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Simulated crop rotation systems control swede midge, *Contarinia nasturtii*

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Abstract

Contarinia nasturtii (Kieffer) (Diptera: Cecidomyiidae), a common insect pest in Europe and a new invasive pest in North America, causes severe damage to cruciferous crops. Many counties in Canada and the USA, in which *C. nasturtii* has not been previously reported, are at risk of being infested by *C. nasturtii*. Effectiveness of chemical control is limited, especially under high population pressure in fields. Alternative management strategies against *C. nasturtii* are sorely needed in order to protect crucifers. Under controlled laboratory conditions, the effectiveness on *C. nasturtii* control by 11 simulated cauliflower-sweet corn and cauliflower-kidney bean crop rotation systems was evaluated, with and without the presence of cruciferous weeds as alternative hosts. Our results indicated that when soil was infested with *C. nasturtii* pupae, the emergence pattern from the soil was very similar regardless whether the soil was later planted to host or non-host plants. As emergence was not affected, we examined whether manipulating host availability for oviposition through crop rotation would be effective. Our results indicated that the simulated cauliflower-sweet corn and cauliflower-kidney bean rotation systems provided full control of *C. nasturtii*. The effectiveness of one cycle of non-host crop rotation was reduced when cruciferous weeds were present. However, the *C. nasturtii* population in a one-cycle non-host rotation system with cruciferous weeds present was significantly lower than that in a non-rotation system. Two consecutive cycles (simulating a cropping season) of non-host plant crop rotations provided full control of *C. nasturtii*, regardless of the presence of the cruciferous weeds. The importance of cruciferous weed management and how to implement a successful crop rotation in fields to control *C. nasturtii* are discussed.

Introduction

In North America, the swede midge, *Contarinia nasturtii* (Kieffer) (Diptera: Cecidomyiidae), was first discovered in Ontario, Canada, in 2000 (Hallett & Heal, 2001) and has been subsequently identified in 32 counties in Ontario, 33 counties in Quebec, one location in Nova Scotia, and three locations in Saskatchewan in Canada (CFIA, 2007). In the USA, *C. nasturtii* was first reported in Niagara County, New York, in 2004 (Kikkert et al., 2006). By the end of 2005, *C. nasturtii* was detected in five major cabbage-producing counties (Erie, Genesee, Monroe, Orleans, and Wyoming) in New York State (Chen et al., 2007, 2009). However, in 2005 and 2006, our laboratory confirmed by molecular analyses (Frey et al., 2004) that *C. nasturtii* was present in 13 more counties in New York and one each in Massachusetts (Hampshire) and New Jersey (Sussex). By the end of 2007, *C. nasturtii* had been detected in a total of 25 counties in New York, in addition to the two counties in surrounding states. An additional *C. nasturtii* infestation was recently reported in New Haven County, Connecticut (http://www.hort.uconn.edu/ipm/general/biocntrl/swedemidge.htm).

Contarinia nasturtii adults emerge in early to middle May in North America from pupae, which have overwintered in the soil (Kikkert et al., 2002). Mating occurs soon after emergence and females then begin to search for suitable hosts. Host plants in the family Brassicaceae (= Cruciferae) are available in fields in mid-May (including many cole crops with a few leaves and winter annual

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cruciferous weed species) and they are at risk of being infested by *C. nasturtii*. Eggs are laid in clusters of 2–50 eggs on meristematic tissue of their host plants (Barnes, 1946), with each female laying about 100 eggs during her short (1–5 day) lifetime. The larval stage is the only life stage that can damage cruciferous plants. Damage symptoms include misshapen plants with twisted stems, crumpled leaves, swollen growing tips, multiple heads, and the formation of galls on leaves and flowers (Bardner et al., 1971; Kikkert et al., 2002), which can severely reduce product quality and marketability (Hallett & Heal, 2001). *Contarinia nasturtii* can have 3–5 overlapping generations per year in North America with the last adult flights in late September to early October (Kikkert et al., 2002; Hallett et al., 2008).

