A Single Swede Midge (Diptera: Cecidomyiidae) Larva Can Render Cauliflower Unmarketable

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Abstract

Swede midge, Contarinia nasturtii Kieffer (Diptera: Cecidomyiidae), is an invasive pest causing significant damage on Brassica crops in the Northeastern United States and Eastern Canada. Heading brassicas, like cauliflower, appear to be particularly susceptible. Swede midge is difficult to control because larvae feed concealed inside meristematic tissues of the plant. In order to develop damage and marketability thresholds necessary for integrated pest management, it is important to determine how many larvae render plants unmarketable and whether the timing of infestation affects the severity of damage. We manipulated larval density (0, 1, 3, 5, 10, or 20) per plant and the timing of infestation (30, 55, and 80 d after seeding) on cauliflower in the lab and field to answer the following questions: 1) What is the swede midge damage threshold? 2) How many swede midge larvae can render cauliflower crowns unmarketable? and 3) Does the age of cauliflower at infestation influence the severity of damage and marketability? We found that even a single larva can cause mild twisting and scarring in the crown rendering cauliflower unmarketable 52% of the time, with more larvae causing more severe damage and additional losses, regardless of cauliflower age at infestation.

Key words: damage threshold, marketability threshold, invasive pest, Brassica production, Cecidomyiidae

Swede midge (Diptera: Cecidomyiidae) is an invasive pest threatening Brassica production in the Northeastern United States and Eastern Canada (Hallett and Heal 2001; Olfert et al. 2006; Chen et al. 2011). Feeding by swede midge larvae results in a range of damage, from slight swelling of plant tissue to scarring, twisting, branching, and most severely, the complete loss of the apical bud (Chen and Shelton 2007; Chen et al. 2009). Once established in an area, swede midge is extremely difficult to remove (Chen et al. 2011). A decade after the midge invaded Ontario, Canada, losses to broccoli and cauliflower (Brassica oleracea Gp. Botrytis and Gp. Italica, respectively) can exceed 85% annually (Hallett and Heal 2001). Swede midge outbreaks have reversed previous integrated pest management (IPM) gains in cole crops because growers often resort to calendar-based spraying (Hallett and Sears 2013). There is a critical need for better insecticide treatment thresholds (Hallett and Sears 2013) and alternative management tactics for swede midge (Chen and Shelton 2007; Chen et al. 2009), both of which depend on a comprehensive understanding of pest biology and susceptibility to treatments (Kogan 1998).

Cecidomyiids are challenging insect pests because of their ability to manipulate plant growth, resulting in galls and tumorous formations (Maia et al. 2005; Vitou et al. 2008; Vijaykumar et al. 2009; Hall et al. 2012; Stuart et al. 2012; Uechi et al. 2017). Specifically, swede midge is difficult to control due to: 1) the short adult life span and concealed feeding of larvae (Readshaw 1966; Hallett et al. 2009a, Chen et al. 2011); 2) multiple overlapping generations with irregular emergence phenotypes that are difficult to predict (Hallett et al. 2009b); and 3) all developmental stages of susceptible Brassica hosts seem impacted by herbivory (Hallett 2007).

Although swede midge clearly manipulates plant growth, no studies have examined the relationship between timing of larval feeding and the emergence of market-relevant damage symptoms in Brassica vegetables. Visible damage within an infested cauliflower field could be due to separate infestations from different calendar days. It is unclear whether the ultimate loss of a marketable plant is due to the final infestation, or a compounded effect from multiple infestations. Effective IPM programs depend on accurate damage (the level of infestation that can cause damage; Walker 1983; Hallett and Sears 2013) and marketability thresholds (the level of infestation that renders a plant unmarketable; Hallett and Sears 2013). Unfortunately, typical scout and spray IPM practices are impractical for swede midge because market-relevant damage is difficult to confirm until after
organisms could allow more precision in the timing of pesticide applications rather than calendar-based sprays (Hallett et al. 2009a, Hallett and Sears 2013).

