

University of Natural Resources and Life Sciences, Vienna  
Division of Plant Protection

Master Thesis

EFFECTS OF METHYL SALICYLATE, METHYL JASMONATE  
AND *CIS*-JASMONE ON *THRIPS TABACI* LINDEMAN

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# 1 Abstract

The aim of this study was to evaluate the effects of three plant volatiles *cis*-jasmonone, methyl jasmonate and methyl salicylate on behavioural responses of the onion thrips *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). The substances were tested at a range of concentrations (0.1 % - 10 %) depending on the bioassay. In the olfactometer bioassay *T.tabaci* showed no significant repellent olfactory responses to all test substance odours at 0.1 %, 1 % or 10 % concentration. The contact chemoreceptory responses of *T.tabaci* were investigated on the leek (*Allium ampeloprasum* var. *porrum*) leaf discs treated with the test substances at 0.1 % or 1 % concentration in settling preference, feeding damage and oviposition rate bioassays. The oviposition rate was influenced by the treatment with methyl salicylate at 0.1 %. The feeding damage area was significantly reduced when *cis*-jasmonone at 0.1 % or 1 % concentration was applied on the leek leaf discs. The application of *cis*-jasmonone (1 %) or methyl salicylate (1 %) had a significant deterrent effect on the settling preference of *T.tabaci*. Methyl jasmonate had no deterrent effect on *T.tabaci*. The results obtained in this study are discussed. Further studies under field or greenhouse conditions are needed to investigate the defence potential of *cis*-jasmonone or methyl salicylate against *T.tabaci*.

**Keywords:** *Thrips tabaci*, onion thrips, Thysanoptera, Thripidae, *cis*-jasmonone, methyl jasmonate, methyl salicylate, deterrent, repellent, olfactometer, feeding damage, settling preference, oviposition rate

## 2 Introduction

*Thrips tabaci* Lindeman (Thysanoptera: Thripidae) is a prevalent, polyphagous pest of many cultivated crops throughout Europe and various other parts of the world. It infests a range of glasshouse and field vegetables, including leeks, and primarily causes feeding damage that disfigures the leaf tissue (Foster et al., 2010). Moreover, *T.tabaci* has been identified as a vector of plant viruses (Diaz-Montano et al., 2010). Chemical control of *T.tabaci* is difficult. Thrips are physically protected because they hide between the inner layers of leek, females lay eggs within leaf tissue and prepupae and pupae rest in the soil (Childers and Achor, 1995; Van Rijn et al., 1995). Alternative methods to control *T.tabaci* are necessitated because of its resistance to pyrethroids and organophosphate insecticides (Allen et al., 2005).

Communication by semiochemicals in the Thysanoptera is well established. Thrips use odours of leaf buds and foliage to orient to the host plants (Terry, 1997). Plants vary in their primary and secondary metabolites, which may attract or repel thrips for feeding or oviposition (Terry, 1997). Concentration of volatiles is also important because the optima may be critical for their attraction (Terry, 1997). The use of semiochemicals in organic or integrated pest management is increasingly seen as an alternative to the conventional methods of the pest control, such as pesticides (Moraes et al., 2009).

*Cis*-jasmonone (Birkett et al., 2000), methyl jasmonate (Bruinsma and Dicke, 2008) and methyl salicylate (Turlings and Ton, 2006) are the important herbivore-induced plant volatiles, which effects on insects were observed in several studies.

*Cis*-jasmonone is emitted from plants after insect damage and can trigger defensive responses (Heil, 2008). *Cis*-jasmonone acted as an attractant on *Thrips obscuratus* (El-Sayed et al., 2009), as a repellent on the damson-hop aphid *Phorodon humuli* and as an attractant on an aphid antagonist, the seven-spot ladybird *Coccinella septempunctata* (Pickett et al., 2006).

Methyl jasmonate plays an important role in induced defence in many plants against a wide range of herbivores (Bruinsma and Dicke, 2008). *Frankliniella occidentalis* thrips had no significant olfactory responses to methyl jasmonate odour, but they showed low settling and oviposition rates on chrysanthemum leaf discs treated with methyl jasmonate (Bruhin, 2009).

Methyl salicylate is a stress-related plant semiochemical, to which the most insects show strong electrophysiological responses (Pickett et al., 2006). Synthetic methyl salicylate attracted carnivore species (Snoeren et al., 2010). Methyl salicylate deterred females of *Frankliniella occidentalis* from feeding and egg-laying (Koschier et al., 2007). The results of the study, conducted by Bruhin (2009), indicated that methyl salicylate applied to

chrysanthemums had no clear effect on *Frankliniella occidentalis*. Blande et al. (2010) reported about aphid-repellent qualities of methyl salicylate.

