Sveriges lantbruksuniversitet
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Faculty of Landscape Architecture, Horticulture
and Crop Production Science

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Utvärdering av en push-pull strategi mot sorgmyggor (Diptera: Sciaridae)

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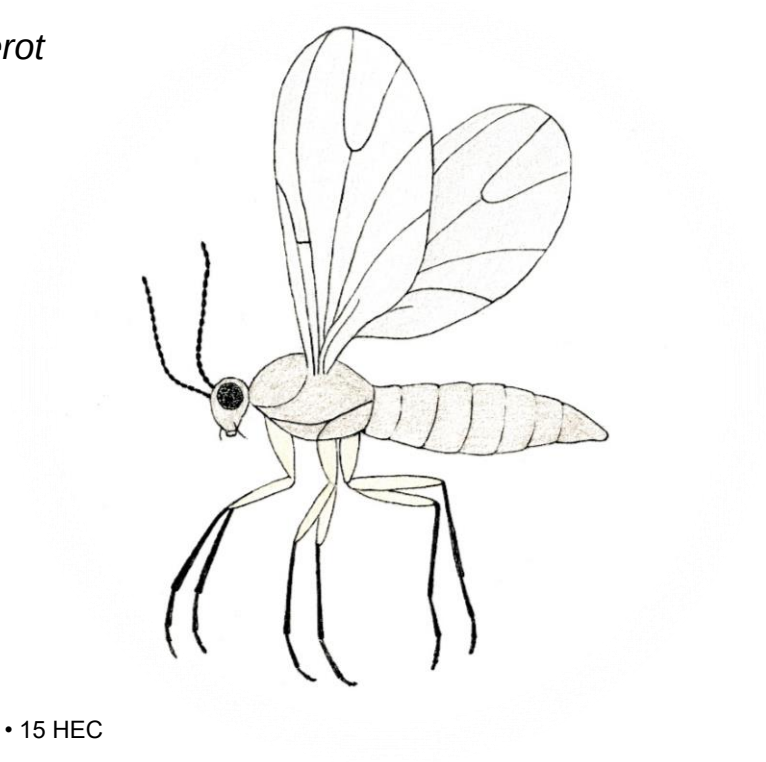

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Faculty of Landscape Architecture, Horticulture and Crop Production Science

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PROJECT INFORMATION

This report is the result of a bachelor thesis (15 ECTS) within the Horticultural Sciences programme at the Swedish University of Agricultural Sciences (SLU). An experiment was conducted at Lödde Handelsträdgård in Löddeköpinge, Sweden. The project was initiated by Klara Löfkvist at JTI, who provided financing and contact with the owner of Lödde Handelsträdgård. Marco Tasin was supervisor and Johan Stenberg the examiner.

ABSTRACT

Fungus gnats (Diptera: Sciaridae) are major insect pests in greenhouse production systems. Their larvae reside in the growing medium and can cause severe damage on the plants when feeding on the root system. When the fungus gnats occur at high population densities, the damage can inhibit plant growth, or in worst-case kill the plant entirely. Moreover, both adult and larva are capable of spreading certain diseases within the greenhouse. There is a need for new methods to supplement the current integrated pest management, and one alternative is to use odours that repel fungus gnats from the growing area.

A push-pull strategy was developed using essential oil of peppermint (*Mentha piperita*) to repel the fungus gnats, and yellow sticky card traps to attract and kill them. An experiment was conducted to evaluate the potential of using this strategy to diminish the fungus gnat population in organic greenhouse production of basil (*Ocimum basilicum*). Furthermore, the experiment tested if it was possible to enhance the effect of the strategy by equipping the yellow sticky cards with green light-emitting diods (LED). The results show that the push-pull strategy was successful in reducing the number of fungus gnats in the treated areas. However, the effect of the strategy was not enhanced by equipping the yellow sticky cards with green LEDs.

SAMMANFATTNING

Sorgmyggor (Diptera: Sciaridae) är allvarliga skadegörare inom växthusodling. Den största skadan orsakas av deras larver som lever i substratet, där de bland annat livnär sig på växternas rötter. När sorgmyggorna förekommer i stort antal kan detta leda till att växternas tillväxt hämmas eller att de i värsta fall dör. Både vuxna och larver kan dessutom sprida vissa sjukdomar inom växthuset. Det finns ett behov av fler metoder som kan komplettera det nuvarande integrerade växtskyddet, och ett alternativ är att använda dofter som repellerar sorgmyggorna från odlingsområdet.

I denna studie utvecklades en push-pull strategi som använde eterisk olja från pepparmint (*Mentha piperita*) för att repellera sorgmyggorna, och gula klisterskivor för att fånga och döda dem. Ett experiment utfördes för att undersöka om denna strategi kunde användas för att minska antalet sorgmyggor i ekologisk växthusodling av basilika (*Ocimum basilicum*). Vidare undersöktes också ifall metoden kunde förbättras genom att utrusta klisterskivorna med gröna lysdioder (LED). Resultaten pekar på att den utvecklade push-pull strategin lyckades i att minska antalet sorgmyggor. Däremot förbättrades inte metoden genom att utrusta klisterskivorna med gröna LED.

ACKNOWLEDGEMENTS

I would like to start by thanking my supervisor, Marco Tasin, who provided valuable insight and expertise throughout the project, and also offered an extra pair of hands (and brain) during the preparations of the growing benches before the experiment. I would also like to thank Klara Löfkvist who gave me the golden opportunity to do this project and also provided much appreciated thoughts and ideas. Thanks also to LRF who made this project possible by their financing. Furthermore, I would like to show my gratitude toward Bengt Jönsson at Lödde Handelsträdgård who welcomed me into his greenhouse and showed an inspiring enthusiasm for my experiment, in which he offered a lot of help and expertise. I also want to show my gratitude toward Jan-Eric Englund who helped me by discussing the statistical analysis, offering valuable suggestions and insights. At last, I thank my family for great support, especially my dad who helped me with the LEDs, even engaging co-workers whose knowledge were much appreciated.

