Detection of *Contarinia nasturtii* (Diptera: Cecidomyiidae) in New York, a New Pest of Cruciferous Plants in the United States

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J. Econ. Entomol. 99(4): 1310–1315 (2006)

ABSTRACT The midge *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae) was first confirmed in North America in Ontario, Canada, in 2000. The insect is now distributed throughout many counties in the provinces of Ontario and Québec. Nearly 1,200 farms in the northeastern United States that grow cruciferous vegetables are at risk for *C. nasturtii* infestation if this insect were to spread to that region. Over a period of 3 yr (2002–2004), ≈3,000 ha of crops on 94 farms in western New York State was scouted for *C. nasturtii*, but none were found. In 2004, 42 experimental pheromone traps were placed in fields of cruciferous vegetables in eight counties. *C. nasturtii* males were captured at low levels (1–50 per trap/8 wk) on four farms in Niagara County, but not at any other site. *C. nasturtii* larvae were found in plant tissue at one of the four farms. Insect specimens were identified by morphological methods, molecular methods, or both. This is the first confirmation of *C. nasturtii* in the United States, which we believe was made possible by the combined use of pheromone traps, morphological characters of trapped adults, and molecular methods. The early detection in New York presents an opportunity to implement measures to limit the spread and establishment of *C. nasturtii* across the state and into other regions of the United States.

KEY WORDS *Contarinia nasturtii*, Brassicaceae, cruciferous vegetables, molecular identification, pheromone trap

The midge *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae) is a serious pest of plants in the Brassicaceae. The insect is widespread and endemic in regions of Europe and southwestern Asia where cruciferous crops are grown. Female *C. nasturtii* oviposit their eggs in clusters on young plant tissue. The larvae feed gregariously at the growing tips of a plant and result in gall-like distortions, deformed plant tissue, and corky brown scars (Barnes 1946, Gagné 1989). Most commonly cultivated cruciferous vegetables such as *Brassica oleraceae* L. (broccoli, cauliflower, Brussels sprouts, cabbage, kale, and gai lan), *Brassica napus* L. (rutabaga and swedes), and *Raphanus sativus* L. (radish) can be severely damaged. *C. nasturtii* also has been reported on canola (*B. napus* L.) and common weed species, including pennycress (*Thlaspi arvense* L.), wild radish (*Raphanus raphanistrum* L.), and shepherd's purse [*Capsella bursa-pastoris* (L.) Medikus] (Barnes 1946, Stokes 1953, Nijveldt 1969, Darvas et al. 2000).

In 2000, *C. nasturtii* was identified in Ontario, Canada, the first record of occurrence in North America (Hallett and Heal 2001). Damage on broccoli plants presumably caused by this pest was observed as early as 1996, but it was erroneously attributed to a nutrient deficiency. Crop losses on some Canadian farms due to *C. nasturtii* have been reported to be as much as 85% (Hallett and Heal 2001). In 2001, researchers surveyed cruciferous crop fields with yellow sticky cards (Hallett and Heal 2001). Although *C. nasturtii* is not attracted to colored sticky cards and it turned out to be difficult to identify small midge species on the cards, good evidence for the presence of *C. nasturtii* was found. In 2002, the Canadian Food Inspection Agency (CFIA) began a nationwide survey of crucifer fields for *C. nasturtii.* Surveyors scouted fields to look for symptoms of crop damage from *C. nasturtii.* A plant was considered infested if *C. nasturtii* larvae were found in the plant tissue. After 3 yr of surveys (2002–2004) by the CFIA, *C. nasturtii* was found on crops in 14 counties in Ontario and four counties in Québec (CFIA 2005) (Fig. 1).