In this study the aim was to investigate responses of *Thrips tabaci* to plant volatiles *cis*-jasmane, methyl jasmonate and methyl salicylate at different concentrations. The effects of these substances were examined in olfactory and contact chemoreceptory (settling preference, feeding damage and oviposition rate) bioassays.

The objectives of the present study were to determine:

1. Do methyl salicylate, methyl jasmonate and *cis*-jasmane applied to leek have a deterrent effect on the settling preference, feeding and oviposition behaviour of *Thrips tabaci*?
2. Do methyl salicylate, methyl jasmonate and *cis*-jasmane as olfactory (odour) cues have a repellent effect on *Thrips tabaci*?



### 3 Material and Methods

#### 3.1 Material: Test insect: *Thrips tabaci* Lindeman

##### 3.1.1 Taxonomic Classification (Mound and Kibby, 1998)

Class:	Insecta
Subclass:	Pterygota
Order:	Thysanoptera
Suborder:	Terebrantia
Family:	Thripidae
Subfamily:	Thripinae
Genus:	Thrips
Species:	<i>Thrips tabaci</i> Lindeman

Common name: onion, tobacco or potato thrips

##### 3.1.2 Geographical Distribution and Dispersal

*T. tabaci* is a cosmopolitan pest of numerous plants between sea level and 2000 m and can tolerate a broad range of climatic conditions (Lewis, 1997). This species is known in Eurasia, North and South America, Africa, as well as in Australia (Mound, 1997; Jenser and Szenasi, 2004). However, it is usually rare in the humid tropics and subtropics, but is often abundant on warm, dry locations (Mound, 1997).

This highly polyphagous species can feed and propagate on many different wild and cultivated plants. It was recorded on leek, onion, garlic, cotton, tobacco, cabbage, cucumber and on many other plants (Belder et al., 2000; Jenser and Szenasi, 2004; Trdan et al., 2005).

The strong affinity of *T. tabaci* for onion and leek may indicate Eastern Mediterranean origin, the apparent region of origin for these crops (Leigh, 1995; Jenser and Szenasi, 2004).

Thrips are limited in their natural ability to spread over long distances (Vierbergen, 1995), but they can be transported by the winds or through the international agricultural trade.

The attributes, such as high reproduction capacity, short generation time, early maturity, parthenogenesis and polyphagy, contribute greatly to their pest status and distribution all over the world (Mound and Teulon, 1995).

### 3.1.3 Morphology and Life Cycle

*T. tabaci* is a relatively small species, the female being 0.8-1.0 mm long (<http://www.agf.gov.bc.ca/cropprot/thrips.pdf>, 25.08.2011) and light (20 to 50 µg) (Lewis, 1997). They vary considerably in body size, and the largest individuals are often darker than the smallest. Such variation in colour and size may be determined largely by environmental factors (Mound and Kibby, 1998). *T. tabaci* adults are larger and darker in cold areas than in warm areas (Mound, 1997).

The body of adults can be differentiated into a head, a thorax and an 11-segmented abdomen (Moritz, 1997). The slender membranous wings are covered in minute microtrichia and are fringed with long cilia (Lewis, 1997). The wings and genital appendages occur only in adults (Moritz, 1997). The forward-directed antennae of *T. tabaci* are inserted into a socket on the head capsule (Moritz, 1997) and comprise seven segments (<http://www.agf.gov.bc.ca/cropprot/thrips.pdf>, 25.08.2011). The chemoreceptors and other sense organs occur on the antennae (Moritz, 1997).

Thrips have piercing-sucking mouthparts. They use the single mandible to penetrate the cell wall and suck up the liquids of the cell through the maxillary stylets (Childers and Achor, 1995; Kirk, 1997).

Thrips locate host plants using colour, shape, size and volatiles. For detecting olfactory, gustatory and morphological differences between plants thrips use tactile, mechanical and chemical receptors on antennae and mouthparts. They may use the same cues for detection of feeding sites and hosts for oviposition (Terry, 1997).

The large and well developed ovipositor is used to pierce plant tissue and deposit singly eggs, which tend to be laid in older, non-expanding leaves to avoid eggs being crushed as cells enlarge (Terry, 1997).

In *T. tabaci*, sexual and asexual populations are common (Moritz, 1997). Mound (1997) notes, that *T. tabaci* exist as two independent subspecies, one parthenogenetic and the other bisexual. However, in many parts of the world males of this species are rare except when populations are very high.

According to Van Rijn et al. (1995) the thelytokous parthenogenesis of *T. tabaci* in which females are produced from unfertilized eggs implies an advantage. All females contribute to population growth without availability of males.

The life cycle of a thrips involves six developmental stages: an egg, two actively feeding larval stages, two non-feeding stages prepupa and pupa and the imago (Fig. 1).

Van Rijn et al. (1995) described differences between the developmental stages as follows:

- Eggs: oval to kidney shaped, whitish, laid in plant tissue.
- Larvae 1 & 2: there are no clear morphological differences between the two larval stages, except in their size.
- Prepupae: can be recognized by their short wing sheaths and erect antennae.
- Pupae: have long wing sheaths, the antennae are bent backwards along the head.
- Adults: can be recognized by their wings.

