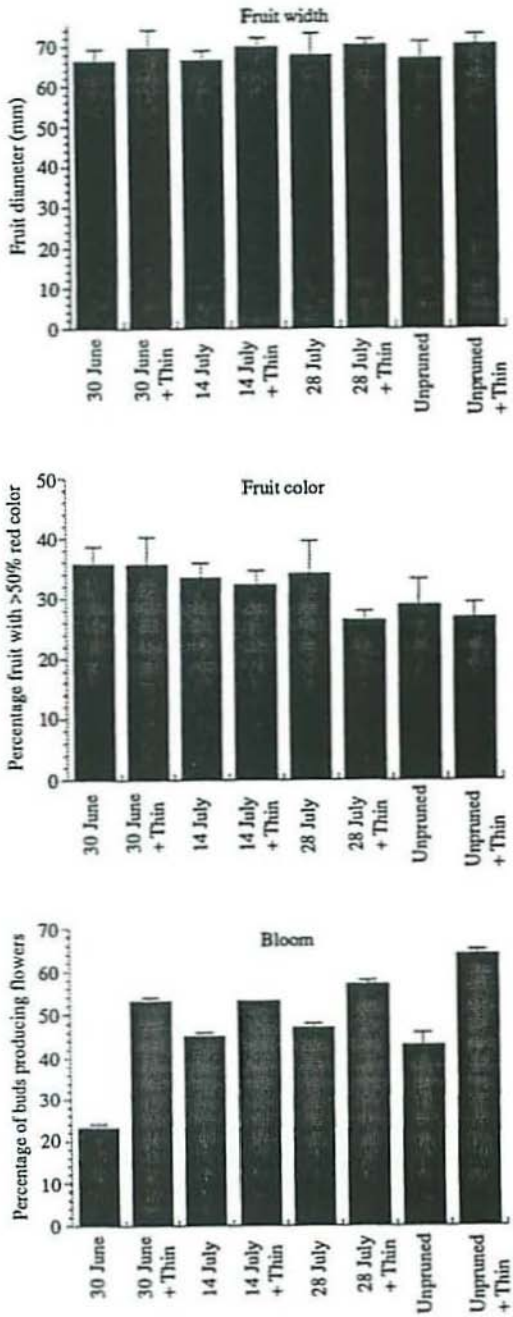

Fig. 1. Effect of summer pruning and fruit thinning (29 June) in small 'Jersey Mac' trees treated in 1994 on fruit width, color, and return bloom the following spring. Histogram bars represent mean ± SE.

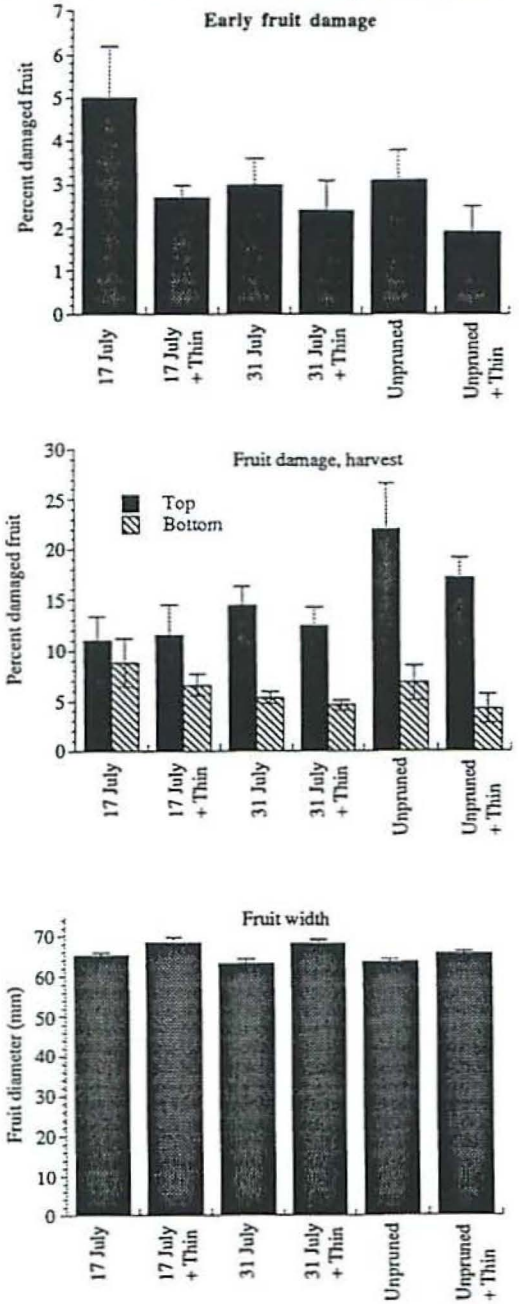

Fig. 2. Effect of summer pruning and fruit thinning (6 July) in large 'Red Delicious' trees on obliquebanded leafroller fruit damage early in the season (14 August), at harvest in the top and bottom of the tree canopy, and on fruit width during 1995. Histogram bars represent mean ± SE.

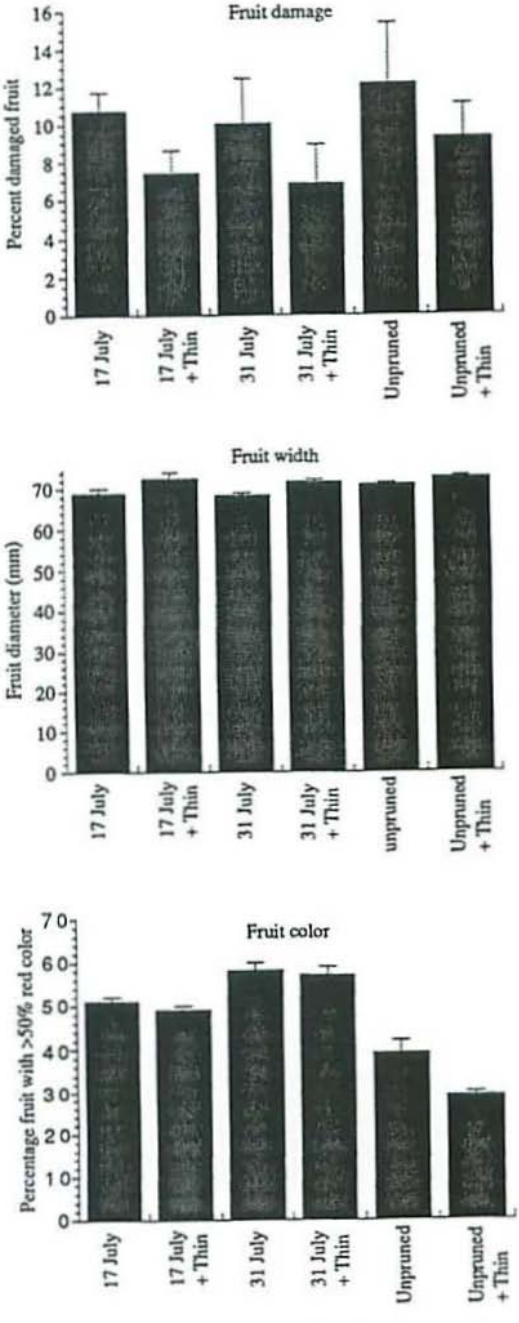

Fig. 3. Effect of summer pruning and fruit thinning (6 July) in small 'Paula Red' trees on obliquebanded leafroller fruit damage (15 August), width, and color during 1995. Histogram bars represent mean ± SE.

In 1994, fruit thinning treatments in the small 'Jersey Mac' trees did not significantly affect damage from the summer generation of obliquebanded leafrollers. However, in all of the summer pruning treatments tested in 1995, obliquebanded fruit damage was consistently less when fruit was thinned (an average of 2–4% reduction) in both large and small trees, but the reductions in damage caused by fruit thinning were significant only in the smaller trees. The variability associated with these results from year to year and between large and small trees may be attributable to the effectiveness of chemical thinners applied to all orchards before our studies were initiated. In orchards where chemical thinning applications caused fruits to be separated from one another, our hand thinning only increased the distance between single fruits and occasionally removed clustered fruits. However, in orchards where many fruits remained clustered after chemical thinning, hand thinning removed clustered fruits leaving only single fruits, which probably resulted in the greatest benefits from hand thinning. When multiple fruits are clustered together, an individual leafroller larva can damage multiple fruits without leaving the feeding site. However, if fruit clusters are thinned to one fruit, larvae must move to damage other fruit.

We expected that pruning would remove the preferred food of obliquebanded leafroller larvae at critical points in their development and would prevent females from ovipositing, due to lack of suitable oviposition sites or food for developing larvae; remove food before larvae start feeding, causing them to leave the tree or feed elsewhere; remove the larvae with the branches being pruned; or modify refugia. Which, if any, of these mechanisms worked is unknown. Only in the large-tree study in 1995 did we observe any significant effect of summer pruning on fruit damage (Fig. 2). During the early fruit evaluation, it appeared that the 17 July pruning increased fruit damage. This damage may have occurred because much of the foliage preferred by larvae was removed, causing them to move onto the fruit to feed. However, by harvest, damage among the pruned treatments was the same, but was 4–9% less in control plots. These large trees were much more vegetative than the smaller trees used during both years. Under these conditions, we removed an estimated 70% of branches that had terminals that were still growing, compared with 55% in the smaller trees. Perhaps only such an extreme tree modification could bring about decreased obliquebanded leafroller-damaged fruit.

This study has shown that a slight modification of the normal horticultural practices of fruit thinning and summer pruning can not only increase fruit quality and tree performance but also significantly reduce fruit damage from the obliquebanded leafroller. Fruit thinning reduced damage in both large and small trees apparently by decreasing the ability of larvae to damage multiple fruits from the same feeding site. Summer pruning was effective in reducing obliquebanded damage only in the tops of large 'Delicious' trees with an abundant amount of vegetative growth.

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Dosage-Mortality Responses of Third Instars of Beet Armyworm (Lepidoptera: Noctuidae) to Selected Insecticides[1]

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ABSTRACT Susceptibility of third instars of beet armyworm, *Spodoptera exigua* (Hübner), larvae to registered and experimental insecticides was evaluated in a diet overlay bioassay. Larvae were progeny of beet armyworms originally collected from cotton fields in Georgia, Louisiana, and Mississippi. Several field strains exhibited reduced susceptibility to the registered insecticides chlorpyrifos and thiodicarb compared with the ECOGEN laboratory strain. In chlorpyrifos bioassays, all field strains had significantly higher LC_{50}s than the ECOGEN strain. In thiodicarb bioassays, five of the eight field strains had significantly higher LC_{50}s than the ECOGEN strain. Five of the eight field strains had significantly higher LC_{50}s than the ECOGEN strain in chlorfenapyr bioassays. In emamectin benzoate bioassays, five of the seven field strains had signficantly lower LC_{50}s compared with the ECOGEN strain. Although considerable variation was observed among field strains in methoxyfenozide bioassays, only the St. Joseph strain from Louisiana had a significantly higher LC_{50} than the ECOGEN strain. In both the spinosad and tebufenozide bioassays, 95% confidence limits (CL) could not be obtained for the ECOGEN laboratory strain; thus, comparisons were based on 90% CL. All field strains responded similarly to the ECOGEN strain in the spinosad bioassays, except for the strain from Tallulah, Louisiana, which had a significantly higher LC_{50}. The strains from Tift County, Georgia, and Winnsboro, Louisiana, were the only field strains to have significantly higher LC_{50}s than the ECOGEN strain in tebufenozide bioassays.

KEY WORDS *Spodoptera exigua*, insecticide toxicity, resistance, diet bioassays, cotton

The beet armyworm, *Spodoptera exigua* (Hübner), has emerged as an important economic pest throughout most of the cotton, *Gossypium hirsutum* (L.), producing regions of the United States. During the past decade, population

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outbreaks have occurred in Alabama, Georgia, Mississippi, and Texas almost annually. During 1993, 60% of the cotton acreage in the mid-south (Arkansas, Louisiana, and Mississippi) and southeastern (Alabama, Georgia, and South Carolina) states was infested with beet armyworms, and on 35% of this acreage, infestations were above the economic injury level (Smith 1994). Nationwide, the beet armyworm was the third most destructive cotton insect pest in 1995 and was responsible for an estimated 15% of the total damage attributed to insects (Williams 1996). In Louisiana, beet armyworm infestations historically have been sporadic and generally less severe than in other states (Burris et al. 1994); however, in 1995, statewide outbreaks were reported. Population outbreaks of this pest in 1995 were a significant factor contributing to the complete destruction of 75% of the cotton crop in some regions of Texas (Sparks et al. 1996). The estimated acreage infested by beet armyworm in the United States has steadily increased since the beginning of this decade. In 1990, an estimated 1.6 million acres was infested with beet armyworms, which increased to 3.5 million acres in 1993 and 6.8 million acres by the 1995 growing season (Head 1991; Williams 1994, 1996).

A growing concern involving the pest status of beet armyworms includes not only its rapid expansion throughout the cotton acreage in the United States but also its tolerance to many insecticides (Layton 1994). Reduced insecticide efficacy has been reported in many cotton-growing regions of the southeastern United States (Layton 1994, Smith 1994). Chlorpyrifos and thiodicarb are the only insecticides presently recommended for beet armyworm control in Louisiana (Bagwell et al. 1997). Thiodicarb was the most effective insecticide against beet armyworm in 1984, providing control in excess of 95% (Burris 1983). However, by the mid 1990s, control was highly variable and in many cases unsatisfactory (Burris et al. 1994, Graves et al. 1995, Mascarenhas et al. 1996). Variable beet armyworm control also has been reported for chlorpyrifos in much of the southeast and mid-south (Elzen 1989, Sparks et al. 1996). In Louisiana, control with chlorpyrifos ranged from 87% (Graves et al. 1995) to 90% (Mascarenhas et al. 1996), although much lower (38%) control has been reported previously (Burris et al. 1994).

Decreased susceptibility of beet armyworm populations to insecticides has been associated with reduced efficacy against field infestations. Variations in susceptibility of beet armyworm to several insecticides have been reported for field-collected strains (Cobb & Bass 1975, Brewer & Trumble 1989, Aldosari et al. 1996, Chandler & Ruberson 1996) and among four laboratory reference strains (Wolfenbarger & Brewer 1993).

Several experimental compounds have exhibited excellent control of numerous lepidopteran pests, including the beet armyworm. Chlorfenapyr, the lead chemical in a new class of insecticides called pyrroles, has shown satisfactory activity against beet armyworm (Farlow et al. 1992, Burris et al. 1994, Wier et al. 1994a, Wiley et al. 1995) and has been available to growers in several states under a section 18 label (Mascarenhas et al. 1996, Sparks et al. 1996). Emamectin benzoate, a nerve poison in the avermectin class of insecticides, is very active against beet armyworms at low rates (Wier et al. 1994b, Sparks et al. 1996). Methoxyfenozide (RH-2485), an insect growth regulator (IGR) affecting the molting process of target insect species, also has been effective against beet armyworms and may become labeled for its control in the future. Spinosad, a

nerve poison in the naturalyte class of insecticides, is toxic to beet armyworms (Mascarenhas et al. 1996, Sparks et al. 1996, Hendrix et al. 1997) and received a section 3 registration in 1997. Tebufenozide, another IGR that accelerates the molting process in certain insect species, also is effective against beet armyworms and has been successfully used in recent years under a section 18 label (Walton et al. 1995).

This study was initiated to determine the susceptibility of field populations of beet armyworm to registered insecticides as well as to experimental insecticides that may become labeled for the control of this pest. This information will serve as a historical record that can be used to monitor insecticide susceptibility of beet armyworm populations.

Materials and Methods

Field-collected beet armyworm larvae were transported to the laboratory and placed on an artificial wheat-germ and soybean protein diet (King & Hartley 1985). Larvae were reared at 28 ± 3°C, 60 ± 8% RH, and a photoperiod of 14:10 (L:D) h until pupation. Pupae were washed in 10% sodium hypochlorite solution (Clorox® bleach) for 2 min, rinsed under running water for 3 min, and allowed to surface-dry at room temperature. Pupae were placed in 3.87-liter cardboard containers lined with wax paper oviposition sheets and allowed to eclose. Moths were fed a 10% sucrose solution and held under the same rearing conditions as the larvae. Oviposition sheets were replaced daily, and eggs were held and allowed to hatch within inflated plastic bags. Upon hatching, 30–40 neonate larvae were transferred into 295-ml paper cups containing artificial diet and held until they reached the appropriate size for bioassay (30–45 mg).

Nine field strains of beet armyworms collected from cotton in three states (Georgia, Louisiana, and Mississippi) were used in bioassays. Field strains and their collection sites are presented in Table 1. In addition, a beet armyworm strain obtained from Ecogen, Inc. (Langhorne, Pennsylvania) was included in these bioassays as a laboratory strain that had not been exposed to insecticides since 1993. The ECOGEN strain originated from a laboratory colony at the USDA–ARS Southern Insect Management Laboratory at Stoneville, Mississippi, and has been in laboratory culture over 10 yr.

A diet overlay bioassay, similar to that described by Joyce et al. (1986), was used to evaluate the activity of selected insecticides against third instars of beet armyworm. Three milliliters of hot diet was dispensed into 30-ml plastic cups by using an Eppendorf repeater pipette (Brinkmann Instruments Co., Westbury, New York) and allowed to cool at room temperature. Serial dilutions of each insecticide were prepared in distilled water based on percentage of active ingredient (AI) of formulated insecticides. Formulated insecticides evaluated in bioassays are presented in Table 2. Each insecticide concentration was applied (100-µl aliquots) onto the diet surface of individual cups and cups were rotated by hand to evenly distribute the insecticide solution over the diet. Diet treated with distilled water was used as the control. After the insecticide solutions had dried (approximately 45 min), one larva was placed in each cup, and cups were capped. All bioassays were conducted under constant light, 23 ± 3°C, and 57 ± 8% RH. If insufficient (<30 individuals per concentration) larvae were available

Table 1. Laboratory and field strains of beet armyworms used in bioassays, 1996.

Strain, location of collection	Collection date
ECOGEN, Ecogen, Inc., Langhorne, Pennsylvania	Laboratory
Tift County, Georgia	20 June
Bayou Macon, Louisiana	15 September
Macon Ridge, Louisiana	27 August
St. Joseph, Louisiana	7 July
Tallulah, Louisiana	9 September
Tensas Parish, Louisiana	25 August
Winnsboro, Louisiana	9 September
Benton County, Mississippi	18 September
Starkville, Mississippi	1 August

at a given time, or if confidence limits (CL) were not obtained, multiple bioassays were conducted using larvae from different generations (i.e., F_1 and F_2). In such cases, data presented are the result of combined bioassays.

Because of the relatively slow mode of action of the IGRs methoxyfenozide and tebufenozide, mortality in all bioassays was scored 120 h after larvae were placed on treated diet. Larvae were considered dead if they did not respond to prodding with a dissecting probe. Data were corrected for mortality observed in the control group (Abbott 1925) and analyzed by probit analysis using POLO-PC (LeOra Software 1987). Beet armyworm mortality on the control diet was generally <10%. The LC_{50}s of field strains were considered to be significantly different from that of the ECOGEN laboratory strain if their respective 90% or 95% CL did not overlap. Toxicity ratios (TRs) were calculated based on estimates of the intercepts and slopes of the probit lines representing the beet armyworm strains and estimates of their variance-covariance matrixes (Robertson & Preisler 1992).

Results

The LC_{50}s of field strains ranged from 73.2 to 295.2 ppm for chlorpyrifos, a 4-fold difference (Table 3). All field strains had significantly higher LC_{50}s than the ECOGEN laboratory strain (28 ppm). The TRs ranged from 2.6 to 10.5 for the Winnsboro and Benton county strains, respectively.

In thiodicarb bioassays, LC_{50}s for the field strains ranged from 956.2 to 7,181.3 ppm, a 7.5-fold difference (Table 4). All field strains for which 95% CL were obtained had significantly higher LC_{50}s than the ECOGEN strain. Ninety-five percent CL were not obtained for the Winnsboro strain; however, its

Table 2. Common name, trade name, percentage of active ingredient (AI), and manufacturer for the insecticides included in the bioassays.

Common name (trade name)	% AI (wt/wt)	Manufacturer
	Registered insecticides	
Chlorpyrifos (Lorsban® 4EC[a])	40.7	DowElanco, Indianapolis, Indiana
Spinosad (Tracer® 4F)	44.2	DowElanco, Indianapolis, Indiana
Thiodicarb (Larvin® 3.2F[b])	32.5	Rhone-Poulenc Ag. Co., Research Triangle Park, North Carolina
	Experimental insecticides	
Chlorfenapyr (Pirate® 3F)	36.1	American Cyanamid, Princeton, New Jersey
Emamectin benzoate (Proclaim® 5SG[c])	5.0	Merck Research Lab., Rahway, New Jersey
Methoxyfenozide[d] (Intrepid® 80WP[e])	80.0	Rohm & Haas Co., Philadelphia, Pennsylvania
Tebufenozide (Confirm® 2F)	22.7	Rohm & Haas Co., Philadelphia, Pennsylvania

[a]EC, emulsifiable concentrate.
[b]F, aqueous flowable.
[c]SG, soluble granular.
[d]Proposed common name.
[e]WP, wettable powder.

LC_{50} was significantly higher than the LC_{50} of the ECOGEN strain based on the 90% CL (281.3–427.6 ppm) of the ECOGEN strain. Although CL could not be calculated for the St. Joseph and Tensas Parish strains, their LC_{50}s fell within the range of the other field strains and were 11.3 and 9.3 times higher than the LC_{50} of the ECOGEN strain. The TRs ranged from 2.5 to 18.8.

A narrow range in LC_{50}s (20.2 to 27.6 ppm) was observed among field strains in chlorfenapyr bioassays (Table 5). Strains from Bayou Macon, St. Joseph, Tallulah, Tensas Parish, and Tift County had significantly higher LC_{50}s compared with the ECOGEN strain, although the TRs were low (1.3 to 1.8).

Emamectin benzoate was the most active insecticide evaluated in this study with LC_{50}s for the field strains ranging from 0.2 to 0.6 ppm (Table 6). All field strains for which 95% CL were obtained had significantly lower LC_{50}s than the ECOGEN strain. The Winnsboro strain had a significantly lower LC_{50} than the ECOGEN strain based on the 90% CL (1.7–3.4 ppm) of the ECOGEN strain. Although CL could not be calculated for the Tift County strain, its LC_{50} fell within the ranges of the other field strains. The TRs ranged from 0.01 to 0.2.

Methoxyfenozide LC_{50}s for field strains ranged from 5.7 to 80.0 ppm, a 14-fold difference (Table 7). The St. Joseph strain was the only field strain with a

Table 3. Toxicity of chlorpyrifos-treated diet to third instars of beet armyworm 120 h after exposure.

Strain	Generation tested	n	Slope (SE)	LC_{50} (95% CL)[a]	χ^2	TR (95% CL)[b]
ECOGEN	Laboratory	260	3.5 (0.40)	28.0 (15.0–46.2)	3.5	
Tift County, Georgia	F_5	190	3.3 (0.50)	168.1 (132.7–203.2)	0.9	6.0 (4.6–7.8)
Bayou Macon, Louisiana	F_2	150	3.3 (0.50)	240.2 (195.5–292.0)	1.3	8.6 (6.7–11.0)
Macon Ridge, Louisiana	F_1	250	4.5 (0.51)	268.0 (163.7–456.9)	3.8	9.6 (7.8–11.7)
St. Joseph, Louisiana	F_3	285	3.0 (0.52)	275.3 (199.7–347.5)	0.6	9.8 (7.3–13.2)
Tallulah, Louisiana	F_1	315	2.3 (0.29)	179.6 (78.7–285.0)	2.8	6.4 (5.0–8.3)
Tensas Parish, Louisiana	F_1, F_2	300	2.0 (0.27)	156.9 (100.8–224.6)	3.5	5.6 (4.5–7.0)
Winnsboro, Louisiana	F_2	270	2.5 (0.40)	73.2 (52.3–91.4)	0.2	2.6 (0.4–18.4)
Benton County, Mississippi	F1	190	2.2 (0.35)	295.2 (234.0–374.4)	0.1	10.5 (8.2–13.6)
Starkville, Mississippi	F2	150	2.7 (0.45)	207.3 (160.2–258.9)	0.7	7.4 (5.6–9.8)

[a] LC_{50} expressed in ppm.
[b] Toxicity ratio (TR) calculated according to Robertson & Preisler (1992).

Table 4. Toxicity of thiodicarb-treated diet to third instars of beet armyworm 120 h after exposure.

Strain	Generation tested	n	Slope (SE)	LC_{50} (95% CL)[a]	χ^2	TR (95% CL)[b]
ECOGEN	Laboratory	240	3.0 (0.48)	356.9 (265.3–441.5)	1.9	
Tift County, Georgia	F_5	190	2.9 (0.41)	4,392.5 (2,641.9–10,393.1)	2.5	11.5 (8.5–15.5)
Bayou Macon, Louisiana	F_2	190	3.1 (0.42)	2,314.3 (1,921.4–2,756.8)	0.1	6.1 (4.6–8.0)
Macon Ridge, Louisiana	F_1	225	2.5 (0.34)	956.2 (767.8–1,150.9)	1.1	2.5 (1.9–3.3)
St. Joseph, Louisiana	F_3	360	3.9 (0.87)	4,039.5[c]	6.5	10.6 (7.8–14.4)
Tallulah, Louisiana	F_1	190	2.8 (0.40)	2,279.5 (1,153.4–3,921.7)	2.4	5.9 (4.5–7.8)
Tensas Parish, Louisiana	F_1, F_2	180	2.5 (0.59)	3,307.6[c]	6.2[d]	8.6 (6.2–12.0)
Winnsboro, Louisiana	F_1	230	2.3 (0.38)	1,868.9 (826.8–3,818.0)[e]	4.0	4.9 (3.7–6.5)
Benton County, Mississippi	F_1	190	4.5 (1.29)	7,181.3 (5,978.5–9,367.5)	0.2	18.8 (14.4–24.4)

[a] LC_{50} expressed in ppm.

[b] Toxicity ratio (TR) calculated according to Robertson & Preisler (1992).

[c] Unable to calculate CL. Index of significance of potency estimator (g) > 0.5.

[d] Significant χ^2 ($P = 0.05$).

[e] 90% CL reported.

significantly higher LC_{50} than the ECOGEN strain. The TRs were highly variable. Three field strains (St. Joseph, Tift County, and Winnsboro) had TRs ranging from 7.1 to 9.3, whereas the remaining four strains (Bayou Macon, Benton County, Macon Ridge, and Tallulah) had TRs ranging from 0.7 to 2.9.

In spinosad bioassays, LC_{50}s of field strains ranged from 47.0 to 164.7 ppm, a 3.5-fold difference (Table 8). The 95% CL for the ECOGEN strain could not be obtained due to variability, thus 90% CL were reported for all strains. Toxicity of spinosad was similar among all field strains and the ECOGEN strain, except for the Tallulah strain. The Tallulah strain had a significantly higher LC_{50} than the ECOGEN strain. The TRs ranged from 0.9 to 3.2.

Tebufenozide LC_{50}s of field strains ranged from 39.7 to 176.3 ppm, a 4.4-fold difference (Table 9). The responses of the ECOGEN strain were highly variable and 95% CL could not be determined. Therefore, all comparisons between field strains and the ECOGEN strain were based on 90% CL. The Tift County and Winnsboro strains had significantly higher LC_{50}s than the ECOGEN strain. As with methoxyfenozide, TRs of tebufenozide were highly variable. Four strains (Bayou Macon, St. Joseph, Tift County, and Winnsboro) had TRs ranging from 8.1 to 10.0, whereas the remaining three strains (Benton County, Macon Ridge, and Tallulah) had TRs ranging from 2.3 to 3.1.

Discussion

For most field strains, the order of toxicity of the insecticides evaluated from least toxic to most toxic was thiodicarb < chlorpyrifos < tebufenozide < spinosad < methoxyfenozide < chlorfenapyr < emamectin benzoate. The average LC_{50} (0.38 ppm) obtained for emamectin benzoate was 6296, 545, 292, 232, 109, and 61 times lower than the average LC_{50}s obtained for thiodicarb, chlorpyrifos, tebufenozide, spinosad, methoxyfenozide, and chlorfenapyr, respectively. The toxicity of the IGRs methoxyfenozide and tebufenozide compared with the other insecticides was dependent on the endpoint of the bioassay. The slower mode of action of the IGRs compared with that for the other insecticides required that the bioassays be extended to 120-h exposure to treated diet instead of the typical 72 h. At this shorter time interval, 50% larval mortality would not have occurred even at the highest doses of the IGRs. Although methoxyfenozide and tebufenozide have similar modes of action, the former appears to be more active against beet armyworm larvae with an average LC_{50} 2.7 times lower than the average LC_{50} for tebufenozide.

The experimental insecticides evaluated in this study showed significant activity against the beet armyworm, although considerable variation in the responses of the field strains was observed to the new compounds. The field strains actually appeared to be more susceptible to emamectin benzoate than the ECOGEN strain and resulted in a wide range of TRs (0.01–0.2). In bioassays of the IGRs methoxyfenozide and tebufenozide, considerable variation in the responses of field strains also was observed, resulting in elevated TRs of some strains (St. Joseph, Tift County, and Winnsboro). Both of these compounds have not been extensively used in the field and differences reported herein may, in part, be due to natural variation (Robertson et al. 1995). The shallower slopes of the dose-mortality lines observed in both the methoxyfenozide

Table 5. Toxicity of chlorfenapyr-treated diet to third instars of beet armyworm 120 h after exposure.

Strain	Generation tested	n	Slope (SE)	LC_{50} (95% CL)[a]	χ^2	TR (95% CL)[b]
ECOGEN	Laboratory	240	7.0 (1.15)	15.1 (13.2–17.0)	0.5	
Tift County, Georgia	F_5	190	4.4 (0.79)	27.6 (21.7–32.7)	0.6	1.8 (1.5–2.3)
Bayou Macon, Louisiana	F_2	190	5.9 (0.85)	24.8 (21.8–28.0)	0.7	1.6 (1.4–2.0)
Macon Ridge, Louisiana	F_1	250	3.5 (0.50)	20.6 (16.6–24.4)	0.6	1.3 (1.0–1.7)
St. Joseph, Louisiana	F_3	190	4.3 (0.69)	21.9 (17.9–25.9)	0.5	1.5 (1.2–1.8)
Tallulah, Louisiana	F_1	190	3.8 (0.71)	24.3 (17.7–30.2)	0.6	1.6 (1.2–2.1)
Tensas Parish, Louisiana	F_1, F_2	275	4.2 (0.65)	21.5 (17.2–25.3)	0.3	1.4 (1.1–1.8)
Winnsboro, Louisiana	F_1, F_2	425	4.2 (0.41)	20.2 (12.0–29.1)[c]	9.6[d]	1.3 (1.1–1.8)
Benton County, Mississippi	F_1	190	3.3 (0.46)	23.3 (14.1–34.5)[c]	4.0	1.5 (1.2–1.9)

[a] LC_{50} expressed in ppm.
[b] Toxicity ratio (TR) calculated according to Robertson & Preisler (1992).
[c] 90% CL reported.
[d] Significant χ^2 ($P = 0.05$).

Table 6. Toxicity of emamectin benzoate-treated diet to third instars of beet armyworm 120 h after exposure.

Strain	Generation tested	n	Slope (SE)	LC_{50} (95% CL)[a]	χ^2	TR (95% CL)[b]
ECOGEN	Laboratory	230	1.4 (0.19)	2.4 (1.6–3.7)	0.2	
Tift County, Georgia	F_5	190	1.2 (0.15)	0.4[c]	7.5[d]	0.2 (0.1–0.4)
Bayou Macon, Louisiana	F_2	210	2.1 (0.50)	0.5 (0.2–0.7)	0.2	0.2 (0.1–0.4)
Macon Ridge, Louisiana	F_2	190	1.6 (0.30)	0.4 (0.2–0.7)	1.4	0.2 (0.1–0.4)
St. Joseph, Louisiana	F_4	190	2.7 (0.50)	0.2 (0.1–0.3)	0.5	0.01 (0.004–0.1)
Tallulah, Louisiana	F_2	190	2.4 (0.80)	0.6 (0.2–0.9)	0.3	0.2 (0.1–0.5)
Winnsboro, Louisiana	F_2	210	1.4 (0.20)	0.3 (0.1–1.0)[e]	4.5	0.2 (0.1–0.3)
Benton County, Mississippi	F_1	190	1.4 (0.30)	0.3 (0.1–0.5)	0.7	0.1 (0.04–0.3)

[a]LC_{50} expressed in ppm.
[b]Toxicity ratio (TR) calculated according to Robertson & Preisler (1992).
[c]Unable to calculate CL. Index of significance of potency estimator (g) > 0.5.
[d]Significant χ^2 ($P = 0.05$).
[e]90% CL reported.

Table 7. Toxicity of methoxyfenozide-treated diet to third instars beet armyworm 120 h after exposure.

Strain	Generation tested	n	Slope (SE)	LC_{50} (95% CL)[a]	χ^2	TR (95% CL)[b]
ECOGEN	Laboratory	230	1.2 (0.16)	8.7 (4.3–15.3)	1.5	
Tift County, Georgia	F_5	190	0.9 (0.13)	61.5 (14.1–358.5)[c]	4.5	7.1 (3.1–16.5)
Bayou Macon, Louisiana	F_2	190	0.8 (0.12)	20.4[d]	6.3[e]	2.4 (1.0–5.8)
Macon Ridge, Louisiana	F_2	190	1.0 (0.13)	14.6 (1.5–87.4)	2.8	1.7 (0.7–3.9)
St. Joseph, Louisiana	F_4	190	0.6 (0.11)	80.0 (28.4–247.0)	0.3	9.3 (3.0–28.8)
Tallulah, Louisiana	F_2	190	0.9 (0.12)	24.8 (3.6–145.6)	5.1	2.9 (0.5–15.5)
Winnsboro, Louisiana	F_1	230	0.8 (0.10)	79.5 (12.2–1365.4)	3.1	9.2 (3.8–22.2)
Benton County, Mississippi	F_1	190	0.9 (0.13)	5.7 (2.7–10.3)	1.9	0.7 (0.1–3.77)

[a] LC_{50} expressed in ppm.

[b] Toxicity ratio (TR) calculated according to Robertson & Preisler (1992).

[c] 90% CL reported.

[d] Unable to calculate CL. Index of significance of potency estimator (g) > 0.5.

[e] Significant χ^2 ($P = 0.05$).

Table 8. Toxicity of spinosad-treated diet to third instars of beet armyworm 120 h after exposure.

Strain	Generation tested	n	Slope (SE)	LC_{50} (90% CL)[a]	χ^2	TR (95% CL)[b]
ECOGEN	Laboratory	360	2.0 (0.25)	52.2 (34.7–88.6)	4.4	
Tift County, Georgia	F_5	190	1.2 (0.36)	118.9 (76.4–302.1)	0.2	2.3 (1.1–4.6)
Bayou Macon, Louisiana	F_2	190	1.4 (0.32)	71.9 (53.7–112.2)	0.1	1.4 (0.9–2.2)
Macon Ridge, Louisiana	F_1	150	1.4 (0.44)	70.5 (45.6–127.7)	0.1	1.4 (0.8–2.4)
St. Joseph, Louisiana	F_3, F_4	295	1.4 (0.35)	88.5 (62.9–140.6)	0.2	1.7 (1.1–2.7)
Tallulah, Louisiana	F_1	190	1.1 (0.37)	164.7 (93.8–781.4)	0.1	3.2 (1.2–8.3)
Winnsboro, Louisiana	F_1	275	1.8 (0.30)	55.2 (44.9–69.3)	0.7	1.1 (0.8–1.5)
Benton County, Mississippi	F_1	190	2.1 (0.37)	47.0 (37.1–59.0)	0.5	0.9 (0.7–1.2)

[a] LC_{50} expressed in ppm.
[b] Toxicity ratio (TR) calculated according to Robertson & Preisler (1992).

Table 9. Toxicity of tebufenozide-treated diet to third instars of beet armyworm 120 h after exposure.

Strain	Generation tested	n	Slope (SE)	LC_{50} (90% CL)[a]	χ^2	TR (95% CL)[b]
ECOGEN	Laboratory	260	0.9 (0.11)	17.6 (3.1–76.5)	5.3	
Tift County, Georgia	F_5	190	1.1 (0.15)	143.3 (91.1–235.8)	0.6	8.1 (3.7–17.8)
Bayou Macon, Louisiana	F_2	190	0.9 (0.14)	176.3 (59.7–810.1)	2.8	10.0 (1.2–81.8)
Macon Ridge, Louisiana	F_2	190	0.8 (0.12)	47.1 (27.9–80.9)	1.8	2.7 (1.2–6.2)
St. Joseph, Louisiana	F_4	190	0.8 (0.12)	160.0 (54.6–756.6)	2.2	9.1 (3.7–22.8)
Tallulah, Louisiana	F_1	190	1.0 (0.13)	54.0 (11.5–277.5)	4.7	3.1 (1.4–6.7)
Winnsboro, Louisiana	F_1, F_2	275	1.2 (0.23)	155.6 (91.8–241.5)	0.5	8.9 (4.1–19.1)
Benton County, Mississippi	F_1	190	0.7 (0.11)	39.7 (7.3–238.5)	3.6	2.3 (0.9–5.6)

[a] LC_{50} expressed in ppm.

[b] Toxicity ratio (TR) calculated according to Robertson & Preisler (1992).

(0.6–1.0) and tebufenozide (0.7–1.2) bioassays also indicate a high degree of heterogeneity in the responses of these field strains to the IGRs (Chilcutt & Tabashnik 1995). Considerable variation in the responses of field strains also was observed in spinosad bioassays. Although spinosad was made available for commercial use during 1997, its use has been limited to experimental plots. Thus, significantly higher LC_{50}s observed for the Tallulah strain may be due to natural variation in that population (Robertson et al. 1995).

The most consistent responses from the field strains, in terms of range of LC_{50} values, were obtained in chlorfenapyr bioassays. The consistency of LC_{50}s obtained with this insecticide compared with the other experimental insecticides is likely related to the greater steepness (3.3–5.9) of the slopes of the dosage-mortality lines, which indicates a high degree of homogeneity in the responses of field strains to this insecticide. Similar results have been reported by Wier et al. (1994a), where chlorfenapyr LC_{50}s, ranged from 13.3 to 31.0 ppm, and slopes of dose-mortality lines ranged from 2.9 to 7.2. In their bioassay, Wier et al. (1994a) evaluated mortality of third instars of beet armyworms at 72 h after exposure to treated soybean leaves. Although LC_{50}s reported herein are based on 120-h exposure of larvae to treated diet, these results are comparable to Wier et al. (1994a) because chlorfenapyr is a relatively quick-acting insecticide with minimal additional mortality occurring beyond 72 h of exposure. Although chlorfenapyr is available under a section 18 label against beet armyworms, its use has been limited. Thus, significantly higher LC_{50}s observed in five of the eight field strains does not necessarily indicate the development of resistance in these populations. To date, this product has performed very well against native populations of beet armyworm in field tests (Farlow et al. 1992, Wiley et al. 1995, Mascarenhas et al. 1996, Sparks et al. 1996).

The data presented in this study show considerable variability in the responses of field strains to the commercial insecticides chlorpyrifos and thiodicarb. All field strains from Louisiana, for which 95% CLs were obtained, required significantly higher concentrations of chlorpyrifos and thiodicarb to provide mortality comparable to that of the ECOGEN strain (Tables 3 and 4). In addition, the steep slopes of dose-mortality lines obtained in chlorpyrifos (2.0–4.5) and thiodicarb (2.3–4.5) bioassays suggest that these field strains are becoming more homozygous in their responses to these insecticides, indicating a rightward shift of the dose-mortality lines compared with the laboratory strain (Lande 1981, Falconer 1989). The combination of higher LC_{50}s, a rightward shift in the dose-mortality lines, and reports of reduced field efficacy indicate that beet armyworms have developed resistance to chlorpyrifos and thiodicarb. New insecticides along with appropriate resistance management strategies must be implemented to prevent economic losses associated with future population outbreaks of this pest.

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Royer, T. A., J. V. Edelson & B. Cartwright. 1988. Onion thrips control, 1987, p. 129. *In* Insecticide and acaracide tests, vol. 13. Entomological Society of America, College Park, Maryland, 459 pp.

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Kristen Greene van den Meiracker

J. H. Cochran Graduate Student Award and Scholarship

Kristen Greene van den Meiracker is the 1997 recipient of J. H. Cochran Graduate Student Award and Scholarship given by the South Carolina Entomological Society. Kristen received a B. S. in Biology from the University of South Carolina – Spartanburg in 1988, and a M.Ed. in Secondary Science Education from Converse College in 1989. She taught middle and high school biology for five years before returning to graduate school to study termites as part of the Urban Entomology Research Laboratory at Clemson University. Kristen has received many honors during her graduate career. She was a three-time recipient of the prestigious Wade Stackhouse Fellowship, recipient of a W. C. Nettles Memorial Grant, and a Phi Chi Omega scholar. She has held leadership positions in the Entomology Club in her department and has served regularly on department committees as the student representative. In addition to her own research, she has been involved in extension and teaching programs at Clemson, having given presentations and lectures to a variety of audiences. Kristen has been a key personality in the outreach programs conducted by the Clemson University Entomology Department, talking to school children and participating in community festivals to teach the public about entomology. Her doctoral research focused on the impact of temperature on the efficacy of hexaflumuron bait for subterranean termite control. Kristen received her Ph.D. in Entomology in May 1998, and is currently a postdoctoral fellow at the Urban Entomology Research Laboratory at Clemson University where she will continue to study household and structural insects.

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Susceptibility of Conventional and Transgenic Cotton Bolls Expressing the *Bacillus thuringiensis* CryIA(c) ∂-Endotoxin to Fall Armyworm (Lepidoptera: Noctuidae) and Beet Armyworm (Lepidoptera: Noctuidae) Injury[1]

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ABSTRACT Fifth-instar fall armyworms, *Spodoptera frugiperda* (J. E. Smith), and third-instar beet armyworms, *Spodoptera exigua* (Hübner), were caged on conventional 'DP 5415' and transgenic *Bacillus thuringiensis* (Bt) 'NuCOTN 33B' cotton bolls of various ages to define the period of boll susceptibility to larval injury. Larval mortality, incidence of feeding, and boll penetration were examined on both cultivars. There was no significant linear relationship between incidence of feeding and boll age for either species caged on DP 5415 and NuCOTN 33B. However, a significant linear relationship between larval mortality and boll age was observed for both species caged on NuCOTN 33B (mortality increased as bolls matured), but no such linear relationship was found on DP 5415. A significant linear relationship between boll penetration and boll age was observed for both species caged on NuCOTN 33B (boll penetration decreased as bolls matured). Similarly, a significant linear relationship between boll penetration and boll age was observed for beet armyworms caged on DP 5415 (boll penetration decreased as bolls matured), whereas no relationship was found for fall armyworms caged on DP 5415. Fall armyworms penetrated ≥60% of DP 5415 bolls regardless of their age, but these bolls were tolerant (≤10% boll penetration) to beet armyworms at 390 heat units. The NuCOTN 33B bolls were tolerant to fall armyworm damage at 864 heat units, but these bolls were tolerant to beet armyworm damage at 361 heat units. These data suggest that fall armyworms and beet armyworms are able to successfully penetrate bolls of 350 heat units at unacceptable (≥10%) levels.

KEY WORDS *Spodoptera frugiperda, Spodoptera exigua*, transgenic cotton, insecticide termination, pest management

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), and the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), are destructive migratory pests of many agroecosystems in the

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southeastern United States (Sparks 1979, Smith 1985). These pests are considered sporadic, but significant pests of cotton, *Gossypium hirsutum* L., from North Carolina to Texas (Smith 1985). Recently, outbreaks of beet armyworm populations contributed to the severe cotton yield losses reported in the Lower Rio Grande Valley of Texas in 1995 (Summy et al. 1996). In addition, local outbreaks of the fall armyworm were reported in 1996 in both conventional cotton and transgenic cotton expressing a *Bacillus thuringiensis* Berliner (Bt) ∂-endotoxin in southern Alabama and Georgia (Hood 1997, Smith 1997).

Because the developmental rate of a cotton boll is closely associated with temperature, boll development is based on heat unit accumulation rather than on age (Bagwell & Tugwell 1992). Cotton development is often determined by counting the nodes above the uppermost, white flower in the first fruiting position of the cotton plant (nodes above white flower, NAWF) (Bernhardt et al. 1986) and by combining these data with boll age (heat unit accumulation) (Bagwell & Tugwell 1992, Torrey et al. 1997). Bagwell (1994) showed that conventional cotton bolls that had accumulated 350 heat units attained maximum resistance to third-instar bollworm, *Helicoverpa zea* (Boddie). Their data suggested that treatments to control this pest on cotton could be terminated when the last population of bolls on cotton plants contributing to yield had accumulated at least 350 heat units. Although cotton boll susceptibility data have been obtained for the boll weevil, *Anthonomus grandis grandis* (Boheman) (Bagwell 1994); bollworm, and European corn borer, *Ostrinia nubilalis* (Hübner) (Ellsworth & Bradley 1992), data for other cotton pests are lacking. Late-instar fall armyworms (4th-6th) feed almost exclusively on bolls low in the plant canopy (Ali et al. 1990), whereas late-instar beet armyworms (3rd-5th) continue to feed on foliage in addition to squares, blooms, and bolls (Smith 1985). Studies with individual late-instar fall armyworms caged on cotton bolls indicated that injury to small bolls resulted in significant reductions in their probability of harvest (Ali et al. 1990). However, the stage of development at which both conventional and transgenic Bt cotton bolls may be resistant to fall armyworm as well as beet armyworm injury is unknown. Therefore, boll tolerance data must be obtained for each lepidopteran pest that can damage bolls to establish recommendations for termination of insect control strategies in conventional and transgenic Bt cotton. This study was conducted to define the period of boll susceptibility to fall armyworm and beet armyworm injury on conventional as well as transgenic Bt cotton.

Materials and Methods

All experiments were conducted from June-August during 1995–1997. Transgenic Bt cotton 'NuCOTN 33B' containing the Bollgard™ gene (Monsanto Co., St. Louis, Missouri) and one of its conventional cotton parental lines 'DP 5415' used in these experiments were grown at the Macon Ridge Location of the Northeast Research Station (Louisiana State University Agricultural Center, Louisiana Agricultural Experiment Station) near Winnsboro, Louisiana. Plots consisted of 4–8 rows by 80–100 ft. Field collections of beet armyworms were made from cotton near Evelyn, Louisiana, and Tift County, Georgia, in 1995 and 1996, respectively. Field collections of fall armyworm were made from field

corn, *Zea mays* L., near Brownsville, Texas, and Baton Rouge, Louisiana, in 1996, and from the Macon Ridge Location of the Northeast Research Station in 1997.

Both species were reared for a minimum of two generations in the laboratory to eliminate diseases and parasitoids before infesting cotton bolls. Larvae of both species were reared according to the methods described in Perkins (1979) by using a soybean and wheat germ meridic diet (King & Hartley 1985). Approximately 60 pupae of each species were placed into individual cardboard cylinders (3.79 liter) lined with wax paper and covered with cheesecloth. Moths were fed a 10% sugar water solution. Eggs were harvested daily from sheets of cheesecloth and wax paper, and held in an environmental chamber at 27 ± 1°C and a photoperiod of 14:10 (L:D) h. Neonate larvae were transferred to 29.6-ml cups containing artificial diet, capped, and reared as described above.

Boll infestations. Yellow "snap on tags" (A. M. Leonard, Inc., Piqua, Ohio) were labelled with the date of anthesis, and placed around the petioles of the corresponding first position white flowers on cotton plants. The daily growth of tagged bolls was recorded in heat units described in Bagwell & Tugwell (1992) as follows: (maximum + minimum daily temperature/2) - 60, where 60 is considered the minimum temperature at which development occurs. The age class of cotton bolls at infestation was calculated by accumulating heat units from the initial date of anthesis to time of infestation.

Fifth-instars (300–400 mg) were used for fall armyworm infestations, and third-instars (30–45 mg) were used for beet armyworm infestations. In 1995, beet armyworms were infested on DP 5415 bolls, and NuCOTN 33^{B} bolls were infested in 1996. In addition, fall armyworms were infested on both DP 5415 and NuCOTN 33^{B} bolls in 1996, but only NuCOTN 33^{B} bolls were infested in 1997. Because beet armyworm tend to feed on bracts of fruiting forms (Smith 1985), the bracts were removed before infestation to ensure that larvae fed only on bolls. A single larva was placed into a 15 cm by 11.5 cm cloth #280 mesh bag with drawstrings and placed over DP 5415 or NuCOTN 33^{B} bolls in various stages (heat units) and closed tightly to minimize escape. After 72 h, the bagged bolls were excised from the plant and larval mortality, incidence of feeding, and boll penetration were recorded. Larval mortality was defined as no movement after 10 s of prodding with blunt forceps. Incidence of feeding was defined as evidence of larval feeding on the external boll wall. Boll penetration was defined as the ability of a larva to completely penetrate the boll walls (i.e., exocarp and endocarp) into the seed or fiber (White 1995).

Data analyses. For each species and cultivar, regression analysis (PROC REG, SAS Institute 1985) was used to determine if there was a significant relationship between larval mortality, incidence of feeding, or boll penetration, and accumulated heat units (boll age). In all regression analyses, linear relationships were used for all regression models, because higher order polynomials did not improve the fit of the model.

Results and Discussion

Fall armyworm. For DP 5415, the mean number of fall armyworm larvae infested per heat unit was 24.3 with a total sample size of 267 larvae for 11 different

heat units (39.5–852 heat units). Initial regression analysis for fall armyworms infested on NuCOTN 33^B bolls revealed no significant differences ($P > 0.05$) between the slopes and Y-intercepts for all observations collected during 1996 and 1997. Therefore, these data were combined to obtain a larger sample size. For NuCOTN 33^B, the mean number of fall armyworm larvae infested per heat unit was 33.5 with a total sample size of 1,172 larvae for 35 different heat units (20.5–780 heat units).

Fall armyworms caged on DP 5415 penetrated ≥60% of bolls regardless of their age (Fig. 1A). Larval mortality was low, ranging from 0 to 4.5%, with a mean of 2.3% (Table 1). Incidence of feeding was high regardless of boll age ranging from 72.7 to 100%, with a mean of 88.4%. There was no significant linear relationship ($P > 0.05$) describing larval mortality ($r^2 = 0.003$; $F = 0.026$; df = 1, 9; $P = 0.876$), incidence of feeding ($r^2 = 0.001$; $F = 0.001$; df = 1, 9; $P = 0.974$), or boll penetration ($r^2 = 0.145$; $F = 1.53$; df = 1, 9; $P = 0.248$) as a function of accumulated heat units.

The regression equation indicated NuCOTN 33^B bolls were tolerant (≤10% boll penetration) to fifth-instar fall armyworm damage at 864 heat units. However at this age, cotton fiber would be mature and bolls are normally opening (Landivar & Benedict 1996) (Fig. 1B). In addition, ≥11% of larvae was able to penetrate mature NuCOTN 33^B bolls that were near opening (between 750 and 850 heat units). Larval mortality was low, ranging from 0 to 14.8%, with a mean of 3.1% (Table 1). Incidence of feeding was high, regardless of boll age, ranging from 57.1 to 100%, with a mean of 81.5%. There was no significant linear relationship ($P > 0.05$) describing incidence of feeding ($r^2 = 0.019$; $F = 0.625$; df = 1, 33; $P = 0.435$) as a function of accumulated heat units. However, there was a significant linear relationship ($P < 0.05$) describing larval mortality ($r^2 = 0.429$; $F = 24.8$; df = 1, 33; $P \leq 0.0001$) as a function of accumulated heat units, indicating that larval mortality increased as NuCOTN 33^B bolls developed. In addition, there was a significant linear relationship ($P < 0.05$) describing boll penetration as a function of accumulated heat units ($r^2 = 0.634$; $F = 57.1$; df = 1, 33; $P \leq 0.0001$), indicating that boll penetration decreased as NuCOTN 33^B bolls developed.

Beet armyworm. For DP 5415, the mean number of beet armyworm larvae infested per heat unit was 27.8 with a total sample size of 333 larvae for 12 different heat units (22.5–360.5 heat units). For NuCOTN 33^B, the mean number of beet armyworm larvae infested per heat unit was 26.1 with a total sample size of 469 larvae for 18 different heat units (17.0–460.5 heat units).

The regression equation indicated DP 5415 bolls were tolerant (≤10% boll penetration) to third-instar beet armyworm damage at 390 heat units (Fig. 2A). Larval mortality ranged from 0 to 25%, with a mean of 9.6% (Table 2). Incidence of feeding on bolls ranged from 73.3 to 100%, with a mean of 83.7%. There was no significant linear relationship ($P > 0.05$) describing larval mortality ($r^2 = 0.223$; $F = 2.87$; df = 1, 10; $P = 0.121$) or incidence of feeding ($r^2 = 0.029$; $F = 0.295$; df = 1, 10; $P = 0.599$) as a function of accumulated heat units. However, there was a significant linear relationship ($P < 0.05$) describing boll penetration as a function of accumulated heat units ($r^2 = 0.523$; $F = 10.9$; df = 1, 10; $P = 0.008$), indicating that boll penetration decreased as DP 5415 bolls developed.

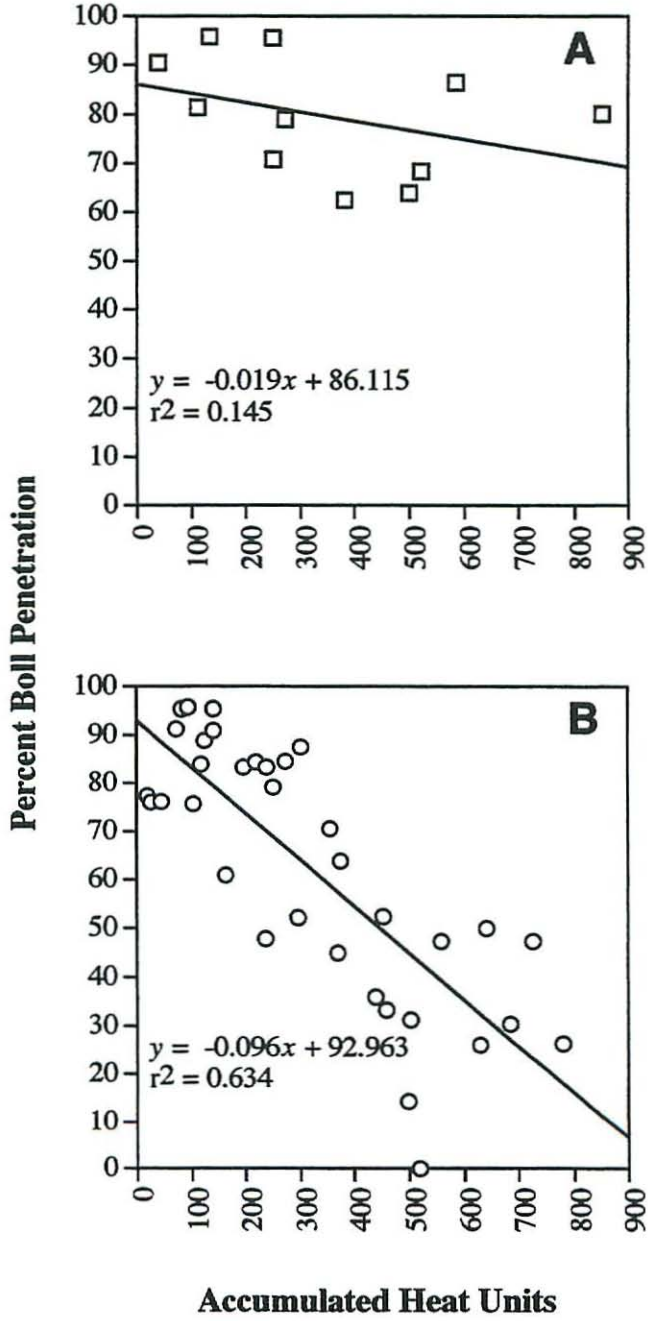

Fig. 1. Percentage of boll penetration versus accumulated heat units for fifth-instar fall armyworms caged on (A) DP 5415 and (B) NuCOTN 33[B].

Table 1. Mortality and incidence of larval feeding for fall armyworm larvae caged on conventional (DP 5415) and transgenic Bt cotton bolls (NuCOTN 33[B]) of various ages.

No. larvae	Heat units[a]	Mean ± SE	
		% Mortality	% Incidence of larval feeding
		DP 5415	
267	39.5–852.0	2.3 ± 0.7	88.4 ± 3.0
		NuCOTN 33[B]	
1172	20.5–780.0	3.1 ± 0.7	81.5 ± 1.7

[a] Method to classify cotton boll age based on heat. (Heat units = [daily high temperature + daily minimun daily temperature/2] - 60).

The regression equation indicated NuCOTN 33[B] bolls were tolerant (≤10% boll penetration) to third-instar beet armyworm damage at 361 heat units (Fig. 2B). Larval mortality ranged from 0 to 25%, with a mean of 7.8% (Table 2). Incidence of feeding was high, ranging from 46.4 to 100%, with a mean of 79.7%. There was no significant linear relationship ($P > 0.05$) describing incidence of feeding ($r^2 = 0.003$; $F = 0.047$; df = 1, 16; $P = 0.831$) as a function of accumulated heat units. However, there was a significant linear relationship ($P < 0.05$) describing larval mortality ($r^2 = 0.351$; $F = 8.65$; df = 1, 16; $P = 0.010$) as a function of accumulated heat units, indicating that larval mortality increased as NuCOTN 33[B] bolls developed. There also was a significant linear relationship ($P < 0.05$) describing boll penetration ($r^2 = 0.880$; $F = 117$; df = 1, 16; $P \leq 0.0001$) as a function of accumulated heat units, indicating that boll penetration decreased as NuCOTN 33[B] bolls developed.

Research on late-season insecticide termination strategies against cotton pests suggests that when plants are at NAWF = 5 and the last effective boll population has accumulated at least 350 heat units, harvestable yield is tolerant to injury by boll weevil or tobacco budworm [*Heliothis virescens* (F.)], and bollworm (Bagwell & Tugwell 1992, Torrey et al. 1997). Unlike these insects, fall armyworms and beet armyworms damaged bolls that accumulated 350 heat units at unacceptable levels. If insecticide applications are terminated when bolls have accumulated 350 heat units, without concerns for other pests such as fall armyworms and beet armyworms, yield losses may result. Thus, when using the insecticide termination rules for tobacco budworms bollworms, and boll weevils, frequent scouting of cotton fields for additional pests is necessary to determine if further integrated pest management control strategies are needed.

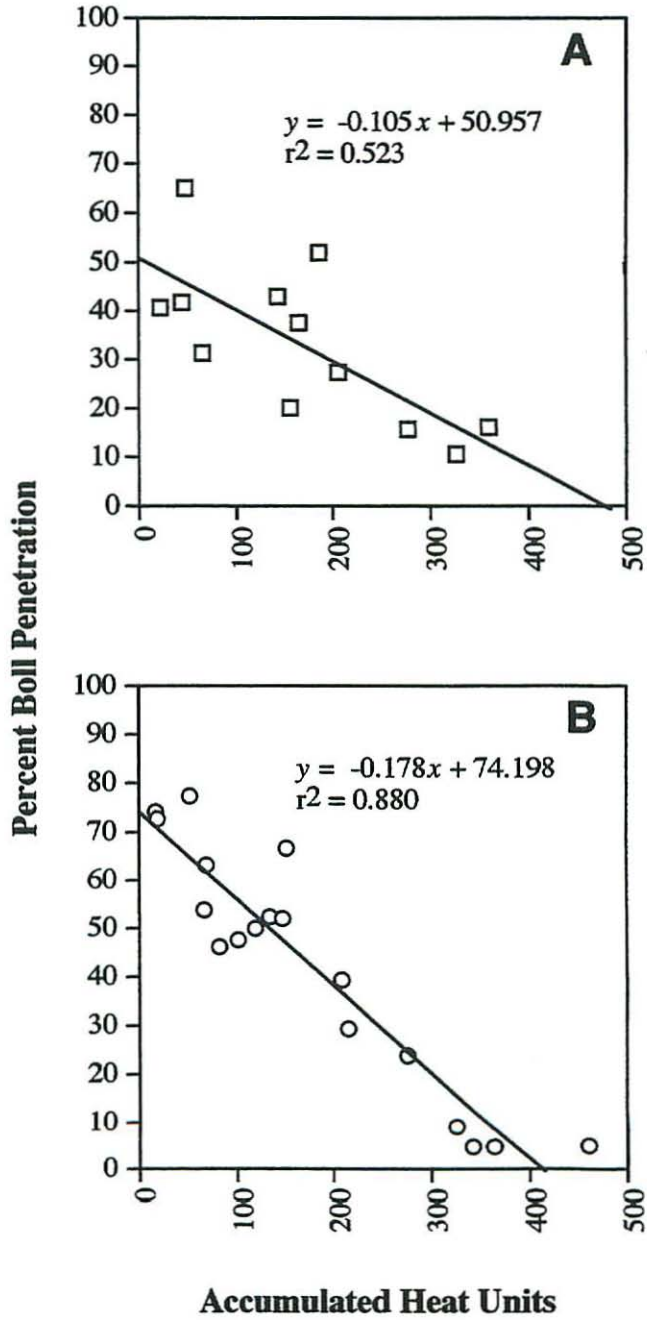

Fig. 2. Percentage of boll penetration versus accumulated heat units for third-instar beet armyworms caged on (A) DP 5415 and (B) NuCOTN 33[B].

Table 2. Mortality and incidence of larval feeding for beet armyworm larvae caged on conventional (DP 5415) and transgenic Bt cotton bolls (NuCOTN 33[B]) of various ages.

No. larvae	Heat units[a]	Mean ± SE	
		% Mortality	% Incidence of larval feeding
		DP 5415	
333	22.5–360.5	9.6 ± 2.6	83.7 ± 2.3
		NuCOTN 33[B]	
469	17.0–460.5	7.8 ± 2.0	79.7 ± 3.2

[a] Method to classify cotton boll age based on heat. (Heat units = [daily high temperature + daily minimun daily temperature/2] - 60).

Producers should exercise caution when using insecticide termination strategies. Further data are needed to determine boll tolerance levels for other larval stages of fall armyworms and beet armyworms, because all larval stages will feed on fruiting forms (Ali et al. 1990, Smith 1985). This study also indicates that boll tolerance differs among noctuid pests. Therefore, boll susceptibility at various developmental stages should be determined for each pest to refine insect control termination strategies.

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Survey of Montana Grasses for Wheat Curl Mite (Acari: Eriophyidae), the Vector of Wheat Streak Mosaic Virus[1]

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ABSTRACT Annual and perennial grasses from a major winter wheat producing area in north central Montana were surveyed for wheat curl mites, *Aceria tosichella* (Keifer), during the summers of 1995, 1996, and 1997. More than 4,800 grass plants representing 16 species were individually inspected for wheat curl mites. *Aceria tosichella* was collected from *Bromus inermis* Leys, *Poa interior* Rydberg, *Poa pratensis* L., and winter and volunteer *Triticum aestivum* L. plants. Few wheat curl mites were collected from *B. inermis, P. interior,* and *T. aestivum* L.; *P pratensis* supported a low population of wheat curl mites all 3 years. In addition, five *Aceria* n. sp., three *Aculodes* n. sp., and *Aculodes dubius* Nalepa were collected from six wild grass species. Results indicate wheat curl mites do not develop to epidemic proportions in wild host grasses.

KEY WORDS wheat curl mite, Acari, Eriophyidae, *Aceria tosichella*, Gramineae, cultivated wheat, volunteer wheat, *Triticum aestivum*

Wheat streak mosaic is a very serious virus disease of wheat in the Western Plains of the United States and Canada. Although annual wheat damage is estimated at 2% each year, complete crop losses are not uncommon in some fields (Appel et al. 1991). In Kansas, an epiphytotic in 1987–1988 resulted in a statewide yield loss of 13%, valued at $150 million (Harvey et al. 1994). Since 1954, when the first case of wheat streak mosaic was observed in Montana, major outbreaks have occurred in 1964, 1981, 1993, and 1994 (Bamford et al. 1996). The most recent wheat streak epidemic caused severe crop losses in Montana valued at $50 million (J. Riesselman, personal communication).

The virus is solely vectored by the wheat curl mite, *Aceria tosichella* Keifer (Slykhuis 1955). Wheat curl mites acquire the virus by feeding on infected hosts, and later transmit it to healthy plants as they feed on them. Under favorable environmental and cultural conditions, mite populations can build up to enormous numbers. Geske & Riesselman (1988) reported 150,000 mites per volunteer wheat plant in the field. The mites are small (mean adult length = 250 µm) and can be easily dispersed by wind from plant to plant. Thus, susceptible wheat fields planted near diseased plants are infested. Warm, dry, windy conditions in the spring, and especially in the fall cause mite populations to increase and spread further into wheat fields (McMullen 1991).

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High incidence of wheat streak mosaic also is associated with early planting of winter wheat, use of susceptible cultivars, and hailstorms. Willis (1984) reported that wheat streak mosaic is most severe in early fall-planted wheat, and that late planting reduces disease incidence dramatically. Wheat cultivars such as 'TAM 107' have been shown to be resistant to the mite. Harvey et al. (1995) reported that wheat curl mites can adapt to this variety. Volunteer wheat caused by grain shattering and sprouting in hailstorms usually serves as the principal reservoir for the mite over the summer (Slykhuis 1953). Because the mites can only survive 4 to 6 d without a green host (Slykhuis 1955), destroying all volunteer wheat, either with herbicides or tillage, 2 wk before planting dramatically reduces the likelihood of a wheat streak mosaic outbreak (Watkins et al. 1989).

In 1992, a combination of early-season drought, disease-stressed crops, shattered wheat heads, and late-season moisture contributed to excessive volunteer wheat populations in north central Montana. These conditions were thought to contribute to the wheat streak mosaic epidemic in 1993. Since 1995, weather conditions and cultural control practices by producers have greatly reduced the incidence of volunteer wheat in this region. It has been suggested that the mite may reside in noncultivated host grasses. Previous studies have shown several annual and perennial grasses as potential hosts for the wheat curl mite under field conditions (Slykhuis 1955, Connin 1956, Staples & Allington 1956, Somsen & Sill 1970).