Current conventional management recommendations for swede midge are to use systemic neonicotinoids at transplant, followed by regular sprays of foliar insecticides for the remainder of the growing season (Chen et al. 2011; Hodgdon et al. 2017), disrupting decades of effective IPM in Brassica systems (Rodríguez Salamanca 2014). An alternative approach is to use an action threshold based on the capture of five males per pheromone trap per day with a minimum 7-d pesticide retreatment interval (Hallett and Sears 2013). This strategy reduces damage to acceptable levels in cabbage, but not cauliflower, possibly because cabbage heartleaves protect the developing meristem from feeding larvae (Andaloro et al. 1983). Also, organic growers are limited to using large-scale crop rotations (Chen et al. 2009) and/or covering crops with specialty insect netting (Hodgdon et al. 2017), both expensive strategies.

Swede midge damage differs across and within groups of B. oleracea vegetables (e.g., cabbages, cauliflower, broccoli, etc.) (Chen et al. 2011). Infestations result in the lack, reduced size, or distorted growth of the marketable portion of cauliflower composed usually of white inflorescence meristem. Here, we studied the relationship between swede midge infestation and plant damage on cauliflower to test how the timing and severity of swede midge infestation influences market-relevant damage. We applied first-instar larvae to cauliflower to ask: 1) How many swede midge larvae does it take to cause visible damage to cauliflower? 2) How many larvae render cauliflower crowns unmarketable? and 3) Does the age of cauliflower at infestation influence the likelihood that it will be unmarketable?

Materials and Methods

Plant Production and Colony Rearing

A laboratory-reared colony of swede midge was used for both laboratory and field trials (origin: Swiss Federal Research Station for Horticulture, Wädenswil, Switzerland). Midges were reared on cauliflower plants, Brassica oleracea group Botrytis ‘Snow Crown’ (Harris Seeds, Rochester, NY), due to midge preference (Hallett 2007) and large bud size. Seeds were planted in 128-cell trays filled with Fafard 3B soilless potting media (Sun Gro Horticulture, Agawam, MA). Seedlings were transplanted into 10 cm pots after 4 wk and returned to the greenhouse. Plants were grown in the University of Vermont greenhouse under a 16:8 (L:D) h photoperiod at ~22°C and ~30% RH until six to eight true leaves had developed.

Each day, four cauliflower plants were placed into one of two oviposition cages (0.6 m² wooden frame, covered in 400 μm nylon mesh) in the lab. Plants were exposed to adults for 1 d, after which they were moved to another cage, where eggs hatched and larval development continued. Eggs hatch ca. 1 d after oviposition in the inner folds of the leafy crown of cauliflower (Hallett and Sears 2013). This design successfully restricted larvae from reaching the soil where they pupate. Therefore, infested cauliflower meristems were cut 3 cm below the crown and inserted back into the planting pots after ~14 d of larval development. Pots with pupating larvae were placed back in the ovipositional cages, where adults emerged and mated ~14 d later (Chen et al. 2011).

Artificial Infestation Procedure

Standard quantities of larvae were applied to plant buds to determine the relationship between larval density and damage intensity. Swede midge larvae are difficult to work with because of their small size (<2 mm), feeding location within the bud, and transparent color. Eggs are nearly invisible, so first instars were used for trials. Most larvae have entered the first instar about 3 d after oviposition based upon Readshaw (1996) and our observations. Larvae were collected from four plants exposed to gravid females for 24 h then grown for 72 h in the greenhouse. All larvae and eggs were separated from the plant by dissecting the apical bud and rinsing the fragments with deionized water in a petri dish.

First instars were aspirated and gently applied in the tightly folded leaves developing off the primary meristem using a 200-μl micropipette. The same quantity of deionized water was applied to control plants as a check for any damage caused by the procedure.

