# Effects of plant-fungal interactions on volatile emission of *Hordeum vulgare* and aphid preference

Påverkan av växt-svampinteraktioner på volatiler från *Hordeum vulgare* och preferens hos bladlöss

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Agriculture Programme – Soil/Plant
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## Abstract

Barley is one of the most economically important cultivated crops in the world. However, barley is a host for many insect pests and plant pathogenic fungi which is a threat to the production. *Rhopalosiphum padi* is an economically important insect pest that leads to large economical losses in agricultural crops such as barley. An increased understanding of interactions between insects, plants and microorganisms is an important step in developing non-chemical control strategies to prevent major outbreaks of aphids and other pests. This study firstly aims to study the effect of barley inoculated with the beneficial fungi *Trichoderma atroviride* and the plant pathogen *Fusarium graminearum* on emission of volatile organic compounds. Secondly, it aims to analyse aphid behavior and preference to odours from barley plants inoculated with *T. atroviride, F. graminearum* and non-inoculated controls through olfactometer tests. Volatile organic compounds were collected on volatile traps from barley-plants inoculated and grown under controlled conditions in a growth chamber. The volatile organic compounds were analysed and quantified in a Gas chromatography – mass spectrometry (GC-MS) and then in the Automated Mass Spectral Deconvolution and Identification System (AMDIS). The result of the volatile analysis showed no significant differences in the total volatile organic compound (VOC) composition between plants inoculated with *F. graminearum*, *T. atroviride* and non-inoculated plants. The olfactometer test result shows no significant difference in aphid preference between plants inoculated with *F. graminearum* and non-inoculated plants. However, R. padi did significantly prefer non-inoculated plants over plants inoculated with *T. atroviride*. As a conclusion, based on the results in this study, VOCs induced by *T. atroviride* could possibly work as repellents for *R. padi.* However, further studies on the interaction between plants, insects and microorganisms are needed to develop new control strategies and possibly use it as biological control and in Integrated Pest Management (IPM).

*Keywords:* Volatile organic compounds, chemical communication, *Trichoderma atroviride, Fusarium graminearum, Rhopalosiphum padi,* biological control

## Sammanfattning

Korn är en av de ekonomiskt viktigaste odlade grödorna i världen och är värd för flera skadeinsekter och växtpatogena svampar, vilka utgör hot mot produktionen. Havrebladlusen *Rhopalosiphum padi* är en ekonomiskt viktig skadeinsekt som leder till stora ekonomiska förluster i jordbruksgrödor som exempelvis i korn. En ökad förståelse för interaktioner mellan insekter, växter och mikroorganismer är ett viktigt steg för att utveckla andra icke-kemiska kontrollstrategier för att förhindra stora utbrott av bladlöss. Denna studie syftade för det första till att studera hur inokuleringen av korn med nyttosvampen *Trichoderma atroviride* och den växtpatogena svampen *Fusarium graminearum* påverkar volatila ämnen från plantan. För det andra syftade den till att analysera bladlössens beteende och preferens för volatiler från kornplantor inokulerade med *T. atroviride, F. graminearum* och ej inokulerade kontrollplantor genom olfaktometer tester. Volatiler samlades in på fällor från kornplantor som hade växt under kontrollerade förhållanden i en tillväxtkammare. Volatilerna analyserades och kvantifierades med GC-MS och AMDIS. Resultaten visade inga signifikanta skillnader i den totala sammansättningen av volatiler från plantor inokulerade med *T. atroviride, F. graminearum* och ej inokulerade plantor. Olfaktometertestet visade ingen signifikant skillnad i bladlössens preferens mellan plantor inokulerade med *F. graminearum* och ej inokulerade plantor. *Rhopalosiphum padi* föredrog däremot ej inokulerade plantor framför plantor inokulerade med *T. atroviride*. Som en slutsats, baserat på resultaten i denna studie, kan volatiler inducerade av *T. atroviride* möjligen fungera frånstötande för *R. padi*. Det behövs dock ytterligare studier om interaktionen mellan växter, insekter och mikroorganismer för att utveckla nya kontrollstrategier och för att använda denna strategi inom biologisk kontroll och i IPM-strategier.



*Nyckelord:* Flyktiga ämnen, kemisk kommunikation, *Trichoderma atroviride, Fusarium graminearum, Rhopalosiphum padi,* biologisk kontroll

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# Abbreviations

| | |
|---|---|
| BCA | Biological Control Agent |
| VOC | Volatile Organic Compound |
| DW | Dry weight |
| AMDIS | Automated mass spectral Deconvolution and Identification System |

# 1. Introduction

Barley (*Hordeum vulgare)* is one of the most important cultivated crops for food security in many parts of the world (Wiegmann et al. 2019). The largest part of the production of barley is used for animal feed. Even though the production for direct human consumption is lower, it is still a very important food source in developing countries (Aldughpassi et al. 2016). Furthermore, barley has high tolerance against abiotic stress, which can make it an even more important crop in the future with an increase in negative impacts from climate change (Wiegmann et al. 2019). However, barley is a host for many insect pests and plant pathogenic fungi which is a threat to the production. The bird cherry-oat aphid, *Rhopalosiphum padi* is an example of a destructive insect pest on cultivated plants, including on barley. *Rhopalosiphum padi* is not only causing damage on the plants by feeding on the phloem sap with piercing sucking mouth parts, it is also transmitting viruses such as the barley yellow dwarf virus (Wiest et al. 2021). Pathogenic microorganisms and insect pests are biotic stressors that lead to a decrease in yield and are a current challenge in agricultural crop production all over the world (Pappas et al. 2020).

Chemical control is often used to prevent and manage outbreaks of pests. Application of chemical pesticides are however known to have negative effects on beneficial insects, such as natural enemies (Conboy et al. 2020). Additionally, intensive use of chemicals can lead to the development of resistance of insects to pesticides. Hence, other control strategies than applying chemicals are needed, partly because of the fast evolution of insecticide resistance and the conceivable harm to the environment (Conboy et al. 2020).

There is a need to study the impact of microorganisms on plant volatile organic compounds (VOCs), since, plants and insects produce and perceive semiochemicals which lead to important interactions and behavioural responses between organisms and their environment (Nordlund & Lewis 1976). Therefore, it is important to understand more about the plant-insect-microorganism relationships for the development of new control strategies other than chemical control to possibly prevent major outbreaks of pests in the future (Schoonhoven 2005).

## 1.1 Chemical communication

Plants interact with many different organisms both below and above ground via chemical compounds. The outcome of these interactions can be indirect and direct as well as beneficial or detrimental depending on the organisms involved. Plants can have direct defence interactions against insects such as barriers or chemicals. The indirect outcomes can be based on for example the induction of chemicals that attract natural enemies of insect pests (Battaglia et al. 2013). The chemicals that are used in the communication between individual organisms are called semiochemicals. Semiochmicals used in intraspecific interactions (interactions between the same species) are called pheromones. Allelochemicals are on the other hand used in interspecific interactions, they are further classified into allomones, synomones, and kairomones. This division is based on the impact on the receiver, emitter, or both in the interaction. Nordlund & Lewis (1976) classified signals that are beneficial for the emitter as allomones. An example of an allomone is a plant compound that functions as a feeding deterrent for an herbivorous insect. On the other hand, kairomones are beneficial for the receiver and unfavourable for the emitter. A compound is classified as a kairomone when mediating in interactions where the compound is stimulating phytophagy, for example. Synomones are signals that are beneficial for both the receiver and the emitter, for example VOCs that are emitted from plants in response to herbivore feeding and attract predators and parasitoids (Nordlund & Lewis 1976). Plants can communicate with many different organisms such as other plants, fungi, and insects through the receiving and emission of VOCs (Coppola et al. 2019). Volatiles released from plants are important for insects such as aphids to find a suitable host plant for feeding and reproduction. Microorganisms such as fungi can induce emission of VOCs from the plants (Bruce & Pickett 2011).

## 1.2 Volatile organic compounds

Plants can release VOCs both constitutively or in response to biotic or abiotic stress factors (Dudareva et al. 2013). VOCs can serve as signalling molecules due to their high vapor pressure and a low molecular weight that enables diffusion through the gas phase and exchange of VOCs between- and within organisms (Siddiquee 2014).

Insects and plants can be dependent on the chemical interaction between them (see chapter 1.1). Herbivorous insects are able to sense which host plant is most suitable for feeding or laying eggs by detecting volatile plant metabolites with their olfactory system (de Bruyne & Baker 2008; Bruce & Pickett 2011). There are a lot of different volatiles in the environment and the sensitivity and specificity of the olfactory system are very important, for example when aphids need to locate a suitable host. The volatiles can be in different concentrations and combinations

(Bruce & Pickett 2011). The function of VOCs as signalling molecules (semiochemicals) can be attractive or repellent to insects (Siddiquee 2014). Additionally communication via VOCs can occur between plants and microorganisms in the rhizosphere as well as in the phyllosphere (D'alessandro et al. 2014).

Plant emitted volatiles are secondary metabolites that are synthesized from resources gained from primary plant metabolism, due to different biosynthetic pathways (Dudareva et al. 2013). Green-leaf volatiles and terpenes are examples of classes of volatiles released from plants that have commonly been shown to be important in interactions. Plants can release terpenes to attract beneficial organisms. Green-leaf volatiles (GLV) are compounds that are emitted due to damage or stress factors such as mechanical damage (Dudareva et al. 2013). GLVs are released constantly from almost all plants, but in lower concentration when the plants are not stressed. Induction or changes in VOC composition can carry important information for the receiving organisms such as other plants or insects. The host-insect recognition may occur by specific volatiles connected to specific host plants. However, the recognition can also be from specific blends from numerous volatiles (Bruce et al. 2005). Identification of hosts by insects such as aphids can be implemented through visual cues and olfactory systems, such as VOCs released from the plants (Sudderth & Sudderth 2014).

## 1.3 Aphid host location

Aphids are small insects directly causing both physical and structural damage to the plant through insertion of their stylets into the plant tissue, the release of substances into the tissue with the aphid's saliva, and sustained feeding on the phloem sap (Hogenhout & Bos 2011; Wiest et al. 2021). Aphid feeding behavior is controlled by factors such as mechanical barriers as well as the presence or absence of essential nutrients and secondary compounds that are detrimental in the phloem (Singh et al. 2020). The aphid acceptance or rejection of a host plant is a result of the puncturing of epidermal cells with the stylet, which is done to evaluate the suitability of the plant due to its chemistry (Singh et al. 2020). Additionally, substances in the saliva of aphids that are injected in the plant phloem can alter the physiology of the plant as they can be toxic or lead to chlorosis and necrosis (Hogenhout & Bos 2011; Wiest et al. 2021).

Furthermore, aphids are one of the most common vectors for plant viruses important in agriculture (Singh et al. 2020). Aphids can transmit a variety of different viruses to different plant species during phloem feeding. Aphid transmitted viruses are easily spread by winged aphids, and by non-winged as they walk or when the aphids are spread by the wind or rain. Different plant quality

combined with environmental factors such as temperature, humidity, light, and rain affect the feeding and movement behaviour of the aphids (Sudderth & Sudderth 2014).

Aphids can have both sexual and asexual forms and a holocyclic or anholocyclic lifecycle. Wingless females can go through parthenogenesis, reproduction without fertilization (anholocyclic), often during spring and summer, which produces nymphs that are genetic clones. In temperate regions, both females and males are produced in late summer and fall where the sexual males are produced and eggs can be laid (part of holocyclic lifecycle). Eggs, which can overwinter, are laid on the host plant. The population growth and rapid adaptation to the environment is a consequence of the genotypical diversity of aphids. The diversity can be a result of mutations or recombination after sexual reproduction. Fast adaptation and mutations can lead to insecticide resistance. Insecticides have been used against aphids since the late 1940s (Simon & Peccoud 2018). Nowadays aphids show resistance to multiple classes of the exposing insecticides (Singh et al. 2020). Therefore, non-chemical management approaches are important and should be used more frequently. For example, aphid natural enemies, such as parasitoid wasps, can be used in biological control as well as beneficial fungi (Simon & Peccoud 2018).

### 1.3.1 *Rhopalosiphum padi*

*R. padi* is a devastating pest on crops all over the world (Wiest et al. 2021). The lifecycle of *R. padi* is including bird cherry trees as their primary host (Peng et al. 2017). The females lay eggs in the buds of bird cherry trees, where they can over-winter. Cereals, for example wheat, barley, and oat, and other *Poaceae* species such as maize are the second host of *R. padi*. Migration to cereals and grassland occurs in the summer, mostly to young plants since *R. padi* prefers them. By the end of summer, the winged males and viginoparae are migrating to the bird cherry tree again where the viginoparae are reproducing and the product is both males and females (Peng et al. 2017). The lifecycle can also be anholocyclic, where females produce nymphs that are clones through pathogenesis (Simon & Peccound 2018).

The *Poaceae* family is the most economically important plant family since it includes important plant species such as wheat and barley, provides humans with food sources and are used as feed for animals. Aphid infestation and feeding can weaken young plants by the disposing of nutrients from the plant, but less root growth, lower quality and a decrease in yield is also common consequences. Hence, aphids can cause damages in all stages of the hosts from the *Poaceae* family (Papp & Mesterházy 1993).

Indirect damage caused by *R. padi* can be the transmission of pathogens such as barley yellow dwarf virus (BYDV) which causes one of the most important diseases of cereals called the yellow dwarf disease (Thackray et al. 2009; Wiest et al. 2021).

BYDV is a single-stranded RNA-virus and it is only aphids that transmit the virus from plant to plant. Control measures such as aerial sprays and seed treatments with insecticides are often applied to avoid direct damage or large yield losses caused by the virus (Girvin et al. 2017).