CONTENTS

INTRODUCTION

Fungus gnats are small mosquito-like flies, which often are found surrounding pots with soil or other growing media. It is common to encounter them in the home or in the grocery store, where they usually reside around potted plants, and can be seen flying around with jerky movements. However, the damage made on the plants are in these cases often minimal. Much more severe damage can occur in greenhouse production systems, where they can become a serious problem if they appear at high population densities (Dennis 1978). The larvae can damage the plant by feeding on the roots and other parts of the plant in contact with the growing media. They can also transmit soil-borne pathogens while feeding on the plants, thus spreading diseases (Gardiner 1990; Jarvis 1993; Gillespie & Menzies 1993; Cloyd 2015). The adults do not constitute as big of a problem as the larvae, but can be a real nuisance for the workers and make the plants unattractive for customers. In some cases, the adults can also vector fungal diseases (Cloyd 2015; Gillespie & Menzies 1993; Kalb & Millar 1986; James et al. 1995; El-Hamalawi & Stangellini 2005). Greenhouses are ideal breeding grounds for the fungus gnat, so measures have to be taken to avoid extensive economical losses (Dennis 1978). Some effective methods against fungus gnats are available, but there is a need for more methods that can be used together with current practices to strengthen the integrated pest management (Cloyd 2015). Recent research have provided interesting results that could be implemented in the management of fungus gnats. One of these results is the repelling effect of menthol, a major constituent of the essential oil of peppermint (*Mentha piperita* L.) (Cloyd et al. 2011). There is a potential of using menthol as part of a *push-pull strategy*. The term push-pull was first coined in Australia in 1987, and regards a strategy for manipulating the behaviour of insect pests or their natural enemies through different stimuli (Cook 2007). The strategy can be used to reduce the abundance of a pest. The main concept involves stimuli that causes the pest to be repelled from the area with the protected resource (push), while other stimuli simultaneously works to attract the pest (pull). In this way, the pest can accumulate at the attractive source and then be removed. There are many different ways of manipulating the insect's behaviour, for example through visual- or volatile stimuli. The strategies are generally nontoxic and well suitable for implementation in an overall integrated pest management strategy.

Objective and Setup

The purpose of this study was to examine the potential of using peppermint oil in the management of fungus gnats (Sciaridae: Diptera). To do this, an experiment was conducted in organic greenhouse production of basil (*Ociumum basilicum*). A push-pull strategy was developed, using the odour from the peppermint oil to repel fungus gnats from the growing area, while yellow sticky cards were installed to attract and kill the pest. The experiment also tested whether it would be possible to increase the attractiveness of the sticky card traps by equipping them with LEDs (light emitting diodes). Growing benches with basil were divided into plots, comprising two treatments and the control. In the plots with the first treatment, dispensers emitting peppermint odour were placed in the middle, while the sticky card traps were placed at the perimeter. The second treatment had the same setup as the first, but also included green LED-strips (530 nm) which were placed above the yellow sticky cards.

Theoretical background

Fungus gnats

Fungus gnats belong to the order Diptera and the family Sciaridae. They exists in most parts of the world, with the commonly encountered species being *Bradysia impatiens* Johannsen and *B. coprophila* Cornstock (Cloyd 2015; UF 2014). The fungus gnat larvae lives in the growing media where they can cause damage to the plants by feeding on the roots, preferably the root hairs (Bethke & Dreistadt 2013; Cloyd 2010; Manners 2014). By damaging the roots, the larvae affects the plant's ability to absorb water and nutrients from the soil. This disturbs the health of the plant and its ability to grow and develop. In more severe cases the growth can be stunned entirely, and the damage can even become so severe that the plant dies (Bethke & Dreistadt 2013; Dennis 1978). Seedlings and young plants are the ones most vulnerable to the feeding damages made by the larvae, but more established plants can also take considerable damage (Bethke & Dreistadt 2013; Dennis 1978).

Another important problem with the fungus gnat larvae is their ability to spread soil-borne pathogenic fungi when feeding on the plants (Cloyd 2015; Gardiner 1990; Gillespie & Menzies 1993; Jarvis 1993). This includes notorious species such as *Pythium* spp., *Fusarium* spp. and *Verticillium* spp., and studies have even shown that oospores of certain *Pythium* spp. can survive passage through the digestive tract of the *B. impatiens* larvae.

Adult fungus gnats have not been shown to vector *Pythium* spp. because they live aboveground, and therefore do not come in contact with reproductive structures present belowground (Cloyd 2015). However, they have been shown capable of spreading aerial conidia from certain foliar and soil-borne pathogenic fungi, including *Botrytis* spp., *Verticillium* spp., *Fusarium* spp. and *Thielaviopsis* spp. (Cloyd 2015; El-Hamalawi & Stangellini 2005; Gillespie & Menzies 1993; James et al. 1995; Kalb & Millar 1986). The adults' ability to fly, although they are seen as relatively bad flyers, enables them to spread diseases much further than the larvae, which makes them a considerable threat in greenhouse production systems (El-Hamalawi & Stangellini 2005). The fact that both larvae and adults have the potential of spreading plant diseases makes the tolerance level for their presence low, and suggests that intensive pest management practices needs to be implemented in order to avoid considerable economical losses in greenhouse production systems (Cloyd 2015).

BIOLOGY

The fungus gnat lifecycle consists of four different stages: egg, larvae (with four instars), pupa and adult. One lifecycle takes about 20-28 days to complete depending on temperature, with warmer temperatures causing faster development (Bethke & Dreistadt 2013; Cloyd 2010). Optimal temperatures for fungus gnats range between 15-30°C; however, temperatures above 32-35°C and below 10°C are considered unsuitable (Manners 2014). Females live for about 3 days, and during this time they can lay about 50-300 tiny eggs in moist areas of the growing media or other organic material (Bealmer 2010; Cloyd 2010; Manners 2014).

The larvae can grow up to about 6 mm and have a clear-to-white, legless body and a black head. They tend to live in the top 3 cm of the potting soil, where they feed on a wide range of organic matter, including parts of the plant such as root hairs (Bethke & Dreistadt 2013; Cloyd 2010; Manners 2014). However, they prefer to eat fungi, which is essential for their development. The larvae can feed on a wide range of fungi, but some seem to be more beneficial for their development than others (Frouz & Nováková 2000).

The adult fungus gnats are about 3-5 mm in size and have long, thin legs and antennae (Bethke & Dreistadt 2013; Cloyd 2010; Manners 2014). Their wings are clear or light grey in colour and have a characteristic Y-shaped vein pattern that makes them easy to identify

(Bealmer 2010; Bethke & Dreistadt 2013). The adults feed on water and plant nectar (Bealmer 2010). They are relatively bad flyers, and when disturbed they tend to run across the soil or fly in jerky movements around the plant (Bethke & Dreistadt 2013; Dennis 1978). However, they prefer to rest on the soil or on the plant foliage, where they can be hard to notice because of their small size and dark colour.

When finding a place to lay their eggs, the females try to choose a location that enhances the chances of larval survival and development (Braun et al. 2012; Frouz & Nováková 2000). Porous growing media with high content of moisture and organic matter and with a high microbial activity is ideal for fungus gnats (Lindquist et al. 1985; Manners 2014; Cloyd 2010). Certain species of fungi are preferred over others; however, the preferred fungi is not always offering the best conditions for larval development (Frouz & Nováková 2000). Some species of fungi have evolved in a way that increases their attractiveness. In this way, they can use eggs and trapped adults as sources of nutrients, or use the adults as a means of spreading their spores.

