Effects of Imidacloprid Seed Treatment of Corn on Foliar Feeding and *Erwinia stewartii* Transmission by the Corn Flea Beetle

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ABSTRACT

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The effects of imidacloprid seed treatment (a systemic insecticide) on corn flea beetle leaf feeding and transmission of *Erwinia stewartii* to corn were studied in greenhouse experiments. Seed of corn inbred A632 was treated with imidacloprid at 6.0, 3.0, 1.5, or 0 g a.i./kg seed and planted in 15-cm pots. Corn flea beetles were allowed to feed on *E. stewartii*–infected corn plants for 9 to 10 days and were transferred to insect cages containing the 2- or 3-week-old seedlings grown from treated seeds. Beetles were allowed to feed on the treated plants for 2 to 4 weeks. Flea beetle feeding damage, Stewart's disease symptoms, *E. stewartii* infection (detected by enzyme-linked immunosorbent assay), and plant growth were evaluated. Imidacloprid seed treatment at 6.0 and 3.0 g a.i./kg seed significantly reduced the total number of flea beetle feeding scars, the number of feeding scars >3 mm in length, the number of leaves with Stewart's disease symptoms, and the number of plants infected by *E. stewartii*, compared with the control plants. Results indicate that imidacloprid seed treatment at ≥3.0 g a.i./kg seed can be an effective control practice for Stewart's disease in young corn plants.

Additional keywords: *Pantoea stewartii,* Stewart's bacterial wilt

Stewart's disease of corn (*Zea mays* L.), or Stewart's bacterial wilt, incited by *Erwinia stewartii* (*Pantoea stewartii* subsp. *stewartii*) is a wilt and leaf blight disease. It caused major losses to the corn crop in North America in the 1930s (19), but only sporadic outbreaks now occur on dent corn. Effective control of the disease can be attributed to the incorporation of disease resistance into modern corn hybrids (2,12, 19). Some sweet corn hybrids can still suffer substantial damage on a regular basis; in 1995, Stewart's disease caused heavy losses even in moderately resistant sweet corn hybrids in Illinois (18). The causal organism is seedborne (8,11,22,25) but recent research indicates that seed transmission of the pathogen is extremely rare (3, 4). *E. stewartii*, however, remains on phytosanitary regulations for many countries throughout the world and assurance that seed lots are free of this pathogen is an economic concern for seed corn companies involved in export markets. Outbreaks of the disease in 1990 and 1992 caused substantial losses to the seed corn industry in Iowa (C. A. Martinson, personal communication).

It has long been recognized that the corn flea beetle (*Chaetocnema pulicaria* Melsh.) is the most important inoculum source and vector of *E. stewartii* (8–10, 19–22). Various control strategies have been directed at the insect vector. Disease forecasting can be based on estimates of *C. pulicaria* survival derived from a winter temperature index (5,6,26). Spread of the disease can be reduced substantially by foliar insecticides (27), but this strategy is rarely economical and spray timing is difficult to optimize. Systemic soil-applied insecticides have been shown to be effective for a limited time (1,7,24).

Imidacloprid, a chloronicotinyl insecticide, has been shown to have contact and systemic activity against a wide range of insects, primarily members of Orders Hemiptera and Homoptera, but including Coleoptera, Diptera, Hymenoptera, Isoptera, Lepidoptera, and Orthoptera (17). Many of these insects are vectors of plant diseases. Imidacloprid seed treatment has been effective in reducing insect feeding and/or disease transmission by insects on barley, cotton, oats, rice, sorghum, sugar beet, wheat, and other crops (13,14,17). Its activity in the plant can last up to several weeks (17).

The sporadic occurrence of Stewart's disease and its impact on international exchange of corn seed justifies the need for additional approaches to managing the disease. The extended protection provided by imidacloprid in contrast to that of other seed-applied insecticides may increase imidacloprid's potential as a useful deterrent to flea beetle feeding and subsequent *E. stewartii* infection of corn. The objective of this research was to determine the effect of imidacloprid seed treatment on corn leaf feeding and transmission of *E. stewartii* by the corn flea beetle.