## 1.4 Plant pathogenic fungi

Plant pathogenic fungi are species that utilize nutrients from plants and affect plants negatively. The infection leads to the cause of many different diseases in plants and large economic losses. Crop losses due to pathogenic fungi can be a result of decreased seed qualities, such as size, weight, and composition (Argyris et al. 2003). Additionally, plant pathogenic fungi can produce toxins called mycotoxins in infected plant seeds, which leads to economic losses, due to lower quality of the crop. Seed contamination of the mycotoxin DON can cause risks of human and animal health and is often a result of an infection caused by the plant pathogenic fungus *F. graminearum (Argyris et al. 2003).*

One of the reasons for the wide distribution of plant pathogenic fungi in the world is that the dispersal of fungal spores can occur by water, wind, or insect vectors (Doehlemann et al. 2017). Various strategies are used by the fungi to infect plants. Therefore, they can be categorized by how they feed on the host and their pathogenic lifestyle. Dependent on the strategies and lifestyle, fungi can be divided into necrotrophs and biotrophs. Necrotrophs kill their hosts and feed on dead plant tissue, whereas biotrophs get their nutrients and colonise the tissue of a living plant. However, both strategies interfere with the primary plant defence and can lead to yield losses (Doehlemann et al. 2017).

### *1.4.1 Fusarium graminearum*

The Ascomycete *F. graminearum* is a plant pathogenic fungus causing the diseases damping-off, root and crown rot, and Fusarium head blight (FHB) on cereals such as barley and wheat. Grain infection leads to a reduction in storage protein, amylose, cellulose, and seed germination which decreases the grain quality (Argyris et al. 2003). Root and crown rot lead to problems with water and nutrient uptake (Taheri 2018).

The fungus produces asexual spores (conidia) and sexual spores (ascospores) when overwintering in crop debris (Manstretta & Rossi 2015). Debris leads to the initiation of an epidemic of FHB, and the amount of residue is related to the density of the inoculum and residue infestation. However, other microbes in the same environment can be antagonists or influence the decomposition rate of the residue (Pérez et al. 2007).

Tillage is a type of management used to decrease the spread of inoculum because it allows the residue to decompose in the soil. Other control practices are crop rotation, fungicide application, and selection of varieties that are somewhat resistant since there are no varieties that have a total resistance. The wide host range together with the ability to spread inoculum over a long distance makes it difficult to control diseases caused by *Fusarium spp.* (Pérez et al. 2007).

## 1.5 Biological control agents

Biological control is a method to manage plant pathogenic organisms with other living organisms such as beneficial microorganisms (Pappas et al. 2020). Biological control agents are often included and complemented in IPM strategies. The use of beneficial organisms can lead to positive responses by plants which can lead to decreases in the negative effects of plant pathogens. Biological Control Agents (BCAs) can suppress diseases in plants as a result of the interactions between plants, the microbial community, and insects (Vinale et al. 2008).

Examples of the function of a BCA can be the ability to parasitize fungi, compete for nutrients (Coppola et al. 2019), promote plant growth or plant defence responses. This can be used against fungal pathogens and are the basis of the antagonistic nature of *Trichoderma spp.* (Brotman et al. 2010).

### 1.5.1 Beneficial fungi, *Trichoderma atroviride*

*Trichoderma spp.* are mycoparasites that have the ability to antagonize phytopathogenic fungi (Brotman et al. 2010), and are one of the most studied BCAs (Coninck et al. 2020). *T. atroviride* is a species that is studied for use as a BCA and has its natural habitat in the rhizosphere in the soil where it can penetrate the plant roots and colonize the plants. The symbiosis between *Trichoderma spp*. and plants are based on the ability of the fungi to get nutrients from the plant and the response of the plant which leads to the initiation of a defence mechanism against pathogens. The interaction can also affect the plant growth positively (Konappa et al. 2020).

It is shown, that the beneficial fungus *T. atroviride* induces plant defence mechanisms in tomato plants (Coppola et al. 2019). In the same study, Coppola et al. (2019) found a negative impact on pest insect fitness due to changes in VOC composition. The emission of VOCs can therefore be a part of the indirect defence of the plants since it can affect the behavior of the aphids. Emission of plant VOCs can be induced by fungi such as different *Trichoderma* species. *Trichoderma spp.* can release a lot of different VOCs, whereas more than 480 are identified (Speckbacher et al. 2020). One of the first volatiles studied and isolated from *Trichoderma spp.* is 6-pentyl-2H-pyran-2-one (6PP) and is connected to the

interaction between *Trichoderma* and plants. The induced plant VOCs are affected by the stage of the fungus as well as the environment and can be both species- and strain-specific (Speckbacher et al. 2020).

# 2. Aims and objectives

This thesis aims to study the effect of fungal colonization (plant pathogenic or beneficial) on plant volatile organic compounds and aphid behavior on barley. The objectives were firstly to determine differences in VOC composition released by plants inoculated with fungi. This was conducted by analysing and comparing VOC profiles from barley plants inoculated by the plant pathogen *F. graminearum* or the beneficial fungus *T. atroviride.* Secondly, to study aphid preference to plants inoculated with *F. graminearum* and *T. atroviride* separately compared to non-inoculated plants in olfactometer tests.

The identification of compounds possibly connected to plant defence and aphid preference can lead to an understanding and new strategies for control such as BCAs and semiochemicals in IPM strategies. Three hypotheses have been tested in this thesis:

1. There are differences in composition of VOCs emitted by plants inoculated with *F. graminearum*, *T. atroviride,* and non-inoculated plants due to induction of different volatile compounds.

2. Inoculation of barley with *T. atroviride* results in less aphid preference because of the induction of plant defence and emission of different insect repellent volatiles.

3. Inoculation of barley with *F. graminearum* results in less attraction of aphids due to induction of different volatiles emitted from the plants.

# 3. Material and methods

## 3.1 Plants

The plant used was Barley *Hordeum vulgare* variety, Salome. The variety Salome is spring barley that has two rows of seeds (two-row barley) and is used for malting. Resistance to powdery mildew and high yields are two varietal characteristics of Salome. Salome has gone through official tests in many parts of Europe and is suitable for regions in Europe (*SALOME Spring malting barley* 2022).

### 3.1.1 Sowing and inoculation of plants

For this experiment, cultures of the plant pathogen *F. graminearum* isolate VPE91 and the beneficial fungus *T. atroviride* strain 206040 were used. The cultures were grown at 20 °C for seven days on ½ strength Potato dextrose agar (PDA), composed of 19.5 g PDA (Merck, Germany) and 7.5 g Bacto agar (SWAB, Sweden) per L deionized water. The barley seeds were firstly surface sterilized with 70 % ethanol for 20 seconds, the ethanol was then washed off the seeds with water in three different water baths and left to air dry for min. 24 h.

Seeds were sown in plastic pots (400 ml volume, 8.5 cm in diameter) in 300 ml of dry non sterilized sand (SIBELCO, Molndal, Sweden) with six seeds per pot. The pots were transparent and black plastic foil was added around each pot to prevent light from reaching the roots. The control was inoculated with an agar plug (only PDA) made by a 5 mm in diameter cork-borer and added close to the seeds in the first pots. The agar plugs were placed between the seeds without touching the seeds. Then, 5 mm agar plugs of *F. graminearum,* and 5 mm agar plugs of *T. atroviride* were added to other pots with seeds (Fig. 1). Agar plugs from *T. atroviride* were added to pots last to avoid contamination through spores spreading through the air. The procedure was repeated until 6 pots were inoculated with each treatment. Thereafter, 50 ml of sand was added to cover both seeds, and plugs and 50 ml of water was added to the pots to prevent the plugs from drying. The same method was used when preparing the plants for the olfactometer test. Seeds were

inoculated with *T. atroviride* and with *F. graminearum* in six pots each, and agar plugs only containing PDA used as non-inoculated controls were added to 12 pots.

All plants for the olfactometer test showed unexpected symptoms in all treatments (see appendix 4 for symptoms). The first batch of plants were only used in the experiment between plants inoculated with *T. atroviride* and non-inoculated plants since the plants inoculated with *F. graminearum* the next day showed more symptoms. The experiment was therefore repeated, and the second approach followed the same method as the first except for the sterilization of the sand in an oven at 140 °C for 24 hours before use.


*Figure 1. Planting of six barley seeds (cv. Salome) per pot and nine agar plugs of* T. atroviride *for the inoculation.*

### 3.1.2 Maintenance and environmental condition

The pots were randomized within blocks and put into clear exposing cages (each chamber 10x10x40 cm) in a growth chamber with additional light with the intensity of 200 $W*m^{-2}$. The cages were connected to a pump that pulled air over the plants and out of the room with an airflow of 1.3 L/min to prevent the risk of

contamination between the treatments. 50 ml fertilized water with 2 ml fertilizer/1 L water (Wallco plant nutrition, 51-10-43 + micro) was directly added through an automated dripping system after sowing. On the second day after inoculation, 10 ml of water was added through the dripping system. Watering and nutrition were continuously repeated every time the sand on the surface had dried. All pots were further watered and fertilized with the same amount. Ten days after sowing, a ten times higher concentration of fertilizer was added continuously to the plants with a 20 ml syringe. Specific amounts of applied water and fertilizer are reported in appendix 3. Environmental conditions for the sown plants with inoculated fungi were a temperature of 20 °C ± 2 °C and a photoperiod of 16:8 hr (day: night).

## 3.2 Volatile organic compound

### 3.2.1 VOC collection

To investigate the impact of plant associated fungi on plant semiochemistry, volatiles emitted from non-inoculated barley plants and plants inoculated with *T. atroviride* or *F. graminearum* were collected after 22 days. Volatiles from twelve pots (six pots with plants inoculated with *T. atroviride* or six pots with plants inoculated with *F. graminearum* and six pots with non-inoculated plants) with six plants each were collected for 24 hours.

Polyethylene terephthalate oven plastic bags (Toppits Melitta) were used to enclose the barley plants (cv. Salome). The bags were baked at 140 °C for two hours before the volatile collection The volatiles were collected for 24 h with a push-pull system. Charcoal-filtered air was pushed into the oven bags with a flow of 600 ml/min and the air was pulled out over a volatile trap with 400 ml/min. Self-packed sample tubes (glass liner for Optic Injector) containing 50 mg adsorbent (Tenax TA) were used to trap the VOCs (Fig. 3). Before sampling, contaminants were removed from the Tenax tubes at 250 °C for 2 hours, under a flow of nitrogen. Contaminated air was prevented from entering the bag because of the difference in the flow rate. After volatile collections, symptoms and plants weights were evaluated as described in 3.2.2 and 3.2.3.

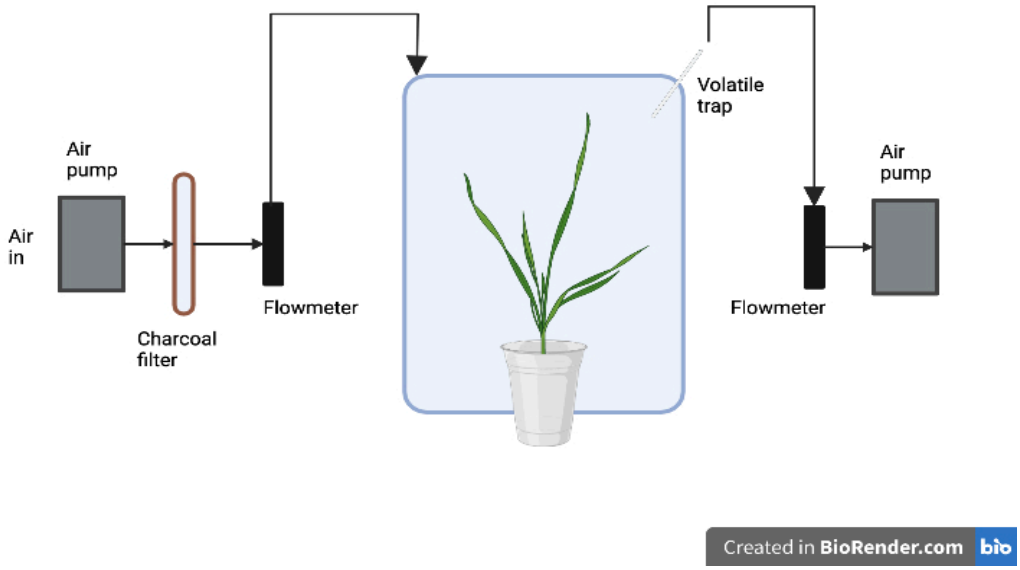


*Figure 2. The set up for the collection of volatiles from barley inoculated with the plant pathogenic fungi* F. graminearum*, beneficial fungus* T. atroviride *or non-inoculated plants. Volatiles were collected with a push-pull system on sampling tubes that were added through a hole in the oven bags sealed over each pot.*

### 3.2.2 Biomass

After volatile collection, the plants were cut directly above the sand and weighted. The dry matter biomass of the barley plants was then measured by taking the plants and weighing them directly after drying for 5 days at 70 °C.

### 3.2.3 Gas Chromatography – Mass Spectrometry

The headspace of barley plants was collected and analysed separately to determine differences in the compound composition of emitted volatiles within and between treatments. The volatile compositions from barley plants, inoculated with the beneficial fungus *T. atroviride* or the plant pathogen *F. graminearum* as well as non-inoculated control plants, were analysed by Gas Chromatography-Mass Spectrometry (GC-MS).

