Effect of Insect Density, Plant Age, and Residue Duration on Acetamiprid Efficacy Against Swede Midge

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J. Econ. Entomol. 103(6): 2107–2111 (2010); DOI: 10.1603/EC10223

ABSTRACT The Swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae), a common insect pest in Europe, is a newly invasive pest in North America that constitutes a major threat to crucifer vegetable and field crops. Chemical control of Swede midge with synthetic insecticides under laboratory conditions indicated that insecticides generally could provide very effective control; however, insecticide treatments in the field were rarely able to maintain damage levels within marketable limits. In the current study, factors affecting insecticide efficacy were investigated using a neonicotinoid insecticide, acetamipird, as a foliar spray on cauliflower plants. Our results indicated that Swede midge density did not affect the efficacy of acetamirpid, although it significantly increased the subsequent Swede midge population on the unsprayed cauliflower plants. Additionally, cauliflower plant age did not significantly affect spray coverage and acetamipird efficacy on Swede midge. However, acetamiprid only provided 6-d control of Swede midge and its efficacy was reduced by up to 50% 9 d after spraying. Implications of our results on the development of an overall integrated pest management (IPM) program for Swede midge also are discussed.

KEY WORDS *Contarinia nasturtii*, acetamiprid, invasive species, spray coverage, insecticide residue

Swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae), is a gall-forming pest of plants in the family of Brassicaceae, including most cultivated cruciferous vegetables (e.g., broccoli, cabbage, cauliflower, Brussels sprouts, kale), cruciferous weeds, and canola (Barnes 1946; Stokes 1953a,b; Darvas et al. 2000; Hallett 2007; Chen et al. 2009a). As a common and endemic pest in Europe and southwestern Asia, Swede midge can severely reduce yield and marketability (Barnes 1946, Darvas et al. 2000). In many parts of Europe, Swede midge is considered a major pest with frequent crop losses in spite of regular chemical treatments (den Ouden et al. 1987). In June 2000, the first Swede midge in North America was discovered using yellow sticky traps in Ontario, Canada (Hallett and Heal 2001). Subsequently, Canadian researchers launched a Swede midge survey in cruciferous crops and found that Swede midge occurred in nine counties in Ontario and one county in Quebec in 2001. The number of infested counties in Canada increased from 18 in 2005 to 49 in 2006 and 65 in 2008, plus one location in Nova Scotia and three locations in Saskatchewan (Chen et al. 2009b). In the United States, Swede midge was first reported in Niagara County, NY, in September 2004 (Kikkert et al. 2006). By the end of 2005, Swede midge was detected in five major cabbage-producing counties in New York state. An additional 13 counties in New York and one each in Massachusetts, New Jersey, and Connecticut were reported having Swede midge based on molecular and morphological identifications (Chen et al. 2007, 2009a). To date, Swede midge has been detected in a total of 26 counties in New York, in addition to the three in surrounding states. An additional Swede midge infestation in Ottawa county, OH, was confirmed by our group based on Swede midge mitochondrial cytochrome oxidase subunit I gene (*COI*) sequence data (M.C. et al., unpublished data).

In the past 10 yr, Swede midge infestations in North America have spread rapidly and created a major threat to crucifer vegetable and canola farmers, especially in some areas of Canada. Crop losses due to Swede midge on some Canadian farms in Ontario have been reported to be as high as 85% (Hallett and Heal 2001). One of the first actions taken for immediate control of Swede midge and prevention of further spread in North America was to screen synthetic insecticides labeled for crucifer vegetables. Twenty insecticides belonging to 12 different classes were evaluated using foliar sprays, soil drenches or seed treatments against Swede midge adults and larvae under laboratory conditions and several of them provided excellent control (Wu et al. 2006, Chen et al. 2007). However, under field conditions in Canada, insecticide treatments were rarely able to maintain damage levels within marketable limits (Hallett et al.

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2009a). Low efficacy of synthetic insecticides in the field was initially assumed to be associated with insecticide resistance. However, Hallett et al. (2009a) found similar susceptibilities to synthetic insecticides between field-collected and laboratory-susceptible Swede midge colonies. Other possible reasons causing field control failure, such as spray coverage and high population pressure, have long been speculated; however, we are unaware of any data to validate such claims. To achieve better chemical control of Swede midge, factors affecting insecticide efficacy, such as pest density, plant age, spray coverage, and the residual life of insecticide, were evaluated on cauliflower plants in the current study.

