

Control of *Contarinia nasturtii* Keiffer (Diptera: Cecidomyiidea) by foliar sprays of acetamiprid on cauliflower transplants

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Received 2 October 2006; received in revised form 6 January 2007; accepted 25 January 2007

Abstract

Swede midge (*Contarinia nasturtii* Keiffer) is a serious gall-forming insect pest of cruciferous plants in Europe and southwestern Asia. In North America, it was first identified in Ontario, Canada, in 2000 and in Niagara County, New York, US, in 2004. The insect is now rapidly spreading in Canada and the US. To date, infestation of *C. nasturtii* has been confirmed in 46 counties in Canada. The US Department of Agriculture has confirmed the presence of *C. nasturtii* in 6 counties in the US, although our surveys using pheromone traps have identified 19 counties in New York and one county in Massachusetts and New Jersey. Currently, hundreds of farms in the northeastern US that grow cruciferous vegetables are at risk for infestation of *C. nasturtii* through movement of vegetable seedlings and harvested produce from infested areas. To prevent the spread of *C. nasturtii*, the efficacy of acetamiprid, the first labeled insecticide in the US for *C. nasturtii* control, was evaluated by using foliar sprays on cauliflower seedlings. Results indicated the efficacy of acetamiprid on *C. nasturtii* was 99.52% 100% and 99.83% when cauliflower seedlings were sprayed before inoculation with *C. nasturtii*, 0 and 4 d after inoculation, respectively. The efficacy of acetamiprid was reduced to 69.89% when seedlings were sprayed 8 d after inoculation, and *C. nasturtii* larvae could successfully pupate and emerge after the spray. These results indicate that acetamiprid can effectively control *C. nasturtii* on cauliflower seedlings, especially in the early stage of insect occurrence. Based on these results, we suggest that seedlings be treated with acetamiprid as a foliar spray before shipment of seedlings from *C. nasturtii* infested areas.

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Keywords: *Contarinia nasturtii*; Invasive species; Foliar spray; Acetamiprid

1. Introduction

Swede midge (*Contarinia nasturtii* Keiffer), a insect pest of *Brassicaceae* plants, is widely distributed in Europe and southwestern Asia where cruciferous crops are grown. In North America, it was not known to occur until its first nearctic record in Ontario, Canada, in 2000 (Hallett and Heal, 2001). *C. nasturtii* larvae, the stage causing damage on crops, are tiny yellowish maggots living gregariously inside the growing tips of a plant. They produce a secretion that breaks down plant tissues, which are then consumed without gnawing on the plants directly (Barnes, 1946). The typical damage symptoms of *C. nasturtii* are swelling and twisting of the growing tips, multiple heads or no heads (Kikkert et al., 2002), all symptoms that can severely

reduce the product quality and marketability. Yield losses in some Canadian farms caused by *C. nasturtii* have been estimated to be more than 85% between 1996 and 1999 (Hallett and Heal, 2001).

In 2001, researchers in Canada launched a *C. nasturtii* survey in cruciferous crops using yellow sticky cards, and good evidence was found that *C. nasturtii* occurred in 9 counties in Ontario and 1 county in Quebec. Subsequently, the Canadian Food Inspection Agency (CFIA) started a nationwide investigation for *C. nasturtii*. After 3 yr of intensive field scouting (2002–2004), the presence of *C. nasturtii* was confirmed in 14 counties in Ontario and 4 counties in Quebec (CFIA, 2005). The total number of regulated counties for *C. nasturtii* in Ontario and Quebec was 46 in 2006 (CFIA, 2006). In the US, *C. nasturtii* adults were first captured in September 2004 in Niagara County, New York using pheromone traps and *C. nasturtii* larvae were also identified at the same time (Kikkert et al., 2006).

