Occurrence of the New Invasive Insect *Contarinia nasturtii* (Diptera: Cecidomyiidae) on Cruciferous Weeds

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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. In the United States, *C. nasturtii* was first reported in western New York in 2004. From 2005 to 2007, field surveys were conducted in western New York to investigate the occurrence of *C. nasturtii* in weeds that might serve as a reservoir for this pest. The results indicate that 12 cruciferous weed species were found in and around commercial vegetable crucifer plantings, and *C. nasturtii* emergence was detected from most of them. The number of *C. nasturtii* that emerged from the weeds was low and varied by species, year, and the timing of sampling. Peak emergence from weeds in fallow fields occurred in June. Nonchoice tests in the laboratory showed that significantly fewer larvae were found on cruciferous weeds than on cauliflower plants, although *C. nasturtii* could lay eggs on the weeds. When weeds and cauliflower plants were simultaneously exposed to *C. nasturtii* adults for egg laying (choice tests), 97.3% of the *C. nasturtii* larvae were found on the cauliflower plants 8 d after oviposition, 2.7% on *Sinapis arvensis* L., and none on the other five weed species tested. Our results suggest that cruciferous weeds can serve as alternative host plants of *C. nasturtii* but are less suitable than cauliflower. A method of detecting *C. nasturtii* on weeds and control of *C. nasturtii* through weed management are discussed.

KEY WORDS *Contarinia nasturtii*, swede midge, Brassicaceae, weed

Swede midge, *Contarinia nasturtii* (Kieffer) (Diptera: Cecidomyiidae), is a serious pest that feeds on plants in the Cruciferae (=Brassicaceae) family and is widely distributed in Europe and southwestern Asia (Barnes 1946, Darvas et al. 2000). In North America, *C. nasturtii* was first identified in Ontario, Canada, in 2000 (Hallett and Heal 2001). To date in Canada, *C. nasturtii* has been detected in 32 counties in Ontario and 33 counties in Quebec and in one location in Nova Scotia and three locations in Saskatchewan (CFIA 2007). In the United States, *C. nasturtii* was first reported in Niagara County, NY, 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. These discoveries were officially confirmed by the United States Department of Agriculture (USDA), based on both morphological and molecular evidence (Chen et al. 2007). However, in 2005 and 2006, our laboratory confirmed by molecular analysis (Frey et al. 2004) that *C. nasturtii* was present in 13 more counties in New York and one 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, CT (http://www.hort.uconn.edu/ipm/general/biocntrl/swedemidge.htm).

The main fresh cruciferous vegetables (broccoli, Brussels sprouts, cabbage, and cauliflower; all cultivars of *Brassica oleracea* L.) were grown on ≈121,400 ha in the United States and had a total value of $1.23 billion in 2006 (USDA ERS 2007). Based on temperature and rainfall data, many of the major cruciferous vegetable growing regions in the United States are suitable *C. nasturtii* habitats and at risk of being infested by *C. nasturtii* (Ellis 2005, Olfert et al. 2006). Plant damage results from larval feeding. 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. In Canada, *C. nasturtii* damage has accounted for up to 85% loss in market yield of Ontario crucifer vegetable crops (Hallett and Heal 2001). In some areas of Europe, *C. nasturtii* infestations may account for 100% loss in a grower's crop, despite treatment with insecticides (Ellis 2005).

Control of *C. nasturtii* has proved difficult because of its wide host range and high reproductive potential.

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Its host range includes all varieties of *Brassica oleracea* L., *Brassica napus* L., *Brassica rapa* L., and *Raphanus sativus* L., and many common weed species, such as mustard (*Brassica* spp.), wild radish (*Raphanus raphanistrum* L.), shepherd's purse [*Capsella bursa-pastoris* (L.) Medik.], field pepperweed [*Lepidium campestre* (L.) R. Br.], field pennycress (*Thlaspi arvense* L.), and yellow rocket (*Barbarea vulgaris* R. Br.) (Stokes 1953a,b; Kikkert et al. 2002; CFIA 2006; Hallett 2007). However, it is not clear what role cruciferous weeds serve in terms of the occurrence and population dynamics of *C. nasturtii* under field conditions and whether cruciferous weeds and vegetables are equally preferred by *C. nasturtii.* Furthermore, although *C. nasturtii* has a wide range of cruciferous food plants (Stokes 1953b), different host species may have different susceptibility to *C. nasturtii* damage (Hallett 2007).