Artificial Infestation Procedure—Method Validation

To validate that larvae remained intact after applications to treated plants, additional trials testing larval survival were performed. Larvae (0, 1, 3, 5, 10, or 20) were applied to plants with 8–10 true leaves, using 20 replicate plants for each larval density. Infested plants were grown in mesh cages (1 m x 0.7 m x 0.7 m, BioQuip, Rancho Dominguez, CA) for 10 d under rearing conditions (described above). After 10 d of development, young leaves were removed using a scalpel and larvae were gently rinsed from the leaves using deionized water. A dissecting microscope was used to count the number of larvae that physically responded to a gentle touch with a probe. Summary statistics from these trials were used to estimate the proportion of larvae that survived the artificial infestations. We found that half the larvae survive the procedure, with 0.4 ± 0.11, 1.5 ± 0.25, 2.25 ± 0.34, 5.7 ± 0.63, and 10.4 ± 0.77 (mean ± SE) moving larvae for 1, 3, 5, 10, and 20 larvae, respectively.

What Is the Damage/Marketability Threshold for Swede Midge?

To test the relationship between larval density and damage severity, 0, 1, 3, 5, 10, or 20 larvae were applied to uninested cauliflower with 8–10 true leaves, replicated over 25 plants for each treatment density. The cauliflower plants tested were grown for 8 wk using the conditions described above, with the exception that they were transplanted into 15 cm circular planting pots, rather than 7 cm pots. Following the artificial larval infestation, treated plants were returned to the greenhouse in large pop-up cages and grown for ~21 d. To prevent larvae from completing their life cycle and reinfecting treated plants prior to data collections, circular, flat, acetate sheets were fastened around plant stems with cotton filling the extra space between the sheets and the stem. This design successfully restricted larvae from reaching the soil where they pupate.

Cauliflowers were evaluated using a scale adapted from Hallett (2007), described in Table 1. Cauliflower plants with a score ≥1 were unmarketable using this scale (C.A.S., personal observations). To determine how many swede midge larvae cause damage to cauliflower (damage threshold), the frequencies of plant damage ratings across larval treatment densities were tested using a log-linear regression. The relationship between larval density and marketability (marketability threshold) was tested using a binary-logistic regression. Using the models fitted from our data, the lowest numbers of larvae that cause damage and render cauliflower unmarketable were estimated.

Does the Age of Cauliflower at Infestation Influence the Likelihood That It Will Be Marketable?

We tested if plant age and the number of larvae influenced market-relevant plant damage using a potted plant experiment from

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July 1, 2015 to August 30, 2015 at the Bio-Research Complex at the University of Vermont. We chose the site because it was at least 5 km away from any commercial Brassica plantings, minimizing background midge populations that could influence the study. The study site was situated between outdoor hoop-house structures. Due to the low background population of midges in this area, our study design allowed all plants, regardless of infestation date, to be grown outside for the entire experimental period. In order to minimize disturbance to the study and control weeds, we covered the study area (3 × 15 m) with black landscape fabric. We raised cauliflower in 15 cm circular planting pots between Jackson traps with a swede midge pheromone lure (Solida Distributions, Saint-Ferreol-les-Neiges, Québec) to verify that midges remained absent from the field.

To test whether plant age has an effect on plant damage and the likelihood of marketability, 0, 1, 5, 10, or 20 larvae were applied to plants of three age groups (30 [2 true leaves], 55 [4–6 true leaves], and 80 [6–8 true leaves] d after seeding [DAS]), replicated across 30 cauliflower plants in a randomized complete block design. After hardening off 4 wk old seedlings for 2 d, we placed randomly assigned plants to the different treatments at the start of the study, so each plant remained in the same location for the entire experiment. Each block consisted of two trays (0.3 × 0.5 m) that hold six pots each in a 2 × 3 grid. We placed six plants randomized by treatment date and larval density in a zig-zag pattern in the trays. We were concerned that the circular acetate sheets may constrict cauliflower stems through the course of this trial, so instead we loosely fastened fine mesh around the base of the stem to restrict larvae from reaching the soil.

We inoculated midge larvae on the treatment plants at 30, 55, and 80 DAS. On each inoculation date, we brought the subset of pre-assigned plants into an onsite hoop-house. Under a dissecting microscope, we infested cauliflower using the same micropipette method, but the outermost layer of the waxy cuticle was also gently abraded using Kimwipes (Uline, Pleasant Prairie, WI), so the water droplets could adhere to the small meristems of the youngest plants. In order to control for any damage this may have caused, we also abraded older plants.