Volatiles were released from the adsorbent in the packed glass liners (sample tubes) with an Optic Injector by thermal desorption. 1-nonene was used as Internal Standard, 1 µl (1-nonene 20 ng/µl in hexane), was added with a 10 µl syringe to the Tenax tube shortly before the GC analysis. A Tenax tube with a sealing O-ring (GL Science) was inserted into the injector and helium, with a flow of 1.3 ml/min, was used as a carrier gas (Helium 6.0). The injector was heated from 40 °C to 250 °C

by 30 °C/sec. An Agilent 7890N GC system equipped with an HP-1MS capillary column (30x0.25 mm id x 0.25 µm film thickness, 100 % Dimethylpolysiloxane) coupled to an Agilent 5975C mass spectrometer was used to separate the thermal desorbed compounds.

The temperature program in the GC started with an initial oven temperature of 30 °C held for 2 min that increased at a rate of 5 °C /min to 150 °C, followed by a rate of 10 °C/min to the final temperature of 250 °C, held for 15 min. GC inlet temperature was 250 °C as well as the ion source temperature. The electron impact (EI) mode, at 70 eV at 150 °C, was used to operate the quadrupole mass detector and the gain factor was set to 10. All data were produced by the collection of the full-scan mass spectra within the range of 40–500 m/z. The chromatogram from the GC-MS was recorded in ChemStation software, ChemStation was also used to operate the GC-MS system.

### 3.2.4 VOC identification and quantification

VOC emissions from barley plants were analysed to identify and quantify volatile compounds. The analysis was performed with the Automatic Mass Spectral Deconvolution and Identification System (AMDIS, V. 2.66; National Institute of Standards and Technology NIST, Boulder, CO). The extraction of ions in the GC-MS is based on a common chromatographic shape and is excluding background signals. AMDIS finds similarities and matches the collected volatile compounds with libraries. If there are differences in the search and library spectra for a compound it can be below the threshold and lead to a false, negative finding (Stein 2012). Low concentration can lead to spurious peaks due to noise which can have been affecting the spectrum of a compound to differentiate from the library. Therefore, compounds with a signal-to-noise ratio (S/N) lower than 100 were excluded from the analysis.

A target library and target library file were created for all compounds found in the volatile samples run in the GC-MS to be able to define the compounds that AMDIS matches with. Samples were randomly checked to find compounds for the target library. Mass spectra of compounds where no reference standard was available, were compared to the NIST library. The Retention Index (RI) values were then also checked and added. Appropriate RI values were extracted from the NIST Chemistry WebBook (Linstrom & Mallard 1997). RI values from analysis with comparable GC columns and temperature programs were added to the target library. Compounds with insecure identification were added as “known unknowns” to the target library to enable their comparison, without further identification.

The internal standard (IS) was checked and the ones outside of the expected range were excluded as well as impurities and compounds found in less than 10 %

of the chromatograms. Whole samples that did not show expected IS peak were also excluded.

## 3.3 Aphid rearing

Lab cultures of the bird cherry-oat aphid, *R. padi,* reared on oat plants were used for the olfactometer test. The aphids were reared on oat and not barley, which is used in the experiments, to reduce the risk of aphids choosing a plant they recognise and are used to later in the olfactometer test. The rearing room and growth chamber (for the plants) had the conditions of 16:8 hr (day: night), 20 ± 2 °C and a humidity of 60 %. The aphids were fed once per week with one week old oat-plants (*Avena Sativa,* variety Belinda). About 40 seeds were sown per pot (8x8x6 cm), in soil (S-soil, Hasselfors garden). Wingless adults and individuals in the fourth nymph state of *R. padi* were used for the tests.

## 3.4 Olfactometer test

Olfactometer tests were conducted to determine the influence of volatiles on the preference of *R. padi* for barley plants inoculated with *F. graminearum*, *T. atroviride* or non-inoculated plants. With the used two-way olfactometer, preferences of insects to odours can be investigated and enables a comparison of two different odours at the same time. Experiments were only conducted between 09:00 and 16:00 during bright sunny days with high air pressure since aphid behavior is depending on weather conditions.

The room was kept dark, except for a light source 30 cm over the olfactometer. The light conditions over the olfactometer were regulated and set to 60 $W*m^{-2}$. The 18 days old plants were put in different two-chamber cages. Pots with plants inoculated by either *T. atroviride* or *F. graminearum* was connected to the two-way airflow olfactometer through one arm (tube) whereas the other arm was connected to a non-inoculated control plant (Fig. 4).

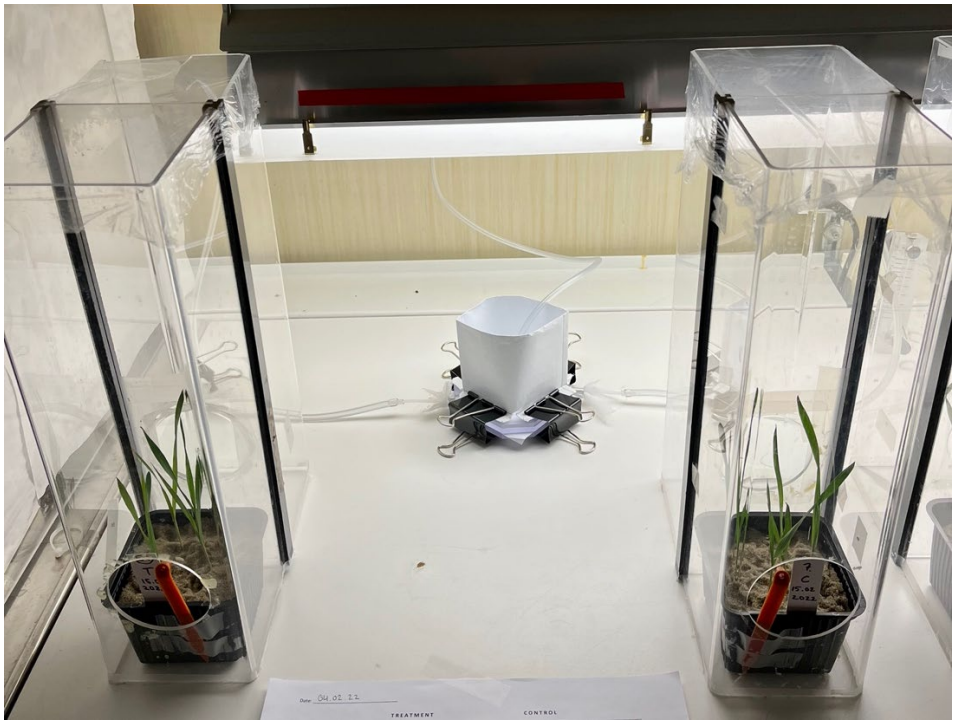


*Figure 3. Two-chamber cages with non-inoculated plants and plants inoculated with* F. graminearum *or* T. atroviride *connected to the two-way airflow olfactometer.*

The olfactometer (10.5x10.5 cm) was divided into three zones: one zone for each plant odour, and a neutral zone between them, (Fig. 5). A vacuum pump pulled air from the centre of the olfactometer, which create an air current in the side arms connected to the cages with the plants. A flow meter at the centre inlet was used to regulate the airflow in to the olfactometer (120 ml/min). A paper was attached around the olfactometer as a cover to reduce the risk of visual influence on the aphid's behavior.

Aphids were moved from the rearing and left in the olfactometer room for 30 minutes for acclimatization before the conduction of the test. The largest (fourth nymph state to adult) and most active individuals of the aphids were chosen for the experiments. A randomly chosen aphid was carefully placed through a hole in the lid in the middle of the olfactometer with a paintbrush. The aphids were given a period of 10 minutes to adapt in the olfactometer. Individuals that did not move after 10 minutes were excluded from the test.

Thereafter, the position of the aphids was recorded for 30 minutes every 3 minutes. The olfactometer was rotated before each test to make sure that the aphid behavior was not affected by the position. The test was repeated 23 times on five different pots with plants inoculated with fungi paired with five different pots with non-inoculated plants. A new aphid was used for every test (23 different aphids). The olfactometers were sterilized with 70% ethanol between the tests. The olfactometer test was conducted one time with plants inoculated with *T. atroviride*

showing stress symptoms and repeated a second time with new plants without stress symptoms, to confirm the results. The experiment with plants inoculated with *F. graminearum* was only conducted with the plants without stress symptoms.

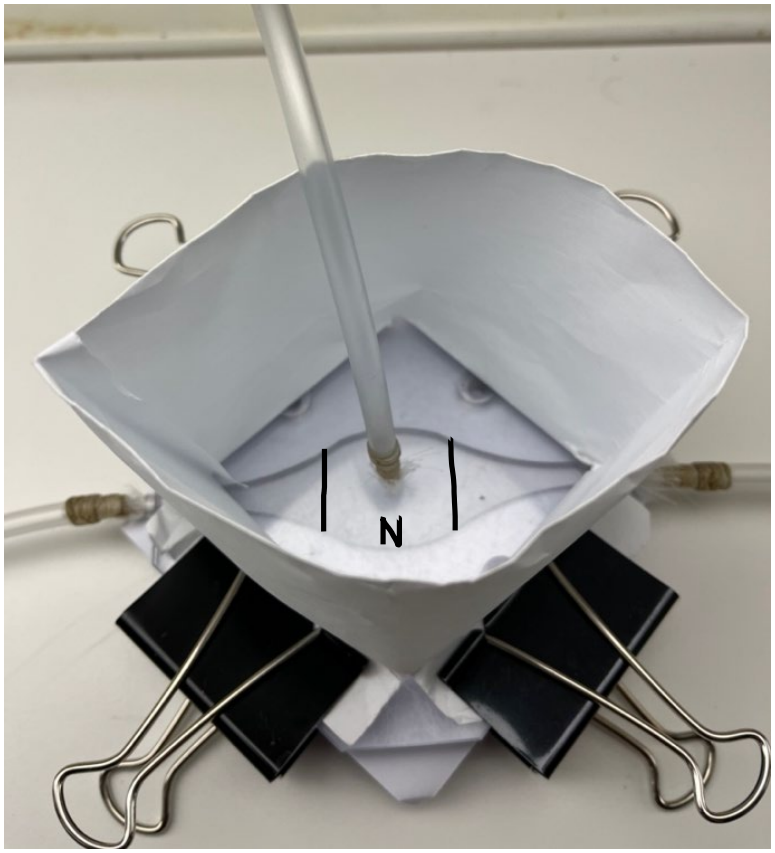


*Figure 4. Olfactometer with one arm connected to the odour sources of barley inoculated with* F. graminearum *or* T. atroviride *and the other connected to non-inoculated plants. The lines mark the neutral zone (N) in the centre and the zone for inoculated plants and non-inoculated plants each side.*

## 3.5 Symptom evaluation

Symptom development was assessed by visually grading on barley stem bases in all treatments directly after the volatile collection and olfactometer tests. Symptoms were graded as follows: 0 for stems without any symptoms, 1 for small visible lesions, 2 for lesions covering more than half of the circumference, and 3 for lesions covering the whole circumference of the stem base, (Fig. 2). A disease index was then calculated, n is the number of plants with each grade of infection, for the different treatments using the equation (Matusinsky et al. 2016):

$$\text{Disease index} = [(n1 + 2n2 + 3n3) \times 100]/[3 \times (n0 + n1 + n2 + n3)]$$

Where *n* is the number of plants with each grade of infection, for the different treatments.


*Figure 5. Disease symptoms showing browning on the coleoptile, dark brown lesions on the stem base, and darkened roots. Symptoms graded from the left: 1, 2, and 3.*

## 3.6 Statistical analysis, multivariate statistics

Statistical analyses for the volatile collection were performed in R version 4.0.3 (R Core Team 2020), with the package "vegan" (Oksanen et al. 2020). The Kruskal-Wallis test was used to analyze if single VOCs were emitted in different amounts from barley plants inoculated with *F. graminearum, T. atroviride* and non-inoculated plants. Permutational multivariate analysis of variance (PERMANOVA) was conducted to analyze differences between the VOC composition of plants inoculated with *F. graminearum, T. atroviride* and non-inoculated plants, with the *adonis* function (N permutations = 10.000). The variances were further analyzed through Permdisp to confirm that the variance was homogenetic between treatments. Non-metric multidimensional scaling (NMDS) plot was used to visualize Bray-Curtis dissimilarities (calculated with *vegdist* function) with the *metaMDS* function and the scaling was done by Wisconsin double standardization. The responses of the aphids in the olfactometer test were analyzed with the Wilcoxon signed rank test for matched pairs. Differences in the disease index and biomass data were analyzed by one-way analysis of variance, ANOVA followed by a post-hoc test.

# 4. Results

## 4.1 Symptom evaluation

Disease symptoms were observed and documented for the plants after the volatile collection and olfactometer test. The symptoms observed were browning on the coleoptile and dark brown lesions on the stem base.

The result from the one-way Anova test showed that there was a significant difference in disease index between plants inoculated with *F. graminearum, T. atroviride* and non-inoculated plants (Anova, F= 73.7, df =32, p= 2.66E-12). The calculated disease index for the plants used in the volatile collection shows that the plants inoculated with *F. graminearum* had the highest average disease index of 46.06 (+/- 9.26), and the non-inoculated plants had an average disease index of 12.31 (+/- 6.09), and the *T. atroviride* had the lowest average value 11.56 (+/-7.22) (Fig. 6). Plants used in all olfactometer tests show that inoculation with *F. graminearum* leads to the highest average disease index of 37.22 (+/-3.04) and *T. atroviride* the lowest average disease index of 4.07 (+/- 5.29) (Fig. 7). Post-hoc analyses, of the disease index in plants used for both tests, showed that there was a significant difference in disease index between plants inoculated with *F. graminearum* and plants inoculated with *T. atroviride* (p= 9.52E-10) as well as between *F. graminearum* and non-inoculated plants (p= 1.30E-8). However, there was no significant difference in disease index between non-inoculated plants and plants inoculated with *T. atroviride* (p= 0.79).