MATERIALS AND METHODS

Infected source plants. Nontreated seeds of inbred corn (A632) were planted in 15-cm-diameter pots placed in a greenhouse. This inbred is highly susceptible to Stewart's disease; it was the most susceptible inbred of 49 inbreds evaluated in 1992 by the North Central Regional Plant Introduction Station in Ames, IA (3). Plants were watered and fertilized as needed. Greenhouse temperatures ranged from 20 to 30°C during the experiment. Natural light was supplemented by high-intensity sodium greenhouse lights for 14 h/day. *Erwinia stewartii* was grown for 4 days on yeast extract–dextrose–calcium carbonate (YDC) agar (15). Plants, 2 to 4 weeks old, were inoculated by wounding leaves (two per plant) 5 to 10 times with a sterile needle and flooding the wounded area with a suspension of *E. stewartii*. The concentration of bacterial cells was approximately 2.0×10^8 cells/ml for experiment 1. For experiment 2, plants were inoculated twice, 4 days apart, with bacterial concentrations of approximately 2.5×10^8 cells/ml and 5.0×10^8 cells/ml. Cell concentrations were determined by optical density at 600 nm, compared with a standard curve developed for *E. stewartii*. Inoculated plants were then placed in 76 × 76 × 91 cm insect cages with 42 × 42 mesh (Lu-Mite Division of Synthetic Ind., Gainesville, GA) in the greenhouse. Caged plants were watered by a drip irrigation system.

Infestation of corn flea beetles with *E. stewartii*. Corn flea beetles were collected from natural populations on corn plants in research plots in Champaign County, IL, on 26 and 27 August 1994 for use in experiment 1. The plots contained field corn and sweet corn exhibiting symptoms of Stewart's disease. Additional beetles were collected from oat plants in research plots in Washington County, IA, on 14 September 1994 for use in experiment 2. These plots were surrounded by a large area of hybrid dent corn, but no Stewart's disease

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symptoms were observed. Beetles were collected by sweep net, aspirated by mouth into 125-ml flasks, and stored in a cooler until they were released into insect cages containing corn plants that had previously been inoculated with an isolate of *E. stewartii* as described above. Beetles were allowed to feed on infected plants for 9 to 10 days to acquire the bacterium. In experiment 1, plants were inoculated with *E. stewartii* on the same day the beetles were released in the cage. In experiment 2, plants were inoculated 8 and 12 days before beetle release and plants were showing symptoms of Stewart's disease at the time of beetle release.

Imidacloprid-treated plants. Corn seed (A632) was slurry treated with imidacloprid (Gaucho 480FS [50% active ingredient], Gustafson, Inc., Dallas, TX) in a Gustafson BLT Batch Lab Treater, at the rate of 6.0, 3.0, or 1.5 g a.i./kg seed for treatments 1, 2 and 3, respectively. Control seed (treatment 4) was treated with water only. Seeds were planted in 15-cm-diameter pots (one seed per pot) and grown in a greenhouse to growth stage V3 (23) (experiment 2) or V5 (experiment 1) before beetle infestation. Plants were then placed in insect cages described above. The experimental design was a randomized complete block with four treatments and three blocks (total of 12 insect cages). Each cage contained 10 plants grown from seed treated with a single rate of imidacloprid (or with water) and 10 plants grown from nontreated seed intermixed randomly in the cage. The plants from nontreated seed were included in each cage to provide a feeding alternative for the insects. In this study, control plants refer to those grown from seed treated with water only; nontreated plants refer to those grown from nontreated seed. Treated plants refer to those grown from seed treated with imidacloprid or water. Corn flea beetles were removed from the infected source plants by aspiration and 50 beetles were released into each cage containing imidacloprid-treated plants in both experiments. In experiment 2, only 40 beetles were released per cage in one replicate due to insufficient beetle numbers. Plants were incubated with the beetles for 2 weeks for the first experiment and 4 weeks for the second experiment. At the end of the feeding period, plants were removed from the cages and placed on a greenhouse bench for 1 week to allow symptom development. Feeding damage, determined by number and size of feeding scars, was evaluated on each plant. Feeding scars were rated as large if they were >3 mm long. The number of leaves per plant and the number of leaves with Stewart's disease symptoms also were recorded for each treatment. Symptoms appeared as yellow, purple, or water-soaked streaks surrounding the feeding scars.

