Effect of Imidacloprid on Nonflight Movement of *Rhopalosiphum padi* and the Subsequent Spread of Barley Yellow Dwarf Virus

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**ABSTRACT**


The bird cherry-oat aphid (*Rhopalosiphum padi*) is an important vector of the barley yellow dwarf luteovirus, BYDV-PAV-IL. Insecticides used to reduce the abundance of the vector on small grains can result in an increase in aphid activity and a subsequent increase in disease incidence. The transmission characteristics of viruliferous wingless (nymphs and aperous adults) *R. padi* after access to oats treated with different rates of imidacloprid, a seed-treatment insecticide, were compared. After access to treated plants, aphid fecundity was reduced and aphids walked and fed atypically and often abandoned the host plant. The spread of BYDV from a focus of infestation to individual imidacloprid-treated oats planted in a grid was followed by observation of symptoms and by enzyme-linked immunosorbent assay. Aphids transmitted the virus to both treated and untreated plants, but the percentage of infected insecticide-treated seedings was one-half that of untreated seedlings.

Barley yellow dwarf luteoviruses (BYDV) cause the most economically important viral disease of cereals worldwide. They are phloem-limited plant pathogens obligately spread by several species of aphids (20). Five isolates of the virus have been identified that cause yellow dwarf symptoms in cereals and grasses.

Although pesticides can effectively control aphids, they may induce a change in aphid activity and toxicity may interfere with virus transmission efficiency (17). Insecticides, including synthetic pyrethroids, organophosphates, and carbamates, reduce the abundance of aphids on small grains (6,21,24,28), but aphids have developed resistance to some commercial insecticides (2,16,25).

Contact (pyrethroids) and systemic (organophosphates and carbamates) aphicides can enhance virus spread by stimulating aphid movement (13,14,23,30). Villacarlos (36) used the term “restless” to describe the behavior of the green peach aphid (*Myzus persicae* (Sulzer)) exposed to a range of systemic insecticides. Gabriel et al (15) and Rice et al (31) reported that organophosphates and carbamate aphicides can induce alarm pheromone release and trigger dispersal of aphids.

Insecticides with active ingredients belonging to the chemical class nitroguanidine are an alternative for the control of aphids, thrips, leafhoppers, leaf miners, and leaf beetles (3,10,12,34). Imidacloprid (Gaucho) is a nitroguanidine with both contact and systemic properties and is applied as a seed treatment that provides protection from the time of sowing until well into the growing period (27). The mechanism of action of imidacloprid differs from that of the organophosphates and carbamates, which are acetylcholinesterase inhibitors, and the pyrethroids, which act on certain nerve fiber membrane proteins. Like acetylcholine, a naturally occurring signal substance, imidacloprid stimulates certain nerve cells by acting on a receptor protein in the nerve fiber membrane (27).

Unlike acetylcholine, which is quickly degraded by acetylcholinesterase, imidacloprid is either not degraded or only slowly degraded. This prolonged action fatally disrupts the operation of the insect’s nervous system (27).

Because synthetic systemic insecticides induce atypical behavioral responses in cereal aphids (17,23,36), the effect of different concentrations of imidacloprid applied as a seed treatment on the behavior of the bird cherry-oat aphid (*Rhopalosiphum padi* (Linnaeus)) and the subsequent spread of BYDV were investigated.

**MATERIALS AND METHODS**

**Virus isolate and aphid vector.** In the midwestern United States, *R. padi* is the most important and efficient vector of the BYDV-PAV variant (7,8,11). Laboratory clones of *R. padi* maintained on barley (*Hordeum vulgare* L.) cv. Hudson infected with BYDV-PAV-IL were provided by the USDA Cereal Virolology facility at the University of Illinois at Urbana-Champaign. Late instar or aperous viruliferous adults infested with the BYDV-PAV-IL isolate were used.

The BYDV-PAV-IL isolate has been characterized (19).

**Laboratory study.** To examine the behavior of *R. padi*, aphids were allowed to feed on oat (*Avena sativa* L.) seedlings grown from seed treated with imidacloprid; two cultivars, Ogle and Don, and four rates of imidacloprid were used. Ogle is tolerant to BYDV-PAV (4,30) and Don is sensitive to BYDV-PAV (5). The rates of imidacloprid were 0.0 (control), 0.6, 1.2, and 1.8 g a.i./kg of seed. These rates will be referred to as 0X, 1X, 2X, and 3X. Late instars or adult aperous aphids were placed individually on treated or control plants at the three-leaf stage and confined with a small (1.5-cm-diameter) cylindrical plastic cage fitted with mesh windows for aeration.

The cage was clipped onto a portion of the second leaf and supported with a small stake. Observations were made hourly for 12 hr and then at 29, 32, 35, 38, 50, 78, and 83 hr after the aphids had been transferred to the plants. Observations determined if the aphids were alive, if they had reproduced, their position in the cage, and any atypical behavior. There were four replications for each combination of cultivars and chemical rates. Two plants per replication were included for each of the insecticide-treated plants, but during the experiment, some of the plants were accidentally destroyed and the total numbers of plants for insecticide treatments differ. The objective of this preliminary study was to plan the greenhouse study by determining approximately when aphids became restless after feeding on insecticide-treated plants.