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At the end of 2005, *C. nasturtii* infestations in five additional counties (Erie, Genesee, Monroe, Orleans and Wyoming) in New York State were officially confirmed by the USDA, based on both morphological and molecular evidence (http://www.aphis.usda.gov/ppq/ep/emerging_Pests/Swedemidge.html). However, in 2005 and 2006, our laboratory confirmed by molecular analysis that *C. nasturtii* was present in 13 more counties in New York (Allegany, Cattaraugus, Chautauqua, Chenango, Franklin, Jefferson, Lewis, Madison, Onondaga, Oswego, St. Lawrence, Suffolk and Wayne) and one in Massachusetts (Hampshire) and New Jersey (Newton). It is obvious that this tiny pest is spreading in North America with dramatic speed. However, Wyss and Daniel (2004) reported that *C. nasturtii* generally spreads only within the crop or only a few centimeters above it. Fraser et al. (2005) also suggested *C. nasturtii* adults were not strong fliers. The evidence above suggests that dispersal by flight might not be the key reason for its rapid distribution. Shipment of vegetable seedlings, which have *C. nasturtii* larvae on seedlings or pupae in soil, may be the main reasons for its rapid distribution. A similar situation has been well documented with movement of crucifer seedlings infested with *Plutella xylostella* (L.) resulting in rapid interstate movement and widespread control problems (Shelton et al., 1996).

If *C. nasturtii* are found in a county, the county may become regulated and be put under quarantine conditions with restriction of shipment of all cruciferous vegetable products or seedlings to isolate and control the damage from *C. nasturtii*. However, such quarantine restrictions could result in dramatic economic losses to those vegetable growers. The Great Lakes region of the US is a major crucifer production region and borders Canada. Canada is a major supplier of crucifer transplants to this area and local transplants also are commonly moved within the region. Therefore, movement of *C. nasturtii*-infested transplants will cause a large risk to crucifer vegetable growers as well as to those who grow canola (*Brassica napus*), which is grown on 469 kha in the US (NASS, 2005). Prior to the discovery of *C. nasturtii* in the US, our lab had evaluated, under laboratory quarantine conditions, several insecticides against *C. nasturtii* (Wu et al., 2006). Based on these tests and field data from Canada, the US label for acetamiprid (Assail 70WP) was expanded to include *C. nasturtii*. However, no tests had been conducted with this product on crucifer seedlings. Crucifer seedlings are normally shipped when they are ca. 5 wk old, and under greenhouse conditions it takes 1 wk for plant emergence. Hence, there are 4 wk in which an infestation can occur. Even using a pheromone trap, the transplant grower will not be able to precisely time a spray so that all *C. nasturtii* are in the same stage. Hence, it is important to determine the effectiveness of the spray against different times of infestation. The objective of this research is to test the efficacy of spraying cauliflower seedlings at different times with acetamiprid for *C. nasturtii* control in order to

develop a procedure for treating seedlings before they are shipped to the field for transplanting.

2. Materials and methods

2.1. Insect culture and tested plant

An initial population of *C. nasturtii* was kindly received from Robert Baur in Switzerland in 2004 and was subsequently reared in a chamber under quarantine conditions at 22 °C, RH 75–78% and 16 h L:8 h D (Wu et al., 2006). The food plant was cauliflower, *Brassica oleracea* var. *botrytis*, “Snow crown” cultivar, with 8 to 10 true leaves. Cauliflower seeds were planted in a 128-cell tray every other week, and 3-wk old seedlings were transplanted in pairs into 13 cm dia. pots and maintained in Cornell University/NYSAES greenhouses. Two pots of plants were put into a 50 × 50 × 50 cm wooden cage containing about 100 adults, males and females (ca. 1:2), for egg laying, and plants were changed daily. The growing tips of the plants were misted with tap water daily after larvae emerged from the eggs. The above ground parts were cut off 15 d after oviposition, and placed on the soil to let older larvae in the tips pupate and eclose for the next generation. *C. nasturtii* tested in this experiment were from the 27th generation. Cauliflower seedlings with 3 true leaves (the common stage for transplanting) were used for the following experiments.

