**Master project in the Horticultural Science Programme**
**2008-02, 20 p (30 ECTS)**

# Responses of the swede midge, *Contarinia nasturtii*, to volatile compounds from *Arabidopsis thaliana* and *Brassica oleracea* var. *botrytis*

Photo: Linda-Marie Rännbäck

by


## Johannes Albertsson

**Fakulteten för landskapsplanering, trädgårds- och jordbruksvetenskap**

**SLU-Alnarp**

## PROJECT INFORMATION

This report is a result of a master thesis (30 ects) carried out at the department of Plant Protection Biology within the Horticultural programme at the Swedish University of Agricultural Sciences. Tina Boddum and Ylva Hillbur were supervisors and Peter Anderson was the examiner.

**ABSTRACT**

The swede midge, *Contarinia nasturtii*, is a serious gall-forming insect pest of most cultivated Brassicaceae such as cauliflower and cabbage. *Arabidopsis thaliana,* also a Brassicaceae, is a well-known model plant for which the genome is sequenced and several well-characterized mutants and transgenic genotypes are available. Previous results indicate that odours from cauliflower attract *C. nasturtii*. In this study the responses of *C. nasturtii* to volatiles from cauliflower and Arabidopsis were tested in a four-arm and a y-tube olfactometer. The results indicate that females are attracted to volatiles both from cauliflower and Arabidopsis. Males on the other hand were not attracted. This study also suggests improvements for the y-tube as well as a four-arm olfactometer setup.

**SAMMANFATTNING**

Kålgallmyggan, *Contarinia nasturtii,* är en skadegörare på flertalet odlade grödor tillhörande familjen Brassicaceae så som blomkål, vitkål och broccoli. *Arabidopsis thaliana*, som också tillhör denna familj, är en viktig modellväxt eftersom dess genom helt är kartlagt och att en mängd väldokumenterade mutationer och transgena genotyper finns tillgängliga. Tidigare studier har indikerat att *C. nasturtii* är attraherad av doft från blomkål. I det här arbetet undersöker vi, med hjälp av en fyr-arms olfaktometer och en y-rörs olfaktometer, om *C. nasturtii* är attraherad av flyktiga ämnen från blomkål och Arabidopsis. Resultaten från detta arbete pekar på att honor attraheras av doft från båda dessa växter. Hannarna attraherades däremot inte. Detta arbete föreslår också modifikationer och förbättringar för y-rörs samt fyr-arms olfaktometrarna.

## INTRODUCTION

The swede midge, *Contarinia nasturtii* (Kieffer) (Diptera: Cecidomyiidae) is a serious pest on cruciferous crops in many brassica-growing regions (Kikkert et al., 2006). Host plants include cruciferous weeds and most cultivated crucifers such as broccoli, cauliflower, cabbage and Brussel sprouts (Stokes, 1953; Hallett, 2007). The larva causes the damage when it feeds near the growing point of the plant. Symptoms include gall-like distortions, deformed plant tissue, and corky brown scars. Adult gall midges are yellow to light brown and 1.5-2 mm long. Larvae are white to yellow and 2-2.5 mm long (Readshaw, 1966).

### *Life cycle*

*C. nasturtii* overwinters in the soil as larva in spherical cocoons (Readshaw, 1966). At the end of May or beginning of June the larvae start to pupate and emerge. After emergence the adults mate and the female starts to search for a suitable host plant for oviposition (Madsen & Hansen, 2006). The female lays about 100 white eggs in clusters of 2-50 that hatch after a few days. The larvae feed on the plant tissue for 4-10 days and then leave the plant by falling down to the soil. Depending on temperature, humidity and day length the larvae either pupate to develop the next generation, or enter diapause (Readshaw, 1966). In Sweden there are three overlapping generations each year (Jönsson et al., 2007).

### *Host plant finding*

Insects use a predictable sequence of behaviour when selecting a host plant (Schoonhoven et al., 2005). The sequence usually begins with random flying or walking and ends with the acceptance of the host plant. As the sequence progresses the intensity and number of cues from the host plant increase. Host location of phytophagous insects is mediated by a combination of visual and olfactory cues (Schoonhoven et al., 2005). The relative importance of the two varies between species and distance to the plant. Currently there is no clear evidence that *C. nasturtii* uses plant volatiles to mediate host finding. However, in a previous study, C. nasturtii males responded strongly to plant volatiles

from cauliflower (Möllerström, unpublished data). Mated females were also attracted to cauliflower volatiles but not as strongly as males. Host plant volatiles have also been shown to play an important role in host finding for several other *Cecidomyiidae* species e.g. *Dasinuera tetensi* (Crook & Mordue, 1999), *Dasinuera mali* (Galanihe & Harris, 1997), *Dasinurea brassicae* (Pettersson, 1976) and *Sitodiplosis mosellana* (Birkett et al., 2004). In crucifers and related plant families glucosinolates are thought to serve as a first line of defence against a variety of invading insects (Schoonhoven et al., 2005). Hydrolysis of glucosinolates, forming volatile isothiocyanates, takes place at low rate during normal catabolism but increase rapidly when plant tissues are ruptured or wounded (Schoonhoven et al., 2005). These compounds are not only acting as defence compounds, they also play a key role in host selection for several crucifer specialists like the cabbage aphid, *Brevicoryne brassicae* (Nottingham et al, 1991), and the seed weevil, *Ceutorhynchus assimilis* (Blight et al., 1995). Additional, not yet identified compounds in crucifers may also play an important role in host plant selection for insects (Alan & Renwick, 2002).

