SAMPLING FOR PINE SHOOT MOTHS (LEPIDOPTERA: TORTRICIDAE): BIOLOGICAL PRINCIPLES AND PROCEDURES

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ABSTRACT

Insect species in the genera *Rhyacionia* and *Eucosma* (Lepidoptera: Tortricidae) are common pests of pines (*Pinus* spp.). The larvae of some of these insects feed within stems of host trees, stunting or killing the growing shoot tips. Because of their similar life histories, these insects present common problems for sampling. Selection of an appropriate sampling method depends upon clearly defined study objectives. The various sampling methods can be divided into two broad classes; (1) extensive sampling (to determine relative abundance) and (2) intensive sampling (to determine absolute abundance). Specific sampling methods include sex-attractant or pheromone traps, host damage assessments, direct counts through host inspection and dissection, and radiographic techniques. The sampling methods are described and the biological bases for their development are discussed.

Key Words: Tortricidae, pine shoot borers, pine tip moths, sampling, pine shoot moths.

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A number of insect species in the family Tortricidae are common pests of pines (*Pinus* spp.). The larvae of some of these insects feed within stems of host trees near the growing shoot tips. Larval feeding stunts, deforms or kills infested shoots, resulting in reduced tree growth and/or poor form. The most important of these species are found in the genera *Rhyacionia* and *Eucosma*. Throughout this paper these insects will be referred to collectively as shoot moths, although they are also commonly called tip moths and shoot borers. Details of the biology, ecology, and control of these insects are summarized in several publications (Furniss and Carolin 1977, Coulson and Witter 1984, USDA 1985, Johnson and Lyon 1988).

Because of their similar life histories and feeding habits, shoot moths present common problems for sampling. A variety of methods have been developed to sample shoot moth populations to achieve specific objectives such as delineation of species ranges, identification of natural mortality factors, determination of damage thresholds, and evaluation of control treatments. This review discusses the methods that have been used to sample shoot moth populations and the biological considerations underlying their development. It is based primarily on literature dealing with *R. frustrana* and *E. sonomana*, although references to other species are included. I have specifically avoided discussing the statistical aspects of sampling which can be found in more general papers (Morris 1955, Waters and Henson 1959) and books devoted to that topic.

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Prior knowledge about the distribution of an insect population can be used to increase the efficiency and accuracy of sampling. Much is known about the temporal and spatial distributions of shoot moths which can be used in the design of a sampling program.

Shoot moth phenology varies considerably among the different species. For example, *R. buoliana* completes one generation every 12 months overwintering as larvae, *R. adana* and *Eucosma* spp. complete one generation per year overwintering as pupae, and *R. frustrana* can have from two to six generations per year depending upon the climate. The general phenology of most shoot moths is known, although regional variations occur and can influence population sampling, particularly with multivoltine species. The sex pheromones or attractants for six *Rhyacionia* spp. (Berisford 1982) and *E. sonomana* (Sower et al. 1979) have been identified, and some have been used to study seasonal phenology of shoot moths in specific locations (Berisford 1974, Canales and Berisford 1981, Malinoski and Paine 1988).

A map of *R. frustrana* phenology in Georgia based on mean thermal requirements for development has been constructed (Ross et al. 1989). Where shoot moth seasonal phenology is uncertain, precautions must be taken (e.g., preliminary studies) to ensure that sampling is conducted at the appropriate stage of moth development.

Shoot moths exhibit unique spatial distributions at all levels from geographic ranges to within tree patterns. On the broadest scale, each shoot moth species has a characteristic range. Some shoot moth species are sympatric and others are allopatric. The two North American species of *Eucosma* that bore in the shoots of pines are apparently allopatric. The distribution of *E. gloriosa* corresponds closely to the natural range of eastern white pine (*P. strobus* L.), while *E. sonomana* follows that of ponderosa pine (*P. ponderosa* Douglas ex Laws.) (DeBoo et al. 1971). Some species of *Rhyacionia* are very similar morphologically, but have distinct ranges and attack different pine species. *R. frustrana* and *R. bushnelli* are one such pair of allopatric species that are considered by some to be different races of the same species (Berisford 1988). In contrast, the range of *R. frustrana* overlaps with at least four other common species of *Rhyacionia* (Powell and Miller 1978). *R. frustrana* and *R. rigidana* are often found in the same stands, trees, and even the same shoot tips (Miller and Wilson 1964, Yates 1967a, Baer and Berisford 1975).