2.2. Insecticide

Acetamiprid (Assail 70WP, Cerexagri, Inc., PA), a neonicotinoid which was the only insecticide labeled for *C. nasturtii* control on cole crops in the US, was tested as a foliar spray. We used the label rate of acetamiprid for *C. nasturtii* (34 g AI/ha) which converted to 8.4 mg AI/m² when sprayed onto transplant trays.

2.3. Foliar spray tests on eggs and larvae

Cruciferous vegetables are commonly seeded into trays containing cells filled with soil. A 128-cell styrofoam tray with cauliflower seedlings was cut into 6-cell trays, and each 6-cell tray containing 6 seedlings was used as one unit in the formal experiment. For all treatments, each tray was placed into a wood oviposition cage (50 × 50 × 50 cm) containing 18 *C. nasturtii* adults (12 female+6 male) collected from the colony using a mouth aspirator. The position of each tray in the oviposition cage was changed after 24 h to increase random oviposition, and the trays remained in the cage for 48 h.

The foliar spray test had 4 different treatments: (1) spraying seedlings immediately before putting them into the oviposition cage; (2) spraying seedlings immediately after taking them out of the oviposition cage; (3) spraying seedlings 4 d after removing them from the oviposition cage; (4) spraying seedlings 8 d after removing

them from the oviposition cage. These treatments were chosen to determine the most effective time to provide a spray against *C. nasturtii*. In treatment 1, one 6-cell tray was used as one replication, and 5 sprayed replications with 5 unsprayed control replications were put into 10 oviposition cages. In treatments 2–4, two 6-cell trays (one for spray, the other for the unsprayed control) were used as one replication and were put into the same oviposition cage. There were 5 replications per treatment.

Spraying was conducted in a track chamber (Allen Machine Works, Midland, MI, USA), using a single nozzle (800 3VS) 50 cm above the seedlings and spraying at 40 psi, 3.2 km/h and 28 ml/m². After the seedlings were sprayed, they were put into a wood cage (50 × 50 × 50 cm) and placed in a rearing chamber and watered as needed. In treatment 1–3, the number of eggs or larvae on each seedling was counted 7 d after oviposition by dissecting the growing tips under a stereomicroscope. In treatment 4, the number of eggs or larvae on each seedling was counted 8 d after oviposition in the control group and 10 d after oviposition (2 d after spray) in the spray group. Preliminary tests had indicated *C. nasturtii* larvae need 10–15 d feeding on a plant to finish the larval stage in the rearing chamber, and then jump from the plant and pupate in soil. To ensure no *C. nasturtii* larvae had jumped into the soil before the spray in treatment 4, it was necessary first to check the control plants 8 d after oviposition (i.e. the same day as the spray treatment in treatment 4). Also, the trays with soil from the treatment and control plants in treatment 4, after the seedlings were cut at the soil level and removed, were kept and checked for adult emergence from larvae which might have moved into the soil.

2.4. Data analysis

Comparisons of the number of eggs and larvae per plant, the hatching percentage, and adult mortality and emergence between sprayed and unsprayed were analyzed by Student's *t*-test. The overall efficacy (%) of each treatment was calculated as the number of dead eggs and larvae in the spray treatment/total eggs laid in the spray treatment. All percentage data were transformed into arcsine square-root before being subjected to statistical analysis. All statistical calculations were performed using SPSS package (version 11.5 for Windows).

3. Results

In treatment 1, a foliar spray of acetamiprid on cauliflower seedlings providing high mortality of *C. nasturtii* adults on cauliflower seedlings with averages of 84.44% and 100% at 24 and 48 h after a spray, respectively, compared with the control ($t = 23.912$, $df = 4$, $P < 0.001$ and $t = 51.454$, $df = 4$, $P < 0.001$) (Fig. 1). The number of eggs laid on seedlings sprayed with acetamiprid in treatment 1 was greatly reduced ($t = 5.976$, $df = 4$, $P = 0.004$) (Table 1). There was greater than three-fold the