*Control of C. nasturtii*

*C. nasturtii* is hard to control due to its short lifecycle and many generations. Insecticides, like pyrethroids can be applied to control them, but there are also many other cultural strategies available to lower the damage (Jönsson et al., 2007). The Swedish Board of Agriculture recommend the following measures; at least three year crop rotation, no rape seed next to cabbage fields, and as long a distance as possible (several hundred meters) between early and late batches of cabbage (Jönsson et al, 2007). Another culture strategy to decrease *C. nasturtii* damage is to use exclusion fences. An experiment made with these fences in Switzerland reduced damage caused by *C. nasturtii* significantly (Wyss & Daniel, 2004). Since the sex pheromone of *C. nasturtii* is identified there is also a possibility to develop an easy-to-use monitoring system (Hillbur et al., 2005).

Other control methods, including resistant cultivars and transgenic plants can and may be explored in the future (Wu et al., 2006). Resistance to insects in crop cultivars is caused either by biochemical or morphological features (Diarisso et al., 1998). One example of a morphological feature is the resistance of some sorghum cultivars to the

sorghum midge, *Stenodiplosis sorghicola*. In these genotypes the spikelets are closed when the female sorghum midges are active and oviposition is thus prevented (Diarisso et al., 1998). The resistance of several wheat cultivars to the wheat midge, *Sitodiplosis mosellana* is however caused by biochemical features. When attacked by the larvae the resistant cultivars react by increasing the production of phenolic acids in combination with a local hypersensitive reaction on the seed surface (McKenzie, 2002). The defence mechanism reduces survival up to 99 % of the first-instar wheat midges (Ganehiarachichi & Harris, 2007). An investigation on host plant susceptibility to *C. nasturtii* showed differences in susceptibility both between various cruciferous species and between cultivars (Hallett, 2007). The cause of the differences is not yet understood, but the fact that differences exist suggests possibilities to find genotypes conferring resistance to *C. nasturtii*.

Another possible control method is the use of transgenic plants. The best example of this method is the engineered crop plants expressing *Bacillus thuringiensis* (Bt). Bt crops exhibit resistance to major insect pest in both corn and cotton and in 2004 such crops were grown on more than 22 million hectares (Ferry et al., 2006). Recent studies have also demonstrated that genetic transformation can be used to alter the emissions of a plant and render it more attractive to beneficial arthropods (Kappers et al., 2005; Schnee et al., 2006).

*Arabidopsis*

*Arabidopsis thaliana* belongs to the Brassicaceae family, which includes around 3350 species worldwide (Koch et al., 2001). It is an important model plant for plant sciences and potentially also for ecological research (Schoonhoven et al., 2005). The genome has been fully sequenced and well-characterized mutants and transgenic genotypes are available. *C. nasturtii* host plants, like cauliflower and oilseed rape, are the closest agricultural relatives to Arabidopsis (Kliebenstein et al., 2001; Mitchell-Olds, 2001). In response to herbivores, Arabidopsis use similar transduction pathways as other crucifer plants, both when activating induced direct and indirect defence (Schoonhoven et al., 2005).

## HYPOTHESIS

The properties of Arabidopsis and the indication of *C. nasturtii* attraction to host plant volatiles suggest a possibility for a new approach when searching for host plant volatiles. The aim of this study is therefore to verify the response of *C. nasturtii* to cauliflower and to test our hypothesis that plant volatiles from Arabidopsis attract *C. nasturtii* males and females. To test our hypothesis a bioassay suitable for testing *C. nasturtii* attraction to host plant volatiles had to be developed and evaluated.

## MATERIALS AND METHODS

### *Insect material*

All the *C. nasturtii* used in the experiments originated from Switzerland. A *C. nasturtii* population had been kept at the Department of Crop Science, SLU Alnarp, since August 2004. A new batch of *C. nasturtii* was incorporated into the rearing system in September 2007 by collecting infested cauliflower plants from a field in Wädenswil, Switzerland. The infested plants were packed in plastic bags and transported to Sweden by car. In Alnarp, all plants were placed in large plastic boxes filled with 5-7 cm peat substrate. The boxes were placed in a climate chamber (25°C, 70% RH, LD 18:6 h) and covered with a white cotton cloth. Fourteen days after collection of the plant material the first midges emerged from the substrate. Some midges were used for experiments but most were used to infest new cauliflowers plants for the rearing.

All midges were reared in ventilated glass cages (30 x 30 x 35 cm) on cauliflower, *Brassica oleracea* var. *botrytis*, in the climate chamber described above. Every third day a new pot with cauliflower plants, with 6-9 true leaves, was placed in the cages and midges were allowed to mate and oviposit on the plants. The infested plants were then removed, replaced with a new batch of plants, and incubated under the same climate condition as described above. Infested plants were misted every day with tap water. Approximately two weeks after oviposition, the larvae left the plants and pupated in the pot substrate. One-day prior to emergence, 17 to 18 days after oviposition, all the

aboveground parts of the cauliflower plants were cut off. The pots with substrate and pupae were then placed in a cage where midges were allowed to emerge.

Males, one day old, and mated females were used in the bioassays. The females had been allowed to mate during the day before the bioassay. If some females exposed their ovipositor in the afternoon, indicating calling behaviour (Harris, & Foster, 1999), they were considered as unmated and discarded.