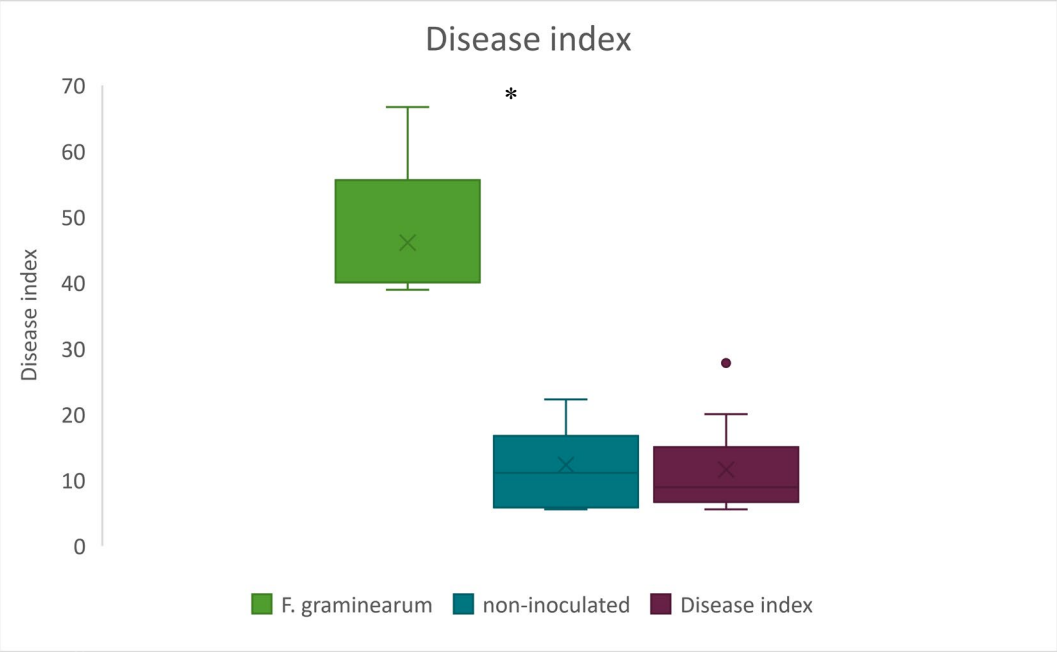


*Figure 6. Boxplot of the disease index of non-inoculated plants (blue, n=12) plants inoculated with* F. graminearum *(green, n=11) and* T. atroviride *(lilac, n=10) used in volatile collection. Points outside of the boxes representing outliers and the line in the boxes is the median, whereas the x is the mean values. There was a significant difference between non-inoculated plants and plants inoculated with* F. graminearum *or* T. atroviride *(Anova, F=73.7, df=32, p= 2.66E-12). The star (*) represent a significant difference in disease index between plants inoculated with* F. graminearum *and plants inoculated with* T. atroviride *as well as non-inoculated plant (post-hoc test).*

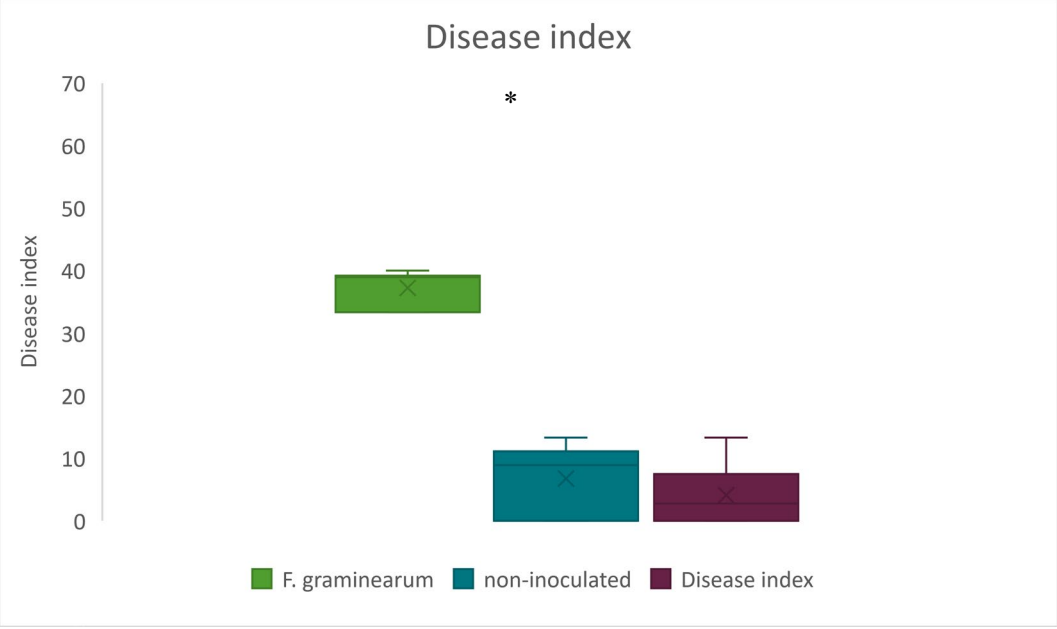


*Figure 7. Boxplot of the disease index of non-inoculated plants (blue, n=12) plants inoculated with* F. graminearum *(green, n=6) and* T. atroviride *(lilac, n=6) used in olfactometer tests. The line in the boxes is representing the median and the x is the mean values. There was a significant difference*

*between non-inoculated plants and plants inoculated with* F. graminearum *or* T. atroviride *(Anova, F=92.0, df=23, p= 4.07E-11). The star (*) represent a significant difference in disease index between plants inoculated with* F. graminearum *and plants inoculated with* T. atroviride *as well as non-inoculated plant (post-hoc test).*

## 4.2 VOC identification and quantification

In total 68 compounds were annotated in the chromatograms and compared between plants inoculated with *F. graminearum, T. atroviride* or non-inoculated plants, including 37 unknown compounds. The volatile compounds were identified using reference standards. In cases where no standards were available, compounds were identified by comparison to the mass spectrum library NIST and retention index values from the NIST webbook (NIST Informatics 2022). Internal Standard was used to check the quality of GC-MS analysis. Samples with lower peak areas for IS than expected were excluded from the analysis.

Visualization by NMDS plots did not show any clear distinction between plants inoculated with any of the fungi and non-inoculated plants (Fig. 8). The low stress value (< 0.14) indicated a good representation in reduced dimensions in the NMDS. The differences in VOC composition were not significant for plants inoculated with fungus and non-inoculated plants and the variance within groups was the same between groups (Permanova, p=0.95).

Undecane was the only collected VOC that showed a difference between groups since the Kruskal-Wallis test showed a significant difference between *Fusarium* and the non-infected plants. Plants inoculated with *F. graminearum* significantly emitted more Undecane than the non-inoculated plants (Kruskal-Wallis test, p= 0.048). Undecane was emitted in different amounts from the barley plants inoculated with *F. graminearum, T. atroviride* and non-inoculated plants, with the lowest peak areas in the non-inoculated (Fig. 9). However, there was no significant difference in emission on undecane between plants inoculated with *T. atroviride* and non-inoculated plants.

The emission of Linalool from plants inoculated with *F. graminearum* or *T. atroviride* was reduced compared to non-inoculated plants (Fig. 10). However, differences in the peak area per dw were not significant (Kruskal-Wallis rank sum test, p=0.46, chi-squared=1.55). The compound (E)-2-hexenal was emitted from all plants (Fig. 11). It was emitted in different amounts from barley inoculated with fungus and non-inoculated, but the difference was not significant (Kruskal-Wallis test, p=0.30). The identification of Cis-3-hexenylacetate did not show a difference (Kruskal-Wallis, p=0.90) between and within samples or inoculation with fungi and non-inoculation (Fig. 12), and the compound was identified in all treatments but not all samples.

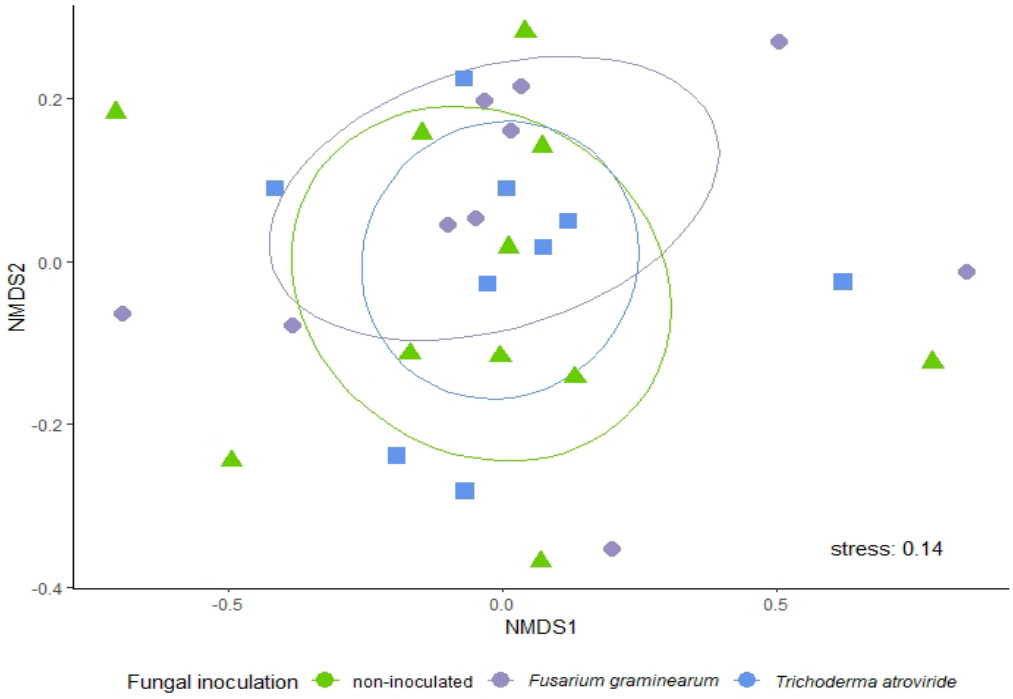


*Figure 8. Non-metric multidimensional scaling (NMDS) showing no clear distinction in volatile compositions between non-inoculated plants (green triangles, n=11), plants inoculated with* F. graminearum *(lilac circles, n=10) or* T. atroviride *(blue squares, n=9). Stress < 0.14.*

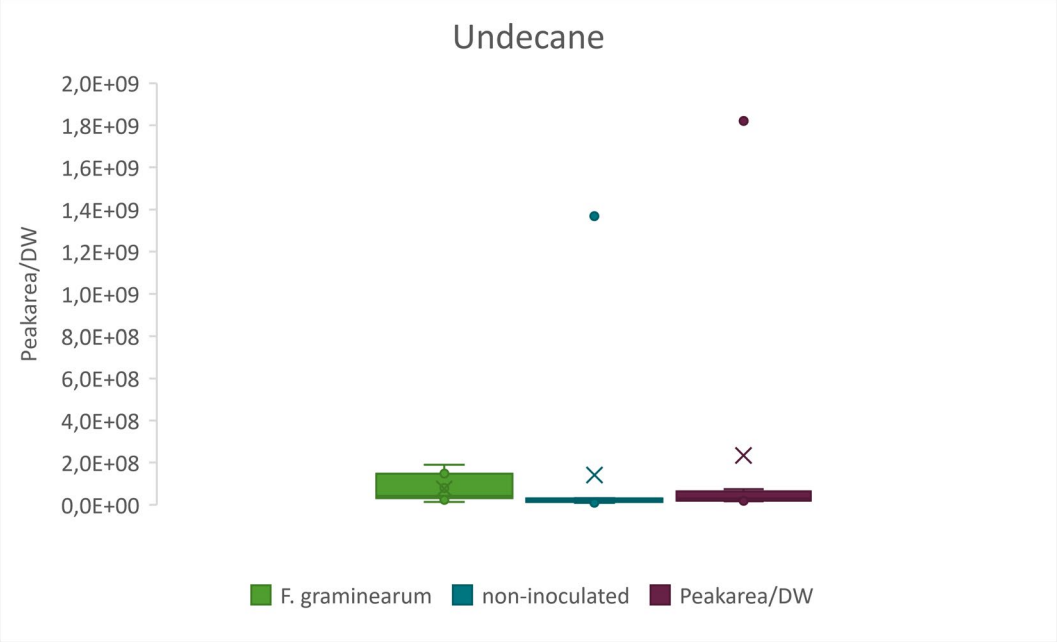


*Figure 9. Box plot of peak area per dw for emission of Undecane from non-inoculated barley plants (blue) and plants inoculated with* F. graminearum *(green) or* T. atroviride *(lilac). The line in the boxes is representing the median, the x the mean value, and the points outside of the box are outliers.*