Our objectives were to determine which species of grasses were infested with wheat curl mites in grass areas bordering wheat fields, if infested grass hosts could support mite populations from year to year, and whether early detection of commonly infested grasses can facilitate predicting a wheat streak mosaic outbreak in Montana. We also report on the occurrence of other eriophyid species collected from grasses during this study.

Materials and Methods

Field survey. Annual and perennial grasses that were found growing naturally in borrow pits and fields bordering winter wheat crops were examined for the presence of *A. tosichella*. The four collection sites were located within an 8-km radius in the northwest section of Chouteau County, Montana (latitude 48° 2′–8′N and longitude 111° 16′–23′W) at a mean elevation of 3,500 m. In 1995, grass samples were collected twice a month beginning 5 May and ending 25 August. In 1996 and 1997, samples were collected on 13 July and 16 August, and on 23 May, 19 June, 26 July, and 26 August, respectively. Grasses with inflorescence were identified to species and collected in randomly selected plots (0.5 m diameter) throughout each site. Immature grass plants (without inflorescence) were transported back to the laboratory after collection and identified with a Gramineae taxonomic key (Hitchcock et al. 1950). Depending on the year, the number of plots at each site varied from four to eight. In each plot, 10 to 25 whole plants of a single Gramineae species were collected and stored on ice and taken to the laboratory, where they were preserved at 4°C for no more than 5 d until examined for mites. In addition, 25 to 50 wheat plants were randomly collected from fields adjacent (1–5 m) to the grass collection sites and

processed identically as the grass samples. Grass composition at each site was determined by using the Daubenmire frame technique (Daubenmire 1959).

Eriophyid mite detection. Grass and wheat plants from each site were examined for the presence of eriophyid mites by using a dissecting microscope (16×). Initially, only the ligule area was examined, but it was determined early in the investigation that the majority of the eriophyid mites was found over the entire abaxial surface of the leaves (all leaves for each plant sample were inspected). Grass and wheat plants that harbored eriophyid mites were stored at -20°C for later mite species classification. Two to 30 eriophyid mites from each grass and wheat plant were mounted for species identification. Mites were cleared in sorbitol:chloral hydrate (1:2), and mounted on microscope slides in gum arabic (Jepson 1975). Representative specimens were sent to J. W. Amrine, Jr. (Division of Plant and Soil Sciences, West Virginia University, Morgantown) for verification of identifications. Voucher specimens are deposited in the Montana Entomology Collection, Montana State University, Bozeman.

Results

1995 Survey. Population densities of grass species differed among sites. Site 1 had western wheatgrass [*Pascopyrum smithii* (Rydberg) Love], slender wheatgrass [*Agropyron trachycaulum* (Link) Malte], Kentucky bluegrass (*Poa pratensis* L.), and green needlegrass (*Stipa viridula* Trinius) comprising 85% of the grass composition. *Pascopyrum smithii,* smooth brome (*Bromus inermis* Leys), and *P. pratensis* were the predominant perennials at site 2 and composed 80% of the species. At site 3, crested wheatgrass [*Agropyron cristatum* (L.) Gaertner], *B. inermis,* and *S. viridula* composed 50% of the species, and at site 4, 65% of the species composition was made up of *P. smithii* and *S. viridula.*

Fifteen grass species totaling 1,780 plants were individually inspected for the presence of *A. tosichella*. Wheat curl mites were identified on *B. inermis* and *P. pratensis* (Table 1). Five mites were found on 175 *B. inermis* and 130 were found on 120 *P. pratensis* plants. The number of wheat curl mites on *P. pratensis* was less than half the total number of mites observed because an *Aceria* n. sp. that was morphologically similar to the wheat curl mite was present on 50% of the plants examined. In addition, the eriophyid species *Aculodes dubius* Nalepa was identified on *P. smithii*. Also, *Aceria* n. sp. was discovered on collections of *P. smithii*, *A. trachycaulum,* and *S. viridula*; *Aculodes* n. sp. was found on each of *P. pratensis, Stipa comata* Trinius & Ruprecht, and *S. viridula* (Table 2). No eriophyid mites were observed on wild oats (*Avena fatua* L.), blue grama [*Bouteloua gracilis* (Humboldt) Lagasca], Japanese brome (*Bromus japonicus* Thunberg), cheatgrass brome (*Bromus tectorum* L.), Canada wild rye [*Elymus canadensis* (C.L. Hitchcock) Jensen], foxtail barley (*Hordeum jubatum* L.), sandberg bluegrass (*Poa secunda* Presl), or wheat plants. All grass and wheat plants examined were asymptomatic for wheat streak mosaic virus.

1996 Survey. Two additional grass species, inland bluegrass (*Poa interior* Rydberg) and volunteer wheat, along with *B. inermis* and *P. pratensis,* were found to harbor wheat curl mites in 1996. A total of 815 plants was examined for *A. tosichella*. *Poa interior* supported small populations of *A. tosichella* mites on 50% of the plants inspected. Eight wheat curl mites were observed on

Table 1. Wheat curl mite (WCM) infestation on annual (A) and perennial (P) grasses collected in Chouteau County, Montana, 1995–1997.

	1995			1996			1997		
		WCM			WCM			WCM	
Grass host	No. plants	% Infest.	Mean[a] ± SD	No. plants	% Infest.	Mean[a] ± SD	No. plants	% Infest.	Mean[a] ± SD
P. interior (P)	—	—	—	10	50	8 ± 7 (12)[b]	—	—	—
P. pratensis (P)	120	41	3 ± 2 (18)	200	35	4 ± 6 (56)	1,150	8	6 ± 8 (85)
B. inermis (P)	175	1	1	150	5	1 ± 2 (2)	475	5	1 ± 2 (2)
T. aestivum (A) (Cultivated wheat)	320	0	0	85	0	0	550	1	1
T. aestivum (A) (Volunteer wheat)	70	0	0	25	1	1	350	1	1

[a]Mean (± SD) number of wheat curl mites per total number of infested plants examined.

[b]Number in parentheses equals the maximum number of eriophyid mites found on a single plant for that particular year.

Table 2. Eriophyid species infestation on annual (A) and perennial (P) grasses collected in Chouteau County, Montana, 1995–1997.

	1995		1996		1997		
Grass host	No. plants	% Infest.	No. plants	% Infest.	No. plants	% Infest.	Eriophyid species
A. cristatum (P)	120	< 1	20	0	75	0	Unidentified
A. trachycaulum (P)	315	14	110	10	25	40	*Aceria* n. sp.
A. fatua (A)	35	0	20	0	50	0	
B. gracilis (P)	10	0	—[a]	—	—	—	
B. japonicus (P)	30	0	—	—	—	—	
B. tectorum (A)	45	0	—	—	125	0	
E. canadensis (P)	20	0	—	—	—	—	
H. jubatum (A)	35	0	20	80	50	0	*Aceria* n. sp., *A. dubuis*
P. secunda (P)	20	0	—	—	—	—	
P. smithii (P)	115	44	40	25	50	0	*Aceria* n. sp., *A. dubuis*
S. comata (P)	30	45	—	—	—	—	*Aculodes* n. sp.
S. viridula (P)	240	29	135	75	50	0	*Aceria* n. sp., *Aculodes* n. sp.

[a]No plants were collected.

150 *B. inermis* plants, whereas 140 wheat curl mites were found on 200 *P. pratensis* plants. The composition of other eriophyid species identified on *P. pratensis* included an *Aculodes* n. sp. (50%) and *Aceria* n. sp. (15%). In addition, *A. dubuis* and an *Aceria* n. sp. were identified on *H. jubatum*. All wheat and grass plants examined were asymptomatic for wheat streak mosaic virus.

1997 Survey. Because 1995 and 1996 results indicated that *B. inermis* and *P. pratensis* were the most suitable hosts for wheat curl mites, survey and sampling efforts focused on these species. In 1997, *B. inermis* and *P. pratensis* comprised 79% of all of the grass individuals examined. Although five times (ca. 1,150 plants) as many *P. pratensis* plants were examined compared with the previous year, only 357 eriophyid mites (30% *A. tosichella*, 40% *Aceria* n. sp., and 30% *Aculodes* n. sp.) were observed on this species. All 2,050 grass plants examined were asymptomatic for wheat streak mosaic virus.

The first symptomatic volunteer wheat plants were collected from site 4 on 19 June. The plants showed distinct wheat streak mosaic viruslike symptoms of greenish-yellow dashes and streaks parallel to the axis of their leaves. However, only a few wheat curl mites were found on 50 volunteer wheat plants that were examined from this site. As the season progressed, wheat streak mosaic virus symptoms also were observed at sites 1 and 3 in the cultivated winter wheat fields. Winter wheat plants collected on 26 July were selected based on wheat streak mosaic leaf symptoms. Winter wheat plants that displayed symptoms were dispersed randomly within the cultivated field. Only three wheat curl mites were found on 150 symptomatic winter wheat plants collected from sites 1, 3, and 4 on this date.

Discussion

Six of the grass species sampled in our study have been reported to be naturally infested with wheat curl mites; all grass species except *P. secunda* have been reported to be susceptible to the mite in the greenhouse (Somsen & Sill 1970, Staples & Allington 1956, Slykhuis 1955). *Aceria tosichella* was recovered from *P. pratensis*, *P. interior*, and *B. inermis*. We collected *A. tosichella* from *P. interior*, but because of the low number of *P. interior* samples collected, it is unknown whether this species can support large populations of wheat curl mites from year to year. Our results suggest that *P. pratensis* is a suitable host for the wheat curl mite. Slykhuis (1963) reported wheat curl mites can become abundant on *P. pratensis* in the field.

We do not know whether *P. pratensis* can maintain sufficient populations of wheat curl mites to be a serious epidemiological source of viruliferous mites in the field. However, *P. pratensis* is a common perennial that remains relatively green from May to September (Hitchcock 1950), and the natural folding of its leaf blade provides a protective microhabitat for eriophyid mites. Also, the low number of *A. tosichella* found on many *P. pratensis* plants could be associated with interspecific competition between *A. tosichella*, *Aceria* n. sp., and *Aculodes* n. sp. The number of new species of eriophyid mites collected on several of the grass species is not unusual because an estimated 70% of the species in the Eriophyidae have yet to be identified (Amrine & Stasny 1994). Several greenhouse studies have shown *P. pratensis* to be susceptible to wheat curl mites, but

resistant to wheat streak mosaic virus (Sill & Connin 1953, Slykhuis 1965, Somsen & Sill 1970, Bremer 1971).

Bromus inermis harbored wheat curl mites all 3 yr. However, the extremely low number of wheat curl mites found on *B. inermis* supports other work that suggests this species is a poor host for the mite. Laboratory reports by Connin (1956) found *B. inermis* to be a very poor host for the mite and immune to the virus. McMullen & Nelson (1989) found *B. inermis* to be positive for wheat streak mosaic virus in the laboratory but did not indicate whether wheat curl mites could survive on this species.

Because of the limited number of *A. fatua*, *B. gracilis, B. japonicus, E. canadensis,* and *S. comata* collected, we are unable to draw conclusions about the relative importance of these species as alternate hosts for the wheat curl mite. However, it is interesting to compare our results with other studies in the northern Great Plains of the United States and Canada. In Nebraska, a large, thriving population of wheat curl mites was found on a few *E. canadensis* plants, but only a couple of mites were found on *S. comata* plants, whereas none was discovered on *B. gracilis* (Staples & Allington 1956). In southern Alberta, Slykhuis (1955) reported *B. japonicus* and *A. fatua* to be naturally infected with wheat streak mosaic virus but did not indicate whether wheat curl mites were found on these species.

Source of wheat streak mosaic virus infection in wheat fields in 1997 was unknown. Although a few mites were found on the 880 wheat plants that were examined, the spread of the disease from these plants to adjacent wheat plants was not indicative of mite transmission (field edge effect). Rather, symptomatic wheat plant patches (clusters of five to 10 plants) suggest virus transmission through leaf abrasions. Transmission has been reported between infected and healthy wheat plants through abrasive leaf action during high winds (Sill 1953). Our observations suggest an early spring infestation of seedlings (2–3-leaf stage) by viruliferous wheat curl mites. However, for unknown reasons these mites were unable to develop a significant population on these plants. The absence of large wheat curl mite numbers on volunteer wheat suggests that weather conditions such as high temperatures, low relative humidity, and extended dry periods may have contributed to the suppression of the wheat curl mite population. Possible infestation sources other than grasses may be mites blown in from other regions or early spring volunteer wheat plants that survive near susceptible wheat fields. Slykhuis (1955) captured wheat curl mites as far as 3.2 km from the nearest wheat field. According to Shahwan & Hill (1984) there is always a low level of wheat curl mites in wheat fields every year. Therefore, grass hosts may serve as long-term reservoirs for the wheat curl mite, whereas wheat streak mosaic outbreaks are almost always associated with the presence of volunteer wheat (McMullen 1991).

In conclusion, our survey indicates that wheat curl mites originating from *P. pratensis* and *B. inermis* do not develop to epidemic levels during nonoutbreak years. Rather, they may provide the background wheat curl mite populations found in wheat fields every year. The results of our study indicate that annual and perennial grasses are of limited importance as alternate hosts for wheat curl mites.

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Survival of Hessian Fly (Diptera: Cecidomyiidae) Larvae on Wheat Cultivars Carrying Different Genes for Antibiosis[1]

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ABSTRACT Field and growth chamber tests were conducted to determine which of the wheat (*Triticum*) genes that condition antibiosis to Hessian fly, *Mayetiola destructor* (Say), allow for larval survival on resistant plants. The results showed that the *H*1*H*2 genes allowed the highest larval survival and the *H*7*H*8 genes conditioned an intermediate level of larval survival. Wheats carrying the *H*3, *H*5, *H*6, *H*9, and *H*10 genes had low (<15%) larval survival. No larvae survived on resistant plants carrying the *H*13 gene. Larvae that survived on resistant plants were smaller than those on susceptible 'Newton' plants. Resistance genes that allow avirulent genotypes to survive on resistant plants should be deployed to reduce selection for biotype development.

KEY WORDS *Mayetiola destructor*, resistance genes, wheat, larval survival, antibiosis, *Triticum*

The Hessian fly, *Mayetiola destructor* (Say), is a destructive pest of wheat (*Triticum* spp.), throughout most of the production areas of the world. In the United States, the insect has been a serious pest of common wheat since its accidental introduction in the late 1700s. During the last 50 yr, genetic resistance present in known wheat cultivars has been used to reduce losses (Hatchett et al. 1987). Twenty-five resistance genes, previously designated *H*1 through *H*20 (Patterson et al. 1992), *H*22 through *H*24 (Raupp et al. 1993), *H*26 (Cox & Hatchett 1994), and *H*27 (Ohm et al. 1997), have been identified in *Triticum* species for use in breeding resistant cultivars. Two resistance genes, designated *H*22 and *H*25 (Hatchett et al. 1993), were transferred from rye, *Secale cereale* L., to common wheat via wheat-rye chromosomal translocations (Friebe et al. 1990, 1991). With the exception of recessive *h*4, all other genes are partially to completely dominant in expression.

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Although a large number of genes is available, breeding wheat for resistance to Hessian fly has become more complex because of the highly specific gene-for-gene relationship between the host and insect (Hatchett & Gallun 1970). Currently, 16 biotypes of Hessian fly (designated Great Plains and A through O) have been isolated from field populations and are distinguished only by their ability (virulence) or inability (avirulence) to survive on and stunt wheats carrying resistance genes *H*3, *H*5, *H*6, or *H*7*H*8 (Ratcliffe & Hatchett 1997). Some of these virulent biotypes are now prevalent in populations where those genes have been deployed for several years in widely grown cultivars and are assumed to have evolved as a result of selection by resistant cultivars (Ratcliffe & Hatchett 1997).

All resistance genes confer larval antibiosis, whereby first instars die soon after they begin feeding on plants. The presumed chemical basis of the antibiosis is not known for any gene. Shukle et al. (1990) proposed that the functional basis of antibiosis may involve an incompatible interaction of a specific gene product in the plant and a gene product in the insect that results in the death of larvae. All resistance genes, however, do not confer the same level of mortality among first instars. Sosa (1981) observed various levels of larval survival on resistant plants carrying the *H*3, *H*5, or *H*6 gene. Because these larvae did not permanently stunt the plants and appeared to develop normally, he suggested that some of the wheat plants resistance genes may confer a plant tolerance response against those larvae that survive antibiosis factors. Because reduced larval mortality may result in reduced overall selection by the host plant, these genes slow the rate of change of populations towards virulence and may be more durable than those that confer complete mortality. The objective of this study was to quantify survival of Hessian fly larvae on wheats carrying different genes for antibiosis and thereby determine which of these genes exert the strongest viability selection on larvae in heterogeneous populations.

Materials and Methods

Wheat cultivars and resistance genes. We used 11 common wheat cultivars, each carrying one or two of 13 different resistance genes known to confer antibiosis to Hessian fly larvae: 'Big Club 60' (*H*1*H*2), 'Arkan' (*H*3), 'Arthur 71' (*H*5), 'Caldwell' (*H*6), 'Seneca' (*H*7*H*8), 'Ella' (*H*9), 'Joy' (*H*10), 'Kay' (*H*11), 'Lola' (*H*12), 'KS89H98' (*H*13), and 'Brule' (*H*18). 'Newton' wheat, which has no known genes for resistance, was used as a standard susceptible check. Genes *H*1, *H*2, *H*3, *H*5, *H*7, *H*8, and *H*12 were identified in common wheat, *Triticum aestivum* L.; *H*6, *H*9, *H*10, *H*11, and *H*18 were derived from durum wheat *Triticum turgidum* L.; and *H*13 was derived from the wild wheat *Triticum tauschii* (Coss.) Schmal. Genes *H*1 through *H*8 and *H*18 have been used widely in numerous cultivars that have provided protection against Hessian fly damage (Ratcliffe & Hatchett 1997). Genes *H*9 through *H*13 have not been deployed.

Source of Hessian flies. The insects used for the infestations were descendants of ≈500 randomly mated females that emerged from infested plants collected in 1985 from a field of susceptible 'Eagle' wheat in Ellis County, Kansas. The population was reared on Newton wheat in a greenhouse and propagated

for five generations from a minimum of 500 randomly mated females at each generation. Between generations, the population was stored between 6 and 8 mo as diapausing larvae at 5 ± 1°C. The complete biotype composition of the population was unknown, although analysis of the third generation indicated that the population was comprised predominately of avirulent Great Plains biotype individuals (Black et al. 1990). We assumed that the insects used in the tests were heterogeneous and genetically similar to those of the field population and that most plants of the resistant cultivars would express antibiosis.

Field study. This study was conducted during the fall growing seasons of 1988 and 1990 at the Kansas State University's Rocky Ford experimental farm in Manhattan, Kansas. The soil type was chase silty clay loam. A randomized complete design was used with four and three replications in 1988 and 1990, respectively. Replications of the 12 cultivars were seeded 1 wk apart, beginning on 16 and 25 September in 1988 and 1990, respectively. The wheats were hand-seeded in separate hills (50 cm apart) at a rate of 50 seeds per hill. When the plants of each replication were in the two-leaf stage, they were thinned to 30 plants per hill. Cages constructed from cloth-ventilated, plastic buckets (40 cm in height and 35 cm in diameter) (Rice & Wilde 1988) were placed over the hills to contain Hessian fly females. Six newly enclosed females of similar size were released into each cage for oviposition. Only females that had their ovipositors retracted, a behavior indicating recent mating (McKay & Hatchett 1984), were released. All releases were made between 0730 and 0830 hours (CST). The cages were removed 6 d later, by which time females had died and the eggs had hatched.

About 2 wk after the eggs hatched, when larvae on Newton plants were late-second instars or puparia, all plants of each cultivar were removed, taken into the laboratory, and examined for infestation. Individual plants were separated and recorded as being resistant or susceptible based on their reaction to larval feeding. Susceptible plants were stunted and dark blue-green, whereas resistant plants were not stunted and light green. Five susceptible plants of Newton and five resistant plants of each resistant cultivar also were selected at random from each replicate and examined under a stereoscopic microscope for presence of live or dead larvae. The number of live larvae was recorded, and the length of each larva was measured using an ocular micrometer.

Plant growth chamber study. A study similar to the field study was conducted in a plant growth chamber by using the same wheat cultivars and the same Hessian fly culture for infestation. The objective was to determine if resistant cultivars expressed the same level of antibiosis in a controlled environment as in the field. The experimental design was a randomized complete block with three replications. About 50 seeds of each cultivar were seeded in separate plastic pots (15 cm diameter) containing a standard soil mixture. When the plants were in the two-leaf stage, they were thinned to 30 plants per pot and held in a growth chamber set at 20 ± 1°C with a photoperiod of 12:12 (L:D) h and 70–90% RH. Infestation and plant evaluation procedures were similar to those used in the field study, except that small cheesecloth cages (12.5 cm in height and 25.0 cm in diameter) were used to confine females on pots, and only three females were used to infest the 30 plants in each pot. Plants were examined 15 d after infestation for presence of live and dead larvae. The

numbers of live larvae on resistant plants and on susceptible Newton plants were recorded, and the lengths of the larvae were measured to determine if feeding on resistant plants affected larval growth. To determine if the growth of resistant plants was affected by larval feeding, we measured the heights of a maximum of five resistant plants that had live larvae and a maximum of five resistant plants that had only dead larvae for the cultivars carrying *H1H2*, *H3*, *H7H8*, or *H18* genes. Measurements were taken from the base of the stem to the tip of the longest leaf.

Data were analyzed using analysis of variance (PROC ANOVA) (SAS Institute 1988), and the means were separated using Fisher's least significant difference (LSD) test. Percentage data were transformed by arcsine square root before analysis, but pretransformed means are presented.

Results

Infestation levels were high in both the field and growth chamber tests. Newton plants were 100% infested and had high larval densities in all tests (Tables 1–3). Based on the number of live larvae on Newton plants, all resistance genes, with the exception of *H12*, provided effective control of the Hessian flies used in these tests. Nearly all cultivars carrying resistance genes, however, had a small percentage of susceptible plants, indicating that the population was heterogeneous for virulence against these genes.

Plant genotype had a significant effect on the percentage of resistant plants with live larvae. Big Club 60 (*H1H2* genes) had the highest percentage of resistant plants with live larvae in both the field and growth chamber tests. Arthur 71 (*H5* gene) had the second highest percentage of resistant plants with live larvae in the 1988 field test, but the number did not exceed 5% in the 1990 field test and growth chamber test. This large difference in larval survival may have been caused by high temperature that occurred during the infestation period of the 1988 field test. Seneca (*H7H8* genes) had an intermediate level of larval survival on resistant plants, averaging 17% in the field tests and 29% in the growth chamber test. In general, cultivars having the *H3*, *H5*, *H6*, or *H9* gene had a relatively low percentage (<16%) of resistant plants with live larvae, whereas cultivars with the *H10*, *H11*, or *H13* gene had an extremely low percentage (<6%) or no resistant plants with live larvae.

The response of Brule (*H18* gene) was different in field and growth chamber tests. The percentage of resistant plants with live larvae averaged 28.5% in the field, whereas only 13.3% of the resistant plants had live larvae in the growth chamber. Because the *H18* gene has reduced expression of resistance at temperatures above 20°C (Maas et al. 1987), the higher larval survival in the field may have been due to high temperature.

Larvae that survived on resistant plants of all cultivars were smaller than those on susceptible Newton plants in all tests, but they were not always significantly ($P < 0.05$) smaller (Tables 1–3). Larvae on plants with genes that allowed for higher larval survival, such as *H1H2*, were only about 80% as large as those on Newton plants. However, differences in the size of larvae on Newton and on resistant plants may have been greater if their densities had been similar.

Table 1. Response (mean ± SD) of resistant wheat cultivars and susceptible Newton wheat to infestations of Hessian fly, field test 1988, Manhattan, Kansas.

Cultivar, resistance gene	% Plants resistant[a]	% Resistant plants with live larvae[b]	No. live larvae per resistant plant and susceptible Newton plant[c]	Length (mm) of live larvae[d]
Big Club 60, *H1H2*	88.5 ± 13.5cd	50.3 ± 10.9a	2.6 ± 1.6b	3.6 ± 0.6b
Arkan, *H3*	98.1 ± 3.8cd	9.7 ± 5.3ed	2.1 ± 0.8b	2.7 ± 0.6cd
Arthur, *H5*	93.6 ± 7.4cd	43.2 ± 13.1ab	2.8 ± 0.8b	3.2 ± 0.4cd
Caldwell, *H6*	100.0 ± 0.0d	13.1 ± 4.7d	2.3 ± 0.6b	2.5 ± 1.0cd
Seneca, *H7H8*	94.0 ± 3.1a	28.2 ± 9.3c	2.3 ± 0.8b	2.9 ± 1.0cd
Ella, *H9*	100.0 ± 0.0d	0.0 ± 0.0g	0.0 ± 0.0c	-
Joy, *H10*	95.8 ± 2.9ab	2.5 ± 1.8f	1.3 ± 0.6bc	2.8 ± 0.3bcd
Kay, *H11*	100.0 ± 0.0d	5.2 ± 1.9ef	1.3 ± 0.5bc	2.3 ± 0.6d
Lola, *H12*	40.8 ± 6.5e	1.5 ± 0.7gf	1.5 ± 1.0bc	2.5 ± 0.3cd
KS89H98, *H13*	98.6 ± 2.7cd	0.0 ± 0.0g	0.0 ± 0.0c	-
Brule, *H18*	82.6 ± 10.1bc	35.4 ± 5.3bc	3.0 ± 1.0b	3.4 ± 1.1bc
Newton, none	0.0 ± 0.0f	-	11.2 ± 3.5a	4.7 ± 0.4a

Means (± SD) within columns followed by the same letter are not significantly different (Fisher LSD test; $P > 0.05$ [SAS Institute 1988]).

[a] F test ($F = 14.22$; df = 11, 33; $P = 0.0001$).

[b] F test ($F = 51.66$; df = 10, 30; $P = 0.0001$).

[c] F test ($F = 22.35$; df = 11, 33; $P = 0.0001$).

Table 2. Response (mean ± SD) of resistant wheat cultivars and susceptible Newton wheat to infestations of Hessian fly, field test 1990, Manhattan, Kansas.

Cultivar, resistance gene	% Plants resistant[a]	% Resistant plants with live larvae[b]	No. live larvae per resistant plant and susceptible Newton plant[c]	Length (mm) of live larvae[d]
Big Club 60, *H1H2*	100.0 ± 0.0ab	34.1 ± 9.5a	2.6 ± 0.9bc	2.5 ± 0.5
Arkan, *H3*	100.0 ± 0.0ab	6.3 ± 1.1c	1.7 ± 1.1bc	2.4 ± 0.6
Arthur 71, *H5*	98.9 ± 1.9a	0.0 ± 0.9e	0.0 ± 0.0d	-
Caldwell, *H6*	100.0 ± 0.0ab	5.0 ± 3.5cd	1.8 ± 0.7bc	2.4 ± 0.6
Seneca, *H7H8*	100.0 ± 0.0ab	5.2 ± 2.7c	2.0 ± 1.0bc	2.3 ± 0.5
Ella, *H9*	99.1 ± 1.6a	0.9 ± 0.1de	1.0 ± 0.5cd	2.2 ± 0.4
Joy, *H10*	99.9 ± 1.6a	3.7 ± 1.6cd	3.2 ± 1.0b	2.7 ± 0.2
Kay, *H11*	100.0 ± 0.0ab	0.0 ± 0.0e	0.0 ± 0.0d	-
Lola, *H12*	48.6 ± 1.3c	8.3 ± 4.5c	1.5 ± 0.5cd	2.2 ± 0.4
KS89H98, *H13*	98.2 ± 3.1a	0.0 ± 0.0e	0.0 ± 0.0d	-
Brule, *H18*	87.2 ± 9.9a	21.5 ± 6.6b	2.4 ± 1.1bc	2.1 ± 0.4
Newton, none	0.0 ± 0.0c	-	12.1 ± 2.1a	3.3 ± 0.6

Means (± SD) within columns followed by the same letter are not significantly different (Fisher LSD test; $P > 0.05$ [SAS Institute 1988]).

[a] F test ($F = 14.56$; df = 11, 22; $P = 0.0001$).

[b] F test ($F = 23.78$; df = 10, 20; $P = 0.0001$).

[c] F test ($F = 33.35$; df = 11, 22; $P = 0.0001$).

[d] F test ($F = 1.56$; df = 8, 16; $P = 0.214$).

Table 3. Response (mean ± SD) of resistant wheat cultivars and susceptible Newton wheat to infestations of Hessian fly, growth chamber test.

Cultivar, resistance gene	% Plants resistant[a]	% Resistant plants with live larvae[b]	No. live larvae per resistant plant and susceptible Newton plant[c]	Length (mm) of live larvae[d]
Big Club, *H1H2*	90.3 ± 9.5ab	45.9 ± 11.2a	3.3 ± 0.9b	3.2 ± 0.2ab
Arkan, *H3*	100.0 ± 0.0b	12.7 ± 4.0cde	2.0 ± 1.0bc	2.7 ± 0.3bc
Arthur, *H5*	100.0 ± 0.0b	5.0 ± 3.0f	2.0 ± 1.0bc	3.3 ± 0.3ab
Caldwell, *H6*	98.3 ± 2.9ab	6.7 ± 2.9ef	2.3 ± 0.6bc	2.5 ± 0.7c
Seneca, *H7H8*	100.0 ± 0.0b	29.0 ± 9.6b	2.3 ± 1.4bc	3.0 ± 0.1abc
Ella, *H9*	90.7 ± 10.1ab	14.7 ± 10.1ab	2.0 ± 1.5bc	2.8 ± 0.2abc
Joy, *H10*	95.0 ± 5.0a	3.3 ± 0.8f	2.2 ± 1.0bc	2.3 ± 0.4c
Kay, *H11*	100.0 ± 0.0b	0.0 ± 0.0g	0.0 ± 0.0d	-
Lola, *H12*	42.7 ± 8.7c	7.2 ± 1.8def	1.3 ± 0.6cd	2.3 ± 0.5c
KS89H98, *H13*	100.0 ± 0.0b	0.0 ± 0.0g	0.0 ± 0.0d	-
Brule, *H18*	84.3 ± 5.1ab	13.3 ± 3.5cd	1.3 ± 0.6cd	2.8 ± 0.4abc
Newton, none	0.0 ± 0.0d	-	7.8 ± 2.5a	3.5 ± 0.3a

Means (± SD) within columns followed by the same letter are not significantly different (Fisher LSD test; $P > 0.05$ [SAS Institute 1988]).

[a] F test ($F = 27.3$; df = 11, 22; $P = 0.0001$).

[b] F test ($F = 33.52$; df = 10, 20; $P = 0.0001$).

[c] F test ($F = 10.12$; df = 11, 22; $P = 0.0001$).

The Hessian fly larvae that survived on resistant plants of Big Club 60 (*H*1*H*2 genes) and Arkan (*H*3 gene) significantly reduced leaf elongation compared to resistant plants that had only dead larvae (Table 4). Larvae that survived on resistant plants of Seneca (*H*7*H*8 genes) and Brule (*H*18 gene) did not significantly effect leaf elongation.

Discussion

Results of field and growth chamber tests showed that some resistance genes in wheat allow for high survival of Hessian fly larvae on resistant plants. That larvae surviving on resistant plants were avirulent genotypes indicates resistance genes, such as *H*1*H*2, *H*7*H*8, and *H*18, would reduce selection pressure for Hessian fly populations to develop virulent biotypes. Thus, cultivars carrying these genes should be more durable than cultivars carrying genes that confer high larval mortality. Avirulence alleles will be maintained at high frequencies in populations, because large numbers of larvae will survive on the resistant plants. This will dilute virulence alleles in populations and should slow down the evolution of virulent biotypes. Among the resistance genes tested, *H*1*H*2 probably would exert the least amount of selection, because of high larval survival on resistant plants. Also, because these larvae were similar in size to those on susceptible Newton plants, the adults of these larvae should be as competitive as those from Newton.

Wheat genes such as *H*3, *H*11, and *H*13 and other genes from wild or related species that condition high levels of larval mortality (Hatchett et al. 1981, Gill et al. 1987; Friebe et al. 1990, 1991) may result in high selection pressure on Hessian fly populations. If there is genetic variation in the Hessian fly population that confers increased survivalship on those cultivars, virulent biotypes may evolve. Cox & Hatchett (1986) proposed that when neither homozygous avirulent nor heterozygous avirulent larvae survive on resistant wheat plants, virulence alleles become fixed rapidly. Thus, knowledge of genetic parameters such as the survival of avirulent phenotypes on wheats carrying specific resistance genes is essential to predicting their durability.

Table 4. Effect on plant growth of Hessian fly larvae that survived on resistant plants of four wheat cultivars, growth chamber test.

Cultivar	Resistance gene	Mean LSD[a] leaf length (cm) of resistant plants with dead larvae	Mean LSD[a] leaf length (cm) of resistant plants with live larvae
Big Club 60,	*H1H2*	42.0 ± 4.8a	34.1 ± 4.3b
Arkan,	*H3*	42.8 ± 2.8a	33.3 ± 1.0b
Seneca,	*H7H8*	43.3 ± 5.6a	40.6 ± 3.4a
Brule,	*H18*	39.0 ± 4.2a	33.2 ± 7.7a

Means (± SD) within a row followed by the same letter are not significantly different ($P > 0.05$; LSMeans, SAS Institute 1985).

[a]F test ($F = 14.23$; df = 1, 14; $P = 0.0021$).

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Sequential Sampling Programs for Leafhoppers (Homoptera: Cicadellidae) on Wine Grapes in Washington[1]

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ABSTRACT Wine grape vineyards in south central Washington were sampled to collect data to develop sampling programs for leafhoppers. Most of the vineyards had a mixture of two species: the western grape leafhopper, *Erythroneura elegantula* Osborn, and the Virginia creeper leafhopper, *E. ziczac* Walsh. Both species had aggregated spatial distributions with *E. ziczac* the more aggregated of the two. Two sequential sampling programs based on Wald's sequential probability ratio test were developed for the two leafhopper generations for both species combined. The sampling programs were validated by simulation using the Monte Carlo method. The maximum number of samples, which was limited to 50 or 100 samples, had little effect on the operating characteristic curves. Reducing the maximum number of samples reduced the average number of samples but increased the percentage of terminal decisions. Terminal decisions, made after taking the maximum number of samples had unacceptable error levels, especially at means above the critical density. However, a terminal decision indicated that leafhopper numbers were near the critical density and sampling should be repeated in a few days. Alternatively, sampling history or other information could be used to help make an informed pest control decision.

KEY WORDS Homoptera, Cicadellidae, *Erythroneura elengantula*, *Erythroneura ziczac*, *Vitis vinifera*, sampling, Monte Carlo method

Wine grape, *Vitis vinifera* (L.), vineyards in south central Washington are often sprayed with insecticides to control two leafhopper pests: the western grape leafhopper, *Erythroneura elegantula* Osborn, and the Virginia creeper leafhopper, *E. ziczac* Walsh. *Erythroneura elegantula* is also a major pest of wine, raisin, and table grapes in parts of California (Wilson et al. 1992). *Erythroneura ziczac* occurs throughout the United States and southern Canada (Metcalf 1968) and is the major pest of wine grapes in British Columbia (McKenzie & Beirne 1972). *Erythroneura elegantula* has two or three generations per year in California (Wilson et al. 1992) and *E. ziczac* has two in

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British Columbia (McKenzie & Beirne 1972). In Washington, both leafhopper species appear to have two complete generations per year. The second generation, if unsprayed, is usually larger and hence more damaging than the first generation. A management guideline for California vineyards recommends treating 'Thompson Seedless' vineyards when *E. elegantula* numbers reach 15 to 20 nymphs per leaf or no more than 20% leaf loss, but Thompson Seedless vines are probably more resistant to leafhoppers than many other varieties (Wilson et al. 1992). In Washington, pest managers have found that treating the first generation at a mean leafhopper density of about five nymphs per leaf prevents the second generation from reaching damaging numbers. Although this tactic works well, sampling should continue for the second generation. The provisional threshold for controlling second-generation leafhoppers is about 15 nymphs per leaf. There is no evidence that the leafhopper species differ in the damage they cause. Therefore, pest managers treat the two species the same. Grape variety influences leafhopper abundance: varieties with less hairy leaves generally have more leafhoppers (McKenzie & Beirne 1972, Olsen 1995). Also, late-maturing varieties tend to have more leafhoppers than the early maturing varieties (Wilson et al. 1992). Adult leafhoppers fly well and take flight at the slightest disturbance, making counting adults on the leaves difficult. Therefore, leaf sampling is directed at the nymphs, which feed on the underside of leaves (McKenzie & Beirne 1972, Wilson et al. 1992). Adult leafhoppers can be monitored with yellow sticky boards (McKenzie & Beirne 1972, Wilson et al. 1992), by counting fifth-instar exuviae, which are the only ones that adhere to the leaves, and by suction (Olsen 1995). Leafhoppers are most abundant on the shade leaves (Wilson et al. 1992), which are found underneath the outer, sunlit parts of the grape canopy.

A sampling program is a method that is used to evaluate the number of pests in a field in relation to a critical density (action threshold, economic injury level). The sampling information helps pest managers make informed pest control decisions. Sequential sampling programs, such as Wald's sequential probability ratio test method, are especially well suited to agriculture because they generally require less effort than fixed sample size programs (Binns 1994). No sampling program has been developed for Washington grape leafhoppers. A monitoring program was developed for California vineyards (Wilson et al. 1992) but the precision of that program has apparently not been determined. Our objective was to develop sequential sampling programs for first- and second-generation leafhopper nymphs in wine grape vineyards.

Materials and Methods

Field sampling. Vineyards in south central Washington were sampled to obtain the data for developing the sampling plan. In 1991, three vineyards were sampled at about 2-d intervals starting on 21 July and ending on 20 August. Sampling continued in 1992 at 3-d intervals in three vineyards (two were the same as in 1991), starting 10 July and ending 31 August. In 1993, one vineyard was sampled starting on 16 June and ending 1 November. The sampling was done at 3-d intervals until the end of August and weekly thereafter. The grape varieties sampled were 'Riesling' in all years and 'Chardonnay' in

1991 and 1992. The vines sampled in small vineyards were adjacent to one another in a block. In larger vineyards there were two or three vines between sampled vines. A total of 110 field samples was collected. One shade leaf was sampled from each of 100 vines per vineyard. The leafhopper nymphs were identified to species, and the number on each leaf was recorded. The vineyards were separated from each other by at least 36 km. The 1991 to 1993 data were originally collected in a competition study and are used herein principally to determine if the distribution of leafhoppers within vineyards was consistent through time.

Sampling continued in 1997 to obtain data from a larger number of vineyards and varieties, to get more samples of first-generation nymphs, to select vines randomly in a pattern more suitable for field sampling, to record sampling time, and to determine the location of nymphs on grape shoots. Sampling started on 11 June 1997 and ended on 27 August 1997. A total of 31 vineyards was sampled. The vines within a vineyard were randomly selected while walking through the vineyard in a diamond-shaped pattern. Leafhopper nymphs were counted on main stem leaves 1, 3, 5, 7, 9, and 11 (beginning at the base of the shoot) on 25 shoots per vineyard on separate vines to determine the distribution of nymphs on the shoots. The number of leaf nodes on each shoot also was counted. In addition, 75 vines were sampled in each of the vineyards by selecting from each vine one leaf that grew near the base of a shoot (shade leaf). The sampling time was determined in 16 vineyards by recording the time spent taking the 75 leaf samples. The 75 leaf samples were combined with one leaf from each vine sampled near the base of each of the 25 shoot samples for a total of 100 individual leaf samples per vineyard. These data were used to determine the leafhopper variation among vines and develop the sampling program. The number of leafhoppers of each species was recorded separately in 29 of the 31 vineyards sampled. Varieties represented were Chardonnay, 'Sauvignon blanc', 'Pinot noir', 'Semillon', 'Muscat Canelli', 'Cabernet Sauvignon', Riesling, 'Merlot', and 'Chenin blanc'. Vineyards ranged in size from 0.53 to 23.59 ha, averaging 4.10 ha. Most of the vineyards were in the Lower Yakima Valley of Washington but several were in the Paterson (Columbia River) area.

The mean location of nymphs on the shoot nodes was calculated as a measure of the within-shoot distribution of the nymphs. Twenty-six vineyards were used in this analysis because five of the vineyards had leafhopper numbers that were too low to use for the within-shoot distribution data.

Sampling program. Taylor's power law (Taylor 1961) was computed to determine if the dispersion of the nymphs among vines was consistent through time and place and to test if the two species differed from each other in their dispersion patterns. The power law parameters were determined by linear regression of $\ln(v)$ on $\ln(m)$, where v is the variance and m is the mean of each vineyard sampled. The difference between the slopes of the lines was tested by a homogeneity-of-slopes model. If no significant difference was found, an analysis-of-covariance model was used to test if the lines were parallel but had different intercepts. The computations were done using the general linear model (GLM) procedure of SAS (SAS Institute 1988).

A sequential sampling program was developed using Wald's sequential probability ratio test method (Binns 1994). To construct the sequential sampling

program, a common k (k_c) (Davis 1994) was calculated from the 1997 data in which the species were not separated. Only the 1997 data were used for the sampling program because the pattern of the 1997 sampling and the diversity of the vineyards was considered to be better for field sampling than the earlier method. The fit of the k_c was tested by regression of $1/k$ on $\ln(m)$: a slope not different from zero indicates that k_c adequately describes the data. The critical density for first-generation leafhoppers was set at five nymphs per leaf. Therefore, the lower mean, m_0 (mean density at which no control is indicated), was set at four nymphs per leaf and the upper mean, m_1 (control indicated), of the sampling program was set at six nymphs per leaf. The other two parameters of the sampling program, α and β, were each set at 0.1. The provisional threshold for second-generation leafhoppers was set at 15 nymphs per leaf with $m_0 = 12$, $m_1 = 18$, and $\alpha = \beta = 0.1$. The lower decision line values were rounded down to the nearest whole number and the upper line values were rounded up.

Sampling program validation. The performance of the sampling programs was evaluated by a computer simulation method suggested by Nyrop & Binns (1991). The method used Taylor's power law statistics to calculate a stochastic v from which k was computed. The Monte Carlo method (Rubinstein 1981) was used to generate data sets that had distributions of values similar to 1997 field counts (LCW, unpublished data). The computer program generated 10,000 numbers for each data set and 500 data sets were generated at each mean density and sampled with the sampling plans. Mean densities of the species combined ranged from 2.0 to 9.0 to simulate first-generation sampling and from 8 to 22 for the second generation. The maximum number of samples was limited to 50 or 100 samples. If the maximum number of samples had been taken and no sequential decision had been made, a terminal decision was made by comparing the mean of the samples to the critical density. The simulations allowed us to determine the average sample number (ASN) required to reach a decision (sequential and terminal) over a range of population means (μ). The error in the sampling program was determined by calculating the operating characteristic (OC), which is the proportion of no-action decisions over a range of means (Binns 1994).

Results and Discussion

Species composition. Most of the vineyards contained a mixture of the two leafhopper species. In 1991, 1992, and 1993, *E. elegantula* was found in all 110 field samples and *E. ziczac* was found in 91. In 1997, 16 vineyards had both species, 14 had only *E. elegantula*, and one had *E. ziczac* alone. Wolfe (1955) found *E. ziczac* in a vineyard in the Yakima Valley but Wells & Cone (1989) found *E. ziczac* only in the areas surrounding the valley. We found both species in every Yakima Valley vineyard sampled in 1991 through 1993. In 1997, we found *E. ziczac* in 12 of the 21 field samples taken in the Yakima Valley and it was always found with *E. elegantula*. *Erythroneura ziczac* appears to outcompete *E. elegantula* (Wells & Cone 1989, Olsen 1995), but *E. elegantula* was more numerous than *E. ziczac* in all but two vineyards in 1997. Comparing our results with those of Wells & Cone (1989) suggests that *E. ziczac* may be increasing in importance as a pest of wine grapes in this region, but the evidence is not conclusive.

Leafhopper distribution. The leafhoppers were found near the bases of the shoots throughout the season but moved distally along the shoots as the season progressed. The average location of nymphs was near the third node from the shoot base in June and was around the fifth node in August (Fig. 1). The average location was biased downward late in the season because we sampled only to the 11th node and some vineyards, especially ones with high leafhopper numbers, had moderate numbers on the 11th node, indicating that more distal nodes also were infested. The leaves at the nodes near the shoot bases were on the inside of the foliage canopy and were usually in the shade, indicating that the leafhoppers prefer the shady areas and that the shade leaves should be sampled. Leafhopper developmental rates decline above 33°C (Olsen et al. 1998). Summer daytime air temperatures in central Washington often exceed this level and the temperatures of surfaces exposed to the sun would be even higher. Leafhoppers probably prefer the shade leaves because the cooler temperatures in the interior of the foliage canopy would be a better environment for development than the warmer, sunlit leaves.

Both leafhopper species had aggregated or clumped spatial distributions, with *E. ziczac* the more aggregated of the two species. The regression line statistics for *E. ziczac* in 1991 to 1993 were as follows: $\ln(a) = 2.076$, $b = 1.577$, $r^2 = 0.972$, $n = 91$; and in 1997: $\ln(a) = 2.363$, $b = 1.549$, $r^2 = 0.946$, $n = 15$. No significant difference was found between the slopes($F = 0.09$; df = 1; 102; $P = 0.763$) and the difference in the line intercepts was marginal ($t = 1.96$, df = 103, $P = 0.053$). The regression line statistics for *E. elegantula* in 1991 to 1993 were as follows: $\ln(a) = 1.021$, $b = 1.401$, $r^2 = 0.973$, $n = 110$; and in 1997: $\ln(a) = 1.086$, $b = 1.455$, $r^2 = 0.972$, $n = 28$. The two *E. elegantula* regression lines were not significantly different (slopes: $F = 0.98$; df = 1, 134; $P = 0.323$; intercepts: $t = 0.98$: df = 135, $P = 0.329$). The regression lines of the two species had significantly different slopes in the 1991 to 1993 data ($F = 23.82$; df = 1, 197; $P < 0.0001$) but not in 1997 ($F = 0.90$; df = 1, 39; $P = 0.348$). However, in 1997 the intercept of the *E. ziczac* line was larger than the *E. elegantula* intercept ($t = 7.75$, df = 40, $P < 0.0001$). All of the Taylor's power law regression lines had slopes greater than 1.0 ($P < 0.001$) indicating that both leafhoppers had aggregated distributions (Davis 1994). Although we sampled first- and second-generation nymphs (the second generation appeared in mid-July), the good fits of the regression lines indicate that the functional relationships of variances to means were consistent through time. The more aggregated distribution of *E. ziczac* may be partially explained by its habit of laying eggs singly and in clusters, whereas *E. elegantula* lays its eggs only singly (Wells & Cone 1989). When the number of both species in each sample was combined (species ignored) in 1997, the line was $\ln(a) = 1.195$, $b = 1.551$, $r^2 = 0.974$, $n = 31$. Because the damage caused by the two leafhoppers is considered the same and separating the two species (especially the small nymphs) in the field is difficult and time-consuming, we developed the sampling program based on the two species considered as one.

The common k, k_c, for both species combined in 1997 was 0.907. The slope of the test of the fit of k_c was not significantly different from 0 ($t = -0.1367$, df = 29, $P > 0.50$) indicating that the common k is relatively independent of the mean and can be used to develop a sampling program (Davis 1994). The k_c was >0, indicating that the data fit a negative binomial distribution. Therefore, a

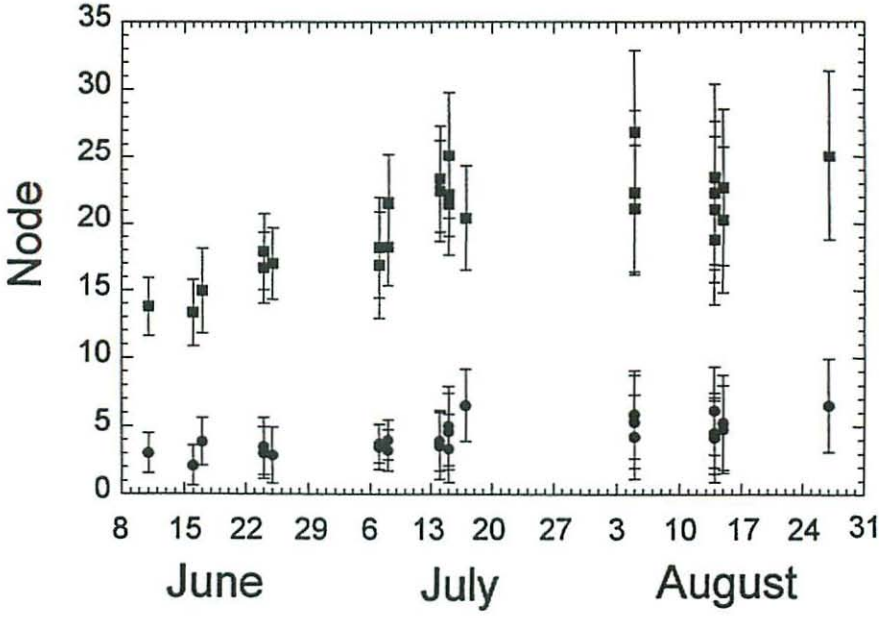

Fig. 1. Mean position of grape leafhopper nymphs on grape shoots (circles) and mean number of nodes per shoot (squares) through the 1997 season. Nodes were numbered starting at the basal node of the shoots. Error bars represent standard deviations.

sequential probability ratio test sampling plan was developed using the negative binomial distribution (Binns 1994).

Sampling plan and validation. Table 1 presents the data for the sequential sampling programs. The equation for the first-generation lower decision line was $-34.55 + 4.876n$ and $34.55 + 4.876n$ for the upper line, where n is the sample size. For the second-generation sampling plan the lower line was $-92.7 + 14.61n$ and the upper line was $92.7 + 14.61n$.

The ASN for the sampling program is presented in Fig. 2. Fewer samples were taken when the number of samples was restricted to 50 than when a maximum of 100 samples was taken. However, the maximum number of samples had little effect at means in the tails of the curves.

The mean sampling time in 1997 was 34.8 s per sample (SEM = 0.026 min, n = 16). Sampling time was recorded from the first leaf sample to the last; no travel time was included. For the first-generation plan and an ASN of 25 sequential samples at $\mu = 4$ and 50 samples maximum, about 14.5 min would be required. Sampling time increased with the number of leafhoppers, the size of the vineyards, and the difficulty of walking through the vineyards.

Reducing the maximum number of samples from 100 to 50 resulted in a large increase in the number of terminal decisions (Fig. 3). Considering all means generated from 2 to 9, 12.0% of the 50 sample maximum and 2.4% of the 100 maximum were terminal decisions in the first-generation sampling plan.

The maximum number of samples had little effect on the sequential decision OC curves except that the 100-sample maximum had a slightly better OC curve than the 50-sample maximum in the second-generation program (Fig. 4). The terminal decisions were wrong more often than the sequential decisions, usually at means above the critical densities (Fig. 4). Below the critical density, the 50-sample terminal decisions had about the same error as the sequential decisions and the 100-sample maximum decisions had less error. Therefore, the wrong terminal decisions usually indicated no need to apply a control when a control was needed. This type of error would lead to crop damage. Over all means sampled with the first-generation plan, the percentage of wrong terminal decisions was about the same regardless of the maximum number of samples: 50-sample maximum, 37.3% were wrong; 100-sample maximum, 36.7% were wrong. In contrast, 11% of the first-generation sequential decisions was wrong in the 50-sample maximum trials and 13.1% in the 100-sample maximums. In the second-generation sampling plan (means 8 to 22), 34.0% of the terminal decisions was wrong in the 50-sample maximums and 36.6% was wrong in the 100-sample maximums. Of the 50- and 100-maximum sample second-generation sequential decisions, 9.4% and 9.5% were wrong, respectively. Generally, the terminal decisions had about three times more error than the sequential decisions. Therefore, the terminal decisions had too much error to be reliable. However, concluding the sampling without reaching a sequential decision alerts the vineyard manager that the leafhopper mean density is close to the critical density. Sampling should be repeated within a few days or 1 wk. Or a decision to treat could be made based on the population trend established by previous sampling, weather, time of the season, economic conditions, horticultural practices, or experience with the particular vineyard or variety.

Leafhopper management is aimed at controlling first-generation nymphs with the intention of keeping the larger, more damaging second generation from growing to injurious levels. If vineyards are sampled regularly and controls are effective, no controls should be needed for the second generation. However, unexpected events can occur, so sampling should be continued for the second generation.

Although a separate sampling program could be developed for each species from the data we have collected, any gain in efficiency would probably be offset by the extra time spent identifying the leafhoppers. In addition, no difference in the damage caused by the two species is known. Therefore, at this point in the development of the pest management of leafhoppers on grapes, it seems that one sampling program is adequate. The parameters of the sampling program can be changed to adjust the critical density and the ASN and OC curves.

Table 1. Sequential sampling programs for first- and second-generation *E. elegantula* and *E. ziczac* on wine grapes.

	Cumulative number of leafhoppers			
	First-generation		Second-generation	
Sample number	Lower limit	Upper limit	Lower limit	Upper limit
1	-	40	-	108
2	-	45	-	122
3	-	50	-	137
4	-	55	-	152
5	-	59	-	166
6	-	64	-	181
7	-	69	9	195
8	4	74	24	210
9	9	79	38	225
10	14	84	53	239
11	19	89	68	254
12	23	94	82	269
13	28	98	97	283
14	33	103	111	298
15	38	108	126	312
16	43	113	141	327
17	48	118	155	342
18	53	123	170	356
19	58	128	184	371
20	62	133	199	385
21	67	137	214	400
22	72	142	228	415
23	77	147	243	429
24	82	152	257	444
25	87	157	272	458
26	92	162	287	473
27	97	167	301	488
28	101	172	316	502
29	106	176	330	517
30	111	181	345	532
31	116	186	360	546
32	121	191	374	561
33	126	196	389	575
34	131	201	404	590
35	136	206	418	605
36	140	211	433	619
37	145	215	447	634
38	150	220	462	648
39	155	225	477	663
40	160	230	491	678
41	165	235	506	692
42	170	240	520	707
43	175	245	535	721
44	180	250	550	736
45	184	254	564	751
46	189	259	579	765
47	194	264	593	780

48	199	269	608	794
49	204	274	623	809
50	209	279	637	824
51	214	284	652	838
52	219	289	667	853
53	223	293	681	868
54	228	298	696	882
55	233	303	710	897
56	238	308	725	911
57	243	313	740	926
58	248	318	754	941
59	253	323	769	955
60	258	328	783	970
61	262	332	798	984
62	267	347	813	999
63	272	342	827	1,014
64	277	347	842	1,028
65	282	352	856	1,043
66	287	357	871	1,057
67	292	362	886	1,072
68	297	367	900	1,087
69	301	371	915	1,101
70	306	376	930	1,116
71	311	381	944	1,131
72	316	386	959	1,145
73	321	391	973	1,160
74	326	396	988	1,174
75	331	401	1,003	1,189
76	336	406	1,017	1,204
77	340	411	1,032	1,218
78	345	415	1,046	1,233
79	350	420	1,061	1,247
80	355	425	1,076	1,262
81	360	430	1,090	1,277
82	365	435	1,105	1,291
83	370	440	1,119	1,306
84	375	445	1,134	1,320
85	379	450	1,149	1,335
86	384	454	1,163	1,350
87	389	459	1,178	1,364
88	394	464	1,192	1,379
89	399	469	1,207	1,393
90	404	474	1,222	1,408
91	409	479	1,236	1,423
92	414	484	1,251	1,437
93	418	488	1,266	1,452
94	423	493	1,280	1,467
95	428	498	1,295	1,481
96	433	503	1,309	1,496
97	438	508	1,324	1,510
98	443	513	1,339	1,525
99	448	518	1,353	1,540
100	453	523	1,368	1,554

The critical density is five nymphs per leaf for the first generation and 15 for the second generation. Sampling continues until the cumulative number of leafhopper nymphs sampled is less than the lower limit, indicating no treatment is needed, or greater than the upper limit, indicating a treatment is required.

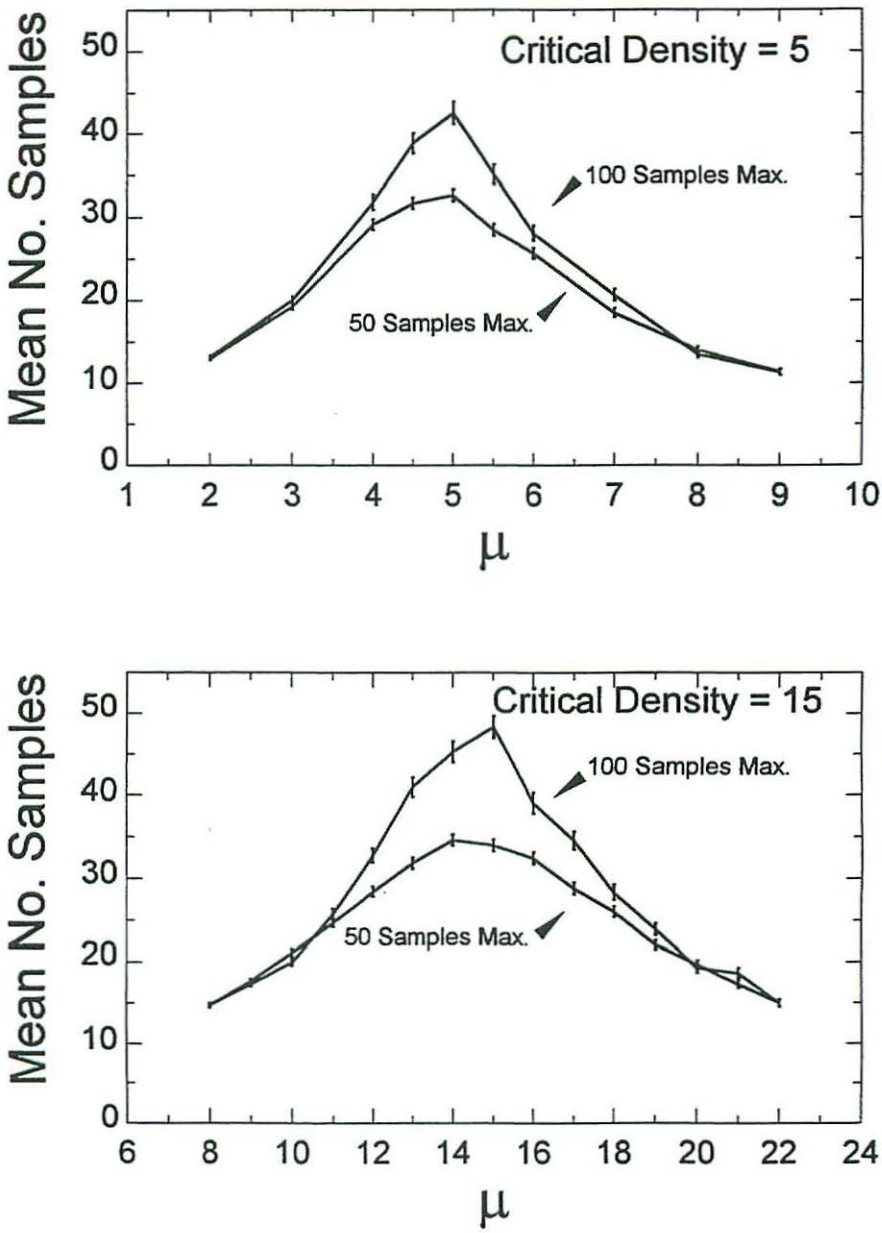

Fig. 2. Average sample number for first- (critical density = five nymphs per leaf) and second-generation (critical density = 15 nymphs per leaf) sequential sampling programs for leafhoppers on wine grapes. The population mean is indicated by the μ symbol. The maximum number of samples was limited to 50 or 100. Error bars represent standard error of the means.

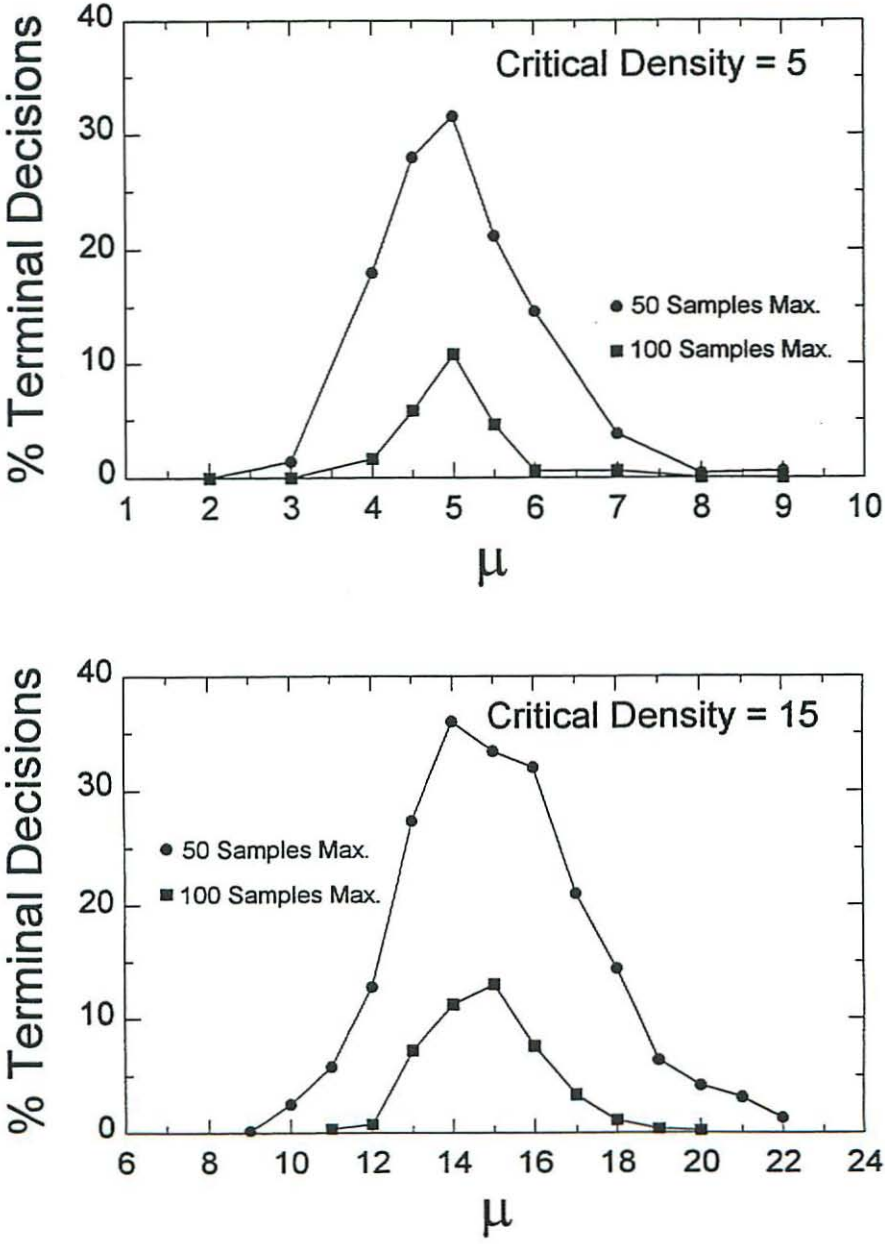

Fig. 3. Percentage of sampling occasions that reached the maximum of 50 or 100 samples at which point a terminal decision was made. The first-generation sampling plan had a critical density of five nymphs per leaf and the critical density for the second-generation plan was 15. The population mean is indicated by the μ symbol.

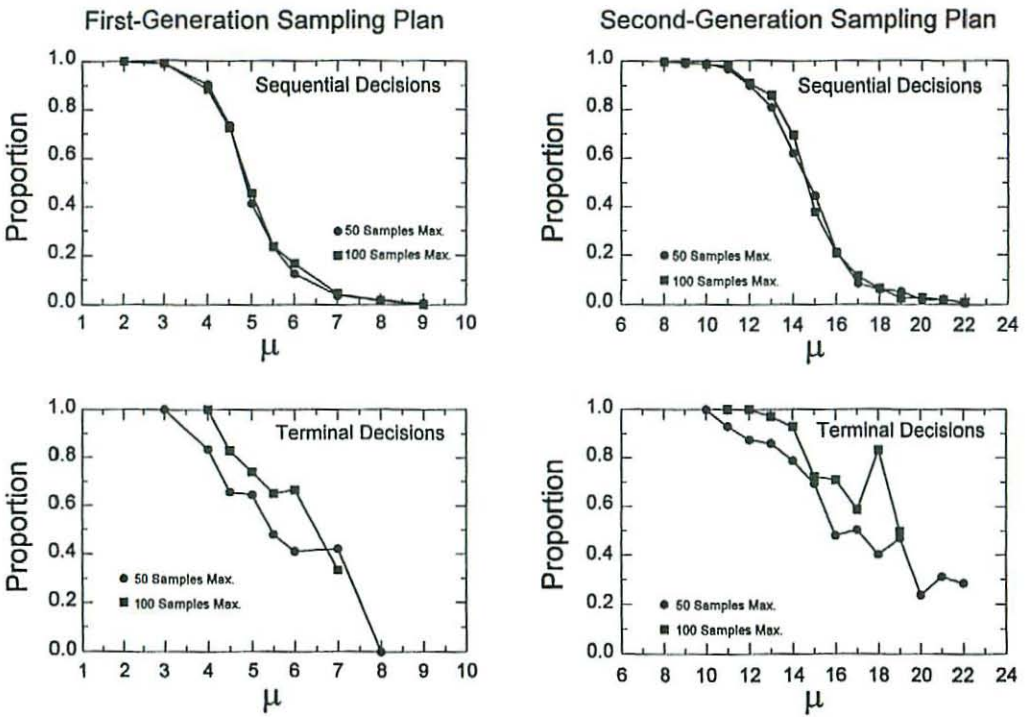

Fig. 4. Operating characteristic (the proportion of decisions indicating that no control application was necessary) curves for sequential and terminal decisions of the first- and second-generation leafhopper sequential sampling plans. The maximum number of samples was limited to 50 or 100. The population mean is indicated by the μ symbol.