Contarinia nasturtii infestations have rapidly spread in North America, and control of *C. nasturtii* has proved difficult because of its wide host range, high reproductive potential, cryptic feeding behavior, and other biological features. Different control strategies have been tested in the USA and Canada (Wu et al., 2006; Chen et al., 2007; Chen & Shelton, 2007; Shelton et al., 2009; Hallett et al., 2009). Under laboratory conditions, synthetic insecticides provided good control of *C. nasturtii* larvae and adults (Wu et al., 2006; Chen et al., 2007). However, multiple-year/site insecticide field trials conducted in Canada and the USA indicated that chemical control provided unreliable and generally poor control of *C. nasturtii*, especially when *C. nasturtii* populations are high, though no insecticide resistance was detected (Hallett et al., 2009). In addition, one of our previous studies documented that soil manipulations, involving regulating soil moisture and depth of burying pupae, may help control *C. nasturtii* in fields; however, it is clear that soil management alone will not be sufficient (Chen & Shelton, 2007).

Golightly & Woodville (1974) reported that outbreaks of saddle gall midge, *Haplodiplosis equestris* Wagner, on wheat plants could be avoided in most years by means of crop rotation in conjunction with selection of less susceptible crops and early sowing. Similarly, Faheemah & Sulaiman (1990) also concluded that the number of cucurbit gall midge, *Lasioptera chichindae* Grover, in fields could be reduced by crop rotation. Control of *C. nasturtii* using crop rotation has also been suggested (Taylor, 1912; Rygg & Braekke, 1980; Theunissen et al., 1997; Kikkert et al., 2002; ISMTF, 2005); however, no data exists to confirm this.

During unfavorable environmental conditions, such as the absence of the preferred host plant, insects may enter diapause to increase the likelihood of survivorship, although the presence or absence of food is not a common cue for diapause induction or termination (Robbins, 1972; Denlinger, 1986). Carlisle et al. (1965) reported that terpenoids released from the buds of *Commiphora* shrubs could trigger reproductive maturation in the desert locust, *Schistocerca gregaria* Forsskål, further leading to a new generation of the insect. Based on the information above, in this paper, we first investigated whether the absence of host plants could alter *C. nasturtii* adult emergence pattern from infested soil. In addition, we simulated 11 crop rotation systems involving host and non-host plants under laboratory conditions to investigate whether crop rotation could be an effective strategy for *C. nasturtii* control.

Materials and methods

Insect culture and tested plants

A *C. nasturtii* population was initially received from Switzerland (from R. Baur, Swiss Federal Research Station for Horticulture, Wädenswil) in 2004 and was subsequently reared in a chamber under quarantine conditions at 22 °C, 75–78% r.h., and L16:D8 (Wu et al., 2006; Chen et al., 2007). The food plant was cauliflower, *Brassica oleracea* L. var. *botrytis*, cv. 'Snow crown' (Brassicaceae), with 8–10 true leaves.

The same cauliflower cultivar was also used as the *C. nasturtii* host plant in the simulated crop rotation systems. Cruciferous weeds, shepherd's purse, *Capsella bursa-pastoris* L., and wild mustard, *Sinapis arvensis* (DC.) L.C. Wheeler (both Brassicaceae), were used as alternative host plants (Chen et al., 2009), and sweet corn, *Zea mays* L. (Poaceae), and kidney beans, *Phaseolus vulgaris* L. (Fabaceae), were used as non-host plants for *C. nasturtii* in the simulated crop rotation systems. Previous studies have identified these plants as hosts or non-hosts (Stokes, 1953; Kikkert et al., 2002; Ellis, 2005; Hallett, 2007; Chen et al., 2009). All tested plants were seeded in 128-cell trays, and 3-week-old seedlings were transplanted in pairs into 13-cm-diameter pots and maintained in Cornell University/NYSAES greenhouses. Cauliflower, cruciferous weeds, sweet corn, and kidney beans used in the formal experiments below were ca. 3–4 weeks old after transplanting.

Impact of non-host plants on *Contarinia nasturtii* emergence pattern

First, we investigated whether different plants (host or non-host) can alter *C. nasturtii* adult emergence from infested soil and the impact of sweet corn, kidney beans, cruciferous weeds (shepherd's purse and wild mustard), and cauliflower on *C. nasturtii* emergence patterns.

Moistened Cornell Mix soil (http://www.backyardgardener.com/soil/soil12.html) (7–10 cm deep) was placed on the bottom of a wood-framed cage with netted sides (50 × 50 × 50 cm). Twenty sweet corn seeds were evenly seeded in the soil contained in each cage. Five cages were set up as five replicates and cages were placed in the rearing chamber. When sweet corn seeds started germinating, 50 *C. nasturtii* larvae (10 days old after oviposition) were moved with a tiny brush from the insect culture to the growing tip of an uninfested cauliflower plant (ca. 4 weeks old after transplanting). The plants were kept in the rearing chamber for 2 days, then cut at soil level. The growing tips of the plants were put on the surface of the soil in each cage and the soil was moistened with tap water using a hand sprayer as needed. One yellow sticky card (12 × 11 cm) was placed in each cage. The sticky card and the inner walls of each cage were checked daily and the number of *C. nasturtii* adults emerged in each cage was recorded until no more *C. nasturtii* adults emerged for 30 days.