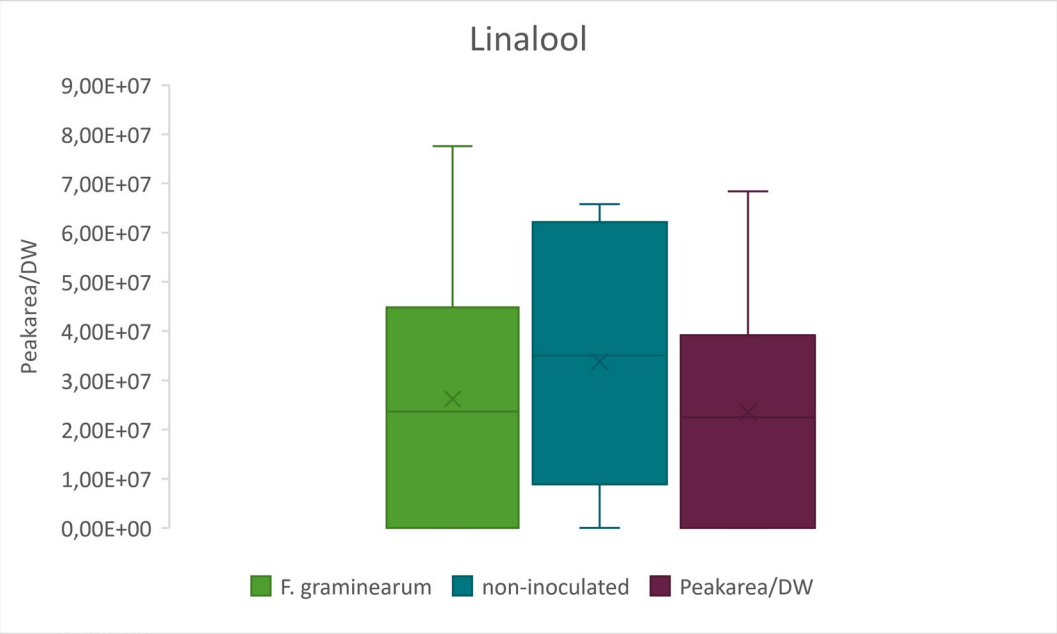


*Figure 10. Box plot of peak area per dw for emission of Linalool from non-inoculated barley plants (blue) and plants inoculated with* F. graminearum *(green) or* T. atroviride *(lilac). The line in the boxes represents the median and the x the mean value.*

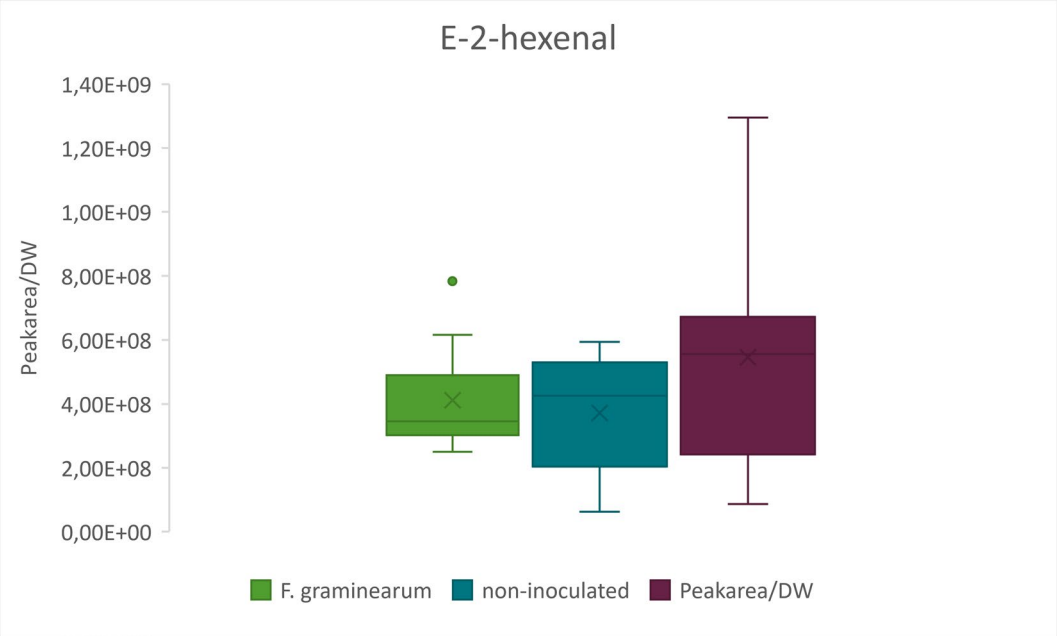


*Figure 11. Box plot of peak area per dw for emission of E-2- hexenal from non-inoculated barley plants (blue) and plants inoculated with* F. graminearum *(green) or* T. atroviride *(lilac). The line in the boxes represents the median, the x is the mean value, and the points outside of boxes outliers.*

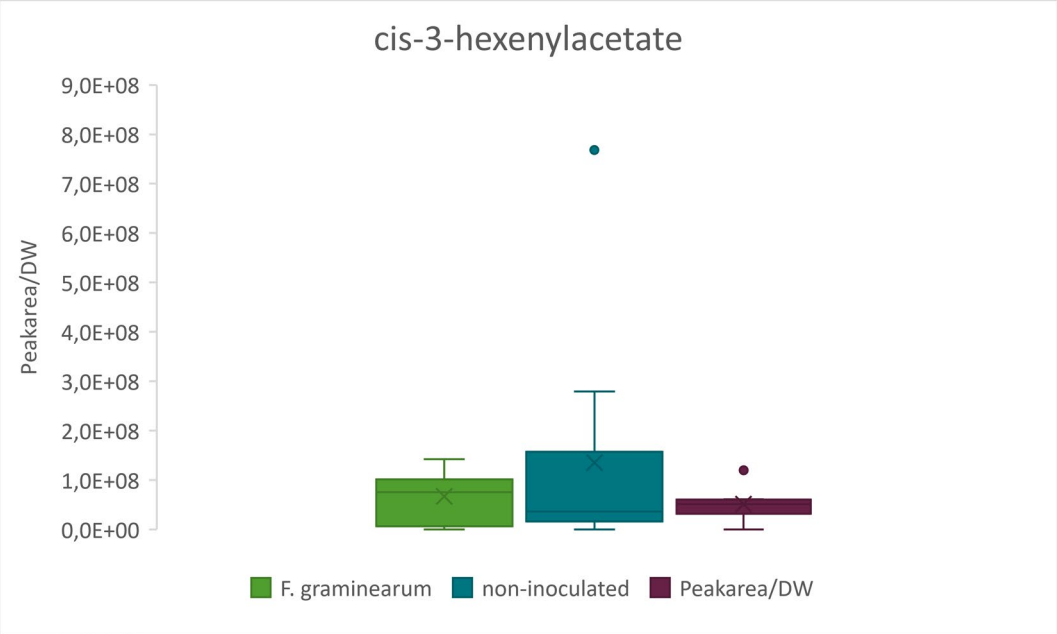


*Figure 12. Box plot of peak area per dw for emission of cis-3-hexenylacetate from non-inoculated barley plants (blue) and plants inoculated with* F. graminearum *(green) or* T. atroviride *(lilac). The line in the boxes represents the median, the x is the mean value, and the points outside of boxes outliers.*

## 4.3 Olfactometer test

The result from the first olfactometer test (grown in non-sterilized sand) between plants inoculated with *T. atroviride* and the non-inoculated plants showed that R. *padi* preferred the odour from the non-inoculated plants. The aphids visited the zone with the odour of non-inoculated plants significantly more often (Fig. 13, Wilcoxon signed rank test, W=48). With an average number of visits of 2.13 (+/- 1.36) in the zone with odour from *T. atroviride* inoculated barley plants and 4.04 (+/- 1.55) in the zone with odour from non-inoculated plants. The result from the repeated olfactometer test (grown in sterilized sand) for plants inoculated with *T. atroviride* and non-inoculated plants also showed a significant difference between treatments (Fig. 14, Wilcoxon signed rank test, W=53). The aphids preferred the odour of non-inoculated plants over the odour of *T. atroviride* inoculated plants, they visited zones 83 against 63 times with mean visits 3.61 (+/- 0.94) and 2.83 (+/- 1.12), respectively.

The result from the olfactometer test for plants inoculated with *F. graminearum* or non-inoculated plants (grown in sterilized sand) showed no significant difference in aphid preference (Fig. 15, Wilcoxon signed rank test, W=135). The aphids did not prefer any odour source more than the other. They choose the odour from *F. graminearum* inoculated barley plants 61 times and the non-inoculated 62 times, with the mean visits of 2.65 (+/- 1.99) and 2.70 (+/- 1.72), respectively.

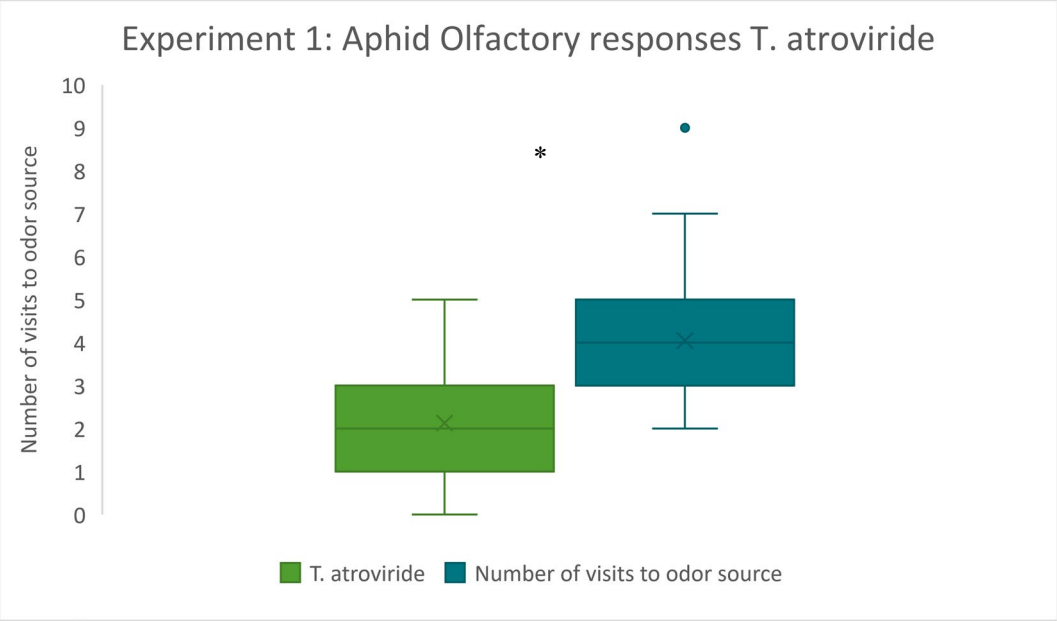


*Figure 13. Box plots of responses of* R. padi *to plants inoculated with* T. atroviride *(green) and non-inoculated plants (blue) in olfactometer tests (non-sterilized sand). The line in the boxes represents the median, the x the mean value and, the point outside the box is an outlier. Aphids preferred non-inoculated plants (n=93) more than plants inoculated with* T. atroviride *(n=49). The star (*) represent a significant difference in olfactory responses between plants inoculated with T. atroviride and non-inoculated plants (Wilcoxon signed rank test).*

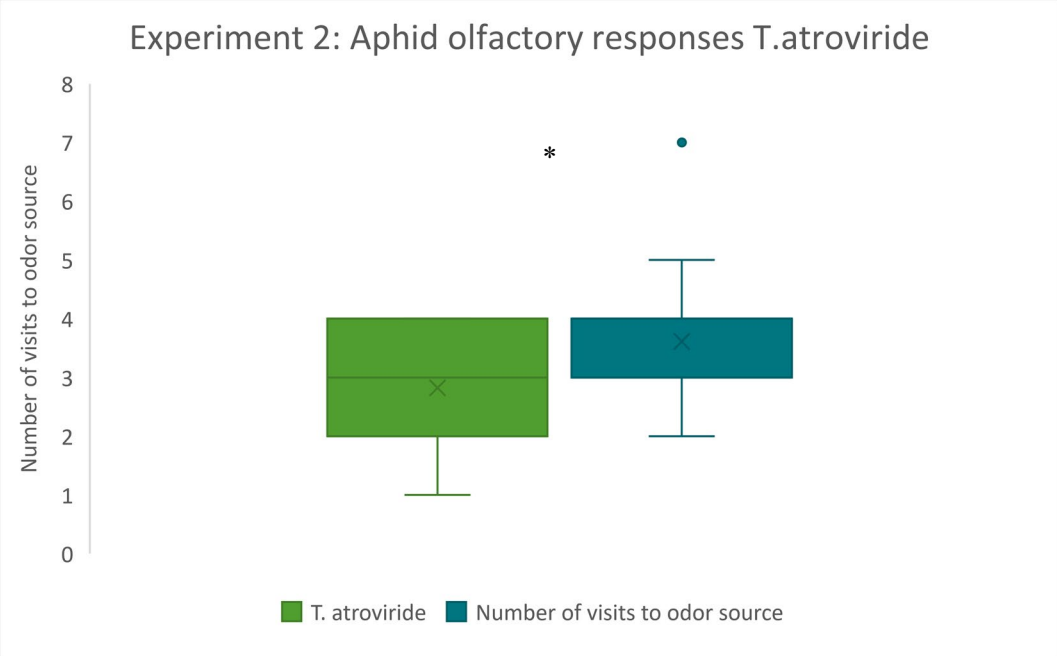


*Figure 14. Box plots of responses of* R. padi *to plants inoculated with* T. atroviride *(green) and non-inoculated plants (blue) in olfactometer tests (sterilized sand). The line in the boxes represents the median, the x the mean value and the point outside the box an outlier. Aphids preferred non-inoculated plants (n=83) more than plants inoculated with* T. atroviride *(n=63). The star (*) represent a significant difference in olfactory responses between plants inoculated with T. atroviride and non-inoculated plants (Wilcoxon signed rank test).*