After the larval inoculations, we assessed the treatment plants for damage every 4 wk. Plants infested at 30 DAS were assessed 4, 8, and 12 wk after transplanting (WAT), plants infested at 55 DAS were assessed at 8 and 12 WAT, and plants infested at 80 DAS were evaluated 12 WAT. We were able to visually differentiate swede midge damage from other herbivores including diamondback moth (Plutella xylostella L.) (Lepidoptera: Plutellidae) and imported cabbageworm (Pieris rapae L.) (Lepidoptera: Pieridae) that feed on foliage rather than the developing meristem (see Table 1 for swede midge damage descriptions). We recorded plant marketability at the end of these trials using standards developed from discussions with a local vegetable grower (A.J., personal communication). Cauliflowers with scarring or twisting within the inner petioles of the crown were unmarketable.

We used a log-linear and binary-logistic regression to test how larval density, plant age, and their interaction influenced damage and marketability, respectively. We used the model output to predict the larval density and plant age that had the highest impact on damage and marketability. All statistics were performed using R version 3.3.

Table 1. Damage scale and associated symptoms used to assess cauliflower artificially infested with 0, 1, 3, 5, 10, or 20 swede midge larvae

<table>
<thead>
<tr>
<th>Damage value</th>
<th>Cauliflower symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No damage</td>
</tr>
<tr>
<td>1</td>
<td>Mild twisting to 1 leaf or florets</td>
</tr>
<tr>
<td>2</td>
<td>Mild twisting of stem, 2–3 leaves, or florets and/or mild swelling of petioles</td>
</tr>
<tr>
<td>3</td>
<td>Severe twisting of 2–3 leaves or florets and/or severe swelling of petioles</td>
</tr>
<tr>
<td>4</td>
<td>Severe twisting and/or crumpling of stem, 3+ leaves, or florets; severe swelling and/or scarring of petioles and/or florets</td>
</tr>
<tr>
<td>5</td>
<td>Severe twisting of stem, leaves, and florets; severe scarring of stem, leaves, petioles, and florets</td>
</tr>
<tr>
<td>6</td>
<td>Death of apical meristem and/or multiple compensatory shoots</td>
</tr>
</tbody>
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Fig. 1. (a) Counts for cauliflower damage scores of different larval densities applied to plants in the laboratory. Twenty plants per treatment were assessed for damage at 10 d postlarval infestation using a modified scale from Hallett (2007) (described in Table 1). Larval density was positively correlated with plant damage ($z = 4.16; P < 0.001$). (b) Binomial logistic regression testing the effect of larval density on the likelihood that infested cauliflower will be marketable. Histograms indicating the number (n) of marketable ($P = 1$) and unmarketable ($P = 0$) cauliflower are also reported for each treatment density. Larval density was negatively correlated with marketability ($z = -3.40; P < 0.001$).
Fig. 2. (a) Counts for cauliflower damage scores of different larval densities applied to plants at 30 DAS. Thirty plants per treatment were assessed for damage at 4, 8, and 12 wk postlarval infestation using a categorical damage scale described in Table 1. Damage at 12 wk is shown. Larval density was positively correlated with plant damage ($z = 3.418; P < 0.001$). (b) Binomial logistic regression testing the effect of larval density on the likelihood that cauliflower infested 30 DAS will be marketable. Histograms indicating the number of marketable ($P = 1$) and unmarketable ($P = 0$) cauliflower are reported for each treatment density. Larval density was negatively correlated with marketability ($z = −3.364; P < 0.001$). (c) Counts for cauliflower damage scores of different larval densities applied to plants at 55 DAS. Thirty plants per larval density were assessed for damage at 4 and 8 wk postlarval infestation. Damage at 8 wk is shown. Larval density was positively correlated with plant damage ($z = 5.455; P < 0.001$). (d) Binomial logistic regression testing the effect of larval density on the likelihood that cauliflower infested 55 DAS will be marketable. Histograms reporting marketable and unmarketable cauliflower are reported for each treatment density. Larval density was negatively correlated with marketability ($z = −5.032; P < 0.001$). (e) Counts for cauliflower damage scores of different larval densities applied to plants at 80 DAS. Thirty plants per larval treatment were assessed for damage at 4 wk postlarval infestation. Larval density was positively correlated with plant damage ($z = 5.907; P < 0.001$). (f) Binomial logistic regression testing the effect of larval density on the likelihood that cauliflower infested 80 DAS will be marketable. Histograms indicating the number of marketable ($P = 1$) and unmarketable ($P = 0$) cauliflower are reported for each treatment density. Larval density was negatively correlated with marketability ($z = −5.852; P < 0.001$).
Results

