A method for induction and quantification of diapause entry in the swede midge (Diptera: Cecidomyiidae)

Lauren E. Des Marteaux,[1] Marc B. Habash, Jonathan M. Schmidt, Rebecca H. Hallett,[1]

Abstract—Induction of diapause under laboratory conditions is a valuable tool for the study of dormancy in economic pests such as the swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae). In the present study, diapause in larval swede midge was achieved via manipulation of rearing photoperiod and temperature. Frequency of diapause was assessed by sieve separation of diapause cocoons from pre-sifted peat substrate following emergence of pupating individuals. Mean diapause frequency for swede midge larvae reared under cool conditions with short day length or cool conditions with decreasing day lengths were 45.2% and 19.5%, respectively. Only 1.2% of swede midge reared under warm, long day length conditions entered diapause. A small percentage of larvae neither pupated nor entered diapause and remained in substrate long after other individuals had emerged as adults. This behaviour was more prevalent under cool and short or decreasing day length rearing conditions. Approximately 76% of the larvae used for diapause induction were recovered with the present larval and cocoon retrieval method, and premature (larval and pupal) mortality averaged 18.2%. Although diapause occurred in the present study, conditions resulting in higher diapause frequencies should be investigated and attempts should be made to improve survival and recovery of individuals.

Résumé—L'induction de la diapause dans des conditions de laboratoire est un outil précieux pour l'étude de la dormance chez les insectes ravageurs d'importance économique, tels que la cécidomyie du chou-fleur, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae). Dans notre étude, des manipulations des photopériodes et des températures d'élevage ont provoqué la diapause chez les larves de la cécidomyie du chou-fleur. La récupération des cocons en diapause par tamisage du substrat de tourbe pré-tamisée, après l'émergence des individus qui ont complété la nymphose, a permis d'estimer la fréquence de la diapause. La fréquence moyenne de la diapause est de 45,2% chez les larves de la cécidomyie du chou-fleur élevées en conditions fraîches et en photophase courte et de 19,5% en conditions fraîches avec durée décroissante de la photophase. Seulement 1,2% des larves de la cécidomyie du chou-fleur élevées dans des conditions de jours longs et chauds entrent en diapause. Un petit pourcentage de larves n'entre ni en nymphose ni en diapause et demeure dans le substrat longtemps après que les autres individus aient émergé comme adultes. Ce comportement est plus fréquent sous des conditions d'élevage à température fraîche et à photophase courte ou décroissante. Nous avons retrouvé environ 76% des larves utilisées dans les expériences d'induction de la diapause avec notre méthode de récupération des larves et des cocons; la mortalité avant la maturité (des larves et des nymphes) est en moyenne de 18,2%. Bien que la diapause se soit produite durant notre étude, il reste nécessaire de rechercher des conditions menant à des fréquences plus élevées de diapause et d'essayer d'améliorer la survie et la récupération des individus.

Introduction

The swede midge, *Contarinia nasturtii* Kieffer (Diptera: Cecidomyiidae), is an invasive pest of Brassicaceae in North America and is of increasing economic concern (Kikkert *et al*. 2006). In the 16 years since the first symptomatic plants were found in Ontario in 1996 (Hallett and Heal 2001), the swede midge has become established across southeastern Canada (Canadian Food Inspection

Received 4 March 2012. Accepted 30 April 2012.

L.E. Des Marteaux,[1] M.B. Habash, J.M. Schmidt, R.H. Hallett,[1] School of Environmental Sciences, University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada N1G 2W1

[1]Corresponding author (e-mail: ldesmart@gmail.com; rhallett@uoguelph.ca).
doi:10.4039/tce.2012.81

Agency 2009). Swede midge populations have spread to some regions of the Prairies and north-eastern regions of the United States (Chen *et al*. 2009; Hallett *et al*. 2009). In southern Ontario, four generations of swede midges occur from May to October (Hallett *et al*. 2009). The swede midge has a facultative diapause. This form of diapause occurs in multivoltine species with variable diapause induction frequency based on environmental conditions (Mansingh and Smallman 1966). Swede midges overwinter in the third (last) larval instar within spherical cocoons in the soil (Readshaw 1961). Adults emerge during the spring and summer following diapause termination and pupation (Readshaw 1961). Our understanding of diapause physiology and factors regulating diapause entry or termination in swede midge and many other insects is incomplete (Denlinger 2002; Emerson *et al*. 2009). Knowledge of diapause systems in insects (especially for species of beneficial or detrimental economic importance) may be useful for population management and may allow us to elucidate selection pressures influencing the evolution of plasticity in diapause behaviour (Danks 1987; Biron *et al*. 1998; Masaki 2002; Fournet *et al*. 2004; Emerson *et al*. 2009).