*Plant material*

In all bioassays with plant material, cauliflower plants (*Brassica oleracea* var. *botrytis* 'Vito') with 7-10 true leaves, or 5-6 weeks old Arabidopsis (*Arabidopsis thaliana* 'Columbia') served as odour sources. Cauliflower plants were grown two and two in pots (13 cm diam, 8 cm height) in a substrate consisting of 92% peat and gravel. Arabidopsis plants were grown two and two in pots (6 x 6 cm width, 5 cm height) in the same substrate. All pots were placed in a climate chamber (20°C, 60 % RH, and LD 16:8 h, 250 μmol $m^{-2}$ $s^{-1}$) and treated with *Steinernema feltiae,* to control sciarid flies. In bioassays with plant material one cauliflower pot or two Arabidopsis pots were used. To minimize the volatiles released from the soil surface and the pot itself, they were covered with aluminium foil.

Cauliflower plants for insect rearing were grown two and two in pots (13 cm diam, 8 cm height) in a substrate consisting of 92% peat and gravel in a greenhouse with a temperature of 18-25°C and 10 hours of additional light (kb 1141, 400 W).

*Four-arm olfactometer bioassay*

The four-arm olfactometerer was based on the six-arm olfactometer described by Turlings et al. (2004). It consisted of a four–armed central choice chamber with one insect trapping bulb connected to each arm (Figure 1). Air was pumped into the olfactometer through two 250 ml gas-wash bottles, one with granulated activated charcoal and one with distilled water. After the gas-wash bottles, the inflow of air was divided into four lines of Teflon tubing via four flowmeters (BA-4AR, Kytölä, Muurame, Finland) to the test plants. The test plants were enclosed in polyethylene cooking bags

(45 x 55 cm, Toppits, Melitta) and connected via Teflon tubes to each olfactometer arm. To avoid changes in air pressure a vacuum pump (Micro pump NMP 30 KNDC, 12 V, KNF Neuberger, Germany) was connected to the insect release point. The flow of incoming air was 0.9 l/min in each of the four Teflon tubes and the flow at the release point was 3.6 l/min. To get a uniform illumination and eliminate any visual distractions, the central choice chamber was covered with a white cotton cloth, placed 35 cm above the chamber and 35 cm to the sides. The olfactometer was illuminated with a mercury lamp (Tungsram 9L, HgMIF, 400/DM) placed 1.2 meter above the central choice chamber. After each experiment, all glassware was heated for 8 hours in 350 °C and all Teflon tubing and connections were washed once with 70 % ethanol to remove any odour contamination. The olfactometer was completely rebuilt after 10 days. The entire setup was disconnected and connections were, if spare ones existed, replaced with new ones. Tubing and all connections were placed over night in a box with 70 % ethanol. To get a more uniform illumination a white cylinder (50 cm diam and 20 cm high) was placed around the central choice chamber.

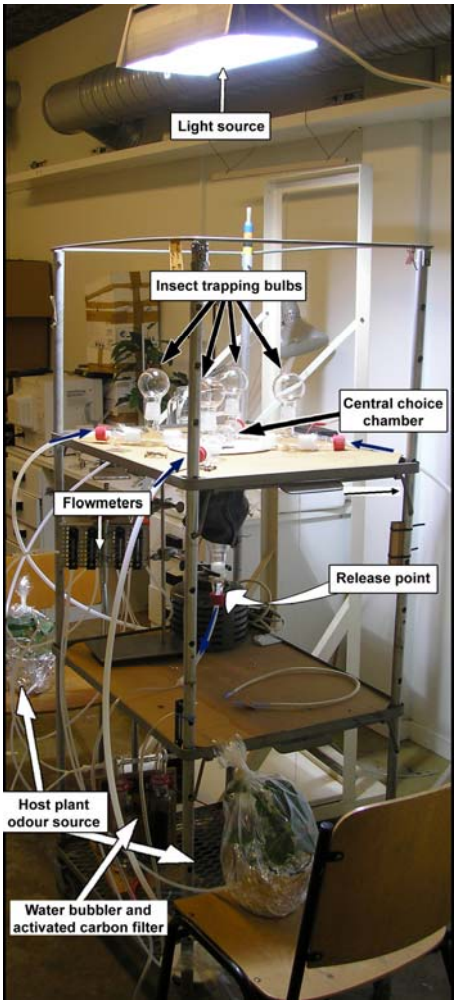


**Figure 1**. The four-arm olfactometer bioasssay setup. Photo: Linda-Marie Rännbäck & Thilda Nilsson.

To evaluate the four-arm olfactometer setup, two experiments were made. In the first experiment, *C. nasturtii* males were tested against four empty bags. This experiment was made both with the initial and the rebuilt setup. In a second experiment, *C. nasturtii* males were tested against one bag, containing a cauliflower pot and three empty bags. The second experiment was only made using the rebuilt setup. In both experiments males were

released simultaneously using a glass tube with removable cotton plugs at each end. All experiments lasted for two hours and were made between 10 pm to 2 am. Insects were only tested once.

*Y-tube olfactometer bioassay*

The y-tube olfactometer consisted of a y-shaped glass tube with an entry arm (14 cm long, 22 mm i.d) and two side arms (14 cm long, 22 mm i.d.). Both arms of the y-tube were connected to a polyethylene cooking bag (45 x 55 cm, Toppits, Melitta) which contained the odour source. Air was pumped into the olfactometer through two 250 ml gas-wash bottles, one with granulated activated charcoal and one with distilled water. After the gas-wash bottles the inflow of air (0.6 l/min) was divided into two lines of Teflon tubing via two flowmeters (BA-4AR, Kytölä, Muurame, Finland) to the bags. Visual cues were excluded in the olfactometer setup by placing the y-tube in a box (40 x 40 x 40 cm) covered with a white cotton cloth. The olfactometer was illuminated with a lamp (Massive, 906609, 400 W) placed behind the cloth and 30 cm in front of the two side arms.