Adults and larvae 1 & 2 are agile and cause the feeding damage on host plant. In prepupal and pupal stages they do not feed and use sheltered places like soil for their pupation (Deligeorgidis and Ipsilandis, 2004).

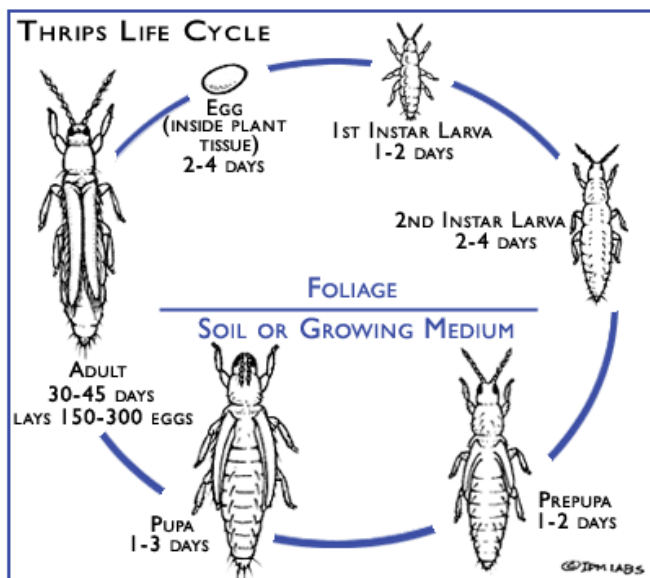


Fig. 1: Thrips life cycle (Source: <http://www.ipmlabs.com/cs4a.php>, 25.08.2011)

### 3.1.4 Thrips Damage and Control

*T. tabaci* is regarded as an economically harmful pest, which has spread to all continents (Fournier et al., 1995; Liu and Sparks, 2003; Sparks et al., 2011). It is recognized as a pest on many cultivated plants such as leek, onion, garlic, cabbage, cotton, tobacco and cucumber (Trdan, 2005). *T. tabaci* can cause damage on the host plants directly through feeding and indirectly through transmission of plant viruses (Childers and Achor, 1995).

Thrips feeding and oviposition induce a range of symptoms in plant tissue. As parenchyma feeders, they suck out the cell sap and cause the silverying of plant tissue due to air entering into empty cells (Mound and Kibby, 1998). Leek leaves develop silvery spots that enlarge and coalesce into white areas (Childers, 1997). Oviposition injuries may predispose bacterial or fungal diseases (Childers and Achor, 1995).

The feeding of *T. tabaci* adults and larvae on plant leaves causes the disturbance of photosynthesis and water retention, which results in the production of smaller and less valuable plants (Nault and Shelton, 2010).

The within plant distribution of *T. tabaci* on leek varies with their life cycle stages due to their different preferences for plant organs. Adults prefer to lay eggs where they feed, moving continually towards the younger tissue (Terry, 1997).

Beside the direct damage *T. tabaci* can cause as well indirect damage such as virus transmission. They are the vectors of a series of plant viruses that are known as tospoviruses, a genus in the virus family Bunyaviridae. The relationship between thrips and tospoviruses is complex. Thrips can acquire the virus only through the feeding as a first instar larva and transmit the virus subsequently as adult feeding on the next suitable plant (Mound and Kibby, 1998).

*T. tabaci* can transmit Iris yellow spot virus (Diaz-Montano et al., 2010) as well as tomato spotted wilt virus (Trjapitzin, 1995).

Pesticides are the main option for control, but this approach is challenging because of problems with delivery and contact (Foster et al., 2010). *T. tabaci* lives and breeds well protected between the inner leaves. That makes them difficult to treat with insecticides (Kirk, 1997). There is also the risk of resistance development. The loss of control with organophosphates against *T. tabaci* since the early 1990s can be illustrated as an example (Foster et al., 2010). Pesticide resistance is one of the major concerns of pest management (Mound and Teulon, 1995; Allen et al., 2005; Herron et al., 2008; Foster, 2010).

As a consequence of this the alternatives to pesticides are important. Some genotypes of plants possess the features making them resistant to *T. tabaci*. Leaf structure, growth form,

presence of wax and colour might affect thrips landing as well as subsequent feeding (Terry, 1997; Yousefi, 2011).

Several studies show the reducing of thrips feeding symptoms in intercropped leeks (Theunissen and Schelling, 1998; Belder et al., 2000).