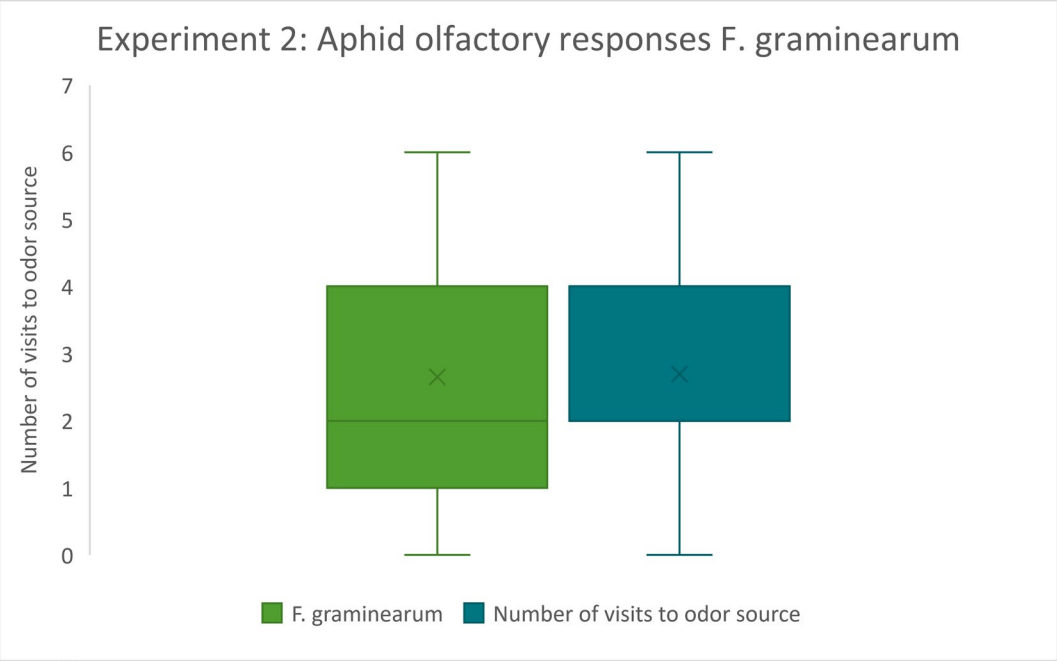


*Figure 15. Box plots of responses of* R. padi *to plants inoculated with* F. graminearum *(green) and non-inoculated plants (blue) in olfactometer tests (sterilized sand). The line in the boxes represents the median and x the mean value. Aphids did not prefer non-inoculated plants (n=62) more than plants inoculated with* F. graminearum *(n=61).*

## 4.4 Biomass

There was not a significant difference between treatments for biomass (Anova, F=1.33, df=32, p= 0.280). Plants inoculated with *T. atroviride* had the highest dry weight of the above ground plant material, with a mean value of 0.221 g (+/- 0.0331), followed by the non-inoculated plants with a mean value of 0.213 g (+/- 0.0316). Plants inoculated with *F. graminearum* produced the lowest amount of above ground biomass with a mean of 0.194g (+/- 0.0472) per 6 plants (Table 1). *Trichoderma atroviride* also had the highest fresh weight whereas *F. graminearum* had the lowest. For details of weight for all plants see appendix 2.

*Table 1. Mean DW (g) and standard deviation (SD) for non-inoculated plants and plants inoculated with the plant pathogenic fungus F. graminearum or the beneficial fungus T. atroviride.*

| **Inoculation** | **Mean DW (g)** | **Standard deviation SD (g)** |
|---|---|---|
| *F. graminearum* | 0.194 | +/- 0.0472 |
| *T. atroviride* | 0.221 | +/- 0.0331 |
| non-inoculated controls | 0.213 | +/- 0.0316 |

# 5. Discussion

There was no difference in the total VOC composition released from plants inoculated with *T. atroviride*, *F. graminearum* and non-inoculated plants. Bird cherry-oat aphids did not show a preference between plants inoculated with *F. graminearum* and non-inoculated plants. However, they did prefer the odour from non-inoculated barley plants before plants inoculated with *T. atroviride.*

This finding was in accordance with other studies as it is shown that *T. atroviride* can induce plant defence and emissions of different insect repellent volatiles (Battaglia et al. 2013). According to Lee et al. (2016). *Trichoderma* spp. can trigger plant defence against phloem sucking pathogens by activation of salicylic acid and Jasmonic acid signalling pathways. Aphids are phloem-sucking, and they therefore often activate the salicylic acid pathway, whereas *Trichoderma* spp. activates the Jasmonic acid signalling pathway in the plant because of the release of different VOCs. A diversity of systems and pathways is probably being used by plants to be able to respond to volatiles in different ways. At the same time as plants need to regulate the interactions with beneficial organisms, they also need to regulate the interactions for defence (Pineda et al. 2013).

An indirect defence for plants can be the emission of VOCs that attracts enemies of for example aphids (Battaglia et al. 2013). A study on the interaction between Tomato, *Trichoderma longibrachiatum,* and *Macrosiphum euphorbiae* showed a higher attractiveness of predators and parasitoids after colonization of *T. longibrachiatum (Battaglia et al. 2013)*. At the same time, the development and reproduction of the aphids were significantly improved. The beneficial consequence of nutritional access for the plant after colonization may also lead to the positive development of aphids. Plants colonized by *T. longibrachiatum* showed differences in VOCs released compared to then non-colonized plants. *T. longibrachiatum* was significantly stimulating the release of volatiles such as cis-3-hexen-ol, methyl salicylate, and β-caryophyllene. Studies have shown that these compounds can attract aphid parasitoids such as *Aphidius ervi* by affecting the orientation of their flight. Cis-3-hexen-ol, methyl salicylate, and β-caryophyllene were released from the plants inoculated with *T. atroviride* in this study as well, but there was no significant difference between treatments and non-inoculated plants. However, the same methods were not used for the volatile collection since Battaglia et al (2013) collected volatiles for 3 hours with tenax as adsorbent after 5 weeks

and did not use *T. atroviride*. The volatiles were collected for 24 hours from 3 weeks old plants in this study. The consequence of attracting parasitoids could be a more sustainable solution than chemical control to prevent economical losses due to aphid outbreaks (Battaglia et al. 2013). It can therefore be interesting to investigate those interactions involving VOCs in further studies.

However, the volatile analysis only showed a significant difference between plants inoculated with *F. graminearum* and non-inoculated plants for one compound, the alkene hydrocarbon Undecane. Undecane is a compound emitted by plants, but it has not been found to be connected to any biological interactions in the literature. However, this does not mean that Undecane is not of importance or not affecting the interactions between plants, insects, and microorganisms. Compounds of importance could possibly be under the level of detection which could be due to the low amount of biomass. The plant size and biomass were expected to be higher, and the low amount of biomass can be a consequence of less nutrients in the sand than in soil.

Furthermore, I detected a high amount of Linalool in the headspace of non-inoculated plants, as well as in fungi-inoculated plants. Linalool is a compound that is induced by abiotic and biotic stress factors and therefore further indicates a general stress factor affecting all plants (Piesik et al. 2011). A study investigated the induction of VOCs from cereals due to *Fusarium spp.* infection and showed an increased release of Linalool, (E)-2-hexenal, and (Z)-3-hexenylacetate after *Fusarium spp.* Infection (Piesik et al. 2011). Unfortunately, their volatile collection method differed from the one in this study, since they used a different adsorbent (Super Q) and collected the volatiles for a shorter period (4 hours), which lowers the comparability of the results.

Other studies have shown that host acceptance of *R. padi* can be reduced after being exposed to the volatile compound methyl salicylate (Ninkovic et al. 2021). The release of methyl salicylate can be increased by sap-feeders such as aphids as a part of the salicylic defence pathway. The same plant responses could be the effect of different pathways such as the interaction between plants and aphids or plants and microorganisms (Battaglia et al. 2013). However, the volatile analysis in this study did not show a difference between treatments in the release of methyl salicylate.

Drakulic et al. (2015) showed in an olfactometer bioassay that aphids (*Sitobion avenae*) were significantly repelled by headspace samples of VOCs induced by the colonization of *F. graminearum* on wheat plants. This indicates that *F. graminearum* colonization in hosts affects host colonization by aphids negatively. Aphids preferring non-colonized plants can be a strategy used by them to avoid hosts of lower quality. However, aphids in the olfactometer test conducted in this thesis did not prefer the odour from non-inoculated barley or barley inoculated with *F. graminearum.*

The high disease index of plants inoculated with *F. graminearum* in both experiments indicated a successful inoculation with the fungi. There were also symptoms of disease on the non-inoculated plants and plants inoculated with *T. atroviride*, which indicates an infection from other species of fungi that could have been present in the seeds. There could be VOCs induced by other fungi than those inoculated since the seeds were only surface sterilized. Some of the identified unknown compounds emitted by the plants might have been induced by unidentified fungi already present inside the seeds. Also, it cannot be ruled out that some of the unknown compounds can be of importance in the interaction in the olfactometer test. The plants used in the volatile collection showed some symptoms of stress as did the plants used for the first olfactometer test. Less stress symptoms for plants used in olfactometer test experiment 2 can be due to the sterilization of the sand.

Another factor to take into consideration in further studies is that the volatile collection and GC-MS analysis only show volatiles released at a specific time, plant stage, and environmental condition. An important consideration after the olfactory test is that the plants inoculated with *T. atroviride* did not show any significant differences in biomass compared to plants inoculated with *F. graminearum* and non-inoculated plants, and no certain indication of colonization. Roots of inoculated plants can be analysed for the presence of. *T. atroviride* by taking parts of the roots and adding them on petri dishes with PDA to analyse the growth (Battaglia et al. 2013), but also through molecular tools such as PCR. For future studies on plant-*Trichoderma*- aphid interaction further analyses would be preferred since there is no certainty of a successful inoculation and colonization in this study. Additionally, it would also be interesting to further study the preference of *R.* padi by conducting a two-way olfactometer test between plants inoculated with *T. atroviride* and clean air. In that case to analyse if volatiles induced by *T. atroviride* has such a strong repellent effect that starvation is more preferred. To analyse the interactions further, aphid host acceptance instead of preference can be tested with a settling test where the aphid can probe and test the plant before choosing a host.

Implementation of Integrated pest management (IPM) and less pesticide-input is expected to be used by the member states in the European Union (EU) according to the European Commission (Hillocks 2012). An alternative to chemical control, and a part of IPM strategies, is biological control using biological control agents such as the fungus *Trichoderma spp*. Another possible alternative for use in IPM strategies could be volatile organic compounds (VOCs), which can act directly as insect repellents or as elicitors of plant defence (Conboy et al. 2020). Furthermore, it is of great importance to increase the understanding of chemical communication between plants and insects by analyses and determination of VOCs in tritrophic interactions (Cai et al. 2015). This study has shown that *T. atroviride* can have a positive effect on plant health regarding aphid infestations and therefore possibly affect the plant - insect interaction since the aphids significantly preferred the non-

inoculated plants. Therefore *T. atroviride* may have a great potential to be useful in non-chemical control strategies, but further studies are needed before implementation as control management in IPM.

# 6. Conclusions

The experiments in this study have shown that the composition of volatile organic compounds emitted by barley plants does not differ between treatments of the beneficial fungi *T. atroviride*, plant pathogenic fungi *F. graminearum,* or non-inoculated plants. The bird cherry-oat aphid prefers non-inoculated barley plants before barley plants colonized with *T. atroviride.* However, the aphids did not show any preference between non-inoculated plants and plants infected by *F. graminearum.* As a conclusion based on the results in this study, barley inoculated with *T. atroviride* could possibly emit repellent semiochemicals to *R. padi*.

A possible alternative for use in IPM strategies could be strategies involving VOCs, since studies have shown that VOCs induced by fungi such as *T. atroviride* can act as insect repellents or as elicitors of plant defence. However, further studies on the interaction between plants, insects and fungi are needed to develop new control strategies, such as for example biological control, to prevent outbreaks of pests in the future.

# References


Aldughpassi, A., Wolever, T.M.S. & Abdel-Aal, E.S.M. (2016). Barley. I: Caballero, B., Finglas, P.M., & Toldrá, F. (red.) *Encyclopedia of Food and Health*. Oxford: Academic Press, 328–331. https://doi.org/10.1016/B978-0-12-384947-2.00055-6

Argyris, J., van Sanford, D. & TeKrony, D. (2003). Fusarium graminearum Infection during Wheat Seed Development and Its Effect on Seed Quality. *Crop Science*, 43 (5), 1782–1788. https://doi.org/10.2135/cropsci2003.1782

Battaglia, D., Bossi, S., Cascone, P., Digilio, M.C., Prieto, J.D., Fanti, P., Guerrieri, E., Iodice, L., Lingua, G., Lorito, M., Maffei, M.E., Massa, N., Ruocco, M., Sasso, R. & Trotta, V. (2013). Tomato Below Ground–Above Ground Interactions: Trichoderma longibrachiatum Affects the Performance of Macrosiphum euphorbiae and Its Natural Antagonists. *Molecular Plant-Microbe Interactions®*, 26 (10), 1249–1256. https://doi.org/10.1094/MPMI-02-13-0059-R

Brotman, Y., Kapuganti, J.G. & Viterbo, A. (2010). Trichoderma. *Current Biology*, 20 (9), R390–R391. https://doi.org/10.1016/j.cub.2010.02.042

Bruce, T.J. & Pickett, J.A. (2011). Perception of plant volatile blends by herbivorous insects – Finding the right mix. *Phytochemistry (Oxford)*, 72 (13), 1605–1611. https://doi.org/10.1016/j.phytochem.2011.04.011

Bruce, T.J.A., Wadhams, L.J. & Woodcock, C.M. (2005). Insect host location: a volatile situation. *Trends in Plant Science*, 10 (6), 269–274. https://doi.org/10.1016/j.tplants.2005.04.003

Cai, X.-M., Xu, X.-X., Lei, B., Zongxiu, L. & Chen, Z. (2015). Measurement of volatile plant compounds in field ambient air by thermal desorption-gas chromatography-mass spectrometry. *Analytical and bioanalytical chemistry*, 407. https://doi.org/10.1007/s00216-015-9076-5

Conboy, N.J.A., McDaniel, T., George, D., Ormerod, A., Edwards, M., Donohoe, P., Gatehouse, A.M.R. & Tosh, C.R. (2020). Volatile Organic Compounds as Insect Repellents and Plant Elicitors: an Integrated Pest Management (IPM) Strategy for Glasshouse Whitefly (Trialeurodes vaporariorum). *Journal of Chemical Ecology*, 46 (11), 1090–1104. https://doi.org/10.1007/s10886-020-01229-8