The impact of kidney beans and cruciferous weeds (shepherd's purse and wild mustard seeded in the same cage, 10 seeds of each species) on the *C. nasturtii* emergence pattern was evaluated with the same experimental design and conditions as above. For all trials, five soil cages with 20 cauliflower seeds planted in the soil in each cage were set up as a positive control and another five soil cages without any plants in the soil (simulating fallow land) were set up as a negative control.

Simulated crop rotation systems tested for control of *Contarinia nasturtii*

In total, 11 rotation systems were set up, including host (cauliflower and the cruciferous weeds) and non-host plants (sweet corn and kidney beans) (Table 1) to assess the effectiveness of crop rotation on control of *C. nasturtii*. In order to evaluate whether and how long *C. nasturtii* could survive in a non-host crop cycle (henceforth referred to as a season), one and two planting seasons of non-host crops (sweet corn and kidney beans) were included in the simulated crop rotation systems either with or without the cruciferous weeds. Cauliflower plants used in three consecutive simulated seasons were used as a positive control, i.e., no non-host plant rotation was applied, in comparison with other rotation systems.

In each rotation system for simulated season 1 (Table 1), two potted plants were introduced into a wood-framed cage with netted sides (50 × 50 × 50 cm) filled 7–10 cm deep with moistened Cornell Mix soil. Ten *C. nasturtii* female adults and five male adults were introduced into the cage. The *C. nasturtii* adults and plants were kept in the cage for 15 days, then the plants were cut and placed on top of the soil in the cage to allow *C. nasturtii* larvae, if any, on the plants to pupate in the soil. *Contarinia nasturtii* emergence was checked daily. When first emergence occurred, two potted plants used in simulated season 2 (Table 1) were introduced into the cage (in cases where no *C. nasturtii* emergence was observed, the plants used in season 2 were introduced into the cage ca. 25 days after the plant introduction in season 1). No additional *C. nasturtii* adults were added into the cage and the plants of season 2 were again kept in the cage for 15 days, then the plants were cut and placed on top of the soil in the cage to allow *C. nasturtii* larvae, if any, on the plants to pupate in the soil. *Contarinia nasturtii* emergence in the cage was checked daily. When first emergence occurred from season 2 (in cases where no *C. nasturtii* emergence was observed, the plants used in season 3 were introduced into the cage ca. 25 days after the plant introduction in season 2), two potted cauliflower plants used in simulated season 3 (Table 1) in each rotation system were introduced into the cage. The cauliflower plants used in season 3 were taken out ca. 12 days after their introduction and the number of *C. nasturtii* larvae on the plants in each rotation system were counted under a stereomicroscope. For each rotation system, four cages were set up as four replicates. During the course of the above experiments, soil and plants in the cages were moistened/watered as needed

Table 1 Simulated crop rotation systems tested in the laboratory for *Contarinia nasturtii* control

Rotation system	Simulated season 1	Simulated season 2	Simulated season 3
1	Cauliflower	Cauliflower	Cauliflower
2	Cauliflower	Sweet corn	Cauliflower
3	Cauliflower	Kidney beans	Cauliflower
4	Cauliflower	Cruciferous weeds	Cauliflower
5	Cauliflower	Sweet corn + cruciferous weeds	Cauliflower
6	Cauliflower	Kidney beans + cruciferous weeds	Cauliflower
7	Sweet corn	Sweet corn	Cauliflower
8	Kidney beans	Kidney beans	Cauliflower
9	Cruciferous weeds	Cruciferous weeds	Cauliflower
10	Sweet corn + cruciferous weeds	Sweet corn + cruciferous weeds	Cauliflower
11	Kidney beans + cruciferous weeds	Kidney beans + cruciferous weeds	Cauliflower

and *C. nasturtii* populations, if any, were allowed to cycle in the cages without any additional *C. nasturtii* introduction. If cruciferous weeds were included in a rotation system, one pot each of shepherd's purse and wild mustard were concurrently placed in a cage alone or with other crops used in the simulated season (Table 1).