PEST MANAGEMENT

For a long time, insecticides has been the main means of managing fungus gnat infestation in greenhouse production systems (Manners 2014). However, the use of many insecticides has become restricted due to negative effects on for example health or the environment. Biocides based on the toxins produced by the bacteria *Bacillus thuringiensis* var. *israelensis* have been used successfully in both conventional and organic production systems (Bealmer 2010). Such a toxin is capable of causing destruction of the midgut cells of the digestive system, so that the insect ceases eating and thus starve to death. However, there is a risk of the fungus gnat developing resistance to such toxins when used extensively, rendering them useless in the pest management (Lacey & Mulla 1977; Lindquist et al. 1985). More sustainable alternatives include several biological control agents that are currently being used successfully when managing fungus gnat populations (Bealmer 2010; Cloyd 2015). These include a soil-dwelling predatory mite (*Stratiolaelaps scimitus* Womersley), commonly known as Hypoaspis, which feeds on fungus gnat eggs and larvae; and a nematode (*Steinernema feltiae* Filipjev) which enters the body of the larvae and releases a symbiotic bacteria that kills the larva, resulting in the nematode feeding of the bacteria and the dead host.

Even if there are both chemical and biological strategies for managing fungus gnats, these must be implemented together with alternative management strategies, such as cultural, physical and sanitation, in order for them to be effective when managing fungus gnat populations (Cloyd 2015). Some important measures include eliminating excess moist and avoiding contamination from the outside. A common recommendation is to let the upper part of the growing medium to dry out occasionally in order to create an unattractive egg-laying site for the fungus gnats which requires moist conditions (Cloyd 2010). However, this is not always feasible is greenhouse production because the plants need sufficient moist in order to grow and develop properly. Another variant of this solution is to place a layer sand or similar material above the growing medium. In some production systems it may be viable to use such physical barriers to decrease the numbers of fungus gnats by implementing it as a part of the overall management strategy (Cloyd 2015). Drawbacks of this strategy include it being unsuitable for production systems using small pots, and that layers up to 3 mm have been shown ineffective in preventing females from laying eggs or adults from emerging (Cloyd 2010).

In Sweden both conventional and organic production systems use integrated pest management against fungus gnats, combining the biocide Gnatrol® (with toxins from *B. thuringiensis*), Hypoaspis and nematodes together with alternative management strategies. The strategy is usually sufficient for managing fungus gnats in conventional production systems, but not always in organic ones. This may be caused by the use of more favourable growing media, for example with higher moisture content and more microbial activity (Löfkvist 2015).

An interesting field of research concerns the potential of repelling fungus gnat adults from the growing medium, making it an unattractive site for the females to lay their eggs. In one study by Cloyd et al. (2010) researchers examined the repelling effect of Bounce® dryer sheets, and found that these were actually successful in repelling fungus gnats under laboratory conditions, so much so that the repelling effects even exceeded the attracting properties of growing medium with excessive moisture. When analysing the constituents, the researchers found linalool to be the major volatile compound. Linalool occurs naturally in the essential oil of some plants, including lavender (*Lavandula angustifolia*), basil (*Ocimum basilicum*) and marjoram (*Origanum majorana*) (Cloyd et al. 2010). Some greenhouse producers actually mix these dryer sheets into their potting mix

as a measure in their management of fungus gnats. However, the authors of the study (Cloyd et al. 2010) point out an important aspect, namely that the fungus gnats are not killed directly and therefore can migrate to other parts of the greenhouse, and suggests that the dryer sheets should be placed throughout the greenhouse in order to be effective. Another study examined the repelling capacity of 10 naturally occurring, volatile alcohols on the fungus gnat *B. coprophila* (Cloyd et al. 2011). Menthol turned out to be the most repellent among the compounds tested; the experiment used a two-armed experimental arena, where about 6% of the total amount of fungus gnats chose the compartment containing menthol, compared with about 36% choosing the control. Although other studies were done to examine the repelling effects of certain plant derived essential oil constituents, the study of Cloyd et al. (2011) was the first to make a quantitative evaluation of the repellent activity of some essential oil constituents against fungus gnats. Essential oil of peppermint (*Mentha piperita* L.) could be a viable candidate for pest control of fungus gnats, because it consists of a high percentage of menthol and also trace amounts of linalool (Yang 2010).

Beside volatiles with a repellent effect, it has been suggested that it would be possible to use yellow sticky card traps in a higher density for control of fungus gnats, and some studies have examined the possibility of enhancing their capturing ability by equipping them with LEDs (light emitting diodes) (Löfkvist 2015). One study made by Chu et al. (2004) found that yellow sticky cards equipped with lime green LEDs (with a wavelength of 530 nm) were successful in catching 377% more fungus gnats (*B. coprophila*) than unlit yellow sticky cards. In a similar study made by Chen et al. (2004) the LED-equipped yellow sticky cards captured well over 500% more *B. coprophila* than ordinary yellow sticky cards.

HYPOTHESIS

The indicated repellent effect of menthol on fungus gnats suggests that essential oil of peppermint (*Mentha* x *piperita* L.), which contains a high percentage of menthol, can be used to repel fungus gnats in greenhouse production systems. The fact that fungus gnats are drawn to yellow is well established, and the use of yellow sticky card traps has for a long time been implemented successfully in greenhouse production, but mainly as a way to estimate the magnitude of the fungus gnat infestation. However, it has been suggested that a higher density of yellow sticky cards can be used as a method to control the pest.

Some studies have also indicated that equipping the yellow sticky cards with LEDs of the wavelength 530 nm will increase their attraction of fungus gnats. Therefore, the aim of this study is to test the following two hypotheses:

- The developed push-pull strategy (using volatiles from essential oil of peppermint and yellow sticky card traps) will be successful in lowering the amount of fungus gnats.
- Equipping the yellow sticky cards with green LEDs (530 nm) will further reduce the fungus gnat population in the treated area.

MATERIALS AND METHOD

METHOD

The experiment was conducted during spring 2016 at Lödde Handelsträdgård, located in Löddeköpinge, Sweden. Two treatments against fungus gnats were tested in greenhouse production of basil. The treatments were constructed as a push-pull system, where peppermint oil worked as the repelling agent and the yellow sticky cards as the attracting agent. One treatment had ordinary sticky cards, while the other had sticky cards equipped with green LEDs. The experiment was repeated two times, the first round during period 1 (week 15-18) and the second round during period 2 (week 18-20). Each experiment started one week after the establishment of the basil, when the covering plastic had been taken of, and lasted until the basil was ready for disposal (a period of approximately three weeks).

The basil was grown on growing benches measuring 9x1.25 m. Two benches were used in both period 1 and 2 of the experiment. Each of the two growing benches were divided into plots, which arrangement is illustrated in figure 1 below. There were two plots of treatment and two of control on each bench, and a buffer zone were placed in between the plots in order to minimize the impact of the treatments on the control. In total, four replicates were made of each treatment, together with eight replicates of the control. Picture 1 and 3 on page 9 provides an illustration of how this looked in practice.