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Host Choice and Offspring Sex Allocation in the Aphid Parasitoid *Aphelinus abdominalis* (Hymenoptera: Aphelinidae)[1]

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ABSTRACT The influence of host size on proportion of male and female offspring of the polyphagous aphid parasitoid *Aphelinus abdominalis* (Dalman) was investigated. The proportion of males decreased with increasing mummy weight. This was due to preferences of mated females which, given a choice, tended to allocate male eggs to small hosts and female eggs to large hosts. Male larvae also had a tendency to kill the host at an earlier developmental stage than female larvae of the parasitoid. Size-dependent sex allocation was similar in four host aphids: potato aphid, *Macrosiphum euphorbiae* (Thomas); *Metopolophium dirhodum* (Walker); English grain aphid, *Sitobion avenae* (F.); and bird cherry-oat aphid, *Rhopalosiphum padi* (L.). The pattern of sex allocation varied among the different-sized aphid species, but regardless of their size, parasitoid females preferred to lay male eggs in smaller hosts and female eggs in larger hosts, and host size corresponding to a 1:1 sex ratio was near the median size (except for small *R. padi*). Females constrained life-long to hosts of small size increased their sex ratio during the period of oviposition. Virgin females produced only male eggs and altered their host size preferences during the course of the oviposition period. Initially, they chose small hosts, but gradually allocated more eggs into large hosts.

KEY WORDS *Aphelinus abdominalis, Macrosiphum euphorbiae,* Hymenoptera, Aphelinidae, Homoptera, Aphidae, cereal aphids, parasitoid, body size, sex ratio, development period, biocontrol

Females of several hymenopteran species in the suborder Apocrita can determine the sex of an offspring at oviposition. That the sex ratio in some species varies with host size, being usually male biased in small hosts and female biased in the large ones, has been known since the early 1900s (reviewed by Godfray 1994). Females can discriminate between small and large hosts and preferentially lay male (haploid, unfertilized) eggs into small hosts and female (diploid, fertilized) eggs into large hosts (Nguyen & Sailer 1987, Heinz &

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Parrella 1990, Tillman & Cate 1993). This behavior may have adaptive significance as female fecundity is directly proportional to body size (Honěk 1993), and decreasing body size negatively affects female fitness. In nature, males are smaller than females and their fitness may be less affected by variation in body size. Hence, depositing male eggs into small hosts and female ones into large hosts may allow optimal exploitation of available hosts (Charnov et al. 1981). Primary sex ratio determined by female choice may be further modified by differential mortality of the sexes (secondary sex ratio). Mortality should be checked when interpreting parasitoid sex ratio data where pre-adult survival and female decision at oviposition may be confounded.

Aphelinus species (Hymenoptera: Aphelinidae) are aphid parasitoids that lay eggs into host larvae and adults. Compared with aphidiid parasitoids of aphids, the whole pre-adult development of *Aphelinus* is short (Lajeunesse & Johnson 1992, Bernal & Gonzalez 1993), but the pupal stage is relatively long (Trimble et al. 1990). In the final instar the larva pupates within the exoskeleton of the dead host aphid (i.e., the mummy), and the mass of the pupa is close to the size of the mummy. Asante & Danthanarayana (1993) demonstrated that *Aphelinus* species require the presence of large hosts (aphids) to ensure optimum sex ratio. *Aphelinus abdominalis* (Dalman) is an important component of the parasitoid complex of cereal aphids in the field (Dean et al. 1981) and a candidate for aphid biocontrol in greenhouse crops (Schelt 1994, Jarošík et al. 1996). In many parasitoid species, maintenance of optimum sex ratio (in the sense of equal investment to male and female progeny) may be of crucial importance to mating success (Hardy 1994) and optimum population growth. In addition, host size influences parasitoid sexual dimorphism (Salvo & Valladares 1995, Mackauer 1996), adult fecundity, and body size (Sequeira & Mackauer 1992, 1994; Mackauer & Sequeira 1993), which are influenced by host-size-dependent variation in larval developmental rate. Thus, we initiated a study of the effects of host size on the biology of *A. abdominalis*. We also investigated the relationship between mummy size and sex ratio, the factors that affect female preferences for small or large hosts during oviposition, and the differences in developmental periods of males and females.

Materials and Methods

Insects. Laboratory colonies of *A. abdominalis* were reared on the potato aphid, *Macrosiphum euphorbiae* (Thomas), and on three species of cereal aphids: *Metopolophium dirhodum* (Walker); English grain aphid, *Sitobion avenae* (F.); and bird cherry-oat aphid, *Rhopalosiphum padi* (L.). Colonies were maintained at Laboratoire de Biologie des Invertébrés in Antibes, France. The parasitoid colony was held at a photoperiod of 16:8 (L:D) h and 23–25°C (maintained on *M. euphorbiae*). Cereal aphids were reared in nylon-covered 30 by 30 by 45 cm cages on winter wheat (*Triticum aestivum* L.) seedlings and *M. euphorbiae* in 50 by 50 by 90 cm cages was reared on young eggplant (*Solanum melongena* L.). To maintain aphid colonies, young potted host plants were introduced into the cages, and cut shoots of old host plants infested with aphid populations of a single species were added. The aphids dispersed to young plants where, due to constant survivorship and fecundity in a stable environment

(Mertz 1970), dense populations with stable age structure established within 4 or 5 d. In parasitoid cultures, aphid populations were then exposed to 300–400 *A. abdominalis* composed of 60% females.

Sex ratio dependence on mummy size. Single-species populations of *S. avenae, M. dirhodum, R. padi,* and *M. euphorbiae* were established and exposed to the parasitoids as described above. Our balances were unable to weigh individuals for establishing the regressions of sex ratio and mummy mortality on mummy weight. Instead, groups of the smallest, largest, and medium-sized individuals were selected visually from a large sample of mummies. The size of groups depended on the total number of mummies available and their size variation. This procedure resulted in establishing groups of mummies of similar size. Earlier tests of the accuracy of this method by using eggs of *Pyrrhocoris apterus* L. (Honěk 1987) revealed that the differences between the smallest and largest individual of a group was 1/10 of the diameter of the former (Honěk, unpublished data). Groups of 15 *R. padi* or 20 individuals for the other hosts were weighed and their mean weight calculated. The mummies were separated in gelatine capsules, kept at 25°C, and the sex of parasitoids was determined at emergence. Mummy mortality was assessed by counting dead mummies.

Multi-choice experiments with female preferences. Multi-choice, dual-choice, and no-choice experiments with female preference for depositing male and female eggs into aphids of a particular size were conducted at a photoperiod of 18:6 (L:D) h and 26.3°C by using *M. euphorbiae* as the host. In multi-choice experiments, nymphs of different instars and adults of *M. euphorbiae* were exposed to the parasitoid as described previously. Parasitoids were left exposed to the same aphids for 3-d intervals, and nymphs and adults were sampled each day from the beginning of *A. abdominalis* parasitism. The daily samples were sorted by instar and placed on eggplant leaf discs floated on a thin layer of water just covering sand in 8-cm-diameter Plexiglas petri dishes covered with nylon fabric for ventilation. The aphids were kept on leaf discs until mummification, at which time they were placed individually in glass tubes for adult emergence. The sex of the adults was determined.

Dual-choice experiments with female preferences. Parasitoid females were exposed to mixtures of small (first instar) and large (fourth instar) hosts. The parasitoids were reared from mummies kept separately in small glass tubes. The experiments started with females <48 h old, without any previous experience with aphids. Thirty first and 30 fourth instars were placed on eggplant leaves in petri dishes, and each replicate with the mixture of 60 nymphs was exposed to a virgin *A. abdominalis* female (12 replicates) or a male-female pair (18 replicates) established at the start of the experiment. The parasitoids were removed after 24 h. Small and large aphids in each replicate were separated and placed on floating eggplant leaf discs for mummification. The sex of the parasitoids was determined after adult emergence.

No-choice experiments with female preferences. Parasitoid females were provided either small first-instar or large fourth-instar hosts. The aphids on eggplant leaf discs were exposed to male-female pairs of *A. abdominalis* reared as in the dual-choice experiments. Pairs were provided with 40–50 first instars daily (six replicates), or with the same number of fourth instars (six replicates). After 24 h the parasitoids were transferred into new petri dishes

with fresh aphids. Dead males were replaced, and transfers were continued until the female died. Parasitized aphids were held on leaf discs until mummification. The mummies were then stored and the sex of adults was determined upon emergence.

Developmental period. Twelve groups of 30 newborn first instars of *M. euphorbiae* were exposed for 5 h to five mated *A. abdominalis* females. The aphids were then placed on floating eggplant leaf discs at 25°C. After mummification, the instar of the aphid when killed was determined using the length of antennae and the length of siphunculi. The identification was made by A. M. Geria from Laboratoire de Biologie des Invertébrés in Antibes. The antennae of mummified aphids are 2/5 longer in the fourth than in the third instar, and the ratio of the (4th + 5th):6th antennal segment is 1:1.12 and 1:1.45 in the third and fourth instar, respectively. The siphunculi are 400 µm long in third and 500 µm long in fourth instars. Single mummies were placed into glass tubes and the sex of the parasitoid was determined at emergence.

Statistical analysis. Data were analyzed with logit proportions as a response variable. The logits are the natural logarithms of the odds ratio [i.e., ln(p/1-p)] where p is proportion. The data were weighted for sample size, and it was assumed that the distribution of the errors in the response variable followed a binomial distribution (Cox 1970).

Calculations were made using general linear modeling (Francis et al. 1994). The aim of each analysis was to determine the minimal adequate model. In this model, all parameters were significantly ($P < 0.05$) different from zero and from one another. This was achieved by a stepwise process of model simplification, beginning with the maximal model (containing all factors, interactions, and covariates that might be of interest), then proceeding by the elimination of nonsignificant terms (by using deletion tests from the maximal model), and the retention of significant terms (Crawley 1993).

After the minimal adequate model was fitted, the data were checked for overdispersion, and the logits were transformed to proportions. McCullagh & Nelder's (1989) treatment of overdispersion was followed; that is, for overdispersion, the data were rescaled using a dispersion factor that was calculated by dividing Pearson's χ^2 by the residual degrees of freedom.

Sex ratio dependence on mummy size. Sex ratio and mummy mortality in relation to mummy size were examined by analysis of covariance (ANCOVA). In the maximal model, logit proportion of males or logit proportion of mummy deaths was the response variable, whereas mummy weight was a covariate, and aphid species was a factor.

The modeling of logit proportions started by fitting the maximal model where each species was regressed on mummy weight with a different intercept and a different slope. The parameters of the maximal model were inspected, and the least significant term was removed in a deletion test. If the deletion caused a nonsignificant increase in deviance, the term was left out of the model. The inspection of parameter estimates was repeated, and the least significant term remaining in the model was removed in the following deletion test. If the deletion caused a significant increase in deviance, the term was put back into the model. The deletion tests were repeated until the minimal adequate model that contained nothing but significant terms was determined.

To test if the response variable changed linearly with increasing mummy weight the squares of the covariate were added to the models. If the addition caused a significant reduction in deviance, there was evidence of nonlinearity.

Multi-choice experiments with female preferences. The data were tested using a two-way analysis of variance (ANOVA) without replication. In the maximal model, logit proportion of males or logit proportion of mummies formed was the response variable, and aphid developmental stage and exposure time (d) from the start of parasitization were factors.

Dual-choice experiments with female preferences. Sex allocation by mated females was analyzed by one-way ANOVA by using logit proportion of males as response variable and aphid host instar as a factor. The influence of mating status on host size preferences was analyzed by one-way ANOVA separately for mated and virgin females. In these analyses, logit mummies formed was the response variable, and aphid instar was a factor.

No-choice experiments with female preferences. The no-choice experiments were investigated by ANCOVA by using logit proportion of males as response variable, maternal age in days as covariate, and aphid developmental stage as a factor. In the maximal model, logits were regressed on maternal age with a different intercept and a different slope for each stage. The model was simplified by deletion tests as described previously.

Developmental period. To test for difference in the aphid instar at mummification the data were evaluated by one-way ANOVA with logit proportion of males as response variable, and the instar when mummies formed as a factor.

Results

Sex ratio dependence on mummy size. In all host species, the sex ratio of *A. abdominalis* was related to mummy size ($F_{\text{slopes}} = 129.57$; df = 2, 98; $P < 0.01$). Female allocation of male progeny decreased linearly ($F_{\text{residuals in test for nonlinearity}} = 2.93$; df = 2, 96; NS) with increasing mummy weight (Fig. 1). The decrease did not differ significantly ($F_{\text{residuals for differences in slopes}} = 0.74$(df = 2, 98; NS) within the smaller host species, *R. padi* and *S. avenae,* and within the larger host species, *M. dirhodum* and *R. euphorbiae*. *Rhopalosiphum padi* produced the smallest mummies, and the proportion of females was also lowest and did not approach 100% (Fig. 1). The decrease was significantly steeper in *R. padi* and *S. avenae* than in *M. dirhodum* and *M. euphorbiae* (deletion test for differences in $F_{\text{slopes}} = 43.56$; df = 1, 99; $P < 0.01$). *Metopolophium dirhodum* and *M. euphorbiae* were not only larger on average but also more variable in size. Mean mummy size corresponding to a 1:1 sex ratio varied among hosts: 0.16 mg in *R. padi* and *S. avenae* compared with 0.26 mg in *M. dirhodum* and *M. euphorbiae*.

In *R. padi*, *M. dirhodum*, and *M. euphorbiae*, mortality did not vary with mummy weight ($F_{\text{residuals for slopes}} = 1.11$; df = 3, 96; NS). However, mortality in *S. avenae* significantly decreased with mummy weight ($F_{\text{slope}} = 6.36$; df = 1, 97; $P < 0.05$), and the minimal adequate model predicted a decrease in mortality from 23.4 to 8.7% with an increase in mummy weight from 0.1 to 0.2 mg. The mortalities in *R. padi*, *M. dirhodum*, and *M. euphorbiae* differed significantly between species ($P < 0.05$ in deletion tests), and declined from the species with

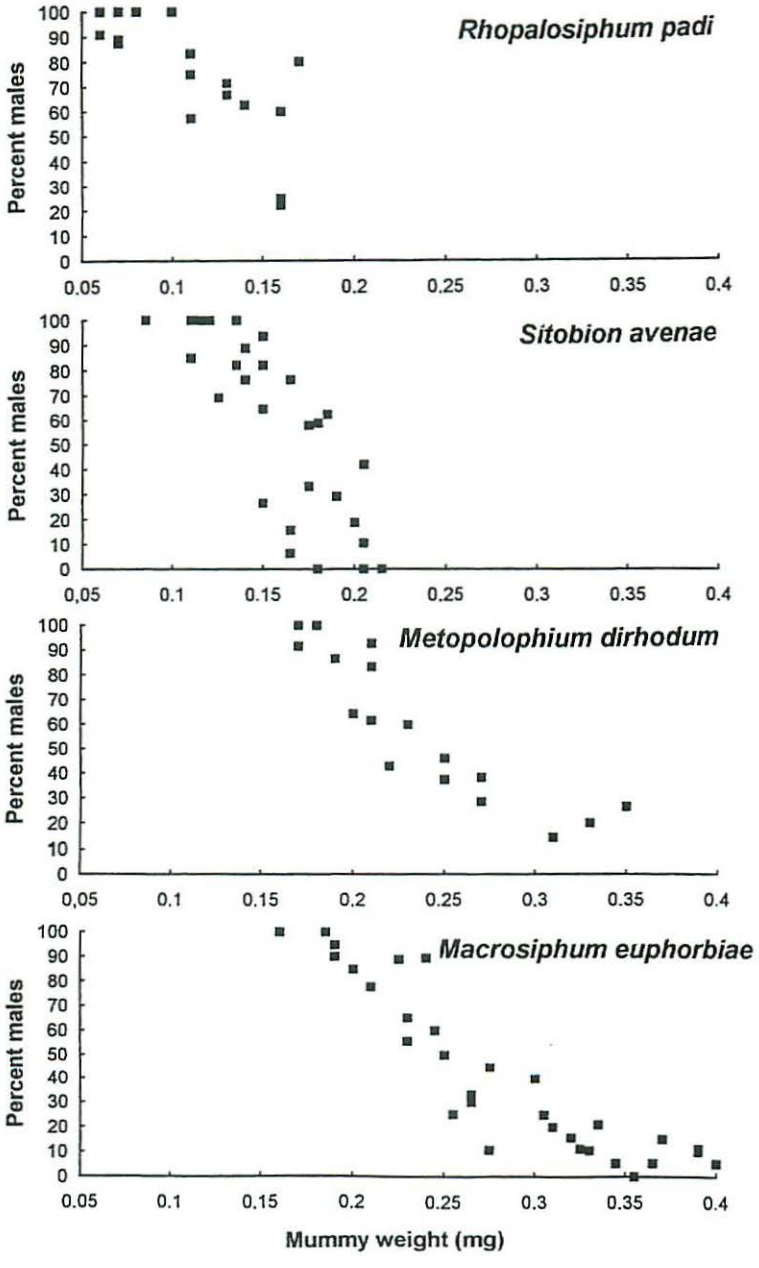

Fig. 1. Sex ratio of *A. abdominalis* in relation to mummy weight. For *R. padi* and *S. avenae*, the minimal adequate model is ln (p/1-p) = 6.09 (± 0.53) - 37.03 (±3.37) mummy weight, and for *M. dirhodum* and *M. euphorbiae* ln (p/1-p) = 6.09 (± 0.53) - 23.55 (± 2.01) mummy weight. R^2 = 72.6%, df = 2, 96.

the lightest mummies, *R. padi* (45.5%, SE 41.8–49.2), to *M. dirhodum* (15.0%, SE 12.0–18.7), and *M. euphorbiae* (3.9%, SE 2.9–4.9), which produced the heaviest mummies (Fig. 1).

Multi-choice experiments with female preferences. In the multi-choice experiments, a large number of *M. euphorbiae* was exposed for 3 d to a population of adult parasitoids. A similar proportion of parasitization appeared among second through fourth instars and adults. This proportion was significantly higher than for the first instars (Table 1). Moreover, the proportion of parasitization increased significantly each day of exposure in all stages, including the first instar.

Table 1. Multi-choice experiment: proportion of *M. euphorbiae* mummies (mean ± SE, %) formed by *A. abdominalis* in different host stages after different times of exposure to parasitism (d).

Stage	Day 1		Day 2		Day 3	
	n	Mean (SE[a])	*n*	Mean (SE[a])	*n*	Mean (SE[a])
N1	85	1.5 (0.9–2.4)	103	6.1 (4.2–8.7)	80	16.9 (12.2–22.8)
N2-N4, adults	358	7.2 (4.9–10.5)	315	25.3 (18.7–33.4)	317	51.6 (42.2–60.8)

The table shows all statistically significant ($P < 0.05$) differences between developmental stages or their groups as indicated by minimal adequate model in two-way ANOVA without replication. The pooled developmental stages did not differ significantly in deletion tests. n = total of investigated aphids.

[a]Standard errors are asymmetrical due to transformations of logits to proportions.

There was a tendency to allocate male eggs too small and female eggs into large aphids (Table 2). Males prevailed in small first and second instars, and females in large fourth instars. Only female progeny were produced in adult aphids. During the first 2 d of oviposition, first-instar aphids produced only males, but on the third day some females were produced in first instars. Across all larval instars, the proportion of emerging males significantly increased between 1–2-d and 3-d exposure to the parasitoids.

Dual-choice experiments with female preferences. In the dual-choice experiments, a mixture of small (first instar) and large (fourth instar) aphids was offered to either mated or unmated *A. abdominalis* females. As expected, all progeny from unmated females produced males. In mated females, similar proportions of both sexes ($F_{residuals} = 0.05$; df =1, 22; NS) emerged from small aphids (44.5% males, SE 40.1–49.1). However, the progeny emerging from large aphids consisted of significantly ($F_{difference} = 34.57$; df = 1, 33; $P < 0.01$) more females than males (9.4% males, SE 6.5–13.4).

Table 2. Multi-choice experiment: proportion of *A. abdominalis* males emerged from *M. euphorbiae* mummies (mean ± SE, %) in relation to aphid host stage and exposure time (d).

Stage		Day 1–2		Day 3
	n	Mean (SE[a])	*n*	Mean (SE[a])
N1-N2	35	55.7 (47.9–63.3)	50	76.8 (70.0–82.7)
N3	36	31.6 (24.0–40.3)	28	54.9 (45.4–64.1)
N4	18	3.7 (2.2–6.3)	51	9.2 (6.4–13.4)
Adults[b]	24	0.0	48	0.0

The table shows all statistically significant ($P < 0.05$) differences between developmental stages and exposure times, or their groups as indicated by the minimal adequate model in two-way ANOVA without replication. The pooled developmental stages did not differ significantly in deletion tests. n = total of investigated aphids.

[a]Standard errors are asymmetrical due to transformations of logits to proportions.

[b]Only female progeny were produced (not included in statistical analysis due to zero variance).

Mating significantly affected parasitoid preferences for host size. Mated females parasitized the same proportion ($F_{\text{residuals}} = 0.07$; df = 1, 33; NS) of small and large aphids (23.6%, SE 21.4–25.9). However, virgin females parasitized a significantly ($F_{\text{difference}} = 7.75$; df = 1, 22; $P < 0.05$) larger proportion of large fourth instars (31.2%, SE 25.1–38.1) than small first instars (16.3%, SE 13.5–19.6).

No-choice experiments with female preferences. In no-choice experiments, *A. abdominalis* females were provided life-long with either small first instar or large fourth instar aphids. Females exposed to fourth instars did not modify their oviposition preference with age ($F_{\text{residuals for slope}} = 0.16$, df = 1, 172; NS), and maintained 22.5% (SE 20.5–24.5) of males among their progeny throughout their lifetime (Fig. 2A). When provided first instars, newborn females started at the same rate of male production as those exposed to fourth instars ($F_{\text{residuals for common intercept}} = 2.25$; df = 1, 171; NS). However, the percentage of male progeny increased significantly ($F_{\text{slope}} = 28.84$; df = 1, 173; $P < 0.01$) with age (Fig. 2B). Within 14.6 d of adult life, females supplied with first instars attained the same proportion of males in their progeny (44.5%) as females supplied with a mixture of first and fourth instars in the dual-choice experiment.

Developmental period. When newborn nymphs of *M. euphorbiae* were exposed to *A. abdominalis* females for 5 h, mummification invariably occurred in the third or fourth instars. The mummies formed in third instars gave rise to significantly ($F_{\text{difference}} = 23.07$; df = 1, 10; $P < 0.01$) more males (78.5%, SE 73.3–82.9) than females. The sex ratio in *A. abdominalis* progeny emerged from mummies formed during the fourth instar was significantly female biased (39.5% males, SE 30.9–48.7).

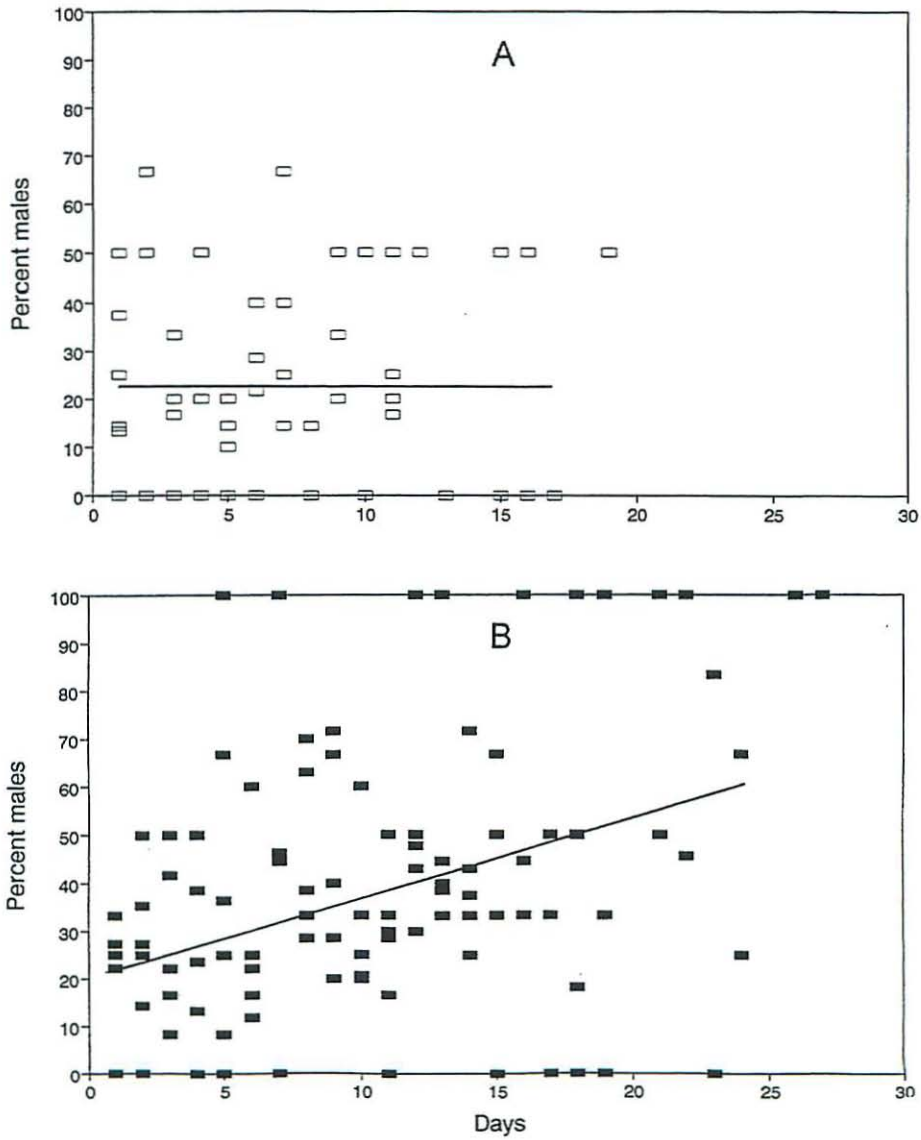

Fig. 2. Percentage of *A. abdominalis* males in relation to maternal age (d) when (A) fourth instars or (B) first instars of *M. euphorbiae* were offered for parasitization. For the fourth instars, the minimal adequate model is ln (p/1-p) = -1.24 (± 0.11), and for the first instars ln (p/1-p) = -1.24 (± 0.11) + 0.069 (± 0.012) day. R^2 = 16.7%, df =1, 172.

Discussion

Our experiments showed that sex ratio of *A. abdominalis* is correlated to mummy size. The proportion of males decreased as mummy weight increased. This represents indirect evidence for size-dependent sex allocation because the host size at death (measured as mummy weight) was used as a surrogate for host size at oviposition in the four host species. Direct evidence for size-dependent sex allocation is given in the experiments with female preference for depositing male and female eggs into aphids of a particular size by using *M. euphorbiae* as a host. The sex allocation was a consequence of female parasitoid preferences for small and large hosts when depositing male and female eggs, respectively, and a shorter developmental period of male parasitoid larvae compared with females in similar-sized hosts.

The experiments also showed that the wasp is able to adjust oviposition preferences and sex ratio. In multi-choice experiments, acceptance of first instars increased with decreasing availability of nonparasitized large aphids. Also, in dual-choice experiments virgin females selected large aphids for males as there was no need to save them for female progeny. In the absence of female progeny, laying male eggs into large aphids will improve exploitation of available range of host sizes without a risk of suboptimal resource allocation for males and females. In no-choice experiments, changes in offspring sex allocation were revealed when naive females were provided only with first instars. These females started laying mostly female eggs but gradually increased the proportion of males. Because the design of no-choice experiments with first and fourth instars was identical, the increase could be hardly due to depletion of sperm. Copulations were not checked but the males were present during the whole female life. Another demonstration of oviposition plasticity was the matching of sex allocation to particular host size ranges when different aphid species were offered for parasitization. That is, males were allocated to small hosts and females to larger hosts. This plasticity enables optimal resource allocation of male and female progeny when wasps are constrained to a particular size range within a single host species.

The experiments were not designed to detect the effects of two factors that may confound the relationship between female choice and host size at parasitization — mortality of parasitoid sexes in small and large hosts and host age — which may contribute to the effect of different size, through physiological variation between young larvae, old larvae, and adults. This is because variation among developmental stages was the most important source of host size variation (within species).

The results provide indirect evidence that differential mortality among sexes and the effects of host age are of minor importance. In *M. euphorbiae* where female oviposition preferences were studied in detail, the overall mummy mortality was too low (3.9%, SE 2.9–4.9) to influence the results on sex allocation significantly. Neglecting parasitoid egg and larval mortality appears justified because it is probably not greater than mummy mortality (cf. Hagvar & Hofsvang 1986, Steenis 1993). Low mortality of parasitoid developmental stages makes the natural selection of sex ratio much simpler than when significant egg-pupal mortality is a strong selection factor (Nagelkerke & Hardy 1994).

The age structure of aphid populations was similar in all species. The regression of sex ratio on mummy weight in *R. padi* and *S. avenae*, however, was steeper and host size range smaller than in *M. dirhodum* and *M. euphorbiae* (Fig. 1). This indicates a minor importance of host age compared with host size for parasitoid preference during sex allocation. Moreover, sex ratio was not associated with absolute host size, as aphids of similar size belonging to different species provided different sex ratios. Thus, the minimal adequate models for 0.2-mg mummy weight predicted 21.1% males for *R. padi* and *S. avenae* but 79.8% males for *M. dirhodum* and *M. euphorbiae*. This variation due to relative preferences of parasitoid females was apparently little affected by aphid age within species.

The preferences of *A. abdominalis* were without a fixed threshold for transition from male to female production. The mummy size corresponding to 1:1 sex ratio was near the median of the size range provided by a particular host species (except in *R. padi* where mummy size for 1:1 sex ratio was 45% above the median value). The congruence of median host size with 1:1 sex ratio suggests a precise estimation of host size by the parasitoid, little affected by temporal variance in female preferences or by uncertainty of estimating host size (Godfray 1994, cf. King 1994). In *R. padi*, assuming equal encounter and oviposition rate in large and small aphids, high mortality rather than parasitoid difficulties in estimating host size may cause a biased sex ratio (cf. van Emden 1995). Other evidence of flexible decision in estimating host size when laying male or female eggs is indicated by the change of sex ratio in no-choice experiments. Naive *A. abdominalis* females exposed to first or to fourth instars started to lay the same proportion of male eggs (22.5%) but later decreased the proportion of females when confined with small nymphs. In contrast, the proportion of males remained the same over time when *A. abdominalis* were confined with large nymphs. This indicated the existence of initial allocation values, with variation possible during oviposition.

The dependence of sex allocation on host size is most likely to be found in species that either attack nongrowing host stages, or prevent their hosts from continuing to grow (Waage 1982, Werren 1984, Wellings et al. 1986). This may occur because it is difficult for the female parasitoid to estimate final host size with sufficient precision to allocate offspring sexes differentially to hosts that vary both in size and age. *Aphelinus abdominalis* is one of the less frequent contrary examples (King 1989) of a species that allocates male and female eggs into growing hosts of different size. This may be due not only to plasticity of sex allocation as revealed in our experiments but also to the relatively small difference in size between neonate larvae and adults in larviparous aphids compared with a greater one existing in oviparous insects. Also, the short larval development of *A. abdominalis* can make the host size at mummification predictable.

Killing the host at an earlier instar due to the shorter developmental period of parasitoid males also contributes to smaller size of male than female mummies.

Earlier emergence of males than females (protandry) is a common occurrance (Honěk 1997), and could increase male fitness by providing them an advantage of early mating. It also could be a consequence of a tendency to increase female body weight and fecundity (Wiklund & Fagerstrom 1977, Wiklund & Solbreck 1982).

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Parasitoids Found in On-Farm Shelled Corn in Kentucky[1]

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ABSTRACT Shelled corn (*Zea mays* L.) in metal grain storage bins was sampled between June and September 1990 for insects on farms in the three westernmost crop reporting districts in Kentucky to identify, enumerate, and determine geographic distributions of pest and beneficial insects. A deep bin cup probe sampler was used to remove 0.3 liters of corn from three different depths in the center and at the edge of grain masses to detect and estimate insect density in representative composite samples. Each composite sample was sifted within 1 wk after sampling to remove all living and dead insects. The samples were sifted again after incubating them at 27 ± 1°C and ≥70% RH for 30 d to collect adult insects that had completed their development during the month-long period. Seven species or species complexes of pest insects were found representing 92% of the total number of pests captured. Eight species or species complexes of parasitoids were identified from the samples. *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae) was dominant. Other species present were *Pteromalus* sp. (Hymenotpera: Pteromalidae), *Cephalonomia waterstoni* Gahan (Hymenoptera: Bethylidae), *Cephalonomia tarsalis* (Ashmead) (Hymenoptera: Bethylidae), *Cephalonomia* spp. (Hymenoptera: Bethylidae), *Habrobracon hebetor* Say (Hymenoptera: Braconidae), *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae), and a member of the family Eurytomidae. *Anisopteromalus calandrae* and *Pteromalus* sp. were found in greater numbers in the center versus the edge of grain masses. Results are discussed in the context of parasitoid importance in stored-grain ecosystems.

KEY WORDS *Anisopteromalus calandrae, Pteromalus* sp., *Cephalonomia tarsalis, Cephalonomia waterstoni*, Hymenoptera, Pteromalidae, Bethylidae, Braconidae, stored corn

On-farm storage of corn (maize, *Zea mays* L.) in Kentucky ranged from 60 to 85 million bu from 1993 to 1997 (Kentucky Agricultural Statistics 1996–1997). Despite the enormous value of this commodity and decreasing efficacy and availability of synthetic pesticides, little is known of the parasitic insect species present in shelled corn in on-farm metal storage facilities in Kentucky (Barney et al. 1989, Sedlacek et al. 1998).

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During a 1990 survey of pest species present in on-farm stored corn, 34 species or species complexes of insect pests were found in shelled corn (Sedlacek et al. 1998). Frequently, swarms of small wasps, 1–3 mm in length, were observed on the surface of the grain or flying in the headspace of the grain bins. These insects attack and kill several of the insect pest species that infest grain. However, information about the species of beneficials present in on-farm stored corn in Kentucky is not available. Thus, the objective of the research reported herein was to identify parasitoid species present in on-farm stored corn, determine their relative abundance in storage, and determine their geographic distributions in Kentucky.

Materials and Methods

Grain bins containing shelled corn in the three westernmost crop reporting districts of Kentucky, where most of the corn is grown and stored in the state, were selected for sampling insects. One hundred sixty-four samples in 85 bins were taken at 75 farms in 24 counties. The objective was to maximize detection of species and determine relative abundance and geographic distribution; therefore, as many farms as possible were sampled and no effort was made to standardize bin conditions (e.g., bin size, volume of grain per bin, duration of storage). Bin volume ranged from 1,000 to 60,000 bu; corn was in storage from 6 mo to 2.5 yr; and grain mass temperature, moisture content, and volume varied among bins.

A deep bin cup probe sampler was used to remove 0.3 liter of corn from three different depths (top, middle, and bottom) in the grain mass at the center and near the edge of bins to estimate insect density in representative samples (Hagstrum 1994). A small number of bins had grain mounded up only in the center or near the bin edge. Representative composite samples were taken in these situations as well by using the cup sampler to sample from the top, middle, and bottom of each mound. The three samples taken from each location within a bin were combined to form a composite sample and placed in a 0.9-liter Mason jar with a ventilated lid. Thus, two composite samples (center and edge) were derived from most bins sampled. Sampling began in June in the westernmost counties and progressed eastward through September.

Samples were stored in an environmental growth chamber at $15 \pm 1^{o}C$ until sifted. Each sample was sifted through 5-mm followed by 2-mm sieves within 1 wk after sampling to remove living and dead parasitoids. The samples were sifted again after incubating them at $27 \pm 1^{o}C$ and $\geq 70\%$ RH for 30 d to collect adult parasitoids that had completed their development during the month-long period. Values presented represent the sum of individuals from both siftings. Insects were initially preserved in 70% EtOH. However, a reference collection was established by point mounting individual parasitoids and subsequently identifying them to genus, and species when feasible, by using the taxonomic key by Gordh (1991). Identification and enumeration of all species were based on individuals in the reference collection.

Descriptive statistics, Wilcoxon's sign rank test, and correlation and regression analyses were used to analyze data (SAS Institute 1988). Only bins for which both center and edge samples were taken had data analyzed for effect of

location on number caught. Wilcoxon's sign rank test was used to determine differences in numbers of parasitoids caught between center and edge samples when transforming the data failed to homogenize variances. Correlation analysis was used to ascertain the relationship between number of parasitoids and pests caught among the three crop reporting districts and to test associations between pest and parasitoid species. Regression analysis was used to test associations between number of parasitoids and number of pests within bins.

Results and Discussion

Eight species or species complexes of parasitoids were found in this survey (Table 1). Five species were found in this commodity in a long-term study conducted by Arbogast & Mullen (1988) in southeastern Georgia and only one species by Horton (1982) in an extensive survey of stored corn in South Carolina. Finding fewer species could have been due to Arbogast & Mullen (1988) sampling a single, nontraditional storage structure (an aluminum shed lined with plywood and styrofoam) for the entire duration of their study. Similarly, Horton (1982) primarily sampled corn that had been in storage for a short period or that may have been treated with insecticides. In the current study, the most abundant and widely distributed species or species complexes of parasitoids were *Anisopteromalus calandrae* (Howard), *Pteromalus* sp., *Cephalonomia tarsalis* (Ashmead), and *Cephalonomia waterstoni* Gahan (Table 1; Fig. 1). Maize weevil, *Sitophilus zeamais* Motschulsky; Angoumois grain moth, *Sitotroga cerealella* (Olivier); flat, rusty, and flour mill beetles, *Cryptolestes* spp.; saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.); foreign grain beetle, *Ahasverus advena* (Waltl); red and confused flour beetles, *Tribolium* spp.; and hairy fungus beetle, *Typhaea stercorea* (L.), were the most abundant and widely distributed pest species encountered. They accounted for 92% of the total number of pest insects captured (Sedlacek et al. 1998).

Of those farmers providing information on duration of storage, corn was in storage from 6 mo to 2.5 yr. Interestingly, one of the most abundant and widely distributed pests was the maize weevil, and the majority of parasitoids found was *Anisopteromalus calandrae* (Table 1), the primary parasitoid of the maize weevil. Horton (1982) found the maize weevil to be the most abundant pest found in a survey of stored corn, yet *C. tarsalis* was the only parasitoid species reported. A survey of farmers in that study revealed that the sampled corn was in storage an average of 7.3 mo. Interestingly, *C. tarsalis* was usually found in empty grain bins containing corn residues that were in poor condition (Horton 1982). Arbogast & Mullen (1988) sampled one bulk of shelled corn for 8 yr and found maize weevil to be a dominant pest and *A. calandrae* to be a dominant parasitoid after 16 and 32 mo, respectively. In addition, abundance and frequency of occurrence of parasitoids were low in 9-mo-old corn stored on farm in western South Carolina (Arbogast & Throne 1997). Thus, it appears likely that duration of storage may be an important factor accounting for the abundance and even presence of beneficials.

Significantly more maize weevils, *Cryptolestes* spp., *Tribolium* spp., hairy fungus beetles, foreign grain beetles, and total beetles were found in the center than at the edges of grain masses in the bins sampled (Sedlacek et al. 1998).

Table 1. Incidence and average density per sample (± SE) of parasitoids caught in on-farm stored corn in western Kentucky during 1990.

Species	Counties present (%)	Bins present (%)	Avg. no./sample District 1 (range)	Avg. no./sample District 2 (range)	Avg. no./sample District 3 (range)
Anisopteromalus calandrae	41.7	17.2	0.28 ± 0.23 (0–7)	5.96 ± 4.50 (0–218)	11.28 ± 4.20 (0–205)
Pteromalus sp.	25	9.2	0.00 ± 0.00 (0)	1.40 ± 1.40 (0–73)	2.21 ± 1.34 (0–76)
Cephalonomia tarsalis	29.2	11.5	0.00 ± 0.00 (0)	0.21 ± 0.15 (0–7)	6.65 ± 0.22 (0–11)
Cephalonomia waterstoni	41.7	17.2	0.00 ± 0.00 (0)	0.08 ± 0.06 (0–3)	0.69 ± 0.19 (0–7)
Cephalonomia spp.	12.5	3.4	0.00 ± 0.00 (0)	0.04 ± 0.04 (0–2)	0.13 ± 0.09 (0–6)
Theocolax elegans	4.2	1.1	0.00 ± 0.00 (0)	0.00 ± 0 .00 (0)	0.47 ± 0.44 (0–32)
Habrobracon hebetor	8.3	2.3	0.00 ± 0.00 (0)	0.04 ± 0.03 (0–1)	0.01 ± 0.01 (0–1)
Eurytomidae	4.2	5.3	0.00 ± 0.00 (0)	0.00 ± 0.00 (0)	0.01 ± 0.01 (0–1)
unidentifiable [a]	45.8	5.8	0.09 ± 0.05 (0–1)	0.23 ± 0.16 (0–7)	1.17 ± 0.29 (0–12)

[a]Defined as broken pieces, body parts, or individuals damaged so as to make identification impossible.

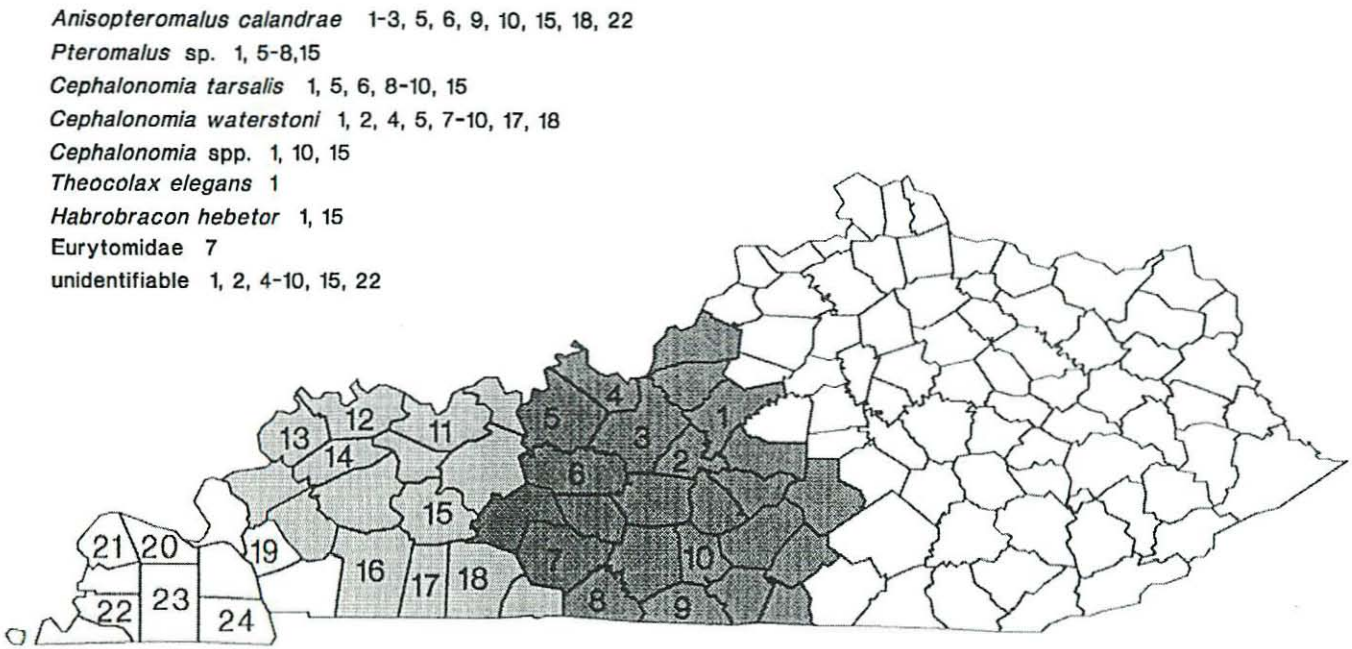

Fig 1. Map of Kentucky identifying counties in which shelled corn was sampled and showing distribution of parasitoids. County numbers correspond to county names as follows: 1-Nelson, 2-Larue, 3-Hardin, 4-Meade, 5-Breckinridge, 6-Grayson, 7-Warren, 8-Allen, 9-Monroe, 10-Metcalfe, 11-Daviess, 12-Henderson, 13-Union, 14-Webster, 15-Muhlenberg, 16-Christian, 17-Todd, 18-Logan, 19-Lyon, 20-McCracken, 21-Ballard, 22-Henderson, 23-Graves, 24-Calloway. Crop reporting districts 1–3 are denoted by progressively darker shading.

Hagstrum et al. (1985) also found higher densities of the most abundant insects in the center zone than edge zone in on-farm wheat. This was thought to be due to higher concentrations of fines located in the mass center. Similarly, more *A. calandrae* and *Pteromalus* sp. were recovered from centers than edges of shelled corn grain masses (Table 2). The reverse was found for *C. tarsalis* and *C. waterstoni,* but the magnitude of these differences was so small that realistically they have little biological significance. The spatial differences in number of parasitoids were largely explained by the differences in pest density; the regression between total pest insects and total parasitoids recovered from each sample was highly significant ($r^2 = 0.5972$, df = 154, $P < 0.0001$).

Table 2. Effect of sample location on number of parasitoids caught in center versus edge samples of corn stored on farm in grain bins.

Species	n	Mean ± SE Center	Mean ± SE Edge	Pr > |S|
Anisopteromalus calandrae	78	9.15 ± 4.19a	5.35 ± 2.60b	0.0001
Pteromalus sp.	78	2.06 ± 1.35a	0.91 ± 0.76b	0.0078
Cephalonomia tarsalis	78	0.35 ± 0.17b	0.40 ± 0.15a	0.0020
Cephalonomia waterstoni	78	0.28 ± 0.12b	0.41 ± 0.15a	0.0001
Cephalonomia spp.	78	0.03 ± 0.03a	0.11 ± 0.08a	0.1250
Theocolax elegans	78	0.03 ± 0.03a	0.41 ± 0.41a	1.0000
Habrobracon hebetor	78	0.03 ± 0.02a	0.01 ± 0.01a	1.0000
Eurytomidae	78	0.01 ± 0.01a	0.00 ± 0.00a	1.0000
unidentifiable	78	0.63 ± 0.24a	0.55 ± 0.15a	—

Means followed by different letters within a row are significantly different ($P < 0.05$; Wilcoxon's sign rank test [SAS Insitute1988]).

Importance of insect pests and parasitoids in on-farm shelled corn storage can be determined by examining relative abundance and distribution. Data obtained from this survey indicate a relatively broad spectrum of parasitoids associated with on-farm stored shelled corn. Two species of parasitoids were found in large numbers and four species were distributed widely (i.e., in crop reporting district 3) (Table 1). Parasitoids found in higher densities that were associated with pest species were *A. calandrae* with maize weevil ($r = 0.8493$, df = 155, $P < 0.0001$), *Pteromalus* sp. [probably *cerealellae* (Ashmead)] with Angoumois grain moth ($r = 0.8215$, df = 155, $P < 0.0001$), and *C. tarsalis* with sawtoothed grain beetle ($r = 0.6718$, df = 155, $P < 0.0001$). These findings correspond well with known host associations (Pederson 1992).

Dominant parasitoids were not as widely distributed in the three crop reporting districts of the western half of Kentucky as were their primary hosts. However, the density of parasitoids was higher than we anticipated. For example, *A. calandrae*, *Pteromalus* sp., *C. tarsalis*, and *C. waterstoni* were found in 41.7, 25.0, 29.2, and 41.7% of the counties sampled, respectively. Their primary hosts, maize weevil, Angoumois grain moth, sawtoothed grain beetle, and *Cryptolestes* spp., were found in 75, 83.3, 58.3, and 91.7% of the counties sampled, respectively. The overall average number of pest insects and associated parasitoids caught per sample was as follows: maize weevil (10.7 per 0.9 liter), *A. calandrae* (6.9 per 0.9 liter), Angoumois grain moth (6.4 per 0.9 liter), *Pteromalus* sp. (1.4 per 0.9 liter), sawtoothed grain beetle (4.2 per 0.9 liter), *C. tarsalis* (0.4 per 0.9 liter), *Cryptolestes* spp. (9.3 per 0.9 liter), and *C. waterstoni* (0.3 per 0.9 liter). A greater number of parasitoid species and number of each species were caught in crop district 3 than in the other districts (Table 1). Differences among the crop districts could be due to presence or absence or abundance of prey, time of year sampled, or geographic differences in grain storage conditions. For example, infestations of maize weevil or parasitoids may not be detected if corn is sampled too early in the storage year because the grain has not yet warmed enough to permit significant insect development, movement, or population growth when sampled (Sedlacek et al. 1998). The greater number of parasitoids present in the eastern counties sampled is probably explained by thermal accumulation when sampling was initiated (June) in the westernmost counties and when it was terminated (September) in the easternmost counties. In Paducah (representing the westernmost counties sampled), 1,082 degree-days ($DD_{10°C}$) had accumulated by 1 June 1990 whereas in Bardstown (representing the easternmost counties sampled), 3,033 DD had accumulated by 1 September 1990. Geographic differences in grain storage practices and end use may be other contributing factors to parasitoid distribution and abundance. Crop reporting district 3 has a greater percentage of small farms, smaller or low-storage-capacity farms, corn cribs, farms maintaining livestock and, thus, farms feeding corn to livestock (Barney et al. 1989). Based on our observations, the condition of corn stored in crop district 3, especially on farms with livestock operations, was poorer than that in districts 1 and 2, where corn was more likely destined for sale at a country elevator or kept in federal grain reserve programs.

The relatively narrow host specificity of the parasitoid species caught, combined with the relatively high percentage of counties and bins in which they were found and the high average number per sample, suggests that controlled

field-scale experiments should be conducted on the potential of parasitoids for limiting pest population growth in bulk grain. Augmentation and inundation of the commodity with multiple, frequent releases of parasitoids would be appropriate for use in these farm ecosystems because rapid response by parasitoids to growing populations of pests is necessary to preserve the value of the commodity (Brower 1996). However, this would require stringent quality control for mass rearing and development of economical techniques for mass rearing, storage, transportation, and application of live natural enemies.

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NOTE

Monitoring for European Corn Borer (Lepidoptera: Crambidae) Resistance to *Bacillus thuringiensis*: Logistical Considerations When Sampling Larvae[1]

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Corn, *Zea mays* L., is one of several crops that has been successfully transformed to express protein toxins derived from the bacterium *Bacillus thuringiensis* var. *kurstaki* (i.e., Bt corn) (Koziel et al. 1993, Armstrong et al. 1995). Since 1995, several seed companies have received approval to market Bt field corn, with the first Bt corn in the United States planted during the 1996 field season (Ostlie et al. 1997). Sweet corn seed companies also are conducting research to seek approval for marketing Bt sweet corn (Hutchison et al. 1997). With most Bt corn events, the primary target pest is European corn borer, *Ostrinia nubilalis* (Hübner). *Ostrinia nubilalis* is the major insect pest of field and sweet corn in the midwestern United States, with annual losses in U. S. field corn alone estimated to exceed $1 billion (Tollefson & Calvin 1994).

Because of the high selection pressure created by Bt corn, there is concern that *O. nubilalis* could develop resistance to Bt toxins (Alstad & Andow 1995, Bolin et al. 1995, Huang et al. 1997). For a successful proactive resistance management plan (Ostlie et al. 1997), it will be critical to develop cost-effective resistance monitoring programs for *O. nubilalis*. Such programs are necessary for increasing the odds of detecting resistance (Roush & Miller 1986) and thereby implementing a timely management response by growers, extension personnel, and seed companies. Presently, the most common monitoring approach is to sample Bt corn plants for larvae (usually late instars), mate the surviving adults, and bioassay their progeny. Although baseline susceptibilities to the Bt Cry1Ab protein have been published for some *O. nubilalis* populations

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(Siegfried et al. 1995, Huang et al. 1997), bioassays of field-collected *O. nubilalis* from Bt corn have not yet been reported in the United States. The purpose of the current study was to characterize the dose-mortality response of the progeny of *O. nubilalis* larvae collected from Bt corn in Minnesota during 1995, and to assess the cost-effectiveness of larval bioassays for resistance monitoring.

Late-instar *O. nubilalis* were collected from 1 September to 11 October 1995 at the Rosemount Agricultural Experiment Station, Rosemount, Minnesota (Dakota County), from replicated variety trials designed to assess the efficacy of selected Bt field and sweet corn varieties (Hutchison et al. 1997). All Bt hybrids in this study were developed to express the Cry1Ab gene of *B. thuringiensis* var. *kurstaki* but used different events and promoters. One field corn hybrid, 'Ciba 1134Bt' ('Ciba'), was provided by Novartis Seeds, Inc. (formerly Ciba Seeds, Inc.), and developed by using 'Event 176' and the PEPC promoter (Koziel et al. 1993), in which Bt expression is limited primarily to green leaf tissue and pollen. The three sweet corn hybrids used in this study ('Jubilee') were provided by Novartis Seeds, Inc., and developed by using 'Event BT11' and the 35S promoter (Armstrong et al. 1995), which allows for high Bt expression in green leaf and reproductive tissues. Event 176 is known to result in a gradual reduction of Bt protein following anthesis, whereas the BT11 event provides high levels of Bt expression up to plant senesence (Ostlie et al. 1997).

The Bt field and sweet corn hybrids were sampled as part of larger variety trials, arranged in separate, randomized complete block designs with 4 replications of each hybrid. Field corn hybrids were planted on 14 June to 4-row plots (91-cm row width), 6.8 m in length. Sweet corn varieties were planted on 19 June to 1-row plots (76.2 cm row width), 7.6 m in length; seed for both Bt field and Bt sweet corn was limited. Field corn plots were separated by 2 rows on each side and 1.83-m alleys on either end; sweet corn rows were separated by single skip (non-planted) rows on each side and 1.83 m on each end, throughout each block. For both studies, various non-Bt hybrids were used as controls, including a near-isogenic non-Bt hybrid check that was planted for each Bt hybrid in each trial.

Larval collection and processing. Larvae from Bt sweet corn were collected on 1 September during the ear evaluation of the efficacy trial (n = 100 ears per hybrid), and on 6 and 18 September during stalk dissection evaluation (n = 20 stalks per hybrid). Larvae from Bt field corn were collected on 8 and 29 September, and on 11 October during whole-plant dissection and efficacy evaluation (n = 20–40 stalks per hybrid, depending on larval density). For all hybrids, larvae were collected from plants with obvious ear, leaf, or stalk-feeding damage. Larvae from sweet and field corn events were held separately and placed on a wheat germ meridic diet (Reed et al. 1972, Guthrie 1990) in 254.5-cm^3 plastic dishes; dishes were placed in a cooler for transport to the laboratory.

In the laboratory, larvae (n = 88) were individually surface sterilized in phenylmercuric nitrate (0.0001% solution) for 2 s. Larvae were held in individual 35-ml diet cups. After 3 wk, each larva was placed on an agar cube and subjected to diapause conditions (5–8°C, photoperiod of 24:0 [L:D] h) on 4 October 1995. Diapause was terminated on 2 February 1996. Because of high parasitism rates (≈75%), primarily by *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae), and additional natural mortality, only seven adults emerged (three from Jubilee, four from Ciba). Adults were designated J1-3 and C1-4.

Larval increase. To expose potential resistance genes for identification and to increase the number of individuals available for bioassay, a modification of an F_2 screen was used (Andow & Alstad 1998). Each field-collected adult was mated to an adult of the opposite sex (males or virgin females) selected from an *O. nubilalis* colony maintained in the Department of Entomology, University of Minnesota. This colony was field collected from Le Sueur, Minnesota, during 1993. It is considered susceptible to Bt (P.C.B., unpublished data), and has been maintained on artificial diet for 20 generations. Pairs were placed in small wire-mesh cages, 9 cm in height by 5.5 cm in diameter. If the field-collected parent had a single resistance allele at some locus, half of the F_1 progeny would be heterozygous for the resistance allele. These progeny were then randomly sib-mated. In the resultant F_2 generation, any resistance allele at any locus will be expressed in a homozygous condition in 1/16 of the F_2 offspring. For example, P_1: SS × Sr; F_1 progeny: SS (0.5) + Sr (0.5); F_1 sib matings: Sr × Sr (0.25) + SS x SS (0.25) + SS x Sr (0.50); and F_2 progeny: SS (0.5625) + Sr (0.375) + rr (0.0625), with S = susceptible and r = resistant (recessive). If the resistance allele were dominant, 0.4375 of the F_2 larvae would be phenotypically resistant. Of course, if the field-collected parent did not have any resistance allele, none of the F_2 larvae would be phenotypically resistant. Progeny from each of the seven moth lines were reared separately.

Mass screening. For mass screening of the F_2 progeny, an initial discriminating dosage of 42 µg of toxin per milliliter of diet was selected. In preliminary 7-d assays with the LeSueur colony, mortality of first instars averaged 81.3% at 21 µg of toxin per milliliter of diet (P.C.B., unpublished data). For all assays, Cry1Ab was obtained as formulated product (Mycogen Corp., San Diego, California), where the Bt protein crystal is encapsulated within the bacterium *Pseudomonas fluorescens* (Trevisan); this formulation does not contain spores (Bolin et al. 1996). Cry1Ab was blended into a preservative-free wheat germ diet (Warnock et al. 1997) and poured into 254.5-cm^3 mass-rearing dishes to cool. Hatching *O. nubilalis* egg masses were pinned just above the diet surface. Approximately 20 egg masses, averaging 25 eggs per mass, were placed in each dish, with one dish per line (average 500 eggs per line). Diet dishes were held at 27°C (photoperiod 24:0 [L:D] h; ≈70% RH). After 7 d, all dishes were examined for surviving larvae.

Bioassay. For a more complete analysis of the dose-mortality response of the progeny from six of the parental strains, a serial dilution of nine concentrations of Cry1Ab was established to provide a range of 10–90% mortality. A full bioassay was not done on moth line C-2, because this line had much greater susceptibility to Cry1Ab in preliminary assays than did the other six lines. For these assays, Cry1Ab was incorporated into the wheat germ diet (Warnock et al. 1997), and dispensed into BioAssay trays (CD International, Pittman, New Jersey) with ≈1 ml of diet per well. Egg masses were allowed to hatch over a 4-h period; one neonate was then placed in each well for each Bt concentration, for a total of 32 larvae per concentration. The entire dose range was replicated two times. All assays were evaluated after 7 d for larval mortality. Data were analyzed by using probit analysis within POLO-PC (LeOra Software 1994).

There were no survivors from the initial discriminating dose-screening procedure (i.e., zero from an average of 450 neonates per line). Survival of 1/16 of

the F_2 offspring would have indicated the presence of a resistance allele in its homozygous state. Survival greater than 1/16 would have indicated the additional survival of some individuals heterozygous for the resistance allele. Resistance ratios (RR) from all bioassays, for progeny from field-collected parental larvae, were all <1.0 (Table 1). Many of the slopes for the field-collected lines were also <1.0, or less than that of the Le Sueur nonselected check colony, indicating that the field-collected larvae were no more tolerant to Bt than were larvae from the susceptible colony. Differences in LC_{50}s between the check colony and the field-collected larvae could have resulted from natural variation (Robertson et al. 1995). In addition, whereas the laboratory colony was maintained free of pathogens, such as *Nosema pyrausta* Paillot, the field-collected larvae were not screened for disease. The effects of *N. pyrausta* infection could have an impact on resistance monitoring in terms of increased field-collected larval mortality and decreased fecundity from the pair-matings coupled with increased mortality on their progeny (Zimmack et al. 1954), as well as an impact on the possible initiation of a *B. thuringiensis*-resistant colony.

Table 1. Bioassay results for progeny (first instars) of *O. nubilalis* collected as parental larvae during September 1995, Rosemount, Minnesota.

Source[a]	Total no. larvae	LC_{50}[b]	95% CI Upper	95% CI Lower	Slope	χ^2	g-Value (0.95)[c]	RR[d]
MN-Susc	512	3.02	2.13	4.27	1.16	15.2	0.064	—
J-1	448	0.28	0.02	0.71	1.24	37.6	0.35	0.09
J-2	450	0.08	0.002	0.31	0.69	17.7	0.23	0.03
J-3	447	2.23	1.30	3.45	0.96	6.81	0.05	0.74
C-1	448	1.38	0.75	2.21	1.07	11.2	0.06	0.46
C-3	416	0.58	0.08	1.54	0.51	10.9	0.20	0.19
C-4	447	0.20	0.01	0.63	0.83	25.7	0.24	0.07

[a]Minnesota susceptible (MN-Susc) colony developed from 1993 field collection of *O. nubilalis* larvae, near Le Sueur, Minnesota; J and C-1, 2, and 3 refer to individual moth lines developed from field-collected larvae from Bt sweet corn (Jubilee) and Bt field corn (Ciba) events, respectively.

[b]Expressed as micrograms of toxin per milliliter of diet.

[c]A measure of goodness-of-fit (LeOra Software 1994)

[d]Resistance ratio: LC_{50} from the transgenic survivors divided by that of the susceptible (Le Sueur, Minnesota) colony.

Survival of *O. nubilalis* found in a Bt cornfield may not be a sign of resistance. In field corn, up to 4% of a given seed bag may contain non-Bt off-types (Ostlie et al. 1997), and growers and researchers also must be aware of non-Bt volunteer corn from the previous year. In our study, with a small-plot design, the presence of larvae on Bt corn probably resulted from inter-row larval movement (Ross & Ostlie 1990) from nearby nontransgenic plants. Also, with Event 176, Bt expression is known to decline as the season progresses, and there is virtually no expression in female reproductive tissues (ear, kernel, or silk) (Koziel et al. 1993). Larvae could have initially established in these non-Bt sites, then later moved into the stalks as late instars. For example, at the first field-corn dissection (8 September), 69% of surviving larvae was found in the ear tissue; by the last dissection (11 October), all larvae were late instars and were found in the stalk. Movement of late instars to Bt corn tissues also is possible because large larvae are able to tolerate 400× higher concentrations of Bt compared with neonates (Davis & Coleman 1997).

The larval collection and assay protocol used herein is a modification of the F_2 screen (Andow & Alstad 1998), involving deliberate matings of the P_1 generation with a known Bt-susceptible laboratory colony. The method has several advantages over several alternative approaches. If all adults from collected larvae were mass mated, it would not be possible to know that all larval genotypes were evaluated, and if they were pair mated as they emerged, the loss of one pair-mating from early death, infertility, or inviability of eggs would result in the loss of two collected larvae. By mating to susceptible individuals, multiple mates can be provided that increase the probability of continuation of the line.

The variable costs associated with this method were high. The time required to dissect 100 plants for an initial sample of 88 late instars required ≈5 h by two people. At $10/h for labor at 10-person h, the cost ($100) was estimated to be $1 per plant. This cost is incurred whether or not larvae are found, or if all larvae survive for subsequent assay. We do not include transportation costs to the field as this will vary considerably from site to site. Laboratory costs included labor for setting up field-collected larvae (4 h), monitoring larvae during diapause (1 h per wk for 18 wk), setting up appropriate matings (4 h), and collecting F_1 eggs, rearing F_1 families, and collecting F_2 eggs (7 h). At $10/h for labor, this amounts to $330. Materials cost $50. Consequently, each of the lines cost ≈$54 to handle in the laboratory, which does not include transportation costs for larval collection, or the costs of screening. Clearly, the loss of larvae from parasitism and other mortality significantly reduces the economic efficiency of this method, not to mention the possible loss of resistance genes. If all 88 larvae had survived to be screened, field and laboratory handling costs would have been $1.20 and $18.86 per larva, respectively (total of $1,765), again not accounting for transportation and screening. This is still more expensive than the F_2 screen (Andow et al. 1998).

Given the costs associated with the collection and processing of seven field-collected larvae over a 7-mo period, this procedure may not be practical for widespread resistance monitoring, particularly for late-season *O. nubilalis* generations (entering diapause). Alternatively, the methods used herein could be used only for instances of alleged Bt corn failures. The approach may be cost-effective for widespread monitoring if diagnostic doses can be developed (Huang

et al. 1997) and if the assay is limited to a few regional laboratories that are funded for this purpose (Siegfried et al. 1995). However, the major obstacle with larval sampling is that only a limited number of individuals from any given population can be assayed. The efficiency of larval assays will need to be improved to handle the larger sample sizes necessary for resistance detection, rather than the minimum samples needed only to document resistance (Roush & Miller 1986).

There may be several cost-saving options for *O. nubilalis* larval assays. These include the collection of moths for oviposition in the laboratory, or direct collection of egg masses in the field, for more rapid Bt assays of neonates. Field collection of egg masses, however, also can be time-consuming at low densities (e.g., <1 egg mass per plant) and can be hindered by natural mortality and variable hatching times. Another possibility would be the development of a late-instar assay that could significantly increase assay speed. This could require more toxin per assay but would eliminate the need for long-term rearing and colony maintenance. Finally, new alternatives include screening field-collected adults by using a F_2 screen (Andow et al. 1998) or direct screening of field populations by using tissue-specific sampling of Bt sweet corn (Andow & Hutchison 1998). Direct in-field monitoring warrants further investigation because it does not require extensive processing in the laboratory, and therefore should be more cost-effective for multiple *O. nubilalis* populations throughout the Corn Belt. As more transgenic corn is deployed over more corn-producing states, the need for practical resistance monitoring will become more critical.

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Parasitoids of Insects Associated with Soybean and Vegetable Crops in Indonesia[1]

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ABSTRACT Field surveys were carried out in Indonesia on the islands of Java, Sumatra, Sulawesi, and Bali from November 1994 to October 1997 to determine the parasitoid species associated with vegetable crops and soybean. Lepidopteran larvae were placed on artificial diet and brought to the laboratory for parasitoid emergence. Egg masses of sucking bugs also were collected or eggs from colony-reared bugs were placed in the field and collected later. Leafminer-infested leaves were held in containers until emergence of parasitoids or adult flies. Eighty-five species of parastoids from 62 genera emerged from the collections. Most (92%) of the species were from four superfamilies: Ichneumonoidea (37 species or 44%), Chalcidoidea (31 species or 37%), Proctotrupoidea (9 species or 11%), and Bethoidea (1 species). There were seven species of parasitic Diptera. Many of the parasitoid species, such as *Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae) on *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), were clearly controlling host larvae. *Omiodes indicata* (F.) (Lepidoptera: Pyralidae) and *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) yielded 15 and 11 species of parasitoids, respectively. Several undescribed species of parasitoids were found from a range of hosts. Several of the parasitoids are illustrated.