Data analysis

Data on *C. nasturtii* emergence rate, emergence time, and the number of *C. nasturtii* larvae per plant in different crop rotation systems were analyzed using Proc GLM in SPSS for Windows (SPSS, Chicago, IL, USA). Percentage data were transformed into arcsine √x before being subjected to statistical analysis, but untransformed means are presented. All statistical calculations were performed using SPSS package (version 11.5 for Windows).

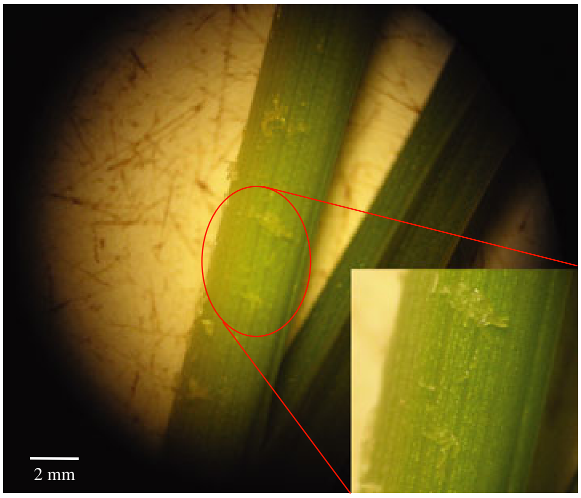

Figure 1 *Contarinia nasturtii* eggs laid on sweet corn plants.

Results

Impact of non-host plants on *Contarinia nasturtii* emergence pattern

Plant type did not significantly affect *C. nasturtii* emergence rate ($F_{4,20}$ = 0.066, P = 0.99) from previously infested soil (Table 2). Emergence time of *C. nasturtii* in the soil planted with the host plants (cauliflower or cruciferous weeds) and the non-host plants (sweet corn or kidney beans) was very similar to the fallow treatment ($F_{4,20}$ = 0.210, P = 0.93) (Table 2).

Simulated crop rotation systems tested for control of *Contarinia nasturtii*

No *C. nasturtii* larvae or feeding damage were found on the non-host plants (sweet corn or kidney beans) used in the different rotation systems, though *C. nasturtii* eggs were found on sweet corn plants (Figure 1). When the alternative host plants (cruciferous weeds) were absent in a rotation system, one planting season with the non-host plants (sweet corn or kidney beans) eliminated the *C. nasturtii* population in the following planting season of cauliflower plants (rotation systems 2 and 3 in Table 3). However, when the cruciferous weeds were present in a non-host crop season in a rotation system, the *C. nasturtii* population could survive to the next host crop season (rotation systems 5 and 6 in Table 3). Rotation with non-host crops effectively controlled *C. nasturtii* populations in comparison with consecutive plantings of the host crop (cauliflower) ($F_{10,33}$ = 49.384, P< 0.001). The number of *C. nasturtii* larvae in season 3 of three consecutive planting seasons of cauliflower plants (system 1) resulted in an average of 1 245.8 per cauliflower plant, whereas no *C. nasturtii* larvae were found in season 3 of the rotation systems including one non-host plant season without the cruciferous weeds present (systems 2 and 3), or two non-host plant seasons with (systems 10 and 11) or without (systems 7 and 8) the cruciferous weeds present (Table 3). In comparison with the control (i.e., no rotation applied; system 1), 101.3, 69.2, and 55.4 times fewer *C. nasturtii* larvae were found in season 3 of the rotation systems of cauliflower → cruciferous weeds → cauliflower (system 4), cauliflower → sweet corn + cruciferous weeds → cauliflower (system 5), and cauliflower → kidney beans + cruciferous weeds → cauliflower (system 6), respectively (Table 3).

Table 2 Impact of host and non-host plants on *Contarinia nasturtii* emergence pattern

Plants	Days to first emergence[1]	Emergence rate (%)
Cauliflower	11.8 ± 0.8a	30.8 ± 4.7a
Sweet corn	12 ± 1.2a	30.4 ± 5.5a
Kidney beans	11.6 ± 1.1a	32.8 ± 4.9a
Cruciferous weeds	11.2 ± 1.9a	30 ± 3.5a
Fallow	11.6 ± 1.8a	31.6 ± 5.2a

Means (± SE) followed by the same letter within a column are not significantly different (Fisher's protected LSD means separation test: P>0.05).