A number of site and stand factors influence the occurrence and abundance of a given species of shoot moth within its geographic range. Each shoot moth species attacks some pine species more often than others. For example, in the Southeast, *R. frustrana* favors loblolly and shortleaf (*P. echinata* Mill.) pines, slash pine (*P. elliottii* Engelm.) is sometimes attacked, and longleaf (*P. palustris* Mill.) and eastern white pines are apparently immune to attack (Yates et al. 1981). High shoot moth populations have been found to be associated with pure even-aged stands (Berisford and Kulman 1967, DeBoo et al. 1971), wide tree spacings (Grant 1958, Berisford and Kulman 1967, DeBoo et al. 1971), low levels of competing vegetation (Warren 1963, Berisford and Kulman 1967, Miller and Stephen 1983, Ross 1989), intensive mechanical site preparation (Hertel and Benjamin 1977, White et al. 1984, Hood et al. 1988), low site quality (Hood et al. 1988), and xeric habitats (Stoszek 1973). Some shoot moths only attack young trees, and populations...
decrease rapidly following crown closure (DeBoo et al. 1971, Berisford 1988). Other species attack trees regardless of their size or age, although they are less apparent in larger trees (Grant 1958, Miller 1967, Sower and Shorb 1984).

Hedden (1979) found that a major source of variation in *R. frustrana* infestations was between trees. This may be due to differences in the apparent or acceptability of different trees, since *R. frustrana* females lay more eggs on vigorous trees than on stressed trees when both are equally available (D. W. R., unpublished data). Similarly, *E. sonomana* is more likely to attack shoots arising from the largest spring buds (Sower and Mitchell 1987), which would be associated with the most vigorous trees in a stand. Differential oviposition is also apparently responsible for the aggregated distribution of *R. buoliana* among trees in a stand (Miller 1967).

Waters and Henson (1959) found that the distribution of *R. frustrana* in trees, whorls of branches, and shoot tips followed the negative binomial, indicating a clumped distribution for each sample unit. Within trees, the uppermost whorls were most likely to be attacked by *R. frustrana*. Numerous studies have reported analogous results (Berisford and Kulman 1969, Stephen and Wallis 1978, Hedden 1979, Andersen et al. 1984). Since larval *R. frustrana* migrate very little during development (Yates 1967b), the clumped distribution of attacks must be due to the ovipositional behavior of adult females. In contrast to *R. frustrana*, the initial vertical distribution of *R. buoliana* eggs and young larvae within a tree is roughly uniform, with the exception that the top and bottom branch whorls have the lowest densities (Miller 1967). In the spring, overwintering larvae migrate extensively and become concentrated in the tops of trees. The aggregation of shoot moths in the upper crown during at least part of the life cycle seems to be universal among the species that have been studied.

**SAMPLING PROCEDURES**

The methods that have been used to sample shoot moth populations can be classified into two broad categories. Extensive sampling includes those methods that involve simple collecting or that provide only estimates of relative populations, while intensive sampling includes those methods that provide estimates of absolute populations (i.e. numbers of insects per unit area). Selection of an appropriate sampling method requires clearly defined study objectives. The simplest method which provides the necessary information to answer the proposed question(s) should be used. Since intensive sampling requires a substantial investment of resources, it can only be justified when extensive methods are judged to be inadequate to meet the project objectives.