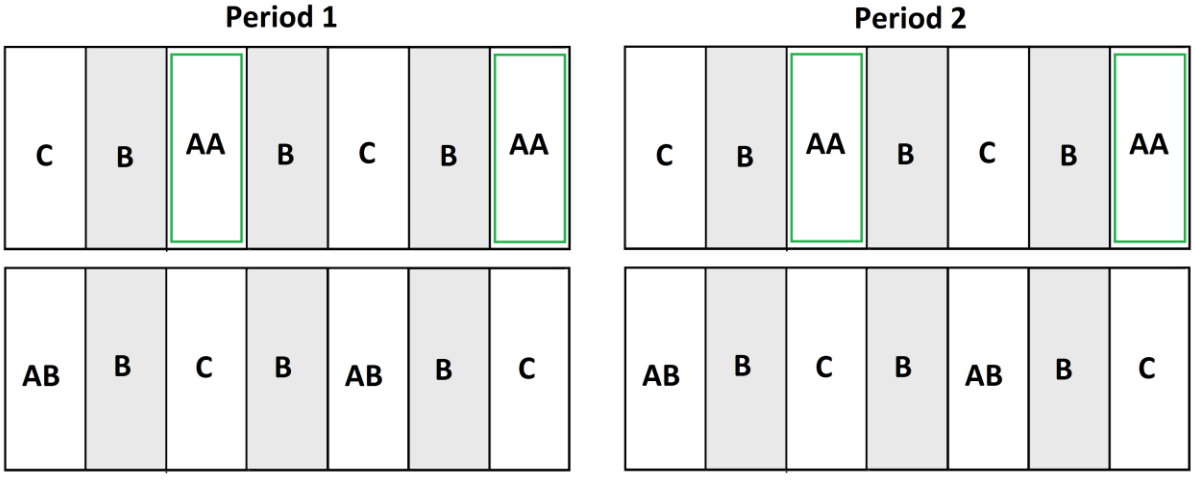


Figure 1. Two growing benches were divided in to plots measuring approximately 1x1.25 m. **AA** peppermint dispensers and LED-equipped yellow sticky cards; **AB** peppermint dispensers and yellow sticky cards; **C** control; **B** buffer area. The experiment was conducted two times (period 1 and 2), using the same growing tables and arrangement of the treatments.

Picture 1. The experiment during period 1 with all the plots visible. *Therese Diderot, 2016*.

MATERIALS

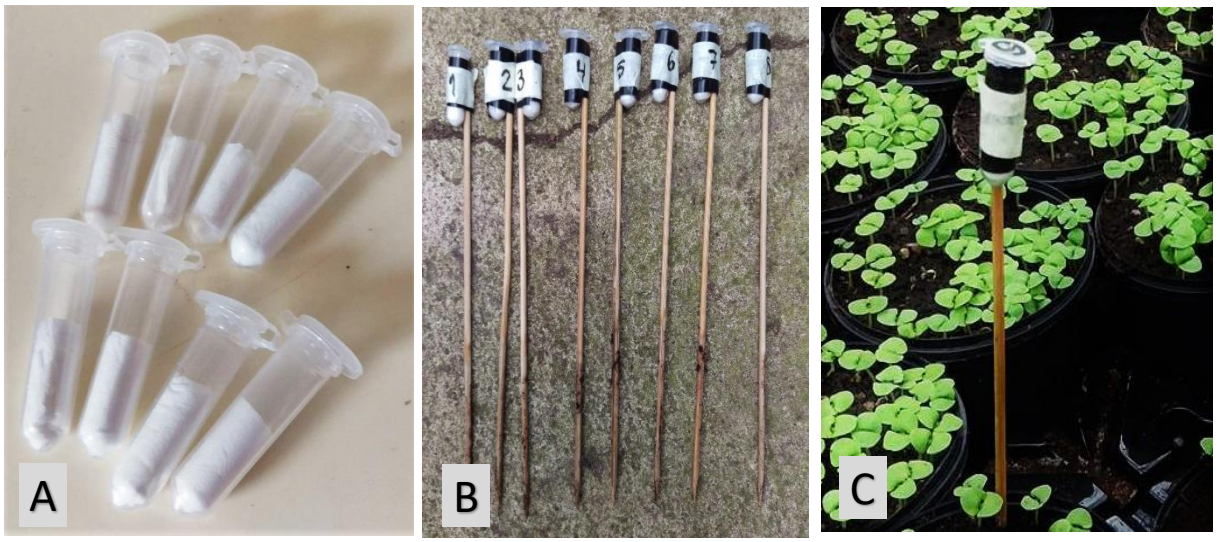
PLANT MATERIAL

The basil were cultivated in approximately 18°C in organic growing medium. During the first week the basil seedlings were covered with plastic. Thereby, the experiment started approximately one week after the establishment, when the plastic was removed. Furthermore, the basil was watered from above during the first week, but after that, and throughout the experiment, the basil was watered from underneath.

Normally, the basil is treated with *B. thuringiensis* and nematodes. In this case, however, none of these treatments against fungus gnats were used, thus avoiding the risk of them disturbing the results of the experiment. Yet, there were some predatory mites in the greenhouse which could not be kept away from the experimental area. There was also a stripe of yellow sticky trap in the ceiling, running through the whole greenhouse.

DISPENSERS

The dispensers that emitted the peppermint odour were constructed of small Eppendorf vials (2 ml). Half of a cotton dental wick was placed in each vial and 500 µl of peppermint oil (100%, MS Biredskapsfabriken AB, Töreboda, Sweden) were added and allowed to sink in to the cotton. Then 50 µl mineral oil (Sigma Aldrich, St. Louis, Missouri) were added on top of the peppermint oil in order to slow down the release rate. The lid of the tube was then closed, and just before placement in the experimental area a small hole (1 mm in diameter) was drilled in the lid in order to further limit the emission from the vial. This way it could be an even and steady flow of volatiles emitted during the whole experiment. The dispensers were made during the morning the same day the experiment started, so they were not in need of any storage. They were attached to 25 cm long plant supporting sticks with black electrical tape, which then were placed in the pots with basil (see picture 2 below).



Picture 2. The 8 dispensers (A) were attached to 25 cm long plant supporting sticks (B) and then placed in pots with basil (C). *Therese Diderot, 2016*.

The amount of peppermint oil was estimated by comparing the volatility of menthol with the ones of other substances that had been used in similar dispensers. Methyl salicylate was found to be have a similar KI value (Kovats retention index) to that of menthol (Nicolić et al. 2013; Sun et al. 2014). By examining studies in which dispensers with methyl salicylate had been used, the conclusion was reached that 0.5 ml of essential oil would be enough to last during one experimental period (James et al. 2006; Jones et al. 2011; Mallinger et al. 2011; Orre-Gordon et al. 2013; Woods et al. 2011).