A single *C. nasturtii* male was introduced into the tube and observed until he made a choice or until 5 min had elapsed. Males that did not choose a side arm within 5 min were recorded as 'no choice'. When half of the males had been tested in each batch the y-tube and the odour source was turned to avoid any directional effects. To test if *C. nasturtii* males were attracted to cauliflower or Arabidopsis, the attraction of a bag containing a cauliflower pot or a Arabidopsis pot was compared to the attraction of an empty bag. To evaluate the y-tube olfactometer

**Figure 2.** The female y-tube olfactometer bioassay setup. The male setup did not have the insect trapping bulbs.

setup a control experiment was made with one empty bag connected to each side arm. All the experiments with males were made between 10 am and 1 pm.

In the female y-tube olfactometer setup an insect-trapping bulb was connected to each side arm (Figure 2). The modification was made to be able to run experiments during several hours. The females were released simultaneously by placing a glass tube, with 20-25 females, in the entry arm. To avoid females from escaping the glass tube was covered with an insect net at the end facing outwards. The attraction of *C. nasturtii* females to cauliflower or Arabidopsis was tested by comparing a bag containing cauliflower or Arabidopsis to an empty one. All the experiments with females lasted for 16 hours and started 5 pm. The light was on between 5 pm and 8 pm. After each experiment, all glassware was heated for 8 hours in 350 °C and all Teflon tubing and connections were washed once with 70 % ethanol to remove any odour contamination.

## STATISTICS

Only those insects that made a choice were included in the statistical analyses. To test the difference between the arms in both the y-tube olfactometer and the four-arm olfactometer a chi square test was performed on the data from each bioassay (Zar, 1999).

## RESULTS

*Four-arm olfactometer*

In both the initial and the rebuilt four-arm olfactometer setup, most responding males made the choice within the first five minutes of the bioassay. In the initial control experiment there was a significant difference between the arms ($chi^2 = 23.59$, $p < 0.001$). There was still a significant difference between the arms when the experiment was repeated in the rebuilt setup ($chi^2 = 12.79$, $p < 0.01$) although another arm was preferred (Figure 3B). When cauliflower was tested against three controls, significantly more males ($chi^2 = 7.91$, $p < 0.05$) choose one of the controls (Figure 3C). Only 8 out of 54 males chose the cauliflower arm. The sample size and percentages of responding *C. nasturtii* in the four-arm olfactometer bioassays are presented in Table 1.

**Table 1.** Sex, sample size (N) and percentages of responding *C. nasturtii* for the four-arm olfactometer bioassay.

| Bioassay (number of arms) | Sex | N | Responding (%) | Figure 3 |
|---|---|---|---|---|
| Initial Control (4) | male | 141 | 79 | A |
| Rebuilt Control (4) | male | 29 | 66 | B |
| Rebuilt Control (3) - Cauliflower (1) | male | 54 | 85 | C |

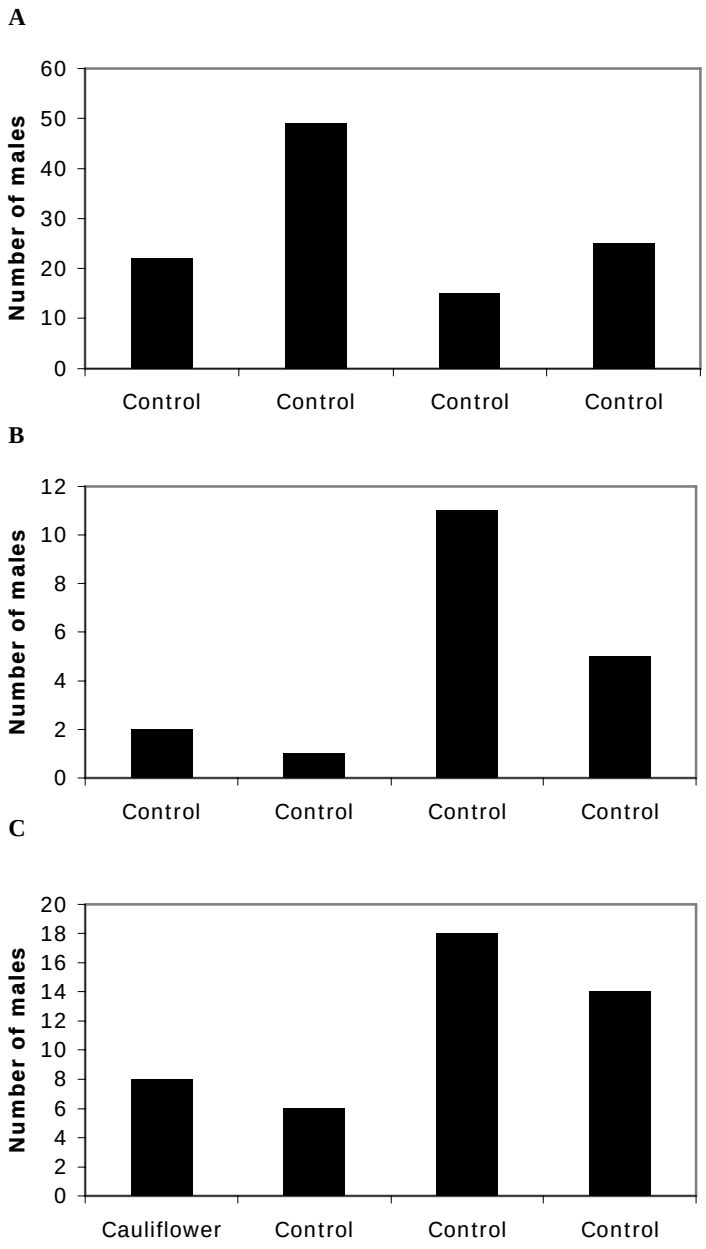


**Figure 3**. Response of male *C. nasturtii* in the four-arm olfactometer bioassay. **A)** Initial control, $chi^2$ = 23.59, $p < 0.001$, **B)** Rebuilt control, $chi^2 = 12.79$, $p < 0.01$, **C)** Rebuilt setup with one cauliflower arm and three control arms, $chi^2 = 7.91$, $p < 0.05$.