**Extensive Sampling**

Extensive sampling methods can be used for most purposes when estimates of absolute population are not necessary. For example, they can be used to determine shoot moth occurrence in an area or stand, species composition of populations, stage of development, presence of parasites, degree of parasitism, response to environmental factors, or general level of infestation (e.g., high, medium, or low).

Some sampling objectives can be met by simply collecting a number of shoot moths or infested shoot tips from one or more stands of trees. Miller and Wilson
(1964) collected infested pine shoot tips from 22 stands in seven southeastern states to determine the species composition of shoot moth infestations. The same method has been used in northeast Georgia (Baer and Berisford 1975) and Maryland (Staines et al. 1984). Freeman and Berisford (1979) collected ca. 15,000 *R. frustrana*-infested loblolly pine shoots which they held in a rearing cage to determine the degree of parasitism, the species composition of the parasitoid population, and emergence time of parasitoids relative to adult moths. Jennings (1974) collected an arbitrary sample of *R. neomexicana* cocoons to determine the relationship between female pupal size and potential fecundity. The temporal and spatial restrictions on sample collection will depend upon the objectives of a particular study and the potential sources of variation that were discussed earlier.

Sex attractant-baited (pheromone) traps have been particularly useful for sampling pine shoot moths. They have been used to determine the natural geographic distribution of shoot moths, the suitability of potential host tree species (Sartwell et al. 1980), to monitor the spread of an introduced shoot moth (Daterman and McComb 1970, Daterman 1974), to determine the seasonal activity of shoot moths (Berisford 1974, Canals and Berisford 1981, Sower et al. 1982, Malinoski and Paine 1988), and to identify critical stages in shoot moth development (Canals et al. 1983). Webb and Berisford (1978) used pheromone traps to determine the effects of ambient temperature on the flight of *R. frustrana* males. Trap catches may also provide reliable start points for phenological models used to time insecticide applications for shoot moth control (Gargiullo et al. 1983a, 1984, 1985, Malinoski and Paine 1988).

Sampling for shoot moths often involves counting infested shoot tips as an index to population density. The external symptoms of a shoot moth attack are recognizable only for a limited amount of time which varies with the species of shoot moth and host pine. For all of the shoot moths, larval feeding is not readily apparent until the late instars when infested shoot tips show signs of reduced growth, abnormal growth, or turn yellow and then red if they are killed. An exit hole and swelling in the stem surrounding it provide evidence of a successful *Eucosma* attack for several years following infestation (Stevens and Jennings 1977). In contrast, loblolly pine shoot tips killed by *R. frustrana* may only be distinguishable for one to two months following attack because of rapid tree growth and shedding of the dead tissue. Consequently, shoot moth damage should be assessed during late larval and pupal stages of development or as soon after adult emergence as possible. Pheromone traps provide a reliable method of distinguishing the end of the pupal stage and the beginning of adult activity.

Assessing shoot moth damage caused by multivoltine species involves some special considerations. Timely sampling is essential to clearly distinguish damage caused by different shoot moth generations. Fast growing trees shed shoot moth-killed tips soon after adult emergence, while slow growing trees retain dead tips which quickly become indistinguishable from tips killed by previous shoot moth generations. Therefore, sampling at only one time late in the season may considerably underestimate damage.

Since the number of growing shoot tips on young pine trees increases exponentially with increasing height (Lashomb et al. 1980), the time required to inspect all tips on a tree for shoot moth infestation is prohibitive, except on very small seedlings. Consequently, methods have been developed to reduce the time required to assess shoot moth damage on individual trees. Fox and King (1963)
proposed using counts of infested leaders, or lateral shoots in the uppermost whorl of branches, or both to assess *R. frustrana* infestations. They found that leaders alone were a better predictor of infestation in the top level of the tree than were laterals alone. They calculated that inspecting only leaders, as opposed to leaders and first whorl laterals, would result in an 80% savings of time with only a 10% loss in accuracy.