Symptom diagnosis was confirmed by enzyme-linked immunosorbent assay (ELISA). One leaf was selected from each plant. Symptomatic leaves were tested whenever symptoms were present; otherwise, leaves with flea beetle feeding scars were chosen. The entire leaf was ground and extracted; the extract was tested for *E. stewartii* by ELISA (16), using a commercial detection kit (AgDia, Inc., Elkhart, IN). The mean number of infected plants per treatment, as determined by ELISA, was recorded.

The effects of imidacloprid rate on the total number of feeding scars, the number of large feeding scars, total number of leaves, number of leaves with symptoms, and the number of plants testing positive for *E. stewartii* by ELISA were determined by analysis of variance and mean separation was performed with the procedures of SigmaStat Statistical Software (Jandel Scientific, San Rafael, CA). Data for treated plants (including controls) were analyzed separately from data for nontreated plants.

RESULTS

Beetle feeding caused typical feeding scars and transmission of *E. stewartii* (determined by visual Stewart's disease symptoms and ELISA results) in both experiments. Plants grown from seed treated with imidacloprid at 6.0 or 3.0 g a.i./kg seed had significantly fewer flea beetle feeding scars, fewer large feeding scars, and fewer leaves with symptoms than control plants grown from seed treated with water only. Frequency of *E. stewartii* transmission also was reduced significantly by imidacloprid treatment. In the first experiment, the 1.5 g a.i. rate of imidacloprid significantly reduced beetle feeding damage, Stewart's disease symptoms, and *E. stewartii* infection. Treatment had no detectable effect on plant growth or number of leaves per plant (Table 1). Similar results were obtained in both experiments, but fewer feeding scars occurred in experiment 2, in which the plants were at an earlier growth stage at the beginning of the experiment. It is likely that the concentration of imidacloprid was higher in these plants. Block effects were not significant.

The plants from nontreated seed had more feeding damage and Stewart's disease symptoms than did the plants from treated seed. Among the nontreated plants, those placed in cages with plants treated with 3.0 or 6.0 g/kg imidacloprid suffered less feeding damage and disease than those in cages with the control plants, but the differences were not significant in most cases (Table 1).

ELISA confirmed the presence of *E. stewartii* in all leaves with symptoms that were tested (Table 1). Some asymptomatic leaves with flea beetle feeding scars reacted positively for presence of the bacterium.

Table 1. Flea beetle feeding and transmission of Stewart's disease in corn plants grown from seed treated with several rates of imidacloprid

	Experiment 1					Experiment 2				
Imidacloprid treatment[t]	Total feeding scars[u]	Large feeding scars[v]	Leaves per plant	Leaves with symptoms[w]	Plants infected[x]	Total feeding scars[u]	Large feeding scars[v]	Leaves per plant	Leaves with symptoms[w]	Plants infected[x]
1	193.7 a[y]	73.7 a	7.2 NS	0.0 a	0.7 a	41.3 a	30.1 a	6.1 NS	0.0 a	0.0 a
2	126.3 a	71.0 a	7.1	0.0 a	0.7 a	89.0 a	56.0 a	6.4	1.0 a	0.7 a
3	327.3 b	170.3 a	6.9	2.3 a	2.3 a	308.0 b	262.7 b	6.2	4.3 b	2.3 a
Control	506.3 c	364.7 b	7.1	3.0 b	4.7 b	341.0 b	279.7 b	6.6	3.7 b	5.0 b
Nontreated[z]										
With treatment 1	327.7 NS	222.3 NS	7.1 NS	1.7 NS	2.3 ab	144.3 NS	71.0 NS	6.1 NS	2.3 NS	2.3 NS
With treatment 2	269.3	210.7	7.0	0.3	1.0 a	183.5	155.2	6.7	1.3	1.7
With treatment 3	393.7	255.0	6.9	4.3	4.3 b	282.0	229.3	6.2	4.3	2.0
With Control	439.3	345.7	6.9	3.7	4.7 b	333.0	276.0	6.3	2.7	3.0

[t] Treatment 1 = 6.0 g a.i./kg seed; treatment 2 = 3.0 g a.i./kg seed; treatment 3 = 1.5 g a.i./kg seed. Control seed treated with water only.
[u] Number of feeding scars per 10 plants (mean of three replications of 10 plants each)
[v] Large feeding scars were >3 mm long.
[w] Number of symptomatic leaves per 10 plants (mean of three replications of 10 plants each)
[x] Mean number of plants positive for *Erwinia stewartii* of 10 per treatment per replication. Estimated by sampling one leaf per plant.
[y] Within a column, values followed by the same letter are not significantly different according to the Student-Newman-Keuls test ($\alpha = 0.05$); NS = treatment had no significant effect ($P > 0.05$).
[z] Nontreated plants were grown from nontreated seed and placed in cages with plants treated with the indicated imidacloprid rate.