In this article, we describe a 3-yr field survey in western New York to investigate the presence of *C. nasturtii* on cruciferous weeds in and around commercial fields of cruciferous vegetables to better understand the importance of weed hosts for *C. nasturtii* during cruciferous cropping and noncropping periods. In addition, we studied the oviposition preference of *C. nasturtii* to some common cruciferous weed species in North America, in comparison with a common crucifer vegetable plant (cauliflower), under choice and nonchoice conditions. These studies were undertaken with the hope that information on the role of cruciferous weeds could lead to improved management practices.

Materials and Methods

Field Survey of *C. nasturtii* on Cruciferous Weeds. *2005 Studies.* To investigate whether *C. nasturtii* could use cruciferous weeds as host plants under field conditions, field surveys were conducted in four different fields in Niagara and Erie counties in New York in September 2005, where *C. nasturtii* infestation had been reported previously (Chen et al. 2007). Cruciferous weeds, in and on the borders of each field, were collected, sorted into species, and placed in black plastic bags, which were then placed in a cooler and returned to the laboratory. One collection per field was made, and plants were selected randomly in and around the field with the goal of collecting ≈300 total plants per field. The presence of *C. nasturtii* on different cruciferous weeds was checked after the black plastic bags containing weeds had been left in the sun for several hours, which causes the larvae to leave the plants and crawl on the inside of the bag where the white larvae can be distinguished on the black plastic (Kikkert et al. 2002).

2006 and 2007 Studies. To complement the survey of 2005, more intensive field surveys were conducted monthly in two of the same fields (fields 1 and 2) in Niagara County plus a new field (field 3) in the same County in 2006 (June to August) and 2007 (May to October). Field 1 was planted to cabbage in 2005, planted to cabbage and broccoli in 2006, and was fallow in 2007. Field 2 was planted to cabbage in 2005 and was fallow in 2006 and 2007. Field 3 was fallow from 2005 to 2007. On each sampling date, weed samples were collected as in 2005. Weeds of a single species were placed on moist Cornell Mix soil (7–10 cm in depth) contained in a wood-framed cage with netted sides (50 by 50 by 50 cm), which served as a pupation site for larvae when they left the foliage. The weeds and soil were misted with tap water daily for 15 d. Two yellow sticky cards (each 15 by 15 cm) were placed in each cage to catch emerging *C. nasturtii* adults. All the weed samples were kept in the cages for 2 mo, and then they were dried in a hot air oven at 37°C overnight. Dry weight of each plant sample was recorded to provide a standard unit of measurement for density per plant of *C. nasturtii.* Adult midges captured by yellow sticky cards were examined under a dissecting microscope to identify *C. nasturtii*, and those identified to be *C. nasturtii* based on morphological characteristics were further confirmed by the molecular diagnostic technique described previously (Kikkert et al. 2006). For molecular identification, the mitochondrial cytochrome *c* oxidase subunit I gene (*COI*) sequence of *C. nasturtii* (GenBank accession no. EU812560) was used as the diagnostic molecular marker. Briefly, a 488-bp *COI* gene fragment was amplified from the specimens by a polymerase chain reaction (PCR) by using a pair of universal primers (5′-GGATCACCTGATATAGCATTCCC-3′ and 5′-CCCGGTAAAATTAAAATATAAACTTC-3′) and then a *C. nasturtii* diagnostic PCR was followed using the 488-bp PCR fragment as the temperate with a pair of diagnostic primers (5′-CAATTATTGGAGATACTCGAAGATGA-3′ and 5′-ATTCGAACTCCTGCTCCTATTCGATCTAGG-3′) (Frey et al. 2004, Kikkert et al. 2006). Positive amplification of a 286-bp, specific *C. nasturtii* PCR fragment indicates the positive identification of a *C. nasturtii* specimen.