Photoperiod is one of the most ubiquitous and reliable environmental cues employed by insects to avoid adverse conditions (De Wilde *et al*. 1959; Dingle *et al*. 1977; Bingxiang *et al*. 1998; Han and Denlinger 2009). Methods for diapause induction using photoperiod manipulation in laboratory settings have been developed for a number of species including the pest insects *Delia radicum* (Linnaeus) (Diptera: Anthomyiidae) (De Wilde *et al*. 1959; Biron *et al*. 1998), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (De Wilde *et al*. 1959), and *Sarcophaga bullata* (Parker) (Diptera: Sarcophagidae) (Denlinger 1972), but have not been conducted for the swede midge. Entry into diapause in the swede midge is influenced by photoperiod (in the field) and temperature (in the field and laboratory) (Readshaw 1961); however, photoperiod induction of diapause for swede midges reared in laboratory has only been briefly reported for individuals under natural light regimes (Readshaw 1961; Readshaw 1966), and methodological details are sparse. Furthermore, isolation of diapausing swede midge in field-collected soil samples is often difficult and time-consuming, involving extensive searches through water-suspended debris (Bevan and Uncles 1958).

In this paper, we present a rearing method and development of a technique for inducing and quantifying diapause for swede midge in the laboratory. Pre-sifting pupation and diapause media to particle sizes smaller than cocoons expedites recovery of swede midge when quantifying diapause frequency. Washing field-collected soil through appropriate sieve sizes may also alleviate the often cumbersome task of separating swede midge larvae and cocoons from soil (Golightly 1952; Bevan and Uncles 1958).

Materials and methods

Swede midge rearing

Swede midges were reared on 'Snow Crown' cauliflower (*Brassica oleraceae* Linnaeus; Brassicaceae) (Stokes Seeds, Thorold, Ontario, Canada) according to the methods of Chen and Shelton (2007). Cauliflower was seeded into 128-cell flats and allowed to germinate in a greenhouse at the University of Guelph, Guelph, Ontario, Canada. Flats were fertilised weekly with 10-52-10 plant starter fertiliser (Plant Products Co. Ltd., Brantford, Ontario, Canada). After ~3 weeks, seedlings were transplanted in pairs to 13 cm diameter pots. Transplants were fertilised weekly with 20-20-20 all purpose fertiliser (Plant Products Co. Ltd.) until ~4 months of age. Plants were used for swede midge rearing when they had 8–10 true leaves with substantial heartleaf formation (i.e., just prior to head formation).

Swede midges for initiation of the University of Guelph colony were kindly received from Dr. Anthony Shelton at the New York State Agricultural Experiment Station (Cornell University, Geneva, New York, United States of America) from a colony established in 2004. The colony originated from the Swiss Federal Research Station for Horticulture, Wädenswil, Switzerland (Chen and Shelton 2007). Adults were kept in Plexiglas® chambers (48 × 35.5 × 46 cm) with mesh on three sides, while larvae were kept in Plexiglas® chambers (61 × 91.5 × 61 cm) with mesh on two sides to maintain humidity. Both larvae and adults were kept at 28 ± 2.5°C and relative humidity 65% ± 15% on a 16:30L:7:30D light cycle. Chambers were lit with four 40-watt fluorescent bulbs; three standard white light bulbs

https://doi.org/10.4039/tce.2012.81 Published online by Cambridge University Press

and one full spectrum bulb (Sylvania® Gro-Lux or General Electric® Plant and Aquarium Ecolux). During the emergence period, pots of cauliflower were provided to adults for 24 hours each before placement in a larval chamber for larval development (~7–8 days). Cauliflower heads containing late third-instar larvae were cut and laid on the soil in their respective plant pots. Pots with cut heads were placed into empty adult chambers where pupation and emergence occurred after ~7–8 days. The total life cycle from egg to adult was ~15 days under these conditions.

Cocoon size and shape

Swede midge diapause cocoons from the field were measured to determine an appropriate sieve mesh size for separating cocoons from the substrate used for the diapause induction methods described below. Soil samples containing larval cocoons were collected from the field at the University of Guelph Elora Research Station during Fall 2008 and stored in a refrigerator at 4°C in lidded plastic containers until April 2009. Soil samples were transferred to individual watch glasses and submerged in tap water. The floating cocoons were then retrieved under a dissecting microscope. Cocoons were placed on a P8 Fisherbrand® filter paper (Fisher Scientific Company, Ottawa, Ontario, Canada) in a 10 cm Petri plate and allowed to dry in a refrigerator until November 2010. The widest and narrowest aspect of each cocoon was measured using an ocular micrometer, and the average diameter of each cocoon determined ($n = 34$).