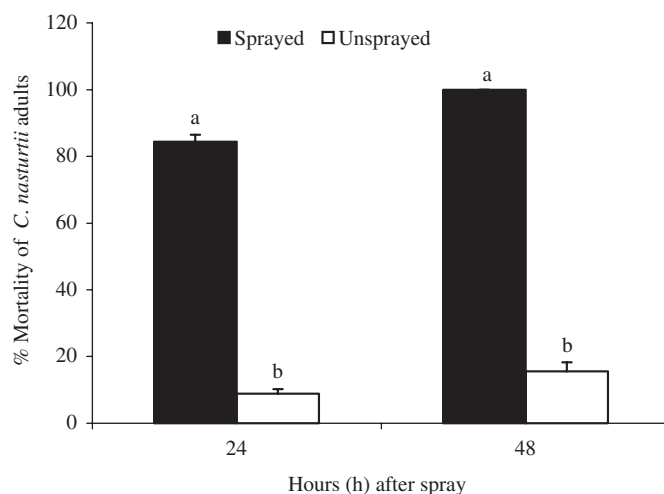


Fig. 1. Mortality of *Contarinia nasturtii* adults at 24 and 48 h after plants were sprayed with acetamiprid. Means (\pm S.E.) marked with different letters are significantly different when analyzed by Student's *t*-test ($P < 0.05$).

Table 1
Efficacy of acetamiprid as a foliar spray on *Contarinia nasturtii* eggs and larvae occurring on cauliflower seedlings

Indicator	Trt.1	Trt.2	Trt.3	Trt.4
No. of total eggs/plant				
Spray	9.27 ± 2.15 b	23.80 ± 6.69 a	12.97 ± 2.28 a	16.21 ± 1.91 a
Control	34.70 ± 5.53 a	25.90 ± 5.28 a	10.77 ± 0.78 a	18.07 ± 4.58 a
% Egg hatching				
Spray	0.48 ± 0.21 b	0 b	100 a	100 a
Control	100 a	100 a	99.73 ± 0.27 a	100 a
No. of dead eggs/plant				
Spray	9.23 ± 2.16 a	23.80 ± 6.70 a	0 a	0 a
Control	0 b	0 b	0.03 ± 0.03 a	0 a
No. of dead larvae/plant				
Spray	0 a	0 a	12.94 ± 2.26 a	11.37 ± 1.71 a
Control	0 a	0.03 ± 0.03 a	0.03 ± 0.03 b	0 b
No. of live larvae/plant				
Spray	0.03 ± 0.03 b	0 b	0.03 ± 0.03 b	1.13 ± 0.19 b
Control	34.70 ± 5.53 a	25.86 ± 5.30 a	10.70 ± 0.75 a	18.07 ± 4.58 a
% Overall efficacy				
Spray	99.52 ± 0.48	100	99.83 ± 0.17	69.89 ± 2.90

Trt.1: sprayed before oviposition; Trt.2: sprayed immediately after oviposition; Trt.3: sprayed 4 d after oviposition; Trt. 4: sprayed 8 d after oviposition. Means (\pm S.E.) within a column for the same treatment followed by different letters are significantly different when analyzed by Student's *t*-test ($P < 0.05$).

mean number of eggs laid on unsprayed seedlings (34.70) than that on sprayed ones (9.27). All eggs laid on the sprayed seedlings died except for one. Conversely, eggs laid on unsprayed seedlings had a 100% hatch rate. In treatment 2, after maintaining seedlings in the oviposition cages for 48 h, the mean number of eggs laid on the seedlings by *C. nasturtii* adults was very similar between the treatment (23.80) and the control (25.90) ($t = 0.530$, $df = 4$,

