Introducing *Pandora neoaphidis* (Zygomycetes: Entomophthorales) Into Populations of *Myzus persicae* (Homoptera: Aphididae) on Flue-Cured Tobacco

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**ABSTRACT**

The entomophthoralean, *Pandora neoaphidis* (Remaudière & Hennebert) Humber, was successfully introduced into populations of the tobacco-adapted form of the green peach aphid, *Myzus persicae* (Sulzer), on flue-cured tobacco in the field and in the greenhouse. In the field study, the pathogen was introduced three ways: 1) as a combination of infected live aphids and sporulating cadavers of fungus-killed aphids, 2) infected live aphids alone, and 3) triturated cadavers of fungus-killed aphids. These treatments were compared with an untreated control. Two weeks after introduction, 33–37% of the aphids in plots treated with infected live aphids alone or in combination with aphid cadavers were killed or had symptoms of infection compared with 2% infection in untreated control. Infection was only 6% in those treated with the triturated aphid cadavers and it was not significantly different from the infection in untreated control. However, the *P. neoaphidis* treatments had little impact on aphid populations. In the greenhouse experiment, releases of infected live or cadavers of fungus-killed aphids were equally effective in introducing the pathogen into aphid populations. Although infections progressed up to 21% at 2 weeks after introduction, *P. neoaphidis* did not reduce aphid populations. This study demonstrates that artificial introduction of *P. neoaphidis* establishes the pathogen in aphid populations. Such an introduction early in the season might aid in preventing aphid population build up.

**KEY WORDS** Aphid, artificial introduction, entomophthorales, microbial control, tobacco

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1A tobacco-adapted form of the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae), is a major insect pest of tobacco reducing the yield and quality of the cured leaf (Mistric & Clark 1979, Cheng & Hanlon 1985, Reed & Semtner 1992). Aphid damage caused by premature yellowing and necrosis of leaves reduces the yield by 10–30% (Dominick 1949, Reed & Semtner 1992). Sooty mold development on honeydew secretions further reduces the quality of tobacco leaves (Dominick 1949, Kulash 1949). In addition to the direct damage to the plant, *M. persicae* also transmits disease-causing viruses such as tobacco etch

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Materials and Methods

Field experiment. The field experiment was conducted on the regrowth of 'K-326' flue-cured tobacco at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, Virginia, in 1993. Experimental tobacco was transplanted on 20 May and the stalks were cut back to approximately 15 cm high in mid July to produce a ratoon crop. Excess secondary shoots were removed from the ratoon, and regrowth was allowed from a single axillary leaf bud. Normal and ratoon tobacco are similar in their physical and chemical characteristics as well as the abundance of different pests (Jackson et al. 1987). The experiment was initiated on 13 August when the regrowth was 45–60 cm high and the plants were naturally infested with populations of M. persicae that were building up. Before the treatments were applied, aphids in the test plots were checked thoroughly and appeared to be free of natural infections of P. neoaphidis or other entomopathogens.

Pandora neoaphidis was introduced into aphid populations in three forms: triturated (powdered) cadavers of fungus-killed aphids, infected live aphids, and a combination of infected live aphids and sporulating cadavers of fungus-killed aphids on tobacco leaf fragments. The three treatments and an untreated control were arranged in a randomized complete block with four replications. Each plot had a 6-m long single row of approximately 12 tobacco plants with two untreated buffer rows on either side spaced 1.2 m apart. Treatments were applied to five randomly selected plants within each plot between 1700 and 1830 h on 13 August. Plants were sprayed with water (700 L/ha) just before administering the treatments so that the triturated and whole cadavers of the fungus-killed aphids would adhere to the leaf surfaces and to promote favorable conditions for sporulation and conidial germination of the pathogen.

To prepare the triturated form of P. neoaphidis, cadavers of fungus-killed aphids were collected from natural infections in the source field. Aphid cadavers were air-dried and ground by sieving through a No. 45 strainer (355-μm opening) within 72 h of application. As the P. neoaphidis multiplies and invades host tissues, infected aphids turn pale or pink before they die and turn golden brown
when they die. These two stages are easy to identify. Infected live aphids and cadavers of fungus-killed aphids were collected in a tobacco field located approximately 1 km from the test site. The two fields were separated by meadow and woodland, and prevailing winds were generally from the southwest across the test field toward the source field.