YELLOW STICKY CARDS & LEDS

The yellow sticky cards were used to trap the repelled fungus gnats, preventing them from moving over to other parts of the greenhouse. To do this they were placed around the outer border of each plot. A construction was built in order to create a frame on which the sticky cards could be hung. There were in total 12 sticky cards around each plot. On the plots treated with LED-equipped sticky cards, a wooden frame was constructed above the sticky cards, on which a LED-strip was placed. A black fabric was also attached to the construction. This was to avoid any impact from the LEDs being made outside the plots. The same fabric was also applied around the plots without LEDs to create as similar conditions as possible in both treatments. The constructions were approximately 25 cm high.

The LED-strips (LEDshopen i Sverige AB, Sjöbo, Sweden) were of a green colour (530 nm) and the diodes had a distance of 10 cm between them. The diodes emitted 12 V of light 24 hours a day during the whole experiment, except the last day of the second period, when the LED-strips had stopped working for unknown reason.

READINGS

The evaluation of the treatments was made in three different ways; I) with white sticky card traps, II) by counting the visible fungus gnats in each plot, and III) by carefully examining soil for fungus gnats larvae.

Six white sticky cards were attached to 25 cm long plant support sticks and placed in each plot as shown in figure 2. They were left there for three days before they were collected and the fungus gnats counted. The measurement was made one week after the start of each of the two experimental periods. A loupe was used to identify the fungus gnats by their characteristic wing pattern. The traps were of a white colour so that they would not attract fungus gnats from outside the plot, but rather just those

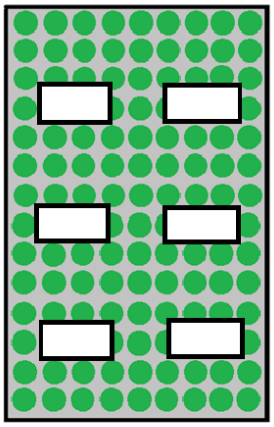


Figure 2. The placement of the six white sticky cards in each plot.

that were in the plot and accidentally flew in to them. Picture 3 below gives an illustration of how the sticky cards looked when placed in the plots.

Picture 3. The measurement during period 1 with 6 white sticky cards per plot. *Therese Diderot, 2016*.

The visual evaluation of fungus gnats was made during three minutes in each plot. In the beginning of the inspection, seedlings were stroked so that their movement would stimulate flight among the fungus gnats. The evaluation were made at the same day the white sticky cards were collected, 10 days in to the experiment.

At last, two pots of basil from each plot were taken for soil analysis at the last day of the experiment. The soil was inspected carefully and systematically in order to detect any fungus gnats larvae present. First, the soil was taken out of the pot, and the inside of the pot was inspected. The lump of soil was turned upside down with the seedlings facing the floor, and thin layers of the soil were removed while inspected, falling down on a plastic cover underneath. When all the soil and roots had been separated, a second inspection was made by moving small amounts of soil from the pile while spreading it out and then placing it at the side, creating a new pile. Finally, a third inspection was made where all of the soil was spread out and moved around in order to see more angles of the soil particles.

STATISTICS

The data collected from the experiment was analysed using the statistical software Minitab 17. The analytical method used was *general linear mixed models* with a normal distribution. The analysis was "mixed" in the sense that the two factors *group* (treatment) and *block* (position) were set as fixed effects, while the factor *period* was set as a random effect. Fixed effect factors means that the analysis only includes the specific factors given in the data, in this case the treatments used (*group*) and their position in the greenhouse (*block*); it doesn't try to make any assumptions about other treatments or positions. Random effects factors, however, means that the analysis estimates the effects of all different levels of the factor. This enables the analysis to estimate how the fixed effect factors (treatment and position) affect the number of fungus gnats during all periods, not only the ones during which the data were collected. Because the position of the *blocks* were the same during both periods, the period was nested with the block so that the analysis would take that fact into consideration. Illustrating column charts were made in Microsoft Excel.

RESULTS

The amount of fungus gnats in each plot were estimated using three different methods; I) white sticky card traps, II) visual inspection and III) counting larvae. However, only one of these methods, namely the one using white sticky cards, was successful in collecting the sufficient amount of data needed for analysis. Therefore, the results featured in this report only come from the data collected using that method. The raw data is featured in table 1 (appendix 1), while the results from the analysis is presented in table 3 (appendix 2).

Figure 3 on page 15 features three different column charts showing the average amount of fungus gnats captured on a white sticky card from the different treatments during period 1, period 2 and both periods, respectively. There were more fungus gnats during period 2 compared to period 1, but the proportions of fungus gnats were very similar. The treatment with sticky cards and the one with LED-equipped sticky cards had very close average values. When the data from period 1 was merged with the data from period 2, these treatments even showed the same average value.