To determine quantitatively the shape difference between diapause cocoons (spherical) and pupal cocoons (ovoid) (Readshaw 1966), pupal and diapause cocoons of swede midge reared in laboratory were measured. Midges were laboratory reared under warm, long day conditions of 28 ± 2.5°C, 16:30L:7:30D light cycle ($n = 59$), or cool, short day conditions of 13.3 ± 1.4°C, 10:00L:14:00D light cycle ($n = 49$). These conditions corresponded to those previously shown in the field to result in low and high diapause entry, respectively (Readshaw 1961). Cocoons were collected from cups containing ground peat moss as described below and allowed to dry at 4°C for 20–30 days before measuring with an ocular micrometer. The ratio between the narrowest and widest aspects of laboratory-reared cocoons were compared with field-collected diapause cocoons used in cocoon size analysis by analysis of variance (ANOVA) (Statistica 7.0, Tulsa, Oklahoma, United States of America) with $\alpha = 0.05$.

Diapause induction

To determine the critical conditions for diapause entry, swede midge were reared under pupation-inducing or diapause-inducing conditions (Readshaw 1961; Tauber and Tauber 1970). Pupation-induced swede midge were reared in a larval chamber under warm, long day length conditions: 28 ± 2.5°C; $65\% \pm 15\%$ relative humidity; 16:30L:7:30D light cycle. Diapause-induced swede midges were reared under one of two conditions: cool, decreasing day length at 11.9 ± 1.4°C; $65\% \pm 15\%$ relative humidity; light cycle decreasing 10 minutes per day from 16:20L:7:40D to 12:20L:11:40D over 25 days, or cool, short day length conditions: 14.3 ± 1.7°C; $65\% \pm 15\%$ relative humidity; 10L:14D light cycle. Adult midges were allowed to oviposit on cauliflower for 24 hours. The plants were then immediately transferred to larval rearing chambers under pupation-inducing or diapause-inducing conditions, with eight replicated pots per treatment. When larvae reached the late third instar stage (~7–8 days under pupation-inducing conditions and 25 days under diapause-inducing conditions), 100 larvae were selected randomly from the two cauliflower heads in each of the eight replicate pots per treatment and carefully transferred to pupation/diapause containers. On four out of 24 occasions there were fewer than 100 larvae on cauliflower heads within a single pot, therefore all third instar larvae (between 27 and 92) were selected for those replicates.

Pupation/diapause containers consisted of 5 cm wide 30-dram clear styrene vials with snap lids (Bel-Art Scienceware, Wayne, New Jersey, United States of America) containing a small fresh cauliflower heart leaf on top of a layer of peat moss. Ground, sifted peat moss was used as the pupation/diapause medium in order to increase efficiency of cocoon and swede midge retrieval. PRO-moss peat moss (Premier Tech. Ltd., Rivière-du-Loup, Québec, Canada) was ground finely with a coffee grinder and sifted twice through a steel sieve with a number 40 mesh size of 0.425 mm (Fisher Scientific, Ottawa, Ontario, Canada) (see "Results" section). Sifted peat moss

https://doi.org/10.4039/tce.2012.81 Published online by Cambridge University Press

was added to containers to a depth of 3.5 cm (68.7 cm^3 of peat moss). Peat moss was overlain with 0.75 cm tap water (14.7 cm^3 of water) and stirred thoroughly until the peat moss particles were moistened but still well separated (~4.7:1 peat moss to tap water volume ratio). The mixture was gently patted down for a final soil height of 5 cm. Larvae were transferred carefully to the surface of a fresh cauliflower heart leaf, which allowed for continuation of larval feeding, if required for some individuals. Cups with lids were placed individually in mesh BugDorm© cages (24.5 × 24.5 × 24.5 cm) (Megaview, Taiwan) to contain emerging adults and stored in the same conditions under which the larvae were reared. Lids ensured that larvae were contained and each day any larvae found on the lid or cup sides were gently placed back onto the peat moss. Cups were kept lidded until all larvae had burrowed into the soil, after which the lids were removed to allow for emergence. Emerging adult males and females were counted and removed from cages daily.