Berisford and Kulman (1969) subsequently showed that *R. frustrana* infestation in the top whorl of loblolly pine was not a good predictor of infestation in the whole tree. However, Stephen and Wallis (1978) found that the number of infested tips in the top whorl was a good predictor of total infested tips on a tree, if the trees were less than three years old and tip moth density was low. Hedden (1979) found a strong correlation between the percentage of infested leaders per plot and the percentage of all shoots that were infested, although the correlation decreased with increasing stand age. He also found that estimates of percent infested shoots per plot based on a systematic subsample of one branch per whorl agreed closely to those obtained from counts of tips on the entire tree. Percent injured or infested leaders and percent injured or infested trees (at low population densities) were both good predictors of whole tree *R. buoliana* infestations in red pine (*P. resinosa* Ait.) stands (Miller 1967). In some cases, tree height improved the relationship because of its correlation to total number of shoot tips per tree.

Several sampling programs have been developed to estimate relative *R. frustrana* infestations in pine plantations. Hedden (1979) proposed using the percentage of infested leaders as determined on 200-400 trees along two randomly located transects to rank plantations by relative infestation. Andersen et al. (1984) used a sequential sampling technique to estimate, with a predetermined level of precision, the percent of the total shoot tips infested by *R. frustrana* in loblolly and shortleaf pine stands. They suggested that the variation in estimates derived by their method could be reduced to acceptable levels by stratified sampling based on tree size, more clearly distinguishing new and old attacks, and sampling to include more trees per setting with subsampling of the shoots on the trees. Sower et al. (1984) recommended using sex attractant-baited traps for preliminary assessments of *E. sonomana* populations over large areas and visual assessments of infested terminals for surveying populations in specific plantations.

**Intensive Sampling**

Some of the methods used to estimate relative population density of shoot moths can be extended to provide estimates of absolute population density, but only for late larval and pupal stages (Gargiullo et al. 1983b). To generate estimates of absolute population density by these methods, information on stand structure and the number of shoot moths per infested tip is required. Radiographic techniques have been used as an alternative to dissection of infested tips to determine the number of shoot moths present (Yates 1967c, Stephen and Wallis 1978, Andersen et al. 1984). Stephen and Wallis (1978) published a regression equation to estimate the number of *R. frustrana* immatures per tree from the number of apparently infested tips per tree. However, the validity of using this equation for sites and stands other than those for which it was developed has not been determined.

Miller (1967) used the concept of an equivalent-tree to estimate *R. buoliana* population densities in Michigan. Beginning with a randomly chosen tree, single
branches were collected from successive trees in a row such that a group of
branches would represent a typical tree in the stand. The numbers of shoot moths
found on individual branches were summed to estimate the mean number per tree.
This technique was used to disperse the effects of destructive sampling and to
account for inter-tree variation. A disadvantage of this method is that the variance
between trees cannot be estimated, since equivalent-trees are composed of a
number of individuals. Also, the time required to inspect an entire tree for shoot
moths, particularly eggs and young larvae, may prohibit the use of this method
except with very small trees.

A two-stage cluster sampling technique to estimate the absolute density of any
immature stage of Rhyacionia spp. was developed and used to construct life tables
1983b). This technique involves the random sampling of shoots on randomly
selected trees within a stand. The shoots must be carefully inspected for all life
stages of Rhyacionia spp. The resulting data are entered into a FORTRAN
program which calculates numbers of immatures in each life stage per shoot, per
tree, and per area (if necessary stand density data are available) along with
variances for each estimate. The program also calculates the optimum sample size
needed to estimate the mean numbers of immatures per tree within a desired level
of precision. This is the only technique currently available to precisely estimate
absolute population density for shoot moth immatures.

CONCLUSIONS

Many different methods are available to sample shoot moth populations. The
sampling method that is most appropriate for a particular study depends upon the
information desired and the resources available for sampling. During the design of
a sampling program, all of the possible sources of variation in shoot moth
distribution should be considered. Failure to account for major sources of variation
could result in unreliable or misleading conclusions. In all cases, the simplest
method that provides the desired information to meet the study objectives should
be used.

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