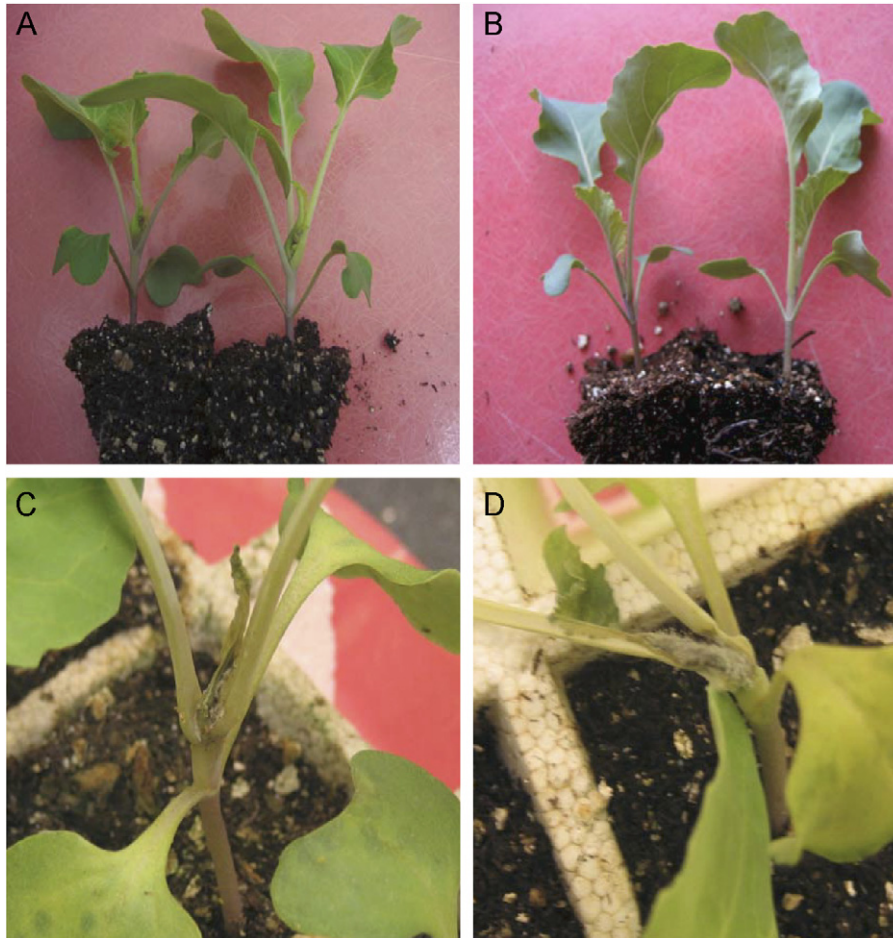


Fig. 2. Damage symptoms on cauliflower seedlings caused by *Contarinia nasturtii* larvae: (A) swollen growing tips on unsprayed seedlings in treatment 2; (B) sprayed seedlings in treatment 2; (C) damage symptom and dead larvae on sprayed seedlings in treatment 4; (D) mold in growing tips after *C. nasturtii* infestation.

$P = 0.624$) (Table 1). However, 100% of eggs laid on the sprayed seedlings died before hatching. Meanwhile, 100% of the eggs laid on the unsprayed seedlings hatched and caused typical damage symptoms (Fig. 2). In treatment 3, *C. nasturtii* adults also laid similar numbers of eggs on the seedlings for the treatment and the control in the oviposition cages ($t = 0.925$, $df = 4$, $P = 0.407$). On day 4 after being taken out of the oviposition cages, all eggs hatched except for one dead egg from the control plants (Table 1). All the eggs from the unsprayed seedlings developed to the larval stage except one. In treatment 4, the eggs laid on different seedlings all hatched 8 d after seedlings were taken out of the oviposition cages ($t = 0.507$, $df = 4$, $P = 0.639$). The *C. nasturtii* larvae caused severe damage to the tips of the seedlings. Also, some seedlings were infested by mold because of the damage of *C. nasturtii* (Fig. 2D). In order to increase the accuracy of the data, the number of *C. nasturtii* eggs or larvae on the unsprayed seedlings was checked 8 d after oviposition, because *C. nasturtii* larvae would crawl out from growing tips and jump to the soil. The mean number of *C. nasturtii* larvae, including the dead (11.37) and live (1.13) ones on the above-ground part of each seedling from

treatment group, was recorded 2 d after spraying, and was lower than that (18.07) from the corresponding unsprayed seedling ($t = 4.412$, $df = 4$, $P = 0.012$), which suggests some old larvae in the treatment group jumped to the soil to pupate earlier (they usually need 10–15 d) because of acetamiprid spraying. After the seedlings were cut at soil level and removed, the trays with soil were put into wood cages for ca. 7 d and 112 *C. nasturtii* adults (including 13 females) emerged from the trays from the acetamiprid treatment, while no *C. nasturtii* adults emerged from unsprayed trays.