Materials and Methods

Insect Culture and Tested Plants. A laboratory population of Swede midge was kindly received from R. Baur from Switzerland (Swiss Federal Research Station for Horticulture, Wädenswil, Switzerland) in 2004 and reared in a chamber under quarantine conditions at 22°C, 75–78% RH, and a photoperiod of 16:8 (L:D) h on cauliflower, *Brassica oleracea* variety *botrytis*, 'Snow crown' plants (Chen et al. 2007). Cauliflower seeds were planted in 128-cell trays, and 3-wk-old seedlings were individually transplanted into pots (13 cm in diameter) and maintained in Cornell University greenhouses for insect rearing and experimental use.

Insecticide. Acetamiprid, (*E*)-*N*1-[(6-chloro-3-pyridyl)methyl]-*N*2-cyano-*N*1-methylacetamidine) (Assail 30SG, Cerexagri, Inc., King of Prussia, PA), a neonicotinoid insecticide with systemic property, was used in the current study because it was the first labeled product for Swede midge control on cole crops in the United States (EPA no. 8033-36-82695) and was documented to be effective against Swede midge under laboratory conditions (Wu et al. 2006, Chen et al. 2007). We used the label rate of acetamiprid for Swede midge (34 g [AI]/acre), which converted to 8.4 mg (AI)/m^2 when sprayed onto the tested cauliflower plants.

Effect of Swede Midge Density on Efficacy of Acetamiprid. Cauliflower plants in pots (≈4 wk after transplanting) were individually placed into 50- by 50- by 50-cm wooden cages with netted sides in which Swede midge adults were allowed to lay eggs. To evaluate the impact of Swede midge density on the performance of acetamiprid, different numbers of Swede midge female adults, i.e., 3, 6, 9, 12, and 15 females per plant, were introduced into each cage. After 48 h of egg laying, cauliflower plants from different density treatment groups were removed and sprayed with acetamiprid at the label rate for Swede midge with 0.1% (vol:vol) Bond spreader sticker (Loveland Industry, Loveland, CO). Cauliflower plants sprayed with water and Bond (0.1% vol:vol) only were used as the control. There were four replicates of each treatment. Sprays were applied in a track chamber (Allen Machine Works, Midland, MI) by using a single nozzle (800 3VS) 50 cm above the plants and spraying at 40 psi and 3.2 km/h. After the plants were sprayed, they were put into larger wooden cages (90 by 90 by 90 cm) with netted sides and placed in a rearing chamber and watered as needed. The number of Swede midge eggs and larvae on each plant from the treatment and control group was counted 10 d after oviposition by dissecting the growing tips under a stereomicroscope.

Aside from the above-mentioned effect of acetamiprid on Swede midge eggs and larvae (i.e., introducing Swede midge adults for egg laying before spraying), the effect of acetamiprid on Swede midge adults (i.e., spraying before oviposition) also was investigated. To test this, cauliflower plants were sprayed with acetamiprid first in the chamber and air-dried at room temperature for 4 h and then placed into oviposition cages with 3, 6, 9, 12, and 15 females per plant. The experimental design, test conditions, and evaluation methods were the same as described for the eggs and larvae.

Effect of Plant Age on Acetamiprid Efficacy and Spray Coverage. Older plants have more leaves and bigger leaves that may inhibit coverage by creating protected areas for Swede midge larvae in the growing tips of the plant. To investigate the impact of plant age on acetamiprid efficacy and spray coverage, cauliflower plants at 15, 30, 45, 60, and 75 d after transplanting were used (Supp. Fig. S1 [online only]). Each plant was first inoculated with six Swede midge adults (four females and two males) for 48 h in an oviposition cage (50 by 50 by 50 cm) and then sprayed with acetamiprid at the label rate with 0.1% (vol:vol) Bond in the spray chamber. Cauliflower plants sprayed with water and Bond (0.1% vol:vol) only were used as the control. There were six replicates of each treatment. The number of Swede midge larvae on each plant from the treatment and control group was counted 10 d after oviposition by dissecting the growing tips under a stereomicroscope.