KEY WORDS *Liriomyza huidobrensis,* parasitoids, Lepidoptera, Pentatomidae, survey, Indonesia, vegetables, soybean

There is a notable lack of information about natural enemies of insects that attack soybean and vegetable crops in Indonesia, especially for parasitoids. Kalshoven (1981) mentioned some parasitoid species in Indonesia but few of them are associated with soybean and vegetable crops. Similarly, a list of parasitoid species in the Bogor Research Institute for Food Crops (now the Bogo Research Institute for Food Crops Biotechnology) collection at Bogor, West Java, Indonesia, was published by Siwi et al. (1989). However, of those species listed, few are from insects that feed on soybean and vegetables.

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Parasitoids of the stem fly, *Melanagromzya sojae* Zehntner (Diptera: Agromyzidae), were monitored in soybean throughout the season from North Sumatra (van den Berg et al. 1995). A complex of seven hymenopteran parasitoids were reported: *Bracon* sp., *Gronotoma* sp., *Eurytoma* sp., *Cholocytus* sp., *Colotrechnus agromyzae* Subba Rao (Hymenoptera: Pteromalidae), *Sphegigaster* sp., and *Syntomopus* sp. Average parasitism levels reached nearly 70% toward the end of the growing season. Djuwarso et al. (1992) reported *Cynipoide* sp., *Eurytoma poloni* Girault (Hymenoptera: Braconidae), *Trigonogastra agromyza* Dodd (Hymenoptera: Braconidae), *Eurytoma,* sp. and *Secondella* sp. from *M. sojae* and *Ophiomyia phaseoli* (Tryon) (Diptera: Agromyzidae). Van den Berg et al. (1997) listed eight and Naito and Djuwarso (1993) reported six parasitoid species from *Etiella* spp. (Lepidoptera: Pyralidae) the most important pod-boring insects in Indonesia.

Several species of egg parasites of sucking bugs [*Nezara viridula* (F.), *Piezodorus hybneri* (Gmelin)] (Hemiptera: Pentatomidae) and *Riptortus linearis* F. (Hemiptera: Coreidae) occur in soybean, mungbean, and other legumes in Indonesia (van den Berg et al. 1995). Only one genus (*Gryon*) has been reported from *R. linearis*. *Piezodorus hybneri* and *N. viridula* eggs also are attacked by *Gryon* spp., along with parasitoids in the genera *Telenomus, Anastatus,* and *Ooencyrtus* (Annual Report 1995). *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) also was reported from eggs of *N. viridula* (Hirose et al., unpublished data).

Liriomyza huidobrensis (Blanchard) (Diptera: Agromyzidae) is a serious pest of potato and other crops and a recent immigrant into Indonesia (Shepard et al. 1996). The major parasitoid of this pest in Indonesia is *Hemiptarsenus varicornis* (Girault) (Hymenoptera: Eulophidae), although others occur less frequently (Shepard et al. 1998).

Diadegma semiclausum (Hellen) (Hymenoptera: Ichneumonidae) was introduced into Indonesia in the 1950s to control *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) on brassica crops (Voss 1953, Sastroiswojo and Sastrodihardjo 1986). This parasitoid is widespread in Indonesia and, where chemical pesticides are not used, provides control of *P. xylostella*. Although *P. xylostella* is under control by *D. semiclausum*, the cabbagehead caterpillar, *Crocidolomia binotalis* (Zeller) (Lepidoptera: Pyralidae), does not have sufficient parasitoids or other natural enemies to keep its populations below damaging levels in Indonesia.

Because of this obvious lack of information about natural enemies of soybean and vegetable insects in Indonesia, we conducted field surveys to determine the most important parasitiods and provide illustrations of some of the more common species.

Materials and Methods

Surveys were carried out in Indonesia on the islands of Java, Sumatra, Sulawesi, and Bali from November 1994 to October 1997 to determine the parasitoid species from insects associated with soybean and vegetable crops. Lepidopteran larvae were collected from plants and placed singly into 30-ml plastic cups containing artificial diet. These were brought to the laboratory at Bogor,

West Java, and held for emergence of parasitoids or adult lepidopterans. Those that died from infection by entomopathogens, or from other causes, also were recorded. These data will not be published in the current paper. Parasitoids were stored in vials of 80% EtOH or pinned as dry specimens until identifications were made.

Sucking-bug egg parasitoids were determined by collecting egg masses from the field and by placing egg masses from colony-reared bugs in the field, collecting them 3 or 4 d later, and holding them in the laboratory in test tubes plugged with cotton. Pest species from which egg parasitoids were collected included *N. viridula*, *R. linearis*, and *P. hyberni*.

Aphid and leafminer parasitoid surveys were carried out by bringing leaves of plants containing the pests to the laboratory and holding them in jars with inverted funnels with small cups fitted to the funnel stems. Methods for monitoring the incidence of parasitism of leafminers are provided by Shepard et al. (1998). Voucher specimens were placed in the parasitoid collection in the Palawija IPM Laboratory at the Bogor Research Institute for Food Crops Biotechnology, Cimanggu Kecil, Bogor, Indonesia.

Results and Discussion

A list of major parasitoids that emerged from insects collected from soybean and vegetable crops is provided in Table 1. Their relative abundance also is indicated. Larval and egg collections were made during field surveys carried out at irregular intervals, thus variable numbers of hosts were collected and parasitoid seasonality was not determined. During the entire collection period, 8,403 larvae of *S. exigua*; 1,493 *S. litura*; 2,361 loopers [mainly *Chrysodeixis chalcites* (Esper) and *Thysanoplusia orachalcea* (L.)] (Lepidoptera: Noctuidae); 356 *H. armigera*; 4,820 *C. binotalis*; 1,095 *P. xylostella*; 302 *Omiodes indicata* (F.); 1, 896 *Etiella* spp.; and 149 *Maruca vitrata* (Meyer) (Lepidoptera: Pyralidae) were collected. There were 85 species of parasitoids belonging to 62 genera that emerged from lepidopteran larvae, sucking bug eggs, aphids, and leafminers. Of the two orders, the parasitic Hymenoptera were dominant with 78 (92%) of the species distributed from four superfamilies: Ichneumonoidea (37 species or 44%), Chalcidoidea (31 species or 37%), Proctotrupoidea (9 species or 11%), and Bethoidea (1 species). The parasitic Diptera had only seven species (8%), all of which were tachinids.

Three families comprised the superfamily Ichneumonoidea: Braconidae with 19 species in 14 genera, followed by Ichneumonidae (16 species in 11 genera), and Aphidiidae (two species in two genera). All of these wasps are primary parasitoids of noctuids (*Spodoptera* spp., *T. orichalcea*, *C. chalcites*, and *H. armigera*), pyralids (*Etiella* spp., *C. binotalis*, *M. vitrata*, *O. indicata*), and the family Yponomeutidae represented by *P. xylostella*. The Chalcidoidea were second in importance with nine families, mostly dominated by the eulophid wasp, *Hemiptarsenus varicornis* (Girault) (Hymenoptera: Eulophidae), the single most important parasitoid of the leafminer *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae). Proctotrupoidea ranked third, consisting mainly of two scelionid genera: *Gryon* and *Telenomus* that commonly emerged from eggs of *N. viridula*, *P. hybneri*, and *R. linearis*.

Table 1. Parasitoids associated with soybean and vegetable crops in Indonesia.

Insect host	Parasitoid	Relative abundance	Crops	Figure number
Lepidoptera				
Pyralidae				
Etiella zinckenella (Treitschke)	*Zygobothria* sp.		soybean	1
E. hobsoni (Butler)	(Diptera: Tachinidae)	+	mungbean	
			cowpea	
	Baeognatha (=Agathis) sp. n. A		other legumes	3
	(Hymenoptera: Braconidae)	+		
	Baeognatha javana Bhat & Gupta			4A & B
	(Hymenoptera: Braconidae)	++		
	Unidentified Braconinae			
	(Hymenoptera: Braconidae)	+		
	Bracon sp.			
	(Hymenoptera: Braconidae)	+		
	Cardiochiles szepligeti (Enderlein)			
	(Hymenoptera: Braconidae)	+		
	Chelonus sp. A			5A & B
	(Hymenoptera: Braconidae)	+		
	Chelonus sp. B.			
	(Hymenoptera: Braconidae)	+		
	Phanerotoma philippinensis (Ashmead)			6A & B
	(Hymenoptera: Braconidae)	++		

Table 1. Continued.

Insect host	Parasitoid	Relative abundance	Crops	Figure number
	Pristomerus naitoi Kusigemati (Hymenoptera: Ichneumonidae)	++		12A, B & C
	Temelucha etiellae Kusigemati (Hymenoptera: Ichneumonidae)	++		
	Trichogrammatoidea bactrae bactrae Nagaraja (Hymenoptera: Trichogrammatidae)	++		
Crocidolomia binotalis Zeller	*Colpotrichia* sp. (Hymenoptera: Ichneumonidae)	++	Brassica crops	14
	Diadegma sp. (Hymenoptera: Ichneumonidae)	++		18A & B
	Eriborus argenteopilosus (Cameron) (Hymenoptera: Ichneumonidae)	++		15A, B & C
	Bleparipa (= Sturmia) sp. (Diptera: Tachinidae)	+		
	Palexorista inconspicuoides Baranov (Diptera: Tachinidae)	+		
Maruca vitrata (Geyer)	*Baeognatha* (=*Agathis* sp.) n. sp. B (Hymenoptera: Braconidae)	+	Legumes	
	Cotesia sp. (Hymenoptera: Braconidae)	++		11

Table 1. Continued.

Insect host	Parasitoid	Relative abundance	Crops	Figure number
Omiodes indicata (F.)	*Brachymeria* cf. *lasus* (Walker) (Hymenoptera: Chalcididae)	+	Legumes	
Omiodes indicata (F.)	*Charops brachypterum* Gupta and Maheswary (Hymenoptera: Ichneumonidae)	+		16
	Chelonus sp (Hymenoptera: Braconidae)	+		
	Dolichogenidea inquisitor Wilkinson (Hymenoptera: Braconidae)	8 ++		
	Elasmus sp. (Hymenoptera: Elasmidae)	+		
	Eurytoma sp. (Hymenoptera: Eurytomidae)	+		21
	Goniozus cf. *triangulifer* Kieffer (Hymenoptera: Bethylidae)	+		
	Pristomerus cf. *fumipennis* Wilkinson (Hymenoptera: Ichneumonidae)	+		
	Sympiesis dolichogaster Ashmead (Hymenoptera: Eulophidae)	+		

Table 1. Continued.

Insect host	Parasitoid	Relative abundance	Crops	Figure number
	Tachinid (Unidentified) (Diptera: Tachinidae)	+		
Omiodes indicata (F.)	*Temelucha philippinensis* (Ashmead) (Hymenoptera: Ichneumonidae)	++		13A, B & C
	Temelucha sp. (Hymenoptera: Ichneumonidae)	+		
	Trichomalopsis apanteloctenae Crawford (Hymenoptera: Pteromalidae)	++		22A & B
Yponomeutidae				
Plutella xylostella (L.)	*Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae)	+++	Brassica crops	17A & B
	Diadegma sp. (Hymenoptera: Ichneumonidae)	+		18A & B
Noctuidae				
Spodoptera exigua Hübner	*Diadegma semiclausum* Hellen (Hymenoptera: Ichneumonidae)	+	onions, (scallions shallots)	17A & B
	Eriborus argenteopilosus (Cameron) (Hymenoptera: Ichneumonidae)	+++	peppers brassica crops others	15A, B & C

Table 1. Continued.

Insect host	Parasitoid	Relative abundance	Crops	Figure number
	Charops brachypterum Gupta & Maheswary (Hymenoptera: Ichneumonidae)	+		16
	Euplectrus cf. *chapadae* (Ashmead) (Hymenoptera: Eulophidae)	+		
Spodoptera exigua Hübner	*Stenomesius japonicus* (Ashmead) (Hymenoptera: Eulophidae)	+		
	Microplitis similis Lyle (Hymenoptera: Braconidae)	+++	soybean, brassica crops	9
	Microplitis sp. (Hymenoptera: Braconidae)	+		
Spodoptera litura (F.)	*Microplitis similis* Lyle (Hymenoptera: Braconidae)	++		9
	Rogas sp. (Hymenoptera: Braconidae)	+		
	Brachymeria excarinata (Gahan) (Hymenoptera: Chalcididae)	+		20
	Enicospilus sp. A (Hymenoptera: Ichneumonidae)	+		19A & B
	Charops brachypterum Gupta and Maheswary (Hymenoptera: Ichneumonidae)	+		16

Table 1. Continued.

Insect host	Parasitoid	Relative abundance	Crops	Figure number
	Euplectrus sp. (Hymenoptera: Eulophidae)	+		
	Glyptapanteles sp. (Hymenoptera: Braconidae)	++		
	Metopius rufus javanus Szepligeti (Hymenoptera: Ichneumonidae)	+		
Spodoptera litura (F.)	*Peribaea orbata* Wiedemann (Diptera: Tachinidae)	+++		2
	Telenomus remus Nixon (Hymenoptera: Scelionidae)	++		23A,B & C
	Opius sp. (Hymenoptera: Braconidae)	+		7
	Tachinidae (Unidentified) (Diptera: Tachinidae)	+	carrot onion	
Chrysodeixis chalcites (Esper)	*Copidosoma floridanum* (Ashmead) (Hymenoptera: Encyrtidae)	+++	legumes potato carrot onion	24A & B
	Brachymeria lasus (Walker) (Hymenoptera: Chalcididae)	+		
	Tachinidae (unidentified) (Diptera: Tachinidae)	+		

Table 1. Continued.

Insect host	Parasitoid	Relative abundance	Crops	Figure number
	Cotesia sp. (Hymenoptera: Braconidae)	+++		11
	Diatora prodeniae (Ashmead) (Hymenoptera: Ichneumonidae)	+		
	Glyptapanteles phytometrae Wilkinson (Hymenoptera: Braconidae)	+++		10
	Microplitis similis Lyle (Hymenoptera: Braconidae)	++		9
Chrysodeixis chalcites (Esper)	*Microplitis* sp. (Hymenoptera: Braconidae)	+		
	Trichomalopsis apanteloctenae (Crawford) (Hymenoptera: Pteromalidae)	+		22A & B
	Enicospilus sp. B. (Hymenoptera: Ichneumonidae)	+		
	Argyrophylax sp. (Diptera: Tachinidae)	+		1
Helicoverpa armigera Hübner (Diptera: Tachnidae)	*Argyrophylax* sp. +		polyphagous	
	Eriborus argenteopilosus (Cameron) (Hymenoptera: Ichneumonidae)	+++		15A, B & C"

Table 1. Continued.

Insect host	Parasitoid	Relative abundance	Crops	Figure number
	Diadegma semiclausum Hellen (Hymenoptera: Ichneumonidae)	+		17A & B
	Trichogramma cf. *chilonis* Ishii (Hymenoptera: Trichogrammatidae)	++		25A, B & C
Agrius convolvuli L.	*Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae)	+++	sweet potato soybean	
Agrius convolvuli L.	*Zygobothnia* cf. *atropivora* R. D. (Diptera: Tachinidae)	+		
Diptera				
Agromyzidae				
Melanagromzya sojae Zenhtner	*Bracon* sp. Hymenoptera: Braconidae)	+	soybean mungean	
	Cynipoide sp. (Hymenoptera: Cynipidae)	++		
	Tetrastichus sp. (Hymenoptera: Eulophidae)	+		26
	Sympiesis sp. (Hymenoptera: Eulophidae)	+		
	Eurytoma poloni Girault (Hymenoptera: Eurytomidae)	++		

Table 1. Continued.

Insect host	Parasitoid	Relative abundance	Crops	Figure number
	Eurytoma sp. (Hymenoptera: Eurytomidae)	+		21
	Sphengigaster sp. (Hymenoptera: Pteromalidae)	+		
	Colotrechnus agromyzae Sabba Rao (Hymenoptera: Pteromalidae)			
	Syntomopus sp. (Hymenoptera: Pteromalidae)	+		
Melanagromzya sojae Zenhtner	*Trigonogastra agromyzae* Dodd (Hymenoptera: Eulophidae)	+		
Liriomyza huidobrensis (Blanchard)	*Hemiptarsenus varicornis* Girault (Hymenoptera: Eulophidae)	+++	polyphagous 28 A & B	27A & B
	Euderus sp. (Hymenoptera: Eulophidae)	++		29
	Eucoilidea sp. (Hymenoptera: Cynipidae)	+		
Phytomyza horticola Gour.	*Hemiptarsenus varicornis* Girault Hymenoptera: Eulophidae	+++	peas,beans	27A & B 2A & B

Table 1. Continued.

Insect host	Parasitoid	Relative abundance	Crops	Figure number
	Pediobius sp. (Hymenoptera: Eulophidae)	+		
	Cynipoide sp. (Hymenoptera: Cynipidae)	+		
	Callitula sp. (Hymenoptera: Pteromalidae)	+		
Cedidomyiidae				
Diptera				
Asphondylia sp.	*Eurytoma* cf. *manilensis* Ash. (Hymenoptera: Eurytomidae)	+	chilies	
	Tetrastichus sp. (Hymenoptera: Eulophidae)	+		26
Asphondylia sp.	*Trichomalopsis* sp. (Hymenoptera: Pteromalidae)			
Hemiptera:				
Alydidae				
Riptortus linearis L.	Gryon sp. B (Hymenoptera: Scelionidae)	++	legumes	
	Gryon sp. C (Hymenoptera: Scelionidae)	+		

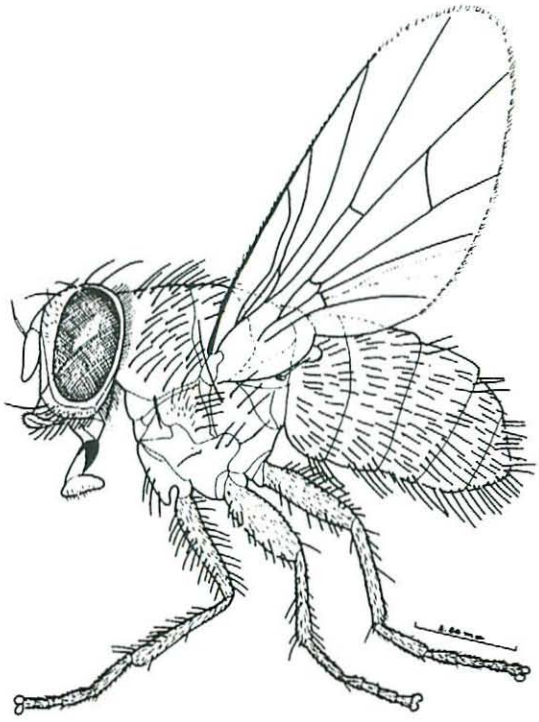
Table 1. Continued.

Insect host	Parasitoid	Relative abundance	Crops	Figure number
	Ooencyrtus sp. (Hymenoptera: Encyrtidae)	+++		31A & B
Pentatomidae				
Piezodorus hybneri (Gmelin)	*Gryon* sp. A (Hymenoptera: Scelionidae)	++	legumes	30A & B
	Telenomus sp. A (Hymenoptera: Scelionidae)	+++		32A & B
	Anastatus sp. (Hymenoptera: Eupelmidae)	+		
	Ooencyrtus sp. (Hymenoptera: Encyrtidae)	++		31A & B
Nezara viridula L.	*Gryon* sp. A (Hymenoptera: Scelionidae)	+++		30A & B
	Telenomus sp. (Hymenoptera: Scelionidae)	+++		33A & B
	Anastatus sp. (Hymenoptera: Eupelmidae)	++		
	Ooencyrtus sp. (Hymenoptera: Encyrtidae)	+ +		31A & B

Table 1. Continued.

Insect host	Parasitoid	Relative abundance	Crops	Figure number
	Trissolcus basalis (Gahan) (Hymenoptera: Scelionidae)	+		
Cicadellidae *Amrasca* sp.	*Eotetrastichus* sp. (Hymenoptera: Eulophidae)	+	legumes chilies brassica crops	
Aphidiidae	*Aphidius* sp. (Hymenoptera: Aphidiidae)	+		34A & B
	Trioxys sp. (Hymenoptera: Aphidiidae)	+		
Coleoptera Coccinellidae				
Epilachna vigintioctopunctata (Boisduval)	*Aprostocetus* sp. (Hymenoptera: Eulophidae)	++	egg plant potato	

1



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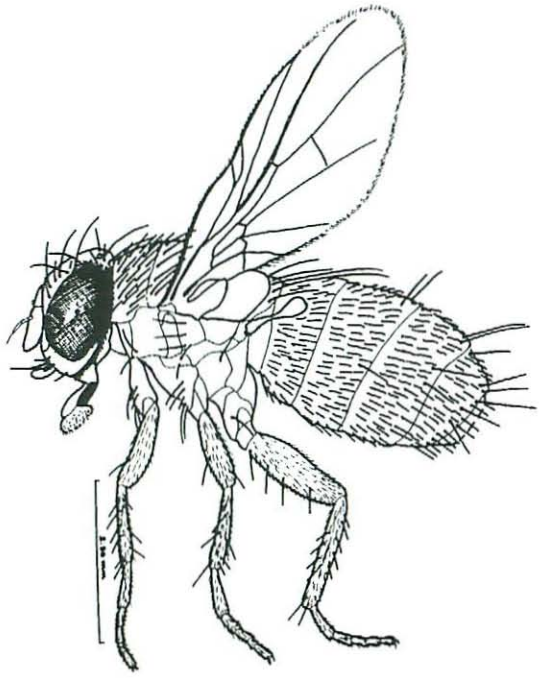


Fig. 1. Medium-size parasitic fly, *Argyrophylax* sp. (Diptera: Tachinidae) reared from soybean pod-borers, *Etiella* spp. (Lepidoptera: Pyralidae). Scale = 1.5 mm.

Fig. 2. Small parasitic fly, *Peribea orbata* (Wiedemann) (Diptera: Tachinidae) reared from larvae of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Scale = 2.5 mm.

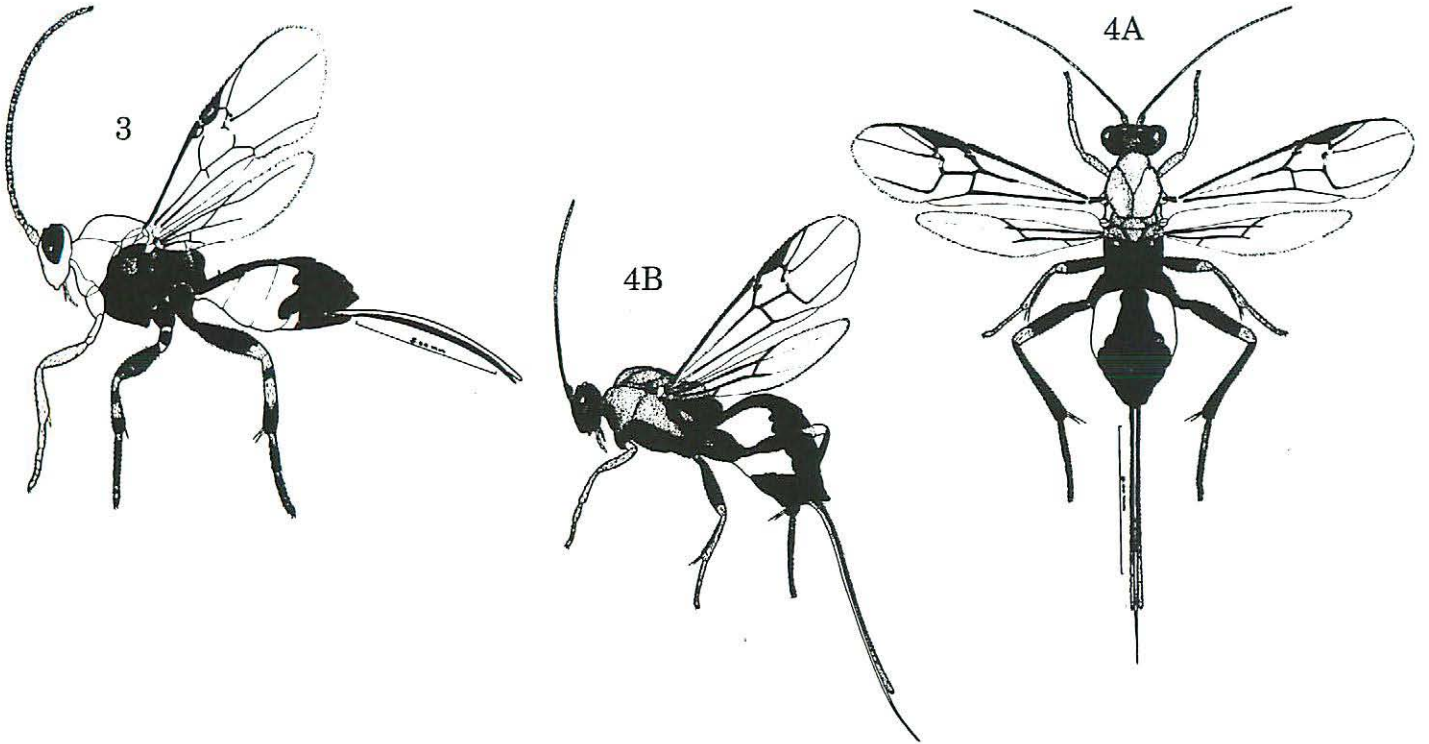
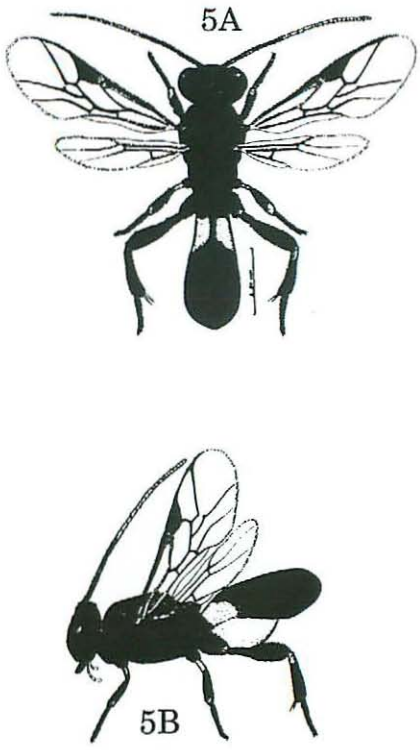


Fig. 3. *Baeognatha* sp. n. A (Hymenoptera: Braconidae) reared from *Etiella* spp. (Lepidoptera: Pyralidae). Scale = 5 mm.

Fig. 4. Dorsal (a) and lateral (b) views of *Baeognatha javana* Bhat and Gupta (Hymenoptera: Braconidae) reared from *Etiella* spp. Scale = 5 mm.



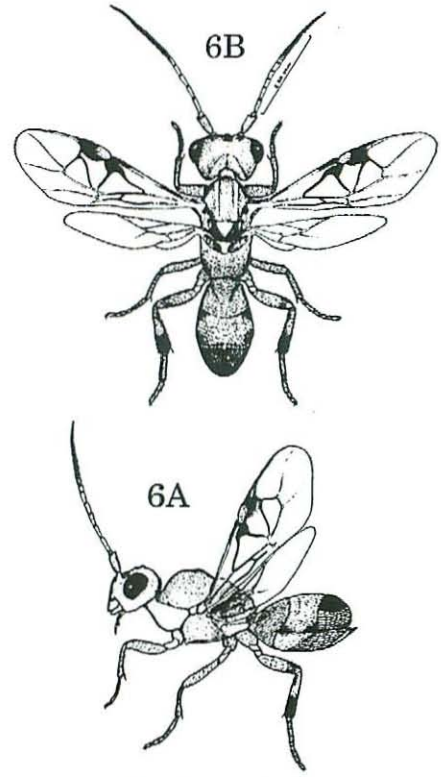
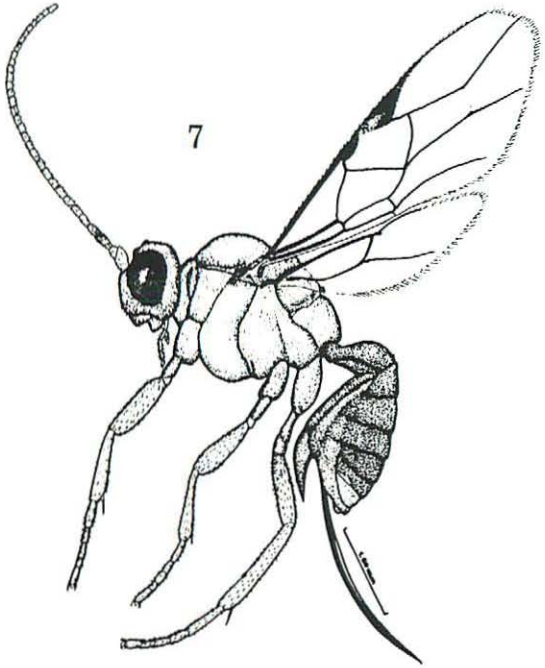


Fig. 5. Dorsal (a) and lateral (b) views of *Chelonus* sp. (Hymenoptera: Braconidae) reared from *Eitella* spp. Scale = 2.5 mm.

Fig. 6. Lateral (a) and dorsal (b) views of *Phanerotoma philippinensis* (Ashmead) (Hymenoptera: Braconidae) reared from *Etiella* spp. Scale = 2.5 mm.



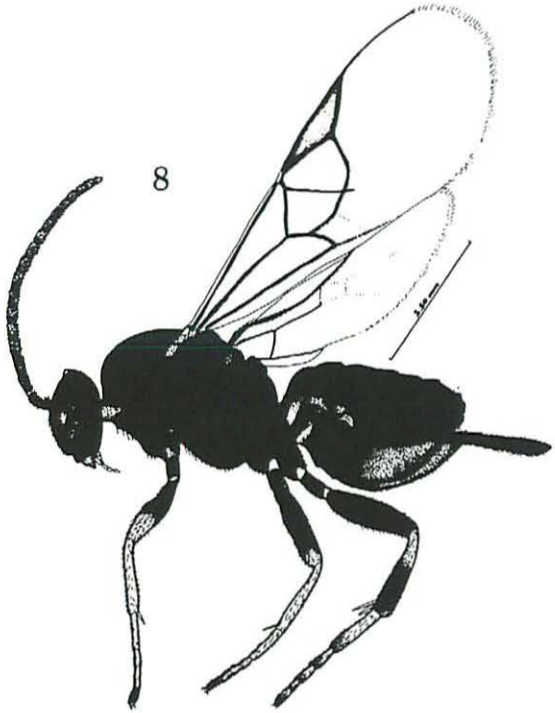
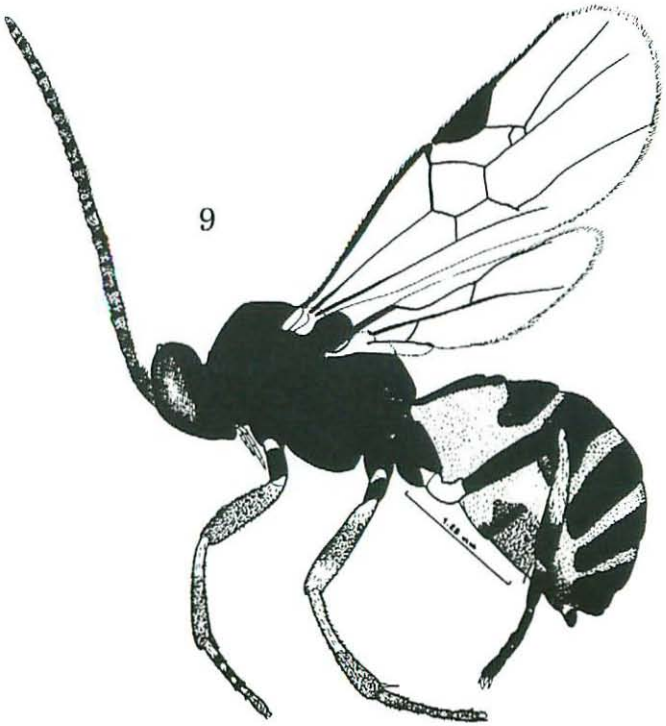


Fig. 7. *Opius* sp. (Hymenoptera: Braconide) reared from *Spodoptera litura* (F.). Scale = 1 mm.

Fig. 8. *Dolichogenidea inquisitor* Wilkinson (Hymenoptera: Braconidae) reared from *Omiodes indicata* (F.). Scale = 2.5 mm.



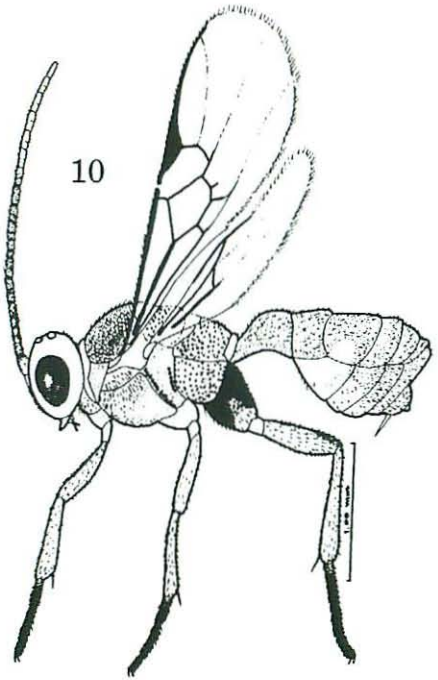
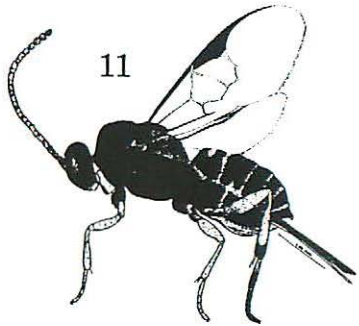
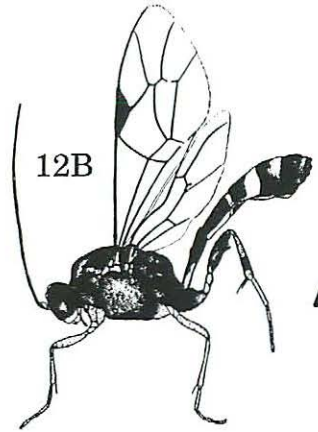
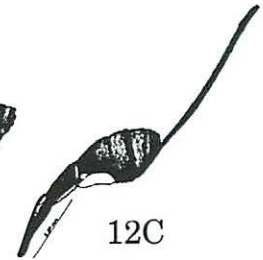


Fig. 9. *Microplitis similis* Lyle (Hymenoptera: Braconidae) reared from *Spodoptera exigua* Hübner, *S. litura*, and *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) (Esper). Scale = 1.25 mm.

Fig. 10. *Glyptapanteles phytometrae* (Wilkinson) (Hymenoptera: Braconidae) reared from *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae). Scale = 1 mm.







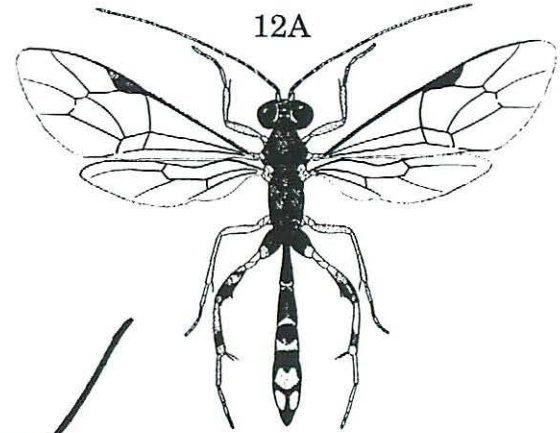


Fig. 11. *Cotesia* sp. (Hymenoptera: Braconidae) reared from *Maruca vitrata* Meyer (Lepidoptera: Pyralidae), *Omiodes indicata* (F.) (Lepidoptera: Pyralidae) and *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae). Scale = 1 mm.

Fig. 12. Dorsal (a) and lateral (b) views of male, and lateral (c) view of female abdomen of *Pristomerus naitoi* Kusigemati (Hymenoptera: Ichneumonidae) reared from *Etiella* spp. (Lepidoptera: Pyralidae). Scale = 2.5 mm.

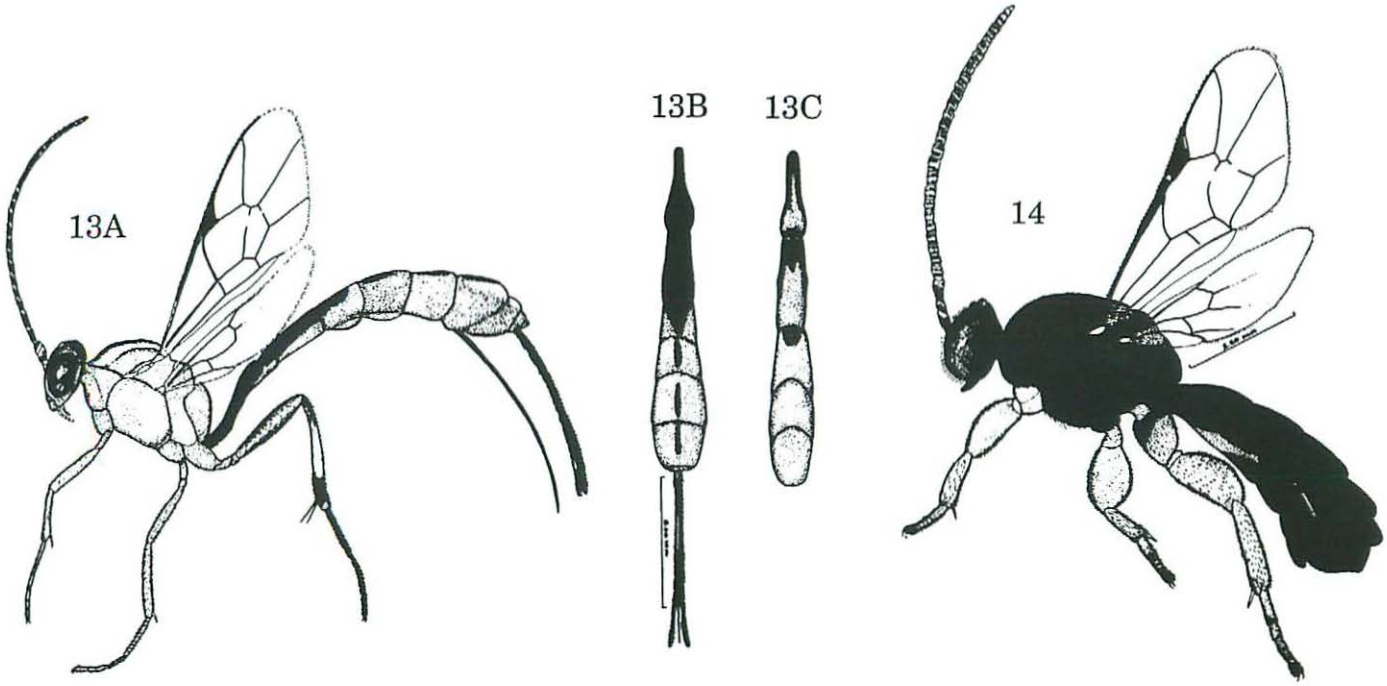


Fig. 13. Lateral (a) and abdominal dorsal (b) view of female and dorsal (c) view of male abdomen of *Temelucha philippinesis* (Ashmead) (Hymenoptera: Ichneumonidae) reared from *Omiodes indicata* (F.) (Lepidoptera: Pyralidae). Scale = 3 mm.

Fig. 14. *Colpotrichia* sp. (Hymenoptera: Ichneumonidae) reared from *Crocidolomia binotalis* Zeller (Lepidoptera: Pyralidae). Scale = 2.5 mm.

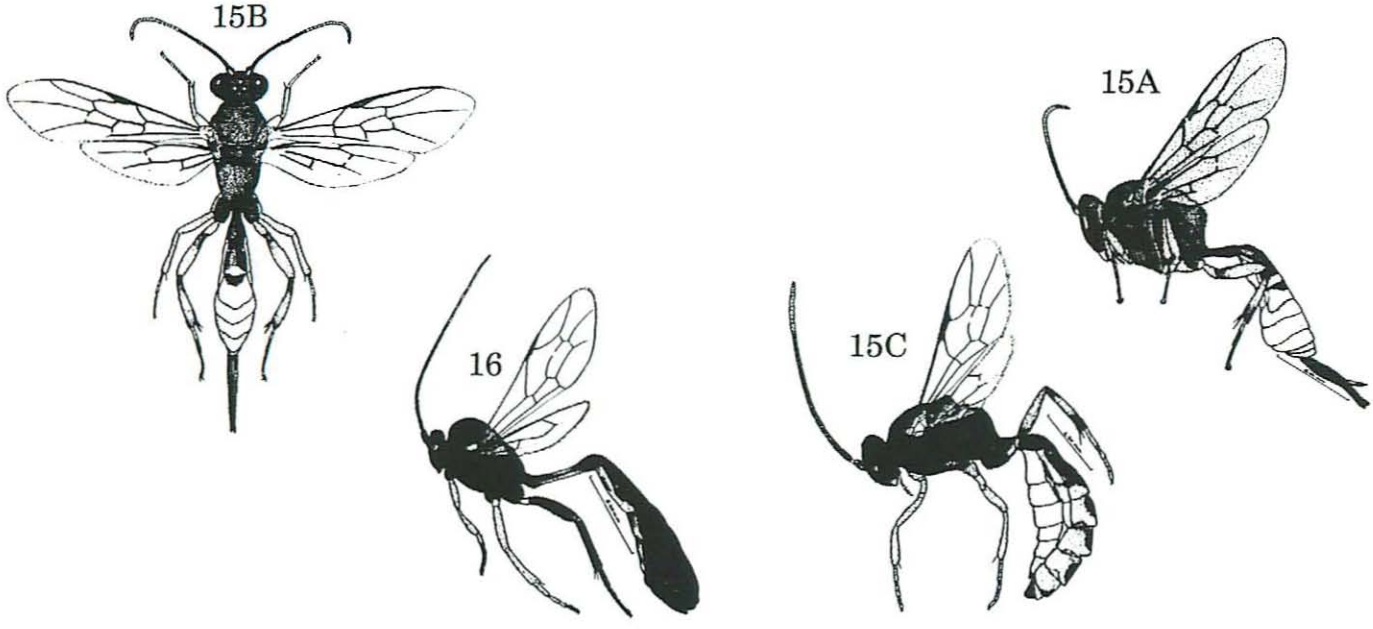


Fig. 15. Lateral (a) and dorsal (b) views of female and lateral (c) view of male *Eriborus argenteopilosus* (Cameron) (Hymenoptera: Ichneumonidae) reared from *Crocidolomia binotalis* Zeller (Lepidotpera: Pyralidae), *Spodoptera exigua* Hübner, and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Scale = 2.5 mm.

Fig. 16. *Charops brachypterum* Gupta & Maheswary (Hymenoptera: Ichneumonidae) reared from *Omiodes indicata* (F.) (Lepidoptera: Pyralidae), *Spodoptera exigua* Hübner and *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Scale = 5 mm.

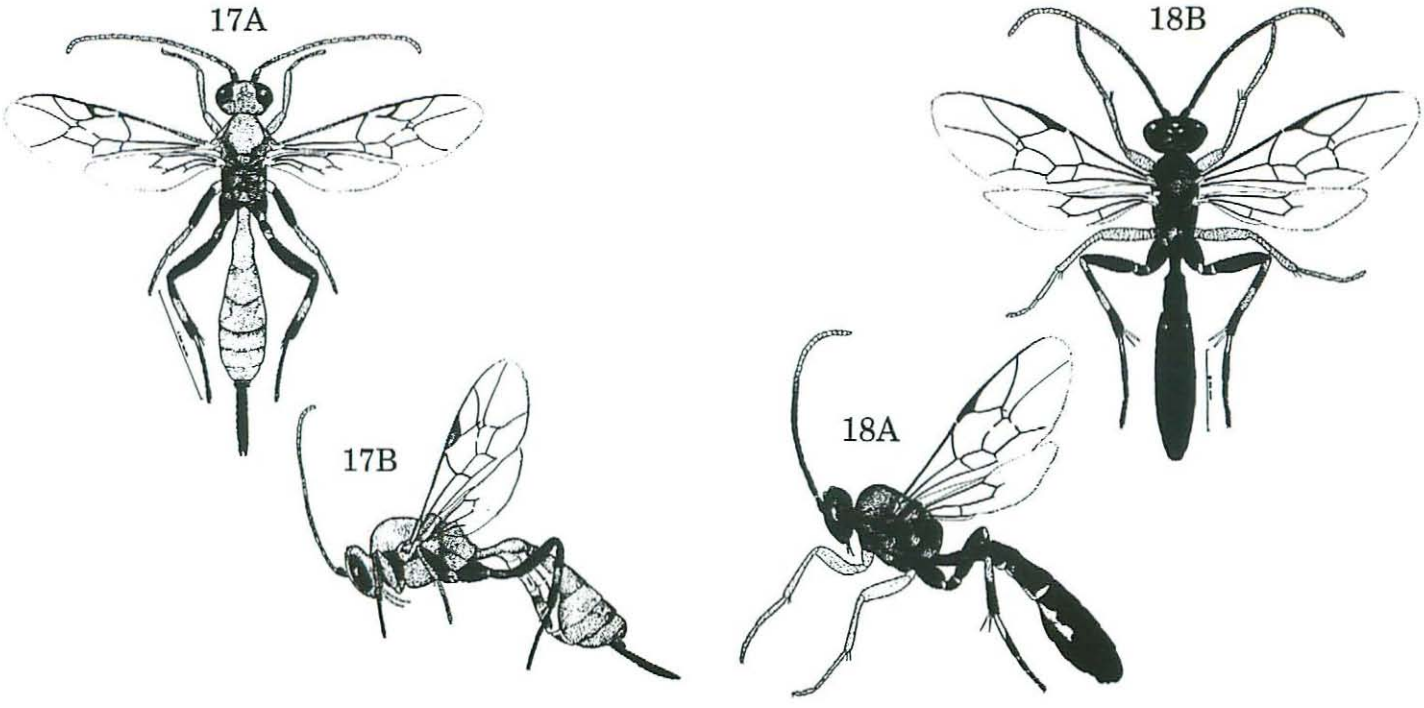
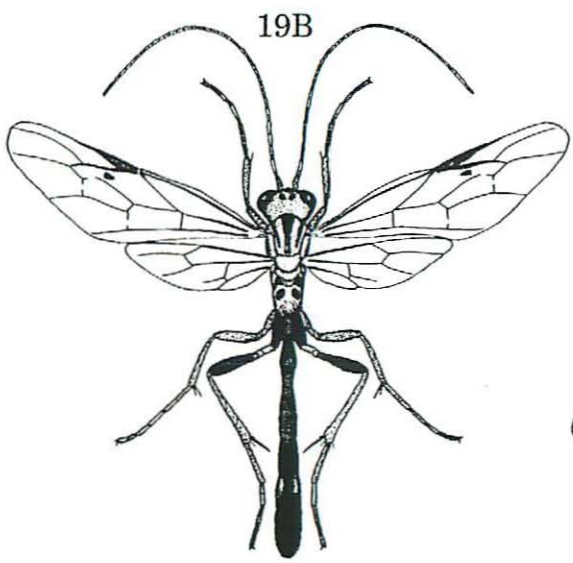
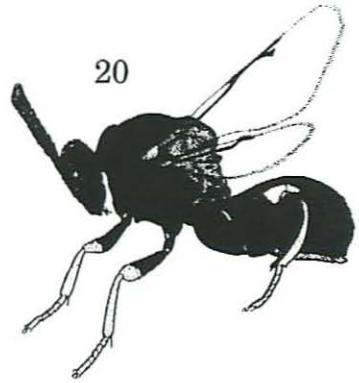


Fig. 17. Dorsal (a) and ventral (b) views of *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae) reared on *Plutella xylostella* (Lepidoptera: Yponomeutidae), *Spodoptera exigua* Hübner and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Scale = 5 mm.

Fig. 18. Lateral (a) and dorsal (b) views of *Diadegma* sp. (Hymenoptera: Ichneumonidae) reared from *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) and *Crocidolomia binotalis* Zeller (Lepidoptera: Pyralidae). Scale = 1 mm.





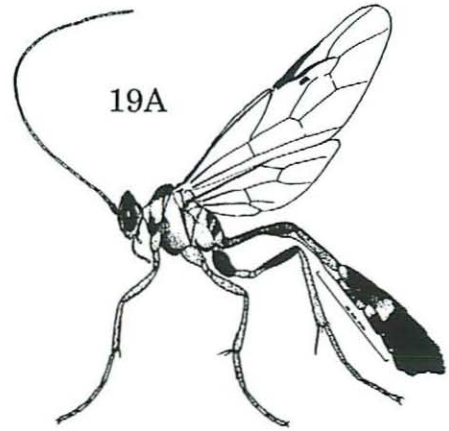
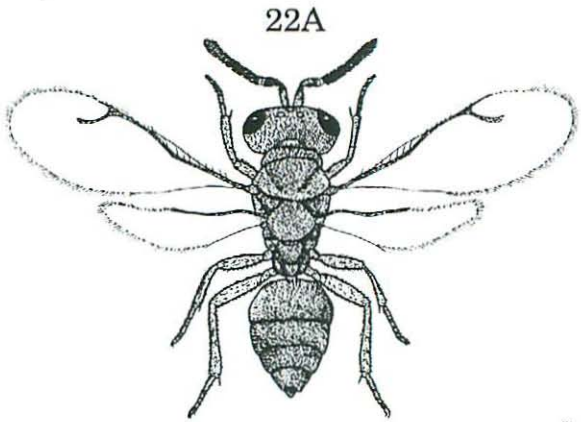
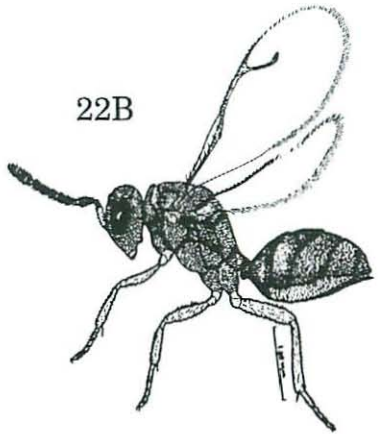


Fig. 19. Dorsal (a) and lateral (b) views of *Enicospilus* sp. n. A (Hymenoptera: Icheumonidae) reared from *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Scale = 10 mm.

Fig. 20. *Brachymeria excarinata* (Gahan) (Hymenoptera: Chalcididae) reared from *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Scale = 2.5 mm.





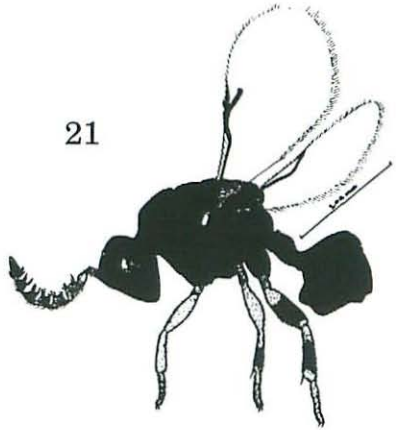
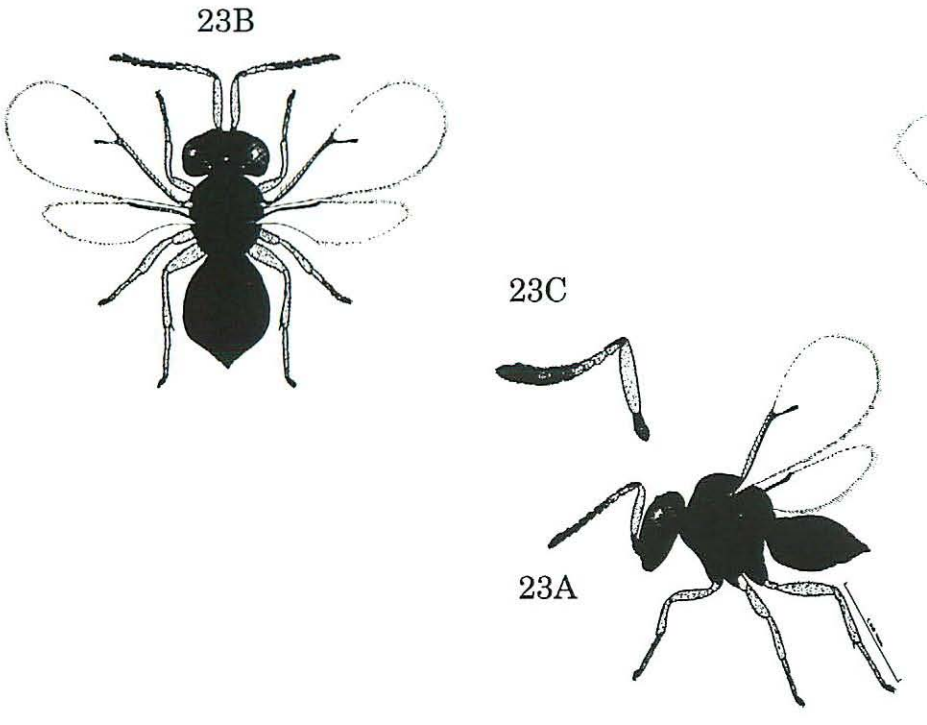


Fig. 21. *Eurytoma* sp. (Hymenoptera: Eurytomidae) reared from *Melanagromyza sojae* Zenhtner (Diptera: Agromyzidae. Scale = 1 mm.

Fig. 22. Dorsal (a) and lateral (b) views of *Trichomalopsis apanteloctenae* Crawford (Hymenoptera: Pteromalidae) reared from *Omiodes indicata* (F.) (Hymenoptera: Pyralidae), *Chrysodeixis chalcites* (Esper), and *Thysanoplusia orchalcea* (L.) (Lepidoptera: Noctuidae). Scale = 1 mm.



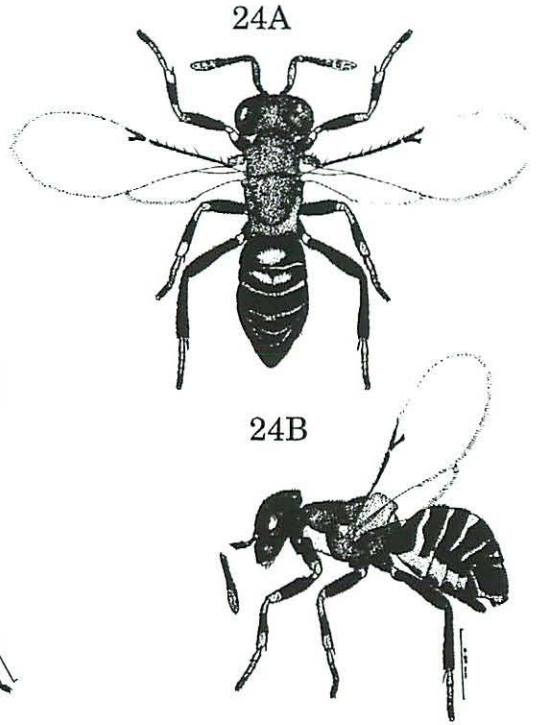
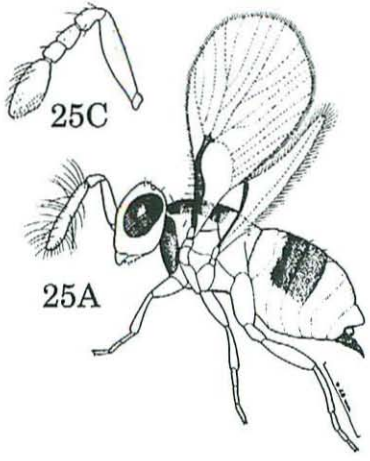
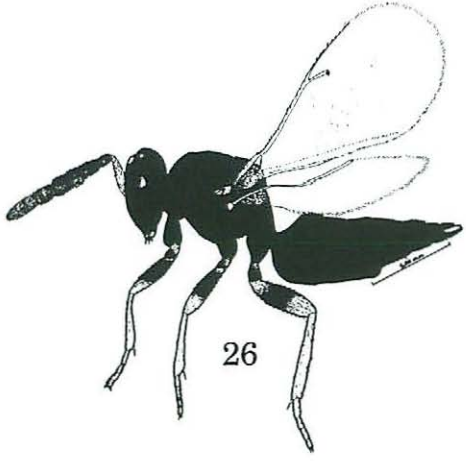


Fig. 23. Lateral (a), dorsal (b), and close-up of female antenna (c) of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) reared from *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Scale = 0.5 mm.

Fig. 24. Dorsal (a) and lateral (b) views of *Copidosoma truncatella* Dalman (Hymenoptera: Encyrtidae) reared from *Thysanoplusia orachalcea* (L.) and *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae). Scale = 0.5 mm.





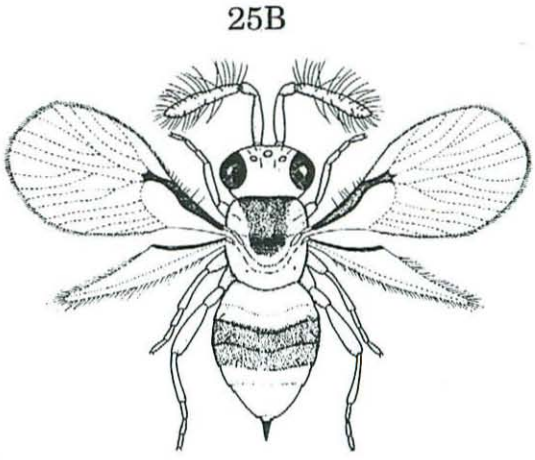
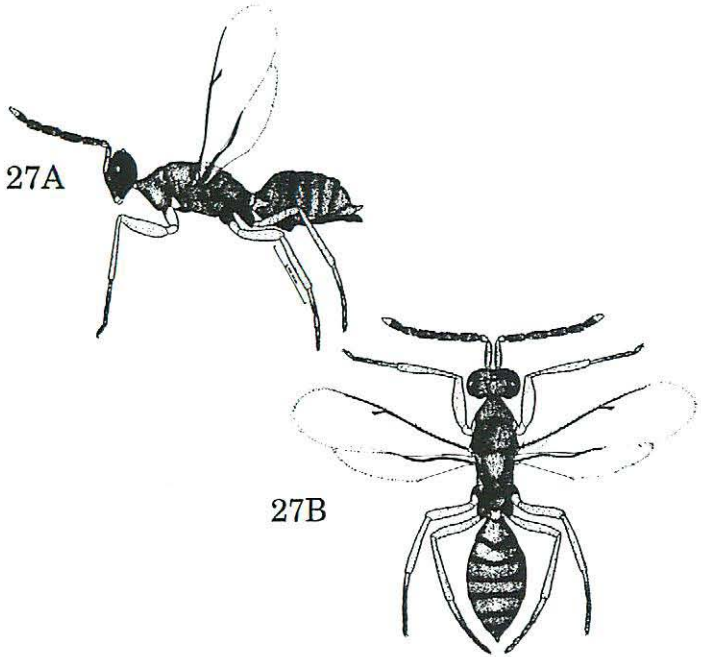


Fig. 25. Lateral (a) and dorsal (b) views of male, and antenna of female (c) of *Trichogramma* cf. *chilonis* Ishii (Hymenoptera: Trichogrammatidae) reared from eggs of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Scale = 0.25 mm.

Fig. 26. *Tetrastichus* sp. (Hymenoptera: Eulophidae) reared from *Melanagromyza sojae* Zenhtner (Diptera: Agromyzidae). Scale = 1 mm.



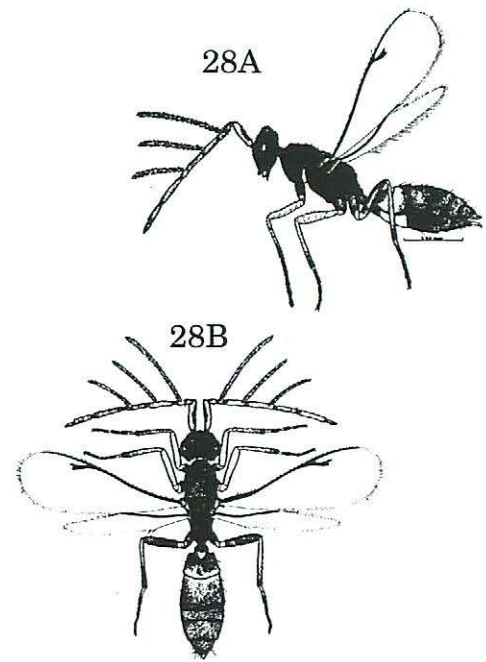


Fig. 27. Lateral (a) and dorsal (b) views of female *Hemiptarsenus varicornis* Girault (Hymenoptera: Eulophidae) reared from *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) and *Phytomyza horticola* (Goureau) (Diptera: Agromyzidae). Scale = 1 mm.

Fig. 28. Lateral (a) and dorsal (b) view of male *Hemiptarsenus varicornis* Girault (Hymenoptera: Eulophidae) reared from *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) and *Phytomyza horticola* (Goursau) (Diptera: Agromyzidae). Scale = 1 mm.

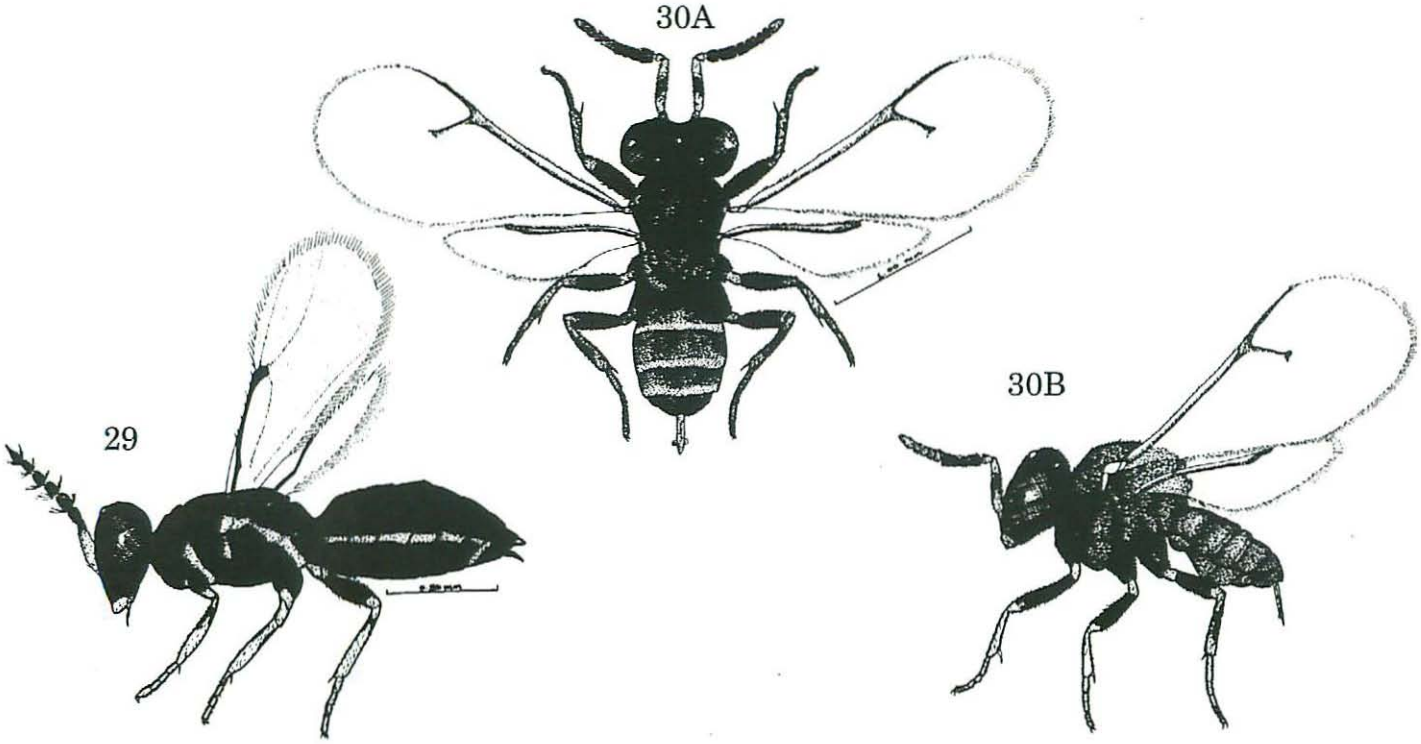
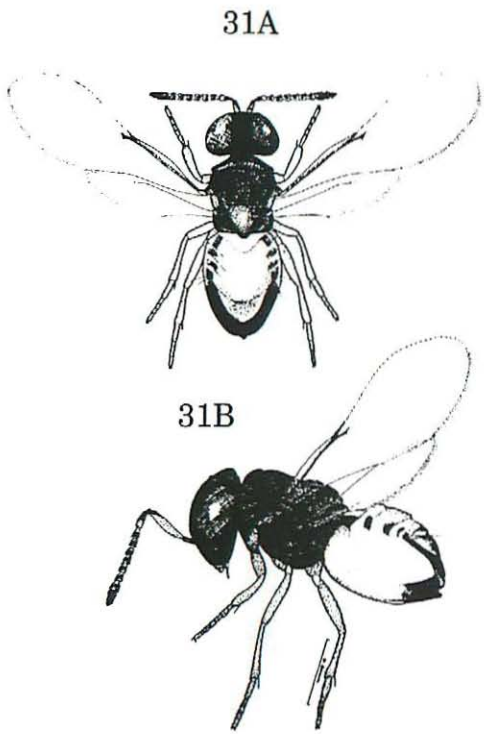


Fig. 29. *Euderus* sp. (Hymenoptera: Eulophidae) reared from *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae). Scale = 0.50 mm.