*Y-tube olfactometer*

Almost all males (90 %) that made a choice made it within the first two minutes. The majority of the responding males flew into the side arms whereas nearly all responding females walked. The sample size and percentages of responding *C. nasturtii* in the y-tube olfactometer bioassays are presented in Table 2.

**Table 2.** Sex, sample size (N) and percentages of responding *C. nasturtii* for the y-tube olfactometer bioassay.

| Bioassay | Sex | N | Responding (%) | Figure 4 |
|---|---|---|---|---|
| Control - Control | male | 20 | 90 | A |
| Cauliflower - Control | male | 61 | 85 | B |
| Arabidopsis - Control | male | 51 | 78 | C |
| Cauliflower - Control | female | 62 | 90 | D |
| Arabidopsis - Control | female | 37 | 86 | E |

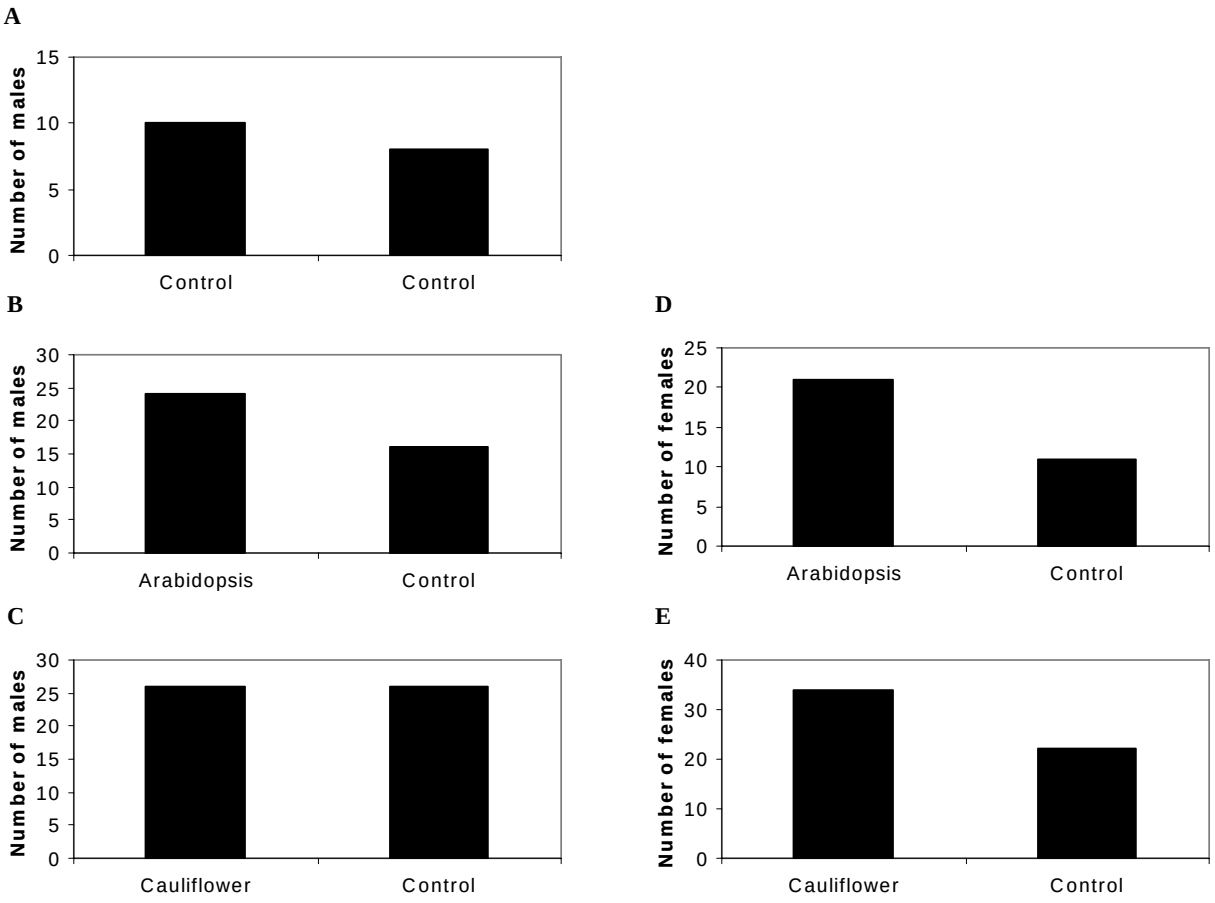


**Figure 4.** Response of *C. nasturtii* in the y-tube olfactometer bioassay. **A)** Control-control (male), $chi^2$ = 0.22, $p > 0.05$, **B)** Arabidopsis-control (male), $chi^2 = 1.6$, $p > 0.05$, **C)** Cauliflower-control (male), $chi^2 = 0$, $p > 0.05$, **D)** Arabidopsis-control (female), $chi^2 = 3.13$, $p > 0.05$. **E)** Cauliflower-control (female), $chi^2$ = 2.57, $p > 0.05$.