Following emergence of adults, diapause frequencies were quantified for cups when no emergence was observed for 5 consecutive days. Cups were rinsed for ~5 minutes under a tap through a 0.425 mm number 40 steel mesh sieve atop a 0.180 mm number 80 steel mesh sieve (Fisher Scientific). The 0.180 mm mesh sieve was used to ensure retention of small larvae that might have passed through the 0.425 mm mesh sieve. Gentle pressure applied to clumped material aided in washing peat moss through the upper sieve. Fine peat particles (the bulk of the material) washed through, leaving a few large particles, cocoons, larvae, pupae, and dead adults on the 0.425 mm sieve. These were washed from the sieve and suspended in water in a watch glass. The lower sieve fraction only occasionally contained small larvae. To avoid excessive searching through large amounts of material, the lower sieve fraction was suspended in water in a watch glass in small portions so that larvae could be easily seen. The contents were viewed under a dissecting microscope and all swede midge life stages were sorted and removed using fine forceps. The majority of cocoons (with or without larvae) were buoyant, whereas pupae would float or sink and larvae would sink. Dead larvae, pupae, and adults were removed and laid on wet P8 filter paper (Fisher Scientific) for counting. Cocoons were removed from the watch glass and placed on a wet filter paper for evaluation and counting. Live larvae in cocoons were bright yellow in colour and clearly visible through the silk and substrate surrounding cocoons. Live larvae in cocoons were considered to be in diapause because they had persisted in the larval stage longer than the mean time for development (see the "Results" section). All other cocoons were opened carefully with fine forceps to remove and enumerate any dead larvae or pupae. Many cups contained a few "free-living" larvae (i.e., those that did not pupate or enter diapause and were not found within cocoons). "Free-living" larvae were also removed and counted. Cocoons could be stored on moist filter paper in lidded Petri dishes sealed with Parafilm in a refrigerator at 4°C for several months. Readshaw (1966) recommended that cocoons be stored 2–5°C for 100 days to complete diapause development before transferring to moist, 20–25°C conditions for diapause termination and pupation.

For each cup, the number of larvae diapausing in cocoons was divided by the number of pupae and adults recovered to determine the proportion of larvae that entered diapause. Dead larvae and larvae not in cocoons were excluded because the potential fate of these individuals (i.e., pupation or diapause) was unknown. The ratios were arcsin-square root transformed prior to ANOVA. Mean separations were evaluated using Tukey's HSD (honestly significant difference) with $\alpha = 0.05$. Untransformed means are presented. Statistical analyses were performed using Statistica© 7 (StatSoft, Inc., Tulsa, Oklahoma, United States of America).

Results

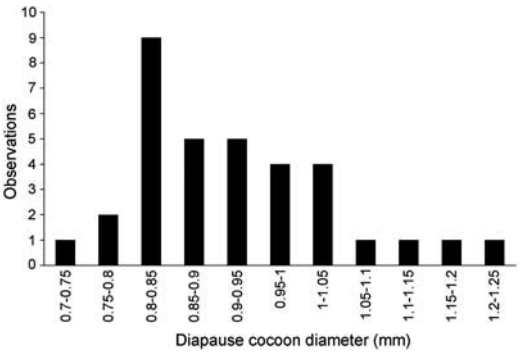
Cocoon size and shape

Field-collected diapause cocoons were 0.92 ± 0.02 mm (mean ± SE) in average diameter (range 0.74–1.23 mm) (Fig. 1). A mesh size of 0.425 mm was therefore chosen for presifting ground peat. Diapause cocoons from the laboratory (Fig. 2A) were larger than those collected in the field, with an average diameter of 1.01 ± 0.02 mm (range 0.60–1.40 mm) ($F = 9.015$, $df = 1, 81$, $P = 0.0036$). Diapause cocoons collected from the field and from the laboratory did not differ from one another in aspect ratio

https://doi.org/10.4039/tce.2012.81 Published online by Cambridge University Press

($F = 0.002$, df = 1, 81, $P = 0.969$). Laboratory-reared pupal cocoons (Fig. 2B) averaged 1.45 ± 0.03 mm in diameter (range 1.10–2.08 mm). Pupal cocoons were significantly less spherical in shape compared with diapause cocoons ($F = 139.8$, df = 1, 140, $P < 0.001$). Mean length-to-width ratios for diapause and pupal cocoons were 1.23 ± 0.04 and 1.72 ± 0.02, respectively (Fig. 3).

Fig. 1. Average diameter of dry, field-collected swede midge diapause cocoons used to determine an appropriate sieve mesh size for cocoon retrieval.