The biological control can also give a contribution to this issue. The main predators of thrips are mites (*Amblyseius* spp.), heteropteran bugs (*Orius* spp.), lacewing larvae (*Chrysoperla* spp.), ladybird larvae (*Coccinella* spp.), hoverfly larvae (*Mesograpta marginata*, *Sphaerophoria ruepelli*) and other thrips (*Aeolothrips intermedius*) (Gillespie, 1989; Sabelis and Van Rijn, 1997). All stages of thrips are attacked by several entomopathogenic fungi (Kirk, 1997).

### 3.1.5 Thrips rearing

For this study the thrips were taken from the stock rearing at the Division of Plant Protection, University of Natural Resources and Life Sciences, Vienna.

This method of thrips rearing was adopted from Loomans and Murrai (1997).

*T. tabaci* was reared in glass jars (Fig. 2) which were covered with a fine mesh for air circulation and to prevent their escape. All the individuals were thelytokous females from the parthenogenetically reproducing strain. Thrips were provided with fresh leek leaves for feeding and oviposition. Twice a week the jars were checked and dry leek pieces were replaced with fresh ones. At the bottom of the jars several layers of filter paper allowed the non-feeding stages of *T. tabaci* (prepupa and pupa) to find a hidden place for their pupation. The thrips were kept at  $24^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  with a photoperiod of 16:8 (L:D) h.



Fig. 2: Thrips rearing in glass jars.

## 3.2 Material: Test Substances

*Cis*-jasmonone, methyl jasmonate and methyl salicylate at a range of concentrations (0.1 % - 10 %) depending on the bioassay were tested in this study.

### 3.2.1 Methyl salicylate (MS)

In this study methyl salicylate with a purity of 99 % was used (Fluka Chemie GmbH, Buchs, Switzerland).

Molecular formula:  $C_8H_8O_3$

Appearance: colourless liquid with an odour of the wintergreen (*Gaultheria procumbens*) essential oil.

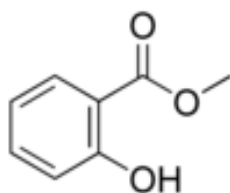


Fig. 3: Methyl salicylate (Source: [http://en.wikipedia.org/wiki/Methyl\\_salicylate](http://en.wikipedia.org/wiki/Methyl_salicylate), 24.08.2011)

### 3.2.2 Methyl jasmonate (MJ)

Methyl jasmonate with a purity of 95 % (Sigma-Aldrich, Vienna, Austria) was used in bioassays of this study.

Molecular formula:  $C_{13}H_{20}O_3$

Appearance: colourless liquid.

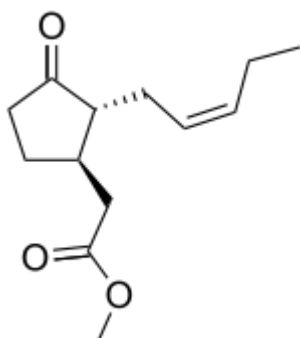


Fig. 4: Methyl jasmonate (Source: [http://en.wikipedia.org/wiki/Methyl\\_jasmonate](http://en.wikipedia.org/wiki/Methyl_jasmonate), 24.08.2011)

### 3.2.3 *Cis*-jasmone (JA)

*Cis*-jasmone with a purity of  $\geq 85\%$  (Sigma-Aldrich, Saint Louis MO, USA) was used in this study.

Molecular formula:  $C_{11}H_{16}O$

Appearance: colourless to pale yellow liquid with odour of jasmine (*Jasminum grandiflorum*).

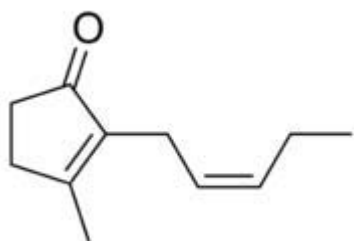


Fig. 5: *Cis*-jasmone (Source: <http://en.wikipedia.org/wiki/Cis-jasmone>, 24.08.2011)

### 3.2.4 Test Substance Dilutions for Olfactometer Bioassays

All test substances (see 3.2.1 – 3.2.3) were diluted in pure paraffin oil (Uvasol, Merck KG, Darmstadt, Germany) at 10 % - 0.1 % concentrations. As a control was used pure paraffin oil only.

### 3.2.5 Test Substance Dilutions for Leaf Disc Bioassays

All test substances (see 3.2.1 – 3.2.3) were diluted in distilled water plus Triton X-100 (0.05 %; Sigma-Aldrich, Vienna, Austria) as wetting agent at concentrations 1 % or 0.1 %. But first ethanol (1:10 v/v) was added to test substances because of their poor water solubility. Triton X-100 (0.05 %) as wetting agent in distilled water plus ethanol was used as a control substance.

## 3.3 Material: Test Plant

*Allium ampeloprasum* var. *porrum* (L.) (Common name: leek) was used as a test plant for leaf disc bioassays. The leaf discs were taken from the inner leaves of the same leek, which was used for the thrips rearing. The leek plants were bought weekly in the supermarket and kept in a plastic box in a refrigerator.