[1]Recorded from the day when cauliflower plants with *C. nasturtii* larvae were cut.

Discussion

Contarinia nasturtii is a serious pest that feeds on cruciferous plants (Barnes, 1946; Darvas et al., 2000) and is rapidly

Table 3 Number of *Contarinia nasturtii* larvae retrieved from cauliflower plants planted in simulated season 3 of a crop rotation system

Rotation system		
System	Planting pattern	*C. nasturtii* larvae/plant
1	Cauliflower → cauliflower → cauliflower	1 245.8 ± 59.3a
2	Cauliflower → sweet corn → cauliflower	0 ± 0c
3	Cauliflower → kidney beans → cauliflower	0 ± 0c
4	Cauliflower → cruciferous weeds → cauliflower	12.3 ± 4.5b
5	Cauliflower → sweet corn + cruciferous weeds → cauliflower	18 ± 13.1b
6	Cauliflower → kidney beans + cruciferous weeds → cauliflower	22.5 ± 15.3b
7	Sweet corn → sweet corn → cauliflower	0 ± 0c
8	Kidney beans → kidney beans → cauliflower	0 ± 0c
9	Cruciferous weeds → cruciferous weeds → cauliflower	0 ± 0c
10	Sweet corn + cruciferous weeds → sweet corn + cruciferous weeds → cauliflower	0 ± 0c
11	Kidney beans + cruciferous weeds → kidney beans + cruciferous weeds → cauliflower	0 ± 0c

Means (± SE) followed by different letters are significantly different (Fisher's protected LSD means separation test: $P<0.05$).

spreading in North America (CFIA, 2007; Chen et al., 2007, 2009). Crop rotation has been suggested as one strategy for *C. nasturtii* control, but no experiment had been conducted to test the effectiveness of crop rotation on control of *C. nasturtii*. In this paper, impact of plant type (host and non-host plants) on *C. nasturtii* emergence patterns was first investigated. The results indicate that *C. nasturtii* have similar emergence rates and emergence times from *C. nasturtii* infested soil subsequently planted to host plants (cauliflower or cruciferous weeds) or non-host plants (sweet corn or kidney beans) or fallow soil (Table 2), suggesting that plant type will not change *C. nasturtii* emergence patterns under the same environmental conditions. It may be inferred that presence or absence of host plants is not a major factor to induce or terminate diapause of *C. nasturtii*.

Levine et al. (1992a,b) reported that a strain of northern corn rootworm, *Diabrotica barberi* Smith & Lawrence, adapted to a corn–soybean rotation and caused damage to corn roots after a 1-year rotation with the soybean crop. Such an adaptive advantage of multiple-year phenotypes would likely diminish the effectiveness of a crop rotation system. In North America, *C. nasturtii* have 2–3 phenotypes with slightly different emergence patterns (Hallett et al., 2008), but it has not been demonstrated that any of the phenotypes has a prolonged diapause. In the present study, we used a strain of *C. nasturtii* originally obtained from Europe where it has been suggested, but not proved, that a proportion of the population does have a prolonged diapause. We were not able to characterize the phenotype of our strain to determine whether some individuals had a prolonged diapause. Thus, although our studies clearly indicate the potential for crop rotation to be a key component in the overall management of swede midge, additional longer term field studies should be undertaken.

Simulated cauliflower-sweet corn and cauliflower-kidney bean rotation systems provided full control of *C. nasturtii* (Table 3). However, the effectiveness of one season of non-host crop rotation on *C. nasturtii* control was reduced when cruciferous weeds were present, although the *C. nasturtii* population in one season of a non-host rotation system with cruciferous weed present was significantly lower than that in a non-rotation system (cauliflower plants only for three consecutive seasons). Under laboratory conditions, oviposition choice tests indicated that shepherd's purse, wild mustard, and other common cruciferous weeds were less-preferred host plants to *C. nasturtii*, i.e., fewer eggs and larvae were found on the weeds in comparison to the cauliflower plants (Chen et al., 2009). Furthermore, a 3-year field survey demonstrated that cruciferous weeds might not be able to maintain a *C. nasturtii* population in fields for >2 years when no preferable host plants, i.e., cruciferous vegetables, were available (Chen et al., 2009). Interestingly, the results of our simulated crop rotation systems indicated that two consecutive seasons of non-host plant rotation provided full control of *C. nasturtii*, even in the presence of cruciferous weeds (systems 10 and 11; Table 3), which agrees with our previous 3-year field survey data (Chen et al., 2009).