DISCUSSION

Results indicate that imidacloprid can be an effective deterrent to flea beetle feeding and transmission of Stewart's disease by the corn flea beetle; imidacloprid is the first insecticidal seed treatment shown to reduce infection by insect-vectored plant pathogens (13,14). Seed treatment with this insecticide at 6.0 or 3.0 g a.i./kg seed significantly reduced flea beetle feeding, *E. stewartii* infection of leaves, and Stewart's disease symptoms. The bacterium was detected in some leaves without visible symptoms; in these leaves either the disease had not completed its incubation period, or the ELISA detected nonviable bacterial cells. Although *E. stewartii* transmission by flea beetles is not known to result in asymptomatic infection, there is an incubation period of 3 to 12 days before symptoms appear, depending on temperature and plant age (3,7). Imidacloprid seed treatment has potential advantages over previous insecticide treatments for control of corn flea beetles in the ease of application, reduced hazards, and possible longer period of protection (17). Foliar insecticide sprays also are less practical because of the need for multiple sprays. Soil-applied insecticides have been reported to cause mortality in beetles feeding on corn up to the four-leaf stage (7), but they are very rarely used to control the corn flea beetle. In the present study, plants were at stage V3 to V5 at the beginning of the experiments, and up to stage V7 at the conclusion of the experiments. The duration of activity was not determined in this study, but the insecticide clearly was effective at the beginning of both experiments (stage V3 or V5).

Reduced numbers of large feeding scars may be particularly important in prevention of *E. stewartii* transmission. Dill (7) reported that Stewart's disease transmission increased from zero to 36% when feeding scars ranged from 3 mm to 18 mm, and only when feeding scars were 9 mm or greater was disease transmission significantly greater than zero.

Mortality of flea beetles also is likely to play a role in Stewart's disease control by imidacloprid (17), although this could not be determined in this study. Beetle populations declined in all treatments including the control to less than 1 beetle per plant by the end of the experiments; mortality was likely due to other factors in addition to the insecticide. There were no significant differences among treatments for beetle survival at the termination of the experiments. Beetle mortality due to the insecticide may have occurred during the first few days, but the time of mortality could not be determined by beetle counts after 2 to 4 weeks, and it was not possible to evaluate beetle survival without removing plants from cages and terminating the experiments.

Feeding damage and Stewart's disease on the nontreated plants may provide some evidence for the effect of increased beetle mortality when the higher rates of imidacloprid were used. The purpose of including nontreated plants in the cages was to provide a feeding alternative for the beetles. In the field, *C. pulicaria* feeds on a wide range of hosts, including small grains and grassy weeds (18) that would not be treated with insecticides. Therefore, it would be unrealistic to provide only imidacloprid-treated plants as a feeding option in the experiments. Reduced feeding damage and Stewart's disease on the nontreated plants in cages with plants treated at the higher rates of imidacloprid may have been the result of increased beetle mortality in these cages due to the insecticide.

The rates of Stewart's disease transmission, measured by symptom development and ELISA results, were considerably lower than those reported in earlier studies in which 57 to 100% of plants became infected (8,22). This may have been due to a lower proportion of beetles carrying the bacterium in our experiments. Although the beetles had more than sufficient time to acquire the bacterium from the infected plants (7), many of them may have been feeding on healthy leaves during that time. Corn flea beetles will feed on a single leaf for at least 72 h if not disturbed. Even after feeding on infected tissue, some beetles do not acquire the bacterium (7).

Subsequent to this research, imidacloprid seed treatment has been reported to significantly reduce the incidence of Stewart's disease in field experiments (D. Berkey, personal communication). Further studies under field conditions with large natural populations of *C. pulicaria* are needed to confirm the effectiveness of imidacloprid seed treatment. This type of study is difficult to conduct because beetle populations in a specific field cannot be predicted accurately, and populations can fluctuate dramatically over short time periods.

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