### 3.4 Methods

#### 3.4.1 Thrips Manipulation

Thrips were collected from the rearing glass jars with a small aspirator. They were transferred individually with a fine slightly moistened brush (size 00) to the leaf discs. To make the handling easier, thrips were put shortly on a cool surface which consisted of a Petri dish bottom lined with a piece of slightly moistened paper towel placed on the crushed ice in an ice box.

#### 3.4.2 Olfactometer Bioassays (choice test)

A glass Y-tube olfactometer as described by Koschier et al. (2000) was used in this study (Fig. 6).



Fig. 6: Y-shaped glass tube olfactometer in a black box.

The olfactometer was placed in a box covered inside with a black cloth in a dark room to avoid the distraction of thrips by the other visual stimuli. To stimulate thrips to move to one of the far ends, the Y-tube was arranged on a black support at an angle of 25° and the Y-junction was illuminated by a small lamp (approx. 160 lux light intensity) attached on the ceiling. The end tubes of the Y-tube were connected to two glass Wheaton Micro Kit adapters attached to two glass vials. The one vial contained the 1 cm<sup>2</sup> filter paper applied with 1 µl pure paraffin oil, the other one had 1 µl diluted test substance. The airstream (5 cm/sec to 10 cm/sec) was supplied to the olfactometer by an electric pump which was connected via silicone tubing to the base of the Y-tube. The airstream was purified by passage through charcoal pellets.



Adult *T.tabaci* of unknown age were randomly collected with an aspirator from mass rearing and left over night (for approx. 15 h) in a glass dish without food. The glass dish was covered with two layers of sealing film (Nescofilm Osaka, Japan) and between them was put a droplet of water. This starvation diet was done to keep the thrips hungry enough to react to the odour cues.

In this bioassay methyl jasmonate, methyl salicylate and *cis*-jasmonone at 0.1 %, 1 % or 10 % concentration were tested (see 3.2.4). Pure paraffin oil was used as a control.

Before starting the experiment, the pump was kept running for 15 minutes to allow the test odour to volatilize. A single *T.tabaci* female was placed at the base of the Y-tube with a fine slightly moistened brush. When the thrips reached the far end of one Y-tube arm, the choice was recorded and the thrips was removed from the Y-tube. If the decision for the direction of movement was not made within three minutes, thrips was replaced with another one. Each individual was used only once. After testing of five individuals, the Y-tube was rotated by 180° to avoid any directional bias. One experiment was finished after 20 scores and had an average duration of one hour. Each experiment was replicated three times. All parts of the olfactometer were cleaned with acetone after each experiment.

### **3.4.3 Leaf Disc Bioassays**

#### General leaf disc bioassay procedure

Leek leaf discs were punched with a cork borer with a diameter of 1.6 cm from inner leaves of leek plants. The leaf discs were sprayed with 2.5 ml test substance (see 3.2.5) by means of Potter Spray Tower (Burkard Manufacturing Co Ltd., Rickmansworth, UK) at a pressure of 4-5 lb/inch<sup>2</sup>. Control leaf discs were treated with control substance (see 3.2.5) and distilled water. In all experimental series the Petri dish bottoms were covered with a thin plastic film after thrips were introduced. To assure the air circulation and to avoid the water condensation the plastic film was perforated by means of insect pins (0.4 mm diameter).

#### Settling Preference (choice test)

For the settling preference test glass Petri dishes (9 cm diameter) were filled with water agar (1%) approx. 4 mm high. After cooling of the water agar two holes of 1.5 cm diameter were punched in it with a cork borer. A treated and an untreated leaf disc were embedded into the solid water agar layer. A piece of filter paper was placed as starting point in the centre of the Petri dish between two discs (Fig. 7).

Ten thrips of unknown age from the stock culture were immobilized on a cool surface (see 3.4.1) and transferred with a brush to the starting point of the Petri dish. The Petri dishes

were placed on white plastic trays in a climate chamber (24°C, L16:D8). After 15, 60, 120, 180, 240, 300 and 360 minutes the number of thrips on the treated or untreated leaf discs was recorded. Each treatment was replicated 10 times.

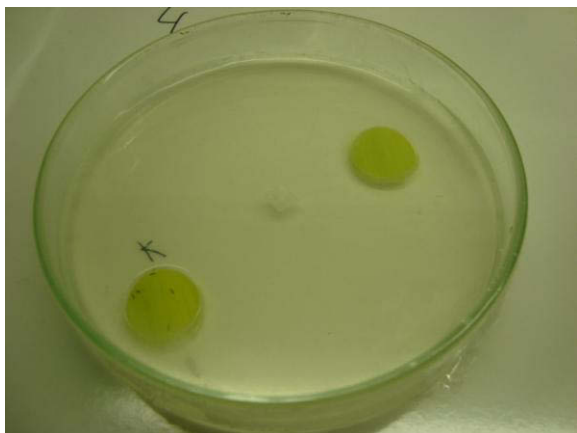


Fig. 7: Petri dish with a treated and an untreated leaf disc embedded in solid water agar (settling preference).