The main fresh market cruciferous vegetables (broccoli, Brussels sprouts, cabbage, and cauliflower) were grown on nearly 121,400 ha in the United States and had a total value of $1.2 billion in 2003 (USDA–ERS 2005). Canola is annually grown on >400,000 ha. Many of the major crucifer-growing regions fall within USDA hardiness zones 4–8, which are considered

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0022-0493/06/1310–1315$04.00/0

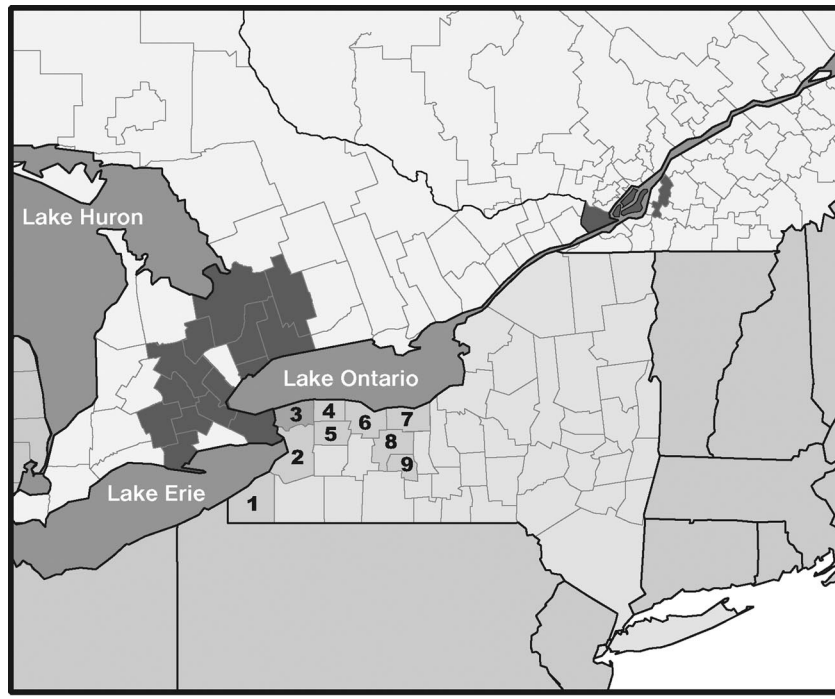

Fig. 1. Map of counties surveyed for *C. nasturtii*. The dark shaded counties in Ontario and Québec, Canada, denote areas where *C. nasturtii* was found on cruciferous crops as the result of the 2002–2004 CFIA survey. The numbered counties in New York were scouted by us in 2002–2004 and had pheromone trap sites in 2004 (except county 1). County names are 1) Chatauqua, 2) Erie, 3) Niagara, 4) Orleans, 5) Genesee, 6) Monroe, 7) Wayne, 8) Ontario, and 9) Yates. *C. nasturtii* were detected in Niagara County in 2004. Original map with county boundaries was obtained from K. Eggleston (Northeast Regional Climate Center, Cornell University, Ithaca, NY).

suitable as *C. nasturtii* habitats (Ellis 2005). Because of the proximity of infested areas in Canada, the northeastern United States is particularly vulnerable to invasion by this pest. By far, cabbage is the most important crucifer in the northeast, accounting for 83% of the total hectares produced and grown on >1,200 farms. New York state ranks second in the country in cabbage production and establishment of *C. nasturtii* in New York, especially in the western region where the majority of cabbage is grown and an area that borders the already infested areas of Ontario, Canada, could lead to substantial economic loss and serve as a source for movement to other U.S. states.

The purpose of this study was to survey fields of cruciferous vegetables in the major production region of western New York state for the presence of *C. nasturtii* and its damage. In 2004, scouting was complemented by the use of a recently developed pheromone trap (Hillbur et al. 2005) and molecular identification of captured insects.

Materials and Methods

Field Scouting. Scouting for *C. nasturtii* in fields of cruciferous vegetables was conducted from mid-July through September 2002, 2003, and 2004, the most likely time to find damage and larvae in the field (Goodfellow 2005). To further increase the likelihood of detecting *C. nasturtii*, growers were asked to identify fields that might be at higher risk for infestation. From the literature and conversations with European and Canadian colleagues, we determined that such fields included sheltered fields bordered by trees or hedgerows, fields where crop rotation out of crucifers was <2 yr, and cabbage fields with the putatively more susceptible red varieties. Most fields were surveyed only once per year because it was considered more important to cover a larger number of fields. Plant damage, even on fully mature crucifers, is most visible if it is caused by infestations initiated before the heading stage, so the time of field scouting was adjusted to maximize the likelihood of detecting plant damage.