Fig. 30. Dorsal (a) and lateral (b) views of *Gryon* sp. A (Hymenoptera: Scelionidae) reared from *Piezodorus hybneri* (Gmelin) and *Nezara viridula* (L.) (Hemiptera: Pentatomidae). Scale = 1 mm.



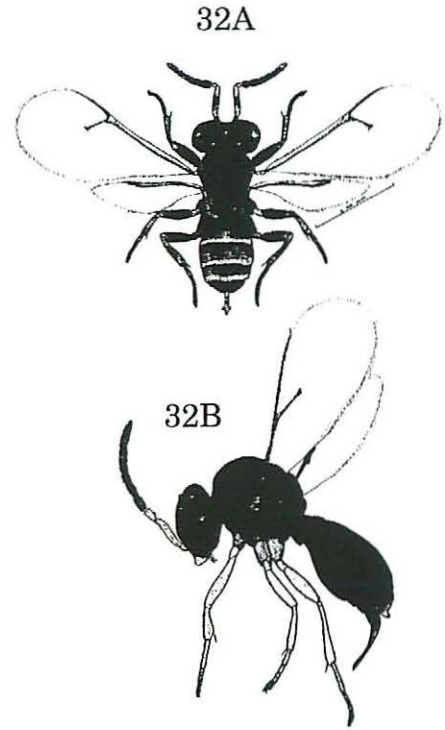
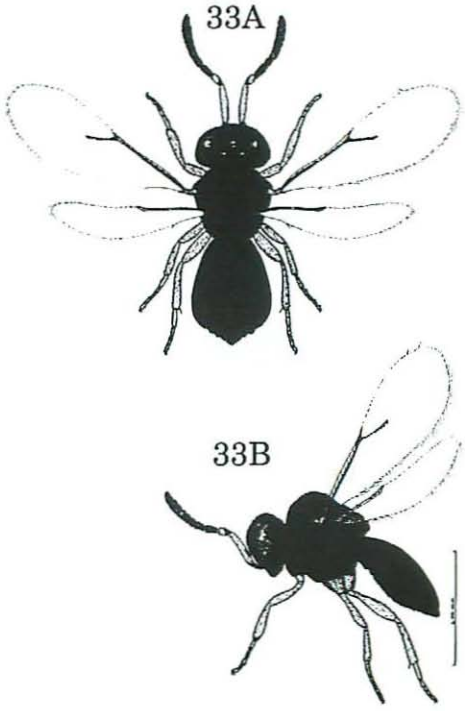


Fig. 31. Dorsal (a) and lateral (b) views of *Ooencytus* sp. (Hymenoptera: Encyrtidae) reared from *Riptortus linearis* L. (Hemiptera: Alytidae), *Piezodorus hybneri* (Gmelin), and *Nezara viridula* (L.) (Hemiptera: Pentatomidae). Scale = 0.50 mm.

Fig. 32. Dorsal (a) and lateral (b) view of *Telenomus* sp. A (Hymenoptera: Scelionidae) reared from *Piezodorus hybneri* (Gmelin) (Hemiptera: Pentatomidae). Scale = 0.50 mm.



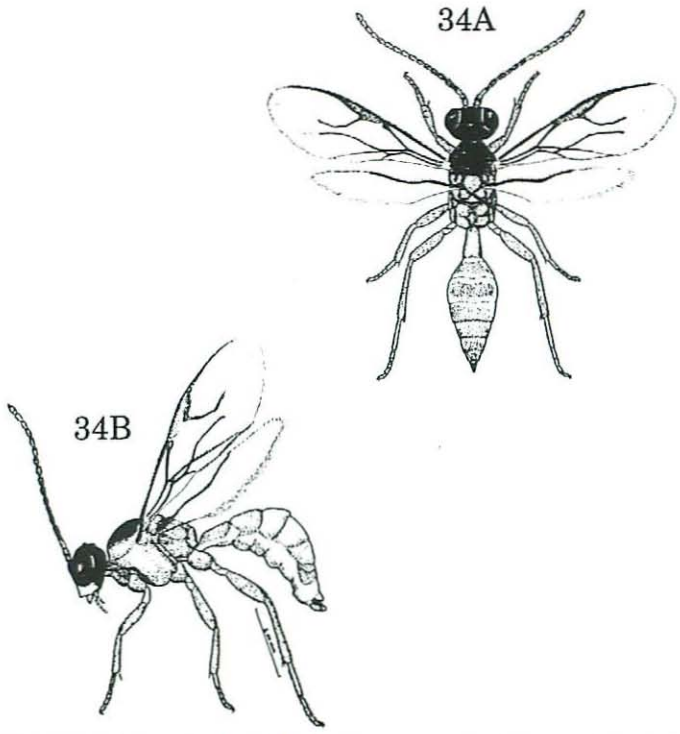


Fig. 33. Dorsal (a) and lateral (b) views of *Telenomus* sp. B (Hymenoptera: Scelionidae) reared from *Nezara viridula* (L.) (Hemiptera: Pentatomidae). Scale = 1 mm.

Fig. 34. Dorsal (a) and lateral (b) views of *Aphidius* sp. (Hymenoptera: Braconidae) reared from *Aphis craccivora* Koch (Homoptera: Aphididae. Scale = 1 mm.

Many of these parasitoids were abundant and were obviously important in suppressing pest populations (Shepard et al. 1998). However, excessive use of pesticides on soybean and especially on vegetable crops, had a major negative impact on parasitoid effectiveness (Shepard & Shepard 1997).

The list of parasitoids reported herein is obviously incomplete and represents only some of the more common species. Additional field surveys and detailed studies to understand the role of these and other species will be important in implementing effective integrated pest management programs in Indonesia.

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Role of Parasitoid Augmentation and Sterile Insect Techniques in Areawide Management of Agricultural Insect Pests[1]

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There is increased interest by pest management scientists and agriculturalists in the areawide management of agricultural insect pests. This increased interest can be attributed to several factors. The development of the sterile insect technique for the eradication and containment of the screwworm, *Cochliomyia hominivorax* (Coquerel), tropical fruit flies (Tephritidae), and tsetse flies (*Glossina* spp.) is a primary reason. The success of the eradication and containment program of the boll weevil, *Anthonomus grandis grandis* Bohemann, in the southeastern cotton-growing regions of the United States and the economic and environmental benefits that have been derived from this successful program provide another important reason. In my view, however, there is a more fundamental reason. It is the gradual realization that managing insect pests on a farm-by-farm basis year after year primarily by the use of insecticides is not a satisfactory solution for major insect pest problems.

Agriculture will be confronted with major responsibilities and challenges in the years ahead. The world's leading scientific academies have projected that the world population will double within 50 yr. This growth in population, together with higher living standards for people in developing countries, will mean that food production will have to be increased by about threefold within the next half century. And it will have to be accomplished on diminishing acres of productive agricultural lands. To meet this goal, agriculture will have to become more productive and efficient in a number of ways.

More effective and less costly insect control procedures will be one of the important ways to meet the responsibilities that agriculture faces. The development of such procedures will be the responsibility of entomologists and scientists in associated disciplines. And we must keep in mind that society will and should demand that insect control be accomplished without compromising the safety of food we eat or the quality of the environment in which we live.

[1]Accepted for publication 14 September 1998.

The issue of safety continues to be a major concern for the insect pest management community. People worldwide are becoming increasingly apprehensive over the environmental hazards and ecological disruptions that are associated with the use of broad-spectrum insecticides. In recognition of this problem, entomologists have devoted major efforts during the past several decades to the development of safer ways to control pests. They have made outstanding progress in this effort.

Insect physiologists and toxicologists have developed a good understanding of physiological processes in insects. They have gained a good understanding of the way insecticides work and how insects develop resistance to insecticides. Entomologists and biochemists have developed a variety of new insect attractants, including sex pheromones. Such attractants are valuable for insect detection and potentially useful for control. Insect microbiologists have discovered many pathogens that could be used as biological insecticides. Biologists working on classical biological control have discovered and introduced new biological control agents and have devoted more effort on the augmentation of biological control agents. Insect behaviorists have learned much about the way that biological agents find their hosts and prey. Plant geneticists and entomologists have concentrated more research effort on the development of host plant resistance to insects and other pests. Insect geneticists and biologists have discovered genetic mechanisms that are potentially more effective than sterile insects for inhibiting insect reproduction.

Insect rearing experts have made outstanding progress in insect rearing methods. Virtually all insect pests and many insect parasitoids and predators can be reared under laboratory conditions. Remarkable progress has been made in the mass production of a number of insects—hundreds of millions per week can be produced and at low cost. I highlight this important development because insect rearing technology is basic to the use of the sterile insect and parasitoid augmentation techniques for areawide management.

Despite such outstanding progress, very little of the new information and technology is being put into practice. After 40 yr of intensive efforts on alternative ways to control insects, growers are still largely dependent on insecticides for the control of insect pests.

Most insecticides have broad-spectrum activity and are therefore potentially hazardous. To minimize the hazard problem a supervised system of insect management evolved that is broadly defined as integrated pest management (IPM). Its primary objective has been to take advantage of the merits of insecticides but to use them more judiciously. This management system has achieved a number of objectives. It has virtually eliminated the unnecessary and indiscriminate use of insecticides. Economic treatment levels have been established for many insect pests and the crops they damage. By following the guidelines farmers gain by not applying control measures until it is profitable to do so. They also avoid the heavy losses that insects can cause if they are not controlled at the proper time. Growers seem to be pleased with the management system that has been developed. Why then has so little of the new technology been put into practice? The reasons become clear when we consider the dynamics of insect pest populations and analyze the nature of the management system that has evolved and the suppression characteristics of the alternative methods of control that have been investigated.

The IPM system is a defensive-reactive management procedure. Under this management procedure the alternative methods cannot accomplish all the things that broad-spectrum insecticides accomplish. Most of the alternative methods of control act too slowly to result in satisfactory control after the pests have already reached damaging levels. Some of the methods such as the parasitoid augmentation technique, sterile insect technique, and pheromone mating disruption are not practical after the pest populations have already reached high levels. Also, few of the alternative methods are available to growers. In essence, the application of fast-acting insecticides is about the only option that is available to growers.

So, where do we stand today and what does the future hold for alternative methods of insect control that might be less hazardous and less costly to use? After analyzing the dynamics of many of our major insect pests and the suppression characteristics of virtually all methods of control, my response to that question is that we must develop and implement new strategies for dealing with the major pests. The reality is that the largely defensive-reactive management system that has evolved, despite its merits, is not a biologically sound management system. It gives insect pests too many advantages. Let's analyze its major shortcomings from the standpoint of the dynamics of insect pests.

Insect pest populations can increase from their normal low level to their normal high level virtually unhampered year after year. Growers are encouraged to use supplemental control measures such as cultural practices and to grow resistant varieties. These methods, however, are not applicable for some pests and are not very effective or dependable if only a small proportion of the growers follows the recommended supplemental procedures.

When a pest population reaches an unacceptable damage level, which will be inevitable every year for many of the pests, about the only option that growers will have is to apply fast-acting insecticides. Because most of the insecticides have broad-spectrum activity, this defeats one of the primary objectives of the IPM system—to take maximum advantage of natural control agents to deal with pest problems. When a high proportion of the natural control agents is destroyed along with the pests, this results in a higher degree of reproductive success of surviving target pests. It also results in a higher degree of reproductive success of nontarget pests that are generally maintained below significant damage levels by natural control agents.

The cost of control by the use of insecticides is high. It is costly not only because of the cost of the treatments but also because it concedes to pests the losses that fall below the economic treatment levels. For many pests such as the *Heliothis/Helicoverpa* complex it is probable that at least half of the losses they cause falls below the cost of control by the application of insecticides. The same is true for the European corn borer, *Ostrinia nubilalis* (Hübner). These two pest problems alone cause losses that amount to about $2 billion per year. What the total losses are for all pests that fall below the current economic treatment levels is difficult to say, but it will amount to several additional billions of dollars per year. These rough estimates should be kept in mind because they give us a good idea of the amount that agriculture could afford to invest on less costly areawide methods of control that will be discussed in this article.

Despite the benefits that are being derived from the current system of insect control, it is not a sound management procedure. It is a no-win strategy. In support of this statement, if a list were made of all the insect pests that were of major importance to agriculture 50 yr ago, before the current insecticides came into being, and a similar list were drawn up today most of the same insects would be on both lists—except I am sure that the current list would be considerably longer.

Only two important pests would not be on the current list—the screwworm in all of the United States and the boll weevil in the southeastern cotton-growing regions. Significantly, they are the only two insect pests that have been subjected to organized areawide management programs during the past 50 yr.

In recognition of the limitations of the current methods of insect pest management, I have long been a proponent of the areawide pest management procedure. With the help of many of my colleagues I have analyzed the dynamics of most of our major insect pests. I have analyzed the suppression characteristics of all known methods of insect control. In the process, I have recognized several principles of insect population suppression and kinds of suppressive actions that previously had not been recognized or were poorly understood. The sterile insect technique and how it works was an outgrowth of such investigations. But I feel that the investigations on the principles of insect parasitism have yielded information of much greater practical significance.

The purpose of this article is to describe the suppressive actions that result from the use of these two procedures. I shall attempt to show why they offer so much promise for dealing with insect pest problems if they are used on an areawide basis. Because of space limitations no effort will be made to review the extensive literature or make reference to the outstanding contributions that many scientists have made that have relevance to the areawide approach to insect pest management by the use of the two biological control procedures alone or when integrated.

Fundamental Principles of Insect Population Suppression

How to manage insect pest populations in the most effective manner requires a good understanding of some of the basic principles of insect population suppression. In a broad sense we can classify insect pest management procedures into the two categories that we have already discussed—the largely defensive-reactive procedure of dealing with the pest problems on a farm-by-farm or crop-by-crop basis as they appear and the preventive strategy that is designed to maintain total insect pest populations below significant damaging levels.

The various methods of insect control that have been devised result in two types of suppressive action—those that achieve about the same degree of control whether the pest populations are high or low, and those that vary in effectiveness depending on the abundance of the pests. Until the sterile insect technique came into being the existence of methods of control that become increasingly more effective with declining pest densities was not recognized. We now know, however, that the effectiveness of the parasitoid augmentation technique also is greatly influenced by the abundance of the pests. The abundance of the

pest also influences the effectiveness of sex pheromone mating disruption, traps, and attracticide baits. This article, however, will not include an analysis of the principles of suppression by the use of insect attractants.

The availability of methods of control that differ in their efficiency depending on the density of pest populations has great practical significance. It offers the science of insect pest management the opportunity to integrate different methods of control and achieve greater efficiency than can be achieved by one method alone.

Three types of suppressive action will result when different methods of insect control are appropriately integrated: (1) additive suppressive action, (2) enhanced suppressive action by one of the methods, and (3) mutually synergistic suppressive action by both techniques.

The concurrent use of two methods of control that have pest-density-independent suppressive action such as the use of insecticides, cultural measures, or other methods will result in an additive effect. The concurrent use of a method of control that has density-independent suppressive action and one that becomes more effective at low densities will result in enhanced suppressive action by the latter method. For example, if sterile insects were to be used alone the release of enough sterile insects to achieve an effective sterile-to-fertile ratio of 4:1 will achieve 80% control. If, however, some simple cultural measures were first used that reduced the total pest population by 50%, the release of the same number of sterile insects would then achieve a sterile-to-fertile ratio of 8:1. This will inhibit reproduction by 89%. The combined control would be 94.5%.

The concurrent use of two methods of control that become more effective with declining pest densities will result in what I have referred to as mutual synergistic suppressive action. The concurrent release of sterile insects and a pest-specific parasitoid will produce this type of action. Much more will be said about synergistic suppressive action. It can result in a very high degree of control.

Dynamics of Insect Pest Populations

A good knowledge of the demographics and dynamics of insect pest populations is fundamental to optimum success of all methods of insect control. It is especially important, however, for managing insect pest populations on an areawide basis. The feasibility of managing insect pests by the parasitoid augmentation and sterile insect techniques is largely dependent on the actual number of the pests that exist per unit area and time during their seasonal or periodic cycles. It is also helpful to know the probable growth rates of insect pest populations from their normal low to their normal high levels. Such quantitative information is not a critical matter for successful control of insect pests by the use of insecticides and other methods of control that have suppressive actions independent of the pest density, but it is of major importance in making use of methods of control that are influenced by the pest density such as the sterile insect and parasitoid augmentation techniques.

Accordingly, I have devoted much time and effort to estimating the actual number of insects that are likely to exist in natural populations for the species that are considered good candidates for areawide management by the two tech-

niques. Obtaining information on the abundance of insects in natural populations has been one of the most neglected aspects of insect population ecology. It has been necessary, therefore, to rely heavily on indirect procedures for obtaining such information for various pest species.

The number of insects that exists in natural populations is highly variable and the growth rates are influenced by many factors. It may seem futile to attempt to quantify the dynamics of a pest by deductive procedures; however, this is not the case. Biologists have obtained considerable information on the abundance of one or more of the life stages of most of the more important insect pests in their investigations on methods of control. Such information together with a recognition of some of the fundamental principles that govern the dynamics of insect pest populations makes it possible to estimate numbers of insects in natural populations that are not likely to deviate from true numbers by a wide margin.

A simple model (Table 1) depicts the estimated number and population trends of many of our major insect pests. Such models may not be highly accurate in some cases, but they are still very useful. They are almost essential for estimating the coexistence pattern and the host-finding efficiency of insect parasitoids. Their primary use, however, is to calculate the influence of different methods of control on the dynamics of the pest populations. I question if it would have been possible to recognize the type of suppressive action to expect from various methods of control when used alone or when integrated without the aid of such models.

Table 1. Demographics and dynamics of a typical insect pest population as it develops during a crop-growing season.

	Generation			
Parameters	1	2	3	4
Normal females	1,000	4,000	10,883	18,937
Normal males	1,000	4,000	10,883	18,937
Larvae produced per female[a]	80	68	58	49
Total larvae	80,000	272,000	631,214	933,594
Percentage of survival, larvae to adults	10	8	6	4.5
Adults next generation, both sexes[b]	8,000	21,760	37,873	42,012
Increase rate	4.0	2.7	1.7	1.1

[a]The density-independent control factors such as weather conditions are assumed to be normal and favorable. The density-dependent factors such as biological agents are assumed to intensify by about 15% each generation as the population grows.

[b]The progeny of the fourth generation are assumed to be the overwintering population. If the probability of winter survival is 5%, the population the next spring would again be near 1,000 adults of each sex. The model is considered representative of a steady density population.

Insect pests differ in the number of generations that occur per year or during periodic cycles. The number of insects per unit area will vary with the species and of course with the conditions that exist in different ecosystems. Authorities on various insect pests could, with suitable modifications, develop models that reasonably represent the demographics and dynamics of the pests in which they are interested. There is usually enough known about the abundance of some of the life stages to develop models that reasonably reflect the dynamics of a pest population in numerical terms.

Several characteristics of insect pest populations are very meaningful. One is the many hazards that all insects experience before they complete their life cycles. Most insect pests have the capability of producing many progeny. Such pests as the corn earworm [*Helicoverpa zea* (Boddie)], various tropical fruit fly species, and others are capable of producing 1,000 or more progeny during their lifetime. Even when conditions for reproduction are favorable, however, the growth rate of well-established species is not likely to exceed an average higher than threefold per generation. The average increase rate of the hypothetical population is only about 2.5-fold per generation. This tells us how important natural control factors are and why it is so important to use control measures that will permit natural control agents to perform their normal function. It also provides a good explanation why the extensive use of broad-spectrum insecticides has created such chaotic conditions in our agroecosystems. It explains why virtually all of our important pests have continued to be major threats to agriculture for the past 50 yr despite the intensive use of insecticides that may kill a high proportion of the pest population each year during one or two of the pest cycles. If this is done by the use of broad-spectrum insecticides the average increase rate of the pest population may be doubled. This is a major difference. A pest population increasing at a threefold average rate per generation for three cycles will grow by 27-fold. A population increasing at a sixfold rate per cycle will increase by 216-fold in three generations. Is there any wonder why we have not reduced the threat of many of our major pests and why new pests such as whiteflies (*Bemisia* spp.) can create such havoc in our vegetable-producing areas? If half or more of the spiders and insect predators cannot survive in an agricultural environment, the reproductive success of many of the pests might be as high as 5- to 10-fold per cycle.

A good understanding of the dynamics of insect pest populations is basic to the logic of dealing with total insect pest populations by methods of control that are target-pest specific. The relatively low increase rate of most insect pests clearly indicates that if we use pest-specific control measures it will not require a high degree of control each pest cycle provided it is applied with consistency and with reasonable uniformity throughout the pest ecosystem. If the increase rate of a pest population is not likely to average more than threefold per cycle and if reproduction is inhibited each generation by as much as 67% the population will not increase during the season. But this is only the first benefit of the total population management procedure. For most pests the natural mortality is very high during the winter or during similar hostile periods in tropical areas. So, if a population cannot achieve its normal increase during a season, the total population at the beginning of the next season might be reduced by as much as 90% or more. Then, if pest-specific suppressive measures that become

more effective as the pest population declines are again used, effective control can be achieved the next year at only a fraction of the first year's costs. In contrast, insecticide applications will cost about the same whether the pest populations are high or low.

The foregoing discussions are elementary, but they are fundamental to sound insect pest management procedures. The point I want to emphasize is that areawide pest management procedures can be used that will not only be low in cost but also will avoid the chaotic conditions that we create in our agrosystems by relying primarily on broad-spectrum insecticides for the control of insect pests.

Suppression Characteristics of Insecticides

In the sections to follow I will describe the influence that different methods of control will have on the dynamics of insect pest populations. The basic model (Table 1) that has already been described will be used for this purpose. I will first show how insecticides influence the dynamics of insect pest populations.

The model in Table 2 depicts the influence of the application of a pest-specific insecticide each pest generation when 90% of the insects is controlled. It may require three to four insecticide applications during each generation to achieve this level of control. If a pest-specific insecticide were used the natural control agents could perform their normal function. And if each reproducing female insect produced 10 adult progeny the pest population would decrease by 50% each generation. On that basis it would be necessary to apply control measures for three to four pest generations before the population would be reduced by 90% and as many more generations to eliminate the population.

Table 2. Influence on the dynamics of a pest population if pest specific insecticides are used to inhibit reproduction by 90% each pest generation.

	Generation			
Parameters	1	2	3	4
Female insects	1,000	500	250	125
Pest control by insecticides	90	90	90	90
Females reproducing	100	50	25	12.5
Adult progeny present per reproducing female[a]	10	10	10	10
Adults next generation, both sexes	1,000	500	250	125

[a]The increase rate of the survivors is assumed to be fivefold.

Table 3. Influence of broad-spectrum insecticide applications on the dynamics of a pest population when used to avoid damage to a crop during the critical growing period.

	Generation			
Parameters	1	2	3	4
Female insects	1,000	4,000	4,000	4,000
Insecticide applications	None	Yes	Yes	None
Percent control	-	90	90	-
Females reproducing	1,000	400	400	4,000
Adult progeny per reproducing female	8	20	20	20
Adult progeny, next generation, both sexes	8,000	8,000	8,000	80,000[a]

[a]If 5% of the potential overwintering population survives the winter, the population the next year would start at a level of 2,000 of each sex instead of 1,000 of each sex as shown for an uncontrolled population in Table 1.

The model shown in Table 3 reflects the results to expect if a broad-spectrum insecticide were used to control a pest during the period when it is most susceptible to damage. Before the new insecticides came into being insecticides were not generally used to control pests such as the boll weevil, tobacco budworm [*Heliothis virescens* (F.)], and the pink bollworm [*Pectinophora gossypiella* (Saunders)]. Without control the yield of cotton probably was reduced by 25 to 33%. After the new, very effective insecticides became available, growers began to apply insecticides, perhaps during the second and third generations. The damage during the first generation when the population is still low was usually not enough to justify the application of control measures. But the application of insecticides during the second and third generations maintained the population below significant damage levels. In the process, however, a high proportion of the natural control agents was destroyed along with the pest; therefore, the reproductive success of the surviving pests was higher, although satisfactory control was achieved. But, after the main crop was produced, the application of insecticides would be discontinued. This permitted reproduction by the pests to continue for one or two generations in an environment devoid of many of the natural control agents. The result would be that the target pest population could increase to a higher level than it would if no control measures were applied (Table 1). Also, because of the reduction of the natural control agents, the reproductive success of certain nontarget pests that normally would be controlled by the natural control agents would be high enough to increase their populations above normal levels.

The model shown in Table 3 is hypothetical, but it demonstrates in principle what has been going on in our agroecosystems for the past 50 yr. The insecticide applications were reducing losses to a minimum. Growers have profited by their use, but it supports the statement that I made earlier. The current management system is not biologically sound. And so long as broad-spectrum insecticides are

a major component in pest management systems ecologically sound insect pest management will not be possible.

Suppression Characteristics of the Sterile Insect Technique

When the sterile insect technique was developed it provided a new principle of insect pest suppression. The degree of suppressive action it causes is influenced by pest density. This is particularly relevant to the areawide management procedure.

Following the success of the pilot test against the screwworm conducted on the island of Curacao, the technique was developed and applied for the eradication of this major pest of livestock and wild animals from the southeastern United States and later from the southwest and all of Mexico. This program has saved the livestock industries in the two countries billions of dollars during the past 40 yr.

The success against the screwworm prompted scientists to investigate the possibility of eradicating or managing other important insect pests. This has led to its use against several tropical fruit fly species, including the Mediterranean fruit fly (medfly) [*Ceratitis capitata* (Wiedemann)], melon fly (*Biosteres cucurbitae* Coquillett), oriental fruit fly (*Biosteres dorsalis* Hendel), and the Mexican fruit fly [*Anastrepha ludens* (Lowe)]. As a complex, the tropical fruit fly species are the world's most damaging pests of fruits and vegetables. The research leading to the use of the sterile insect technique for dealing with tropical fruit flies has been conducted largely by federal and state scientists in Hawaii, by scientists in Japan, and by scientists associated with the International Atomic Energy Agency (IAEA) in Vienna, Austria. The IAEA scientists also have made substantial progress largely due to research on use of the technology for the eradication of tsetse fly populations. Tsetse flies, probably the world's most important livestock insect pests, are a major obstacle to the development of a sound agricultural economy in much of Africa. Progress also has been made against several other insect pests. Federal and state pest control agencies have made use of the sterile insect technique to prevent the establishment of the pink boll worm in the San Joauquin cotton-growing area in California. Canadian scientists are using the sterile method for the eradication of the codling moth, *Cydia pomonella* (L.). Thus, the sterile insect technique has come into use for dealing with a number of important insect pest problems. But its development has been slow. Intensive research was conducted on its possible use against a few other insect pests. A major effort was made against the boll weevil. This effort was not very successful. The sterilization treatment has serious adverse effects on the competitiveness of this pest. Another reason for its slow progress has been the difficulty that scientists have experienced in demonstrating that the technique will be effective. Research budgets are seldom sufficient to conduct trial experiments against total pest populations or on a large scale. Release experiments conducted against unisolated populations will invariably fail to achieve eradication or a high degree of suppression, resulting in a lack of confidence in the technique.

In my opinion, the main reason for the slow progress in developing the technique has been the lack of support and even a great deal of opposition by the

pest management community to the total pest population management concept. This opposition seems to be declining, but it is still a major obstacle to the development and widescale use of the technique.

In my view there are a number of other insect pests that would be good candidates for management on an areawide basis by the sterile insect technique and for eradication if the pest populations are well isolated. I have in mind such pests as the *Helicoverpa/Heliothis* complex (corn earworm and tobacco budworm), European corn borer, sugarcane borer [*Diatraea saccharalis* (F.), all of the tropical fruit fly species, fall armyworm [*Spodoptera frugiperda* (J.E. Smith)], diamondback moth [*Plutella xylostella* (L.)], cabbage looper, [*Trichoplusia ni* (Hübner)], and a number of other species.

When we critically analyze the feasibility of dealing with various insect pest problems, it becomes apparent that there are few options that offer the potential economic and environmental benefits that the sterile insect technique offers if the technology is perfected, and the technique is applied on an areawide basis. The sterile insect technique has its limitations, but it also has far greater potential for dealing with many important insect pest problems in an effective, low-cost, and ecologically acceptable manner than is generally realized.

Although the type of suppressive action that is produced by the sterile insect technique is reasonably well understood, there still seems to be considerable lack of understanding of the way the technique works. Therefore, I developed the model shown in Table 4. It depicts the influence that sterile insect releases will have on the dynamics of a pest population of the nature shown in Table 1. Subsequently, the results can be compared with the influence of the release of parasitoids in pest ecosystems.

It is assumed that 9,000 fully competitive sterile males and 9,000 sterile females will be released during the first generation to achieve a sterile-to-fertile

Table 4. Influence of sterile insect releases on the dynamics of a pest population.

	Generation			
Parameters	1	2	3	4
Normal females	1,000	500	132	10
Normal males	1,000	500	132	10
Sterile insects released, both sexes	18,000	18,000	18,000	400
Sterile-to-fertile ratio	9:1	18:1	68:1	21:1
Normal females mated with normal males	100	26.3	2	0
Expected adult progeny per normal mated female	10	10	10	-
Adult progeny, next generation, both sexes	1,000	263	20	-

ratio of 9:1. Therefore, the probability that a normal female will mate with a normal male is only 10%. This means that only 100 of the 1,000 females will reproduce. Each normal female mating is assumed to result in 10 normal progeny. Therefore, the population during generation 2 will decrease by 50%. Then the release of 9,000 sterile males and 9,000 sterile females will achieve a sterile-to-fertile ratio of 18:1 and the probability of a fertile mating will be only about 5%. This will accelerate the decline in the population and the continued release of 9,000 sterile males and 9,000 sterile females during generation 3 will result in such a high sterile-to-fertile ratio that the probability of a fertile mating will be near zero. The model shown is a classic example of the type of suppressive action that will be achieved by the sterile insect technique as first described by the author. That type of action has been confirmed time and again against a number of insect pests.

It is axiomatic, however, that to achieve strong suppression or eradication of pest populations the technique used must be applied on an areawide basis and with reasonable uniformity and consistency each pest cycle until the objective is achieved. Until this requirement is fully realized the areawide pest management system by any control procedure will be slow to develop.

Fundamental Principles of Insect Parasitism

I will as briefly as possible give my views on the fundamental principles that govern natural parasitism processes and the types of suppressive actions that are involved when parasitoids are used for augmentation purposes. This discussion will be largely a condensation of a more detailed treatment of the subject in the U.S. Department of Agriculture Handbook 693 (Knipling 1992).

Nature has created thousands of parasitoids that are primarily or totally dependent on a given host or closely related host complex for their survival. They are one of agriculture's most important natural resources. Without their presence the control of many crop pests would be much more difficult or even impractical. But developing on their own they are incapable of keeping many pests below damage levels. Therefore, if such parasitoids are to play a prominent role in controlling insect pests their numbers will have to be augmented by artificial means. Most insects attacking crops harbor one or more parasitoid species that depend on the pest for survival. The existence of such parasitoids is tantamount to but potentially much more valuable to agriculture than the discovery of pest-specific insecticides for those pests.

Such parasitoids have existed for millions of years under many variable conditions. Their coexistence with their hosts has been possible because biological and behavioral characteristics have evolved under nature's balancing system that will ensure that the relative numbers of the two organisms will be held within safe limits. These limits permit enough hosts to exist to cause threats to many kinds of crops and livestock. But the science of insect pest management has the capability of altering the relative number of the two organisms in favor of the parasitoids. That is the key to the great potential of the parasitoid augmentation technique.

Simple rationalization tells us that the rates of parasitism that would jeopardize the welfare of the host on which a parasitoid depends for its existence

also would jeopardize the welfare of the parasitoids. That so many parasitoid species have evolved is all of the evidence that is needed to conclude that self-perpetuating parasitoid populations cannot be expected to maintain pest host populations at very low levels. What biological mechanisms come into play to maintain the relative numbers of the two organisms within safe limits is a vitally important question. Indeed, it is essential that we obtain answers to some of the questions if we are to have a good understanding of parasitism processes and how to make optimum use of them for managing insect pests.

I suggest, however, that certain theories that emerged from my investigations go a long way toward a better understanding of parasitism processes, both as natural control agents and when the populations are augmented by artificial means. One of the theories is the very close link between the hazards to survival of the immature parasitoid within the host stage parasitized to the adult parasitoid stage and the hazards to the hosts not parasitized to the adult pest stage. For solitary parasitoid species these relative hazards are likely to be about the same at all host and parasitoid density levels.

Thus, all hazards that cause host mortalities also will cause mortalities of the immature parasitoids within the hosts. Most parasitoids are solitary species (i.e., one host can produce only one parasitoid progeny). Thus, if we know the average rate of parasitism that a certain parasitoid species causes we have a good idea of the relative number of adult parasitoids and adult hosts that normally coexist in natural populations. This then gives us a good idea of the ratio of parasitoids to hosts that will be required to achieve different rates of parasitism and also a good idea of the rates of parasitism to expect from different parasitoid-to-host ratios.

We know, however, that for some species there will be mortalities to the immature parasitoids that will be independent of the mortalities to the host. Hyperparasitism is one such mortality factor. But for most species hyperparasitism is not a common phenomenon. A disease specific for the immature parasitoid but not for the host would be another example. It is probable, however, that few such diseases exist. But granting that significant parasitoid mortalities can occur that are independent of the host mortalities, the parasitoids will have to compensate by parasitizing more of the hosts. If they did not the two populations within a short time would become hopelessly imbalanced. But we know that the two organisms tend to remain in close balance. Therefore, without attempting to explain how it is achieved, we know that this is a natural biological phenomenon. I have adopted the theory that in numerical terms the reproductive success of individual parasitoid females and individual host females in terms of the number of adult progeny they produce has to be about the same when measured over a period of time. We can show by simple models that if parasitoid females consistently produce only 5 to 10% more or fewer adult progeny than are produced by the host females, the two populations will soon become grossly unbalanced. But, as noted, we know that this does not happen.

The adoption of these two logical assumptions has been the foundation of a good understanding of parasitoid–host relationships in numerical terms. It has made it possible to estimate the host-finding ability of individuals of various insect parasitoids with a high degree of confidence that the estimates are approximately correct. It also has made it possible to estimate the number of

parasitoids that will have to coexist with the host populations to achieve various rates of parasitism.

Let's consider some of the other implications of these assumptions. Biologists have obtained a great deal of information on the rates of parasitism caused by the better-known parasitoids of most of the major insect pests. Such data show that the rates of parasitism caused by most parasitoid species will range between 10 and 30%. I question if very many parasitoids cause average rates of parasitism that exceed 50%. In fact, I suggest that if for any reason a parasitoid causes >50% parasitism throughout the ecosystem the two organisms occupy, the host would soon be in serious jeopardy and might not be able to survive. This in turn would then jeopardize the survival of the parasitoid. Consequently, one can assume that natural biological processes have evolved to be certain that such sustained imbalances cannot occur. I will have more to say about this theory and what happens later when large numbers of parasitoids are released in pest ecosystems.

Because the rates of parasitism caused by most parasitoid species are low, we have a plausible explanation for the limited role that parasitoids play as natural control agents. Because of the comparable survival phenomenon we can assume that if a species normally causes an average of about 10% parasitism, which would be typical of many species, the normal ratio of parasitoids to hosts in coexisting populations will be near 10:90 or 1:9. If the species normally causes about 25% parasitism the normal ratios will be near 25:75 or 1:3. Such rates of parasitism cannot be expected to achieve adequate control of most insect pests. What we need to know, however, is the rates of parasitism that will be required to achieve satisfactory control of various insects.

On the basis of the comparable survival theory, we can by extrapolation make a good estimate of the number of parasitoids that will have to coexist with the host populations to achieve various rates of parasitism, if allowances are made for intraspecific competition that involve the reencounter of hosts already parasitized. If a parasitoid-to-host ratio of 1:4 causes 20% parasitism, a ratio of 2:4 or 1:2 will cause about $20 + 0.20(100 - 20)$ or 36% parasitism. A ratio of 1:1 will cause about $36 + 0.36(100 - 36)$ or 59% parasitism. Allowances also must be made, however, for increasing action of density-dependent suppression factors when the two organisms become unbalanced. This is a universal biological phenomenon that applies for all animal populations. To make allowances for this factor, I assume that a parasitoid-to-host ratio of 1:1 will cause about 50% parasitism. On this basis a ratio of 2:1 should cause about 75% parasitism, a ratio of 3:1, 87.5% parasitism, and so forth. Again, these estimates make allowances for intraspecific competition but do not make allowances for changes in density-dependent suppression factors. But ample allowances can be made for these factors.

For solitary species, a given parasitoid-to-host ratio will cause about the same rate of parasitism regardless of the biology and behavior of the parasitoid or the biology and behavior of the host. It also will cause about the same rate of parasitism regardless of the rate of parasitism the species causes as a natural control agent.

But the factor of most practical significance is the assumption that a given parasitoid-to-host ratio will cause about the same rate of parasitism regardless of the host density. There is overwhelming evidence to support this theory.

The highly developed host-detection capability of insect parasitoids permits the individual parasitoids to find their normal lifetime quota of hosts even though the host population may exist at a very low level. At what density host finding becomes a critical survival factor is not known. In my opinion, however, it must fall by 90 to 95% below the normal level before the host-finding ability of the individual parasitoid will be reduced significantly. Even then the reduction will be gradual rather than abrupt. Many biologists assume that rates of parasitism tend to increase or decrease in a linear manner with increasing or decreasing host densities. This assumption, however, has not been confirmed by many other biologists.

Many biologists also assume that if a parasitoid species causes low rates of parasitism this suggests that it is a poor host-searcher and host-finder. This is a false assumption. One can assume with certainty that all parasitoids are diligent host-seekers and remarkably efficient host-finders. Otherwise they would not exist. The normal rate of parasitism a species causes is a reflection of the relative number of the parasitoids and hosts that coexist under nature's constraints when the two organisms have established stability with each other and with the environment. That numerical relationship, however, can be drastically altered by the simple (although perhaps demanding) process of releasing reared parasitoids throughout the ecosystem the two organisms cohabit.

The above in essence describes the basic principles of insect parasitism. It is the foundation for the hypothesis that the parasitoid augmentation technique offers outstanding possibilities for rigidly managing insect pest populations if key parasitoid species are released in adequate numbers throughout the ecosystem occupied by the two organisms. I will examine that hypothesis in more detail in the sections to follow.

To gain a better perspective of the way natural parasitism functions and how efficient a parasitoid is in finding its host, I developed the model shown in Table 5. The basic model shown in Table 1 is again used for this purpose. It is assumed that a parasitoid species exists that is primarily dependent on the host for reproduction. It is a larval parasitoid that parasitizes an average of about 20% of the medium-sized host larvae. The host females are assumed to produce an average of 80 larvae during the first host generation. Thus, a total of 80,000 larvae would be present per unit area during the first generation.

If the rate of parasitism is 20%, 16,000 hosts will be parasitized. On the basis of the comparable survival theory that I have discussed, when the rate of parasitism is 20% the average adult parasitoid-to-host ratio will be about 1:4. Therefore, about 250 female parasitoids will coexist with 1,000 female hosts. Then if 16,000 larvae are parasitized, the individual females will parasitize 64 hosts. Thus, by deduction we have obtained values for two very important parameters, the number of parasitoids that normally coexist with the host population and the average host-finding capability of the individual female parasitoids. The survival rates of the parasitized and unparasitized hosts to the respective adult stage will be variable, but in this case they are assumed to be 12.5%. Therefore, during the first generation both the parasitoid and host populations would grow by fourfold. Because of the increase in the host and parasitoid populations during generation 2, density-dependent suppression factors will become more intense. This phenomenon applies for all animal populations.

Table 5. Estimated coexistence pattern of a larval parasitoid with a host population.

	Generation	
Parameters	1	2
Female hosts	1,000	4,000
Small larvae produced per female	80	68
Total small larvae	80,000	272,000
Coexisting female parasitoids	250	1,000
Adult parasitoid-to-adult host ratio	1:4	1:4
Host larvae parasitized per female parasitoid	64	54
Total host larvae parasitized	16,000	54,000
Percentage of parasitism	20	20
Host larvae not parasitized	64,000	217,600
Survival, unparasitized host larvae to adult hosts, %	12.5	10
Adult hosts, next generation, both sexes	8,000	21,760
Survival, parasitized host larvae to adult parasitoids, %	12.5	10
Adult parasitoids next generation, both sexes	2,000	5,400
Increase rate of host populations	4.0	2.7
Increase rate of parasitoid population	4.0	2.7

The reproductive success of the two organisms will decrease but at the same rate. Therefore, the rate of parasitism during generation 2 will remain constant at about 20%. The population trends for the next few generations are not shown, but they would follow about the same trend as that shown for the basic host population shown in Table 1.

The validity of the estimated host-finding capability of the parasitoid can be tested by modeling procedures. If all other parameter values were held constant, but the assumption were made that the individual parasitoids would parasitize only half the number of hosts assumed, the rate of parasitism during the first two generations would be about 10 and 5%, respectively. The rate of parasitism would continue to decrease by half during the next few cycles. The parasitoid population would soon become extinct. If the assumption were made, however, that the individual females found and parasitized two times as many hosts as assumed the rate of parasitism would soon approach 100% and the host population could not survive. The parasitoid population in time also would become extinct. Such tests convince me that the estimated host-finding efficiency of the hypothetical parasitoid is approximately correct.

Performance of Insect Parasitoids when Released into Insect Pest Ecosystems

I have discussed some of the fundamental principles of natural insect parasitism. I also have postulated basic values for the host-finding efficiency of an insect parasitoid. The practical question is, What influence will the release of various numbers of a parasitoid species throughout its pest ecosystem have on the dynamics of a pest population? The model in Table 6 shows the theoretical influence of such releases on a pest population of the nature described in Table 1.

Any one of a number of well-known parasitoid species could be selected for this analysis. I will assume the release of a parasitoid having the characteristics of the one shown in Table 5. Enough of the parasitoids will be released to achieve an adult parasitoid-to-adult host ratio of 3:1 during generation 1. This ratio would require the release of 6,000 parasitoids of both sexes. Keep in mind that this would be 12 times the number of parasitoids that is estimated to cause 20% parasitism as shown in Table 5. The female parasitoids existing at their normal density levels are assumed to find an average of 64 host larvae during

Table 6. Estimated influence on the dynamics of a host population when adults of a larval parasitoid are released throughout the host ecosystem to achieve an adult parasitoid-to-host ratio of 3:1.

	Generation		
Parameters	1	2	3
Female hosts	1,000	750	166
Host larvae produced per female[a]	80	82	86
Total host larvae	80,000	61,500	14,276
Female parasitoids released, generation 1 only	3,000	4,250	3,985
Adult parasitoid-to-adult-host ratio	3:1	5.7:1	24:1
Host larvae parasitized per female[b]	51	48	49
Host larvae parasitized	153,000	204,00	195,265
Ratio, host parasitized to hosts present[c]	1.9:1	3.3:1	13.7:1
Percentage of parasitism	85	96	99+
Host larvae parasitized, number	68,000	59,040	-
Host larvae not parasitized	12,000	2,460	-
Survival, unparasitized host larvae to adult hosts, %[a]	12.5	13.5	-
Adult hosts next generation, both sexes[a]	1,500	332	-
Survival, parasitized hosts to adult parasitoids, %	12.5	13.5	-
Adult parasitoids, next generation, both sexes	8,500	7,970	-

[a]Some changes in parameter values are made because of changes in parasitoid and host densities.

[b]Females in a natural population are estimated to parasitize an average of 64 larvae. Because of the high density, however, the average has been reduced by 20% to 51 per female.

[c]The rates of parasitism at different ratios of hosts parasitized to hosts present are based on data in Table 1 of USDA Handbook, Number 693 (Knipling 1992).

their lifetime. In view of the abnormally high parasitoid population, however, predation is expected to increase so that the life expectancy of the parasitoids will be shorter. Therefore, I assume arbitrarily that the host-finding ability of the female parasitoids will be reduced by 20% to an average of 51. This efficiency will allow 3,000 females to find 153,000 host larvae during generation 1. It will exceed the estimated 80,000 host larvae present by a ratio of 1.9:1. In theory, only 15% of the larvae will escape parasitism by chance, and 85% will be parasitized one or more times. Thus, 68,000 host larvae will be parasitized, and only 12,000 will escape parasitism.

Then, the theory of comparable survival of the parasitized and unparasitized hosts to the respective adult stages will determine the relative number of adult parasitoids and adult hosts that will coexist during the next generation. The ratio will be 85:15 or 5.7:1. The significance of another key theory that has been discussed then becomes equally important. This is that the host-finding ability of individual parasitoids in numerical terms remains near normal, irrespective of the host density. There is only a reduction of about 25% in the host density during generation 2, but the ratio of parasitoids to hosts almost doubles. Slight adjustments are made in the average number of host larvae produced per female host and the average number parasitized per female parasitoid. These adjustments are not enough, however, to prevent a marked increase in the ratio of hosts found and parasitized to the number of hosts available. This ratio is 3.3:1. In theory this will allow only about 4% of the hosts to escape parasitism by chance and 96% will be parasitized one or more times.

The influence the parasitoids will have during generation 3 continues to be governed by the two theories mentioned. The host population has declined sharply by generation 3, but enough hosts will still be present to permit the parasitoid females to find their normal quota of hosts. The parasitoid-to-host ratio increases to about 24:1. This would be more than 96 times the ratio that causes 20% parasitism. Because it is assumed that the host density is still high enough to permit the individual parasitoids to find their normal quota of hosts, the ratio of hosts found to hosts available will be 13.6:1. This ratio, in theory, will cause nearly 100% parasitism. Therefore, virtually complete collapse of the host population can be expected to occur during generation 3. But, significantly, if any hosts did survive, the ratio of adult parasitoids to adult hosts during generation 4 would be near 100:1. There would seem to be no way for any host larvae to escape ultimate parasitism.

I would like in particular to call attention to the increasing parasitoid-to-host ratio that in theory results from the releases. The ratio increases progressively each cycle and so does the rate of parasitism even though additional parasitoids are not released. In theory, the host population will be eliminated without the release of additional parasitoids. If such action is confirmed it would be a suppression mechanism new to science that has profound practical implications. Even though it might seem to be beyond realism to accept the theoretical results, I cannot envision any biological actions that would prevent the elimination of the pest population. I have noted above that rates of natural parasitism higher than 50% seem to be rare for natural parasitoid populations. Therefore, I raise the question whether biological and behavioral characteristics

have evolved in natural populations to guard against a progressive increase in parasitoid-to-host ratios as depicted in the model. Because of intraspecific competition and the comparable survival rate of parasitized and unparasitized hosts plus the density-dependent control action that is characteristic of all animal populations, I cannot envision any way that a natural parasitoid population on its own could increase enough to achieve a parasitoid-to-host ratio higher than 1:1 and certainly not as high as 3:1. But by augmentation there are no barriers to the attainment of parasitoid-to-host ratios even much higher than 3:1, especially when the host populations exist at abnormally low levels.

This in brief is the rationale for the assumption that the parasitoid augmentation technique holds outstanding promise as a means of regulating or eradicating insect pest populations. I realize that we as scientists are confronted with biological actions that go far beyond the knowledge that we now have concerning the influence of parasitoid releases. Thus, we have no tangible evidence to support the assumptions that have been made. But, no effort has ever been made to determine the influence of parasitoid releases in high numbers throughout a pest ecosystem. No effort has ever been made to estimate the influence of such releases that I have described. I suggest, however, that the probability is near 100% that no insect pest could maintain damaging populations or even survive if subjected throughout its ecosystem to the distorted parasitoid-to-host ratio that can be brought about by artificial means. And there is no question in my mind that we have or can have the technology to achieve even more distorted ratios than I have assumed for this analysis.

Because one of the major purposes of this investigation is to compare the suppressive actions of the parasitoid and sterile insect techniques, I wish again to call attention to the results of sterile insect releases as shown in Table 4. In theory, the release of 6,000 parasitoids of both sexes during the first generation will achieve about the same results as the release of 54,000 sterile insects of both sexes during four pest generations. This estimate is based on the assumption that the sterile insects are fully competitive. For many insects the sterilization process reduces the vigor of the insects. Therefore, for some insects the parasitoid release technique might be even more effective than the sterile insect technique. Also, the higher the ratios the greater will be the advantages of the parasitoid augmentation technique.

Suppression Characteristics of Insect Parasitoids

Although I have already discussed some of the suppression characteristics of insect parasitoids and how they perform when released in pest ecosystems, it is not possible to fully grasp the potential of the parasitoid augmentation technique for managing pest populations unless we critically analyze all of the characteristics of parasitoids that are relevant to the total pest population management concept. This we will do based on the knowledge we have on their biology and behavior and on the type of suppressive actions that they produce when they coexist with their pest hosts. Some comparisons also will be made of the characteristics of other methods of control.

Target pest specificity. The importance of conserving the natural biological control agents normally present in agroecosystems in the efforts to control

insect pests has already been stressed. It also has been emphasized that so long as the use of broad-spectrum insecticides is a major component in IPM systems ecologically sound insect pest management cannot be achieved. Therefore, the use of methods of control that are pest specific should continue to receive high priority in research on the development and use of insect pest management procedures. The release of parasitoids that are primarily or totally dependent on the target pest is among the most pest-specific ways known for managing insect pests. The use of such organisms will cause no harm to plants, animals, or people. All of the natural control organisms can operate in a normal manner.

Pest density. The suppressive action of insect parasitoids is strongly influenced by the abundance of the pest to be controlled. The lower the pest density the more effective a given number of parasitoids will be. This is a negative factor when the pest populations are high. But it becomes a very favorable characteristic when the populations are low, which will be the objective of areawide management systems. The sterile insect technique and the use of insect sex pheromones also have similar advantages over methods of control that achieve about the same degree of control whether the pest populations are high or low.

Mobility factors. When parasitoids are released on an areawide basis for the management of insect pest populations, the ability of the organisms to disperse on their own into all of the pest habitats becomes a very favorable characteristic.

The use of non-mobile control procedures will require much greater precision in their application. Treatment intervals of 33 m will probably be required to achieve effective control when chemical or biological insecticides are used, whereas application intervals of 800–1,600 m (about 1/4 to 1 mi) would probably be adequate for most parasitoids and sterile insects. Thus, there might be as much as a 50-fold difference in the application costs for the two methods of control. Therefore, for insect pests that are widely distributed the parasitoid augmentation and sterile insect techniques may be the only economically and operationally feasible ways to achieve areawide management.

Self-perpetuating populations. Another factor that is unique to the parasite-release technique and which is a major advantage over the sterile insect technique is the ability of the released organisms to maintain self-perpetuating populations from cycle to cycle. This is an inherent characteristic of natural parasitism and is the reason why classical biological control can be so important. But this characteristic is also applicable for the parasitoid augmentation technique.

As previously noted, when enough parasitoids are released into a pest ecosystem to achieve higher than 50% parasitism the parasitoid-to-host ratio and the rate of parasitism can become progressively higher each succeeding generation until, in theory, the pest population is eliminated. For some parasitoid–host associations, such as the oriental fruit fly and the egg-pupal parasitoid *Biosteres arisanus* (Sonan), there is a mechanism that will prevent such progressive increase, as described in Knipling (1992). It involves the influence of superparasitism on the survival of the immature parasitoid. Such a mechanism, however, seems not to exist or is of no importance for many parasitoids. If the increasing suppressive action described is confirmed, it could have a dramatic influence on insect control methodology in the years ahead.

Pest guidance factor. I will now consider a characteristic of insect parasitoids that, in my opinion, makes the parasitoid augmentation technique by far the most effective way now known to manage and possibly to eradicate isolated insect pest populations. When host-dependent parasitoids are released in pest ecosystems, organisms will be released that have the instincts and the propensity to search specifically for the target pest. It is a characteristic of great potential significance in the development and implementation of areawide insect pest management and eradication procedures.

No other method of insect control has this pest guidance factor. When the sterile insect technique is used, both sexes of the insects are usually released. Thus, the sterile insects are no more likely to search for mates among the wild insects than among their sterile siblings. Some researchers have developed mechanisms for rearing males only and others are seeking practical ways to separate the sexes by physical means as they now do for tsetse flies. If they are successful in this effort, this would then also introduce the very important pest guidance factor into the sterile insect technique.

General comments. Thus, when the characteristics of various methods of insect control are analyzed and compared, it becomes apparent that no method of control has all the desirable features of the parasite release technique when it is used to manage total insect pest populations. Its advantages have long been unrecognized because of lack of key information, misconceptions over the way parasitism works, and because the technique has not been considered for the areawide management of insect pests. The technique has so many unique and desirable characteristics when used in areawide preventive programs that it offers almost unlimited potential for dealing with many insect pest populations in an effective, low-cost, and environmentally safe manner. Unfortunately, the advantages the technique holds will continue to remain dormant and unproven until provisions are made for scientists to perfect the technology required for specific pests and to demonstrate that it will perform as anticipated.

Mutual Synergistic Suppressive Action: A Powerful Biological Control Mechanism

In the theoretical appraisals of the influence that different methods of control will have on the dynamics of insect populations when they are integrated, a previously unrecognized mechanism of suppression came to light. This is what I call mutual synergistic suppressive action. If exploited, such biological action could make a further major contribution to more effective and desirable insect pest management methodology. The procedure involves the concurrent use of two or more methods of control that become increasingly effective with declining pest densities. As noted previously, the parasitoid augmentation technique, the sterile insect technique, and insect sex pheromones possess this type of suppressive action. The degree of control that can result from the appropriate integration of two or more such methods of control is exceptionally high.

The model shown in Table 7 is an estimate of the influence of the concurrent release of sterile insects and a primary larval parasitoid into a pest ecosystem. The model is based on the assumption that enough fully competitive sterile insects will be released during the first generation when the adult pests are

Table 7. Influence of the concurrent release of enough sterile insects and parasitoids to achieve ratios of 2:1 during generation 1 of a pest population.

	Generation	
Parameters	1	2
Normal males	1,000	36
Normal females	1,000	36
Sterile insects, both sexes	4,000	None
Sterile-to-fertile ratio	2:1	-
Female reproducing	333	36
Small larvae per female	80	86
Total small larvae	26,640	3,096
Female parasitoids present	2,000	1,631
Host larvae parasitized per female	53	55
Total larvae parasitized	106,000	89,705
Ratio of larvae parasitized to larvae present	4:1	29:1
Percentage of parasitism	98	99+
Larvae parasitized	26,107	2,724+
Larvae not parasitized	533	27
Survival larvae to adults, %	12.5	-
Adult hosts next generation, both sexes	72	-
Adult parasitoids, next generation, both sexes	3,263	-

emerging to achieve a sterile-to-fertile ratio of 2:1 and enough of the parasitoid species will be released to achieve the same ratio. Therefore, a total of 8,000 insects would be released.

The values assigned to the different parameters and the way that the calculations are made are largely self-explanatory. A sterile-to-fertile ratio of 2:1 would permit reproduction by only 333 of the 1,000 female pests, and they would produce 26,640 host larvae. The individual female parasitoids on average are assumed to be capable of finding and parasitizing 53 host larvae. Therefore, a total of 106,000 larvae would be parasitized. This would exceed the total larvae present by a ratio of 3.9:1. In theory, this will result in 98% parasitism. The combined control would be 99.3%.

The host population during generation 2 would be reduced by about 97%. There would be no need to release additional sterile insects or parasitoids. Enough adult parasitoid progeny should be present during generation 2 to exceed the adult host population by 45-fold. Near 100% parasitism could be

expected during generation 2. Thus, in theory, the release of 4,000 parasitoids and 4,000 sterile insects could eliminate all reproduction within two generations. It would require the release of about 200,000 sterile insects alone or the release of about 16,000 parasitoids alone to have a comparable influence on the pest population. Most of the suppressive action is due to the parasitoids.

The degree of suppressive action that is achieved by the concurrent release of the two organisms will depend on the initial ratios that are achieved. If only enough sterile insects and parasitoids were released during generation 1 to achieve ratios of 1:1 for each organism the combined suppressive action during generation 1 would be about 87.5%. If no additional sterile insects or parasitoids are released, enough parasitoid progeny should be present during generation 2 to achieve a parasitoid-to-host ratio of about 4:1 and about 90% parasitism could be expected during generation 2. The rate of parasitism should be higher during generation 3.

To have a comparable influence by the release of sterile insects alone it would be necessary to release about 14,000 sterile insects each generation for three successive generations. Thus, 4,000 insects of both organisms released during one generation would equal the suppressive action of 42,000 sterile insects alone released during three generations. This would be more than a 10-fold difference.

If enough sterile insects and parasitoids were released during generation 1 to achieve a 3:1 ratio for each organism or a total of 12,000 insects, reproduction would be inhibited by more than 99.9%. It would require the release of 2,000,000 sterile insects alone to inhibit reproduction by 99.9%.

Examples given demonstrate the powerful action that can be achieved by integrating the release of parasitoids and sterile insects. Other examples of synergistic suppressive action can be given. The concurrent release of two different parasitoid species that parasitize and complete development in different life stages of the host results in similar synergistic action; for example, the release of an egg parasitoid such as *Trichogramma* spp. and a larval parasitoid. The release of a larval parasitoid and a puparia parasitoid would be another example. Models depicting the suppressive action of such releases are very complicated and difficult to calculate. None will be shown. But in theory, the combined suppressive action would be even greater than the combined action of parasitoids and sterile insects.

It is difficult to fully appreciate what such synergistic suppressive actions could mean to insect pest management. In using the sterile insect technique alone, pest managers think in terms of the production and release of enough sterile insects to achieve starting ratios as high as 10:1 or higher and to continue releases for at least three to four successive pest cycles. The degree of suppressive action that will result from the release of enough sterile insects and parasitoids or enough of two different parasitoid species to achieve such ratios would be difficult to calculate.

But the feasibility of making use of biological organisms in this way for areawide management will still hinge on the feasibility of mass producing the organisms. But when the pest populations are low not many of the organisms will be required to achieve high ratios. Also, we must conduct field release experiments to confirm the theoretical results.

Practical Implications

I recognize that all of the assumptions, hypothetical models, and discussions in support of the areawide approach to the control of insect pests on their own will have little meaning for agricultural executives, farmers, or the general public. What they want to know is how effective the control procedures will be, how dependable they are, and what the costs will be compared with the cost of current methods of control. Many members of the public will be primarily interested in the hazards associated with the control of insect pests by the proposed procedures.

Every insect pest has its distinct characteristics and requirements for its effective areawide management. But the basic principles and the objectives will be the same: to maintain the total pest populations below significant damage levels from year to year or eradicate the pests, if this is a feasible option.

Not all insect pests will be good candidates for areawide management. Several hundred insect pest species that vary in importance can affect agricultural crops and livestock. It will not be advantageous to deal with all of them on an areawide basis. There will be a continuing need for close supervision of insect pest numbers and timely advice to growers as to the best insect control procedures to use. But perhaps 25 to 50 of the more important insect pests can be expected to require control virtually every year. These pests cause perhaps 80% of the losses and are also responsible for up to 80% of the insecticides required for agricultural purposes. The pests that I consider good candidates for areawide management include the corn earworm, tobacco budworm, boll weevil, European corn borer, sugarcane borer, Colorado potato beetle [*Leptinotarsa decemlineata* (Say.)], codling moth, all of the tropical fruit flies, fall armyworm, horn fly [*Haematobia irritans* (L.)], stable fly [*Stomoxys calcitrans* (L.)], face fly (*Musca autumnalis* De Geer), and house fly (*Musca domestica* L.). Even ticks (Ixodidae) may be good candidates for areawide management by the parasitoid augmentation technique.

In a gross way, I have developed models for most of the pests named and have analyzed the influence that releases of known parasitoids will have on the dynamics of the pests when the technique is used alone or when integrated with the sterile insect technique releases.

It is not feasible here to go into much detail on the procedure that could be followed in a management program. I will select several species, however, to indicate in a rough way the nature of the pest, the type of program that might be undertaken, the results to expect, and the probable cost and benefits that could be realized.

Corn earworm and tobacco budworm complex. Corn earworm and tobacco budworm cause damage to a wide range of agricultural crops. They are widely distributed and their dispersal range might be 1,000 mi or more. They are present in most of the nation during the crop-growing season. The losses they cause due to damage and control costs are estimated to exceed $1 billion per year. For the most part insecticide applications are necessary for their control. They are probably responsible for 20–25% of the insecticides used for agricultural purposes each year. The objective would be to manage the total population each year.

With the help of colleagues, I have estimated that about 1 billion adults of both species can be expected to emerge each spring in the overwintering areas in the United States and northern Mexico. Thorough ecological investigations are needed to more accurately determine the numbers of the pests that emerge each spring. But, if we accept this rough estimate and accept the theoretical results that I have presented for the parasitoid and sterile insect techniques, we can make a gross estimate of the costs that might be involved in the management of these two pests in all of the United States and northern Mexico. Parasitoid species are known that are either host specific or that have a high degree of affinity for the corn earworm and tobacco budworm. Their use would provide a highly pest-specific method of control. We have shown by theoretical procedures that an adult parasitoid-to-host ratio of 3:1 achieved during the first generation will in theory effectively control pest populations for several successive generations, or that a combined ratio of parasitoids and sterile insects as low as 2:1 would adequately manage a pest population for a season. But for this analysis I will assume that a parasitoid-to-host ratio of 4:1 or that this ratio of both organisms would be required to manage the populations the first year. The insect releases would be focused on the first generation that emerges from overwintering populations. I make the assumption that given resources, insect rearing experts could develop the technology for mass producing one or more of the known parasitoids of the two pests at a cost of $10 per 1,000 parasitoids or that 1,000 moths for sterilization could be produced at a comparable cost. The cost for distributing the organisms each week at 1-mi intervals for 6 wk in all of the cultivated areas is estimated to be about $5 million per year. A similar cost would be involved in conducting the intensive surveys that would be necessary to guide the operational program.

On the basis of these rough estimates, we could achieve effective management of these two pests nationwide at a cost of about $50 million the first year. If the populations are maintained at a low level the first year, the overwintering population the next year should be greatly reduced. Then much fewer parasitoids or sterile insects would be required to maintain adequate control. The continuing costs may not exceed $20 million per year. The benefits in relation to costs would be on the order of 50:1. The need for ecologically disruptive agricultural insecticides might be reduced by 20% or more.

Many agriculturalists and biologists might be intimidated by these pests and conclude that the implementation and execution of such programs would be out of the question. I totally reject such view. A program of the nature envisioned would not be any more comprehensive than the screwworm program that has been conducted successfully for many years. I grant that I may be overly optimistic and that such management program might cost several times the gross cost estimates that I have made. But even granting this possibility, it is my conviction that we can have the means for dealing with these two pest problems on a year-by-year basis without the need for insecticides at less than one-tenth the losses that the pests now cause under the farm-by-farm basis as practiced by individual growers. I cannot envision any other feasible method of control conducted by individual farmers that would even approach the low cost that has been estimated for the organized areawide procedures described.

European corn borer. The European corn borer is another major insect pest that causes losses amounting to $1 billion or more each year. In areas where it is abundant the losses amount to about $30 per acre. Scientists have developed several ways to control this pest, including growing resistant varieties, releasing *Trichogramma* parasitoids, applying chemical or biological insecticides, and more recently the ingenious method of growing corn that is protected by *Bacillus thuringiensis* Berliner (Bt) biotechnology.

Natural populations of the pest exist at a very high level. I estimate that adults from overwintering larvae in high-density areas are likely to average 2,000 of both sexes per acre. Several insect parasitoids and pathogens exist that achieve some control but not enough to maintain low host populations. Because of the high initial natural populations cost of control by the use of the parasitoid augmentation or sterile insect techniques during the first year would be high. But I believe that if given the resources insect rearing experts could develop mass-production procedures at a cost of $5 per 1,000 parasitoids or adults for sterilization. I also believe that a parasitoid-to-host ratio as low as 2:1 or a combined ratio of one sterile insect and one parasitoid would provide good control the first year. On that basis the cost the first year might be less than the losses. But the major benefits of the proposed management procedure would be the low costs year by year thereafter in all of the areas where it now causes heavy losses.

The probability is very high that the European corn borer could be rigidly managed year by year by ecologically acceptable means at a cost of $2 to $3 per acre or at a cost that would not exceed 10% of the losses it causes under current management procedures. I suggest that we can assume with certainty that this dynamic pest will not readily yield to the farm-to-farm management procedure and that the areawide management approach offers the best way to deal with this major pest in a low-cost and ecologically acceptable procedure.

Boll weevil. This pest has cost cotton (*Gossypium* spp.) growers many billions of dollars during the past century. It has for years caused serious ecological disruptions in our agricultural ecosystems in all of the boll weevil areas because of the intensive use of broad-spectrum insecticides for its control. This has probably doubled the importance of the nontarget pests. An eradication program was undertaken that led to the elimination of the pest from the southeastern cotton-growing region. The economic and ecological benefits that have resulted fully confirmed the claims of the proponents of an eradication program. But the pest is still present in most of its range.

Scientists have made major efforts on alternate ways to control the boll weevil. Outstanding advances were made in the development of a sex attractant. Several host-plant resistant mechanisms have been discovered. Good boll weevil rearing procedures have been developed. Biologists also tried to make use of the sterile male technique to help manage or eradicate boll weevil populations. This effort, however, was not very successful because of the severe damage that the sterilization process does to the competitiveness of the insect. During recent years biologists also have given a great deal of attention to the possibility of controlling the pest by the parasitoid augmentation technique but largely for use on a farm-by-farm basis.

In my appraisal of the potential of the augmentation technique, I have assumed, as with other pests, that the only way this method of control can be highly successful will be to release the parasitoids on an areawide basis. My appraisals considered two parasitoid species: the native parasitoid *Bracon mellitor* (Say) and the exotic parasitoid *Catolaccus grandis* (Burks). The results of the investigation have been published (Knipling 1992). The theoretical results suggest that boll weevil populations can be rigidly managed and possibly eradicated by the mass production and release of a specified number of parasitoids throughout boll weevil ecosystems.