#### Feeding Damage Test (no-choice test)

Adult thrips of unknown age were taken from the stock culture. Small Petri dishes (6 cm diameter) were filled with 8 ml water agar (1%) using bottle-top dispenser Biohit proline® Prospenser (Biohit OY, Helsinki, Finland). After cooling of the water agar a hole (1.5 cm diameter) was punched in it and one leaf disc (treated or untreated) was embedded in it (Fig. 8). Ten thrips immobilized on a cool surface (see 3.4.1) were put into each Petri dish using a brush and covered with a perforated plastic film. After 24 hours in a climate chamber (24°C, L16:D8 h) the thrips were removed, the feeding damage on the leek leaf discs was quantified using a binocular microscope and a transparent grid (with 1 mm<sup>2</sup> fields) (Fig. 9) and the percentage of damaged area on each leaf disc was calculated. The leaf disc of 1.6 cm diameter had an area of 201 mm<sup>2</sup> (100%). The percentage of damaged area was calculated on this basis. Each treatment was replicated 15 times.



Fig. 8: Petri dish with one leaf disc with feeding damage (feeding damage test).

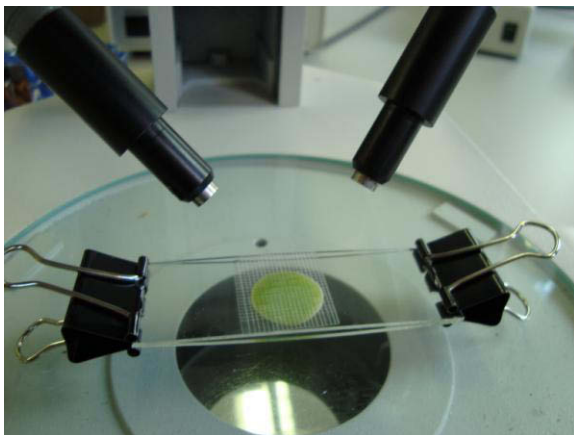


Fig. 9: Recording of feeding damage on a leaf disc using a binocular microscope and a transparent grid (feeding damage test).

#### Oviposition Rate Test (no-choice test)

For the oviposition bioassay synchronized females (at the same age) were obtained by collecting pupae from the stock rearing. The pupae were isolated and put into a Petri dish with a small piece of leek. The Petri dish was sealed with Nesco sealing film (Azwel Inc., Osaka, Japan). After 72 h the emerged adults were separated from pupae and put into another Petri dish. After 48 h the adults were ready for test. According to Van Rijn (1995) the highest ovipositional rate of *T.tabaci* was observed on the second day after their pupation. The Petri dish preparing procedure was similar to that used for the feeding damage test (see above).

Synchronized female thrips were transferred singly with a brush into the small Petri dish. After 24 hours in a climate chamber (24°C, L16:D8 h) the thrips were removed and the leaf discs were blanched in a microwave oven for approx. 3 minutes in order to make the eggs

visible. The eggs in the tissue of leaf discs were counted using a binocular microscope. Each treatment was replicated 15 times.

#### **3.4.4 Statistical Analysis**

All data were analyzed with a confidence limit of 95 % using the software programs Microsoft Excel 2007 and SPSS 15.0 for Windows. Homogeneity of variances was tested using the Kolmogorov-Smirnov-Test. The binomial distribution test was performed to compare the data of the olfactometer bioassays. In case of normal distribution the data from the oviposition rate test were subjected to a one-way ANOVA, the non-normal distributed data were analyzed with a Mann-Whitney-Test. The data of the feeding damage test were compared using an unpaired *t*-test. Because the data obtained in the settling preference test were not normally distributed the results were analyzed by Wilcoxon-Test.

## 4 Results

### 4.1 Olfactometer Bioassays

In the olfactometer study *T.tabaci* showed no significant olfactory responses to *cis*-jasmone odour at 0.1 %, 1 % or 10 % concentration (Table 1). There was a tendency (not significant) that more thrips (63.0 %  $\pm$  2.1) chose *cis*-jasmone odour at 0.1 % concentration. More thrips (60.0 %  $\pm$  1.7 at 1 % or 57.0 %  $\pm$  0.6 at 10 %) also chose *cis*-jasmone odour at the higher concentrations, although differences were not significant.

Table 1: Percentage of individuals choosing an airflow loaded with *cis*-jasmone or clean air in an olfactometer (mean  $\pm$  s.e.). Means are not significantly different at  $P \geq 0.05$  (n.s.).