Scouts closely inspected plants for possible *C. nasturtii* damage while walking the perimeter and a zigzag through the middle of each field. Before initiation of scouting, personnel were trained to identify *C. nasturtii* damage and larvae at infested sites in Ontario, Canada, and scouts carried pictures of damaged plants during field inspections. *C. nasturtii* damage symptoms were noted as abnormal plant growth at the center of the plant, loss of the growing tip, multiple heads or stems, twisted or puckered leaves, swollen petioles, and brown scarring. Plants with suspect *C. nasturtii* damage were examined in the field for *C. nasturtii* larvae by using a hand lens (10×). The growing points

of suspect plants were collected in plastic bags or vials of alcohol and brought back to the laboratory for observation with a lighted magnifying glass (2×) or dissecting microscope (2–20×).

Over a 3-yr period, nearly 3,000 ha of cruciferous crops on 94 farms was scouted for *C. nasturtii* damage symptoms and larvae. The area covered represented 60 townships within nine counties of western New York state (Fig. 1). The percentage of crucifer area scouted was 11.8% in 2002, 28.3% in 2003, and 6.1% in 2004. The crop types in the surveyed fields were cabbage (62%), cauliflower (13%), broccoli (11%), Brussels sprouts (6%), Chinese cabbage (3%), collards (2%), kale (2%), or other (1%).

Pheromone Traps. Pheromone lures and traps were prepared as described in Hillbur et al. (2005). Pieces of dental cotton rolls (length 1 cm), mounted on cardboard holders, were impregnated with the three-component pheromone blend and sealed in gastight bags until use. Delta traps made of brown, waxed cardboard were provided by PheroNet AB (Alnarp, Sweden). The traps were 10 cm in height and were equipped with a white 15.5- by 9-cm insert covered with a thin layer of Tangle Trap insect trap coating (Tanglefoot, Grand Rapids, MI). When traps were assembled, dispensers were positioned 1–2 cm above the insert. Traps were attached to wooden or metal stakes in the field such that the bottom of the trap was 30 cm above the ground, which positioned them within the upper canopy of the crop. The sticky liners were changed weekly and the pheromone inserts were changed every 4 wk. Traps were placed at the edges of crucifer fields that were sheltered by trees or hedgerows, or, to reduce interference with fieldwork, in the border between the crop and hedgerow. Fields selected for pheromone trapping were chosen from the broader samples of fields monitored for plant injury in 2004. Fields were selected to obtain the widest distribution across the cole crop production areas of western New York. In total, 42 pheromone traps were placed in eight western New York counties (Fig. 1). In 38 fields, one trap was placed in each field for 8 wk to correspond with the predicted peak summer flights of *C. nasturtii* (Hallett and Heal 2001, Goodfellow 2005). The initial placement of traps occurred from 15 July through 12 August. In two trap locations (one location in Genesee County and one location in Ontario County), there were two traps per field, and these traps were kept in fields from May through October 2004 as part of a large international study to determine specificity of the pheromone traps (R.B. et al., unpublished).

Insect Identification. Suspected *C. nasturtii* were identified by morphological characteristics, molecular methods, or both.

Morphological Characteristics. Sticky cards were examined for the presence of *C. nasturtii* males by using a dissecting microscope (2–64×). During the initial screening, the antennae and wing venation patterns were used (Harris 1966, Skuhravá 1997). Suspect *C. nasturtii* were mounted on glass slides in Canada balsam, as described by Gagné (1989). Expert identification was provided by Dr. R. Gagné (Systematic Entomology Laboratory, USDA–ARS, Beltsville, MD).