Ovipositional Preference of *C. nasturtii* to Cruciferous Weeds under Laboratory Conditions. For our laboratory studies, we used a colony of *C. nasturtii* (Wu et al. 2006, Chen et al. 2007) reared at Cornell University's New York State Agricultural Experiment Station (NYSAES) in Geneva, NY, in a rearing chamber set at 22 ± 1°C, RH 75–80%, and a photoperiod of 16:8 (L:D) h. Field pennycress (*T. arvense*), wild radish (*R. raphanistrum*), wild mustard (*Sinapis arvensis* L.), field pepperweed (*L. campestre*), shepherd's purse (*C. bursa-pastoris*), and yellow rocket (*B. vulgaris*), common cruciferous weed species in North America (Uva et al. 1997), were used for host plant preference tests for *C. nasturtii* in comparison with cauliflower, *B. oleracea* variety *botrytis* 'Snow crown'. The weed and cauliflower seeds were seeded into 128-cell trays containing cells filled with Cornell Mix soil and 3-wk-old seedlings were transplanted in pairs into 10-cm-diam. pots. All plants were maintained in Cornell University/NYSAES greenhouses and submitted to the following experiments 3–4 wk after transplanting. Thus, all plants were 6–7 wk when tested.

Nonchoice Tests. A single pot containing two plants (either two weed plants of the same species or 2 Snow crown cauliflower plants) was placed in a wood-framed oviposition cage with netted sides (50 by 50 by

Table 1. Survey of *C. nasturtii* on cruciferous weeds in and around cruciferous vegetable fields in Niagara and Erie counties, New York state, in 2005

County	Field	Weed species	Common name	No. weeds	No. *C. nasturtii*
Niagara	1	*E. cheiranthoides*	Wormseed mustard	150	0
		S. officinale	Hedge mustard	11	0
		L. campestre	Field pepperweed	8	0
		S. arvensis	Wild mustard	5	0
		B. vulgaris	Yellow rocket	4	0
		C. bursa-pastoris	Shepherd's purse	2	0
		Berteroa incana	Hoary alyssum	1	0
	2	*E. cheiranthoides*	Wormseed mustard	157	0
		C. bursa-pastoris	Shepherd's purse	47	0
		S. arvensis	Wild mustard	29	0
Erie	1	*E. cheiranthoides*	Wormseed mustard	217	0
		R. raphanistrum	Wild radish	145	0
		S. arvensis	Wild mustard	25	0
		B. vulgaris	Yellow rocket	10	0
		R. islandica	Marsh yellowcress	9	0
	2	*C. bursa-pastoris*	Shepherd's purse	270	0
		B. vulgaris	Yellow rocket	48	0
		E. cheiranthoides	Wormseed mustard	12	0
		R. islandica	Marsh yellowcress	8	0
		B. incana	Hoary alyssum	1	0

Table 2. Survey of *C. nasturtii* on cruciferous weeds in and around cruciferous vegetable fields in Niagara County, New York state, in 2006

Field	Weed species	Common name	No. *C. nasturtii* adults /100 g weed (dry wt)		
			5 June	29 June	30 Aug.
1	*L. campestre*	Field pepperweed	—[a]	8.9	0
	E. cheiranthoides	Wormseed mustard	—	—	—
2	*L. campestre*	Field pepperweed	4	0.6	—
	S. arvensis	Wild mustard	4.2	3.0	—
	C. bursa-pastoris	Shepherd's purse	10.6	5.1	—
	E. cheiranthoides	Wormseed mustard	11.7	—	—
	R. islandica	Marsh yellowcress	—	14.4	0
3	*C. bursa-pastoris*	Shepherd's purse	1.6	25.6	—
	L. campestre	Field pepperweed	2.4	0.5	—
	S. arvensis	Wild mustard	3.0	0.4	—

[a] Weed species not found on the sampling date.