	Percentage of individuals choosing odour or control (mean $\pm$ s.e.)		
Concentration of odour	<b>Cis-jasmone</b>	Control	<i>P</i> -value
0.1 %	63.0 $\pm$ 2.1	37.0 $\pm$ 2.1	0.052 (n.s.)
1 %	60.0 $\pm$ 1.7	40.0 $\pm$ 1.7	0.155 (n.s.)
10 %	57.0 $\pm$ 0.6	43.0 $\pm$ 0.6	0.366 (n.s.)

*T.tabaci* showed no significant olfactory responses to methyl jasmonate odour at 0.1 %, 1% or 10% concentration (Table 2). There was a tendency of slightly less thrips responses (37.0 %  $\pm$  1.5) to methyl jasmonate odour at 10 % concentration, although this effect was not significant. At the lower 1 % concentration less thrips (47.0 %  $\pm$  2.1) chose methyl jasmonate odour, but more thrips (53.0 %  $\pm$  0.6) chose this odour at 0.1 % concentration, although both results were not significant.

Table 2: Percentage of individuals choosing an airflow loaded with methyl jasmonate or clean air in an olfactometer (mean  $\pm$  s.e.). Means are not significantly different at  $P \geq 0.05$  (n.s.).

	Percentage of individuals choosing odour or control (mean $\pm$ s.e.)		
Concentration of odour	<b>Methyl jasmonate</b>	Control	<i>P</i> -value
0.1 %	53.0 $\pm$ 0.6	47.0 $\pm$ 0.6	0.699 (n.s.)
1 %	47.0 $\pm$ 2.1	53.0 $\pm$ 2.1	0.699 (n.s.)
10 %	37.0 $\pm$ 1.5	63.0 $\pm$ 1.5	0.052 (n.s.)

*T.tabaci* showed no significant olfactory responses to methyl salicylate odour at 0.1 %, 1 % or 10 % concentration (Table 3). At all these concentrations more thrips (60.0 %  $\pm$  2.0, 62.0 %  $\pm$  1.5 or 60.0 %  $\pm$  2.0) chose the odour, although differences were not significant.

Table 3: Percentage of individuals choosing an airflow loaded with methyl salicylate or clean air in an olfactometer (mean  $\pm$  s.e.). Means are not significantly different at  $P \geq 0.05$  (n.s.).

	Percentage of individuals choosing odour or control (mean $\pm$ s.e.)		
Concentration of odour	<b>Methyl salicylate</b>	Control	<i>P</i> -value
0.1 %	60.0 $\pm$ 2.0	40.0 $\pm$ 2.0	0.155 (n.s.)
1 %	62.0 $\pm$ 1.5	38.0 $\pm$ 1.5	0.092 (n.s.)
10 %	60.0 $\pm$ 2.0	40.0 $\pm$ 2.0	0.155 (n.s.)

## 4.2 Leaf Disc Bioassays

### 4.2.1 Settling Preference

Application of the control substance had a significant deterrent effect on settling behaviour of *T.tabaci* (Fig. 10). Thrips females significantly preferred settling on the water-treated leaf discs after four hours and for the rest of the observation period.

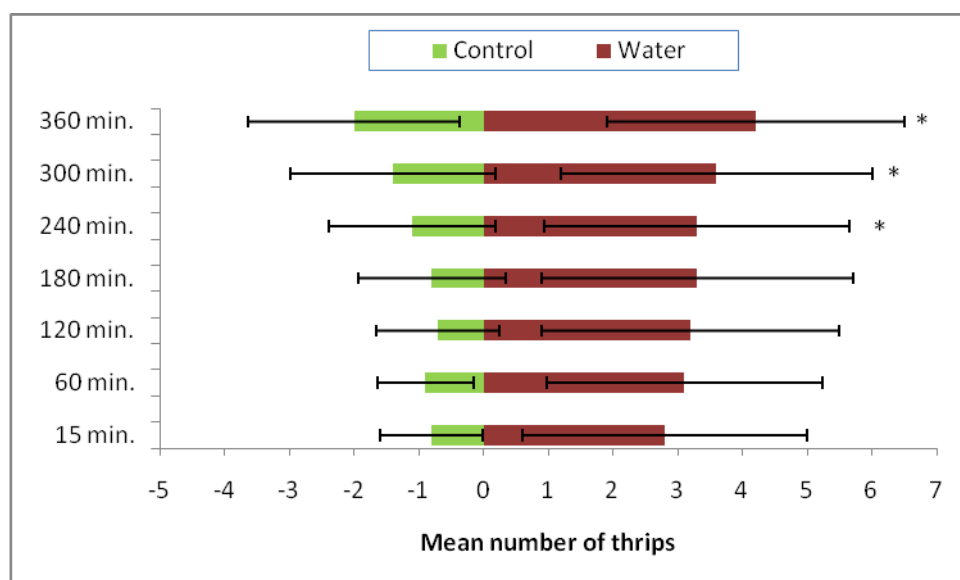


Fig. 10: Settling preference of *T.tabaci* for leek leaf discs treated with distilled water or control substance within a 360 min period (mean number of thrips settled  $\pm$  s.e.). Means are significantly different at  $P \leq 0.05$  (\*).