Molecular Methods. Molecular identifications of *C. nasturtii* were performed using a modified version of the polymerase chain reaction (PCR)-based diagnostic method developed by Frey et al. (2004). DNA from a single specimen or part of a specimen was extracted by homogenization in 10.0 μl of sample lysis buffer [10.0 mM Tris-HCl, pH 8.3, 50.0 mM KCl, 2.5 mM $MgCl_2$, 0.45% (vol:vol) Nonidet P-40, 0.45% (vol:vol) Tween 20, 0.01% (wt:vol) gelatin, and 60.0 μg/ml protease K] and then incubation at 65°C for 15 min. After incubation, the sample homogenate was heated at 95°C for 20 min to inactivate the protease K activity. A 440-bp fragment of the mitochondrial cytochrome *c* oxidase subunit I gene (*COI*) from each specimen was amplified by PCR by using 1.0 μl of sample homogenate and primers C1-J-1751 (5′-GGATCACCTGATATAGCATTCCC-3′) and C1-N-2191 (5′-CCCGGTAAAATTAAAATATAAACTTC-3′) (Simon et al. 1994), as described by Frey et al. (2004). The 440-bp *COI* fragment was then used as the template for a second PCR analysis by using *C. nasturtii COI*-specific primers (SM-FN1, 5′-CAATTATTGGAGATACTCGAAGATGA-3′ and SM-RN1, 5′-ATTCGAACTCCTGCTCCTATTCGATCTAGG-3′) to differentiate *C. nasturtii* from non-*C. nasturtii* specimens (Frey et al. 2004). The PCR was prepared in a 25.0-μl reaction containing 1.0 μl of a 1:50 dilution of the first PCR product as a template, 2.5 μl of 10× *Taq*DNA polymerase buffer, 2.5 U of *Taq*DNA polymerase (New England Biolabs, Beverly, MA), 1.0 μl of 10.0 mM dNTPs, 1.0 μl of 10.0 μM primer SM-FN1, and 1.0 μl of 10.0 μM primer SM-RN1. The PCR reaction was performed in an Eppendorf Mastercycler by a 2-min initial denaturation at 94°C, followed by 40 cycles of 40-s denaturation at 94°C, 40-s annealing at 44°C, and 40-s extension at 72°C, and a final 10-min extension at 72°C. Because nested primers SM-FN1 and SM-RN1 are specific to the *C. nasturtii COI*, amplification of a 280-bp fragment from the second PCR indicated the identification of *C. nasturtii* (Frey et al. 2004).

To confirm the correct identification of *C. nasturtii* by the PCR analysis, at least one PCR-positive specimen from each location (farm) was selected, and its 440-bp *COI* fragment (490 bp including the primers from the first PCR reaction) was sequenced. For DNA sequencing, the 440-bp *COI* PCR fragment was separated by 1.2% (wt:vol) agarose gel electrophoresis and excised from the agarose gel. The DNA fragment was recovered from the gel slice by using the QIAEX II gel extraction kit from QIAGEN (Valencia, CA) and sequenced at the Biotechnology Resource Center (Cornell University, Ithaca, NY). In addition, the *COI* fragment from a laboratory *C. nasturtii* colony, which was imported from Switzerland and maintained in our quarantined *C. nasturtii* rearing facility, was sequenced. The sequence obtained from this laboratory colony together with the *C. nasturtii COI* sequences available from the GenBank sequence database (accession nos. AY485371–AY485378) were used as the

Table 1. Results of insect identification from sticky cards in the *C. nasturtii* pheromone traps

Trap no.[a]	County	2004 trap date	No. suspects[b]	Morphological identification[c]			Molecular identification	
				Negative	Positive	Undetermined[d]	Negative	Positive[e]
2*	Niagara	23 Aug.–3 Sept.	1	0	0	0	0	1
4*	Niagara	13–20 Aug.	3	0	0	2	0	1
		27–30 Aug.	1	0	0	0	0	1
5	Niagara	15–23 July	1	0	0	1	0	0
		23–30 July	2	0	0	1	1	0
8*	Niagara	16–26 July	1	1	0	0	0	0
		26 July–2 Aug.	4	0	0	0	0	1
		9–16 Aug.	2	0	1	0	0	0
		16–23 Aug.	14	0	0	0	0	2
		23–30 Aug.	26	0	1	2	0	4
		30 Aug.–3 Sept.	2	0	0	0	0	0
		3–14 Sept.	3	0	0	0	0	0
9*	Niagara	23–30 Aug.	2	0	0	0	0	2
36	Genesee	6–12 July	1	0	0	1	0	0
OWYS-4	Ontario	27 Aug.–3 Sept.	3	0	0	0	3	0
OWYS-8	Ontario	3–12 Aug.	1	0	0	0	1	0