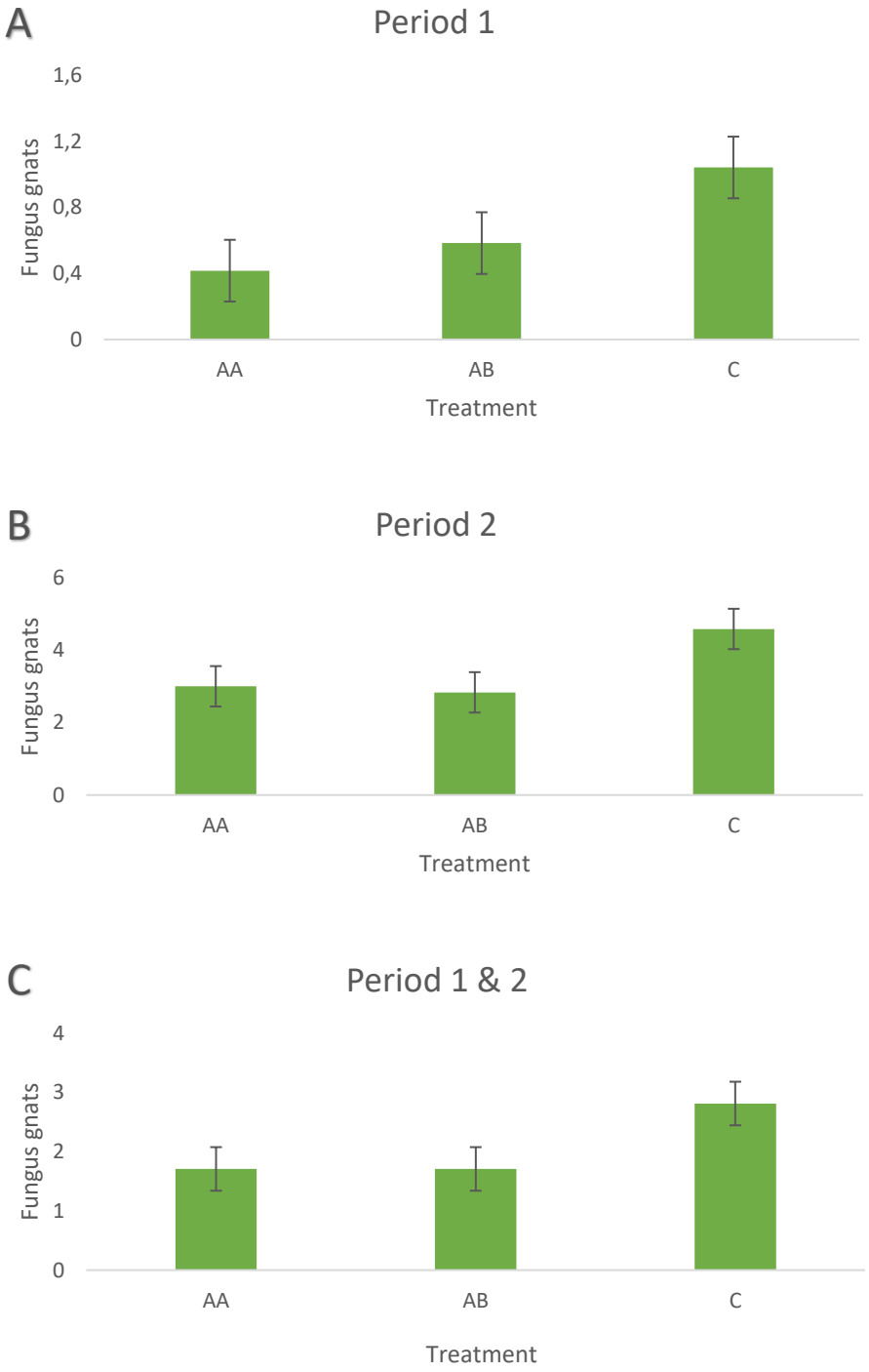


Figure 3. The three column charts shows the average number of fungus gnats captured on a white sticky card trap in plots with the different treatments: AA (peppermint odour and LED-equipped yellow sticky cards), AB (peppermint odour and ordinary yellow sticky cards) and C (control). Observe that the y-axes has different scales. **A)** The average numbers of fungus gnats from the experiment conducted during period 1 (15/4/2016-25/4/2016) **B)** The average numbers of fungus gnats from the experiment conducted during period 2 (6/5/2016-16/5/2016). **C)** The average numbers of fungus gnats from both period 1 and 2 merged together.

By analysing the data in Minitab 17 it was found that there was a significant difference between the treatments and the control. However, there was no significant difference between the treatment with yellow sticky cards and the one with LED-equipped yellow sticky cards. Therefore, it is safe to assume that the LEDs did not have any effect on the numbers of fungus gnats in the plots. Without the LEDs, both treatments were exactly the same, even capturing the same average value of fungus gnats. In order to get a p-value that was as accurate as possible, the data from the two treatments were merged into one dataset. This way the analysis only considered one treatment, comprising peppermint oil and yellow sticky cards. By doing so the numbers of data points were increased resulting in a more accurate result. Thus, at the second analysis in Minitab 17 the experimental setup contained eight replicates of both the treatment and the control respectively, and was divided into four blocks (see figure 4 below).

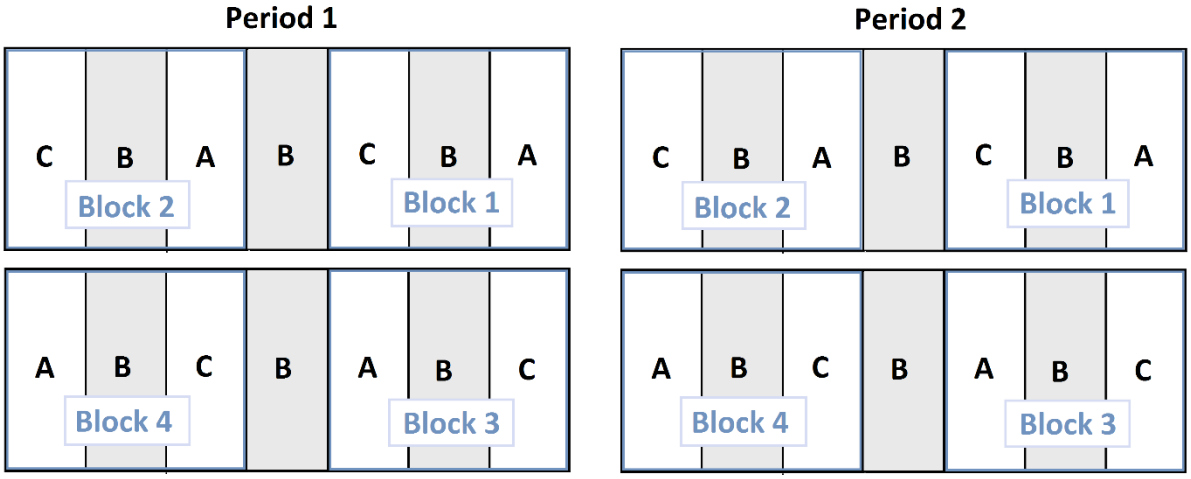


Figure 4. Two growing benches. **A** peppermint dispensers and yellow sticky cards; **C** control; **B** buffer area. The experiment was conducted two times (period 1 and 2), using the same growing tables and arrangement of the treatments.

The data were collected by placing 6 white sticky cards in each plot. An average value were calculated from these, and this average number of fungus gnats captured in each plot were then used when analysing the results. The data used for the analysis is found in table 2 (appendix 1). The analysis in Minitab (appendix 2) showed that there was a significant difference ($p<0.05$) between treatment (A) and control (C). Figure 5 on page 17 illustrates the data, and shows clearly that the data follow a normal distribution, and that the data has little variation and therefore the data points lie close to the trendline. However, one plot with control had higher numbers of fungus gnats and can therefore be seen as an outlier, but it is not that far off so that it needs to be disregarded.