Triturated aphid cadavers were sprinkled by hand at 10 mg/plant over the bud and the four upper leaves of each plant being treated. A camel’s hair brush was used to release infected live aphids on the four upper leaves at 10 aphids/plant. Leaf fragments (approximately 5 cm²) with cadavers of fungus-killed aphids and infected live aphids at 10 aphids/plant were placed on the upper leaves of the plants being treated. Aphid numbers and infection (cadavers and live aphids showing symptoms of infection) were monitored at 2- to 3-day intervals on five plants per plot for about 3 weeks after the test was initiated. On each observation date, five plants were randomly selected in each plot, and aphids and their infection was checked on each leaf of those plants.

Weather data were obtained from a weather station (Omnidata International, Inc., Logan, Utah) located about 0.5 km from the test site. The temperature and relative humidity were measured with a PCRC-11 humidity transducer mounted in an ES-110 sensor (Omnidata International, Inc.). Leaf wetness was measured with an ES-460 leaf wetness sensor (Omnidata International, Inc.).

**Greenhouse experiment.** The greenhouse experiment was initiated on 11 November 1994 with fungus-killed aphids (40/plant) and infected live aphids (40/plant) as treatments along with an untreated control. Each treatment had a 4-week-old, single potted plant of ‘K-326’ flue-cured tobacco replicated four times. Temperature in the greenhouse was variable and maintained at a minimum of 15°C. Plants were arranged in two rows with 0.9 m spacing within rows and 1.2 m between rows. Treatments were arranged in randomized complete block design. Plants were artificially infested with laboratory colonies of *M. persicae* that were allowed to build up for approximately 2 weeks before the treatments were administered. Fungal inoculum, in vivo, was obtained by exposing aphids to the conidial showers from *P. neoaphidis* cultures (Dara 1995). Aphids were placed on a tobacco leaf disc in a 60 × 15-mm petri plate. A sporulating culture of *P. neoaphidis* on Sabouraud maltose egg-milk-agar medium in a 90 × 15-mm petri plate was inverted over the aphids for 4 h to expose aphids to conidial showers from the cultures. Infection was allowed to develop and infected aphids were used as the source of inoculum for the greenhouse release. Infected live aphids and sporulating aphid cadavers were placed on the plants using a camel’s hair brush. A mist system was set up to mist water on the plants for 15–20 sec every 20 min throughout the day. The number of aphids and level of infection were monitored at 2- to 5-day intervals for about 3 weeks after the test was initiated. On each observation day, the number of healthy and infected aphids (cadavers and live aphids showing symptoms of infection) on the entire plant was estimated by checking each leaf.

**Statistical analysis.** Data from each date were analyzed using PROC ANOVA (SAS Institute 1987). The average number of aphids per plant and the proportion of those infected by *P. neoaphidis* were obtained. Aphid numbers were transformed to $\log_{10}(x + 1)$ and the percentages of infection were transformed to arcsine before analysis and significant means were separated using Waller-Duncan k-ratio t-test (k-ratio = 100).
Results

Field experiment. Tobacco treated with infected live aphids alone and in combination with sporulating aphid cadavers had significantly higher \((P < 0.05)\) proportion of infected aphids than those treated with triturated cadavers of fungus-killed aphids or untreated control on 10 and 17 d after treatment (DAT; Fig. 1A). Similar trends were observed at 12, 14 and 20 DAT. Infection by \textit{P. neoaphidis} was first observed in all treatments including possible coincidental natural infection in the untreated control 4 DAT. Level of infection gradually increased in all plots until 17 DAT. The maximum levels of infection resulting from the release of infected live aphids alone and in combination with fungus-killed aphids were 37.2 and 32.2%, respectively. Application of triturated aphid cadavers resulted in a maximum of 6.4% infection, whereas the highest level of infection in untreated control was 2.2%, which were not significantly different from each other throughout the experiment. However, none of the \textit{P. neoaphidis} treatments had significant effect \((P < 0.05)\) on aphid numbers throughout the observation period (Fig. 1A). Aphid populations increased from 550 aphids/plant on 1 DAT to 1100 aphids/plant by 7 DAT.

Greenhouse experiment. The introduction of \textit{P. neoaphidis} did not reduce aphid populations, although infection levels progressed in aphids (Fig. 1B). Infections in aphid populations were seen by 4 DAT. Except for a slight decrease on 9 DAT, the level of infection gradually increased toward the end of the observation period. Both fungal treatments had significantly \((P < 0.05)\) higher levels of infection than untreated control after 4 DAT. However, the number of aphids in treated and control plants was similar \((P > 0.05)\) and aphid populations on treated plants had almost doubled by 19 DAT.