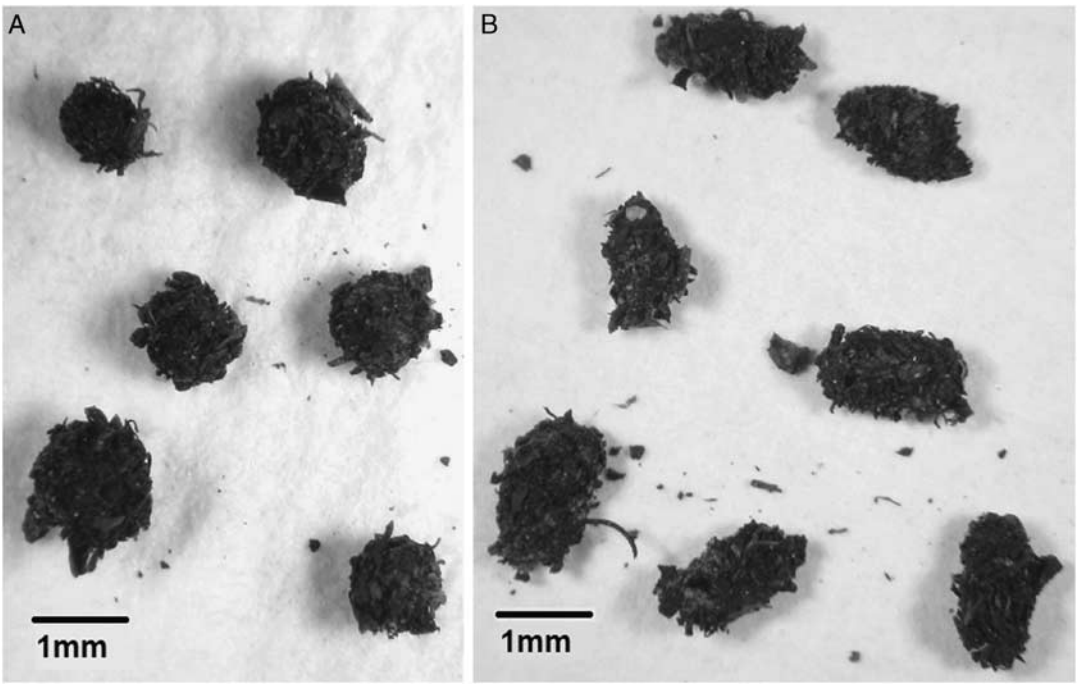


The difference in shape between pupal and diapause cocoons reflects larval position. Larvae and pupae were observed to be extended within pupal cocoons, whereas larvae in diapause cocoons are curled into a "C" shape.

Diapause induction

In this study, $76\% \pm 18\%$ of the total larvae per pupation/diapause container were recovered. Individuals that were not recovered may have died and decomposed prior to sifting. Total premature larval and pupal mortality averaged $18.2\% \pm 4.8\%$ and did not differ significantly between treatments ($F = 1.44$, df = 2, 21, $P > 0.05$). Under warm, long day length conditions, larval feeding lasted 9.6 ± 0.4 days, emergence of adults occurred over 7.6 ± 0.8 days, and lifecycle completion required 21.4 ± 0.5 days. Under cool, decreasing day length conditions, larval feeding lasted 26.0 ± 0.0 days, emergence of adults occurred over 11.1 ± 3.6 days, and lifecycle completion required 57.4 ± 1.8 days. Under cool, short day conditions larval feeding lasted 26.3 ± 0.3 days, adult emergence occurred

Fig. 2. Cocoons of laboratory-reared swede midge: (A) diapausing larvae, (B) pupae. The cocoons pictured were dried at 4°C for ~5 months.



https://doi.org/10.4039/tce.2012.81 Published online by Cambridge University Press

Fig. 3. Length and width of dried swede midge pupal and diapause cocoons. The dashed line indicates a 1:1 relationship between length and width (i.e., spherical cocoons). Diapause cocoons were collected from soil at Elora, Ontario, Canada ($n = 34$) and from a laboratory colony ($n = 49$). Pupal cocoons were collected from a laboratory colony ($n = 59$).