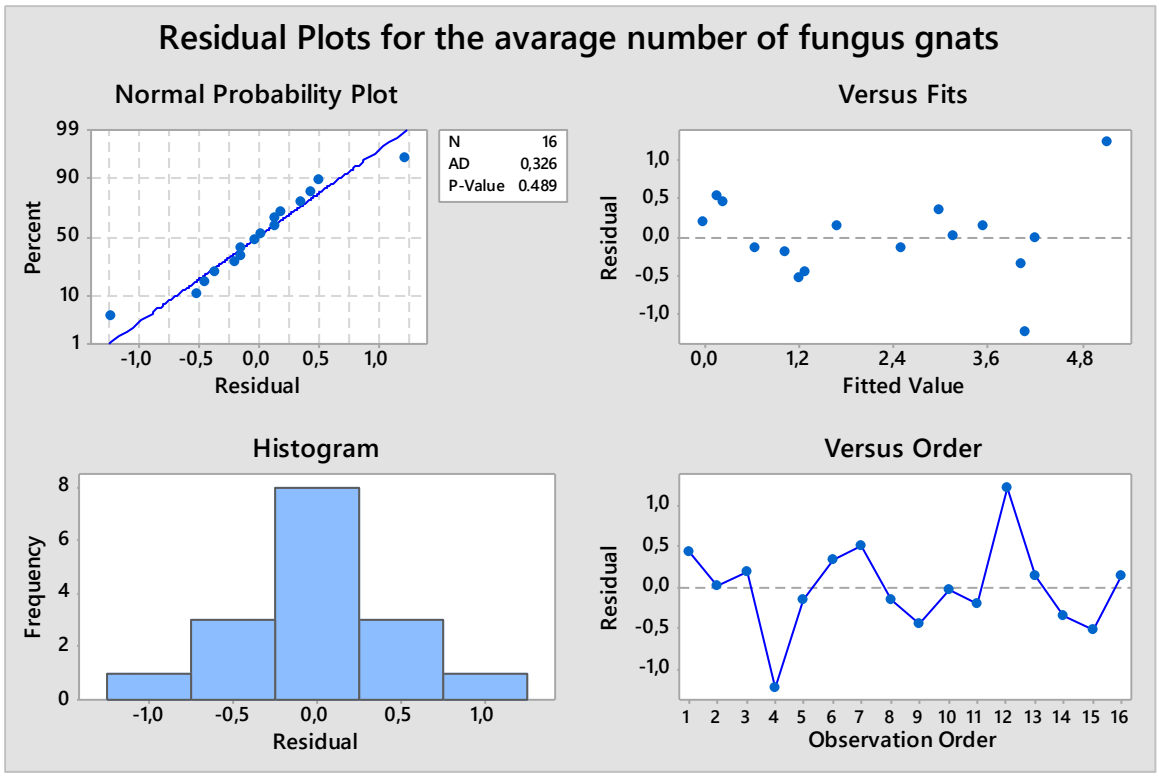


Figure 5. A "four in one" plot that illustrates the data that were analysed in Minitab 17. The plots show that the data fits well into the normal distribution (left bottom corner), and that the data points follow the trendline without any outliers being too far astray (left top corner).

DISCUSSION

LIMITATIONS

There were several aspects that restricted the setup of the experiment, thus limiting the conclusions that could be made based on the results. One of these aspects was the space available at Lödde Handelsträdgård. The time and financing available were also such aspects. Therefore, the experiment was not able to distinguish the effect from the peppermint odour and the yellow sticky cards, thus they have to be seen as parts of a system. Furthermore, the experiment was not able to evaluate how far the potential repelling effect of the peppermint could affect the fungus gnats. Additionally, there were no means within this study to attain details about the dispensers, such as the exact composition of volatiles and their release rates. This also contributed to the difficulty to evaluate the actual effect of the treatments.

DISCUSSION OF THE RESULTS

The results confirm one of the hypotheses made, namely that the push-pull strategy developed in this study (using volatile constituents of the essential oil of peppermint and yellow sticky cards) could diminish the number of fungus gnats in the treated areas. This was expected due to recent studies showing the possible repelling effect of menthol and linalool on fungus gnats, both of which are volatile constituents of the essential oil of peppermint (Cloyd et al. 2010; Cloyd et al. 2011; Yang et al. 2010). It was also expected due to the well-established attracting effect of yellow sticky cards on fungus gnat and similar insects (Löfkvist 2015; Shimoda & Honda 2013). However, linalool only exists in very small amounts in the essential oil of peppermint, and it is therefore unlikely that it would have had any major effect on the results in this study (Yang et al. 2013). Menthol, on other hand, is present in high amounts in peppermint oil. Both menthol and peppermint leaves, that may contain up to 80% menthol, have been shown to repel mosquitos, as well as several beetles and other insects (Cloyd et al. 2011).

As noted above under the heading *limitations*, this study is not capable of determining the exact effect of the peppermint oil versus the yellow sticky cards. The strategy must therefore be seen as a system where the two parts work together; with the peppermint odour repelling the fungus gnat adults from the centre of the plot towards the outer border where the yellow sticky cards attract and captures them. Because there is a lack of

studies examining how far the repelling effect of volatile compounds like menthol and linalool can affect the fungus gnats, there is no way to be certain that the buffer zone between plots with treatment and plots with control (see figure 1, page 8) was enough to avoid that the odour used in the treatments also affected fungus gnats within controls. Moreover, the distance between the two growing tables was limited, also potentially causing the control to be affected. The experimental design was optimized so that the potential drift of the volatiles would be taken into consideration. However, the limited surface available for the trial restricted the distances that could be arranged.

The other hypothesis, however, was not confirmed; the strategy was not shown be more efficient in reducing the numbers of fungus gnats if the yellow sticky cards were equipped with green LEDs (530 nm). This was somewhat surprising considering the promising results obtained in two relatively recent studies, in both of which the amount of fungus gnats caught by the yellow sticky cards increased substantially when equipping them with green LEDs (530 nm) (Chu et al. 2004; Chen et al. 2004). It is also a well-established fact that fungus gnats, as many other insects, are drawn towards light (Bethke & Dreistadt 2013; Shimoda & Honda 2013). Because of practical and statistical reasons, the fungus gnats caught on the yellow sticky cards surrounding the plots with treatment were not counted. However, it would have been an interesting addition to the study, because there is a possibility that the black fabric didn't shield the area good enough, potentially resulting in fungus gnats from outside the plot being drawn towards the lights and thus cancelling out the diminishing effects of the LED-equipped sticky cards. Another reason could be that because the LEDs and the yellow sticky cards were slightly separated (with the LED-strip being placed above the sticky cards), the attraction of the fungus gnat did not result in them getting caught on the sticky cards. In the studies by Chen et al (2004) and Chu et al. (2004) the LEDs were attached onto each yellow sticky card. However, it is interesting that the average values of captured fungus gnats are that very similar in both treatments (see figure 3, page 15), which suggests that the LEDs did not have any effect on fungus gnat attraction at all.

Suggested improvements and future studies

As mentioned before, this study is unable to pinpoint the exact mechanisms behind the obtained results, and it would therefore be relevant with further research looking deeper into these issues. For example, it would be interesting to further investigate the repellent

effect of menthol and other volatile essential oil constituents. There is still much to learn about how they affect the fungus gnats, such as from how long a distance the fungus gnats can detect and react on such volatiles, and for how long time a dispenser can be viable. It would also be relevant to test if menthol can repel other greenhouse pests, such as the shore fly (Diptera: Ephydridae). Furthermore, it would be interesting to determine if there would be any difference in the results when using synthetically produced menthol, compared to plant derived menthol or essential oils (which also contains other volatiles). This can be an important facts when designing both experimental setups as well as implementable strategies for greenhouse production systems. It is also important out of an economic point of view. After all, in order to implement a new strategy, the producers have to assess whether the method is worth the investment by relating it to the money saved by reducing the pest populations.