50 cm). Six *C. nasturtii* adults emerged within 24 h (4 female + 2 male), collected from the colony by using a mouth aspirator, were released into the cage and allowed to lay eggs. The pot was taken from the oviposition cage after 48 h and placed into another *C. nasturtii*-free cage in the rearing chamber. There were six replicates for each treatment. The number of *C. nasturtii* larvae on different cruciferous weeds was checked 8 d after oviposition and compared with that on cauliflower plants. Data on larval counts were analyzed using one-way analysis of variance (ANOVA) and Fisher protected least significant difference (LSD) means separation test (SPSS version 11.5 for Windows, SPSS Inc., Chicago, IL).

Choice Tests. Six pots of different weed species plus one pot of cauliflower plants were placed into an oviposition cage containing 42 *C. nasturtii* adults emerged within 24 h (28 female + 14 male) collected from the colony. Each pot contained two plants of the same species. The position of each pot in the oviposition cage was changed after 24 h to increase random oviposition (Wu et al. 2006), and the pots remained in the cage for 48 h. The choice test was replicated four times. Larval counts and data analyses were conducted as described in the nonchoice test.

Results

Occurrence of *C. nasturtii* on Cruciferous Weeds in Fields. In 2005, a total of 1,159 cruciferous weeds representing nine different species were collected from the four fields in Niagara and Erie counties: hedge mustard [*Sisymbrium officinale* (L.) Scop.], hoary alyssum (*Berteroa incana* L.), marsh yellowcress [*Rorippa islandica* (Oeder) Borbás], *C. bursa-pastoris*, *L. campestre*, *S. arvensis*, *R. raphanistrum*, wormseed mustard (*Erysimum cheiranthoides* L.), and *B. vulgaris* (Table 1). However, we did not find any *C. nasturtii* larvae or damage symptoms on the weeds. In 2006, a total of five different cruciferous weed species were collected from the three fields in Niagara County: *L. campestre*, *R. islandica*, *C. bursa-pastoris*, *S. arvensis*, and *E. cheiranthoides*. *C. nasturtii* adults were subsequently captured from each weed species in the emergence cages (Table 2). *COI* gene fragment PCR analyses of the adults from weeds confirmed the presence of *C. nasturtii* on the cruciferous weeds (Fig. 1). In 2006, *C. bursa-pastoris* generated the highest *C. nasturtii* emergence rate (25.6 *C. nasturtii*/100 g dry weeds) and the lowest rate was from *S. arvensis* (0.4 *C. nasturtii*/100 g dry weeds). In 2007, seven different weed species (*B. vulgaris*, *Arabidopsis thaliana* L., *T. arvense*, *L. campestre*, *S. officinale*, *C. bursa-pastoris*, and *Brassica rapa* L.) were collected from the three fields and *C. nasturtii* emergence was confirmed from *B. vulgaris*, *L. campestre*, *C. bursa-pastoris*, and *S. officinale* (Table 3).

Ovipositional Preference of *C. nasturtii* on Cruciferous Weeds under Laboratory Conditions. Under nonchoice conditions, *C. nasturtii* laid eggs on each

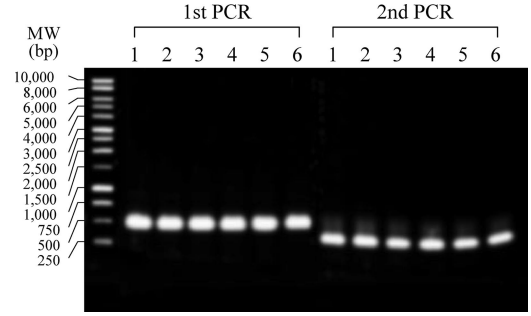

Fig. 1. PCR analysis of *C. nasturtii* adults emerged from cruciferous weed species collected from Niagara County, NY, in 2006. *C. nasturtii* adults were from laboratory colony (positive control; 1), *L. campestre* (2), *S. arvensis* (3), *E. cheiranthoides* (4), *C. bursa-pastoris* (5), and *R. islandica* (6) M, DNA molecular weight markers.