To assess the impact of plant age on spray coverage, yellow florescent dye (provided by South Australia Research and Development Institute, Loxton, Australia) was diluted with distilled water to a 1% solution (vol:vol) and sprayed with 0.1% (vol:vol) Bond on a separate set of cauliflower plants at 15, 30, 45, 60, and 75 d after transplanting. Plants sprayed with distilled water with 0.1% (vol:vol) Bond were used as the control. Spray coverage on different-sized plants was evaluated visually using a portable UV light (Labino AB, Solna, Sweden) in a dark room (Supp. Fig. S1 [online only]) and coverage was ranked on a five point scale, where 0 is no coverage, 1 is coverage ≤25% (of total leaf area), 2 is 25%< coverage ≤50%, 3 is 50%< coverage ≤75%, and 4 is 75%< coverage ≤100%.

Effect of Acetamiprid Residue on Swede Midge Mortality. Cauliflower plants at 3–4 wk after transplanting were sprayed with acetamiprid at the label rate in the spray chamber and then left outside in nylon cages (90 by 90 by 90 cm) to keep them insect free but to expose them to the environment, albeit in a somewhat protected fashion. The cages containing sprayed plants were left outside for 0, 3, 6, 9, and 12 d. The plants were brought back to the lab at the dif-

Table 1. Acetamiprid efficacy on swede midge when used as a foliar spray on cauliflower plants

Treatment	Swede midge density (female/plant)	No. live larvae/plant		No. dead eggs/plant	
		Acetamiprid	Control	Acetamiprid	Control
Oviposition before spray	3	0aB	77.3 ± 7.2cA	>100	0
	6	0aB	92.8 ± 13.6cA	>100	0
	9	3.3 ± 3.3aB	227 ± 21.7bA	>100	0
	12	0aB	401.3 ± 79.1aA	>100	0
	15	1 ± 1aB	417 ± 90.3aA	>100	0
Oviposition after spray	3	0aB	77.3 ± 7.2cA	16.25 ± 9.1aA	0aB
	6	0aB	92.8 ± 13.6cA	22.75 ± 13.6aA	0aB
	9	1 ± 1aB	227 ± 21.7bA	10.25 ± 5.3aA	0aB
	12	0aB	401.3 ± 79.1aA	22.75 ± 8.5aA	0aB
	15	0aB	417 ± 90.3aA	22 ± 5.4aA	0aB

Data in the table are means ± SE; means followed by different lowercase letters in a column or uppercase letters in a row for each parameter are significantly different based on GLM analyses and Fisher's protected LSD mean separation test, $P < 0.05$.

ferent time intervals (i.e., 0, 3, 6, 9, and 12 d) and inoculated with six Swede midge adults per plant (four females and two males) in oviposition cages for 48 h. There were four replicates of each treatment. Plants without acetamiprid spray were used as control. The plants were then transferred from the oviposition cages to larger wooden cages (90 by 90 by 90 cm) and placed in a rearing chamber and watered as needed. The number of Swede midge larvae per plant was checked 10 d after oviposition by dissecting the growing tips under a stereomicroscope.

Data Analysis. Data on pest density effect on acetamiprid efficacy and spray coverage of different sized plants were analyzed using GLM PROC in SPSS for Windows (SPSS Inc., Chicago, IL). The number of Swede midge larvae per plant in the plant age treatment and insecticide residue duration treatment also were analyzed using GLM PROC. All statistical calculations were performed using SPSS package, version 11.5, for Windows.

Results

Effect of Swede Midge Density on Efficacy of Acetamiprid. On the unsprayed cauliflower plants, the number of live Swede midge larvae/plant was positively correlated with adult Swede midge density (female adults/plant) initially introduced in the oviposition cages ($F = 3.877$; df = 4, 30; $P = 0.013$) (Table 1). The number of larvae per plant ranged from a mean of 77.3 when three females per plant were introduced to 417 when 15 females per plant were introduced. However, when cauliflower plants were sprayed 48 h after Swede midge had laid their eggs on the plants, almost no eggs hatched regardless of whether plants were inoculated with three or 15 Swede midge females per plant (Table 1). Thus, acetamiprid sprayed 48 h after oviposition provided effective control of Swede midge eggs and larvae regardless of the initial population density ($F = 41.205$; df = 1, 30; $P < 0.001$) (Table 1). Similarly, when the plants were sprayed 48 h before being exposed to ovipositing adults, the spray resulted in significantly higher numbers of dead eggs/plant and almost no live larvae compared with the control ($F = 43.307$; df = 1, 30; $P < 0.001$) (Table 1). When plants were sprayed before oviposition, a small number of dead eggs were found on the plants, suggesting that acetamiprid did not provide full control of Swede midge adults. However, these eggs failed to develop into viable larvae.