Regarding the yellow sticky cards, it would be relevant to investigate how to apply them in a bigger scale with the same effect as in this study, but without being too much of an inconvenience. An alternative could be using other methods of capturing fungus gnats. This study did not have the time and resources to go into some of the other viable solutions, such as using lures with volatiles that attract the adult fungus gnats. One option could be to attract the females with smells that signals good environment for egg-laying. For example, some fungi seem to have found a way to attract the females, exploiting the female's effort of finding a suitable place for egg-laying (Frouz & Nováková 2001).

CONCLUSIONS

The results from this study showed that the developed push-pull strategy, which used volatile constituents of the essential oil of peppermint as repellent and yellow sticky cards as attractant, was successful in significantly reducing the amount of fungus gnat adults in the treated areas compared with control. However, it was not shown that LED-equipped yellow sticky cards could enhance the effect of the strategy. Because of limitations in the experimental setup, questions still remain about the exact mechanism behind these results, and further research is needed to confirm these findings and to implement them into a commercially viable pest management strategy.

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APPENDIX 1 – DATA

Table 1. The raw data collected by white sticky card traps. The *group* represents the treatment; AA (peppermint odour and LED-equipped yellow sticky cards), AB (peppermint odour and ordinary yellow sticky cards) and C (control), the *response* the number of fungus gnats on each white sticky card, the *block* the position (plot) in the greenhouse, and *period* the time period during which the data were collected.

Group	Response	Block	Period
AA	1	1	period1
AA	0	1	period1
AA	0	1	period1
AA	3	1	period1
AA	0	1	period1
AA	0	1	period1
AA	0	2	period1
AA	0	2	period1
AA	0	2	period1
AA	0	2	period1
AA	0	2	period1
AA	1	2	period1
AB	2	3	period1
AB	0	3	period1
AB	0	3	period1
AB	0	3	period1
AB	0	3	period1
AB	1	3	period1
AB	1	4	period1
AB	0	4	period1
AB	1	4	period1
AB	1	4	period1
AB	1	4	period1
AB	0	4	period1
C	1	1	period1
C	1	1	period1
C	0	1	period1
C	2	1	period1
C	0	1	period1
C	1	1	period1
C	1	2	period1
C	0	2	period1
C	0	2	period1
C	1	2	period1
C	2	2	period1
C	1	2	period1

C	3	3	period1
C	1	3	period1
C	1	3	period1
C	0	3	period1
C	4	3	period1
C	2	3	period1
C	0	4	period1
C	1	4	period1
C	2	4	period1
C	0	4	period1
C	1	4	period1
C	0	4	period1
AA	3	1	period2
AA	1	1	period2
AA	5	1	period2
AA	5	1	period2
AA	2	1	period2
AA	3	1	period2
AA	3	2	period2
AA	3	2	period2
AA	3	2	period2
AA	2	2	period2
AA	4	2	period2
AA	2	2	period2
AB	1	3	period2
AB	2	3	period2
AB	1	3	period2
AB	6	3	period2
AB	4	3	period2
AB	6	3	period2
AB	2	4	period2
AB	4	4	period2
AB	2	4	period2
AB	2	4	period2
AB	2	4	period2
AB	2	4	period2
C	9	1	period2
C	4	1	period2
C	0	1	period2
C	6	1	period2
C	3	1	period2
C	3	1	period2
C	8	2	period2
C	8	2	period2
C	9	2	period2
C	8	2	period2

C	2	2	period2
C	6	2	period2
C	4	3	period2
C	1	3	period2
C	5	3	period2
C	4	3	period2
C	5	3	period2
C	3	3	period2
C	5	4	period2
C	1	4	period2
C	5	4	period2
C	6	4	period2
C	2	4	period2
C	3	4	period2

Table 2. The data used in the analysis in Minitab 17 using *general linear mixed models*. The response is the average numbers of fungus gnats caught on each plot by white sticky cards.

Group	Response	Block	Period
A	0,666667	1	1
A	3,166667	1	2
A	0,166667	2	1
A	2,833333	2	2
A	0,5	3	1
A	3,333333	3	2
A	0,666667	4	1
A	2,333333	4	2
C	0,833333	1	1
C	4,166667	1	2
C	0,833333	2	1
C	6,33333	2	2
C	1,833333	3	1
C	3,666667	3	2
C	0,666667	4	1
C	3,666667	4	2

APPENDIX 2 – ANALYSIS

Table 3. The results from the statistical analysis in Minitab 17 using linear mixed model with a normal distribution.

General Linear Model: response versus group; period; block

```
Method

Factor coding  (-1; 0; +1)


Factor Information

Factor         Type   Levels  Values
group          Fixed       2  A; C
period         Fixed       2  1; 2
block(period)  Fixed       8  1(1); 2(1); 3(1); 4(1); 1(2); 2(2); 3(2); 4(2)


Analysis of Variance

Source           DF  Adj SS   Adj MS  F-Value  P-Value
  group           1   4.340   4.3403     6.98    0.033
  period          1  34.028  34.0278    54.71    0.000
  block(period)   6   3.104   0.5174     0.83    0.581
Error             7   4.354   0.6220
Total            15  45.826


Model Summary

       S    R-sq  R-sq(adj)  R-sq(pred)
0.788684  90.50%     79.64%      50.36%


Coefficients

Term             Coef  SE Coef  T-Value  P-Value   VIF
Constant        2.229    0.197    11.31    0.000
group
  A            -0.521    0.197    -2.64    0.033  1.00
period
  1            -1.458    0.197    -7.40    0.000  1.00
block(period)
  1(1)         -0.021    0.483    -0.04    0.967  1.50
  2(1)         -0.271    0.483    -0.56    0.592  1.50
  3(1)          0.396    0.483     0.82    0.439  1.50
  1(2)         -0.021    0.483    -0.04    0.967  1.50
  2(2)          0.896    0.483     1.85    0.106  1.50
  3(2)         -0.187    0.483    -0.39    0.709  1.50
Regression Equation

response = 2.229 - 0.521 group_A + 0.521 group_C - 1.458 period_1 + 1.458 period_2
           - 0.021 block(period)_1(1) - 0.271 block(period)_2(1)
+ 0.396 block(period)_3(1)
           - 0.104 block(period)_4(1) - 0.021 block(period)_1(2)
+ 0.896 block(period)_2(2)
           - 0.187 block(period)_3(2) - 0.687 block(period)_4(2)

Fits and Diagnostics for Unusual Observations

Obs  response    Fit   Resid  Std Resid
  4     2.833  4.062  -1.229      -2.36  R
 12     6.333  5.104   1.229       2.36  R

R  Large residual
```