Discussion

Weather conditions during the field study were hot and dry. Less than 2 mm of precipitation fell between 1 and 18 DAT and about 6 mm during the last two observation dates. Daily temperatures during the study averaged from 23 to 29°C with the maxima between 31 and 39°C and the minima between 17 and 23°C. The pathogen became established at moderate levels under the prevailing conditions. Periods of high humidity (>90%) are required for sporulation and spread of \textit{P. neoaphidis} in the field (Rockwood 1950, Yu et al. 1995). Periods of leaf wetness for 4 h or longer probably helped the sporulation and germination of conidia. The duration of leaf wetness averaged 6.8 h/night over the study period and ranged from 0 to 13 h/night. The relative humidity was >90% for an average of 5.3 h/d and 15 of the 20 nights had 5–13 h/night of >90% of relative humidity. About 3 h after the application of the treatments on 13 August, the relative humidity was >80% for 13 h (>90% for 1 h) and the leaves were wet for 12 h. Optimum temperatures for conidial production and germination of \textit{P. neoaphidis} were 10–25°C (Yu et al. 1995) and 18–21°C (Morgan et al. 1995), respectively. Formation of appressoria, infective structures formed before the fungus penetrates the host cuticle, was highest at 20 than at 13°C (Dara & Semtner 1998). Minimum temperatures ranged between 17 and 22°C during the observation period in our study. They were between 18 and 19°C for the first 3 days. These initial conditions probably
Fig. 1. Development of artificially introduced *P. neoaphidis* in populations of the tobacco-adapted form of the green peach aphid on flue-cured tobacco in the field, 1993 (A) and the greenhouse, 1994 (B). Number of aphids and proportion of infection (live with symptoms of infection + fungus-killed aphid cadavers) were indicated by lines and bars, respectively. Bars followed by the same letter, at each observation day, are not significantly different (Waller-Duncan k-ratio *t*-test, *k*-ratio = 100).
enabled the pathogen to sporulate and germinate to infect native aphids in the treated plots.

The low incidence of infected aphids in the plots treated with the triturated aphid cadavers might have been related to the lower rates of sporulation and the inability of the pathogen in this form to develop under prevailing weather conditions. The application of triturated aphid cadavers was less successful than that reported in other studies (Wilding et al. 1986, Wilding et al. 1990). Although releasing the infected live aphids has the advantage of those aphids moving around and settling among the native aphids, the triturated form of inoculum, applied to the adaxial leaf surfaces, might be less effective as the persistence of fungal inoculum is adversely affected by the UV light (Inglis et al. 1995, Morley-Davies et al. 1995, Braga et al. 2002). Additionally, we used 10 mg/plant (65 g/ha) of triturated fungus-killed aphids compared with 1.15 kg/ha used by Wilding et al. (1986).

Coincidental natural incidence might have been responsible for natural infection in untreated plots in the field. However, it was significantly lower than the infections in plots treated with sporulating aphid cadavers and/or infected live aphids. Although aphid populations decreased in all plots toward the end of the observation period, *P. neoaphidis* infection did not appear to be responsible for this reduction. Migration of the aphids, as the end of the tobacco season was approaching, might have caused the reduction in aphid populations. In addition, the pathogen was unable to prevent the build-up of aphids after the initial decrease in the greenhouse trial. The number of aphids on treated plants and controls was similar (*P* > 0.05) throughout the observation period. Wilding et al. (1986) reported similar results from treating the black bean aphid, *Aphis fabae* Scopoli (Homoptera: Aphididae) with *P. neoaphidis* and an insecticide on *Vicia* beans. However, artificially introducing *P. neoaphidis* had established infection in aphids in the greenhouse experiment also. Infection in aphids from treated plants spread to those on untreated plants, but the latter had significantly lower proportions of infected aphids after 4 DAT.

Moderate levels of *P. neoaphidis* infection were established in populations of *M. persicae* in experimental plots treated with sporulating aphid cadavers and/or infected live aphids. Although *P. neoaphidis* does not seem to be a practical alternative to chemical control because of its slow rate of spread and high dependence on environmental conditions and host densities, it causes high mortality in aphid populations when conditions are favorable. Therefore, *P. neoaphidis* warrants consideration in designing pest management practices for tobacco, especially for organically grown tobacco for which no effective insecticides are available. Introducing the pathogen early in the season could help it to become established earlier and reduce the potential for aphid infestations. This will help reduce the usage of chemical insecticides and promote natural enemy populations that would otherwise be affected by insecticides. Limited information is available on formulation of *P. neoaphidis* for field application. Formulation of *P. neoaphidis* in alginate matrix was less effective in greenhouse and laboratory trials compared with unformulated fungal applications (Shah et al. 2002). Additional research is needed to find better ways to introduce the pathogen and to determine if the pathogen can be established in aphids in one part of a field, spread to suppress infestations and to reduce the number of foliar insecticides required for aphid control.
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References Cited