The overall efficacy of each treatment reflected the mortality of eggs and larvae. Efficacy was >99.52% for treatments 1–3, but only 69.89% for treatment 4 indicating the difficulty in controlling older larvae.

4. Discussion

C. nasturtii, a new invasive pest in North America (Hallett and Heal, 2001; Kikkert et al., 2006), causes severe damage to cruciferous crops, and is spreading with dramatic speed. To decrease the spread, the USDA has restricted the movement of cole crops, crucifer seedlings,

soil and agricultural machinery from infested areas (Ellis, 2005). Many counties in Canada and the US in which *C. nasturtii* has not been previously reported are facing the threat of being infested by *C. nasturtii* through the shipment of seedlings and through natural dispersal (such as flying) from infested counties. Based on the biology of this insect, we believe transplants may be the main means of movement. Isolation management for this insect might be an effective way to control *C. nasturtii*, such as restricting all vegetable products and seedlings from infested counties. However, such restriction will result in a severe negative economic impact to many growers. Our results indicated that a well-timed spray application of acetamiprid to cauliflower seedlings can provide excellent control of *C. nasturtii* adults after 48 h. Furthermore, spraying plants prior to oviposition resulted in fewer eggs laid. The results of treatments 1–3 demonstrated that acetamiprid could effectively control *C. nasturtii* at the egg and early larvae stage. Similarly, Wu et al. (2006) treated 4–5 wk-old broccoli plants with acetamiprid using the same approach as our treatment 2, and attained 99.9% larval reduction of *C. nasturtii* compared with the unsprayed control. Treating older infestations (e.g. treatment 4) was not effective since ca. 30% of the larvae survived including ca. 20% of the larvae that successfully pupated and emerged after the planting trays were kept in *C. nasturtii*-free cages for several days. Generally, the feeding period of *C. nasturtii* larvae on host plants varies from 7 to 21 d at 15–25 °C (Readshaw, 1966; Kikkert et al., 2002). So, it is possible that some *C. nasturtii* larvae burrowed into the soil before or after the spray. However, the number of larvae on the unsprayed control seedlings checked 8 d after inoculation was much higher than that on the treatment, which suggests *C. nasturtii* larvae burrowed into the soil after the spray. The soil in the trays also had acetamiprid residues after being sprayed in the chamber because the seedlings did not block the spray from reaching the soil. So, we may infer from these results that acetamiprid reduced the number of later instars or pupae, but not to the extent of treatments 1–3. Based on our results, we suggest that acetamiprid can be used to control *C. nasturtii* on crucifer seedlings and that timing is very important for attaining good control. It would not be as effective if acetamiprid were sprayed for later instars of *C. nasturtii*. Thygesen (1966) also reported parathion applied as a spray could effectively kill *C. nasturtii* eggs, larvae and adults if the spray was applied at the beginning of the hatching period.

In the past 2 yr, the *C. nasturtii* population in the US has been very low as determined by our extensive pheromone trapping system, and no *C. nasturtii* were reported in greenhouses. If acetamiprid foliar sprays were deployed in conjunction with pheromone traps (Hillbur et al., 2005), which would help time applications, better control could be attained. Once plants have been treated with acetamiprid, previous results have indicated several weeks of good control of *C. nasturtii* (Wu et al., 2006). Well-timed sprays of acetamiprid may be a viable alternative to restricting the

movement of transplants from an infested area and of reducing its spread within an already infested area.

Acknowledgments

We thank Y.M. Cheung for assistance throughout this study, and H. Collins for helpful comments on an earlier draft of the manuscript.

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