Plants treated with more larvae experienced more severe damage (Fig. 1a; log-linear regression, $z = 4.158, P < 0.001$) and a reduced likelihood that the plant would be marketable (Fig. 1b; binary-logistic regression, $z = -3.400, P < 0.001$). We found that the single larva treatment most often caused minor twisting to leaf stems and florets, rendering cauliflower unmarketable 52% of the time. Ten larvae resulted in a range of damage from mild twisting of leaves to severe swelling and scarring of florets, rendering 68% of the plants unmarketable. Damage from the 20 larvae treatment also varied, but most often resulted in severe swelling and scarring in the developing crown, rendering 82% of the plants unmarketable.

The same trend held in the field potted plant trials, with more larvae causing more cauliflower damage (Fig. 2a, c, and e; log-linear regression, $z = 3.689, P < 0.001$) and a lower likelihood of marketability (Fig. 2b, d, and f; binary-logistic regression, $z = -2.894, P < 0.001$). Larval density and plant age at infestation did not have a significant interaction for damage ($z = -0.540$, NS) or marketability ($z = -0.213$, NS), meaning that similar patterns of damage and marketable losses were present across larval treatments for the different cauliflower age groups. Cauliflower age alone also did not directly influence damage or marketability ($z = 1.464, -0.572$; NS, NS). Altogether, our data suggest that midge larvae cause significant damage and marketable losses regardless of cauliflower age.

Discussion

Our results contribute important findings about swede midge: 1) half of the larvae perish following our inoculation procedure (see Materials and Methods); 2) any quantity of larval feeding can cause noticeable damage on cauliflower (Fig. 1a); 3) a single larva can render cauliflower unmarketable 52% of the time (Fig. 1b); and 4) damage and marketable losses occur regardless of cauliflower age at infestation (Fig. 2).

Given that there is essentially no larval threshold for swede midge on cauliflower, we suggest plants be protected for the entire season. Our trials tested larval densities that are much lower than those with multiple meristems. As mentioned, we still do not know whether larval excretions impact development throughout the plant or only within the infested meristem. If swede midge damage is localized to the developing meristem, plants with multiple growing points, like canola and Brussels sprouts, may only lose a portion of marketable growth. That said, whether the number or severity of infested meristems on crops with multiple growing points influences ultimate damage or marketability has not been tested.

Swede midge poses a major threat to *Brassica* production in their introduced range (Hallett and Heal 2001; Chen et al. 2011; Hallett and Sears 2013). While systemic insecticides and calendar-based foliar sprays effectively manage swede midge conventionally (Hallett et al. 2009a), regular spraying reverses previous gains in IPM programs developed for other *Brassica* pests (Hallett and Sears 2013; Rodriguez Salamanca 2014), like diamondback moth and imported white cabbage worm (Furlong et al. 2013). Furthermore, large-scale crop rotations can provide control for organic producers (Chen and Shelton 2009) but only temporarily on farms with limited acreage because a portion of pupae remain dormant in the soil (Readshaw 1966; Hallett 2007; Stuurt et al. 2012). To avoid dormant populations, growers are recommended to rotate at least 2 km away from previous *Brassica* plantings for at least 3 yr. Tactics that prevent mating between females from finding and ovipositing on host plants (e.g., repellents), males from finding and mating with females (e.g., pheromone mating disruption), and/or physically block midges from contacting *Brassica* crops (e.g., exclusion netting) would be more effective long-term solutions to manage swede midge organically.

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