Contarinia nasturtii pheromone trap captures in or around cruciferous vegetables fields with normal weed management practices, have indicated *C. nasturtii* may have 3–5 overlapping generations per field season in North America (Hallett, 2007; Hallett et al., 2008). However, the number of *C. nasturtii* adults emerged from cruciferous weeds collected from the fields without cruciferous vegetables (their preferred host plants) present for 1–3 consecutive field seasons gradually declined until there was no *C. nasturtii* emergence during the third season (Chen et al., 2009). Pheromone trap captures also indicated that fewer *C. nasturtii* generations were found in the fields without cruciferous vegetables for two consecutive seasons compared with fields with cruciferous vegetables, even when cruciferous weeds were present in fields without cruciferous vegetables (M Chen, AM Shelton & CA Hoepting, unpubl.). Our confined cage studies indicated that *C. nasturtii* could not pass to the next generation when provided with one season of non-host plants (sweet corn or kidney beans), or two consecutive seasons of non-preferable host plants (shepherd's purse and wild mustard). Thus, it is clear that the lack of a suitable food source caused the termination of *C. nasturtii* life cycle in our confined cage studies. However, during a regular field season in North America, there may be staggered cruciferous vegetables and overlapping generations of cruciferous weeds present in a field, which would facilitate 3–5 *C. nasturtii* generations per field season. Our confined laboratory studies demonstrated the potential of achieving a successful cultural control of *C. nasturtii* with crop rotation; however, the short 'season' (with the very controlled presence or absence of host plant) used in our crop rotation simulation experiments necessitates further field studies which attempt to identify possible alternative hosts for *C. nasturtii* in crop rotation systems.

Contarinia nasturtii, long established in Europe, is still a problematic pest there due to a lack of effective control strategies, though chemical and cultural control methods (such as excluding fences, emulsions, soil management) have been tested in different regions (Hornig, 1953; Thygesen, 1966; Rygg & Braekke, 1980; Ouden et al., 1987; Theunissen et al., 1997; Wyss & Daniel, 2004). In the early 20th Century, Taylor (1912) suggested that crop rotation could be explored as a control strategy. Theunissen et al. (1997) reported that high *C. nasturtii* infestations on crucifers in The Netherlands were considerably reduced after a 2-year period without the cultivation of crucifers. However, *C. nasturtii* has a wide range of cruciferous host plants including cruciferous weeds and readily moves from one to another (Stokes, 1953).

The present study of 11 simulated rotation systems under controlled laboratory conditions clearly demonstrates the potential effectiveness of crop rotation on *C. nasturtii* control and the importance of cruciferous weed management when crop rotation is applied. *Contarinia nasturtii* and several other *Contarinia* species have been reported to undergo prolonged diapause for more than one winter season (Rygg & Braekke, 1980; Danks, 1987; Widenfalk & Solbreck, 2005). The *C. nasturtii* colony used in the present study was kept under laboratory conditions for ca. 4 years, although it was initially field-collected. Adaptation to laboratory conditions and limited genetic variability in the colony might have led to an absence of prolonged emergence phenotypes in our *C. nasturtii* colony, which would challenge the success of crop rotation in the field. However, others have argued that soil water capacity, photoperiod, and temperature are the major factors controlling the induction and termination of the diapause of *C. nasturtii* (Readshaw, 1966; Hallett et al., 2008). Olfert et al. (2006) reported that climatic conditions are favorable to *C. nasturtii* in North America, which may suggest that *C. nasturtii* are less likely to undergo a prolonged diapause in the area. However, longer-term field trials are needed to demonstrate successful control of *C. nasturtii* with crop rotation.

The spatial scale of the rotation will also influence the success of crop rotation. Although no studies have documented the distance *C. nasturtii* adults can move, it is likely that rotations of non-host plantings done in a limited space would not be as effective as when implemented over a larger landscape. Vegetable growers in upstate New York traditionally rotate their fields and plant cabbage every 3–5 years. Additionally, non-host crops such as sweet corn and beans are the common rotational vegetable crops and fields are generally >20 ha, a size that might reduce inter-planting colonization compared to smaller fields. These agronomic practices may reduce the likelihood of major outbreaks in this area, compared with sites with smaller, non-rotated crucifer fields or in areas of extensive planting of crucifers, such as canola.

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