There were no significant differences between the arms when two controls – empty arms – were compared ($chi^2 = 0.22$, $p > 0.05$; Figure 4A). When males were given the choice between cauliflower and control exactly 50 % of the males chose the control arm ($chi^2 = 0$, $p > 0.05$ ; Figure 4B). More males chose the arm with Arabidopsis than the control arm (Figure 4C), but the difference was not significant ($chi^2 = 1.6$, $p > 0.05$).

Two y-tube olfactometer bioassays were made with females. In the first, females were given the choice between cauliflower and control. Cauliflower showed a tendency to be more attractive than control (Figure 4D), but the difference was not significant ($chi^2 = 2.57$, $p > 0.05$). In the last bioassay, Arabidopsis attracted far more females than the control, 21 out of 32 (Figure 4E). However, the difference was not large enough to be significant ($chi^2 = 3.13$, $p > 0.05$).

## DISCUSSION

The results from this study suggest that *C. nasturtii* females are attracted to plant volatiles from both cauliflower and Arabidopsis. A new approach for investigating *C. nasturtii* - host plant interactions could thus be developed. The method would test the attraction of *C. nasturtii* to transgenic or mutant Arabidopsis lacking certain volatile compounds. It would not only give the information about *C. nasturtii* attractiveness to a mutant or genetically modified Arabidopsis, but also hopefully the gene responsible for the attraction. This information could in a longer perspective be used for ecological studies and/or for traditional/transgenic breeding purposes (Turlings & Ton, 2006). In contrast to the females, the males did not show any clear attraction to either cauliflower or Arabidopsis.

Initially, a four-arm olfactometer was used to test the behavioural response of *C. nasturtii*. However, the control bioassays showed significant difference between the arms both before and after the setup had been rebuilt. Therefore a decision was taken, two months into the study, to discard the four-arm olfactometer and instead use a y-tube olfactometer. The y-tube olfactometer is more time consuming to use because the insects are released one by one. This was not a problem when working with males since their response time was very short (see result). The females one the other hand had a much

longer response time (data not presented). To be able to test the females within the time frame of this study a modification with insect trapping bulbs connected to each side arm was made. The modification made it possible to release females simultaneously without constant supervision.

Different types of olfactometers have been used in several experiments to test insect behaviour (Pettersson, 1976; Turlings et al., 2004). The reason why the *C. nasturtii* males significantly chose one arm in our four-arm olfactometer setup is not yet clarified. One possible explanation could be that the plastic connections between the Teflon tubing were contaminated with some repellent or attractant. However, the Teflon tubing and the connections were cleaned with 70 % ethanol between each test, so the compounds must then be resistant to such treatment. Another explanation could be visual cues. However, the light intensity was measured several times with a luxmeter (Gossen, 1.70-291, Germany) to verify the uniformity and no difference in light intensity was seen between the four arms.

To get the four-arm olfactometer to work properly the following improvements are proposed; replace the polyethylene cooking bags with glass cylinders, use press-fit connections when connecting the glass cylinder with Teflon tubing, replace plastic connections between Teflon tubing with Teflon connections, use a light bulb as Turlings et al (2004) to get a more uniform illumination, and place the setup in a climate chamber with the possibility to have constant background illumination, temperature, and humidity.

In the y-tube olfactometer setup used in this study, *C. nasturtii* males did not show any behavioural response to plant volatiles from cauliflowers. The result is contradictory to results by Möllerström (unpublished data), where *C. nasturtii* males flew significantly more to arms containing cauliflower plants in a four-arm olfactometer. Since the variety of the cauliflower is unknown in Möllerstöms study, it is not possible to rule out differences in volatile composition between the varieties. It is also possible that the difference between the studies is due to other reasons such as contamination of the setup. However, since we didn't use any females in the y-tube olfactometer prior to the male bioassays and made several control bioassays, it is unlikely that our setup was contaminated.

Growing conditions for the plants were also different between the studies. In our study, the plants used for the y-tube olfactometer bioassays were grown in a climate chamber with no occurrence of any herbivore insects. It is well known that plants attacked by herbivore insects increase the volatile production due to their induced direct and indirect defence (Kessler & Baldwin 2001; Dicke & Van Poecke, 2002). Hence, the contradictory results may depend on differences in herbivore damage of the tested plants.

In other *Cecidomyiidae* species such as *D. tetensi* (Crook & Mordue, 1999), *D. mali* (Harris et al. 1996), and *D. brassicae* (Pettersson, 1976; Williams & Martin, 1986) no male attraction was found to host plant volatiles. However, Murchie et al. (1997) caught *D. brassicae* males in traps baited with synthetic secondary volatiles from crucifers. The conflicting results from the *D. brassicae* studies may indicate that factors, not yet known, could affect the behavioural response of male gall midges to host plant volatiles. Such factors could perhaps be temperature, humidity, time of the day and light conditions since these factors affected male mating activity of *S. mosellana* (Pivnick, 1993). McNeil & Brodeur (1995) have also suggested that differences in mating success of *Aphidius nigripes* could depend on changes in atmospheric pressure. The strong *D. brassicae* male response to the traps could also indicate that the synthetic volatiles used as lures, or a volatile contamination in the trap, could be a component of the *D. brassicae* sex pheromone.

Both *D. tetensi* (Crook & Mordue, 1999) and *D. mali* (Harris et al. 1996) have perennial host plants. Hence, the lack of response to host plant volatiles could be explained by the fact that they emerge directly below their host plants (Crook & Mordue, 1999). However, this argument is not applicable to *C. nasturtii* since most of its host plants are annuals (Stokes, 1953). What benefit would a *C. nasturtii* male then have of detecting host plant volatiles? This question is difficult to answer because there are no studies made on *C. nasturtii* female behaviour after emergence. If the female only mates once, like *Mayetiola destructor* (Harris & Foster, 1999), and stays at the emergence place until she is mated, like *Contarinia oregonensis* (Miller & Borden, 1984), there are no obvious benefits for the male to fly to crucifers. However, if the female mates several times and/or fly to crucifers directly after emergence males would clearly have a better chance to find a mating partner.