**Greenhouse study.** The experiment was carried out in the Agronomy and Plant Pathology greenhouse of the University of Illinois using the oat cultivars Ogle and Don and the four rates of imidacloprid described above. Plants were grown in a greenhouse equipped with metal halide lamps operating on a 14-hr photoperiod with day and night temperatures of approximately 21 and 16°C, respectively. The experimental unit was a flat containing five rows of five plants, separated from each other by 8 cm. Immediately after seeding, each flat was caged and placed in a screenhouse to prevent any accidental infestation by stray insects. At the three-leaf stage, 10 aphids reared on Hudson barley infected with BYDV-PAV-IL were trans-
ferred to the center plant of each flat and confined with the cylindrical plastic cage described above. Results from the preliminary laboratory study indicated that some aphids ceased to feed on the insecticide-treated plants and abandoned their host as quickly as 1 hr after having been caged, while other aphids fed continuously for 2-12 hr before abandoning their host plant (Tables 1 and 2). Therefore, for the greenhouse study, aphids were allowed 3 hr to become acclimatized, after which the small cage was carefully removed. Aphids present on the inner side walls of the cage were removed with a very small artist's brush and transferred to the base of the plant. The aphids were confined in each flat with a large cage and allowed to disperse from the infestation focus to neighboring plants. From the laboratory study (Tables 1 and 2), aphids that fed on insecticide-treated plants had an average life span of 5 days. Therefore, after 5 days, each flat was fumigated and moved to an aphid-free greenhouse for up to 3 wk to allow for symptom development.

Three weeks after infestation, each plant was scored for symptoms and a leaf sample was harvested and examined by triplicate antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) (9). The experiment was replicated five times, and each replication consisted of eight cultivar-insecticide combinations resulting in a randomized complete block design. A total of 1,000 plants were examined by TAS-ELISA. The negative threshold for the ELISA tests was established by taking the mean of the negative controls (from eight wells) plus four standard deviations. On the basis of the negative threshold, any plant sample showing an ELISA value exceeding the threshold was considered to be infected. An average distance (D) of aphid movement for each rate of insecticide was determined by calculating the total distance (sum of the distances, in rows, from the infestation focus to each infected plant in the flat) and dividing by the incidence (N, the number of infected plants). This formula, modified from Southwood (35), can be represented as 

\[ D = \frac{\Sigma d_i}{N} \]

where \( d_i \) = distance from the center of the infestation focus to the \( i \)th concentric zone of each plant. A relative efficiency of transmission for each rate of insecticide was determined by taking the total number of plants infected divided by the total number of plants offered to the aphids during the 5-day inoculation period. Statistical analyses were performed with the general linear model (GLM) procedure of SAS (32).

## RESULTS

### Laboratory study

During the first 12 hr, aphids allowed access to insecticide-treated plants ceased feeding and abandoned their hosts as quickly as 1 hr.

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### Table 1. Behavioral response of *Rhopalosiphum padi* on plants of the susceptible oat cultivar

Don treated with four rates of imidacloprid

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### Table 2. Behavioral response of *Rhopalosiphum padi* on plants of the susceptible oat cultivar

Ogle treated with four rates of imidacloprid

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4OX, 1X, 2X, and 3X indicate 0.0, 0.6, 1.2, and 1.8 g a.i./kg of seeds, respectively.

3Observations were made hourly for 12 hr and then at 29, 32, 35, 38, 50, 78, and 83 hr after the aphids were transferred to the plants.

Aphids were feeding, W = walking, or D = dead; 1 = any atypical behavior such as incapacitation.

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after they were caged (Tables 1 and 2). After the first 3 hr, most aphids were walking on the sides of the cage or on the screen mesh. The aphids walked atypically and were easily dislodged from their location with a light tapping on the cage. Aphid reproduction was very low and decreased with increasing rates of insecticide. Aphids placed on the cultivar Don produced a mean number of nymphs of 1 ± 2, 2 ± 4, and 2 ± 2 for the 1X, 2X, and 3X rates, respectively. The mean number of nymphs for the cultivar Ogle were 1 ± 1, 3 ± 3, and 1 ± 1 for the 1X, 2X, and 3X rates, respectively. In some cases, aphids deposited partially differentiated embryos. Aphids that fed on insecticide-treated plants had an average life span of 5 days (Tables 1 and 2). Aphids placed on control plants (not treated with imidacloprid) settled quickly and were presumed to be feeding throughout the entire observation period. They had a longer life span (>5 days) than aphids that fed on insecticide-treated plants (Tables 1 and 2). They also reproduced abundantly. The mean number of nymphs produced per adult during the observation period were 12 ± 4 and 11 ± 8 for the cultivars Don and Ogle, respectively. These means differed significantly (P = 0.05) from the mean number of nymphs produced on the insecticide-treated plants.