Coninck, E., Scauflaire, J., Gollier, M., Liénard, C., Foucart, G., Manssens, G., Munaut, F. & Legrève, A. (2020). Trichoderma atroviride as a promising biocontrol agent in seed coating for reducing Fusarium damping-off on maize. *Journal of Applied Microbiology*, 129 (3), 637–651. https://doi.org/10.1111/jam.14641

D'alessandro, M., Erb, M., Ton, J., Brandenburg, A., Karlen, D., Zopfi, J. & Turlings, T.C.J. (2014). Volatiles produced by soil-borne endophytic bacteria increase

plant pathogen resistance and affect tritrophic interactions. *Plant, Cell & Environment*, 37 (4), 813–826. https://doi.org/10.1111/pce.12220

Doehlemann, G., Ökmen, B., Zhu, W. & Sharon, A. (2017). Plant Pathogenic Fungi. *Microbiology Spectrum*, 5 (1), 5.1.14. https://doi.org/10.1128/microbiolspec.FUNK-0023-2016

Drakulic, J., Caulfield, J., Woodcock, C., Jones, S.P.T., Linforth, R., Bruce, T.J.A. & Ray, R.V. (2015). Sharing a Host Plant (Wheat [Triticum aestivum]) Increases the Fitness of Fusarium graminearum and the Severity of Fusarium Head Blight but Reduces the Fitness of Grain Aphids (Sitobion avenae). *Applied and Environmental Microbiology*, 81 (10), 3492–3501. https://doi.org/10.1128/AEM.00226-15

Dudareva, N., Klempien, A., Muhlemann, J.K. & Kaplan, I. (2013). Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytologist*, 198 (1), 16–32. https://doi.org/10.1111/nph.12145

Girvin, J., Whitworth, R.J., Rojas, L.M.A. & Smith, C.M. (2017). Resistance of Select Winter Wheat (Triticum aestivum) Cultivars to Rhopalosiphum padi (Hemiptera: Aphididae). *Journal of Economic Entomology*, 110 (4), 1886–1889. https://doi.org/10.1093/jee/tox164

Hillocks, R.J. (2012). Farming with fewer pesticides: EU pesticide review and resulting challenges for UK agriculture. *Crop Protection*, 31 (1), 85–93. https://doi.org/10.1016/j.cropro.2011.08.008

Konappa, N., Krishnamurthy, S., Dhamodaran, N., Arakere, U.C., Ramachandrappa, N.S. & Chowdappa, S. (2020). Beneficial Effects of Trichoderma on Plant–Pathogen Interactions: Understanding Mechanisms Underlying Genes. I: Manoharachary, C., Singh, H.B., & Varma, A. (red.) *Trichoderma: Agricultural Applications and Beyond*. Cham: Springer International Publishing, 41–85. https://doi.org/10.1007/978-3-030-54758-5_3

Lee, S., Yap, M., Behringer, G., Hung, R. & Bennett, J.W. (2016). Volatile organic compounds emitted by Trichoderma species mediate plant growth. *Fungal Biology and Biotechnology*, 3, 7. https://doi.org/10.1186/s40694-016-0025-7

Manstretta, V. & Rossi, V. (2015). Effects of Weather Variables on Ascospore Discharge from Fusarium graminearum Perithecia. *PLoS ONE*, 10 (9), e0138860. https://doi.org/10.1371/journal.pone.0138860

Matusinsky, P., Mikolasova, R., Klem, K., Spitzer, T. & Urban, T. (2016). The role of organic vs. conventional farming practice, soil management and preceding crop on the incidence of stem-base pathogens on wheat. *Journal of plant diseases and protection (2006)*, 115 (1), 17–22. https://doi.org/10.1007/BF03356234

Ninkovic, V., Glinwood, R., Ünlü, A.G., Ganji, S. & Unelius, C.R. (2021). Effects of Methyl Salicylate on Host Plant Acceptance and Feeding by the Aphid Rhopalosiphum padi. *Frontiers in Plant Science*, 12. https://www.frontiersin.org/article/10.3389/fpls.2021.710268 [2022-01-20]

NIST Informatics, N.O. of D. and (u.å.). *NIST Chemistry WebBook*. https://doi.org/10.18434/T4D303

Nordlund, D.A. & Lewis, W.J. (1976). Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. *Journal of Chemical Ecology*, 2 (2), 211–220. https://doi.org/10.1007/BF00987744

Papp, M. & Mesterházy, Á. (1993). Resistance to bird cherry-oat aphid (Rhopalosiphum padi L.) in winter wheat varieties. *Euphytica*, 67 (1), 49–57. https://doi.org/10.1007/BF00022724

Pappas, M.L., Baptista, P., Broufas, G.D., Dalakouras, A., Djobbi, W., Flors, V., Guerfali, M.M., Khayi, S., Mentag, R., Pastor, V., Pereira, J.A., Sánchez-Bel, P. & Papadopoulou, K. (2020). Biological and Molecular Control Tools in Plant Defense. I: Mérillon, J.-M. & Ramawat, K.G. (red.) *Plant Defence: Biological Control*. Cham: Springer International Publishing, 3–43. https://doi.org/10.1007/978-3-030-51034-3_1

Peng, X., Qiao, X. & Chen, M. (2017). Responses of holocyclic and anholocyclic Rhopalosiphum padi populations to low-temperature and short-photoperiod induction. *Ecology and Evolution*, 7 (4), 1030–1042. https://doi.org/10.1002/ece3.2720

Pérez, C., Dill-Macky, R. & Kinkel, L. (2007). Management of soil microbial communities to enhance populations of Fusarium graminearum-antagonists in soil. *Plant and Soil*, 302, 53–69. https://doi.org/10.1007/s11104-007-9455-6

Piesik, D., Pańka, D., Delaney, K.J., Skoczek, A., Lamparski, R. & Weaver, D.K. (2011). Cereal crop volatile organic compound induction after mechanical injury, beetle herbivory (Oulema spp.), or fungal infection (Fusarium spp.). *Journal of Plant Physiology*, 168 (9), 878–886. https://doi.org/10.1016/j.jplph.2010.11.010

Pineda, A., Dicke, M., Pieterse, C.M.J. & Pozo, M.J. (2013). Beneficial microbes in a changing environment: are they always helping plants to deal with insects? *Functional Ecology*, 27 (3), 574–586. https://doi.org/10.1111/1365-2435.12050

*SALOME Spring malting barley* (2022). *Saaten Union*. https://www.scandinavia.saaten-union.com/index.cfm/action/varieties/cul/587/v/4584.html [2022-02-10]

Schoonhoven, L.M. (2005). *Insect-plant biology*. 2nd ed. Oxford ; Oxford University Press.

Siddiquee, S. (2014). Chapter 11 - Recent Advancements on the Role and Analysis of Volatile Compounds (VOCs) from Trichoderma. I: Gupta, V.K., Schmoll, M., Herrera-Estrella, A., Upadhyay, R.S., Druzhinina, I., & Tuohy, M.G. (red.) *Biotechnology and Biology of Trichoderma*. Amsterdam: Elsevier, 139–175. https://doi.org/10.1016/B978-0-444-59576-8.00011-4

Simon, J.-C. & Peccoud, J. (2018). Rapid evolution of aphid pests in agricultural environments. *Current Opinion in Insect Science*, 26, 17–24. https://doi.org/10.1016/j.cois.2017.12.009

Singh, B., Simon, A., Halsey, K., Kurup, S., Clark, S. & Aradottir, G.I. (2020). Characterisation of bird cherry-oat aphid (Rhopalosiphum padi L.) behaviour and aphid host preference in relation to partially resistant and susceptible wheat landraces. *Annals of Applied Biology*, 177 (2), 184–194. https://doi.org/10.1111/aab.12616

Speckbacher, V., Ruzsanyi, V., Wigger, M. & Zeilinger, S. (2020). The Trichoderma atroviride Strains P1 and IMI 206040 Differ in Their Light-Response and VOC Production. *Molecules*, 25 (1), 208. https://doi.org/10.3390/molecules25010208

Stein, S. (2012). Mass Spectral Reference Libraries: An Ever-Expanding Resource for Chemical Identification. *Analytical Chemistry*, 84 (17), 7274–7282. https://doi.org/10.1021/ac301205z

Sudderth, E.A. & Sudderth, E.B. (2014). Quantifying aphid behavioral responses to environmental change. *Entomologia Experimentalis et Applicata*, 150 (1), 7–18. https://doi.org/10.1111/eea.12139

Taheri, P. (2018). Cereal diseases caused by Fusarium graminearum: from biology of the pathogen to oxidative burst-related host defense responses. *European Journal of Plant Pathology*, 152 (1), 1–20. https://doi.org/10.1007/s10658-018-1471-2

Thackray, D.J., Diggle, A.J. & Jones, R. a. C. (2009). BYDV PREDICTOR: a simulation model to predict aphid arrival, epidemics of Barley yellow dwarf virus and yield losses in wheat crops in a Mediterranean-type environment. *Plant Pathology*, 58 (1), 186–202. https://doi.org/10.1111/j.1365-3059.2008.01950.x

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Woo, S.L. & Lorito, M. (2008). Trichoderma–plant–pathogen interactions. *Soil biology & biochemistry*, 40 (1), 1–10. https://doi.org/10.1016/j.soilbio.2007.07.002

Wiegmann, M., Thomas, W.T.B., Bull, H.J., Flavell, A.J., Zeyner, A., Peiter, E., Pillen, K. & Maurer, A. (2019). "Wild barley serves as a source for biofortification of barley grains". *Plant Science*, 283, 83–94. https://doi.org/10.1016/j.plantsci.2018.12.030

Wiest, R., Salvadori, J.R., Fernandes, J.M.C., Lau, D., Pavan, W., Zanini, W.R., Toebe, J. & Lazzaretti, A.T. (2021). Population growth of Rhopalosiphum padi under different thermal regimes: an agent-based model approach. *Agricultural and Forest Entomology*, 23 (1), 59–69. https://doi.org/10.1111/afe.12404

# Popular science summary

Have you ever looked at plants and wondered if they can interact with their environment? Plants can interact and communicate with each other, insects, and other organisms. This communication can lead to interactions such as insects' ability to choose which plant that fits them the best to feed- and live on. Volatile organic compounds are organic chemicals that for example are giving perfumes their odour and scents. Odours released by plants can work as repellents or attractants for insects such as aphids. Aphids are small destructive insects that are sucking fluid from plants and can transport damaging plant viruses between different plants, for example barley. Barley is a cereal that feed both humans and animals all around the world. Chemical control is today often used to avoid large crops losses due to aphids. However, with a higher demand on sustainable food sources, with as little chemical control as possible, we need to find other approaches and control measurements to avoid crops losses. Integrated Pest management (IPM) is a strategy to prevent pests and focus on a more sustainable solution that will work for a longer time. An example of strategies could be the use of beneficial organisms. A beneficial organism, such as a fungus, can grow on the roots of plants and help them to get nutrients from the soil.

This thesis has studied volatiles emitted from barley plants infected by either a beneficial or a plant pathogenic fungus. Additionally, analyse if aphids prefer the chemical signals from fungus colonized plants or plants without any fungus. The aphid had a choice to choose which odour (volatiles) they preferred during a time period. The plants were grown inside climate chambers separately from the aphids and the result from the experiment was analysed through statistics.

The result showed that the aphids did not prefer the odour of plants infected by the beneficial fungus and the volatiles emitted differed between treatments. However, further tests and analyses are needed to be able to evaluate the plant-microorganism- insect pest interaction.