Scientists with the United States Department of Agriculture laboratory at Weslaco, Texas, and their state collaborators have shown that the release of reared *C. catolaccus* parasitoids can achieve 100% parasitism or kill of the boll weevil larvae in heavily infested cotton fields. The results of the field releases strongly support the results obtained by theoretical means. This support has greatly enhanced the confidence I have in the possibility of managing this and other pests by the augmentation procedure. The scientists have not yet had the opportunity to make parasitoid releases against completely isolated populations. This will be an absolute requirement to determine to what level parasitoid releases can suppress pest populations. I suggest the probability is near 100% that boll weevil populations can be effectively managed and possibly eradicated by the parasitoid augmentation technique. Insect rearing experts have made excellent progress on in vitro rearing procedures for the parasitoid at costs <25% of the costs that I estimated would be economically feasible for managing or eradicating boll weevil populations. If an insect pest that is as highly resistant to control by natural biological organisms as is the boll weevil can be effectively managed by the release of parasitoids there is reason to believe that many other pests could be managed in the same way.

Fall armyworm. This pest was selected as an example of a good candidate for total population management because it is representative of a number of insect pests that have highly restricted overwintering areas and exist in relatively low numbers in the overwintering areas. The overwintering populations of such pests can increase by perhaps 25- to 50-fold during the crop-growing season and cause losses on 25 times the acreage of host plants on which they reproduce in the overwintering areas. The loss to agriculture due to the pests that spread each spring from the restricted overwintering areas may amount to $25 to $50 million per year. The use of insecticides is now the only option that growers have for controlling such pests. The fall armyworm is one of the reasons that a two cropping system is not more feasible in the southern regions.

It has long been my view that efforts should be made to manage such pests during the winter months in the restricted overwintering areas. How to accomplish this in an effective, low-cost, and ecologically acceptable manner has been the question. It is now my conviction, however, that the two biological control procedures I have proposed could be perfected to deal with such pests in an ecologically acceptable manner at <10% of the losses they cause under current management procedures.

Looking to the Future: What Needs to be Done

As noted at the outset, agriculture worldwide is faced with the momentous task of producing enough food for the expanding world population. More efficient pest control is one of the ways to meet this responsibility. The few examples I have given indicate the large economic and environmental benefits that can be realized by dealing with major insect pest problems on an areawide basis by the biological procedures proposed.

It is my view that farmers, most of the agricultural executives in both the public and private sectors, and many scientists have no idea of the potential economic and environmental benefits that can be realized by the development and use of areawide management procedures to control insect pests. I hope that this article will help to inform them of the large economic and environmental benefits that could be realized by dealing with major insect pest problems on an areawide basis by the biological procedures proposed. I propose that our agricultural leaderships should strive for a research initiative that is focused on the development of areawide suppression procedures. I estimate that about $25 million per year focused on areawide control procedures would meet the research needs.

It is my view that most of such research funds should be provided by publicly supported institutions. Growers and the agricultural industry should assume most of the cost for implementing such management programs. If it can be demonstrated to farmers that a serious pest problem can be met at costs that are much lower than the losses the pest causes under present methods of control, there should be little difficulty in gaining their full support for implementing such programs. In fact, I suggest they would virtually demand that such programs be implemented as they did after they learned of the successful screwworm eradication experiment on the island of Curocao and after they learned of the success of the pilot boll weevil eradication programs conducted in North Carolina. Certainly the general public would encourage the implementation of programs that could make major contributions to alleviating the environmental hazards associated with extensive use of broad-spectrum insecticides.

The development of information needed to demonstrate the feasibility and advantages of areawide management programs will require major changes in research procedures and objectives. More effort will have to be focused on obtaining basic information on insect population ecology. The more practical aspects will have to be focused on the development of technology for producing and handling the large numbers of the biological organisms that will be required. And provisions must be made for conducting experiments against isolated pest populations or in areas large enough to avoid the adverse influence of insect movement. The conduct of research in limited size pest habitats will not provide the information needed. Indeed, such research is likely to yield results that will make scientists and administrators lose confidence in the proposed management procedures. The cost for research that is focused on the development of areawide management procedures may seem high, but it will be very low considering the large economic and ecological benefits that eventually can be realized. An investment in such research would be one of the best research investments that agriculture could make.

I recommend that our agricultural leadership in both the public and private sectors become more involved in shaping insect pest management techniques and strategies for the future. A good beginning would be to appoint a panel of experts to critically analyze the potential economic and environmental benefits that can be derived from the areawide management approach to dealing with many of the major insect pests versus a continuation of the farm-to-farm management procedure as the need arises.

This is not a minor agricultural issue. Much is at stake both economically and environmentally in considering the two approaches. I am confident that a panel of experts on various aspects of insect pest management would find that technology has been or could be readily developed that would permit the management of many of the major insect pests with little or no need for insecticides at annual costs that would be much lower than the losses the pests now cause under the farm-to-farm management procedure. I would be willing to join with other proponents of the areawide approach to the management of key pest species in efforts to advance this ecologically desirable way of dealing with many of our major insect pest problems.

Acknowledgment

Hundreds of biologists have obtained information relevant to the areawide management of insect pests and to the parasitoid augmentation and sterile insect techniques on which this article is based. It is not feasible to make reference to the contributions they have made that were helpful in my investigations. Also, I have had personal communication with some of my colleagues on aspects of the biology, behavior, and dynamics of the pest hosts and the parasitoids that was helpful in analyzing the principles of insect parasitism processes. I wish to acknowledge the valuable information and assistance they provided. I owe special thanks to my daughter, Edwina K. Lake, for all the typing and editorial assistance that made it possible for me to prepare this long article for publication.

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Sterile Insect Technique for Suppressing and Eradicating Insect Population: 55 Years and Counting[1]

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ABSTRACT The sterile insect technique (SIT) has a long, interesting, but controversial history. The concept, operation, and outcomes of SIT programs have been criticized heavily and acceptance of this areawide approach to insect management is minimal. These criticisms are examined in general and specifically with regard to Mediterranean fruit fly, *Ceratitis capitata* (Weidemann), and screwworm, *Cochliomyia hominivorax* Coquerel. The chief objections reviewed included evolutionary responses to SIT, the occurrence of sibling species, the role of weather in causing pest suppression and outbreaks during SIT programs, and the occurrence of undetected pest populations where eradication has been claimed. There is a paucity of data relating sterile fly releases to sterile mating rates in target populations and sterile matings to target population dynamics. The overkill strategy should be updated, especially in experimental efficacy trials. Despite the carping, it is concluded that SIT is a highly effective method for insect population management. This environmentally benign method of insect pest suppression and eradication is underutilized even though using SIT has eradicated screwworm populations on a continental scale and many tephritid fruit fly infestations throughout the world. It would lend credibility to the efficacy of SIT if sterile mating frquencies were estimated in challenged populations and correlated with target population densities.

KEY WORDS Sterile insect technique, radiosterilization, sterile male hybrids, screwworms, *Cochliomyia hominivorax*, Medfly, *Ceratitis capitata*, tsetse flies, *Glossina* spp.

More than 80 yr has elapsed since Runner (1916) demonstrated that X-rays induced sterility in cigarette beetles, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae). In 1937, E. F. Knipling raised the possibility in conversation with R. C. Bushland that the screwworm, *Cochliomyia hominivorax* Coquerel, might be controlled if the males could somehow be sterilized (Lindquist 1955). Potts (1944) and Vanderplank (1944) suggested that the sterile male progeny of interspecific matings between *Glossina morsitans centralis* Machado and *G. swynnertoni* Austen might be used to control *G. m. centralis*. Using the same

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principle, Davidson et al. (1970) released sterile male hybrids from the cross of male *Anopheles arabiensis* Patton × female *An. melas* Donitz in an effort to suppress a natural *An. gambiae* population in Upper Volta. It has long been known that when two related species or subspecies are crossed it is the heterogametic sex that is sterile—Haldane's rule (Orr 1997). Hybrid sterility remains an attractive option for tsetse control. Because many tsetse species and subspecies are allopatric, one species could be reared, and the males culled and released into a target population of a different species or subspecies.

Most insect pests, however, do not belong to species complexes. A safe and effective means of inducing sterility is afforded by use of ionizing radiation and it was first used by Bushland & Hopkins (1951) to induce dominant lethal mutations in screwworms. It is nearly 50 yr since the first crucial experiment of releasing radiosterilized sterile screwworms on Sanibel Island, not far from Tampa, Florida, was carried out. That experiment almost eliminated screwworms and was followed by a larger trial in which the species was eradicated from Curacao in the Netherlands Antilles (Baumhover et al. 1955). The rest is history.

That history includes the development of new and flourishing lines of research in addition to very real accomplishments in pest management. Investigations into details of insect reproduction from developmental, physiological, genetic, cytological, behavioral, evolutionary, and productivity standpoints are now considered essential to the eventual development of biorational control measures (IAEA 1993, 1998). A good example was the sophisticated and highly productive Commonwealth Scientific and Industrial Research Organization (CSIRO) (Canberra, Australia) research program into the ecology and dynamics of using chromosome rearrangements for control of sheep blow fly, *Lucilia cuprina* Robinou-Desvoidy (Foster et al. 1993).

But sterile insect technique (SIT) operations frequently have been questioned and disputed in high-profile journals. In this article, I examine the chief criticisms leveled against some operational SIT programs. These criticisms include underestimation of the costs of areawide programs and overestimation of the benefits (Meyers et al. 1998) but such assessments lie outside the scope of the current article. The programs discussed are the screwworm (Diptera: Calliphoridae), the Mediterranean fruit fly (Medfly) (Diptera: Tephritidae), and tsetse flies (*Glossina* spp). There are other ongoing SIT programs, for example, codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), in British Columbia; pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) in the Central Valley of California; and onion fly, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae) in the Netherlands. Melon fly (*Bactrocera cucurbitae* Coquillett) has been eradicated from the Ryukyu Islands of Japan (Yamagishi et al. 1993, Ito et al. 1995), and a good review of tephritid fruit fly eradication programs is given by Mitchell et al. (1995). Useful pilot trials have been attempted in many species, but most criticisms leveled against any one project would seem to apply to all. Current activity in SIT and related matters on an international scale are abstracted in Food and Agriculture Organization/International Atomic Energy Agency (FAO/IAEA 1998).

Fundamental principle of SIT. Where strong density-dependent phenomena do not occur and high frequencies of sterile matings occur, target populations

become progressively smaller, the sterile male to fertile male ratio increases, sterile mating rates increase, and the target population eventually collapses. The proportion of sterile matings (P) in a population will be a function of the relative numbers of sterile (mS) and fertile (F) insects such that $P = mS/(mS + F)$, where m is the competitiveness of sterile relative to wild males. Thus, as sterile releases increase, $P \rightarrow 1$.

What is success? Because SIT can lead to eradication, anything less may come to be judged as failure. Thus, 11 yr after full operations began, Steelman (1976) labeled the Southwestern USA Screwworm Eradication Program "...a failure to date." Bush et al. (1976) and Richardson et al. (1982) forcefully argued that genetical explanations accounted for the screwworm outbreaks that had occurred during 1972–1976. They urged stoppage of capital investment required to establish the Mexico program until they could investigate all the problems that they claimed to have detected. Even after no screwworm cases had been detected in the United States or Mexico for 4 yr, Readshaw (1986) predicted that screwworm populations remained and would reappear with the onset of favorable weather. After screwworms had been eradicated from Libya in 1991 (FAO 1992), some commentators suggested that weather, not SIT, was the responsible factor (Krafsur & Lindquist 1996). Carey (1991, 1995) advocated that recurring Medfly detections in the L.A. basin, an area of 5,581 km^2, were manifestations of continuously reproducing populations that remained below an unspecified threshold of detection. Clearly the use of SIT for eradication of pests over large areas has raised expectations and elicited reaction from scientists a few of whom insisted that their particular expertise would answer the very problems they claimed to have uncovered.

The criticisms of SIT include the following: (1) Eradication, in the full sense of the term, is virtually impossible to achieve and even complete suppression is questionable as pest populations continue to exist undetected. Indeed, weather explains all or most of the population dynamics; SIT was not decisive. (2) Evolutionary responses in terms of mating incompatability causes "resistance" to SIT in target populations. (3) The target pest species is a complex of morphologically identical but more or less reproductively isolated sibling species. (4) The correlation between sterile insect releases and decline in target populations is coincidental with little field data to support the concept that sterile matings occur or that they reduce target population densities.

Eradication is virtually impossible and suppression is temporary. Eradication connotes finality and Meyers et al. (1998) discuss the semantics of the term. But many, perhaps most, insect pest species are highly vagile, screwworms and Medfly demonstrably so. Reinvasion from neighboring, infested areas was always a threat in screwworm programs and several introductions have been detected since the United States was eradicated in 1982 (Krafsur et al. 1987, Spradbery 1994). Introduction of Medflies via infested, imported fruit and vegetables into California occurs frequently (APHIS–PPQ, unpublished data; Dowell & Penrose 1995). The result is that there is always a finite, demonstrable risk of reinfestation that creates the expectation that there has been no eradication. How long must a region remain free of a pest before eradication can be claimed? As John Maynard Keynes said, "...in the long run we are all dead" so something less than an average human lifetime would seem appropriate.

"Eradicated" populations exist below the threshold of detection and will reappear when environmental conditions allow. Medflies were detected in the L.A. basin in 1975, 1980–1982, 1984, and yearly from 1986 to 1994. Thus, Carey (1991, 1995) suggested that Medflies had become endemic in the L.A. basin and elsewhere in California at densities usually below the threshold of detection, a position supported by Kaneshiro et al. (1995). Thereby, the onus was placed on the Medfly program to establish heroic levels of sampling in efforts to prove the negative. Such levels included 1,000 yellow sticky traps per square mile.

The reality of screwworm eradication has been questioned. Readshaw (1986) claimed that weather governs pest density and densities will return to "normal" when meteorological conditions become favorable. Because program officials routinely claimed that unusual weather, particularly copious rainfall, was largely responsible for the screwworm outbreaks of 1972–1976 (and always without data; e.g., Novy 1991), the argument was turned round by critics to suggest that years of very low screwworm incidence may have been caused by low rainfall and not by sterile fly dispersions (Richardson 1978). The role of weather in screwworm abundance was reviewed by Krafsur & Lindquist (1996). Readshaw's (1986, 1987, 1989) statistical argument that prevailing rainfall and temperature patterns accounted for the apparent disappearance of screwworms from Texas fell apart when it was demonstrated that serial correlations in screwworm case incidence from one year to the next explained his regression equation. It was shown that only summer temperatures were associated with screwworm case incidence in livestock; rainfall and winter temperatures had undetectable effects in seven climatological zones of Texas (Krafsur et al. 1986a, b; 1987).

Resistance to SIT. Mechanisms that tend to isolate wild females from released, sterile males will be strongly selected, to the extent that such mechanisms have a heritable genetic basis. Such assortative matings were suggested as a likely explanation for a large epizootic of screwworm in the southwestern United States (Bush et al. 1976).

The mating behavior of fruit flies involves male aggregation (lekking) and female choice exercised during courtship. Data show that sterile mating frequencies decline with prolonged exposure to sterile males, leading to the hypothesis that assortative mating patterns had developed (Ito et al. 1992). Kaneshiro et al. (1995) and McInnis et al. (1996) observed an analogous phenomenon in Medfly in Mani and Kauai, Hawaii. At least a partial solution to the problem may be to adopt the "filter rearing system" of Fisher & Caceres (1998) that would allow the continuous introgression of wild material into breeding stocks. Assortative mating has not been detected in other Medfly programs. No mating incompatibility was detected when crossing wild Medfly strains from eight countries on five continents nor was incompatability detected when four genetic sexing strains were reciprocally crossed to the wild strains (Cayol 1998).

Sibling species, gamodemes, etc. It has been suggested that target insect populations are in fact species complexes and the released sterile flies represent at most one of the constituent sibling, or cryptic, species. These interesting hypotheses were based on investigations in speciation among Hawaiian drosophilid flies (Makela & Richardson 1978, Richardson et al. 1982). Thus,

Richardson et al. (1982) offered a complex argument to explain outbreaks in which it was assumed that nominate *C. hominivorax* were in fact a complex of up to nine reproductively isolated, sympatric and allopatric "gamodemes" (i.e., cryptic or sibling species). The hypothesis was used to explain screwworm outbreaks by suggesting that sterile fly releases were effective only against the same gamodeme from which the release strain was obtained. The effectiveness of SIT against one gamodeme in the field allowed compensating increases in numbers of the other gamodemes presumably by density-dependent regulation. Richardson & Vasco (1985) explained ex post facto the apparent eradication of screwworms by suggesting that several gamodemes were unknowingly incorporated by United States Department of Agriculture (USDA) personnel into the factory stocks; thus, multiple gamodemes were released and brought the wild populations under control.

The notion of gamodemes was unsupported by genetic data; no crosses among the putative gamodemes were made and Richardson et al. (1982) ignored published data that showed high frequencies of sterile matings in geographical regions containing purportedly incompatible gamodemes challenged with the same strain of sterile males, and in regions different from that in which the release strain originated; indeed, he ignored any evidence that was inconsistent with his views. Years of such work by the Agriculture Research Service of the USDA has not found instances of mating incompatability in screwworm (Krafsur et al. 1987). Sibling species can usually be detected by the occurrence of hybrid sterility when crossing different strains. Mangan (1986) reported that an isofemale line from Colima, Mexico, showed variable and inconsistent levels of reproductive failure when crossed to 11 other isofemale lines from Colima, Oaxaca, and Michoacan. The outcrossed males were, on average, more semisterile than the females, but this varied from full fertility to complete sterility in replicated crosses and its genetic basis, if any, is obscure. Screwworms were eradicated from Mexico soon after Mangan's work was carried out.

Three lines of evidence exist concerning the possibility of sexually isolated screwworm populations: (1) sterile mating rates among native females after sterile fly releases (Krafsur et al. 1987); (2) banding patterns of polytene chromosomes from isofemale lines and their hybrids (Dev et al. 1986); and (3) limited sequence divergence in mitochondrial DNA in 30 different screwworm lines from diverse geographical locations, including Texas, Jamaica, Mexico, and Guatemala (Roehrdanz 1989). None of this evidence is consistent with a hypothesis of reproductive isolation.

Richardson and colleagues' arguments violate the principle of parsimony; indeed, they have created an unfalsifiable hypothesis that requires USDA to prove the negative—that "gamodemes" did not exist. In a puzzling anecdote, Richardson & Vasco (1985), "without raising the issue of technical competence," castigated the USDA for its failure to adopt the gamodeme scenario and support Richardson's research. Richardson's views have now crept into the tertiary literature. In a well-regarded textbook, Pedigo (1996) summarized some of Richardson's arguments without citing any evidence that contradicts it.

An additional argument advanced to explain screwworm outbreaks was that of laboratory selection of a rare α-glycerophosphate dehydrogenase (α-Gpdh)

allele that conferred a fitness advantage to flies in the production factory but not to the released, sterile flies in the field (Bush 1978, Bush et al. 1976). Assortative matings occurred by default, it was suggested, because "lazy" factory flies were active only in the afternoons, whereas the wild flies could mate in the mornings, thereby avoiding sterile matings. Whitten (1980) and Krafsur & Whitten (1993) showed that the frequency of the presumptive "rare" α-Gpdh allele in natural populations in Texas and Mexico was 0.6–0.7, thereby falsifying the "lazy fly" hypothesis.

Few data exist that correlate sterile fly releases to sterile matings and target population decline. Many criticisms of programs that use SIT could be deflected if such data were available; however, there are two problems in obtaining these data. The first is that in many SIT experimental trials, all manner of control is applied in an effort to isolate and reduce target population densities. The chief criterion for success is that of eliminating the target population—a repetition of the Curacao experiment. Thus, the effects of sterile male challenge are confounded by other treatments and it may be impossible even to relate magnitudes of sterile fly releases to sterile mating frequencies. Much useful information would be gained if sterile mating rates were measured along two or more transects that cross the treated area. In this way heterogeneity in sterile mating rates could assess the effects of dispersal of wild and sterile flies. Of course, the level of sterile matings necessary to achieve a downward trend in target population density will vary spatially and temporally but program efficiencies could, in principle, be increased if an understanding of relationships between sterile fly dose, sterile mating rates in the target population, and target population density responses were better understood (Krafsur 1994).

The second problem is related to ongoing eradication campaigns in which it may be difficult or impossible to gain measures of sterile mating frequencies. Often, as in the case of the L.A. Medfly program, wild females are exceedingly rare so informative and statistically valid sampling therefore impossible to achieve. In screwworm campaigns, however, the necessary sampling can be accomplished but is does not seem that those carrying out the work care to publish their findings. Sterile mating rates in tsetse fly populations subjected to sterile male releases can be assessed by dissecting out their reproductive systems.

Background and current status of programs. *Tsetse flies.* In a long-overlooked article, Vanderplank (1947) attempted in 1942 to eradicate *Glossina swynnertoni* Austen in a region of Tanzania that had experienced a human trypanosomiasis epidemic. *G. swynnertoni* is allopatric to the closely related *G. m. centralis*. Vanderplank released field-collected *G. m. centralis* pupae into a *G. swynnertoni* population. The F_1 females are semisterile and the males are completely so. Presumably a large genetic load had been introduced into the *G. swynnertoni* population. At the end of the trial, *G. swynnertoni* had been replaced by *G. m. centralis*, but *centralis* could not survive long in the region because of its aridity. The result was that the region became essentially tsetse free and was recolonized by the people who had been driven out by the earlier trypanosomiasis epidemic. This was the first field trial of a genetic control method.

Knipling (1955), unaware of Vanderplank's work, suggested that tsetse might prove to be highly susceptible to SIT because of its low reproduction rate

and its comparatively small population densities. Successful application of SIT could, in principle, eradicate tsetse without the environmental costs of habitat destruction and insecticidal use. Indeed, small SIT field trials have been performed in southern Africa, West Africa, and East Africa. *Glossina palpalis palpalis* (Robineau-Desvoidy) was totally suppressed in central Nigeria by a combination of methods, including SIT (Oladunmade et al. 1986). The IAEA and the Ethiopian government are developing an SIT campaign against *G. pallidipes* in southern Ethiopia (Mebrate & Feldmann 1998). Offori (1993) reviewed the research and pilot studies on SIT applied to tsetse flies. The most recent accomplishment is the eradication of *G. austeni* Newstead from Zanzibar in 1996 (Msangi 1998).

Tsetse eradication by SIT is technically and operationally feasible. The chief barriers are political and financial. The magnitude of such problems in the Mexico screwworm program is given by Meyer (1994).

The L.A. Medfly program. After 9 yr of detecting at least one Medfly in the L.A. basin, none was detected from November 1994 to October 1997, when 23 adults were found and that single infestation eradicated. At this writing (September 1998), there have been no further detections. Medfly operations in the L.A. basin were outlined by Dowell & Penrose (1995) and Dowell et al. (1998). This was a large and multifarious program, involving extension, quarantine, surveillance, ground bait sprays, fruit removal, sterile fly production, packaging, and releases, and other activities that involve federal, state, county, and city workers. Until 1994, the program was largely reactive, and the critical element, sterile fly releases, was made in response to local detections rather than over an area large enough to present dispersed, undetected wild females with high probabilities of sterile matings. Sterile fly releases over an area of 5,581 km^2 have been made continuously since 1994 in an effort to prevent the establishment of new introductions (Dowell et al. 1998). The risk of new, low-density infestations in urban Los Angeles remains, due mainly to illegally imported, infested fruit (USDA–APHIS–PPQ, unpublished reports; California Department of Food and Agriculture, unpublished data; Dowell & Penrose 1995). The probability of detecting a Medfly infestation is important to know and constant surveillance has been practiced for years. Lance & Gates (1994) evaluated the sensitivity of trapping regimes for detecting low-level Medfly densities by using release–recapture experiments. Their data convincingly showed that current sampling protocols would detect the low levels of infestation typical of urban foci within three generations. The foregoing research was not cited by Carey (1995), who plotted the geographical distribution and numbers of flies and larvae detected but did not provide an index of sampling intensities in the infested region. Carey's argument assumes that the detectability of Medfly populations is very low, and populations exist beneath the threshold of detection and may have done so since 1925. Carey does not seem to have indicated the magnitude of such populations (i.e., hundreds, thousands, tens of thousands of flies). Neither has he specified in the most approximate terms the chances of detecting an existing Medfly infestation.

It is always prudent to acknowledge that a failure to refute a null hypothesis does not prove that hypothesis. But Medflies, like screwworms, have a high reproductive potential—a single female can generate about 462 daughters over

her lifetime (Carey 1984). Thus, medflies can show boom-and-bust population dynamics (Southwood et al. 1974). Surely, were Medflies endemic, then occasionally sizable populations would have occurred. But no large populations have ever been detected and the most captured in any one year was fewer than 500 (including larvae), obtained by intensive and prolonged sampling. It is telling that in the controversy surrounding the question of endemic Medfly populations in California no reference has been made to basic demographic variables. Probability theory predicts that all populations become extinct sooner or later (Feller 1968), very much sooner when population densities are small. If reproductive success follows a Poisson distribution with a mean of unity (as in a population of constant size), then the probability that a fertile female will give rise to at least one adult daughter is $1 - \exp[-1] = 0.63$. Where all matings are fertile, after 10 generations the probability that there is at least one adult daughter is only 0.16. For a small population of four females, the chance that at least one will produce a surviving daughter after one generation is 0.98, but after 10 generations only a 0.50 probability of at least one female. Thus, it is unlikely that small populations could long persist without detection, especially when vigorous detection and control efforts are made.

Requiring Medfly program managers to prove the negative is illogical and unhelpful. The efficacy of SIT against fruit flies has been demonstrated time and again (Klassen et al. 1994, Hendrichs et al. 1995) and it is hard to see the advantage of declaring failure, abandoning the technology, and allowing California (and now Florida) fruit to be embargoed.

Field studies have shown repeatedly that genetic sexing strains are highly advantageous in terms of sterile mating rates and economics of production and distribution (McInnis 1994, Franz & McInnis 1995, Hendrichs et al. 1995). The USDA is now using the technology in an eradication program in California and Florida (M. Stefan, APHIS–PPQ–USDA, personal communication). It has been suggested that sterile, released females may act as a sperm sink from wild males, thereby assisting SIT effectiveness (Kaneshiro et al. 1995). If valid, it does not compensate for the much greater sterile mating rates achieved with male-only releases because there is always a huge excess of spermatozoa relative to the number of eggs to be fertilized.

Screwworm eradication. Most observers now admit the reality of eradication, at least for the time being. The risk of accidental introduction continues to recede: sterile fly dispersals have begun in Panama, where a new "barrier" is to be established (Wyss 1998) and planning is underway to begin operations in Cuba, Hispaniola, and Jamaica (Grant et al. 1998). It has been suggested that screwworms are especially susceptible to SIT, perhaps even a special case (Meyers et al. 1998), but this view ignores the reproductive biology of the fly. Screwworm females are highly vagile and fecund, their longevity is comparable with other higher Diptera, and their basic reproductive rate is of the order of 135 female offspring per female (Krafsur et al. 1987). It is true, as was suggested by Lindquist and Knipling, that average adult densities are comparatively low, but local densities can become large and the dispersal of fertile females cannot be challenged by the release of however many sterile males; it is only their virgin female offspring that are eligible for sterile matings.

What was the genesis of the screwworm outbreak of 1972–1976 if not assortative matings, weather, or the spread of sibling species? One cause was the reduced labor force on ranches that arose out of the greatly reduced threat of loss to screwworm infestations. Thus, screwworm infestations in livestock were less likely to be detected and treated. Another cause was the adoption of less expensive sterile fly distribution costs in which flies were packaged in larger units and released from C-45 and C-47 aircraft on parallel flight lanes set 5 or 10 miles apart. The change was justified on the basis of trap back of released sterile flies. There was no evidence, however, that sterile males dispersed after release in the same manner as the females. Male screwworms do not respond well to traps; thus, inferences from trap back trials were based on female responses. It was only after operational research showed that the manner of sterile fly distribution was critical (Krafsur & Garcia 1978, Hofmann 1985, after a suggestion by the late B. G. Hightower) that release tactics were adopted that ensured a better coverage of sterile males by maximizing chances of putting them in or near male aggregation sites (Krafsur 1978).

The case for SIT. On economic and biological grounds, the case for SIT for fruit flies is compelling. A number of countries, for example, Japan and member states of the European Union, will not accept fruit from endemic countries without expensive postharvest treatment. Citrus IPM programs cannot be instituted because insecticidal baits and sprays must be applied where Medfly is endemic (Hendrichs et al. 1995). The insecticidal treatments have allowed secondary pests to flourish. Fruit fly suppression by using SIT would allow natural control mechanisms to become reestablished. Indeed, there is no realistic alternative to autocidal control methods for fruit flies although the parasitoid augmentation technique is hypothetically advantageous (Knipling 1992) and could be used together with SIT (Knipling 1998). Fortunately, the economic incentives are such that national and international programs are readily established and funded. Indeed, mass rearing, packaging, and distribution technologies are well advanced. The genetics of Medfly are well understood and there is an international cadre of experienced and knowledgeable experts.

Interest in developing SIT and related methods for insect pests of humans and animals led to strong research programs on numerous *Aedes, Culex*, and *Anopheles* mosquitoes, house flies (*Musca domestica* L.), stable flies [*Stomoxys calcitrans* (L.)], and horn flies [*Haematobia irritans* (L.)]. Some projects advanced to the point where small- or medium-scale field trials were attempted, for example in *L. cuprina*, *Anopheles albimanus* Weidemann, *An. gambiae* Giles, *Glossina morsitans* Westwood, and horn flies. A review of the various programs in which SIT was used is given by Klassen et al. (1994).

But the prognosis for further development of autocidal control methods for human pests is much less favorable than for pests affecting commercially valuable products. Poor people have no political influence but it is they who are at the greatest risk. Conceptually and technically advanced methods of controlling vector-borne disease are in early development in the well-equipped laboratories of the developed world. These methods are predicated on construction of transgenic insects that cannot transmit disease-causing agents (Beard et al. 1994). It seems, however, that there is little or no research on ways to replace indigenous insect vector populations with the desirable genotypes. For many

reasons, vector-borne diseases of humans are difficult to control in the places where the diseases are most prevalent. With regard to livestock insects, stockmen in rich countries tend to avoid the public sector and maintain independence. Their commodity organizations in the United States seem uninterested in research on ecto- and endoparasites. Promising lines of research on pests of man and animals become abandoned as the university and government laboratories that performed the research fail to achieve support to scale up or even to continue their work. Instead, they switch attention to technically advanced and fashionable lines of enquiry (e.g., creating transgenic insects) and to insect pests for which there is some financial support. The American screwworm program was a notable exception, but USDA discontinued most research on genetic methods of controlling other insect pests of humans and other animals years ago. The productive CSIRO sheep blow fly project was canceled in 1992.

Why is this so? Why are SIT and related genetic methods of pest control generally viewed negatively? The reason may well be related to the current fashion of operating public institutions "like a business" in which the long-term view is virtually ignored and chiefly politically attractive, short-term results are acceptable. The SIT requires capital investment, establishment of infrastructure, and appreciation of the likely response time of target populations to continuous sterile male challenge. Another reason for dismissing SIT is that the scale of research required to achieve or show a practical, useful field application is beyond that of a university laboratory. Moreover, cooperative research among principal investigators is often ineffective or inefficient because research in academe is entrepreneurial, highly competitive, and local. Research is highly specialized and focused, and investigators tend to examine broad questions related to areawide control programs chiefly through the lens of their specialties. It is for these reasons, in my opinion, that many good scientists envy the resources awarded to projects such as the Medfly and screwworm programs.

Conclusions and prognosis. There is no credible evidence that evolutionary responses to sterile male challenge have made operational programs ineffective nor was there valid evidence that screwworms or Medflies form species complexes. The likelihood that Medflies have been continuously present (endemic) in the L.A. basin is vanishingly small—a likely red herring. It is hoped that USDA and the California Department of Food and Agriculture will publish in the peer-reviewed literature their studies on illegal importation of fruit-fly-infested fruits. The likelihood that undetected screwworm populations remain in North America is nil nor can weather explain the disappearance of screwworms. In concept and application, SIT is a powerful method of altogether eliminating a pest species from large geographic areas. The method is particularly effective in eliminating scattered residual clusters of pests that otherwise would escape detection. The SIT is not a club with which target populations are simply overflooded into extinction; as E. F. Knipling has often pointed out, variation in age structure, distribution of population densities, phenology, and other matters can greatly effect the outcome in the short term; therefore, pest control managers must take a longer-term view than if insecticides alone were being used. As more is learned about mating behavior, breeding structure and genetics of natural insect pest populations better IPM schemes can be designed in which SIT can play a major and decisive role. Research on pest

bionomics, reproductive biology, and nutritional and ecological physiology leads to continuously improving mass-rearing methods. Much operational experience, including packaging and distribution technologies gained in Medfly (Robinson & Hooper 1989) and screwworm programs (Hoffman 1985, Meyer 1994), can be applied to other species. There is a paucity of published data that relate sterile male releases to population suppression. It would lend much credibility to the efficacy of SIT if sterile mating frequencies were estimated in challenged populations. Numerous models have been constructed that relate sterility and genetic deaths to population density but few were tested with field data. Studies in which target population dynamics are evaluated in terms of sterile mating rates and other covariates are badly needed. In this way, useful mathematical models could be made and tested with actual data.

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Overview of Areawide Management of Insects[1]

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ABSTRACT In 1995 the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) implemented a new areawide pest management initiative. This program, targeted at key pests across the United States, has resulted in renewed discussion of the pros and cons of this integrated-pest-management-related control tactic. This introduction to an areawide pest management symposium presented at the 1997 Entomological Society of America Annual Meeting provides information on the development of areawide pest management as we know it today. Additionally, a comparison of areawide management to conventional pest control strategies, as well as examples of historic and current programs are presented.

KEY WORDS *Heliothis, Helicoverpa*, codling moth, boll weevil, pink bollworm, areawide pest management, IPM, pest suppression

With the initiation of four Agricultural-Research-Service (ARS)-supported areawide pest management programs since 1995, a debate on the merits of these large-scale management activities has been rekindled throughout the United States. Areawide management of insects is certainly not a new concept. Depending upon how one defines areawide pest management, however, the use of the term can produce visions of total pest management, eradication, mandatory control, various integrated management tactics, or even classical biological control. All of these management approaches have at some time in the past been implemented over broad geographical areas with varying degrees of success. Suffice it to say that areawide pest management has developed a substantial following of persons interested in this approach for management of key insect pests.

The recent resurgence of interest in areawide pest management has its origins in the 1960s and 1970s. Many renowned entomologists, including E. F. Knipling, have embraced the idea as an effective method to manage pests of

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economic importance by using an organized and coordinated attack on pest populations over large areas rather than through the use of a field-by-field approach (Knipling 1978, 1979). Knipling (1980) even suggested that virtually all insect pests of major importance to agriculture in the 1950s were still important pests 30 yr later due to the "unorganized farm-to-farm and crop-to-crop method of controlling pests only when and where necessary." In 1993–1994 the United States Department of Agriculture (USDA) Integrated Pest Management (IPM) Working Group developed a framework for collaborative activities and redefined the areawide management concept. A briefing paper was developed following a meeting of key pest management representatives from USDA, university research and extension, and state Departments of Agriculture that clearly outlined goals and expectations of the 1990s USDA areawide management programs (Coppedge et al. 1993). The result of these activities was the development of the current USDA–ARS areawide management initiative. Based on the discussion held by the IPM Working Group we currently define areawide pest management as the systematic reduction of a target key pest(s) to predetermined levels through the use of uniformly applied pest mitigation measures over geographical areas clearly defined by biologically based criteria.

This introductory paper focuses on the characteristics and advantages of areawide pest management compared with conventional strategies, provides historic examples of areawide management programs, and summarizes briefly the current areawide management programs being conducted worldwide. It also serves to set the stage for related papers on current areawide management programs being conducted in the United States. Each location that is conducting areawide management activities (Yakima, Brookings, etc.) has developed or is developing its own website. One example is the Areawide Pest Management Research Unit in College Station, Texas, at http://USDA-APMRU.TAMU.EDU/. The ARS homepage at http://www.ars.usda.gov/ will link to each areawide management site as it becomes available.

Areawide Management Characteristics and Advantages

Kogan (1995) stated that areawide suppression of key pests rests on a set of principles that differ substantially from the tenets of IPM. Kogan (1998) recently put forth the following as a refined definition of IPM: "IPM is a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost-benefit analyses that take into account the interests of and impacts on producers, society, and the environment." Areawide management, although closely related to IPM, has distinct characteristics that, at times, can make it uniquely different. Three key characteristics currently serve as guideposts for development of ARS areawide management programs.

First, the program must be conducted over large geographical areas that are delineated by biological criteria associated with pest colonization and dispersal potential. Kogan (1995) states that the operational unit of an areawide management program should be defined by some geographic entity that encompasses individual farms as well as all other nonfarm components of the landscape.

Second, areawide management programs should be coordinated by organizations (groups of key participants) rather than individuals. It is important to involve as many stakeholders (commodity groups, farm organizations, agribusinesses, local extension agents, growers, and rural residents other than growers) as possible in the formative stages of any program development. Acceptance of the concept by participants is a key component for the program to be successful. Naturally, grower support and guidance is the primary foundation for the entire process. Without the organized efforts of this key group within an area there would be little possibility of developing an effective program. After all, the producers will be the parties who in all likelihood will pay for either putting areawide programs in place or continuing those started by ARS and university partners. Implementation of areawide pest management requires exceptional levels of coordination of all phases of program conduct. The site coordinator(s) and their teams will become the spokesperson(s) for the project and must be able to select appropriate control tactics, monitoring procedures, educational opportunities, and assessment methods. Additionally, coordinated oversight of budgets and personnel is needed to ensure that programs move forward to address important issues and maintain the common goals of the group.

And third, programs should focus on reducing and maintaining a pest population at an acceptably low density. Currently, ARS-supported areawide management programs target suppression of key pests rather than eradication. Suppression of a pest can be accomplished using natural control tactics (e.g., biological control agents, weather) or augmentative suppression measures that are developed using current agricultural research (e.g., mating disruption, insecticides, host-plant resistance). Prevention of significant economic damage can be more easily attained by maintaining pests at a low density.

Based on these characteristics it is readily apparent how areawide pest management could be viewed as different from IPM. As described previously, areawide management focuses on only a single or small group of pests over a large region, whereas IPM incorporates all pests within an agroecosystem into a management program that is primarily conducted on a farm-to-farm basis. Recently, however, areawide management proponents in the United States have started using the term areawide IPM to more accurately describe programs currently being conducted. Areawide programs now not only monitor and attempt to manage a key pest(s) but also evaluate and address secondary pests and other nontargets, including beneficial arthropods, in an attempt to better understand and manage all pests across the landscape. Thus, the term areawide IPM provides a more accurate depiction of current management strategies.

Lively discussions have often been conducted to determine the merits of using areawide pest management for control of numerous pests. From these discussions, six advantages or benefits have been identified: (1) areawide pest management, when interfaced with IPM systems, offers a long-term solution to the pest problem as opposed to quick-fix solutions on small acreages; (2) when properly implemented, areawide pest management can prevent major pest outbreaks and provide a more sustainable management procedure for pests; (3) areawide pest management permits the use of the best and most environmentally benign management techniques; (4) once fully implemented, areawide pest

management can be more cost effective than managing pests on an individual farm basis; (5) areawide pest management permits the use of biorational management strategies for secondary and other key pests; and (6) the basis of an areawide pest management program is an effective monitoring program and a reduction in prophylactic control treatments.

Historic Examples of Areawide Pest Management Programs

A thorough search of the entomological literature can provide numerous examples of large-scale, coordinated programs that can be loosely classified as examples of areawide pest management programs. One of the earliest documented examples of a program that specifically targeted a key pest over a wide geographic region is that of the grape phylloxera, *Daktulosphaira vitifoliae* (Fitch). Suppression of this pest occurred in Europe during the 1870s and 1880s. This prime example of a successful host-plant resistance program resulted in resistant grapevines being distributed throughout France and other European and Mediterranean countries. The pest was completely controlled by 1890 (Balachowsky 1951) and saved the French wine industry (Kogan 1982).

A classical biological control program often used as an example for most entomology IPM students is that of the cottony cushion scale, *Icerya purchasi* Maskell. This pest was accidentally introduced into California in the 1860s (DeBach et al. 1971) and rapidly became a significant pest of citrus. By the late 1880s the infestation threatened the existence of the California citrus industry. To combat the infestation two biological control agents were introduced from Australia: the vedalia ladybeetle, *Rodolia cardinalis* (Mulsant), and the parasitic fly *Cryptochaetum iceryae* (Williston). The vedalia ladybeetle quickly became the foremost biocontrol agent in southern California and brought about the complete suppression of this scale insect by the end of 1889 (Doutt 1958). This example can readily be related to areawide pest management due to the broad and coordinated efforts used to distribute the biocontrol agents.

One of the most striking early successes in an areawide eradication effort involves the cattle ticks, *Boophilus annulatus* (Say), and *Boophilus annulatus* var. *microplus* (Canestrini), which were eliminated from most of the United States by the 1950s (Cole & MacKeller 1956). A cooperative federal and state cattle-dipping campaign began in 1906 across 15 southern and southwestern states. Knipling (1979) attributed the success of this effort to a well-organized program involving animal health agencies and livestock growers.

The highly successful eradication of the new world screwworm, *Cochliomyia hominovorax* (Coquerel), from the United States, Mexico, and portions of Central America has become a classical example of areawide pest management based on the sterile male technique. Bushland & Hopkins (1951) conducted initial laboratory studies demonstrating the utility of sterile males for insect control. A sterile-male release program was initiated in Florida and other southeastern states in 1958 and was successfully completed in 1959 (Knipling 1979). In 1962 a similar program was initiated in the southwestern United States and Mexico (Scruggs 1975). By 1966 self-sustaining screwworm populations were eliminated from the entirety of the United States and a barrier zone of sterile flies was set up along the United States-Mexico border to prevent reinfestation.

In 1972 the United States-Mexico Joint Commission was formed with the goal of eliminating the pest from Mexico. In 1991 Mexico was declared free of screwworms. Since that time, the pest has been eliminated from Belize (1994), Guatemala (1994), El Salvador (1995), and Honduras (1995) (USDA–APHIS 1998). This program, which has been jointly financed by various governments and portions of livestock industries, remains a prime example of the coordination necessary to achieve success in an important program over an expansive geographic area.

Current Areawide Pest Management Programs

Numerous areawide insect management programs are currently being conducted throughout the world. Fruit fly (Diptera: Tephritidae) sterile insect technique programs are being conducted in Argentina, Australia, Costa Rica, Greece, Guatemala, Mexico, Pakistan, Peru, Philippines, Portugal, Thailand, and the United States (Hendrichs & Ortiz 1996). These programs are coordinated by grower associations and government agencies to prevent major economic damage to numerous fruits and vegetables. The sterile insect technique program also is being used to eradicate tsetse flies, *Glossina* spp., from Zanzibar (Feldman et al. 1996).

In the United States several key insects are currently being managed using various areawide-related techniques. Pink bollworm, *Pectinophora gossypiella* (Saunders), has been the target of numerous suppression programs since the 1960s. An areawide management program for this pest has been in place in the San Joaquin Valley of California continuously since 1968 (Henneberry 1996). Most of the current pink bollworm suppression programs that are established or under development use sterile insect releases, cotton plant destruction, mating disruption, and trapping for management of the pest. Likewise, two other key lepidopteran pests of cotton, the tobacco budworm [*Heliothis virescens* (F.)], and the bollworm [*Helicoverpa zea* (Boddie)] have been targeted for an areawide suppression program by using aerial application of a baculovirus across the Mississippi Delta (Hardee & Bell 1996). The ongoing boll weevil *(Anthonomus grandis grandis* Boheman) eradication program, which was initiated in North Carolina, South Carolina, and Virginia in 1977, is another example of a successful, highly coordinated management program (Coppedge 1996). Most funding for this program, currently being conducted in portions of Alabama, Mississippi, Tennessee, and Texas, comes from the cotton producers within each region.

In 1995 the first formal USDA–ARS areawide pest management partnership program was implemented in the northwestern United States against the codling moth, *Cydia pomonella* L. (Calkins et al. 1997). The codling moth is the key pest of pome fruit in the western United States and, if not controlled, causes the majority of damage to the product. The goal of the areawide suppression program for codling moth is to marshal a western-regional, multi-institutional program to assess, test, and implement an integrated strategy for the management of codling moth populations on fruit orchards that will alleviate the impact of neurotoxic pesticides on natural enemies and will open the opportunity for use of more environmentally friendly control tactics for secondary pests

(Calkins et al. 1997). This multistate effort (California, Oregon, and Washington) has been highly successful, continues to expand, and has reduced insecticide use targeted at codling moth in each management site.

The corn rootworm areawide management program was implemented in 1996 and was the second ARS-coordinated program conducted under the current areawide management initiative. This program is being conducted at single sites in Kansas, Illinois and Indiana, Iowa, South Dakota, and Texas and is targeted at managing western, *Diabrotica virgifera virgifera* LeConte; northern, *Diabrotica barberi* Smith & Lawrence; and Mexican, *Diabrotica virgifera zeae* Krysan & Smith corn rootworms by using semiochemical insecticide baits. The mission of this program is successful establishment and implementation of an areawide demonstration program that is the result of a partnership of growers; private consultants; applicators and suppliers; research and extension personnel; and local, state, and federal agencies who have a stake in the development and adoption of improved crop management technologies, and that clearly demonstrates advantages of enhanced grower profits, reduced risks, enhanced environmental compatibility, and superiority of IPM approaches compared with current pest control approaches (Chandler et al. 1998). To date, this program has effectively reduced adult corn rootworm numbers and holds promise for improved population management of this important pest complex.

Two additional ARS-coordinated areawide management programs, leafy spurge (*Euphorbia esula* L.) and stored grain insects, were initiated in 1997. These promising programs will be conducted through 2001 and are examples of new and exciting directions taken by researchers who have embraced the areawide management concept.

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Review of the Codling Moth Areawide Suppression Program in the Western United States[1]

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ABSTRACT A regional, multi-institutional program was implemented in 1994 to assess and test an integrated strategy for suppression of populations of codling moth [*Cydia pomonella* (L.)] in fruit orchards in the western United States. The program was designed to alleviate the impact of neurotoxic pesticides on natural enemies while continuing to control insect pest populations. Reduced use of organophosphate insecticides created the opportunity for exploring more environmentally friendly control tactics for secondary pest control. Five pilot test sites were selected, one each in California and Oregon and three in Washington State, to demonstrate the value of using mating disruption, biological control, and in one site, release of sterile moths to control codling moth and secondary pests with a minimum use of neurotoxic chemicals. Results from the first 3 yr indicated that this latter strategy is an effective control measure. Research at the pilot sites will continue through 1999. Funding for the codling moth program is shared currently by the Agricultural Research Service and the fruit industry. Additional support is provided by state universities in California. Oregon, and Washington. A series of research projects impacting the pilot test sites was funded as well. This program may eventually be adopted and supported entirely by the growers.

KEY WORDS *Cydia pomonella*, codling moth, mating disruption, pest management, areawide, apple, pear, IPM

As part of the United States Department of Agriculture Integrated Pest Management (IPM) Initiative, under the Strategic Implementation Plan, the Agricultural Research Service (ARS) is charged with establishing programs to support needs of integrated pest management through implementation of pest management projects that encompass extensive land areas (Calkins et al. 1997). These projects address the control of pest populations on an areawide rather than an individual-field basis. Suppression of insect populations over large areas is a more permanent solution to pest control than small-scale projects under which populations may rebound quickly because of emigration from adjacent areas (Knipling 1979).

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The ARS provided funding for a series of IPM programs that began in 1994. The mission and goals of these programs are to establish and implement research and action activities for key pests and crop systems that have been identified as high priority. These programs are linked and partnered with state university research and extension departments, state departments of agriculture, federal agencies, and the private sector, including growers and commodity groups. An areawide approach is advantageous for at least six reasons: (1) pests do not "recognize" property lines, especially when similar crops are contiguous; (2) cost of overall control is lowered if the problem is addressed on a large scale; (3) pest populations can be reduced below the economic threshold over a large area and they would take time to build up to economic thresholds; (4) local eradication is possible for a short time; (5) worker safety risk is reduced because fewer pesticide treatments are necessary; and (6) growers with limited acreages adjacent to each other that are using the same treatment will enjoy the same advantages as growers having extensive acreages.

The Codling Moth Areawide Suppression Program (CMAP) was initiated in 1994 with the announcement that the ARS had selected the codling moth [*Cydia pomonella* (L.)] as the first candidate for testing the areawide concept and that they would provide funding for the program. Partners in the program were scientists from Oregon State University, Washington State University, and University of California-Berkeley, as well as participating growers. The codling moth was identified as the key pest on apples (*Malus* spp.) and pears (*Psylla* spp.) in the western United States, a region where 54% of the commercial apples and 95% of the commercial pears are grown. Codling moth control represents the major use of organophosphate insecticides for apple production in the United States. Chemical sprays also control other secondary pests depending on when the sprays are applied. These chemicals kill many of the natural enemies; however, that may play a role in managing some secondary pests. A complete chemical control program on tree fruit includes treatments that are directed at secondary pests that are released from biological control when their parasitoids and predators are removed. Growers become locked into a continuous program of spraying to control the key pest, followed by control of the secondary pests, many of which were not pests before the broad-spectrum neurotoxic controls were applied. Elimination of the pesticide azinphosmethyl (Guthion) used against the codling moth, would reduce the pesticide load substantially in orchards and would free the natural enemies from frequent population crashes. Elimination of azinphosmethyl use also would remove much of the pesticide exposure risk to orchard workers. And finally, reduction or elimination of broad-spectrum pesticides would slow the development of resistance in codling moth and some of the secondary pests.

The movement away from broad-spectrum pesticides forced growers and their consultants to spend more time in the orchards, observing conditions and monitoring populations of both pests and beneficial arthropods. Spraying prophylactically became less common, although certain cover sprays were still needed to reduce codling moth populations early in the program. Most pest controls, when needed, would be of a soft nature (i.e., insect pathogen sprays [*Bacillus thuringiensis* Berliner] [Bt] and insect growth regulators for leafrollers

[Tortricidae], and soaps and oils for leafminers [Agromyzidae] and aphids [Aphididae]). The eight objectives of the CMAP are as follows: (1) enhance the efficacy of the nonpesticide system; (2) demonstrate that mating disruption is more efficient on a large scale than a local scale; (3) develop technology to lower control costs; (4) implement a biological control program that is self-sustaining; (5) develop technology to complement mating disruption; (6) develop monitoring systems for all pests and principal natural enemies; (7) improve worker safety; and (8) improve the perception that fruit is produced with high consumer-safety standards in mind.

The primary goal of the program is to eliminate or reduce substantially the broad-spectrum chemicals used for codling moth control. Conventionally treated orchards in the Pacific Northwest presently use several broad-spectrum pesticides. For codling moth control, azinphosmethyl and chlorpyrifos (Lorsban) are used from two to six times during the growing period; chlorpyrifos and encapsulated methyl parathion (Penncap) are used to control the leafroller complex. Aphids are sprayed as needed, as many as three times a season, with imidacloprid (Provado) and endosulfan (Thiodan). Western tentiform leafminers (*Phyllonorycter elmaella* Doganlar & Mutuura) are similarly sprayed with oxamyl (Vydate), which can result in buildup of mite populations. The white apple leafhopper (*Typhlocyba pomaria* McAtee) and other leafhoppers are treated with carbaryl (Sevin) and endosulfan.

Growers were beginning to realize that their front-line control tool was losing its effectiveness. When azinphosmethyl was first used against codling moth, one or two sprays were sufficient to control the population. As the use of azinphosmethyl became more widespread and more frequent, however, the codling moth seemed to develop resistance to it. The growers were spraying as many as six times a season and their fields were still incurring substantial damage. These frequent sprays also were removing many of the natural enemies (parasitoids and predators) of other pests. In addition, there was the perception that the use of organophosphate and carbamate pesticides left residues on fruits that affect the consumer, especially young children and infants. Although any residues were lower than Environmental Protection Agency limits, this perception still persisted.

During 1996, the Food Quality Protection Act (FQPA) was passed by Congress as a replacement for the Delaney Act. The FQPA called for the reduction of exposure of the public to whole classes of compounds, such as organophosphate and carbamate insecticides. The allowed combined exposure is critical for the fruit and vegetable industries because the minute residues that consumers are exposed to on these foods are combined with exposures from household, lawn, garden, and other nonagricultural pesticide uses. The agricultural uses of these chemicals are threatened at a time when viable nonpesticide controls are not readily available.

To replace the hard chemicals, a strategy involving mating disruption of codling moth was applied. Mating disruption is the use of an insect's sex pheromone communication system to disrupt the reproductive process (Cardé & Minks 1995). This technique consists of placing a synthetic sex pheromone of the codling moth in dispensers located throughout an orchard. The recommended dispenser takes various forms, including ropes and membranes, that

emit varying amounts of pheromone throughout the growing season (Beers et al. 1993) These dispensers, containing ≈1 mg of the synthetic pheromone, are placed near the tops of the tree canopy at the rate of 1,000 dispensers per hectare. Presumably, males have trouble finding the live calling females amongst these dispensers and thus many females remain unmated for most or all of their lives. The result is a dramatic reduction in the number of fertile eggs laid in the orchard. Early mating disruption trials with codling moth have had variable results with most of the failures in small orchards surrounded by conventionally sprayed orchards (Beers et al. 1993). Most of the damage occurs on the perimeter; on the uphill sides; and near bin piles, prop piles, and brush piles. It appears that mated females entering the treated orchards are not affected by the pheromone permeation. Larger orchards had the same problems with damage at the perimeter, but because the ratio of area to perimeter was much greater and these orchards tended to be more isolated from their neighbors, the damage was not as severe. Other types of control compatible with the CMAP are release of sterile moths (Dyck et al. 1993), Bt sprays against leafrollers (Beers et al. 1993), augmentative releases of parasitoids, and orchard sanitation (i.e., making sure there are no fruit remaining on the tree after harvest to facilitate another generation of codling moth).

Many growers with extensive acreages of apples, pears, or a combination were using codling moth mating disruption routinely with satisfactory results at the time the program started. Many growers with smaller acreages of orchard, however, were not satisfied with the results and their failures were causing other growers to forego the use of the technology. It became apparent that the technology needed to be fine-tuned to make it useful for all and to make it available throughout the fruit-growing areas in California, Oregon, and Washington. Obviously, the application of mating disruption in small orchards was not going to work under the original methods. The key to making the technology useful was to apply a focused IPM program against codling moth on extensive acreages by using mating disruption as the primary control method. To test this strategy and to demonstrate its utility, it was necessary to establish a number of pilot test sites in strategic fruit-growing areas.

When the CMAP was being developed, the following criteria were established for selecting the pilot test sites: (1) participants should support the concept of areawide pest management and be willing to allocate resources and people to the project; (2) the large-scale pilot test sites must be typical production areas with representative pest problems; (3) each selected site should be large enough that meaningful data could be produced to determine efficacy, and economic and environmental benefits; (4) populations of key pests should occur consistently so that meaningful data could be collected; (5) producers within the test areas should be cooperative and be willing to share costs; and (6) the site should have the organizational structure to support and establish the enhanced IPM systems in the local community.

The first task was to establish pilot tests where the technology could be demonstrated. An intensive monitoring strategy was built into the program to determine the effects of the removal of azinphosmethyl on codling moth, leafroller, leafminer, leafhopper, and aphid populations. Populations of natural enemies also were monitored to determine their role in pest control. Initially, most growers were

skeptical that the technology would be successful because the early experiences of some growers were negative. Others had a wait-and-see attitude, wanting other growers to take the initial risk. When a series of pilot tests was proposed for the three-state area, some progressive growers were willing to organize their neighbors to start the program. Because there was skepticism among some of the neighbors, however, the ARS wanted to ensure that the program would continue for a minimum of 3 yr. Therefore, a subsidy of $125 per hectare was offered to participating growers for 3 yr to reduce the cost of mating disruption to the level of what some growers were paying for chemical control.

The pilot tests, termed CAMP (codling moth areawide management projects) sites, will be maintained for 5 yr to determine the long-range effects of reduced pesticide use. The California CAMP site, Randall Island, in the delta of the Sacramento River, consisted of 300 ha of pears. The 200-ha Oregon CAMP site was located near Medford. Of the three Washington CAMP sites, Lake Osoyoos abutting the Canadian border north of the town of Oroville had 320 ha of mostly apples; Howard Flat was near Chelan and had 440 ha, whereas Parker Heights near Yakima consisted of 180 ha.

After 1 yr, all of the participants were satisfied that the strategy was viable. During the second year, several of their neighbors wanted to participate as well, therefore many of the areas were expanded. After 3 yr, many other growers were anxious to be included in the program so a series of 1-yr CAMP sites was established in areas of California and Washington that were remote from the current sites. The purpose was to create pockets of mating disruption similar to the original sites that had a reasonable chance of succeeding on their own after the 1 yr of support. The sites were required to meet the following criteria: (1) a site must be composed of at least five growers and 160 ha of mostly contiguous apple or pear blocks; (2) a person is needed to organize the growers; (3) a sponsor such as a packing house, an experiment station, or an agricultural service center is required to provide the infrastructure; and (4) there had to be some reasonable chance that the program would continue after 1 yr when program funds were no longer involved. These test sites were subsidized up to $40,000 per site for 1 yr of participation to hire a coordinator, to pay for labor to monitor populations, and to purchase monitoring traps and lures. There were immediate, positive results of the technology in most of the 1-yr sites. The codling moth damage was lower than expected and natural enemy populations increased dramatically at these sites. Most of the growers are continuing the program on their own. Seven more 1-yr pilot test sites have been selected and subsidized for 1998, one in Colorado and six in Washington.

The first step in a sequence of treatments recommended for control of the codling moth was to install pheromone dispensers in the orchard at or before blossom time at the rate of 1,000 dispensers per hectare or two dispensers per tree. Some growers with low codling moth population pressure have been able to achieve control with half that number but this approach was not recommended by the ARS. The first year, a cover spray of azinphosmethyl was recommended after blossom to reduce the codling moth population because mating disruption is most effective at low population levels (Beers et al. 1993). Monitoring traps baited with 10-ml lures were placed in the orchard at the rate of one trap per hectare. These dispensers were monitored each week throughout the season to

determine the codling moth population level. If the population exceeded five moths per trap per week, the grower had the option of applying a second cover insecticide spray to ensure that damage levels did not exceed the maximum allowed by the packing house.

The damage levels in orchards managed with mating disruption during the first year were comparable to damage levels in conventionally treated orchards. By the second year, the trap catches and the damage levels were much lower than those in the conventional controls. The growers had been warned that the perimeter of the areas had the greatest risk of codling moth infestations; thus, many growers installed extra dispensers on the perimeters, in trouble spots, and on the upwind side of the disruption-managed blocks. If the respective growers were concerned about being on the perimeter, it was suggested that they bring their nonparticipating neighbors into the program and let them be on the perimeter. Addition of early nonparticipants also promoted expansion of the test sites. After 3 yr, participating growers have come to accept mating disruption as a viable technology that allows them to bypass the pesticides without risking damage to their fruit crop.

For codling moth, the CMAP operates most effectively with mating disruption that is used as a prophylactic treatment to keep the population low, thus avoiding azinphosmethyl sprays. This approach leads to a biologically driven IPM program as opposed to the chemically driven program that existed before mating disruption was used. Leafrollers, when present, are controlled with sprays of Bt as needed so that natural enemies would be spared. Leafminers are controlled when necessary by using oils; however, parasitoid populations generally increased and controlled the pest to the extent that oils were usually not needed. Leafhoppers are controlled with soaps and oils but usually no treatment has been needed because natural enemies have kept numbers below economic thresholds. Aphid populations are usually sporadic, but growers are now more tolerant of them when populations of coccinellids and lacewings are present, expecting these predators to attack them before they can cause damage. As a last resort, growers can use imidacloprid without harming the natural-enemy populations.

Although the cost of mating disruption was higher initially than conventional chemical control of low populations of codling moth, as the program progressed, the cost of mating disruption actually became lower when reduced treatments for secondary pests were calculated. Worker safety is a factor when azinphosmethyl is used because all personnel must be kept out of the treated orchards for 72 h after application. This cost is real but difficult to calculate. Cost of conventional control of larger codling moth populations for which growers sprayed four to six times was much higher than for the soft program. Thus, the participants in the areawide program had fewer pesticides sprays, less exposure and risk from chemicals, better labor management, better biological control, and they are in a position to control most of the pests when the FQPA takes effect.

A research component was included in the program to solve problems that arose during the implementation phase. Proposals were submitted from interested scientists and those that impacted directly on the implementation portion were funded. A newsletter covering events of the areawide program is printed by the Washington State University Extension Service at regular intervals to

keep participants and interested persons up to date on the progress of the program. The ARS discovered that the message on the effectiveness of the technology also was communicated among growers: when growers saw their neighbors using and discussing the good points of the technology, they wanted to participate as well.

Many growers with extensive acreages are now conducting their own areawide projects and some are bringing in their neighbors with smaller acreages. Many growers with small orchards are cooperating with their neighbors to create extensive acreages under the same treatment regimes. This participation is what ARS had hoped would happen. The CMAP was designed to be a self-help program to transfer the technology to the growers in a form that they could use profitably with minimal use of hard pesticides. The ARS support of this 5-yr program will end in October 1999.

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Rootworm Areawide Management Project in Kansas[1]

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ABSTRACT Kansas State University initiated a Rootworm Areawide Management Project (RAMP) under the auspices of the United States Department of Agriculture (USDA) in 1997. This 4-yr project is being conducted in north central Kansas with the cooperation of 47 owners or operators. The project is a concerted effort to suppress adult western corn rootworms, *Diabrotica virgifera virgifera* LeConte, over a large contiguous area of continuous corn (*Zea mays* L.) production by using the principles of integrated pest management (IPM), including carefully timed applications of the semiochemical insecticide-bait SLAM®. In addition to this adult suppression program, technology is being developed or modified to determine treatment thresholds and application timing so that private consultants and growers can continue rootworm areawide suppression after the year 2000, when the intensive research program terminates. The managed area is located in 41.4-km^2 (16-mi^2) of intensely irrigated corn–soybean production near Scandia, Kansas. A similar 10.36-km^2 (4-mi^2) area near the town of Norway, Kansas, will serve as a control and will not be treated with Slam throughout the duration of this project. One hundred and twenty-one cornfields were monitored in 1997 with Pherocon AM® yellow sticky traps. Treatment thresholds were ≥35 western corn rootworm adults per sticky trap per week or 0.6 western corn rootworm per plant based upon plant inspections that were used to supplement trap counts. One thousand one hundred and seventy-seven hectares required treatment (68.5% of the total from the managed area). The Slam application resulted in ≥95% adult western corn rootworm control in all treated fields. Treated and untreated fields were selected for 10 additional experiments that were initiated to refine sampling technology necessary for adequate timing of insecticide applications. All experiments will be continued, with appropriate modifications, throughout the duration of the project.

KEY WORDS *Diabrotica virgifera virgifera*, western corn rootworm, SLAM®, cucurbitacin, pest management, sticky trap, lure trap, corn, soybean

Corn rootworms (*Diabrotica* spp.) are among the most economically and environmentally important insect pests of corn (*Zea mays* L.) production

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systems in Kansas and in the United States. Levine & Oloumi-Sadeghi (1991) reported that 50–60% of the total corn acreage in the United States is annually treated with soil insecticides to protect the crop from larval feeding damage. This represents the single largest use of insecticide in the United States with growers generally applying insecticides based on previous field history instead of need. Levine & Oloumi-Sadeghi (1991) suggested that more than half of the fields treated do not have corn rootworm densities that warrant an insecticide application. Foster et al. (1986) concluded, however, that applying soil insecticides prophylactically every year to continuous cornfields was more cost- effective than sampling for adults. Foliar insecticide applications for beetle control, although not as widely used as soil insecticides, can impact nontarget organisms, including beneficial arthropods, and may result in pest resurgence. Insecticide drift also can be problematic.

Due to the problems associated with traditional soil and foliar insecticide use for management of corn rootworms, scientists with the United States Department of Agriculture–Agricultural Research Service (USDA–ARS) and several midwestern state agricultural experiment stations have developed a management concept based on suppression of corn rootworm adults by using an environmentally friendly product applied by either aircraft or high-clearance sprayers. The premise of this strategy is to kill beetles prior to oviposition and thus to prevent root damage if corn is planted in the subsequent year. The tactic uses behavior-modifying chemicals that are specific for corn rootworm beetles; these chemicals induce adults to feed compulsively on a bait formulation. The semiochemical insecticide-baits are composed of cucurbitacins, nontoxic edible carriers, and the toxin carbaryl. The baits use 95 to 98% less active ingredient per acre than widely used soil or foliar insecticides and can be applied using either ground or aerial methods.

Research in South Dakota demonstrated that management of corn rootworm adults with semiochemical insecticide-baits can be successful when done over a relatively large area. Semiochemical insecticide-baits or other management tactics over larger areas (i.e., areawide management) could help eliminate these problems. Growers participating in areawide management as part of a corn integrated pest management (IPM) program would make decisions on need for corn rootworm management (as well as that of other arthropod pests) based on knowledge of existing population densities rather than by managing the pests by using prophylactic insecticide treatments.

The objective of this study is to evaluate the effect of adult western corn rootworm *(Diabrotica virgifera virgifera* LeConte) areawide management by using semiochemical bait technology, and to integrate this semiochemical bait technology into a corn IPM system that takes into account other corn insect pests. Comparisons of corn rootworm and other insect population data collected before (baseline data), during, and after implementation of the study within and among the experimental and control areas will be used to assess the efficacy of the concept.

Materials and Methods

Study site. The first full year for the 4-yr Kansas Rootworm Areawide Management Project (RAMP) was 1997. The study site comprises a managed area and a control area. The managed area consists of 41.4-km^2 (16-mi^2) of intensely irrigated corn–soybean production near Scandia (White Rock and Courtland townships), Kansas. Rootworms in this area will be controlled with SLAM®, an insecticide containing the active ingredient carbaryl and the feeding stimulant cucurbitacin (Hoffmann et al. 1995). A 10.36-km^2 (4-mi^2) area located 12.9 km (8 mi) south of the managed area near the town of Norway (Norway Township), Kansas, is the control area that has not and will not be treated with Slam throughout the duration of this project. Both areas lie within the Republican River valley where the Kansas Bostwick Irrigation District No. 2 supplies irrigation on demand. This study site was selected because of the large amount of continuous corn produced in the area and traditionally high populations of western corn rootworm, the significant rootworm pest in Kansas (Higgins et al. 1995). Eighty-eight percent of the fields surveyed in this area in 1996 exceeded the treatment threshold for western corn rootworm adults. In addition, area corn producers expressed willingness to cooperate in an areawide project.