**Greenhouse study.** There was no significant difference among the two cultivars or cultivar*rate* interaction for the dependent variables total distance, average distance, and incidence (Table 3). However, a significant F test was found for rates of insecticide for total distance and incidence. Therefore, the data presented in Table 3 represent a combined analysis over cultivars for rates of insecticide. The comparison of means (LSD) indicated there were significant differences among control flats and insecticide-treated flats. The mean calculated total distance covered by the aphids and the mean incidence in the control flats always exceeded those of the insecticide-treated flats (P < 0.05). No significant difference was observed among the insecticide-treated flats. When the distances were averaged over the incidence, the four rates of insecticides were not significantly different (Table 3). There was a significant linear relationship (P = 0.05) with the rates of insecticide for the variables total distance and incidence. On the basis of the total number of plants infected for each rate of insecticide, the aphids in the control flats had the highest transmission efficiency. There were more infected plants in the control than in any of the insecticide treatments. Twenty-six percent of 240 plants were infected in the 0X treatment vs. 13, 13, and 10% for the 1X, 2X, and 3X, respectively (Table 3).

**DISCUSSION**

Aphicides may cause aphids to be restless and avoid treated foliage. This may result in movement of aphids to neighboring plants, which may become infected (1,23,36). The spread of the potato leafroll virus increased in disulfoton- and thiofanox-treated potato (Solanum tuberosum L.) crops infected with insecticide-resistant aphids (14). Lowery and Riceau (23), looking at the effects of five insecticides on the probing, walking, and settling behavior of *M. persicae* on potato, concluded that local movement of apterous *M. persicae* was significantly increased by exposure to the insecticides. Similar observations were reported in an evaluation of the effect of imidacloprid on *M. persicae* caged on leaves of sugar beet (Beta vulgaris L.) plants (10) and on *R. padi* and Sitobion avenue (Fabricius) on oat and barley seedlings (22).

In this study, aphids that fed on insecticide-treated plants became incapacitated to some degree after a feeding period of 3 hr. The aphids walked atypically, often dropping from the leaf when the cage was tapped lightly. These observations are similar to those of Gibson et al. (18), who examined the effects of the pyrethroid deltamethrin on the acquisition and inoculation of viruses by *M. persicae* and concluded that although deltamethrin initially stimulated walking, a period of paralysis and incapacitation rapidly followed. Rapid paralysis of the aphids would lower the incidence of disease in a field situation because secondary spread of the virus would be prevented.

In the field, *R. padi* has been found to disperse most frequently within rows and across the canopy where plants are close together and rarely between rows (29). In our greenhouse study, interplant spacing was wider than would be expected within the row in the field. In most cases, leaves from adjacent plants did not overlap sufficiently to allow movement across the canopy. The possibility that aphids traveled from one plant to another by moving from leaf to leaf cannot be excluded, since many plants were not isolated from one another during the inoculation access period and since by the end of the 5-day inoculation access period, the plants were substantially larger than when infested. Nevertheless, we suspect that most of the aphids that fed on the insecticide-treated plants dropped from the plants and dispersed from plant to plant on the soil surface. Montgomery and Nault (26), studying the dispersion of *Hyadaphis erysimi* (Kaltenbach) and *M. persicae* after physical disturbance, observed that aphids dropped from their hosts in response to alarm pheromones were more likely to move to an adjacent plant, while aphids that remained on the host tended to walk to another part of the same plant.

Bailey et al. (1) argued that apterous aphids are not morphologically specialized to travel great distances and generally move only a short distance from the host plant when disturbed. In our greenhouse study, the average distances measured for all insecticide treatments did not differ significantly, but fewer plants were infected in the insecticide-treated plants, indicating that the aphids traveled a greater distance from the infestation focus or the aphids spent more time moving and less time feeding than in the untreated plants. The late instar and adult apterae were able to infect plants located as far as 16 cm away from the infestation focus. Because virus infection was monitored, the distances measured in this study only reflect those plants that the aphids traveled to and fed on long enough to infect. For the insecticide-treated plants, aphids could have visited many more plants than the data indicate. If some plants visited by aphids were not inoculated, the total distances measured for the insecticide treatments may be different from the actual distances. Variability in the dose of insecticide in individual plants may also have influenced whether the aphids fed long enough to cause infection, but insecticide content of individual plants was not measured.

Results from these studies indicate that imidacloprid appeared to stimulate aphid movement and subsequent spread of BYDV-PAV-IL. Aphids transmitted the virus successfully to treated plants,
but the percentage of infected plants was significantly lower in the insecticide-treated seedlings than in the untreated seedlings. Although the insecticide interrupted feeding and increased activity, the rapid (within 4 hr) neurotoxic effect of imidacloprid apparently caused sufficient incapacitation of the vectors so that virus incidence in the oat cultivars was significantly reduced. Imidacloprid applied as a seed treatment to manage aphids in small grains may reduce incidence and spread of BYDV.

ACKNOWLEDGMENTS

We wish to thank G. R. Gregerson for his technical support and particularly for providing and maintaining the virus isolate and vectors throughout the experiment.

LITERATURE CITED