Regardless if females are to be tested with a y-tube olfactometer or with a four-arm olfactometer optimizations could be made to get better results. Evidently, it is important that the females are tested when they are in the right physiological state, hence searching for host plants. One factor influencing this behaviour is probably the mating status since only mated females responded to host plant volatiles in both *D. brassicae* (Pettersson, 1976) and *D. tetensi* (Crook & Mordue, 1999). Our way to decide if a female is mated or not is not very accurate, since a virgin female does not call all the time. Therefore, a more accurate method has to be used which guarantee that mating has occurred. Another factor that could affect the result is the experimental period. Cecidomyiidae species like *S. sorghicola* only search actively for host plants between 8 am and 11 am (Diarisso et al., 1998). Hence, tests or observations that establish the time when the *C. nasturtii* are active should be performed. In our bioassays either two cauliflower plants or four Arabidopsis were used as an odour source. This study did not investigate the suitability of this number of plants, however since dose-response test with other insects have shown that volatile concentration affect their choice (Turlings et al., 2004), this is an important area to improve. Furthermore the plants used in the bioassays should be illuminated since several plants increase the odour release when exposed to increased light intensity (Gouinguene & Turlings, 2002). An additional optimization is to use filters that trap volatiles from the odour sources during the bioassay. If these filters are analysed they will detect variations among bioassays in volatile emissions from the tested plants (Turlings et al., 2004), and make the results more valid. If the suggested improvements and optimizations are made to the olfactometers and if more females are tested my personal opinion is that more convincing results could be achieved.

**ACKNOWLEDGEMENTS**

I would like to thank all the people that helped and inspired me in this project. Special thanks to my supervisors Ylva Hillbur and Tina Boddum. You two are probably the most positive people I ever met. I would also like to thank Elin Isberg and Linda-Marie Rännbäck for practical assistance with bioassays and insect rearing, and Peter Anderson for technical support. Finally I would like to thank Alexandra Nikolic and Joakim Ekelöf who I forced listen to gall midge chats during the whole study.

## REFERENCES


**Alan J., Renwick A.** 2002. The chemical world of crucivores: lures, treats and traps. *Entmologia Experimentalis et Applicata*. 104: 35-42

**Birkett M.A., Bruce T.J.A., Martin J.L., Smart L.E., Oakley J., Wadhams L.J.** 2004. Responses of female orange wheat blossom midge, *Sitodiplosis mosellana*, to wheat panicle volatiles. *Journal of Chemical Ecology* 30: 1319-1328

**Blight M.M., Pickett J.A., Wadhams L.J., Woodcook C.M.** 1995 Antennal perception of oil seed rape, *Brassica napus (Brassicaceae*), Volatiles by the cabbage seed weevil *Ceutorhynchus assimilis (Coleoptera, curculionidae*). *Journal of Chemical Ecology* 21: 1649-1664

**Crook D.J., Mordue A.J.** 1999. Olfactory responses and sensilla morphology of the blackcurrant leaf midge *Dasinuera tetensi*. *Entmologia Experimentalis et Applicata*. 91: 37-50

**Diarisso N.Y., Pendleton B.B., Teetes G.L., Peterson G.C., Anderson R.M.** 1998. Spikelets flowering time: Cause of sorgum Resistance to Sorghum Midge (Diptera: Cecidomyiidae). *Journal of Economic Entomology* 91: 1464-1470

**Dicke M., Van Poecke, R.P.M.** 2002. Signalling in plant-insect interactions: signal transduction in direct and indirect plant defence. In *Plant signal transduction*. pp. 289-316 Oxford University Press, Oxford

**Ferry N., Edwards M.G., Gatehouse J., Capell T., Christou P., Gatehouse A.M.R.** 2006. Transgenic plants for insect pest control: a forward looking scientific perspective. *Transgenic Research* 15: 13-19

**Galanihe L.D., Harris O.** 1997. Plant volatiles mediate Host-finding behaviour of the apple leaf curling midge. *Journal of Chemical Ecology* 23: 2639-2655

**Ganehiarachichi G.A.S.M., Harris M.O.** 2007. Oviposition behavior of orange wheat blossom midge on low- vs. high-ranked grass seed heads. *Entmologia Experimentalis et Applicata*. 123: 287-297

**Gouinguene S.P., Turlings C.J**. 2002. The effects of Abiotic factors on induced volatile emissions in corn plants. *Plant Physiology* 129:1296-1307

**Hallett R.H.** 2007. Host plant susceptibility to swede midge (Diptera: Cecidomyiidae). *Journal of Economic Entomology*. 100: 1335-1343

**Harris, M. O., Foster, S. P.** 1999. Gall Midges, pp. 27-49, in J. Hardie and A.K. Minks (eds.). Pheromones of Non-Lepidopteran Insects Associated with Agricultural Plants. CABI Publishing, Wallingford, UK.