Producer cooperation. Prior to the 1997 growing season, two memorandums of understanding (MOUs) were developed by Kansas State University's Office of Grants and Contracts, with approval of the USDA, for all potential owners and operators within these two sites to sign. Separate MOUs were developed for managed-area cooperators and control-area cooperators based on different goals established for each site. These agreements were necessary to explain the goals and methodology and to avoid any misunderstandings by participants. Public meetings were held with all interested parties to explain the project before the MOUs were mailed to potential cooperators. Ninety-eight percent of the cooperators signed the MOUs. One operator, although not opposed to the project, would not sign the MOU because the landowner was satisfied with the existing western corn rootworm management and did not wish to change existing practices. This did not affect the concept of western corn rootworm adult suppression on an areawide basis as this landowner is engaged in adult suppression with a conventional insecticide. During 1997, 47 producers planted ≈2,247 ha (5,553 acres) of corn (36 growers with 1,719 ha [4,247 acres] in the managed area; 11 growers planted 529 ha [1,306 acres] in the control area).

An areawide project could not be conducted without cooperation by area owners and operators. Two-way communication is very important in keeping all of the participants informed of evaluation results, concerns, and other issues. Kansas State University research and extension entomologists try to have at least two meetings annually with area cooperators. One meeting is held prior to the growing season to inform participants of plans and methodologies for the upcoming season, and the second meeting is held postharvest to inform participants of results. Additionally, newsletters are mailed to all participants periodically noting results, project changes, and methodology.

Scouting protocol. Prior to initiating the RAMP the decision was made not to use local, private consultants for adult rootworm monitoring to avoid

competition with local pest and crop management enterprises. Thus, a program was established to hire students for the summer and provide the training necessary to scout for adult western corn rootworms. Initially, 10 scouts were hired and divided into five teams of two scouts each. The 20 sections of land, encompassing the total project, were divided into five equal regions (four sections per region) and one two-scout team assigned to each region. Having each team responsible for the same region throughout the monitoring period was beneficial because it allowed the cooperators to become familiar with the scouts who were in the field every day. It also allowed the scouts to become acquainted with their smaller region and facilitated noting any changes to the western corn rootworm populations or other factors that may affect the RAMP [i.e., European corn borer, *Ostrinia nubilalis* (Hübner), other insecticide applications, etc.]. Information for individual fields was provided to an individual grower only if requested. Growers could and did continue to employ private consultants if that was a normal practice. Private consultants often shared their scouting information on western corn rootworm and European corn borer.

During 1997, there were 121 separate cornfields monitored by RAMP scouts. All fields were mapped, and the maps were supplied to the aerial applicator to aid in locating the treatment fields. This system seemed to work well and will be used throughout the duration of the project. Crop location will change somewhat between years as crops are rotated. Because this is an irrigated area, however, field size and location will not change considerably and a large percentage of the cropland will remain in continuous corn.

Aerial application. Prior to the 1997 growing season, it was decided to employ one aerial applicator for the entire managed area even though area growers commonly used three or four applicators. A bid process was initiated to allow all interested applicators to compete for this contract. It also was suggested that having only one applicator would reduce response time, increase familiarity with the managed area, and simplify paperwork. This approach worked well given the amount of corn involved, and allowed the aerial applicator to focus on western corn rootworm treatment during the critical adult treatment period. Thus, the administrative network was in place to initiate the field monitoring and treatment program for 1997.

Monitoring protocols. Pherocon®AM/NB yellow sticky traps (Trécé, Inc., Salinas, California) were used to monitor all 121 cornfields. At least 12 traps were placed systematically in four rows of three traps per row in all fields by 7 July 1997. A scouting pattern was established to allow all traps to be checked and changed weekly. Numbers of western corn rootworm adults were recorded and traps placed in a freezer so that adults could be examined and sexes separated at a later date. As fields reached the treatment threshold an attempt was made to remove the traps 24 h prior to SLAM® treatment. All traps were replaced within 24 h after SLAM® application and weekly monitoring was resumed to determine whether retreatment was necessary. All fields approaching the treatment threshold also were visually inspected for western corn rootworm adults by making whole-plant counts of five plants selected randomly from four separate locations (20 plants in total) in each field. Sticky traps or visual plant inspections were used to initiate SLAM® treatments if the treatment threshold was exceeded. Approximately 24 h after application all fields

were visually inspected for SLAM® residue and efficacy as the sticky traps were replaced. This monitoring revealed that the first 315.67 ha (780 acres) treated required retreatment because of apparent product unreliability (SLAM® produced in 1994 and the first batch produced in 1997 did not provide desired or expected efficacy) or droplet size and placement problems (required switching of aircraft). After adjusting for these initial problems, SLAM® appeared to provide excellent (≥95%) control in all treated fields.

Treatment thresholds. The treatment threshold for Kansas in 1997 was established at ≥35 western corn rootworms per sticky trap per week (average of the 12 traps per field) or 0.6 western corn rootworms per plant (field average) based upon visual inspections of whole plants (Hein & Tollefson 1985, Higgins et al. 1995). The SLAM® treatments were applied to all fields exceeding the treatment threshold in the managed area when ≥35% of rootworm females, found on sticky traps or during visual plant inspections, were gravid. Forty-four of the 98 fields (68.5% of the total area) in the managed area eventually exceeded the treatment threshold and received an application of SLAM®. Ten of the 23 fields (72.8% of the total area) in the control area also exceeded the treatment threshold but were not treated. The first application was made on 22 July and the last treatment applied on 20 August 1997 with a total of 1,178 ha (2,910 acre) receiving at least one SLAM® treatment.

Insecticide application. D&H Aviation (Burr Oak, Kansas) treated all fields that exceeded the treatment threshold. There was usually 24 h between the time a field was judged to need treatment (i.e., beetles exceeded threshold with ≥35% gravid females) and the time that the application was completed. All fields were treated with 67.9 g of formulation per hectare (0.37 lb of formulation per acre), which resulted in 88 g of carbaryl being applied per hectare (0.048 lb of carbaryl per acre). The delivery volume of the carrier (water + 0.36 liter [12 oz] of an adjuvant [rain fastener]/378.5 liter [100 gal]) was 3.79 liter per 0.4 ha (1.0 gal per acre) by using D-6 nozzles (with no screens or cores) at a delivery rate of 3.97 liter per min (1.05 gal per min) with a standard pressure of 258.08 g/cm^2 (40 psi) and an airspeed of 193 kph (120 mph). The targeted droplet size was ≈600 µm and provided plant coverage of approximately one droplet per 6.45 cm^2. The swath width was 18.3 m (60 ft) and the spray was delivered at 3.1 m (10 ft) above the corn plants. The same aerial applicator and methodology will be used throughout the duration of the project (through the year 2000).

Discussion

Project objectives. The primary purpose of this project is to determine if an areawide adult rootworm suppression program can be successful when adult western corn rootworms are properly monitored and application of semiochemical-based insecticides (e.g., SLAM®) accurately timed. In addition, attempts are being made to discover new sampling techniques or to modify existing methodology to make surveying for adults more acceptable to corn growers and consultants. Therefore, several experiments were designed to better understand the phenology of the rootworm in Kansas and to compare different sampling techniques to determine which techniques provide the greatest fidelity

and which can be modified, if necessary, for widespread consultant or grower acceptance. Thus, after mapping all cornfields in both the managed and control areas, the following studies were initiated.

Larval rootworm monitoring. Two fields that had substantial adult western corn rootworm populations in 1996 were selected for monitoring larval phenology. Corn plants in both fields were in the 10-leaf stage when this study was initiated on 11 June 1997. Weekly samples of 10 plants were randomly selected from each field, the root system and the soil removed intact, the roots soaked carefully and washed, and all immature western corn rootworms collected for later developmental determination. A total of 350 immature rootworms was collected in this manner. The last larval sample was collected on 3 July 1997, and the first adults were collected 1 July 1997. An attempt will be made to determine rootworm species from these samples; Kansas does have northern [*Diabrotica barberi* (Smith & Lawrence) and southern (*Diabrotica undecimpunctata howardi* Barber)] corn rootworms, although western corn rootworm is currently the major corn pest. Larval monitoring will continue throughout the duration of the project and include fields treated with SLAM® to determine effects of the insecticide on subsequent larval phenology and species complexes. Information of this nature may be useful in developing a phenological model to predict western corn rootworm adult emergence.

In an effort to gather baseline data relative to root damage prior to initiation of the SLAM® areawide treatment program, 23 fields were selected for root rating evaluations. These fields were arbitrarily selected and included all cornfields in the 2 × 2 core sections of the managed site plus one field outside this core area that contained emergence cages and one field from each of the four sections in the control area. Five plants were selected randomly from each of four locations (total of 20 plants), their root systems removed intact, soil washed off the roots, and the roots rated using the standard 1–6 scale with 1 indicating no damage or only a few minor feeding scars, and 6 indicating three or more root nodes destroyed (Hills & Peters 1971). All plants (460 total) were rated during the first week of July while the plants were in the pretassel stage. An overall mean root rating of 2.82 was associated with fields subsequently requiring SLAM® treatment (Table 1). Fields not requiring treatment had a mean root rating of 1.35. In subsequent years of the RAMP (1998–2000) all cornfields will be assessed using root ratings with 12 plants per field removed from the area adjacent to each of the 12 sticky traps. Rating roots from every cornfield should provide another indicator of the effectiveness of the areawide adult suppression program.

Adult emergence monitoring. Determining adult emergence is important to the development of a phenology model and is valuable in initiating timely deployment of sticky or lure traps. It also initiates deployment of field scouts if plant inspections are the sampling method of choice. Therefore, emergence cages were placed in four fields (one first-year cornfield; three continuous cornfields) on 27 June. These fields were selected randomly (three in the managed area and one in the control area) and 12 cages placed randomly throughout each of the fields. Adults were collected from emergence cages in two of the four fields. Neither field received a western corn rootworm insecticide application, either adulticide or planting-time treatment, in 1996 or 1997. Cages were

Table 1. Root rating and subsequent sticky trap count of western corn rootworms from the Scandia, Kansas, study area during 1997.

Field no.	$\bar{x}$ Root rating[a]	$\bar{x}$ Sticky trap count[b]	Field no.	$\bar{x}$ Root rating[a]	$\bar{x}$ Sticky trap count[b]
A-10	2.9	206.5*	C-14	2.9	88.9*
A-9	1.2	4.2	C-10	2.2	28.0
A-18	1.4	16.6	D-9	2.3	51.2*
A-1	1.1	3.5	D-8	3.5	267.4*
B-16	1.9	38.3*	D-10	2.2	38.7*
B-18	1.9	4.4	D-7	1.9	37.7*
B-19	1.1	18.1	E-6	2.1	42.2*
B-22	3.0	103.9*	E-7	1.2	1.8
B-24	4.3	43.2*	E-2	1.2	1.1
B-17	1.9	26.7	E-1	2.1	40.6*
C-19	3.5	136.6*	C-21	3.6	68.8*
C-3	3.3	286.3*			

*Mean sticky trap count exceeded treatment threshold, ≈35 western corn rootworm's per trap per week.

[a]Mean calculated from 20 plants per field.

[b]Mean of 12 sticky traps per field for the week prior to SLAM® treatment.

checked approximately every 3 d after placement (27 June). The first adults were collected on 4 July (Table 2), whereas the first adults were observed in the field on 2 July. Adult emergence was 97 and 80% completed by 21 July in the managed and control areas, respectively. The last adults were collected from emergence cages on 4 and 14 August from the managed and control areas, respectively. Peak emergence for both areas occurred during the 12-d period from 10 to 21 July. Corn plants were silking in both fields on 14 July. All beetles collected were preserved for later examination and sex determination. Sex ratios shifted for both fields after 17 July (≈2 wk after first emergence) with males emerging earlier than females (Table 2).

Nontarget organisms. Treating large areas with a semiochemical-based insecticide for adult rootworm suppression may reduce insecticide use as a planting-time treatment and conventional insecticide use as an adulticide. Reductions of these insecticide applications plus increasing semiochemical-based insecticide use may affect the arthropod complex associated with these cornfields. Thus, a survey for nontarget arthropods was initiated in 1997 in all cornfields prior to the fields reaching the tassel stage and continued until western corn rootworm monitoring was terminated. A total of 100 plants was selected randomly (25 plants from each cardinal direction) for visual detection of nontarget arthropods. All arthropods encountered were identified and recorded. If leaf feeding was noted (i.e., shotholing), the plant was dissected to identify the pest involved. Eggs of European corn borers; corn earworms, *Helicoverpa zea* (Boddie); lacewings (Chrysopidae); and other insects also were recorded. This nontarget arthropod survey will be continued in subsequent years of the RAMP and modified as appropriate.

Adult diurnal activity. In Kansas, the most commonly used western corn rootworm sampling method is to make plant inspections at several locations in each field (Higgins et al. 1995). Most scouts have a weekly route and may be monitoring fields at different times of the day. A study was initiated in 1997 and will be continued at least through 1998 to determine where on the plant western corn rootworm adults are located throughout the day. The first field exceeding the treatment threshold in the control site was used for this survey. This field was selected because of the relative ease of observing western corn rootworm adults and because it was not subject to insecticide treatment. Ten plants were selected randomly and inspected visually at 0800, 1100, 1400, and 1700 hours (CST) for 3 d/wk. Plants selected for inspection were approached cautiously, and beetles counted as soon as the plant came into view so that any beetles that alerted early and took flight would be included. Number of beetles per plant and their location was recorded. Plant phenology and ambient temperature also were recorded. Table 3 summarizes the pooled data for this survey conducted for ≈2 wk until the beetles started dispersing or the population level declined and beetles became scarce in the field. This study will be repeated in subsequent years in a continuing effort to improve field monitoring for consultants and growers relative to decision-making sampling for western corn rootworm adults.

Trapping studies. Two studies also were conducted throughout the western corn rootworm adult monitoring window to evaluate selected trap and lure attractability. Two sites were used for the lure attractability study, one near

Table 2. Western corn rootworm adult emergence profile from the Scandia, Kansas, study area during 1997.

Field C-19[a]				Field B-22[b]			
Julian date	Total[c]	Males (%)	Females (%)	Julian date	Total[c]	Males (%)	Females (%)
183	4	4 (100)		183	10	4 (50)	4 (50)
186	6	4 (67)	2 (33)	186	15	9 (75)	3 (25)
189	32	27 (93)	2 (7)	189	115	75 (79)	20 (21)
193	95	36 (50)	36 (50)	193	225	115 (57)	88 (43)
196	51	22 (49)	23 (51)	196	28	13 (46)	15 (54)
200	99	29 (37)	50 (63)	200	100	25 (25)	75 (75)
203	18	5 (33)	10 (67)	204	11	2 (18)	9 (82)
209	21	5 (24)	16 (76)	211	7	2 (29)	5 (71)
217 (100)	7	1 (14)	6 (86)	214	1		1
224	1		1 (100)				
Total	334	133 (48)	146 (52)		512	245 (53)	220 (47)

[a]Field C-19 located in the control site.

[b]Field B-22 located in the managed site.

[c]Discrepancy between total beetles captured and number sexed is due to inability to accurately determine the sex of each beetle.

Table 3. Western corn rootworm adult location on plant by time of day and date from the Scandia, Kansas, study area during 1997.

Date	Leaves[a] (%)	Silks[b] (%)	Stalk[c] (%)
21 July	68[d]	21	11
23 July	68	16	17
30 July	60	25	15
1 Aug.	62	30	8
5 Aug.	51	41	8
8 Aug.	30	70	0
Time (h)			
0800	69	19	12
1100	56	39	5
1400	57	15	28
1700	69	24	7

[a]Leaves, including area behind leaf collar, when leaves were bent down by hand for examination.
[b]Plants in this field started silking 14 July 1997.
[c]Stalk includes tassel.
[d]Total of 576 observations with peak adult emergence on 21 July.

Wamego in Pottawatomie County and the other in the managed site northwest of Scandia. The latter field also was used for a test comparing different Trécé prototype traps by using the standard lure Trécé #8276. The trap test was initiated 24 July and terminated 18 September. Eight trap prototypes were tested with four replications emptied weekly. Sticky traps, Pherocon AM and Multigard, baited with the standard lure #8276 consistently attracted, or at least captured, more western corn rootworm adults than the other prototypes. This finding may be due to the large trapping area and because kill baits, which require a dual system for the lure traps, were required to actually contain the beetles within the trap. The baited sticky traps also are easier to use as they are tied to the corn plant, whereas the lure traps required a vertical polyvinyl chloride pipe for support. Trécé is testing a modification to the lure trap that allows direct placement on the plant. This test will be modified, probably each remaining year of this project, to determine if direct attachment to the plant alters the lure trap's ability to attract and contain western corn rootworm adults. Some of these traps will be supplied to consultants around the state to test their interest and acceptability in an effort to involve them in initiating other western corn rootworm areawide projects.

Lure comparison study. Tests were conducted to delineate attractivity of the various lures by using the standard lure-trap prototype. Twelve lures were tested at the two areas mentioned previously. Lures #8391, #8337, and #8276 consistently caught more western corn rootworm adults than the other lures. Lure #8276 also was among the lures that seemed to consistently be the most attractive to southern corn rootworms that are not a major corn pest in Kansas. Lures #8391 and #8337 were consistently the least attractive for this species. Therefore, for purposes of the RAMP in Kansas, which is primarily concerned with the western corn rootworm, lures 8391 and 8337 may be more practical, especially for consultants and growers. If an attractant can consistently lure more western corn rootworms with fewer nontarget southern corn rootworms, it would be less time-consuming counting each trap's contents—if the correlation between the different sampling techniques can be established. This trend seemed to be consistent at both sites. This test will be repeated in subsequent years by using other lures with similar chemical constituents in a continuing effort to find the most utilitarian lure for the western corn rootworm.

Sampling techniques study. One of the objectives of the RAMP, and the primary reason these various sampling studies are being conducted, is to develop more reliable, efficient, and inexpensive monitoring techniques for insect pests that can be used by consultants and growers. Thus, western corn rootworm adult populations were monitored by using several different methods (plant inspections, Pherocon AM/NB sticky traps, and traps with lure #8276) to determine if a correlation exists between various sampling methods, larval populations, and damage the following year. A total of six different comparisons was made at various locations across Kansas. Populations were monitored by plant inspections, Pherocon AM sticky traps, and lure traps placed around the field perimeter (external) and the interior (internal) of eight cornfields. Plant inspections were conducted and the various traps placed systematically throughout the fields and monitored weekly. A total of 20 cornfields was used during this study, only eight of which had both internal and external lure traps.

The other 12 fields compared plant inspections, internal lures, and sticky traps systematically placed throughout the fields. Treatment thresholds for the various sampling techniques were compared to determine if a relationship existed among these techniques.

The goal in these comparisons is to determine the relationship among sampling methods for deciding whether or not an insecticide treatment is needed. Based upon existing thresholds for the various methods used in the 20 different fields, a comparison was made to determine if a treatment would be recommended. The three thresholds used were as follows: ≥0.6 mean beetles per plant, ≥5.0 beetles per trap per day, and total accumulation of 100 beetles per lure trap. According to plant inspections, beetle populations exceeded the treatment threshold in 11 of the 18 fields surveyed (Table 4). Seven of the 20 fields exceeded the threshold according to sticky trap catches and 16 of 20 exceeded the lure trap threshold. Plant inspections disagreed with sticky trap counts 4 out of 18 times, whereas plant inspections and lure traps disagreed 6 out of 18 times. Lure traps disagreed with sticky traps 9 out of 20 times. All fields will be monitored for western corn rootworm larval damage (by using root ratings) in 1998 to determine which sampling method is most reliable for predicting the need for western corn rootworm adult insecticide applications. This study will be ongoing as the sampling technology is continually refined prior to its transfer to consultants and growers after RAMP formally terminates.

Soybean sampling. Because crop rotation among corn and soybeans [*Glycine max* (L.) Merrill] is a common practice in the Kansas RAMP area, a survey was initiated in 1997 to determine the incidence of the western corn rootworm in soybeans. This study was conducted in four soybean fields (one in the managed area and three in the control area) by using three different traps, the Pherocon AM and Multigard, both nonbaited sticky traps, and the lure trap. Three rows were systematically located within each field and nine traps per row (three of each type) were randomly placed in each row on polyvinyl chloride stakes immediately above the plant canopy. All traps were emptied or replaced weekly. The study was initiated after corn silks in the adjacent cornfields had senesced and the soybeans were in the R3 stage (pod 40.64 cm [3/16 in.] in length at one of the four uppermost nodes in the main stem with a fully developed leaf) of reproductive development.

The lure traps averaged less than three beetles per week throughout the sampling period from the field in the managed area. This finding was somewhat expected as all cornfields in the managed area exceeding treatment threshold were treated, thus significantly reducing western corn rootworm adults available to move into soybeans. The three soybean fields in the control area, however, had surprisingly high numbers of western corn rootworm adults, but only in the lure traps during the first sampling period with a dramatic decrease in numbers during the second week (i.e., field 1: first week mean per lure trap = 131; second week mean = 7 and field 2: first week mean per lure trap = 469, second week mean = 21; field 3: first week mean per lure trap = 97, second week mean = 9). Thereafter, numbers continued a steady decline. At the last sampling date all lure traps averaged less than two western corn rootworm adults per trap per week, which was the initial mean number collected by nonbaited sticky traps. Field 2 had a slight increase in trap average collections

Table 4. Sampling methods and resulting treatment recommendations for western corn rootworm in Kansas during 1997.

Field	PI	ST	EL	IL
E1	–	T	NT	T
C3	T	T	T	T
C6	T	NT	T	T
D8	T	NT	T	T
A10	T	NT	NT	NT
B13	NT	NT	NT	NT
C14	NT	NT	T	T
C22	T	T	T	T
D5	T	T	—	T
D17	T	NT	—	NT
D18	NT	NT	—	T
D21	NT	NT	—	NT
B22	T	T	—	T
B9	NT	NT	—	T
GCF	—	NT	—	T
GCC	—	NT	—	NT
A-13	T	T	—	T
A-4	T	T	—	T
LT-5	NT	NT	—	T
LT-W	T	T	—	T

PI, plant inspection (threshold 0.6 western corn rootworm per plant); ST, Pherocon AM trap (35 western corn rootworm per trap per week); EL, external lure trap (accumulation of 100 western corn rootworm); IL, internal lure trap (accumulation of 100 western corn rootworm); T, treatment recommended; and NT, no treatment recommended.

the third week after which beetle collections declined steadily. This study will be continued in subsequent years with field monitoring initiated beginning with the R2 stage (open flower at one of the two uppermost modes on the main stem with a fully developed leaf). If corn is planted the following year in any of these soybean fields larval populations will be monitored (by using root ratings) and adult emergence cages placed in these fields to determine and compare the first-year cornfield to populations found in continuous cornfields.

Insecticide resistance monitoring. One of the potential problems associated with the RAMP using cucurbitacin-based insecticides is the selection for resistance to the toxic ingredient in the bait. When such a program is used, the insecticide selection pressure to beetle populations is likely to be high due to limited or no refuges and to the repeated use of a single insecticide formulated in the bait. Decreased beetle response to phagostimulants (e.g., cucurbitacins) may select for resistance to toxic baits. This is an additional risk associated with cucurbitacin-based toxic baits used in the rootworm areawide management programs.

To ensure the effectiveness of RAMP, it is extremely important to know the beetle population level of susceptibility to the toxic ingredient in the baits prior to the application program, and to monitor any changes in the population's response to the insecticide after the application. In Kansas, surveys were conducted to determine the susceptibility of the western corn rootworm adult populations to selected insecticides in several corn-growing regions across the state in 1996 and 1997. There were only small seasonal and regional variations in the susceptibility of the beetles to selected insecticides, including carbaryl, methyl parathion, and bifenthrin, but no conceivable resistance was identified in any beetle population examined in Kansas. These findings suggest that the cucurbitacin-based toxic baits containing one of these insecticides should provide effective control in an areawide management program, at least in the surveyed areas within Kansas. Monitoring will continue throughout the duration of the RAMP in Kansas.

Adult longevity study. A postharvest survey also was initiated in 1997 to determine longevity of western corn rootworm adults. Thus, six lure traps were systematically placed on polyvinyl chloride stakes on each side of one field that had been sampled systematically throughout the growing season. These 24 traps were emptied weekly until no western corn rootworm adults were captured (initiated 2 October, terminated 9 December). Nineteen western corn rootworm adults (18 females, 1 male) were collected during this 2-mo sampling period.

Future work. All studies mentioned previously will be conducted throughout the duration of the RAMP in Kansas with appropriate adjustments made for continual fine-tuning to provide the most valid results possible. These studies and their results will be vital to the successful technology transfer at the termination of the project (after the year 2000).

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Rootworm Areawide Management Program in Iowa[1]

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ABSTRACT The objective of the areawide corn rootworm, *Diabrotica* spp., management project is to determine if corn rootworm populations can be reduced to and maintained below economically damaging levels throughout a large, contiguous area by coordinating the control of the adults before they mate and the females lay eggs. Iowa's Areawide Project is being conducted on 41 km^2 in Clinton County. The county is located in east central Iowa and is bordered on the east by the Mississippi River. The topography is rolling and, because much of the land is erodible, very few soybeans [*Glycine max* (L.) Merrill] are grown in the area. Of the 4,144 ha in the area, >2,400 ha was planted to corn (*Zea mays* L.) in 1997. Approximately 90% of the corn was planted where corn had been planted the previous year. Hay is grown as a strip crop between corn and grass waterways, and terraces are prevalent. During 1997, adult corn rootworm emergence began on 12 July, about 10 d later than normal due to below-normal temperatures in May and June. Pherocon® AM sticky traps were placed in fields between 21 and 25 July to monitor beetle density. Beetle populations were very high in nearly all fields. Threshold beetle numbers of six beetles per trap per day were reached in about 30% of the fields by 28 or 29 July. Applications of the insecticidal bait SLAM® (560 g/ha in 3.8 liters of water) began on 5 August. SLAM contains a feeding stimulate (buffalo gourd powder, 87%) and carbaryl insecticide (13%, 73 g/ha). Of the 2,440 ha of corn planted in 118 fields, 2,269 ha (93% of the corn) was sprayed initially. About 10 d of residual activity was obtained with the SLAM treatments. When beetle densities returned to four beetles per trap per day, the fields were resprayed. Resprays began on 22 August and 1,222 ha (54% of the corn) was retreated. No fields were sprayed more than twice. Larval root-injury ratings and adult emergence from the soil in 1998 will be used to determine the effectiveness of the adult management program.

KEY WORDS *Diabrotica* spp., corn rootworm, areawide management, SLAM®

E. F. Knipling (1980) described the concept of areawide pest management, which he termed regional management. The essence of the paradigm is that management of a single pest is coordinated throughout a large geographical area so that pest abundance is reduced and maintained at an acceptably low

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density by suppressing mobile pests at the sites where they are produced. There may be two advantages of areawide pest management over integrated pest management practiced by individual growers. It might be less expensive and it might facilitate the use of biorational tactics that are less hazardous to humans and to the environment.

For corn rootworms, *Diabrotica* spp., the control tactic that appears to offer reduced negative externalities is the use of semiochemical (behavior-modifying) baits to control the adult female before she lays eggs (Sutter & Hesler 1993). The baits incorporate the feeding stimulant cucurbitacin with a small amount of insecticide. The cucurbitacins cause the beetle to feed compulsively, ingesting the insecticide. When contact insecticides are used to protect corn (*Zea mays* L.) roots from larval injury or to control beetles, they are usually applied at the rate of 1.1–1.4 kg of active ingredient per hectare. Because the semiochemical bait is ingested, it requires only 73 g of active ingredient per hectare to control beetles. Because the most economically important pest *Diabrotica* are univoltine and the larvae are nearly monophagous (surviving almost exclusively on corn roots), the semiochemical baits are applied to corn to reduce the number of females before they lay their eggs. Suppressing adult populations reduces oviposition. It is expected that egg laying will be sufficiently reduced so that there will not be economic larval injury to the following season's corn crop. Meinke (1995) has described the practice of adult corn rootworm management to reduce larval injury.

SLAM® (Micro Flo, Lakeland, Florida) is a commercial formulation of the semiochemical bait that combines a powdered cucurbit (buffalo gourd [*Cucurbita foetidisima* H.B.K.] powder, 87%) with carbaryl insecticide (13%). The purchase and application costs of SLAM are currently equivalent on a per-treated-area basis to the most expensive contact insecticides applied to the soil to control larvae. Compounding the economic challenge is the reality that larvicides are usually applied as preventive treatments, without monitoring pest density; therefore, the user does not incur a scouting cost. To properly time the adulticide for maximum effectiveness, the user should know when oviposition is beginning. Thus, killing female beetles before they lay eggs has the additional cost of scouting. To be acceptable to growers, the cost of the scouting and SLAM application must be less than the cost of the cheapest larvicide the farmers are currently using.

Using the semiochemical bait in a cooperative, areawide management program may make it economically competitive. It is expected that suppressing beetle densities before females lay eggs throughout an area will reduce the average rootworm density (Pruess et al. 1974). Subsequently, the pest need only be controlled in fields where it is unusually abundant and before it disperses to other cornfields. The consequence is that, over time, fewer fields will require treatment, reducing the management costs when they are prorated across the management area. The challenge will be to determine how to get farmers, who have made farm-management decisions independently, to share the scouting and treatment costs.

The objective of the corn rootworm areawide project is to determine if the semiochemical bait will be effective and economically competitive if it is used in a coordinated, areawide manner. This will be determined by comparing the effectiveness and cost of the areawide approach with management by individual farmers.

Site Description

Iowa's Areawide Corn Rootworm Management Project site is 41 km^2 (6.4 by 6.4 km) and is located in Clinton County in east central Iowa. Clinton County is bordered on the east by the Mississippi River. The topography is rolling and, because much of the land is erodible, very few soybeans [*Glycine max* (L.) Merrill] are grown in the area. Of the 4,144 ha in this area, over 2,400 ha was planted to corn in 1997. Approximately 90% of the corn planted was planted where corn had been grown the previous year (continuous corn) and is susceptible to infestation by corn rootworms. Livestock is an important commodity in the area. Because of animal production, the second most common crop is hay, often interplanted with corn strips and rotated with corn. Waterways and terraces are prevalent. Annual rotation of corn with another row crop is uncommon.

The research project contains both an adult rootworm management site and a comparison site, located side by side. Each site is subdivided into core and buffer areas. The central 3.2 by 3.2 km core (1,036 ha) is the corn rootworm management area. A 1.6-km-wide buffer surrounds the core. Corn rootworms are managed in all of the fields of the 41-km^2 management site. In the comparison site, eight fields have been chosen at random from the core management area to determine the cost and effectiveness of rootworm management practiced by individual farmers as they have in the past, usually planting-time or postemergence larvicides.

Conduct of the Project

The Iowa project is one of five replicates (Iowa, Illinois/Indiana, Kansas, South Dakota, and Texas) that compare two treatments, areawide management of corn rootworms with management by individual farmers. All five locations have a management site where SLAM is used to suppress adult corn rootworm numbers throughout the area and a comparison site where farmers are allowed to use the control practice that they choose. SLAM (560 g/ha in 3.8 liters of water) is applied in the management sites when the number of beetles exceeds the number that is expected to produce economically damaging larval populations the following year.

To determine when corn rootworm sampling should begin, adult emergence from the soil is monitored in four fields from the core of the management area (Hein et al. 1985). When emergence is underway, the Pherocon® AM yellow sticky trap (Trécé, Inc., Salinas, California) is used to monitor beetle numbers in all cornfields (Hein & Tollefson 1985). When the number of beetles caught exceeds six beetles per trap per day in a cornfield, SLAM is applied to the field. If beetle numbers return to four beetles per trap per day, the field is retreated with SLAM.

The data that are collected in the management area and the buffer area of the management site differ. Because the function of the buffer area is to prevent the dispersal of female corn rootworms into the management area, only corn rootworm adults are sampled in the buffer area.

Because *Diabrotica* are unique in their attraction to cucurbitacins, SLAM is not expected to impact other species; however, natural enemies and other pests

are monitored in the management area in addition to the corn rootworm. Billy Fuller (South Dakota State University, personal communication) determines natural enemy densities prior to and after application of the insecticidal bait by using pitfall trapping and visual counts.

Corn-plant establishment is recorded shortly after emergence in all of the fields in the management area of the areawide study site and in the eight randomly selected fields from the comparison site. If plants are missing, the field is scouted for seed- and seedling-attacking insects. When the corn exceeds 45 cm, first-generation European corn borer, *Ostrinia nubilalis* (Hübner), injury is recorded. At the end of the season, corn stalks are split and the total amount of corn borer tunneling is recorded.

Results from 1997

There were no measurable infestations of seed- or seedling-attacking insects in either the areawide management site or the companion site. The amount of European corn borer injury was comparable in both areas. Just prior to corn harvest, 75% of the plants was infested with an average of 1.1 larvae per plant in the areawide management site; 77% of the plants in the companion site was infested with an average of 1.4 borers per plant.

During 1997, adult corn rootworm emergence began on 12 July, about 10 d later than normal due to the cooler-than-normal temperatures in May and June. As is typical, western corn rootworm, *Diabrotica virgifera virgifera* LeConte, emergence began before the northern corn rootworm, *D. barberi* Smith & Lawrence, and males began emerging before females. Western corn rootworms were much more common than northern corn rootworms during 1997.

Pherocon AM sticky traps were placed in fields between 21 and 25 July to monitor adult density. Beetle populations were very high in nearly all fields. Threshold beetle numbers of six beetles per trap per day were reached in about 30% of the fields by 28 or 29 July. The insecticidal bait was not applied to control the beetles until at least 10% of the females contained eggs, as determined by rupturing their abdomens and examining the contents in the field.

Aerial broadcast applications of the insecticidal bait SLAM began on 5 August. Of the 2,440 ha of corn planted in 118 fields, 2,269 ha (93% of the corn) was sprayed initially (Table 1). The SLAM treatments killed beetles for about 10 d, determined by daily counts of beetle cadavers that had fallen onto screens stretched on wooden frames and placed between rows of corn. When beetle densities returned to four beetles per trap per day and dead beetles were not found on the screens, the fields were resprayed. Resprays began on 22 August; 1,222 ha (54% of the corn) was retreated (Table 1) and no fields were sprayed more than twice.

Evaluation of Areawide Management

Beginning in 1998, larval root-injury ratings and adult emergence will be used to determine the effectiveness of the adult rootworm control program. In all cornfields in the areawide management site, 12 root systems will be dug

Table 1. Applications of SLAM® to control adult corn rootworms in Iowa (1997).

	No. of cornfields	Total area (ha)	First treatment		Second treatment	
			Area (ha)	% of Total	Area (ha)	% of Total
Management area	32	766	749	98	515	69
Buffer	86	1,672	1,520	91	707	47
Total	118	2,438	2,269	93	1,222	54

from an area that has not been treated with a soil-applied larvicide. In the comparison site, 12 root systems will be dug from an untreated area and an additional 12 roots will be dug from an area that the farmer has treated with a soil-applied larvicide of his or her choice. The soil will be washed from the roots and the corn rootworm larval injury rated on a 1–6 scale, with 1 indicating very little damage and 6 indicating severe feeding injury (Hills & Peters 1971). The average level of root injury in the two sites will be used to contrast the effectiveness of corn rootworm areawide management with management in individual fields.

Emergence cages will be used to collect beetles emerging from the soil throughout the season in four fields from both the management site and the comparison site (Hein et al. 1985). The emergence cages will be placed over plants that have not been treated with a soil-applied larvicide. The emergence from the comparison site will estimate that year's pest density. The emergence from the management site will estimate the amount of suppression that has been achieved by the areawide application of prescription SLAM treatments.

The first beetle-management season of the areawide rootworm management project was in 1997. Beetle management will continue for two more seasons, with root-injury evaluations and beetle-emergence collections to assess the program's effectiveness the following years. For the management tactic to be successful, rootworm populations and the number of fields treated with the semiochemical bait in the areawide management site must decline compared with pest populations and management costs in the comparison site. The decline must be sufficient to compensate for the added cost, on a per-area basis, of the beetle control program.

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Resistance Management Concerns for Areawide Management Programs[1]

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ABSTRACT Areawide pest management involves the application of a pest control strategy over wide geographic areas, requires a high degree of grower compliance, and relies on uniform exposure of pest populations. Therefore, the selective pressures and the associated risk for development of resistance may be extremely high. Some of the attributes of areawide management are incompatible with many conventional resistance management techniques. Reliance on biologically based control tactics such as behavior-disrupting chemicals, however, may provide effective resistance management because of the complexity and number of potential target sites associated with the response. Recognition of the risk of resistance and a proactive stance on resistance management will help to preserve the effectiveness of areawide management programs.

KEY WORDS areawide pest management, insecticide resistance, resistance risk assessment

The development of insecticide resistance among pest populations can best be described as an evolutionary response to a selective pressure. Unlike most evolutionary phenomena, however, insecticide resistance has practical and economic significance (Denholm & Rowland 1992). The evolution of resistance in pest species poses a formidable challenge in view of current difficulties in discovering and, in particular, developing pest control technologies, including areawide management. There is an increasing recognition that resistance is an unavoidable consequence of any pest management strategy, and that unless appropriate measures are taken to delay the onset of resistance, a given technology is likely to have a limited market life.

Given the growing magnitude of the resistance problem, the concept of resistance management has taken on an ever-increasing role in the development of new pest control technologies. Resistance management involves a set of methods designed to extend the number of generations that a given pest population can be controlled economically with a given technology (Roush 1996).

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There is increasing interest in resistance management theory, although it is still in an early stage of development, and tactics to implement resistance management vary with the biological, technical, economic, and sociological constraints that may limit its widespread acceptance and success (Croft 1990).

Historically, resistance management has been applied after a field control failure has become widespread (Forrester 1990). In this situation, resistance management is implemented as a curative measure to cause a resistant population to revert to more susceptible levels and mitigate the immediate economic impact of a resistance event. A number of examples exists where the efficacy of a given product has been extended for several growing seasons (Plapp et al. 1990, Forrester et al. 1993) through the coordinated efforts of commodity, industry, and scientific organizations. The design of such curative approaches is made somewhat easier because resistance is already present in the field and is readily accessible to investigation so that the genetics, ecology, and biochemical or physiological mechanisms of resistance can be readily examined. An understanding of these processes provides critical information to the development and implementation of resistance management programs.

In contrast, development of preventative or proactive resistance management programs (i.e., implementation prior to widespread acceptance of a new technology) presents some unique challenges to acceptance and adoption (Forrester 1990). Ideally, we should try to manage susceptibility or implement a preventative resistance management program before a new pest management strategy is adopted and selective pressures initiated. As stated by McKenzie (1996):

> "The horse has already bolted once resistance is present in measurable frequency in a population. The capacity to manage susceptibility, or at least hopefully close the stable door in the face of resistance, depends on the knowledge of the population biology of the pest and the choice of the operational factors of insecticide delivery."

With newer pest control strategies, including novel chemistries, transgenic insecticidal plants, and behavior-disrupting chemicals such as pheromones and baits, there is an increasing demand and in some instances even regulatory requirements (EPA 1995) for resistance management programs to be implemented and adopted before a new control strategy can be marketed. Because resistance has not yet developed in the field, however, critical data involving the inheritance and the biochemical and molecular mechanisms of resistance are lacking. As a result, preventative resistance management programs are usually based only on theoretical models that are very difficult to verify until the technology is deployed on a large scale. Such is the case for the implementation of areawide management programs that, in particular, require some novel thinking with regard to the practice of resistance management.

Areawide Management Programs

The areawide management concept was developed by various groups in the 1970s and 1980s (Pruess et al. 1974, Rummel 1976, Botrell & Rummel 1978, Rabb 1978, Huber et al. 1979, Knipling 1980, Kunz et al. 1983) to control key pests (i.e., perennially occurring species that in the absence of control tactics will often remain at population levels above economic injury levels [Smith and

van den Bosch 1967]). Key pests regularly cause economic loss, are the primary focus of control programs in a given commodity, and historically have required the use of ecologically disruptive pesticides on an annual basis. The objective of areawide management is to systematically reduce a target pest(s) population to a low residual level through the use of uniformly applied control measures over large geographic areas. The intent is not to eradicate the pest (although this is an option if feasible) but to achieve and maintain an overall reduction in the general equilibrium position of the pest population over a large area and to reduce the pest status from key to secondary (Kogan 1995). Areawide management requires coordination by organizations, decision making by program managers rather than individual producers, and compliance of participants in the areawide management program with established management protocols to ensure success of the program (Kogan 1995).

Integrated pest management (IPM) programs historically have differed from areawide programs in that IPM programs are targeted against a pest complex within the crop community, generally individual growers coordinate the program and are final decision makers (or in concert with a consultant), and programs are often implemented on a field-by-field basis (Leslie & Cuperus 1993). Because growers are the principal decision makers, IPM is based on voluntary participation. A goal of most IPM programs is to use a combination of preventive and therapeutic practices to keep pest populations below economic injury levels (Rabb 1978, Pedigo 1992)

In 1993, several United States Department of Agriculture (USDA) agencies developed a position paper that attempted to mesh complimentary aspects of areawide management and IPM into a single management strategy (Anonymous 1993). The main objective of this approach is to effectively manage key pests in a system through areawide suppression programs that will subsequently enhance IPM options for secondary pests on a regional level. Kogan (1995) refers to this strategy as areawide IPM. To accomplish this goal, current USDA–Agricultural Research Service (ARS) areawide management projects are structured around one or more biologically based tactics or technologies (e.g., mating disruption, behavioral baits, sterile male releases, pathogens) that are used to suppress the key pest species in various commodities (Faust & Chandler 1998). The intent of these programs is to use biointensive tactics to reduce the historically heavy pesticide load directed at primary pest species. The reduced pesticide load also will facilitate management of secondary pests with more biologically intensive tactics (Kogan 1994).

Resistance Risk

It has been proposed that the potential for significant field resistance be identified in target pest species (Keiding 1986, Tabashnik 1992) before a new pest control strategy is introduced for wide-scale field use. Resistance risk assessment is done in an attempt to predict the potential rate and extent of resistance development in response to use of a pesticide in a particular environment (Tabashnik 1992). Specifically, resistance risk assessment is the process of estimating the relative influence of different factors on increasing resistant genotype frequencies in the population exposed to the selective agent.

Estimating the potential for developing resistance can be very difficult, although genetic, biological, and operational factors related to the pest species and the specific pesticide use have been identified (Tabashnik & Croft 1982, Georghiou & Taylor 1986, McGaughey & Whalon 1992, Tabashnik 1994). Critical biological and genetic factors that are inherent to the population include initial frequency of the resistant gene, dominance of the resistance gene, number of resistance genes involved, and various biological and ecological traits of the insect (Georghiou & Taylor 1977a, Roush & McKenzie 1987). Operational factors are those that the grower or applicator has some ability to manipulate and are generally the factors targeted for resistance management (Georghiou & Taylor 1977b, Roush 1990). Operational factors primarily include the history of insecticide applications, insecticide persistence, and application type and frequency. Treatment history can have a tremendous impact on the rate of resistance development if previously used compounds share a common target site or functional group susceptible to metabolic detoxification. Insecticide persistence is also critically important in that products that are persistent tend to exert selective pressures over longer periods. Finally, the method of insecticide application, including frequency of application, degree of coverage, life stage(s) exposed, and residual effects, contributes to the degree of selection that a given pest control strategy exerts on a population.

Given the previously described attributes of areawide management programs and the factors used in assigning risk of resistance development, it would appear that the selective pressures associated with these programs would be very strong, and as a consequence, the risk of resistance development for a given areawide management program is probably high. Because of the requirements for population suppression over large geographic areas, the dose and frequency of application would be expected to be high, and the proportion of a population provided refuge from exposure generally low. Because areawide management programs target pest species that consistently exceed economic thresholds and require repeated pesticide applications, these species have historically exhibited a strong propensity for resistance development. The two species that that are currently being tested in areawide management pilot programs include the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, and the codling moth, *Cydia pomonella* (L.), both of which have a long history of resistance to a variety of synthetic insecticides.

Western corn rootworm resistance to soil-applied organochlorine insecticides first became apparent in the late 1950s and early 1960s (Ball & Weekman 1963). As a result, producers in south central Nebraska began a practice of aerial applications of insecticides to suppress beetle populations and subsequently manage larval damage the following season (Meinke 1995). Various studies demonstrated that adult corn rootworm populations could be greatly reduced by aerial applications of insecticides (Hill et al. 1948, Mayo 1976), and Pruess et al. (1974) documented that the adult corn rootworm management strategy could be used to prevent economic damage from larval feeding the following season. Carbamate and organophosphate insecticides that eventually replaced the organochlorine insecticides were used extensively in Nebraska during the 1970s to early 1990s and have generally provided good adult and larval corn rootworm control. During the last 5 yr, however, reports of insecticide control

failures have increased in parts of Nebraska where beetle spray programs have been used for many years (Meinke et al. 1998), and resistance documentation for methyl-parathion and carbaryl has been reported (Meinke et al. 1998, Miota et al. 1998).

A similar scenario can be described for the codling moth. Development of resistance to lead arsenate was detected as early as 1928 (Hough 1928). The widespread detection of DDT resistance in most of the apple-growing areas of the world (Brown 1983) occurred by the late 1950s, and resulted in a shift to organophosphate insecticides such as parathion followed by azinphosmethyl (Barnes & Moffitt 1963, Brader 1977, Croft 1979). Codling moth control with azinphosmethyl has formed the basis for management systems in apple over the past 25 yr (Varela et al. 1993). Resistance to these compounds, although not widespread, has become well established in a number of apple- and pear-growing areas especially in the northwestern United States (Varela et al. 1993) and is forcing a change in management strategies for this pest species.

Resistance Management and Areawide Management: Are They Compatible?

Several strategies based on population genetic theory and insect ecology have been proposed as a means to decrease the rate at which resistance evolves (Roush & McKenzie 1987, Roush 1989, Tabashnik 1990, Denholm & Rowland 1993). Some involve decreasing the proportion of the pest population exposed to the selective agent either through the maintenance of a refuge from exposure or by using the insecticide only when necessary to prevent economic loss (i.e., management by moderation) (Roush & McKenzie 1987). An apparently contradictory alternative strategy is to use a high enough dose of insecticidal agent so that resistance becomes functionally recessive (i.e., heterozygotes are killed by the field rate of insecticide). This method could potentially work well if there is an untreated refuge from exposure to allow reservoirs of susceptible individuals, and it is the primary strategy being proposed for deployment of transgenic plants expressing the toxin from *Bacillus thuringiensis* (Ostlie et al. 1997, EPA 1998, Gould 1998).

The resistance management strategies described previously are unlikely to be compatible with areawide management programs because of requirements for reduced selective pressures through maintenance of a refuge or through reliance on economic thresholds. Areawide management requires uniform applications over relatively large geographic areas to obtain an overall reduction in the pest population and to reduce the pest status from key to secondary rather than simply preventing economic loss. Furthermore, because areawide management requires a high degree of coverage and high doses of insecticidal agent, it is unlikely and probably impractical for the high-dose refuge strategy to be effectively used for managing resistance in areawide management programs.

Another approach to resistance management involves the availability of two or more different control strategies with independent modes of action used in combination or rotated (Roush 1989, Croft 1990, McKenzie 1996). By combining strategies, the initial frequency of individuals carrying alleles that confer

resistance to each management component is extremely rare in populations (Roush & McKenzie 1987). Areawide management strategies that exploit behaviors under complex physiological control, or that combine such behavioral exploitation with toxicants, are likely to be more compatible with this multiple-attack approach to resistance management.

An existing example of an areawide management program that exploits a complex behavior is that of the codling moth in the northwestern United States. (Washington, Oregon, and northern California). The development of insecticide resistance in Pacific Northwest populations of codling moth (Varela et al. 1993) has favored a move toward management by multiple approaches, with emphasis on mating disruption (Calkins 1998). The use of mating disruption in these programs consists of well-timed applications of synthetic female sex pheromone (Barnes et al. 1992). These efforts have been relatively successful (Welter et al. 1993), and it is believed that resistance evolution to the point of economic impact is unlikely. This is partly because sex-linked mutations would have to occur in both male and female moths, which would involve the production of a mutant (novel) sex pheromone by females and the coincident mutation of antennal pheromone receptors in males. Thus, given that moth populations do not adapt to a nonpheromonal mate location strategy (Foster and Harris 1997), this form of behavioral exploitation can be functionally considered as exploiting multiple targets and is compatible with resistance management. Additionally, the current areawide management programs for codling moth usually do not rely solely on mating disruption. Other management techniques, including chemical, cultural, and biological control and even release of sterile males, have been incorporated into specific management areas (Senft 1995). The use of the areawide management concept for codling moth has been viewed as an approach to managing codling moth resistance to azinphosmethyl because it relies on multiple tactics (Calkins 1998).

Pilot studies of corn rootworm areawide management exploit behavioral adaptations to feeding stimulants and arrestants to deliver high doses of a traditional neurotoxic insecticide (Comis 1997). The management tool in rootworm areawide programs consists of a semiochemical bait formulation (SLAM®) containing movement arrestants and feeding stimulants (multiple forms of cucurbitacin) and the acetylcholinesterase-inhibiting toxicant carbaryl (Comis 1997). Cucurbitacins occur in the Curcurbitaceae and have a close relationship to Diabroticite rootworm biology and evolutionary history (Metcalf et al. 1980). The source of the cucurbitacins contained in the semiochemical bait is buffalo gourd (*Cucurbita foetidissima* H.B.K.) roots (ground to a fine powder), which contain a complex mixture of several cucurbitacins (Talamy et al. 1997, Comis 1997). Selection pressures resulting from exposure to cucurbitacin baits could result in genetic alterations in the response of rootworm beetles to cucurbitacins and could completely undermine the toxic bait technology (Talamy et al. 1997).

Cucurbitacins are detected by rootworm beetles via several types of antennal receptors, each of which is specific to individual cucurbitacins or to a very narrow range of structurally similar compounds (Metcalf 1994, Mullin et al. 1994, Talamy et al. 1997, Cossé & Baker 1999). As a result, the likelihood of rapid behavioral resistance evolution via the alteration of multiple antennal receptors

is likely to be minimal compared with the potential for resistance to a single bait component. The contributions of individual cucurbitacins and other allelochemicals (Hibbard et al. 1997) to the overall efficacy of the buffalo gourd blend, however, needs to be further clarified before more definitive statements are possible. Furthermore, although genetic variation in response to a single cucurbitacin has been identified, the variation occurs only at lower concentrations, and thus it should be possible to forumulate baits with high cucurbitacin concentrations that do not cause selection because they affect all individuals in the population (Talamy et al. 1997).

In areas of Nebraska, western corn rootworm resistance to the active ingredient of the bait formulation, carbaryl, has been identified (Meinke et al. 1998) and appears to be physiologically based (Miota et al. 1998, Scharf et al. 1998). In existing areawide pilot sites, carbaryl resistance is absent (Scharf et al. 1998). Given the documented potential for carbaryl resistance to develop in this species, however, other toxic components of the bait formulation should be identified to be more compatible with a multitactic approach to resistance management.

Conclusions

Areawide management by behavioral exploitation can be considered as compatible with resistance management in the context of a multiple attack approach. This is also true if behavioral exploitation is used in conjunction with control technologies in an integrated format. If behavioral manipulation is used as a delivery system for a traditional synthetic insecticide, multiple toxins should be considered and integrated into a proactive and preventative approach to resistance management. Current areawide pilot programs for codling moth and corn rootworm are of limited scale, but in the future could be used over larger geographical areas. Although difficult to predict, this may increase the risk for the isolated selection of behaviorally or physiologically resistant genotypes (Hoy et al. 1998). Therefore, development of baseline information on the range of responses associated with the management strategies used in areawide programs is essential to document whether selective pressures are favoring evolutionary shifts in population responses. Additionally, alternative toxicants and novel pheromone and attractant blends should be considered to ensure the continued success of these programs.

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Future Programs in Areawide Pest Management[1]

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ABSTRACT The United States Department of Agriculture (USDA) Agricultural Research Service's (ARS) Areawide Pest Management Program is a coordinated partnership between the federal and state systems with active grower participation. Two highly ranked proposals were selected for initiation in 1995 and 1996—one involving the codling moth [*Cydia pomonella* (L.)] and the other, corn rootworms (*Diabrotica* spp.). In 1996, the ARS expanded the program. Based on peer reviews of proposals, followed by full proposal evaluations, two new projects targeting the weed leafy spurge (*Euphorbia esula* L.) and insect pests in stored wheat (*Triticum* spp.) were implemented in 1997. The ARS plans to expand its integrated pest management (IPM) research activities in areawide pest management as funds become available, either from new congressional appropriations or from the completion of ongoing projects. New areawide pest management projects judged to be of highest priority and merit will be implemented for insect, mite, tick, plant pathogen, or weed pests. The vision of the USDA–ARS Areawide Program is to provide farmers, consultants, and local organizations with mature areawide pest management systems that are affordable, operational, and adoptable, and that contribute to the overall goals of the USDA IPM Initiative.

KEY WORDS areawide pest management, insects, mites, ticks, plant pathogens, weeds, biological control, cultural control, chemical control

In 1994, the United States Department of Agriculture (USDA) commenced its Integrated Pest Management (IPM) Initiative (USDA 1993, 1994). This initiative reflects the redirection and combination of old and new resources of the USDA and land-grant university programs into a single coordinated and cooperative effort with farmers, private consultants, and industry to achieve the national goal of IPM on 75% of the crop acres by the year 2000. The Agricultural Research Service's (ARS) major contribution to the initiative is through the agency's Areawide Pest Management Program, as well as through its ongoing base-funded IPM research projects. The Areawide IPM Program focuses on management of pests where existing environmentally sound

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technologies are most effective when used over a multistate or multiregion area. The success of the program is dependent on full partnering and participation between the ARS; Cooperative States Research, Education and Extension Service (CSREES); Economic Research Service (ERS); Animal and Plant Health Inspection Service (APHIS); state agricultural experiment stations and extension facilities, farmers, and ranchers; and other private sector (e.g., consultants, industry) entities.

In 1993, ARS management decided, in concert with a USDA IPM Working Group, to develop a partnership framework for collaborative activities on an areawide pest management approach that would include the federal, state, and private sectors. On 27 September 1993, key pest management representatives from the USDA, university research and extension, and several state Departments of Agriculture participated in an organizational meeting in Beltsville, Maryland. At this meeting, participants identified key pests and cropping systems for which environmentally sound pest management technologies were available for implementation on an areawide basis. A high-priority list of 10 projects was developed, proposals were submitted, and these proposals were evaluated by a peer-review panel. Two of the highest ranked projects were selected for initiation. One of these two projects was implemented in 1995 and involved the codling moth [*Cydia pomonella* (L.)] and the apple (*Malus* spp.) pest management system in the U.S. Pacific Northwest by using mating disruption (Howell et al. 1992, USDA 1995). The second project was implemented in 1996 and involved the management of adult corn rootworms (*Diabrotica* spp.) and the corn (*Zea mays* L.) pest management system in the Midwest by using an adult semiochemical insecticide-bait (Lance & Sutter 1992).

Although the ARS's Areawide Pest Management Program is a part of the framework of the IPM Initiative's strategic plan, its scope actually extends beyond pests of cropping systems. These programs may include both animal and plant pests and may address both preharvest and postharvest problems. The program may encompass insects, mites, ticks, plant pathogens, nematodes, and weeds where existing technologies are available and would be most effective when used over a multiregion or multistate area.

The ARS does have certain expectations for all of the areawide projects, both ongoing and new. They are expected to have a strong stakeholder partnership and collaboration; they are expected to demonstrate the positive impacts and advantages of such a program over a large area; and they are expected to provide farmers, consultants, local organizations, and others with a mature areawide pest management system that is affordable and operational, and that will meet the overall goals of the USDA IPM Initiative, through adoption of the demonstrated strategies.

Components of the USDA–ARS Pest Management Projects

The USDA–ARS Areawide Pest Management Program typically is comprised of four key components within the framework of a temporal–spatial scale and phased implementation: operations, assessment, research, and education. The operational component forms the core of an areawide project and is necessary for the implementation of the other components, with the exception of certain research aspects. It includes a stepwise implementation of the program and the

delineation of baseline information requirements, site selection requirements and candidates, and implementation protocols. The assessment component addresses economic, sociological, and environmental impacts. A research component is included as needed to aid in the improvement of program efficacy or to help circumvent existing barriers to implementation on a regional scale. Finally, an education component is needed to ensure continued and broad adoption (technology transfer) of areawide strategies beyond the local study sites, and to enable technical training, communication, and support targeted to specific categories of potential adopters. The training structure is generally composed of four major parts: (1) direct training programs, such as field days, short courses, and seminars; (2) training materials; (3) communication by using newsletters; electronic decision support tools; and bulletin boards, trade journals, etc.; and (4) educational assessment by using social science tools to measure the benefits of the project, rate of the adoption, and opportunities for improvement. The key to enabling the education component is the regional areawide management extension specialists who will provide educational system management and coordination.

Phases of Implementation

Similar to the USDA IPM Initiative, in which the areawide program plays a substantial role, the Areawide Pest Management Program involves three phases of implementation over the 5-yr lifetime of most of its projects. Phase I includes six parts: (1) establishment of research–implementation partnership teams; (2) development of a conceptual plan and other planning activities, and definition of the scope of the projects implementation; (3) definition of the target pest(s) and secondary pest problems to be solved; (4) selection of sites and definition of the bounds of the implementation area; (5) execution of baseline data collection, feasibility studies, and limited implementation; and (6) definition of the range of research, extension, and education activities; economic, sociological, and environmental impact assessments; and technology transfer activities needed to solve end-user problems. In phase II site proposals developed by the teams in phase I are reviewed, modified as necessary, and funded for mission-linked, adaptive, and developmental research and education; technology transfer; and economic, social, environmental, and public health impact assessment. Also, in phase II the implementation and assessment of the project is begun, and the progress of the project is reviewed on an annual basis. Three expectations for phase II are as follows: (1) the areawide pest management system is fully supported by the private sector, and the public sector remains active in providing mission-linked research, extension, education, and training; (2) the project has demonstrated positive impacts and advantages over a large area through enhanced grower profits, reduced worker risks and enhanced environment, and a proven superiority of an areawide pest management strategy; and (3) the project has provided farmers, consultants, and local organizations with a mature areawide pest management system that is affordable, operational, and adoptable; and that will contribute to the overall goals of the USDA IPM Initiative. Successful projects should be able to demonstrate full implementation and adoption by the public sector in phase III of the program.

Criteria for USDA–ARS Areawide Pest Management Projects

Areawide pest management projects must meet certain criteria, eight of which follow: (1) biointensive or environmentally sound technology must be available and the use of an areawide pest management approach must be justified; (2) the project should increase opportunities for implementation of current or future IPM strategies; (3) management technology should be available after the program, and such technology should be economical; (4) the project should provide positive environmental benefits, food and worker safety, help address the impact of the Food Quality Protection Act of 1996, contribute to rural development, increase potential for exports, and have a high benefit-cost ratio; (5) involvement of federal, state, and private groups should be significant; (6) probability for acceptance and implementation by growers at the end of the project should be high; (7) area (sites) for the project should be appropriate in size, should be typical production settings with representative pest problems, with consistent populations of the key pests, and the state or region where the site is located should have an active extension IPM program; and (8) the overall likelihood of success should be high.

Future Programs in Areawide Pest Management

In fiscal year 1997, Congress increased its support of the ARS areawide pest management initiative. These funds allowed the agency to implement fully (phase II) the corn rootworm project, and proceed with the implementation of additional projects. In October 1996, a request for areawide pest management project preproposals was distributed to ARS scientists and to the state IPM coordinators through the coordinator of the USDA IPM Subcommittee. Scientists submitting preproposals were strongly encouraged to develop them as federal-state-customer partnerships. The ARS received 16 preproposals in response to this request.

Twelve reviewers provided evaluation and recommendations regarding the qualifying criteria, including the quality and scope of the science and the relevance of the preproposals to the areawide pest management concept. An ad hoc USDA Areawide Pest Management Oversight Committee also provided comments on the recommendations of the review panel. Eight preproposals were identified as candidates for development into full proposals.

A panel of five scientists from the federal and state sectors, and a number of ad hoc reviewers provided peer evaluation and recommendations regarding the quality of science and the appropriateness of each of the full proposals submitted. The peer panelists and ad hoc reviewers highly recommended three out of the eight proposals for funding. Only two, however, could be funded within the available resources in fiscal year 1997. It must be emphasized that the ARS is not a granting agency and that the funding for this program is not awarded as a grant. Instead, ARS is authorized to support such programs through specific cooperative agreements with its partners. The ARS, however, does use a peer-review panel process similar to that of other granting organizations to ensure that the most meritorious and relevant science is undertaken. Two other areawide IPM projects were initiated by the ARS in fiscal year 1997. One pro-

ject is directed at insects of stored grain in Kansas and Oklahoma and the other project is directed at the weed leafy spurge (*Euphorbia esula* L.) in Montana, North and South Dakota, and Wyoming.

The ARS scientists and scientists from Kansas and Oklahoma State Universities are the key participants in the stored-grain insects project, with farmers and operators of grain elevators as team members. It was determined that this project met the criteria of an areawide concept because grains are moved from the farm to country elevators or network and satellite elevators, to terminal elevators, to mills, and to overseas export terminals. This is analogous to the movement of insects between fields, except stored grain and the pests associated with them are transported by truck and railcar. Currently, phosphine gas is generally used for pest control in stored grains. The technology to be used for this stored-grain project will include early aeration to cool grain and decrease insect and mold population growth (Cuperus et al. 1986), as well as other technologies such as monitoring models for forecasting, safe grain-storage periods, sanitation, and other tactics as needed. This project will run through fiscal year 2001.

The leafy spurge areawide IPM project is being initiated as a partnership between the ARS in Sidney, Montana; the USDA–APHIS; North and South Dakota State Universities; and Montana State University; in cooperation with ranchers, Forest Service, Cooperative States Research, Education and Extension Service (CSREES), the Bureau of Land Management, National Park Service, and the state Departments of Agriculture. This project uses biological control with emphasis on a beetle herbivore (*Aphthona* sp.) of leafy spurge (Rees 1994), and other technologies such as grazing systems, revegetation, decision aids, geographical information systems (GIS), and judicious use of herbicides, if needed. This project will likewise run through fiscal year 2001.

Several other ongoing areawide projects sponsored by the ARS are worthy of mention. In Mississippi, an areawide project underway since 1990 uses early-season spraying of an insect virus to suppress populations of the bollworm [*Helicoverpa zea* (Boddie)] and tobacco budworm [*Heliothis virescens* (F.)], thus decreasing the need for chemical insecticide treatments for these cotton (*Gossypium* spp.) pests. A community-based field trial study for control of the blacklegged tick (*Ixodes scapularis* Say) and lyme disease in the northeastern United States was implemented in 1997. An areawide management project on Colorado potato beetle [*Leptinotarsa decemlineata* (Say)] and aphids (Aphididae) that uses biological control systems and cultural practices to lessen the reliance on chemical insecticides that will be regulated as a result of the Food Quality Protection Act is underway in the northeastern United States.

Candidate projects, based on meritorious proposals that were submitted to the ARS in 1997, but for which there was not enough funding, could be Russian wheat aphid [*Diuraphis noxia* (Mordvilko)]; silverleaf whitefly [*Bemesia argentifolii* (Perring & Bellows)]; pests in potatoes (Solonaceae) with emphasis on Colorado potato beetle and select potato pathogens; boll weevil (*Anthonomus grandis grandis* Boheman) in southern Texas; fruit flies (Tephritidae) in Hawaiian fruits and vegetables; and an expansion of the heliothine moth areawide project by using early-season applications of baculovirus. As resources become available, either as ongoing projects come to completion or as

new congressional appropriations are received, other areawide projects will be initiated. Potential projects could be those that have been submitted as full proposals, after panel reevaluation, or others that might be submitted and peer evaluated. The earliest that new areawide projects might begin is fiscal year 2000.

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Suppression of Mating by Blackheaded Fireworm (Lepidoptera: Tortricidae) in Wisconsin Cranberry Marshes by Using MSTRS™ Devices[1]

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ABSTRACT We conducted a study to optimize the deployment of our controlled pheromone release system called metered semiochemical timed release system (MSTRS™) and to measure its impact on male blackheaded fireworm, *Rhopobota naevana* (Hübner) (Lepidoptera: Tortricidae). The deployment pattern and pheromone emission rate of the MSTRS™ devices were adjusted to give optimal mating disruption in the widely dispersed cranberry (*Vaccinium macrocarpon* Aiton) beds from a perimeter-only pattern of deployment. During the first flight, disruption of pheromone source location averaged 98, 98, and 40% in the first, second, and third grower sites, respectively. During the second flight, disruption averaged 88% in the first grower site and 86% in the second and third grower sites. More importantly, high levels of mating disruption were achieved, as measured by the frequency of mating by captured free-flying females. About 24 and 15% fewer second-generation blackheaded fireworm females mated in the MSTRS™-treated beds in the first and second grower sites, respectively, compared with the check plots. In the first grower site, the mean number of matings, as measured by the numbers of deposited spermatophores, per female captured during the second flight was 0.75 in the MSTRS™ beds and 1.8 in untreated beds. Number of matings per female in the second grower site averaged 1.0 in the MSTRS™-treated beds and 1.48 in the untreated beds.

KEY WORDS *Rhopobota naevana*, blackheaded fireworm, Tortricidae, sex pheromone, mating disruption, spermatophores, controlled release

The blackheaded fireworm, *Rhopobota naevana* (Hübner) (Lepidoptera: Tortricidae), is a major pest of cranberries (*Vaccinium macrocarpon* Aiton) in the United States (Sutherland 1978). Although a female-produced sex pheromone was identified for this species in the late 1980s (McDonough et al. 1987, Slessor et al. 1987), only recently has significant work been undertaken on the potential control of the blackheaded fireworm by using pheromone mating disruption (Fitzpatrick et al. 1995, Baker et al. 1997).