Table 3. Survey of *C. nasturtii* on cruciferous weeds in and around cruciferous vegetable fields in Niagara County, New York state, in 2007

Field	Weed species	Common name	No. *C. nasturtii* adults/100 g weed (dry wt)				
			29 May	29 June	30 July	1 Sept.	1 Oct.
1	*B. vulgaris*	Yellow rocket	0	15.3	—	—	—
	A. thaliana	Mouse-ear cress	0	—[a]	—	—	—
	T. arvense	Field pennycress	0	—	—	—	—
	L. campestre	Field pepperweed	0	11.8	0	0	0
	S. officinale	Hedge mustard	3.1	9.4	1.4	8.8	0
	C. bursa-pastoris	Shepherd's purse	2.5	9.8	—	—	—
	B. rapa	Field mustard	0	—	—	—	—
2	*L. campestre*	Field pepperweed	0	7.8	0	—	—
	B. vulgaris	Yellow rocket	0	—	—	—	—
	B. rapa	Field mustard	0	0	—	—	—
3	*B. vulgaris*	Yellow rocket	0	—	—	—	—
	L. campestre	Field pepperweed	0	0	—	—	—
	B. rapa	Field mustard	—	0	—	—	—

[a] Weed species not found on the sampling date.

weed species (Table 4); however, the ovipositional potential was significantly different between cruciferous weeds and cauliflower plants ($F = 5.937$, df = 6, 44; $P < 0.001$). Significantly fewer *C. nasturtii* larvae were found on weed plants 8 d after oviposition than were found on cauliflower. Under choice conditions, *C. nasturtii* laid nearly all their eggs on cauliflower plants (Table 4) and showed significant oviposition preference for cauliflower plants rather than cruciferous weeds ($F = 8.768$, df = 6, 21; $P < 0.001$).

Discussion

C. nasturtii is rapidly spreading in North America (CFIA 2007, Chen et al. 2007). Aside from its high reproductive potential, perhaps one of the most important reasons for its spread is the wide range of cruciferous weeds that may serve as reservoirs during cropping and noncropping periods.

The results of our field surveys in 2005, 2006, and 2007 showed that >10 different cruciferous weed species were found in and around cruciferous vegetable crops in western New York. Furthermore, the presence of *C. nasturtii* on most species was confirmed by the emergence cage trials in 2006 (Table 2) and 2007 (Table 3) and the PCR analysis, which is the first molecular evidence confirming the actual presence of *C. nasturtii* on cruciferous weeds (Fig. 1). That no *C. nasturtii* were found on the weed samples collected in 2005 (Table 1) most likely resulted from the September sampling timing and the inability of the "black plastic bag" method to detect small numbers of larvae. The cruciferous weeds we found in our surveys generally had small amounts of foliage and a small growing tip, compared with vegetable crucifers, hence limiting oviposition and feeding sites. Thus, the architecture of the weeds may make them poor hosts as shown in Table 4; however, other chemical or morphological factors also may contribute to this phenomenon. The results of the field surveys of 2006 and 2007 indicated that the *C. nasturtii* emergence peak in weeds in fallow fields occurred in June each year, and this is consistent with emergence trap data for *C. nasturtii* in cole crop fields in 2001–2003 in Canada (Hallett 2007). This suggests that weeds may play a role as a reservoir for *C. nasturtii* in and around commercial plantings of cruciferous vegetables. These species may be particularly important biological bridges for *C. nasturtii* in fall cole crops that may be planted as late as mid-July in New York.

Our results from the choice tests demonstrated that *C. nasturtii* preferred to oviposit and feed on cauliflower rather than the six weed species tested. When cruciferous vegetable and weed plants were simulta-

Table 4. Ovipositional preference of *C. nasturtii* on cruciferous weeds and cauliflower under choice and nonchoice conditions

Species tested	Cultivar or common name	No. *C. nasturtii* larvae/plant	
		Non-choice test	Choice test
B. oleracea	Cauliflower 'Snow Crown'	44.3 ± 6.0a	47.3 ± 10.2a
R. raphanistrum	Wild radish	15.0 ± 8.8b	0b
T. arvense	Field pennycress	11.5 ± 5.9b	0b
C. bursa-pastoris	Shepherd's purse	9.6 ± 5.6bc	0b
L. campestre	Field pepperweed	2.9 ± 1.1c	0b
S. arvensis	Wild mustard	2.8 ± 1.3c	1.3 ± 1.3b
B. vulgaris	Yellow rocket	1.4 ± 0.8c	0b