Effect of Plant Age on Acetamiprid Efficacy and Spray Coverage. The size of the cauliflower plants varies greatly as it grows older (Supp. Fig. S1 [online only]). However, plant age (i.e., 15, 30, 45, 60, and 75 d after transplanting) did not significantly affect the number of eggs laid by the Swede midge adults ($F = 1$; df = 5, 55; $P = 0.51$) (Table 2). After a single acetamiprid spray, no Swede midge larvae survived on the cauliflower plants tested at different growing stages ($F = 24.199$; df = 1, 55; $P = 0.004$) (Table 2). Florescent dye sprayed on the cauliflower plants could be easily detected under the UV light (Supp. Fig. S1 [online only]) and was an effective indicator for checking spray coverage. Different-sized plants were all ranked with the highest coverage point, i.e., cauliflower plant age did not significantly affect spray coverage (Supp. Fig. S1 [online only]).

Effect of Acetamiprid Residue Duration on Swede Midge Mortality. When acetamiprid-sprayed plants were left outside, residue of the insecticide provided nearly 100% control of Swede midge up to 6 d after spraying on the plants. However, ≈50% of Swede midge larvae survived on the sprayed plants 9 d after spraying (Table 3), and this resulted in significant damage to the plants. Acetamiprid efficacy on Swede

Table 2. Effect of cauliflower plant age on the efficacy of a foliar spray of acetamiprid on swede midge

Plant age (d after transplant)	No. live larvae/plant	
	Acetamiprid	Control
15	0aB	72.8 ± 14.3aA
30	0aB	86.7 ± 11.1aA
45	0aB	86.5 ± 19.1aA
60	0aB	84.8 ± 8.9aA
75	0aB	74.9 ± 12.3aA

Data in the table are means ± SE; means followed by different lowercase letters in a column or uppercase letters in a row for each parameter are significantly different based on GLM analyses and Fisher's protected LSD mean separation test, $P < 0.05$.

Table 3. Impact of the residual life of a foliar spray of acetamiprid on swede midge on cauliflower plants

Residual life (days after spray)	No. live larvae/plant
0	0c
3	0c
6	0.8 ± 4.3c
9	36.5 ± 5.6b
12	36.8 ± 4.9b
Unsprayed control	72.5 ± 12.3a

Data in the table are means ± SE; means followed by different lowercase letters in a column are significantly different based on GLM analyses and Fisher's protected LSD mean separation test, $P < 0.05$.

midge was significantly reduced by degradation of insecticide residue ($F = 7.817$; df = 5, 23; $P = 0.008$) (Table 3).

Discussion

Swede midge is becoming an increasingly important pest of crucifers in North America, and its control has proven difficult. Once established in an area, we believe this insect will be virtually impossible to eradicate. Thus, USDA–APHIS changed the status of Swede midge from a reportable/actionable pest to a nonreportable/nonactionable pest in April 2009 (http://blogs.cce.cornell.edu/cvp/archives/63). Considerable effort has been invested to develop an effective control program for Swede midge in North America (www.nysaes.cornell.edu/ent/swedemidge). Chemical control with >20 synthetic insecticides, as an immediate control strategy, was extensively investigated first under laboratory conditions with different application methods, i.e., foliar sprays, soil drenches, and seed treatments (Wu et al. 2006). Acetamiprid and most of other insecticides tested were very effective against Swede midge based on the laboratory trials (Wu et al. 2006, Chen et al. 2007). For example, one of our previous studies indicated that acetamiprid could provide 99.5, 100, and 99.8% control of Swede midge on cauliflower seedlings when sprayed before oviposition and 0 d and 4 d after oviposition, respectively. However, under field conditions, synthetic insecticide treatments were rarely able to maintain Swede midge damage levels within marketable limits, although early season application of neonicotinoid insecticides (e.g., acetamiprid, imidacloprid, and clothianidin) could provide some degree of control for several wk (Hallett et al. 2009a). With insecticide resistance being excluded from the possible reasons of the field control failure (Hallett et al. 2009a), results from the field trials (Hallett et al. 2009a) suggested that low efficacy of synthetic insecticides were more likely associated with poor spray coverage, high Swede midge population pressure, or both.