**Harris, M. O., Foster, S. P., Age K., Dana S.** 1996. Sex pheromone communication in the apple leaf curling midge (*Dasinuera mali*). *Proceedings of the New Zealand Plant protection Conference* 49: 52-58

**Hillbur Y., Celander M., Baur R., Rauscher S., Haftmann J., Franke S., Franke W.** 2005. Identification of the sex pheromone of the swede midge. *Journal of Chemical Ecology* 31: 1807-1828

**Jönsson B., Hillbur Y., Albertsson J.** 2007. Kålgallmyggan är en liten insekt som kan ge stora skador. *Viola* 20: 20-21

**Kappers, I.F., Ahoroni A., van Herpen T.W.J.M., Luckerhoff L.L.P., Dicke M., Bouwmeester H.J.** 2005. Genetic engineering of Terpenoid Metabolism attracts bodyguards to Arabidopsis. *Science* 309: 2070-2072

**Kessler A., Baldwin T.** 2001. Defensive function of Herbivore-induced plant volatile emissions in nature. *Science* 291: 2141-2144

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

**Kliebenstein D.J., Kroymann J., Brown P., Figuth A., Pedersen D., Gershenzon J., Mitchell-Olds T.** 2001. Genetic control of natural variation in Arabidopsis glucosinolate accumulation. *Plant Physiology* 126: 811-825

**Koch M., Bernhard H., Mitchell-Olds T.** 2001. Molecular systematics og the Brassicaceae: Evidence from coding plastidic matK and nuclear Chs sequences. *American Journal of Botany* 88: 534-544

**Madsen, P. M. and Hansen, L. M.** 2006. Krusesygegalmyggen. *Grøn Viden*. 167: 1-6.

**McKenzie R.I.H., Lamb R.J., Aung T., Wise I.L., Barker P., Olfert O.** 2002. Inheritance of resistance to wheat midge, *Sitodiplosis mosellana*, in spring wheat. *Plant Breeding* 121: 383-388

**McNeil J.N., Brodeur J.** 1995. Pheromone-mediated mating in the aphid parasitoid, Aphidius nigripes (Hymenoptera: Aphididae). *Journal of Chemical Ecology* 21: 959-972

**Miller G.E., Borden J.H.** 1984. Reproductive behaviour of the Douglas-fir cone gall midge, *Contarinia oregonensis* (Diptera: Cecidomyidae). *Canadian entomologist*. 116: 607-618.

**Mitchell-Olds T.** 2001. *Arabidopsis thaliana* and its wild relatives: a model system for ecology and evolution. *Trends in Ecology & Evolution* 16: 693-700

**Murchie A.K., Smart L.E., Williams I.H.** 1997. Responses of *Dasinuera brassicae* and its parasitoids *Platygaster subliformis* and *Omphale clypealis* to field traps baited with organic isothiocyanates. *Journal of Chemical Ecology* 23: 917-926

**Nottingham S.F., Hardie J., Dawson G.W., Alastair J.H., Pickett J.A., Wadhams L.J., Woodcock C.M.** 1991. Behavioral and Electrophysiological responses of aphids to host and nonhost plant volatiles. *Journal of Chemical Ecology* 17: 1231-1242

**Pettersson J.** 1976. Ethology of *Dasinuera brassicae* Winn (Dipt., Cecidomyidae). I. Laboratory studies of olfactory reactions to the host-plant. *Symposia Biologica Hungarica* 16: 203-208

**Pivnick K.A.** 1993. Response of males to female sex pheromone in the orange wheat blossom midge, *Sitodiplosis mosellana* (Gehin) (Diptera: Cecidomyiidae). *Journal of Chemical Ecology* 19: 1677-1689

**Readshaw J. L.** 1966. The ecology of the swede midge, *Contarinia nasturtii* (Kieff.) (Diptera, Cecidomyiide). 1.-Life-history and influence of temperature and moisture on development. *Bulletin of entomological research* 56:685-700.

**Schnee C., Köller T.G., Held M., Turlings T.C.J., Gershenzon J., Degenhardt J.** 2006. The products of a sigle maize sesquiterpene synthase form a volatile defense signal that attracts natural enemies of maize herbivores. *Proceedings of the National Academy of Sciences of the United States of America* 103: 1129-1134

**Schoonhoven L.M., van Loon J.J.A., Dicke M.** 2005. *Insect-plant biology*. Second edition. pp 421. Oxford university press, Oxford

**Stokes B.M.** 1953. The hostplant range of the swede midge (*Contarinia nasturtii* Kieffer) with special reference to types of plant damage. *European Journal of Plant Pathology* 59: 82-90

**Turlings T. C. J., Davison, A. C., Keller, M. A.** 2004. A six-arm olfactometer permittingsimultaneous observation of insect attraction and odour trapping. *Journal of Chemical Ecology* 29: 45-55.

**Turlings T. C. J., Ton J.** 2006. Exploting scents of distress: the prospect of manipulating herbivore-induced plant odours to enhance the control of agricultural pests. *Current Opinion in Plant Biology*. 9: 421:427

**Williams I.H., Martin A.P.** 1986. Evidence for a female sex pheromone in the brassica pod midge *Dasinuera brassicae*. *Physiological Entomology* 11:353-356

**Wu Q.J., Zhao J.Z., Taylor A.G., Shelton A.M.** 2006. Evaluation of insecticides and application methods against *Contarinia nasturtii* (Diptera: Cecidomyiidae), a new invasive insect pest in the united states. *Journal of Economic Entomology*. 99: 117-122

**Wyss E., Daniel C.** 2004. The effect of exclusion fences on the colonization of broccoli and kohlrabi by the Swede midge, *Contarinia nasturtii* (Diptera: Cecidomyiidae). *Mitteilungen der Deutschen Gesellschaft für allgemeine und angewandte Entomologie* 14: 387-390

**Zar J.H. 1999.** *Biostatical Analysis*. Fourth edition. pp 663. Prentice Hall, New Jersey