All traps not listed in this table had zero suspected *C. nasturtii.*

[a] Asterisk (*) denotes *C. nasturtii*-positive site.

[b] Initial screening by Q.W.

[c] Determined by R. Gagné.

[d] Determination not possible because of inadequate specimen preparation.

[e] Positive specimens were those that showed the presence of the 280-bp PCR fragment by the diagnostic PCR analysis.

reference sequence for confirmation of the *C. nasturtii* specimens identified by PCR analysis. Comparative analysis of *COI* sequences is a highly effective method to identify animal species, including insects (Hebert et al. 2003). The COI sequence from the swede midge specimens reported in this article was identical to that from the laboratory *C. nasturtii* colony and the known *C. nasturtii COI* sequence from the GenBank, confirming the morphological and PCR identification of *C. nasturtii* from the survey. DNA sequencing of the selected specimens positively identified to be *C. nasturtii* by PCR analysis did not show any specimens falsely identified by PCR analysis.

Results

C. nasturtii-like damage symptoms were found at low levels (<1% of the plants inspected) in some fields throughout the survey area in all years. The symptoms included damaged growing points, multiple stems or heads, distorted leaves, and brown scarring. In total, 1,493 plant samples were collected and examined in the laboratory for larvae. No *C. nasturtii* larvae were found in these samples or when field scouting was the only method of survey.

Most pheromone traps captured only a small total number of insects. Putative *C. nasturtii* were collected at eight of the 40 trap sites (Table 1). Four farms in Niagara County were considered positive after R. Gagné identified two specimens as *C. nasturtii,* and an additional 12 specimens tested positive by using the molecular method. At three of the four farms (2, 4, and 9), less than four putative *C. nasturtii* males were captured per farm, and no larvae were found. At trap site 8, 52 putative *C. nasturtii* in total were caught from 16 July to 14 September. The largest number of *C. nasturtii* adults was caught between 16 and 30 August. Based on the results of the pheromone trapping, site 8 was considered to have the highest probability for detection of *C. nasturtii* larvae on the crop plants. Several attempts to find larvae in this field in August and September failed. However, on 27 September, 10 trained individuals performed intensive scouting of this 0.2-ha broccoli field for a 2-h period. Twenty-nine larvae were recovered from five plants. Five of the larvae were analyzed by the molecular method and found to be positive. Six were preserved in 95% ethanol. Fifteen adult females were reared from the remaining 18 larvae. Eight of the adults were determined to be *C. nasturtii* by molecular methods, and R. Gagné morphologically confirmed three others to be *C. nasturtii.*

The farms that tested positive for *C. nasturtii* were all within 38 km of the Canadian border. Farm 4 is in Ransomville, NY, 8 km due east of the Canadian border and ≈17 km northeast of Niagara Falls, NY. Farm 2 is 12 km directly east of farm 4. Farms 8 and 9 are within 1 km of each other just north of Lockport, NY, and are ≈18 km northeast of farm 4. Two of the farms were small- (<5 ha) to medium (<20 ha)-sized fresh market operations with broccoli (2 and 9), cauliflower (2 and 9), Brussels sprouts (2), Chinese cabbage (9), and green cabbage (9). One farm (8) was a small grower–processor of broccoli. The other farm (4) was large (>20 ha) and produced green and red storage cabbage. All of the *C. nasturtii*-positive farms were under conventional production.