In the current study, our results indicated that although Swede midge population density could significantly affect the subsequent Swede midge population on the unsprayed cauliflower plants, a single acetamiprid spray could effectively suppress the subsequent population regardless of the initial density of Swede midge (Table 1). A single acetamiprid spray provided effective control of Swede midge eggs and larvae (i.e., spraying after oviposition) (Table 1). When the spray was applied before oviposition (i.e., the spray acted on Swede midge adults first), some Swede midge eggs were found on the sprayed plants although the eggs were later killed by the acetamiprid residue (Table 1). This suggests that an acetamiprid foliar spray was more effective on Swede midge eggs and larvae than on adults. Similarly, Wu et al. (2006) also found that an acetamiprid foliar spray reduced Swede midge larvae and eggs by 99.9% but only achieved 42.8% mortality on Swede midge adults after 24 h. Furthermore, our results indicated that cauliflower plant age had no significant effect on the efficacy of acetamiprid on Swede midge (Table 2) nor on spray coverage (Supp. Fig. S1 [online only]). This result was surprising because cauliflower plants had more leaves and larger leaves as they grew from 15 d after transplanting to 75 d after transplanting (Supp. Fig. S1 [online only]). Such excellent coverage and control as we achieved using a track sprayer may not be able to be replicated in the field where a broad range of factors could affect insecticide spray coverage and control, such as droplet size, number of droplets per area, application method and timing, and environmental conditions during and after application (Payne 2000). However, our results suggest that good control of Swede midge can be achieved with acetamiprid, and perhaps other insecticides, if these factors can be addressed in the field.

Acetamiprid is a highly effective neonicotinoid insecticide that can be used against various insect pests on a wide range of crops, especially vegetables and fruits. The half-life of acetamiprid, based on some field trials on various crops, has been reported to be <3 d (Pramanik et al. 2006, Tokieda et al. 1999), which could be a challenge to Swede midge management because overlapping generations of the insect exist in North America (Hallett et al. 2009b). In the current study, our results indicated that acetamiprid maintained nearly 100% efficacy on Swede midge for 6 d on cauliflower plants; however, its efficacy was reduced by up to 50% 9 d after spraying (Table 3). Our result is in agreement with other field dissipation studies on acetamiprid where the half-life has been estimated to be 3–14 d (Pest Management Regulatory Agency 2002–2005).

Seasonal development of Swede midge was studied using pheromone traps and emergence cages, and thee to four generations of Swede midge were detected in 2004 and 2005 (Corlay and Boivin 2008). Based on a new predictive model (MidgeEmerge) developed using DYMEX and trap capture data on Swede midge adults and larvae, Hallett et al. (2009b) reported that there were three to five overlapping generations of Swede midge per year and two to three emergence phenotypes in North America. Therefore, based on the results presented herein and on previous studies (Hallett et al. 2009a), it seems likely that where control failure has occurred it is due to multiple and overlapping generations of Swede midge in conjunction with a shorter than needed insecticide residual life in the field. This is in contrast to our previous ideas that failure was due to discrete but high population pressure, poor spray coverage or insecticide resistance. Thus, it seems that the key for control of Swede midge will be good insecticide application timing during oviposition or immediately thereafter.

Since the first identification of Swede midge in North America in 2000 (Hallett and Heal 2001), different control strategies, such as chemical control, cultural control, biological control, and host plant resistance, have been extensively tested under laboratory, greenhouse and open field conditions by researchers in the United States and Canada. However, no single control strategy by far has been able to provide 100% control of Swede midge (www.nysaes.cornell.edu/ent/swedemidge). The results presented here will provide useful information on the development of an overall integrated pest management program to keep Swede midge populations below economic levels.

Acknowledgments

This work was partly supported by grants from USDA Pest Management Alternatives Program and the New York Vegetable Research Association/Council.

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Received 15 June 2010; accepted 28 August 2010.