Means ± SE followed by different lowercase letters within a column are significantly different based on Fisher's protected LSD means separation test ($P < 0.05$).

neously exposed to *C. nasturtii*, a large number of *C. nasturtii* larvae (47.3 ± 10.3) was found on the cauliflower plants, whereas none was found on the tested weed plants except for *S. arvensis* (1.3 ± 1.3) (Table 4), suggesting that the tested cruciferous weeds were less suitable for *C. nasturtii.* However, it should be noted that under field conditions, several of the weed species tested would typically be larger than the crop during the early spring and therefore potentially more attractive alternative hosts than suggested in the greenhouse choice tests. In particular, the winter annual species *T. arvense* and *B. vulgaris* are often 0.6–0.9 m tall and flowering in spring at a time when many cole crops have only a few leaves. In contrast, *R. raphanistrum* and *S. arvensis* do not typically overwinter in New York. The observed oviposition on these two species in the choice test, as well as on smaller winter annual species (*C. bursa-pastoris* and *L. campestre*) probably more closely reflects what would happen under field conditions.

However, under nonchoice conditions, *C. nasturtii* laid eggs on the cauliflower plants and the different cruciferous weeds, although the number of *C. nasturtii* larvae found on the weeds was significantly lower than that on the cauliflower (Table 4). This suggests that *C. nasturtii* can use cruciferous weeds as host plants when preferable cruciferous vegetable plants are not available in fields. Observations of *C. nasturtii* on cruciferous weeds in the absence of a cruciferous crop (Tables 2 and 3) also support this hypothesis. Based on the above-mentioned results, it may be inferred that, during the vegetable season, cruciferous weeds in fields or on surrounding borders may not be a major source for *C. nasturtii;* however, cruciferous weeds in rotational crops, or winter annuals growing before cole crop establishment may serve as important alternative hosts to *C. nasturtii.* Thus, it is necessary to pay more attention to weeds during the vegetable offseason, because they may serve as a starting host until more attractive cruciferous vegetables become present.

It should also be noted that the number of *C. nasturtii* that emerged from the weed samples collected from each field in Niagara County seemed to be related to the cruciferous vegetable plantings in or around the field. For example, after field 3 was fallow for two consecutive field seasons (2005 and 2006), no *C. nasturtii* emergence from weeds in or around the field was detected in 2007. Similarly, *C. nasturtii* emergence from weeds in or around field 2 was markedly reduced in 2007 after the field was fallow for 1 yr (2006). Along with the fact that *C. nasturtii* adults are weak fliers (Ellis 2005), this supports the recommendation of crop rotation to a nonhost crop or a fallow period as an effective approach to control *C. nasturtii* (Taylor 1912, Rygg and Braekke 1980, Theunissen et al. 1997, ISMTF 2005); however, additional studies are needed to quantify the impact that weed species have on *C. nasturtii* populations when fields are rotated out of cruciferous crops. Golightly and Woodville (1974) reported that outbreaks of saddle gall midge, *Haplodiplosis equestris* Wagner (Diptera: Cecidomyiidae), 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 and Sulaiman (1990) also concluded that the number of cucurbit gall midge, *Lasioptera chichindae* Grover (Diptera: Cecidomyiidae), in fields could be effectively reduced by crop rotation.

Our results and previous reports (Barnes 1946; Stokes 1953a,b; Kikkert et al. 2002; CFIA 2006; Hallett 2007) suggest that many common cruciferous weed species can be used by *C. nasturtii* as host plants, especially when more suitable cruciferous vegetable host plants are not available, although such weeds are less preferred. In addition, our 3-yr field survey results demonstrate that populations of *C. nasturtii* exist in cruciferous weeds in fields. Thus, to achieve better control of *C. nasturtii*, weed control and field sanitation should be considered important components for best management practices of *C. nasturtii.*

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