Discussion

A large-scale effort from 2002 to 2004 to scout fields for damage symptoms and larvae, similar to the survey conducted by the CFIA, failed to detect *C. nasturtii* in New York. Infestations and the presence of *C. nasturtii*

could only be detected with the use of pheromone traps. Problems with scouting for *C. nasturtii* include damage symptoms being similar to other maladies, such as injury from cultivation, insect and animal feeding, molybdenum deficiency, herbicide injury, genetic variation, and heat or cold stress; larvae may be present before symptoms are noticeable and by the time damage is seen, they have often left the tissue; and insects are difficult to find at low population levels (Barnes 1946).

The population of *C. nasturtii* in New York seems to be at low levels as indicated by the difficulty in detecting the insect, and there has been no reported economic damage. How *C. nasturtii* became established in New York is unclear, but it could be the result of movement of contaminated plant material or soil, or aerial movement by adults. Regardless, pheromone traps were useful in identifying fields where *C. nasturtii* adults were present at low levels, and they directed us to a field in Niagara County, NY, where we found larvae. It is uncertain whether the capture of one to four *C. nasturtii* on traps at three other Niagara County farms represents transient insect flights. Additionally, the lack of capture of *C. nasturtii* in other counties does not prove they are not present in the other counties. Lack of capture could be due to extremely low populations, limited flight abilities of *C. nasturtii*, low numbers of traps used, low efficacy of the traps, or a combination. The traps have been commercialized by PheroNet (distributed by Andermatt Biocontrol AG, Grossdietwil, Switzerland) and are in use nationally by USDA–Animal and Plant Health Inspection Service–Plant Protection Quarantine. These efforts should be accompanied by thorough studies on trap placement and longevity of the lure.

In field trials in Canada and several European countries, *C. nasturtii* pheromone traps proved to be very species specific (R.B. et al., unpublished), and we found similar evidence. The suspects on the traps that proved not to be *C. nasturtii* often landed on the liners in such a manner that morphological identification was difficult. Thus, not all midge species that may become caught in the traps can be easily distinguished from *C. nasturtii*. When new regions are being surveyed for this pest, there is a critical need to confirm the identity of suspected *C. nasturtii* in a trap. Currently, there are few individuals in the world trained to identify *Contarinia* species, and there are no keys to identification of the larval stage. These limitations present a challenge to any survey for the presence of *C. nasturtii*. However, we found the molecular method described by Frey et al. (2004) to be accurate, easy, and quick to perform. Their method was able to distinguish *C. nasturtii* from other closely related species in Europe, the center of origin for *C. nasturtii*. Thus, we are assured of the specificity of the PCR test for *C. nasturtii* in the United States. Furthermore, PCR is the only method available to identify the larval stage and adult specimens on sticky cards that are not intact or correctly positioned for morphological identification. We believe the molecular method, combined with pheromone trapping, should become the standard procedure for determining the presence of *C. nasturtii*. We also believe that the early detection of *C. nasturtii* in New York presents an opportunity to implement measures to limit its spread and establishment across the state and into other regions of the United States.

Acknowledgments

We are grateful for our hard-working field scouts N. Abbott, K. Bellows, J. Gibbons, G. Hudson, J. McCabe, and S. Torrey and our grower-cooperators who let us survey their farms. We also thank our colleagues in Canada for training us in *C. nasturtii* detection and for helpful discussions on survey procedures: K. Callow, L. Dumouchel, R. Favrin, H. Fraser, S. Goodfellow, R. Hallett, J. Heal, and M. Wood. S. Rauscher (Agroscope, Wädenswil, Switzerland) provided assistance with the pheromone traps, J. Frey (Agroscope) provided information about the PCR methods, and C. Klass (Cornell University) provided assistance with specimen mounting. The cooperation with USDA (S. Ellis, R. Gagné, D. Jewett, J. Smith, and J. Staples) and New York's Department of Agriculture and Markets (K. Carnes and R. Mungari) also is appreciated. The New York State Integrated Pest Management Program and Cooperative Agricultural Pest Survey Program provided funding for this project.

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Received 23 June 2005; accepted 3 April 2006.