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Fitzpatrick et al. (1995) first recorded significant reductions in incidence of mating of laboratory-reared females of *R. naevana* placed in plots treated with spiral polyvinyl chloride (PVC) dispensers (Scentry/Ecogen, Billings, Montana) loaded with the sex pheromone. The spiral PVC dispenser, however, like many available passive dispensers does not meet many of the requirements of a satisfactory dispenser technology, including fully controlled release of pheromone (Ogawa 1997).

A controlled release dispenser called the metered semiochemical timed release system, or MSTRS™, was described in Mafra-Neto & Baker (1996) and Baker et al. (1997). This dispenser allows the user control over the amount and timing of pheromone release. The use of MSTRS™ devices would be advantageous to cranberry growers because only a few dispensers would be necessary per acre, deployed only around the perimeter of the beds where they can be easily retrieved without incurring crop damage. Furthermore, research on optimizing MSTRS™ efficacy is needed even if treatments such as the PVC system are successful because aerial coverage near woods at the edge of beds is problematic, and MSTRS™ may be able to be used to supplement these formulations.

In 1996 we began testing the MSTRS™ devices against the blackheaded fireworm by using three different MSTRS™ densities and deployment patterns. These first tests were encouraging, in that we were able to reduce pheromone source location by blackheaded fireworm males by 98% in several plots (Baker et al. 1997), a level that was as good as that recorded with PVC dispensers (Fitzpatrick et al. 1995). The objective of this second-year study was to use just one deployment pattern and measure the impact of pheromone treatment in preventing mating of free-flying feral females, while improving the MSTRS™ pheromone mating disruption system for the blackheaded fireworm.

Materials and Methods

Pheromone formulation and dispenser. The machine portion of the MSTRS™(Waterbury Co., Waterbury, Connecticut) used in this study was a modified and updated version of the one described by Mafra-Neto & Baker (1996) and Baker et al. (1997). Briefly, the system consisted of a spray-bottle reservoir, a pump spray dispenser unit, and a timer mechanism to activate the spray discharge mechanism. A pad captured the spray and released the pheromone (Mafra-Neto & Baker 1996). Pads were formed by 0.5-cm-thick circular acrylic padding stretched on a needle point hoop (17.8 cm i.d.) and held in place 3 cm from the spray nozzle. The pump spray bottle contained a solution (maximum of 300 ml) with the desired concentration of pheromone in EtOH. A valve on top of the bottle delivered 50 µl of material per spray, and the bottle was housed in the spray dispenser unit with the valve positioned under a lever controlled by a battery-powered timer mechanism. The timer mechanism pressed the valve, which could be set at 5- to 25-min intervals, delivering a replenishing spray onto the pad. The MSTRS™ devices could produce 6,000 recharging sprays of similar strength. The spray time interval ranged between 5 and 25 min, and the timer mechanism allowed us to program the MSTRS™ to spray pheromone only during a particular time of day, such as during the moths' active period. Therefore, we could prevent pheromone from

being wasted by sprays being discharged during times when adult blackheaded fireworm is not sexually active.

All machines contained pump spray bottles filled with 250 ml of 100% EtOH containing 3.96 g of a blend of (*Z*)-11-tetradecenyl acetate (Z11-14:Ac), (*Z*)-11-tetradecenyl alcohol (Z11-14:OH), and (*Z*)-9-dodecenyl acetate (Z9-12:Ac) in a ratio of 9:3:1 (McDonough et al. 1987, Slessor et al. 1987). These components were purchased from Bedoukian Research, Inc., Connecticut, and each was ≈95% pure. The MSTRS™ devices were activated prior to the emergence of first and second generation of blackheaded fireworm (as determined with pheromone traps). To achieve an early high release rate from the pads, the spray pads were primed with 0.5 g of pheromone at the time of deployment.

Release rates of the major pheromone component, Z11-14:Ac, from pads after different times of emission in the field were measured by placing a circular cutout of the pad, contained within a frame (9.2 cm i.d.), on top of a glass funnel (10 cm i.d.). The stem of the funnel was connected with a Teflon connecting tube to a glass pasteur pipette containing a 7-cm-long plug of packed glass wool. Air was drawn across the pad (2,000 ml/min) and through the glass wool trap from the tip of the pasteur pipette by using a vacuum. After a 15-min collection the vacuum was stopped, the pad removed from the funnel, and 100 µg (1 mg/ml) of internal standard [(*Z*)-11-tridecenyl acetate] was added to the glass wool. The funnel wall and glass wool plug were washed with 3 ml of redistilled HPLC-grade hexane. One microliter of this solution was analyzed for the amount of pheromone relative to the internal standard by capillary gas chromatography-mass spectrometry (GC-MS). All GC-MS analyses were performed by using a Hewlett-Packard 5890 GC with a direct interface to a Hewlett-Packard 5972 mass selective detector (30-m DB-225 capillary column, electron impact, 70 eV).

Care was taken so that the pheromone pads did not come in contact with the glass funnel surface. Trap breakthrough was checked and confirmed to be negative by analyzing collected material in a second, in-series-connected pasteur pipette. Pad collections were performed in triplicate and collected amounts of Z11-14:Ac from the pads were calculated for the original pad diameter.

Blackheaded fireworm is known to mate during daylight hours in a broad period extending from early morning to dusk (S. Fitzpatrick, personal communication). MSTRS™ devices were programmed during the first flight (from 1 July to 1 August) to emit puffs of pheromone every 25 min for 12 h on a given day, between 0600 and 1800 hours (CST). During the second flight (from 5 August to 9 September), a shorter span of diel emission but with higher emission frequency per hour was used by setting the MSTRS™ devices to emit every 5 min between 0200 and 0800 hours. Timing of operation of the devices during the second flight was thereby aimed at loading the pad with the highest amount of pheromone at the start of the day, just before the mating period of adult blackheaded fireworm began.

Experimental plot setup. Treatments as well as untreated plots several hundred meters from treated beds were replicated three times in different grower sites within ≈10 km of each other in the cranberry-growing region near Babcock, Wisconsin. Devices were deployed at a density of ≈10 MSTRS™ per hectare (every 35 m) by using the perimeter-only pattern along cranberry beds

that we selected from the previous year's results. At the first grower site, 30 devices were deployed in three beds, totaling ≈4 ha. The untreated plot at this location consisted of two beds of 3 ha in total. At the second grower site, 35 devices were placed along the perimeter of four beds (≈5 ha) (Fig. 1). The untreated plot was two beds of 1.6 ha in total area. The MSTRS™ plot at the third grower site during the first flight consisted of eight beds, totaling ≈4.4 ha. Thirty-one devices were placed at this site. The check plot consisted of three beds of ≈1.6 ha. Due to excessively high population pressure at this site that was uncharacteristic of normal commercial operations, work was discontinued at this third grower site at the end of the first flight. A new third site was selected for the second flight. At this new site, 30 devices, which were removed from the initial third site, were placed in two beds of ≈2 ha in total area. The untreated plot consisted of two beds, totaling ≈1.2 ha.

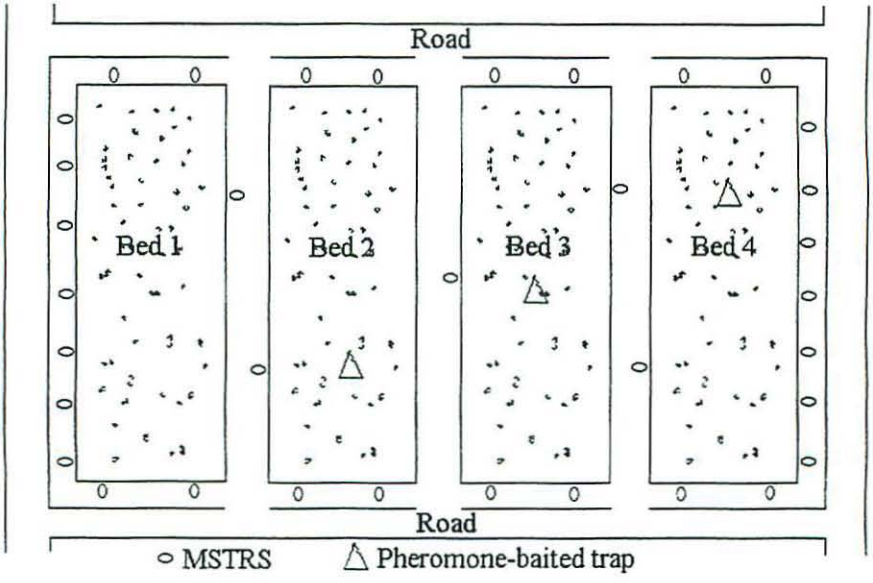

Fig. 1. Schematic representation of the pattern of deployment of MSTRS™ devices along the perimeter of four cranberry beds constituting the pheromone treatment at site 2. Each bed is ≈1.25 ha.

Evaluation of disruption. Mating disruption was assessed by using two parameters: numbers of males captured in Pherocon 1C sticky traps (Trécé, Salinas, California), and mating status and mating frequency of free-flying females captured in treated and untreated fields. Three Pherocon 1C white traps each baited with a rubber septum (Thomas Scientific, Swedesboro, New Jersey) loaded with 10 µg of the pheromone blend used in the MSTRS™ were placed in each treated plot, as well as in the untreated plots. Traps were placed in the interior of each bed and were not closer than 35 m from each machine. The number of males captured was assessed weekly, the males removed, and trap bottoms replaced as needed.

Free-flying, feral female blackheaded fireworm were collected by gently walking near the edges of each bed and capturing with a net any females that were seen flying, or by using a hand-held, battery-powered vacuum cleaner. We also used saturn-yellow Pherocon AM sticky traps baited with 100 mg each of four plant volatiles on a cotton dental wick in treated and untreated plots to try to increase the number of females captured. The components of the flower attractant were benzaldehyde, phenyl acetaldehyde, 2-phenyl ethanol, and α-terpinyl acetate in the ratio of 1:1:1:1. Most of the adults captured in these volatile-baited traps were males, hence only females collected with net or hand-held vacuum cleaner were included in data analysis. Captured females were preserved in glass vials containing 70% EtOH and were later dissected under the microscope at 10×, examining the bursa copulatrices for presence and number of spermatophores.

Mean weekly trap catch was calculated for each site. These means were used to calculate for each site the season-long mean trap catch for the MSTRS™ and untreated plots during first and second flights. Statistical analysis was with a two-way analysis of variance (ANOVA) testing the effect of treatment and site (each site was considered as a replicate) (SAS Institute 1985). Because of inclement weather, off-timing, and the use of insecticides by some growers, collection of feral female blackheaded fireworm was only possible during the second-generation flight, and only in two of the three sites. Even then, only few females were collected at these two sites. Number of mated females was considered as a proportion of the total number of females collected in each plot at each location. Data collected on the mating status of females collected in the treated and untreated plots at each location were analyzed by using the chi-square 2 × 2 test of independence with Yates' correction of continuity (Parker 1979). For the data collected on the mating frequency of feral females, mean number of spermatophores per female was calculated for each plot at each site. These means were analyzed by using a *t*-test, examining differences between the means of two small samples (Parker 1979).

Results and Discussion

A significant disruption of pheromone source location by males was achieved with the MSTRS™ during the first flight ($P = 0.03$). The effect of location was also significant ($P = 0.04$). Disruption of pheromone source location by first-generation male blackheaded fireworm, as measured by male trap catch, averaged 98% in both the first and second grower sites (Fig. 2; Table 1). Only 40%

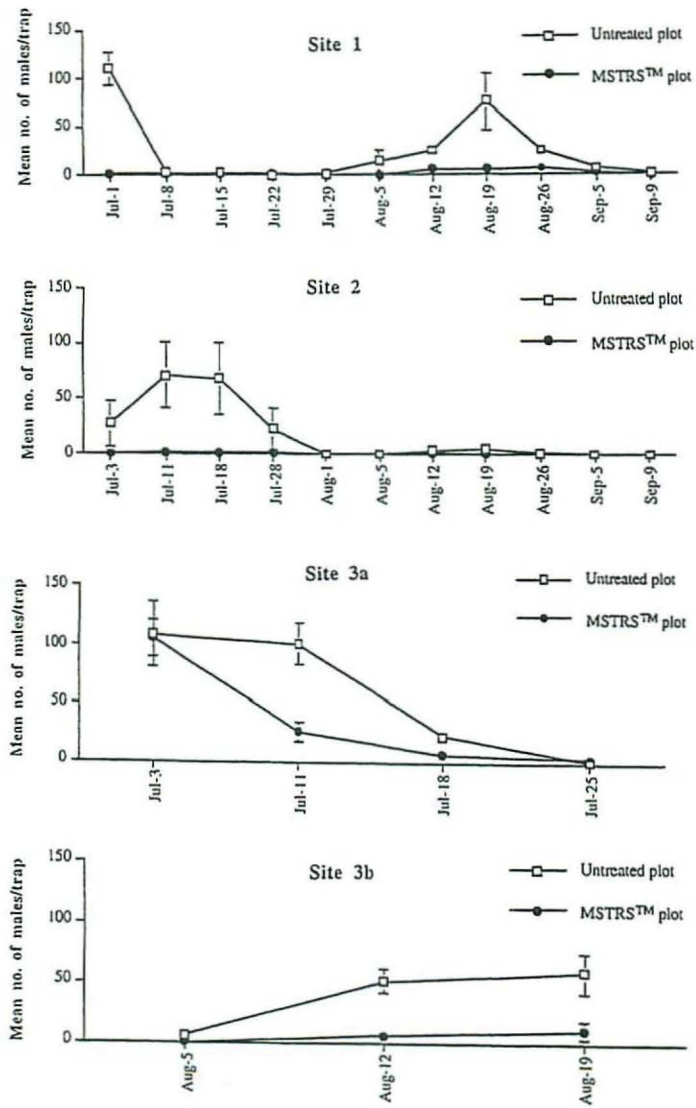

Fig. 2. Mean (± SD) capture per trap of male blackheaded fireworm in traps containing 10 μg of synthetic pheromone in cranberry marshes during first and second flights at the different grower sites (1, 2, 3a and 3b). Three traps were placed in each site. Site 3a was discontinued after the first flight and replaced with a new site (3b).

Table 1. Number of male blackheaded fireworm captured per pheromone trap (mean ± SD) and percentage of disruption of pheromone source location during first and second flights at three grower sites (cranberry plots).

	Site 1	Site 2	Site 3[a]	Mean of 3 sites
1st flight (1 July-1 Aug.)				
Untreated plot	23.20 ± 48.91	37.98 ± 21.23	59.08 ± 54.61	40.12 ± 17.98 a
MSTRS™ plot	0.53 ± 0.51	0.73 ± 0.76	35.68 ± 47.79	12.31 ± 24.0 b
Disruption (%)	97.7	98.1	39.6	69.3
P				0.03
2nd flight (5 Aug.-9 Sept.)				
Untreated plot	24.34 ± 27.13	1.56 ± 2.06	38.45 ± 28.92	21.45 ± 18.61
MSTRS™ plot	3.05 ± 2.67	0.22 ± 0.35	5.47 ± 5.35	2.91 ± 2.63
Disruption (%)	87.5	85.9	85.8	86.4
P				0.18

[a]The initial site 3 was discontinued after the first flight and a new site 3 was selected for the second flight. Means from the same flight having no letters in common are significantly different ($P < 0.05$) according to a two-way ANOVA (SAS Institute1985).

disruption, however, was achieved in the third grower site during this first flight (Fig. 2; Table 1), accounting for the significant effect of location recorded. As noted in Baker et al. (1997), this third grower site has a history of very high populations of fireworm and low yields compared with the industry average in the region. Thus, the use of this third grower site was discontinued at the end of the first flight. The degree of disruption of the ability of first-generation males to locate pheromone source, achieved in all three locations, was generally similar to that recorded during the 1996 study (Baker et al. 1997).

During the second flight, disruption of pheromone source location averaged 88, 86, and 86% in the first, second, and the new third grower sites, respectively (Fig. 2; Table 1), although these levels were not significant ($P = 0.18$). Lower levels of disruption of pheromone source location by second-generation males compared with first-generation males also were recorded during the 1996 study (Baker et al. 1997). In the current study, ≈30% of the machines at each of the three locations broke down for at least 1 wk during the second flight. This also might have contributed to the lower levels of disruption recorded during the second flight.

Although not significant, a considerable reduction in the percentage of female blackheaded fireworm that had mated was recorded for females captured in the MSTRS™ plots compared with the untreated plots at the two sites (sites 1 and 2) in which females were captured (Table 2). At site 1, 91% of the females captured in the untreated plot had mated at least once compared with 68% matings in the MSTRS™-treated beds ($\chi^2 = 0.81$, df = 1, $P < 0.5$). Similarly,

Table 2. Percentage of free-flying, second-generation female blackheaded fireworm that were captured and found to be mated, and the mean number of spermatophores per female at the two sites (cranberry plots) where females were captured.

	No. of females captured	Mated (%)	Mean (± SD) no. spermatophores per female
Site 1			
Untreated plot	11	90.9	1.82 ± 0.87a
MSTRS™ plot	12	66.7	0.75 ± 0.62b
Mating disruption (%)		24.2	58.8
P		< 0.5	< 0.01
Site 2			
Untreated plot	21	95.2	1.48 ± 0.68a
MSTRS™ plot	5	80.0	1.00 ± 0.71b
Mating disruption (%)		15.2	32.4
P		< 0.5	< 0.01

Means from the same location having no letters in common are significantly different ($P < 0.05$) according to *t*-test for two small samples (Parker 1979).

95% of females captured in the untreated plot situated at site 2 had mated compared with 80% matings in the MSTRS™ plot ($\chi^2 = 0.05$, df = 1, $P < 0.5$).

Significant reductions in the frequency of mating (number of spermatophores) per female also were recorded in the MSTRS™-treated plot at both sites. At site 1, the mean number of spermatophores recorded per female captured in the untreated plot (1.82) was significantly greater than the mean (0.75) for females collected in the MSTRS™ beds ($t = 3.44$, df = 21, $P < 0.01$). Similarly, the mean number of spermatophores recorded per female captured in the check plot (1.48) at site 2 was significantly greater that the mean number of spermatophores per female captured in the MSTRS™ plots (1.0) at this same location ($t = 2.82$, df = 24, $P < 0.01$).

Even though very few blackheaded fireworm females were captured during this study, there is potential for the modest reduction in mating frequency of females in MSTRS™-treated plots to translate into significant reductions in the reproductive success of the blackheaded fireworm and increased yield. Rice & Kirsch (1990) showed that modest reductions in the percentage of oriental fruit moth (*Grapholita molesta* Busck) females that mate in disruption plots can translate into some of the most successful and reliable disruption seen in any species. In a study on the effect of multiple mating on the reproductive performance of the European corn borer, *Ostrinia nubilalis* (Hübner), Fadamiro & Baker (1998) recorded a higher reproductive output for multiple-mated females compared with females that mated only once.

The mean emission rate from the MSTRS™ devices is shown in Table 3. After 7 d in the field, the release rate of Z11-14:Ac (the major pheromone component) was ≈6 µg/min and after 28 d it was over 3 µg/min, but this reduction was due to the malfunction of some of the machines due to worn-out dispenser belts in the peak of the summer heat. We have, however, been able to procure a stronger and more durable version of the belt that can withstand high heat. The total amount of pheromone used per hectare of marsh during both flight periods was calculated as 28 g/ha.

Table 3. Pheromone emission rates (mean ± SD) from MSTRS™ pads.

Days in the field	Release rates of Z11-14:Ac in µg/min (mean ± SD)[a]
1	5.29 ± 1.18
7	6.09 ± 1.85
26	-
28	3.08 ± 0.61*

[a]Three replicates were conducted per date. -, no data collected. *, machine breakdown.

The current results demonstrated significant reductions in mating frequency of free-flying blackheaded fireworm females in cranberry beds by using MSTRS™ and confirmed the potential for population control of this important pest species by pheromone mating disruption. It now remains to be seen whether or not this technique can lead to significant reduction of damage by the blackheaded fireworm.

Acknowledgment

We thank Jennifer Remmers, I-Shin Hsu, Jennifer Harris, and Mary Mielke (Ocean Spray Cranberries, Inc.) for their assistance in the field. This research was supported by grants from the Ocean Spray Cranberries, Inc., and the Wisconsin Cranberry Board, Inc. (both to TCB).

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Pheromone-based Management of Codling Moth (Lepidoptera: Tortricidae) in Washington Apple Orchards[1]

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ABSTRACT Pheromone-based management of codling moth [*Cydia pomonella* (L.)] in apple (*Malus* spp.) was investigated over a 3-yr period. The experimental design was a direct comparison of pest control in six orchards treated with pheromone and six adjacent comparison orchards that were under either a conventional program or an organically approved program. Good suppression of codling moth was consistently achieved with single applications of Isomate-C dispensers in three of the six pheromone-treated orchards. Initial densities of codling moth were low at these three sites. Pheromone treatments were less effective at sites with moderate to high codling moth pressure. Population densities of nontarget pests and their natural enemies, as well as the use of insecticides to control them, also were monitored in pheromone-treated and comparison orchards. Leafrollers (Tortricidae) showed the greatest potential for increased pest status in pheromone-treated orchards. Increases in leafroller densities were recorded by the second year in a majority of pheromone-treated blocks and, in the absence of supplemental control with insecticides, two sustained fruit damage >3%. Predatory mites and the leafminer parasitoid *Pnigalio flavipes* (Ashmead) were effective biological control agents in both pheromone-treated and comparison orchards. The densities of some natural enemies were significantly higher in pheromone-treated than in conventional orchards. The observed levels of predators and parasitoids, however, were highly variable from orchard to orchard and it was unclear whether they provided biological control of pests. Aphid predators and the egg parasitoid of white apple leafhopper (*Typhlocyba pomaria* McAtee), *Anagrus* sp., were particularly abundant in pheromone-treated blocks.

KEY WORDS *Cydia pomonella*, Lepidoptera, Tortricidae, mating disruption, pheromone, pest management

Codling moth, *Cydia pomonella* L., is the key pest of pome fruits in Washington, as well as other western states, and is primarily controlled by one or more applicaitons of organophosphate insecticides (Beers & Brunner 1991). These insecticide applications are highly toxic to natural enemies of most pests and are major factors limiting the success of

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biological control in pome fruit. Considerable research has been directed toward developing more selective controls for codling moth. The detection of codling moth resistance to organophosphate insecticides in the western United States (Varela et al. 1993, Knight et al. 1994) has provided a new sense of urgency to this research effort. Several tactics, including insect growth regulators (Burts 1983, Westigard 1979, Westigard et al. 1986), granulosis virus (Westigard & Hoyt 1988), and mass release of sterile males (Proverbs 1966, Dyck et al. 1992) have been demonstrated to be relatively "soft" on beneficial insects and mites. These tactics, however, have been either too expensive, not registered, or unreliable or not sufficiently effective to gain widespread commercial use.

The use of sex pheromones for mating disruption has shown promise as a selective codling moth control (Rothschild 1982, Moffitt & Westigard 1984, Charmillot 1990), but only recently has it become a commercially viable option (Barnes et al. 1992; Gut & Brunner 1991, 1992, 1994; Knight 1995). Research on mating disruption conducted in Australia, Europe, Washington, California, and Oregon has provided the foundation for commercial development of pheromone products for codling moth control (Barnes et al. 1992, Charmillot 1990, Howell et al. 1992). In 1991, Isomate-C® (Pacific Biocontrol, Inc., Vancouver, Washington) was registered as the first mating disruption product for codling moth control in the United States. We report results of a 3-yr study to follow the transition of apple (*Malus* spp.) orchards managed with conventional or organic pest control programs to a pheromone-based pest management program. Pest and natural enemy densities and fruit injury levels at midsummer and harvest were criteria used to assess the success of the pheromone-based management program.

Materials and Methods

Experimental layout. Six north central Washington orchards were included in the study. Five orchards comprised the major apple cultivars in Washington, 'Delicious' interplanted with 'Golden Delicious', whereas the sixth orchard was a planting of the relatively new 'Gala'. Further descriptions of these orchards are outlined in Table 1. The experimental design was a direct comparison of orchards at the same site treated with pheromone or the grower's standard codling moth control program, conventional or organic. Isomate-C was applied at various rates to the pheromone-treated orchards for three consecutive years, 1991–1993 (Table 1). The Isomate-C codling moth control system consisted of a clear polyethylene tube dispenser loaded with 175 mg of active ingredient [63:31:6 blend of (*E*, *E*)-8,10-dodecadien-1-ol, 1-dodecanol, and 1-tetradecanol] and 26 mg of inert ingredients (UV inhibitors and antioxidants). Rates of Isomate-C ranged from 625–1,000 dispensers per hectare and in some cases two applications were made per year. Growers followed their normal horticultural practices and control programs for other arthropod pests, diseases, and weeds in both pheromone and control blocks. Pest management recommendations were provided by private consultants or by field personnel affiliated with a packing shed or agricultural chemical distribution company.

Monitoring of codling moth. Capture of males in pheromone traps and levels of fruit injury were used to evaluate the effectiveness of codling moth

Table 1. Descriptions of orchards and pheromone application information.

	Orchard description			Orchard size, ha		Pheromone treatments[b]		
Site	Main Cultivar	Planting[a] density	Canopy height, m	Pheromone	Comparison	1991	1992	1993
W1	Delicious	270	4 – 5	4.0	4.0	1,000	1,000	1,000
W2	Delicious	502	3 – 4	2.3	3.2	1,000	1,000	1,000
W3	Delicious	602	3 – 4	2.1	2.0	1,000	1,000	1,000(2)
W4	Delicious	500	3 – 4	1.6	2.0	1,000	625	625
W5	Delicious	270	4 – 5	1.8	2.5	1,000(2)	1,000(2)	1,000(2)
W6	Gala	1,135	5 – 6	2.4	1.6	1,000	1,000	1,000(2)

[a]Trees per hectare.
[b]Dispensers per hectare; (2) indicates two applications.

control in test orchards. Pherocon 1CP traps (Trécé, Inc., Salinas, California) were placed in pheromone-treated and comparison orchards at a density of 1 trap per 0.8–1.0 hectare. Each trap in comparison orchards was baited with a red septum containing codlemone [1 mg of (*E*, *E*)-8,10-dodecadien-1-ol], whereas each trap in the pheromone-treated orchards was baited with a red septum containing 10 mg of codlemone (high-load lure). Previous studies had indicated that codling moth activity in pheromone-treated orchards was more effectively monitored with a high-load lure than with a red septum containing 1 mg (standard-load lure) of codlemone (Charmillot 1990, Gut & Brunner 1994). Standard-load and high-load lures were produced by Trécé, Inc. Codling moth injury to fruit at the end of the first generation and at harvest was evaluated by nondestructively examining 3,000 fruit in each orchard. An equal number of fruit from high (>2.2 m) and low (<2.2 m) in the canopy was inspected on 100 trees in each orchard.

Edge effects. Fruit injury samples were taken nonrandomly in an attempt to determine the relative level of fruit damage in the interior and along the borders of pheromone-treated orchards. The border was considered to be an area ca. 30 m wide around the perimeter of each orchard. An equal number of trees (50) from the interior and border was inspected in each orchard at sites designated W1-W6 on each sample date. The same sampling protocols were used to evaluate codling moth fruit injury in an additional 3, 9, and 5 pheromone-treated apple orchards in north central Washington in 1991, 1992, and 1993, respectively.

Monitoring of nontarget species. Population densities of *Typhlocyba pomaria* McAtee (white apple leafhopper), *Aphis pomi* DeGeer (green apple aphid), *Phyllonorycter elmaella* Doganlar & Mutuura (western tentiform leafminer), *Pandemis pyrusana* (Kearfott) (pandemis leafroller), *Campylomma*

verbasci (Meyer) (mullein bug), *Tetranychus mcdanieli* McGregor (McDaniel spider mite), *Panonychus ulmi* (Koch) (European red mite), and their natural enemies were determined at sites W1-W5 from 1991 to 1993 and at site W6 from 1992 to 1993.

Relative levels of white apple leafhopper activity and egg parasitism by *Anagrus* sp. were determined by capturing leafhopper and parasitoid adults on yellow sticky traps (Trécé, Inc.). A sample of five traps per orchard was used during the peak flight of each leafhopper generation. Traps were placed at 2 m in the tree canopy and retrieved after 6 to 9 d.

Aphid abundance was estimated by counting the number of aphid-infested leaves (>4 alates) and the relative number of aphids on the most infested leaf on 60 shoots (5 shoots per 12 trees) per orchard. The latter was estimated by the following rating system: 0 aphids, 0; 1–25 aphids, 1; 26–100 aphids, 2; 101–250 aphids, 3; and >250 aphids, 4. A single count of aphid predators was taken just prior to shoot hardening, when aphid densities were at their peak by counting the number observed per shoot.

Leafminer density was determined by examining 10 leaves from each of 20 trees once per generation and recording the number of tissue-feeding mines. Parasitism of leafminer larvae was measured by gathering the first 10 infested leaves (tissue-feeding mines) encountered on 10 trees once per generation. Samples were taken toward the end of each leafminer generation. To obtain an accurate estimate of parasitoid activity within each generation, leaves sampled were restricted, if possible, to those exposed to egg-laying adults of that generation.

Treatment effects on leafrollers were determined by estimating larval densities of the overwintering and summer generation, as well as fruit injury at harvest. A total of 40 growing points per 25 trees was examined during the overwintering and summer generation, respectively. A sample of 50 fruit on each of 24 trees was inspected for leafroller injury at harvest in each block.

The abundance of mullein bug was estimated by limb tapping. This procedure entailed jarring branches with a rubber-covered piece of wood doweling and recording the number of nymphs falling onto a 46 cm by 46 cm cloth-covered catching frame. A total of 30 trees (1 tap per tree) was sampled in each orchard at bloom.

Densities of spider mites and their predators were determined by leaf brushing. Four samples of 30 leaves per orchard were taken in June and July. Densities of McDaniel spider mite and European red mite were recorded separately, but for purposes of presenting data were combined as a single estimate of spider mite densities. Likewise, predatory mite densities represent a combined count of *Zetzellia mali* (Ewing) and phytoseiid mites, primarily *Typhlodromus occidentalis* (Nesbitt). Both spider and predatory mite data were expressed as the number of eggs and postembryonic individuals per leaf.

Evaluation of control programs. Records of the number and kinds of insecticides used in pheromone-treated and comparison orchards at W1-W6 were obtained for each year. In addition, 1991 and 1992 spray records for an additional 10 paired pheromone-treated and conventionally managed apple orchards in the Yakima Valley, Washington, were provided by Superior Agricultural Products, Inc. (Frank Howell, unpublished data).

Results

Codling moth control. Good suppression of codling moth was achieved for 3 yr in four of the six pheromone-treated orchards (W1, W2, W4, and W5; Table 2). Moth catch in high-load lure-baited pheromone traps was consistently low in pheromone-treated orchards at these sites. In addition, significantly more moths were captured in standard-load lure-baited pheromone traps in conventional orchards than in the high-load lure-baited traps in adjacent pheromone-treated orchards.

Codling moth fruit injury in pheromone-treated orchards at three of the sites was equal to that recorded in conventional (W1, W2, and W4) or organic orchards (W4) (Table 2). Excellent control of codling moth also was achieved at site W5, an organic orchard that, based on fruit packout records, had between 30 and 50% codling moth fruit injury the previous year (1990). In 1991, the organic comparison orchard at the site received 31 applications of ryania plus *Bacillus thuringiensis* var. *kurstaki* and fruit injury at harvest was 10.5%. Two applications of Isomate-C at 1,000 dispensers per hectare accompanied by 16 supplemental treatments of ryania plus B. *thuringiensis* reduced codling moth damage to 0.4% in that same year. In 1992 and 1993, pheromone alone (i.e., without supplemental organic pesticides) was as effective as the conventional program and more effective than the organic program, which incurred 4.7 and 2.3% codling moth fruit injury, respectively (Table 2).

The pheromone treatment was less effective at controlling codling moth at sites W3 and W6. Moth catch in the pheromone-treated orchards was higher at these sites compared with that recorded in pheromone-treated orchards at sites W1, W2, W4, and W5 (Table 2). Moreover, at W3 and W6 similar numbers of moths were captured in the pheromone-treated and comparison orchards.

Greater than 1% codling moth fruit injury was detected at harvest at site W6 in 1992 and 1993. The highest levels of codling moth fruit injury observed in pheromone-treated orchards during the 3 yr of the study occurred at site W3. This was an orchard managed by the Washington State University, Tree Fruit Research and Extension Center, for experimental purposes, and historically codling moth populations had been moderate to high. In 1991 a single application of 1,000 dispensers per hectare in the pheromone-treated orchard provided control equivalent to 3 applications of azinphosmethyl in the conventional comparison orchard (ca. 1% fruit injury). The same pheromone treatment in 1992 was unable to control the codling moth population and 5.9% fruit damage was recorded at harvest. Multiple applications of pheromone in 1993 (2,000 dispensers per hectare total) again did not provide acceptable control under high codling moth pressure and fruit injury at harvest was 7.9%.

Distribution of damage. Codling moth injury to fruit in pheromone-treated orchards was significantly greater along the borders compared with the interior (Fig. 1). Approximately two-thirds of the damage was recorded within 30 m of the perimeter of these orchards.

Effects on nontarget organisms. Densities of nontarget species in pheromone-treated and comparison orchards are compared in Figs. 2–7. In 1991 the density of pandemis leafroller larvae was low in both pheromone-treated and comparison orchards (Fig. 2). Leafroller densities were maintained

Table 2. Codling moth catch in pheromone traps and fruit injury in pheromone-treated and comparison (conventional or organic) orchards at six central Washington locations, 1991–1993.

		Mean seasonal catch, moths/trap			Fruit injury at harvest, %		
Site	Treatment	1991	1992	1993	1991	1992	1993
W1	pheromone	0.3	1.0	2.3	0.00	0.00	0.00
	conventional	3.5	9.3	6.8	0.06	0.00	0.00
W2	pheromone	1.1	4.0	4.5	0.09	0.80	0.05
	conventional	18.8	30.6	22.6	0.78	1.00	0.05
W3	pheromone	1.1	47.0	32.0	1.34	5.90	7.90
	conventional	18.3	23.5	28.5	0.72	0.40	0.00
W4	phermone	2.3	1.0	2.5	0.25	0.10	0.07
	organic	15.8	7.0	3.5	0.38	0.20	0.07
W5	pheromone	4.5	1.5	4.0	0.35	0.20	0.10
	organic	49.8	33.8	37.8	10.46	4.70	2.30
	conventional		172.8	135.3		0.10	0.10
W6	pheromone		14.5	5.3		1.10	2.00
	conventional		15.0	6.0		0.10	0.00

at nondamaging levels in comparison orchards throughout the course of the study. In contrast, leafroller densities increased dramatically from 1991 to 1993 in the pheromone-treated orchards at two sites (W3 and W5), and in both cases leafroller fruit injury constituted a major portion of the cullage (Fig. 2).

The densities of white apple leafhopper adults were not significantly different in pheromone-treated and comparison orchards throughout the study (Fig. 3A). Densities were low at all sites and orchards in year 1 (1991) (data not shown), but increased to high levels by year 3 (1993). Low numbers of the leafhopper egg parasitoid *Anagrus* sp. also were recorded in 1991 (data not shown) and 1992 (Fig. 3B). The density of *Anagrus* sp. increased dramatically in 1993, being significantly higher in pheromone than comparison orchards. The parasitoid, however, seemed to have little effect on subsequent densities of leafhopper adults.

The leafminer density in pheromone-treated orchards was very low, never exceeding one mine per leaf (Fig. 4A). A low density of leafminers also was recorded in comparison orchards in 1991 and 1993. In 1992, however, the mean density of leafminers was significantly higher in comparison orchards than in pheromone-treated orchards. An application of oxamyl was applied for leafminer control in 1992 in two of the six conventional orchards (Table 3).

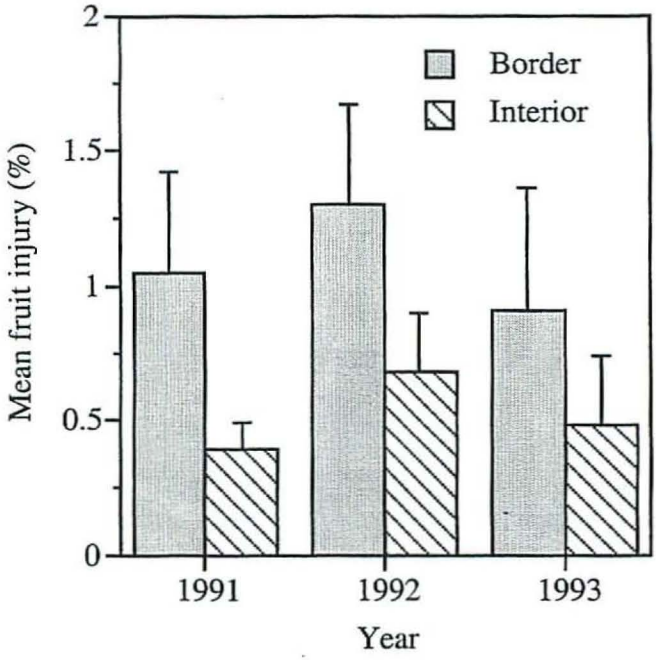

Fig. 1. Comparison of mean codling moth fruit injury (with SEM bars) on the border and in the interior of pheromone-treated apple orchards in north central Washington.

Leafminer parasitism was high (>40%) in pheromone-treated and comparison orchards in 1991 and 1992 (Fig. 4B). In 1993, the level of parasitism was lower in both treatments, probably because of the very low leafminer densities (Fig. 4A). Even so, mean leafminer parasitism was significantly higher in pheromone-treated than in comparison orchards in 1993 (Fig. 4B). Less than 10% parasitism was recorded in the two conventional orchards (sites W3 and W4) that received a summer application of oxamyl the previous year.

The mean number of aphid-infested shoots was not variable among years and no difference was found between treatments (Fig. 5A). In contrast, aphid predator densities were consistently higher in pheromone-treated than in comparison orchards (Fig. 5B). Predator densities were greater in pheromone-treated than in comparison orchards in 1992. *Deraeocoris brevis piceatus* (Knight), *Chrysoperla nigricornis* Burmeister, *C. verbasci*, and syrphid flies, primarily *Scaeva pyrastri* (L.), were the most abundant predators.

Spider mites were maintained at low densities in pheromone-treated orchards throughout the 3-yr study (Fig. 6A). Mean densities of spider mites also were low in comparison orchards in 1991 and 1992, but increased

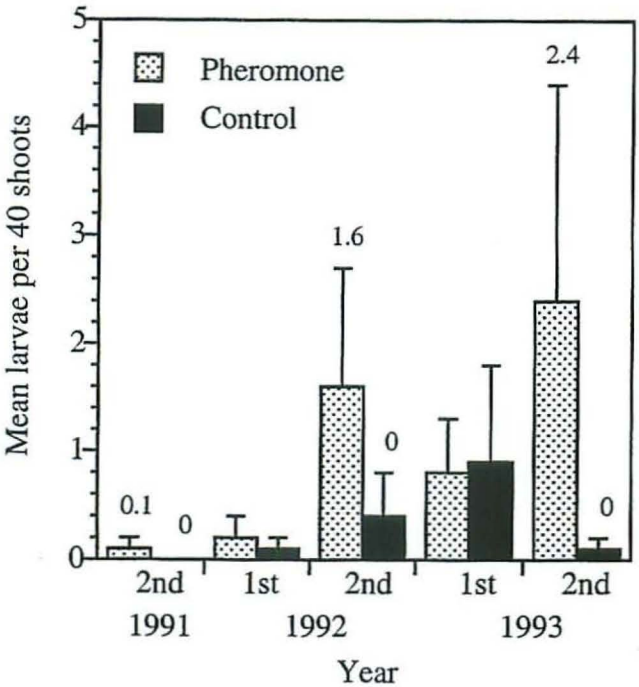

Fig. 2. Mean densities of first- and second-generation pandemis leafroller larvae (with SEM bars) and fruit injury at harvest (percentages shown above bars) in pheromone-treated and conventional or organic (comparison) orchards at six central Washington locations, 1991–1993.

significantly in 1993. Predatory mites were equally abundant in pheromone-treated and comparison orchards throughout the study (Fig. 6B). *Typhlodromus occidentalis* and *Z. mali* were the most abundant predatory mite species.

The mean number of mullein bug nymphs per beating tray was highly variable between sites (Fig. 7). At the two sites where mullein bug was consistently abundant (W3 and W5), however, lower densities were detected in the conventional than in the pheromone-treated orchards. We suspect that in the former, a prebloom application of chlorpyrifos for leafroller control also provided suppression of mullein bug.

The frequency of colonization by other potential pests, such as lygus bug, *Lygus lineolaris* (Palisot de Beauvois) and fall webworm, *Hyphantria cunea* Drury, increased in pheromone-treated orchards but they either did not reach damaging levels or were easily controlled by spot treatment with insecticides (*B. thuringiensis* sprays) or cultural practices (pruning). Fall webworm was the most abundant of these sporadic pests in pheromone-treated blocks, with infestations detected at three of six sites in all 3 yr of the study.

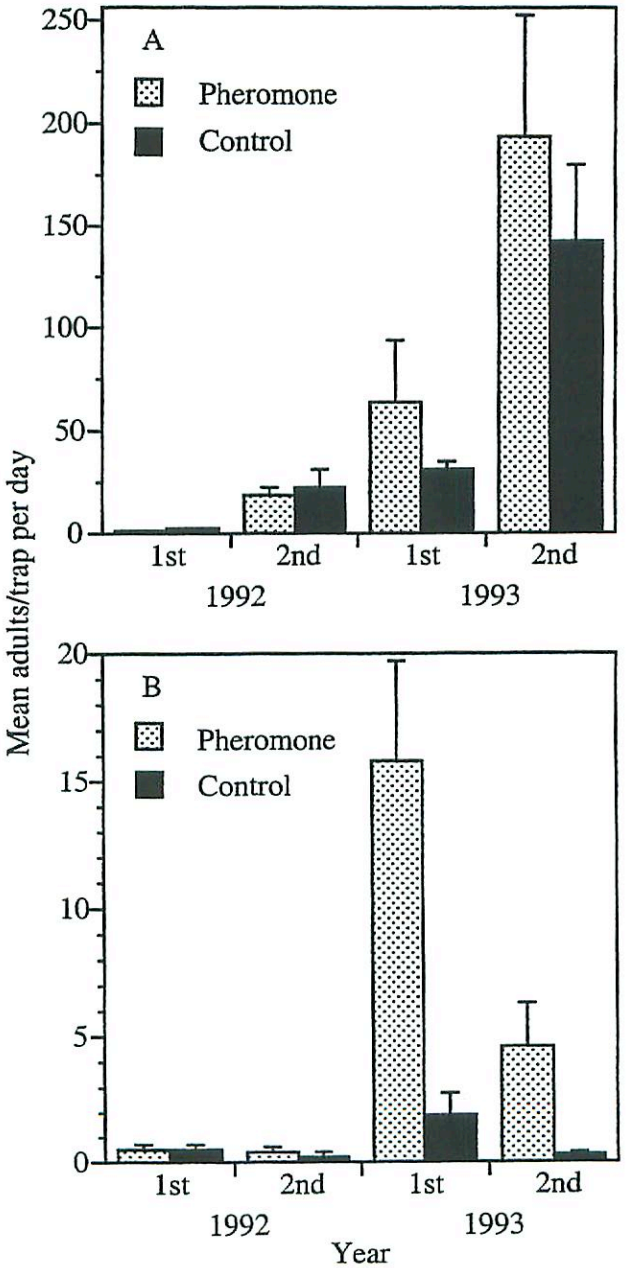

Fig. 3. Mean densities of (A) white apple leafhopper and (B) the leafhopper egg parasitoid *Anagrus* sp. (with SEM bars) in pheromone-treated and conventional or organic (comparison) orchards at six central Washington locations, 1991–1993.

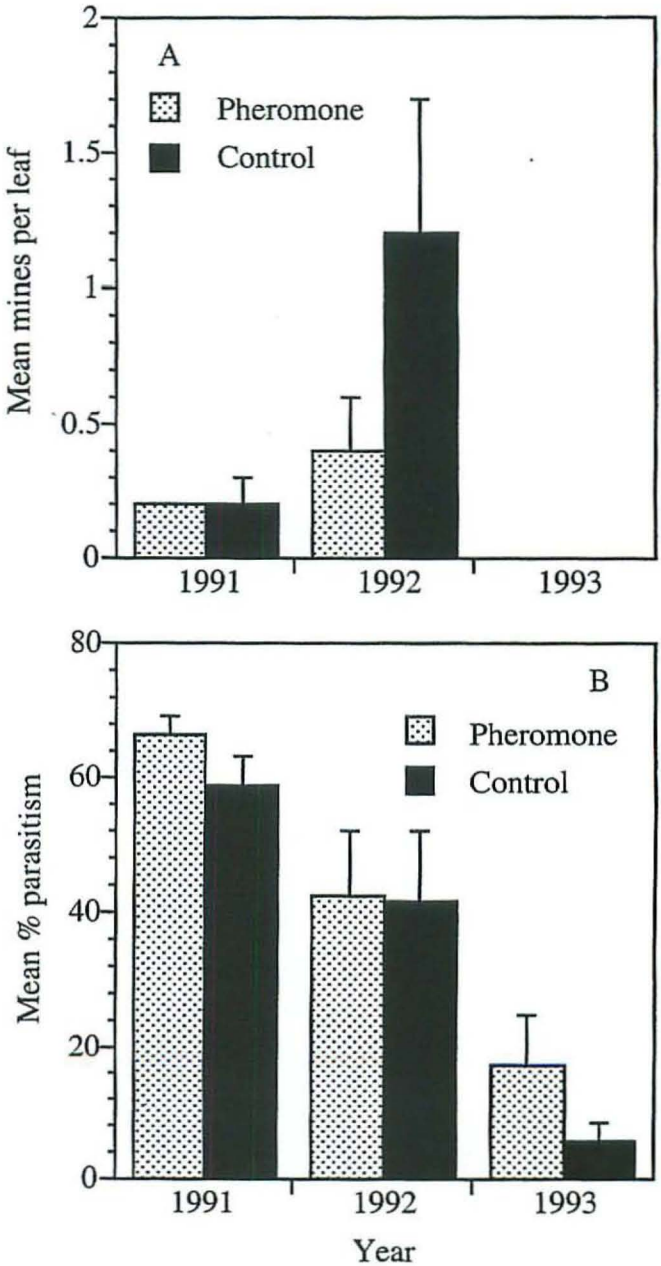

Fig. 4. Mean densities of (A) second-generation leafminer and (B) percentage of parasitism of leafminer larvae (with SEM bars), in pheromone-treated and conventional or organic (comparison) orchards at six central Washington locations, 1991–1993.

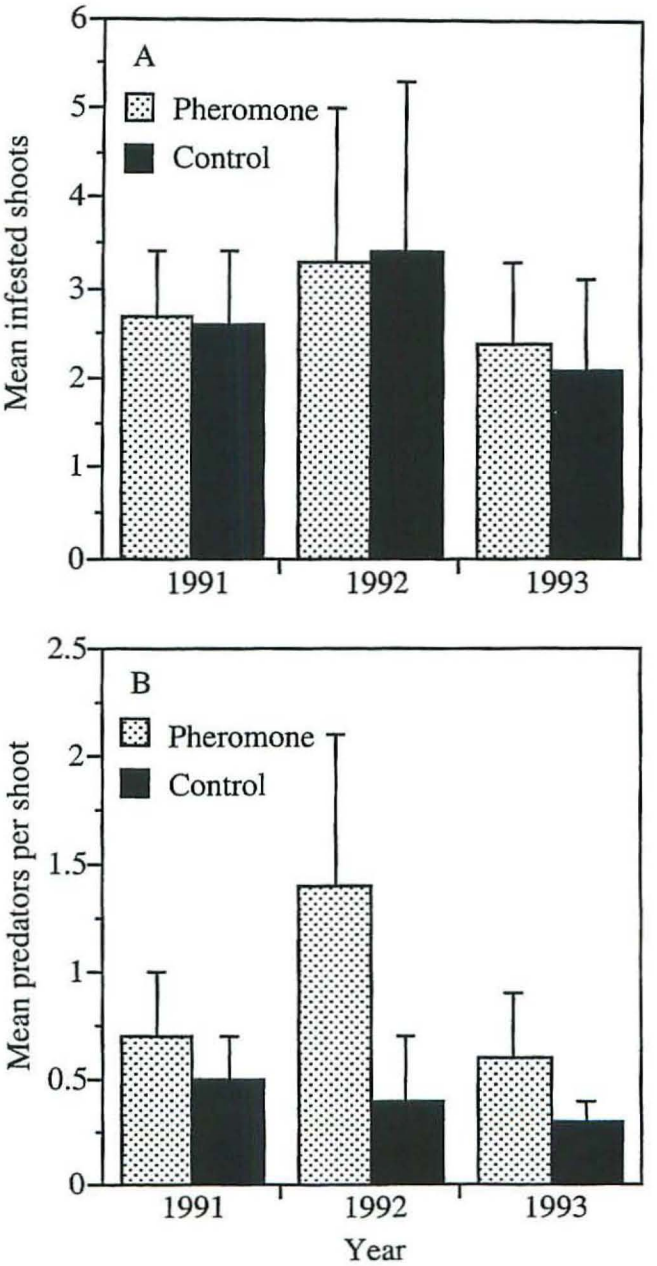

Fig. 5. Mean densities of (A) green apple aphid and (B) aphid predators (with SEM bars), in pheromone-treated and conventional or organic (comparison) orchards at six central Washington locations, 1991–1993.

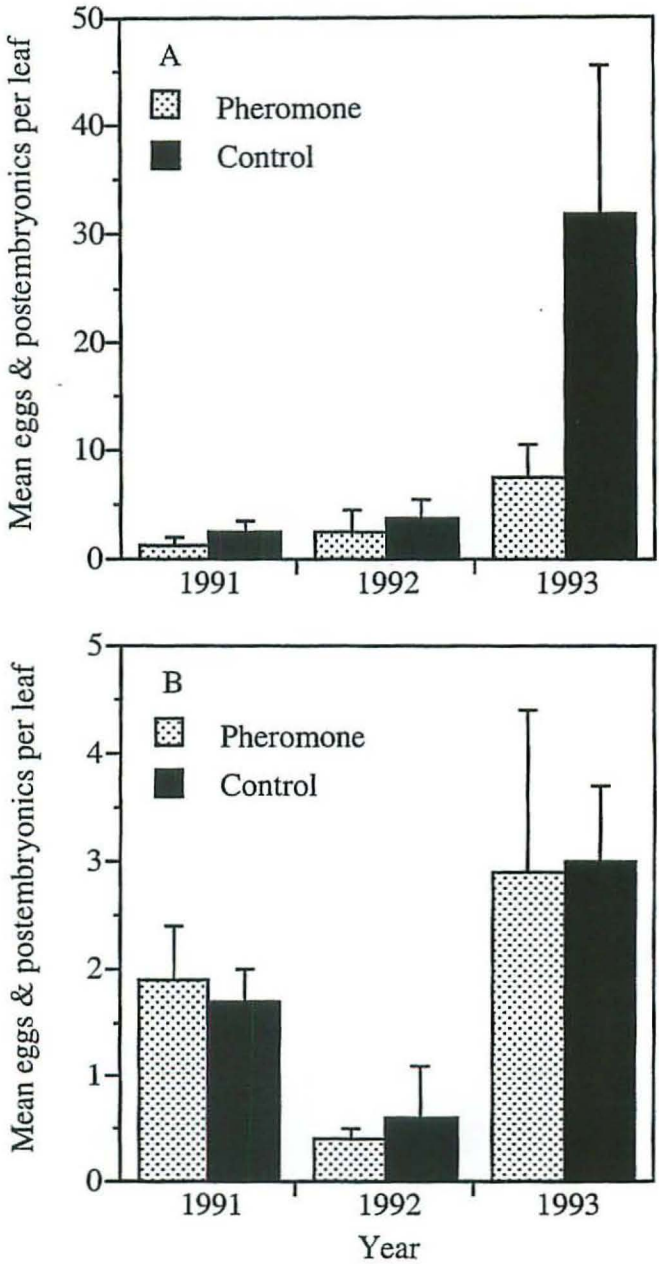

Fig. 6. Mean densities of (A) phytophagous and (B) predaceous mites (with SEM bars) in pheromone-treated and conventional or organic (comparison) orchards at six central Washington locations, 1991–1993.

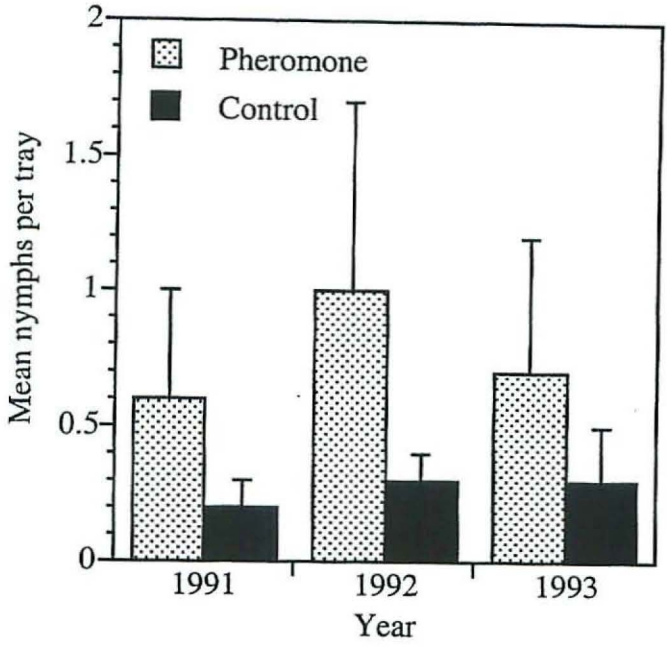

Fig. 7. Mean densities of mullein bug (with SEM bars) in pheromone-treated and conventional or organic (comparison) orchards at six central Washington locations, 1991–1993.

Comparison of control programs. The number and kinds of insecticides used in pheromone-treated and comparison orchards provided a less direct, but valuable means of characterizing different management systems and of their impact on nontarget pests. Spray records for six paired orchards in Wenatchee and 10 paired orchards in Yakima revealed some consistent differences in insecticides applied for pests other than codling moth (Table 3). Leafrollers generally required one or more insecticide applications in both pheromone-treated and comparison orchards. Slightly fewer leafroller control sprays were applied to pheromone-treated orchards in Wenatchee in the first 2 yr of the study but in 1992 two of these orchards sustained unacceptable levels of leafroller fruit injury, indicating more sprays should have been used. More insecticides were applied in Yakima for aphid and leafminer control than in Wenatchee. No sprays for aphids or leafminers were applied to pheromone-treated orchards in Wenatchee and, in Yakima, pheromone-treated orchards received about half the number of sprays as conventional comparison orchards.

Table 3. Comparison of the number of treatments for control of the major nontarget pests in pheromone-treated and comparison (conventional or organic) orchards in Yakima and Wenatchee (W1–W6) from 1991 to 1993.

Treatment or target pests	Mean number of insecticide treatments per block					
	1991		1992		1993	
	Treated	Comparison	Treated	Comparison	Treated	Comparison
Yakima sites[a]						
Leafrollers	1.3	1.5	1.5	2.0		
Aphids	0.6	2.2	1.0	2.1		
Leafminers	0	0.1	0.7	1.4		
Wenatchee sites						
Leafrollers	1.0	1.6	1.0	2.1	2.5	2.8
Aphids	0	0.3	0	0.5	0	0.2
Leafminers	0	0	0	0.3	0	0

[a]Yakima data provided by Frank Howell, Superior Agricultural Products.

Discussion

Results of this 3-yr study on the transition of conventional or organic orchards to a pheromone-based management program demonstrate that marketable apples can be grown in Washington by using mating disruption as a primary means of suppressing codling moth. Pheromone treatments inhibited moth captures in pheromone traps and provided crop protection at least equivalent to that achieved with conventional insecticides or an organic program at four of six orchard sites for three consecutive years. The initial density of codling moth within an orchard or in an adjacent orchard was the single greatest factor influencing the degree of control provided by pheromone. The four orchards in which mating disruption provided good suppression of codling moth had records showing historically low moth captures in pheromone traps and packouts indicating very low levels of fruit injury. In contrast, the two pheromone-treated orchards in which good codling moth control was not achieved by mating disruption had a history of high moth catch in pheromone traps and easily detectable fruit damage at harvest, ca. 1% or more.

Previously reported comparisons of codling moth control in orchards treated conventionally or with various kinds and rates of mating disruption products also indicated the primary importance of initial codling moth population densities in

determining the degree of control (Gut & Brunner 1994, Knight 1995, Thomson 1997). In orchards with a history of low codling moth pressure, control has generally been excellent in pheromone-treated orchards. Gut & Brunner (1994) reported that pheromone rates at or below the recommended level could be used to achieve codling moth control in low-pressure orchards. Codling moth control has been variable in pheromone-treated orchards with moderate codling moth pressure and poor control has consistently occurred in pheromone-treated orchards with a history of high codling moth pressure.

Our findings suggest that increasing pressure from codling moth signals the need for higher rates of pheromone or supplemental treatment with insecticides or both. Control in orchards with low densities can generally be achieved with a single application of Isomate-C even at reduced rates, for example at site W4. Supplemental treatment with insecticides during the first or second codling moth generation or an additional application of Isomate-C at the start of the second flight sometimes will be required to achieve acceptable levels of control. Even increasing Isomate-C application rates will not provide control in orchards with a high density of codling moth. In these situations mating disruption products will need to be used at the maximum label rate and supplemented with insecticides. Supplemental insecticides during the first generation should be a standard part of the codling moth control program in high-pressure and some moderate-pressure orchards.

Orchard borders will require extra attention when implementing a mating disruption control program. The majority of codling moth fruit injury in pheromone-treated orchards was recorded within 30 m of the perimeter. Other researchers have found borders of disrupted orchards to be problematic. For example, Knight (1995) reported low levels of codling moth fruit injury in all but one of seven pheromone-treated orchards. In the orchard where control was poor, 65% fruit injury on one edge was the major contributor to an overall level of damage of only 7.4%. Probably two processes contribute to the development of border infestations in pheromone-treated orchards. Mated females immigrating from nearby sources are free to infest fruit because pheromone provides no protection from invading moths. In addition, pheromone concentrations are suspected to be lower on orchard borders than in the interior, increasing the likelihood that mating and thus damage will occur on borders.

Three tactics can be used to protect orchard borders. Additional pheromone can be applied to border trees or possibly extended into adjacent orchards. Our experience indicates that either tactic is effective when initial codling moth densities are low. In orchards with higher codling moth densities it is best to treat borders with insecticides, a tactic that can be combined with use of higher border rates of pheromone or the extension of pheromone treatments into the neighboring orchard. Treatments (insecticides or extra pheromone) should be applied to an area of ca. 30 m around the perimeter of an orchard to provide sufficient protection of borders.

As a pest control tactic, mating disruption is highly specific. Implementing this tactic for codling moth control, however, will have a significant impact on nontarget arthropods, both pests and their natural enemies. Leafrollers appear to have the greatest potential to become important pests as growers transition from conventional to pheromone-based pest management programs. Leafroller

densities at all six north central Washington orchards were initially low and fruit injury in Isomate-C and conventionally treated orchards was not observed in the first year of the study. In the second year of the study, however, five of eight Isomate-C-treated orchards had measurable pandemis leafroller populations and, in the absence of supplemental control with insecticides, two sustained significant levels of fruit damage, >3% at harvest. In two similar studies conducted in Yakima, Washington, leafroller densities increased in the intitial year of pheromone use. Knight (1995) found that leafrollers caused >3% fruit injury in three of seven pheromone-treated orchards in the first year. Brunner et al (1992) reported that pandemis leafroller was present in eight of eight conventional and mating-disruption orchards in the first year of pheromone use. Mean fruit injury, however, was higher in pheromone-treated than conventional orchards (4.2 versus 0.8%), primarily due to inadequate intervention with chemical controls in the former. In the second year of both Yakima studies leafroller damage was low in both conventional and pheromone-treated orchards due to the use of organophosphate insecticides for leafroller control.

Several potential apple pests, such as fall webworm, lygus, and stinkbugs, are present in nonorchard habitats in Washington but are rarely observed in commercial orchards. The use of azinphosmethyl and other organophosphate insecticides for codling moth control is probably the primary factor preventing these pest species from establishing populations in commercial apple orchards. None of the pests considered as minor or sporadic (Beers et al. 1993) became a serious problem in Isomate-C-treated orchards during this study. Fall webworm, however, colonized three of six pheromone-treated orchards for three consecutive years. Although their distribution in orchards was clumped and developing populations were effectively controlled by pruning infested limbs, the regular occurrence of this pest suggests the need for more careful monitoring of pest species currently classified as minor or sporadic in pheromone-treated orchards.

The abundance of natural enemies was often greater in pheromone-treated than in conventional control orchards. This was especially true where summer insecticides were used in the conventional comparison orchards. The levels of predators and parasitoids, however, were highly variable from orchard to orchard and it was not possible to claim that they consistently provided biological control of pests. For example, densities of the leafhopper egg parasitoid *Anagrus* sp. increased from 1991 to 1993 in Isomate-C-treated orchards but not in conventional orchards. Increased densities of the parasitoid in Isomate-C-treated orchards, however, did not result in reduced densities of white apple leafhopper. It may be that the high dispersal capability of the white apple leafhopper overwhelmed the activity of *Anagrus* sp. A greater abundance of green apple aphid predators was found in Isomate-C-treated than in conventional orchards, but again a direct correlation of their impact on aphid densities was difficult to demonstrate. Fewer insecticides were needed for aphid control in Isomate-C-treated than in conventional orchards, indicating that predators were providing suppression of aphid populations in the former.

Pnigalio flavipes, the dominant parasitoid of tentiform leafminer (Barrett & Brunner 1990) was the most effective biological control agent in Isomate-C-treated and comparison orchards. Leafminer densities were maintained below

damaging levels primarily by *P. flavipes* in most test orchards in Wenatchee. Supplemental control of leafminer with insecticides was required more frequently in Yakima orchards. Isomate-C-treated orchards received fewer aphicides than conventional comparison orchards, providing indirect evidence of more effective biological control in the former. Low levels of parasitism tended to be recorded in orchards with heavy insecticide use patterns, especially where chlorpyrifos or encapsulated methyl parathion, both of which are highly toxic to *P. flavipes* (J.F.B., unpublished data), were applied during summer for leafroller control.

Chemical control of arthropod pests in Washington apple orchards is becoming more difficult and less reliable. The development of pest species resistant to insecticides, withdrawal or cancellation of registered insecticides, and limited prospects for registration of new products have all severely reduced pest control options. It is likely that public concern over pesticide residues on food, environmental contamination, and farm-worker-exposure issues will further reduce the availability of conventional insecticides. Aware of these problems, many orchardists are moving toward greater reliance on mating disruption and other "soft" or selective approaches to pest control. In 1991, only about 600 ha was treated with codling moth mating disruption products in Washington State. By 1995, approximately 6,500 ha was treated with codling moth mating disruption products and we estimate that >12,000 ha was treated with pheromone in 1997. Many of these were large contiguous areas of between 100 and 400 ha.

Three factors appear to be the major obstacles to further adoption of a pheromone-based pest management program in apple—efficacy, selectivity, and cost. Mating disruption is less effective than conventional insecticides as a control for codling moth when densities are moderate to high. Increasing our understanding of the relationship between pheromone concentrations within the orchard and disruption of mating in codling moth will enable us to develop dispensers that consistently provide good control of this key pest, even at higher pressure than is now possible.

Benefits of a pheromone-based codling moth control program are reduced if broad-spectrum insecticides are needed in summer to control leafrollers. Developing alternative controls for leafrollers compatible with codling moth mating disruption will be an important component of a pheromone-based pest management program. Three tactics for leafrollers that do not directly impact natural enemies of other pests are currently being investigated. These include bacterial insecticides, biological control, and mating disruption. Bacterial insecticides that use strains of *B. thuringiensis* as the toxic agent have shown promise as commercially viable controls for leafrollers in apple (Brunner et al. 1995, Knight 1997). Currently, 24% of Washington apple growers reports using *B. thuringiensis* products to control leafrollers (National Agricultural Statistics Survey 1996). Two parasitoid species, *Trichogramma platneri* Nagarkatti and *Colpoclypeus florus* (Walker), hold promise as biological control agents for leafrollers in apple in north central Washington (Brunner 1992). Research on mating disruption of leafrollers has been conducted in Washington since 1990 (Gut & Brunner 1992, Knight et al. 1996) and, although results have been variable, at least two commercial product are available.

The relatively high cost of codling moth mating disruption is a major constraint to its widespread adoption. Williamson et al. (1996) determined that switching from a conventional to a pheromone program in apple resulted in an increase in production costs of $95.91 to $241.22 per hectare. The general trend was that disrupted orchards had higher costs for pheromone dispensers and labor and lower insecticide and machinery costs. The number of non-codling-moth insecticides used can decrease substantially when pheromone rather than organophosphate insecticides are used to control this key pest. Growers in our study orchards used significantly fewer insecticides for leafminer and aphid control in pheromone-treated compared with conventional orchards. Williamson et al. (1996) reported a cost savings of $96.68 per hectare due to reductions in insecticides used to control pests other than codling moth in pheromone orchards.

The cost differential between a strictly conventional and pheromone-based pest management program for codling moth control will probably decrease in coming years. Williamson et al. (1996), however, estimated a cost reduction of 30–73% would be required for mating disruption to be economically competitive. The most cost-effective approach currently used is to combine a low rate of pheromone plus an early-season application of an organophosphate insecticide. The use of Isomate-C at half the label rate or 500 dispensers per hectare supplemented with one application of azinphosmethyl can provide codling moth control equivalent to conventional programs that use three or more azinphosmethyl treatments (Gut & Brunner 1994). Thomson (1997) calculated that applying Isomate-C and one azinphosmethyl spray cost only $26 per hectare more than a conventional program of three azinphosmethyl sprays. New and less expensive technologies, changes in government regulations, and a greater awareness of less tangible benefits gained from the use of selective pest control tactics are factors that will probably lead to adoption of codling moth mating disruption by more growers.

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