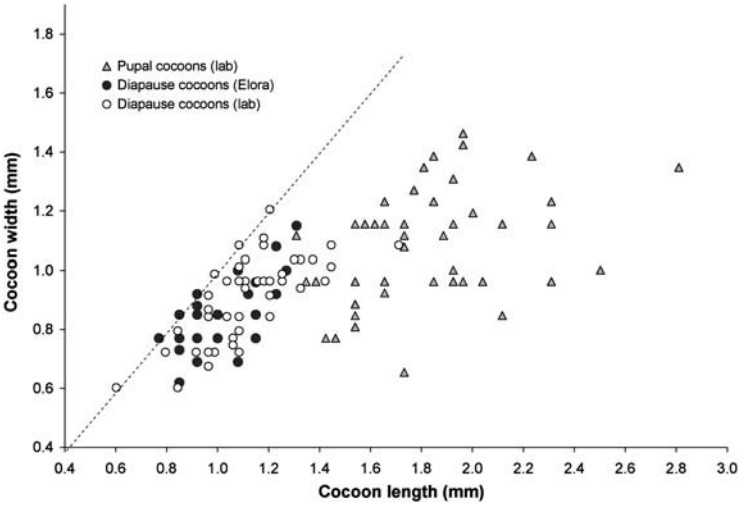
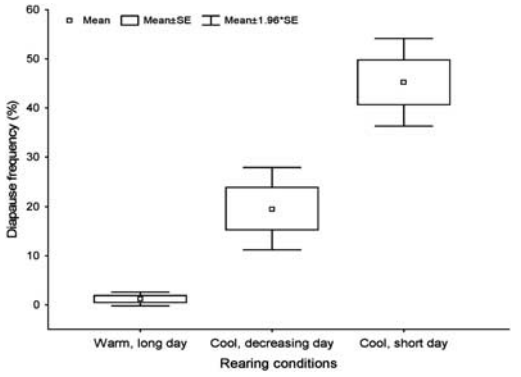


Fig. 4. Proportion of swede midge larvae entering diapause when reared under warm, long day (28 ± 2.5°C, 16:30L:7:30D), cool, decreasing day (11.9 ± 1.4°C, decreasing 10 minutes per day from 16:20L:7:40D to 12:20L:11:40D), and cool, short day 2 (14.3 ± 1.7°C, 10L:14D) conditions.



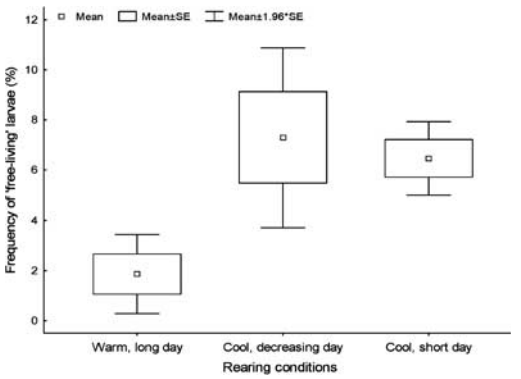
over 13.6 ± 3.3 days, and lifecycle completion required 58.1 ± 1.9 days. Diapause frequencies differed significantly for larvae under control and treatment conditions ($F = 37.37$, df = 2, 21, $P < 0.05$). Cool, short day length conditions resulted in the highest mean incidence of diapause (45.2% ± 4.6%) followed by cool, decreasing day length (19.5% ± 4.2%) (Fig. 4). Only 1.2% ± 0.7% of larvae entered diapause under control conditions. The percentage of larvae that neither pupated nor entered diapause was significantly higher under both diapause-inducing conditions (~6.9%) compared with pupation-inducing conditions (~1.9%) ($F = 10.12$, df = 2, 21, $P < 0.05$), but treatments did not differ from one another (Fig. 5).

Discussion

Washing soil samples through a 0.425 mm mesh sieve increases efficiency when retrieving swede midge larvae, pupae, or cocoons from the field or laboratory. However, some small larvae that pass through a 0.425 mm mesh sieve could be recovered with the addition of a 0.180 mm mesh sieve. Clumps of soil may be gently patted to facilitate washing and the remaining fractions suspended in water within a watch glass for retrieval of swede midges. In previous studies of swede midges by Readshaw (1961, 1966), pupation/diapause media contained larger soil particles (<2.0 mm) and soil was washed through sieves with much smaller mesh sizes (0.149 mm). Because

https://doi.org/10.4039/tce.2012.81 Published online by Cambridge University Press

Fig. 5. Proportion of "free-living" larvae remaining on the surface of peat moss when reared under warm, long day (28 ± 2.5°C, 16:30L:7:30D), cool, decreasing day (11.9 ± 1.4°C, decreasing 10 minutes per day from 16:20L:7:40D to 12:20L:11:40D), and cool, short day (14.3 ± 1.7°C, 10L:14D) conditions.



a mesh size of 0.180 mm retains the vast majority of material, recovery of swede midge would be difficult and time-consuming even after sifting through a 0.149 mm mesh sieve. The present substrate sifting method may therefore facilitate recovery of swede midge (or related species) in small-scale studies and replace more laborious recovery techniques (Golightly 1952; Bevan and Uncles 1958). Substrate sifting may also save time when assessing premature mortality or developmental chronology (Chen and Shelton 2007).