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# Appendix 1

Result of volatile identification and quantification of compounds from all samples in the different treatments. Result showed in average peak area/DW ± SD (standard deviation).

| *Compound* | *Fusarium* *mean area±SD* | *Control* *mean area±SD* | *Trichoderma* *mean area±SD* |
|---|---|---|---|
| trans-Caryophyllene | 1.83E+07±1.57E+07 | 4.79E+06±4.89E+06 | 7.83E+06±4.04E+06 |
| Limonen | 1.23E+08±3.19E+08 | 1.01E+08±3.01E+08 | 7.24E+07±1.97E+08 |
| 1-Hexanol | 3.69E+07±3.80E+07 | 3.14E+07±1.18E+07 | 2.19E+07±1.27E+07 |
| 1-octen-3-one | 5.05E+07±2.17E+07 | 5.98E+07±3.68E+07 | 2.71E+07±1.55E+07 |
| 3-Octanone | 3.59E+07±2.22E+07 | 1.19E+08±1.69E+08 | 4.14E+07±2.33E+07 |
| 6-Methyl-5-hepten-2-one | 3.73E+07±5.42E+07 | 0.00±0.00 | 8.11E+06±4.03E+06 |
| acetophenone | 1.08E+08±5.97E+07 | 8.46E+07±6.92E+07 | 1.03E+08±7.49E+07 |
| alpha-pinene | 1.29E+08±1.61E+08 | 3.25E+08±0.00 | 1.97E+08±0.00 |
| benzaldehyde | 1,03E+09±2.90E+09 | 1.88E+08±2.75E+08 | 1.99E+08±2.66E+08 |
| benzyl-alcohol | 1.38E+08±0,00E+00 | 2.07E+07±1.93E+07 | 8.06E+07±9.40E+06 |
| cis-3-hexen-1-ol | 7.89E+07±5.65E+07 | 7.60E+07±2.72E+07 | 8.35E+07±2.64E+07 |
| cis-3-hexenylacetate | 7.81E+07±3.97E+07 | 8.81E+07±8.59E+07 | 7.85E+07±4.87E+07 |
| Decanal | 1.83E+08±1.66E+08 | 1.99E+08±4.12E+08 | 1.34E+08±2.24E+08 |
| Decane | 2.27E+08±6.25E+08 | 1.15E+08±3.85E+08 | 1.80E+07±2.37E+07 |
| Dodecane | 3.30E+08±7.28E+08 | 2.92E+08±8.17E+08 | 3.33E+08±8.76E+08 |
| Heptanal | 1.60E+08±2.93E+08 | 1.45E+08±2.19E+08 | 1.51E+08±2.67E+08 |
| Hexadecane | 1.25E+09±9.63E+08 | 7.00E+08±4.67E+08 | 6.26E+08±3.63E+08 |

| | | | |
|---|---|---|---|
| Hexanal | 0.00±0.00 | 8.54E+07±1.50E+08 | 1.18E+08±2.32E+08 |
| Linalool | 5.29E+07±2.13E+07 | 4.13E+07±2.08E+07 | 3.53E+07±1.97E+07 |
| methyl-salicylate | 3.65E+07±4.64E+07 | 3.37E+07±6.82E+07 | 2.58E+07±4.60E+07 |
| Nonanal | 1.11E+09±1.54E+09 | 1.07E+09±1.77E+09 | 8.07E+08±1.47E+09 |
| Octane | 2.82E+07±6.09E+07 | 8.53E+06±6.97E+06 | 2.70E+07±5.03E+07 |
| Octane-4-methyl | 1.49E+08±1.12E+08 | 8.51E+07±8.67E+07 | 7.58E+07±5.92E+07 |
| Pentadecane | 1.12E+09±8.49E+08 | 8.22E+08±3.30E+08 | 7.87E+08±5.08E+08 |
| phenol | 5.13E+07±0.00 | 3.64E+07±4.62E+07 | 3.74E+07±2.64E+07 |
| Tetradecane | 1.97E+08±1.05E+08 | 1.16E+08±4.76E+07 | 1.50E+08±6.46E+07 |
| Trans-2-Octenal | 7.22E+07±5.00E+07 | 7.45E+07±4.13E+07 | 5.67E+07±5.07E+07 |
| Trans-2-decenal | 0.00±0.00 | 0.00±0.00 | 5.01E+07±5.56E+07 |
| Trans-2-Hexenal | 4.31E+08±1.69E+08 | 3.71E+08±1.76E+08 | 4.95E+08±2.51E+08 |
| Undecane | 7.73E+07±6.37E+07 | 1.42E+08±4.07E+08 | 1.61E+08±3.77E+08 |
| Undecane-2-methyl | 1.53E+08±1.99E+08 | 8.19E+07±5.39E+07 | 1.12E+08±1.39E+08 |
| Unknown RI=1061.4 | 9.77E+07±5.84E+07 | 9.50E+07±6.22E+07 | 9.19E+07±6.82E+07 |
| Unknown RI=1081.1 | 4.09E+08±1.61E+08 | 2.37E+08±1.31E+08 | 2.19E+08±1.14E+08 |
| Unknown RI=1102.4 | 2.35E+08±4.38E+08 | 4.62E+07±1.77E+07 | 2.57E+08±2.57E+08 |
| Unknown RI=1103.5 | 1.67E+08±2.62E+08 | 7.26E+07±6.61E+07 | 4.69E+07±0.00 |
| Unknown RI=1108.9 | 7.31E+07±6.02E+07 | 6.17E+07±3.95E+07 | 9.91E+07±1.08E+08 |
| Unknown RI=1110.7 | 6.68E+07±9.36E+07 | 3.72E+08±2.47E+07 | 3.76E+07±3.33E+07 |
| Unknown RI=1111.3 | 1.28E+08±1.53E+08 | 1.71E+07±1.46E+07 | 1.06E+08±1.50E+08 |
| Unknown RI=1112.1 | 3.21E+08±1.50E+08 | 2.59E+08±1.48E+08 | 2.44E+08±1.01E+08 |
| Unknown RI=1249.5 | 4.78E+07±1.63E+07 | 9.99E+07±2.08E+08 | 9.38E+07±1.89E+08 |
| Unknown RI=1280.8 | 3.51E+08±1.85+08 | 3.51E+08±2.47E+08 | 3.22E+08±2.24E+08 |

| | | | |
|---|---|---|---|
| Unknown RI=1284.4 | 1.01E+08±2.00+08 | 9.96E+07±1.87E+08 | 1.49E+08±2.67E+08 |
| Unknown RI=1292.6 | 3.22E+07±4.16E+06 | 6.10E+07±7.57E+07 | 1.11E+07±0.00 |
| Unknown RI=1293.1 | 1.02E+08±8.60E+07 | 6.82E+07±3.17E+07 | 6.44E+07±3.07E+07 |
| Unknown RI=1298.6 | 9.42E+05±0.00 | 6.01E+07±0.00 | 0.00±0.00 |
| Unknown RI=1299.1 | 2.22E+08±9.83E+07 | 1.37E+08±4.00E+07 | 1.23E+08±4.79E+07 |
| Unknown RI=1305.5 | 1.94E+08±1.22E+08 | 1.67E+08±1.17E+08 | 1.60E+08±8.64E+07 |
| Unknown RI=1313.0 | 2.86E+08±1.61E+08 | 2.24E+08±9.01E+07 | 2.40E+08±1.34E+08 |
| Unknown RI=1315.6 | 1.54E+08±1.89E+08 | 1.32E+08±9.73+07 | 1.31E+08±7.02E+07 |
| Unknown RI=1324.1 | 1.24E+08±0.00 | 1.79E+08±5.54E+07 | 2.50E+08±0.00 |
| Unknown RI=1329.8 | 6.39E+08±4.47E+08 | 6.84E+08±6.16E+08 | 7.69E+08±6.23E+08 |
| Unknown RI=1336.5 | 1.40E+08±1.79E+08 | 5.98E+07±1.36E+08 | 1.90E+08±1.14E+08 |
| Unknown RI=1337.9 | 8.00E+08±4.54E+08 | 2.00E+08±2.16E+08 | 3.75E+08±4.83E+08 |
| Unknown RI=1338.2 | 6.09E+08±5.26E+08 | 4.38E+08±2.01E+08 | 4.10E+08±1.37E+08 |
| Unknown RI=1339.3 | 3.88E+08±8.61E+07 | 3.57E+08±2.81E+08 | 2.60E+08±1.61E+08 |
| Unknown RI=1346.0 | 1.66E+08±1.03E+08 | 1.96E+08±1.39E+08 | 1.17E+08±1.71E+08 |
| Unknown RI=1347.8 | 0.00±0.00 | 1.34E+08±5.05E+07 | 1.32E+07±0.00 |
| Unknown RI=1349.3 | 1.04E+08±1.42E+08 | 1.18E+08±5.62E+07 | 1.07E+08±7.23E+07 |
| Unknown RI=1357.0 | 2.40E+08±1.76E+08 | 1.34E+08±7.29E+07 | 1.31E+08±8.55E+07 |
| Unknown RI=1395.4 | 6.69E+08±8.02E+08 | 6.02E+08±1.08E+09 | 5.48E+08±9.41E+08 |
| Unknown RI=1463.2 | 3.06E+08±2.88E+08 | 2.07E+08±2.73E+08 | 2.24E+08±3.07E+08 |
| Unknown RI=1501.1 | 1.97E+08±0.00+00 | 3.58E+08±5.05E+07 | 4.31E+08±1.46E+08 |
| Unknown RI=1548.6 | 5.55E+08±2.84E+08 | 5.60E+08±2.37E+08 | 4.25E+08±1.46E+08 |
| Unknown RI=1567.6 | 3.69E+08±2.84E+08 | 1.82E+08±9.71E+07 | 2.98E+08±3.22E+08 |
| Unknown RI=1698.6 | 4.61E+08±3.55E+08 | 2.76E+08±2.23E+08 | 2.88E+08±2.92E+08 |
| Unknown RI=952.4 | 1.16E+08±5.34E+07 | 1.46E+08±1.68E+08 | 2.12E+08±3.86E+08 |

| | | | |
|---|---|---|---|
| Unknown RI=954.7 | 1.27E+08±6.07E+07 | 1.91E+08±1.35E+08 | 9.23E+07±5.65E+07 |
| Unknown RI=980.5 | 3.25E+08±1.48E+08 | 4.51E+08±3.36E+08 | 2.83E+08±2.13E+08 |

# Appendix 2

Result of weight of plants used in volatile collection, fresh weight, and dry weight.

| Treatment | Fresh weight (g) | Dry weight (g) |
|---|---|---|
| ***F. graminearum*** | | |
| F13 | 1.2661 | 0.2761 |
| F14 | 1.1094 | 0.255 |
| F15 | 1.028 | 0.2335 |
| F17 | 0.703 | 0.1844 |
| F18 | 0.7093 | 0.1796 |
| F19 | 0.7106 | 0.1838 |
| F20 | 0.8217 | 0.2004 |
| F21 | 0.442 | 0.1184 |
| F22 | 0.8409 | 0.2094 |
| F23 | 0.4777 | 0.1422 |
| F24 | 0.5015 | 0.159 |
| **Control** | | |
| 1C | 0.8515 | 0.2148 |
| 2C | 0.8618 | 0.1834 |
| 3C | 1.1393 | 0.2669 |
| 4C | 0.9315 | 0.2361 |
| 5C | 1.1661 | 0.2575 |
| 6C | 0.7944 | 0.1704 |
| 7C | 0.7138 | 0.232 |
| 8C | 0.949 | 0.2164 |
| 9C | 0.8105 | 0.2122 |
| 10C | 0.5109 | 0.1696 |
| 11C | 0.6339 | 0.1896 |
| 12C | 0.7323 | 0.2052 |
| ***T. atroviride*** | | |
| T25 | 1.0288 | 0.2872 |
| T26 | 0.7875 | 0.2222 |
| T27 | 0.8543 | 0.2415 |
| T28 | 1.152 | 0.2372 |
| T30 | 0.7322 | 0.2064 |

| | | |
|---|---|---|
| T31 | 0.9361 | 0.2398 |
| T32 | 0.8373 | 0.2098 |
| T33 | 0.7107 | 0.1672 |
| T35 | 0.5762 | 0.1885 |
| T36 | 0.8103 | 0.2087 |

# Appendix 3

Watering and fertilizing scheme of the plants for the volatile collection (batch 1, 2, 3) and for the plants for the olfactometer test (batch 4 and 5).

| Date | Fertilized water (ml)<br>2ml fertilizer to 10L water | Fertilized water (ml)<br>20ml fertilizer to 10L water | Batch |
|---|---|---|---|
| *31.01.22* | 50 | - | 1 |
| *01.02.22* | 50 | - | 2 |
| *02.02.22* | *50* | - | *3* |
| *02.02.22* | *10* | - | *1* |
| *03.02.22* | *10* | - | *2* |
| *05.02.22* | *10* | - | *3* |
| *07.02.22* | *20* | - | *1, 2, 3* |
| *10.02.22* | *20* | - | *1, 2, 3* |
| *14.02.22* | *20* | - | *1, 2, 3* |
| *14.02.22* | *50* | - | *4* |
| *15.02.22* | *50* | - | *5* |
| *16.02.22* | *10* | - | *1, 2, 3, 4* |
| *17.02.22* | - | *10* | *1, 2, 3, 5* |
| *19.02.22* | - | *20* | *1, 2, 3* |
| *20.02.22* | *20* | - | *5, 4* |
| *21.02.22* | - | *20* | *1, 2, 3* |
| *22.02.22* | *10* | - | *4, 5* |
| *23.02.22* | - | *10* | *2, 3* |
| *24.02.22* | - | *20* | *1,2,3,4,5* |
| *25.02.22* | *10* | - | *4, 5* |
| *26.02.22* | *10* | - | *4, 5* |
| *27.02.22* | *10* | - | *4, 5* |
| *28.02.22* | *10* | - | *4, 5* |
| *01.03.22* | - | *20* | *4, 5* |
| *02.03.22* | - | *10* | *4, 5* |
| *03.03.22* | - | *10* | *4, 5* |
| *04.03.22* | - | *20* | *5* |
| *05.03.22* | - | *10* | *4, 5* |
| *06.03.22* | - | *20* | *4, 5* |

| | | | |
|---|---|---|---|
| *07.03.22* | *-* | *10* | *5* |

Water and fertilizing of plants used in olfactometer test experiment 2 with sterilized sand. Batch 6 and 7 sown 14.03.22 and 15.03.22.

| Date | Fertilized water (ml)<br>2ml fertilizer to 10L water | Fertilized water (ml)<br>20ml fertilizer to 10L water | Batch |
|---|---|---|---|
| *14.03.22* | 50 | - | 6 |
| *15.03.22* | 50 | - | 7 |
| *16.03.22* | *10* | - | *6* |
| *17.03.22* | *10* | - | *7* |
| *18.03.22* | - | - | *6, 7* |
| *19.03.22* | *10* | - | *6, 7* |
| *20.03.22* | *10* | - | *6, 7* |
| *21.03.22* | - | *10* | *6, 7* |
| *22..03.22* | - | *10* | *6, 7* |
| *23.03.22* | - | *10* | *6, 7* |
| *24.03.22* | - | *10* | *6, 7* |
| *25.03.22* | - | *10* | *6, 7* |
| *26.03.22* | - | - | *6, 7* |
| *27.03.22* | - | *10* | *6, 7* |
| *28.03.22* | - | *10* | *6, 7* |
| *29.03.22* | - | *10* | *6, 7* |
| *30.03.22* | - | *10* | *6, 7* |
| *31.03.22* | - | *10* | *7* |

# Appendix 4

Figure show symptoms of stress on plants used for the first olfactometer test.

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