By manipulating temperature and photoperiod, diapause could be induced in nearly half of larvae in cool and short or decreasing day length conditions. Conversely, diapause was almost entirely prevented under warm, long day length conditions used in this study. Greater diapause frequency in swede midge may require rearing under colder temperatures than those used in the present study or exposing swede midge cool, short day length conditions at earlier life stages (i.e., embryos). Photoperiods experienced by the last swede midge generation in mid-October exceed 10.5 hours in southern Ontario (~43°64′N, 80°41′W) and 10 hours in northern native regions such as Svalöv, Sweden (55°55′N, 13°07′E) (Barnes 1950; Rogerson 1963; Readshaw 1966; Hallett *et al.* 2007). Rearing under photoperiods less than 10 hours is therefore not expected to increase the diapause induction frequency further. The maximum diapause induction frequency for swede midge reared under laboratory conditions is not currently known. In addition to further manipulation of rearing temperature, determination of other mechanisms contributing to diapause induction (e.g., photosensitive life stage, heritability of diapause, or maternal effects) may be required to increase diapause frequencies in the laboratory. The swede midge used in the present study were reared under warm, long day length conditions for many years (Chen and Shelton 2007), which may have selected against swede midges that would enter diapause under similar conditions in the present study. Because swede midge colonies were not supplemented with wild individuals, diapause frequencies in laboratory populations may therefore not fully reflect those of wild populations under similar conditions. Premature mortality in wild populations has not been documented. Larval and pupal mortality in the present study (18.2% ± 14.2%) is, however, lower than that reported from previous studies, which range from ~32% (Readshaw 1968) to ~70% (Dry 1915).

The occurrence of a prolonged larval stage in 2%–8% of the swede midge (depending on rearing conditions) has not been previously reported, but may represent a delayed pupation phenotype (Biron *et al.* 1998). The higher incidence of prolonged larval stage for individuals reared under diapause-inducing conditions may be a result of slower overall development at colder temperatures. To determine the developmental fate of these larvae it is recommended that individuals be placed back into soil and kept under prior rearing conditions until pupation occurs. Because diapause in swede midge was reported to last a minimum of 100 days (Readshaw 1961), larvae avoiding pupation for 100 days or more are likely to be in diapause in the absence of a cocoon. Whether larvae are able to diapause without a cocoon could be confirmed by comparing gene expression for individuals in a prolonged larval stage with that of diapausing individuals.

Methods of diapause induction and quantification in this study will be useful for future characterisation of diapause physiology, gene expression, or patterns of emergence from diapause in swede midges and related species. Ability to predict entry and termination of diapause in economic

https://doi.org/10.4039/tce.2012.81 Published online by Cambridge University Press

pests is crucial for integrated pest management practices such as crop rotation, seed-sowing, and pesticide application timing.

Acknowledgements

The authors would like to thank Dr. Anthony Shelton and Mao Chen at Cornell University for providing swede midge and rearing information. Thanks to our technician David Makynen and undergraduate research assistants Anne McKee and Michael Schmidt. This research was funded by a Natural Sciences and Engineering Research Council Canadian Graduate Scholarship to L.E. Des Marteaux and the Ontario Ministry of Agriculture, Food and Rural Affairs University of Guelph Sustainable Production Program fund to R.H. Hallett.

References

Barnes, H.F. 1950. The identity of the swede midge, with notes on its biology. Annals of Applied Biology, **37**: 241–248.

Bevan, W.J. and Uncles, J.J. 1958. Studies on soil population of *Contarinia pisi* Winn. in 1957 in Yorkshire and Lancashire. Annals of Applied Biology, **46**: 529–535. doi:10.1111/j.1744-7348.1958.tb02236.x.

Bingxiang, L., Yonglin, C., and Huiluo, C. 1998. Effects of photoperiod on embryonic diapause and reproduction in the migratory locust in three geographical populations. Insect Science, **5**: 342–349.

Biron, D., Langlet, X., Boivin, G., and Brunel, E. 1998. Expression of early and late-emerging phenotypes in both diapausing and non-diapausing *Delia radicum* L. pupae. Entomologia Experimentalis et Applicata, **87**: 119–124.

Canadian Food Inspection Agency. 2009. Review of the pest status of the swede midge (*Contarinia nasturtii*) in Canada. RMD-08-03 [online]. Available from http://www.inspection.gc.ca/english/plaveg/protect/rmd/rmd-08-03e.shtml [accessed 17 October 2011].

Chen, M. and Shelton, A.M. 2007. Impact of soil type, moisture, and depth on swede midge (Diptera: Cecidomyiidae) pupation and emergence. Environmental Entomology, **36**: 1349–1355.

Chen, M., Shelton, A.M., Wang, P., Hoepting, C.A., Kain, W.C., and Brainard, D.C. 2009. Occurrence of the new invasive insect *Contarinia nasturtii* (Diptera: Cecidomyiidae) on cruciferous weeds. Journal of Economic Entomology, **102**: 115–120.

Danks, H.V. 1987. Insect dormancy: an ecological perspective. Biological Survey of Canada, Ottawa, Canada.

Denlinger, D.L. 1972. Induction and termination of pupal diapause in *Sarcophaga* (Diptera: Sarcophagidae). Biological Bulletin, **142**: 11–24.

Denlinger, D.L. 2002. Regulation of diapause. Annual Review of Entomology, **47**: 93–122.

De Wilde, J., Duintjer, C.S., and Mook, L. 1959. Physiology of diapause in the adult Colorado beetle (*Leptinotarsa decemlineata* Say). I. The photoperiod as a controlling factor. Journal of Insect Physiology, **3**: 75–85.

Dingle, H., Brown, C.K., and Hegmann, J.P. 1977. The nature of genetic variance influencing photoperiodic diapause in a migrant insect, *Oncopeltus fasciatus*. The American Naturalist, **111**: 1047–1059.

Dry, F.W. 1915. An attempt to measure the local and seasonal abundance of the swede midge in parts of Yorkshire over the years 1912 to 1914. Annals of Applied Biology, **2**: 81–108. doi:10.1111/j.1744-7348.1915.tb05427.x.

Emerson, K.J., Bradshaw, W.E., and Holzapfel, C.M. 2009. Complications of complexity: integrating environmental, genetic and hormonal control of insect diapause. Trends in Genetics, **25**: 217–225.

Fournet, S., Astier, N., Cortesero, A.M., and Biron, D.G. 2004. Influence of a bimodal emergence strategy of a dipteran host on life-history traits of its main parasitoids. Ecological Entomology, **29**: 685–691.

Golightly, W.H. 1952. Soil sampling for wheat-blossom midges. Annals of Applied Biology, **39**: 379–384. doi:10.1111/j.1744-7348.1952.tb01021.x.

Hallett, R.H., Goodfellow, S.A., and Heal, J.D. 2007. Monitoring and detection of the swede midge (Diptera: Cecidomyiidae). The Canadian Entomologist, **139**: 700–712.

Hallett, R.H., Goodfellow, S.A., Weiss, R.M., and Olfert, O. 2009. MidgEmerge, a new predictive tool, indicates the presence of multiple emergence phenotypes of the overwintered generation of swede midge. Entomologia Experimentalis et Applicata, **130**: 81–97.

Hallett, R.H. and Heal, J.D. 2001. First Nearctic record of the swede midge (Diptera: Cecidomyiidae), a pest of cruciferous crops from Europe. The Canadian Entomologist, **133**: 713–715.

Han, B. and Denlinger, D.L. 2009. Mendelian inheritance of pupal diapause in the flesh fly, *Sarcophaga bullata*. Journal of Heredity, **100**: 251–255. doi:10.1093/jhered/esn082.

Kikkert, J.R., Hoepting, C.A., Wu, Q.-J., Wang, P., Baur, R., and Shelton, A.M. 2006. Detection of *Contarinia nasturtii* (Diptera: Cecidomyiidae) in New York, a new pest of cruciferous plants in the United States. Journal of Economic Entomology, **99**: 1310–1315.

Mansingh, A. and Smallman, B.N. 1966. Photoperiod control of an "obligatory" pupal diapause. The Canadian Entomologist, **98**: 613–616. doi:10.4039/Ent98613-6.

Masaki, S. 2002. Ecophysiological consequences of variability in diapause intensity. European Journal of Entomology, **99**: 143–154.

Readshaw, J.L. 1961. The biology and ecology of the swede midge, *Contarinia nasturtii* (Kieffer) (Diptera: Cecidomyidae). Ph.D. thesis. University of Durham, Durham, United Kingdom.

Readshaw, J.L. 1966. The ecology of the swede midge, *Contarinia nasturtii* (Kieff.) (Diptera, Cecidomyiidae). I. Life-history and influence of temperature and moisture on development. Bulletin of Entomological Research, **56**: 685–700.

Readshaw, J.L. 1968. Damage to swedes by the swede midge, *Contarinia nasturtii* (Kieff.), and a possible method of cultural control. Bulletin of Entomological Research, **58**: 25–29.

Rogerson, J.P. 1963. Swede midge on two Northumberland farms, 1959–61. Plant Pathology, **12**: 161–171.

Tauber, M.J. and Tauber, C.A. 1970. Photoperiodic induction and termination of diapause in an insect: response to changing day lengths. Science, **167** (3915): 170. doi:10.1126/science.167.3915.170.

https://doi.org/10.4039/tce.2012.81 Published online by Cambridge University Press