Application of the test substance *cis*-jasmone at 0.1 % concentration had no deterrent effect on settling behaviour of *T.tabaci* (Fig. 11).

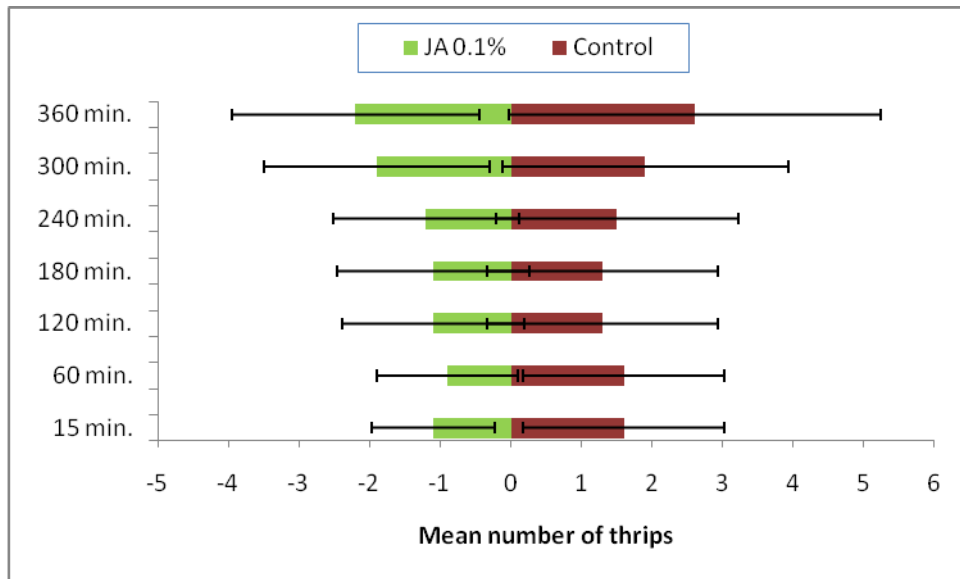


Fig. 11: Settling preference of *T.tabaci* for leek leaf discs treated with *cis*-jasmone 0.1% or control substance within a 360 min period (mean number of thrips settled  $\pm$  s.e.).

Leaf discs treated with *cis*-jasmone at 1 % concentration had a significant deterrent effect on thrips after two hours and for the rest of the observation period (Fig. 12).

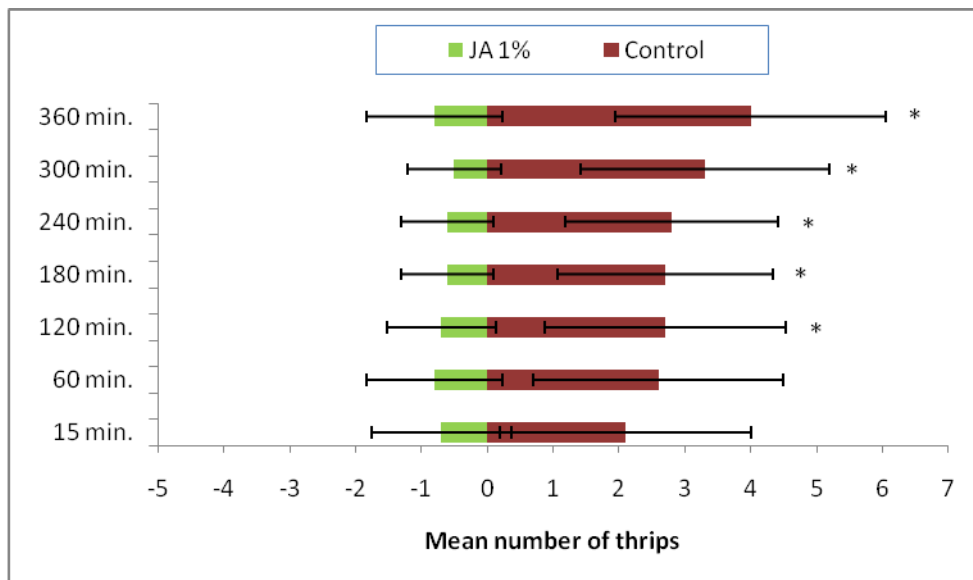


Fig. 12: Settling preference of *T.tabaci* for leek leaf discs treated with *cis*-jasmone 1% or control substance within a 360 min period (mean number of thrips settled  $\pm$  s.e.). Means are significantly different at  $P \leq 0.05$  (\*).

Application of the test substance methyl jasmonate at 0.1 % concentration had no deterrent effect on settling preference of *T.tabaci* (Fig. 13).

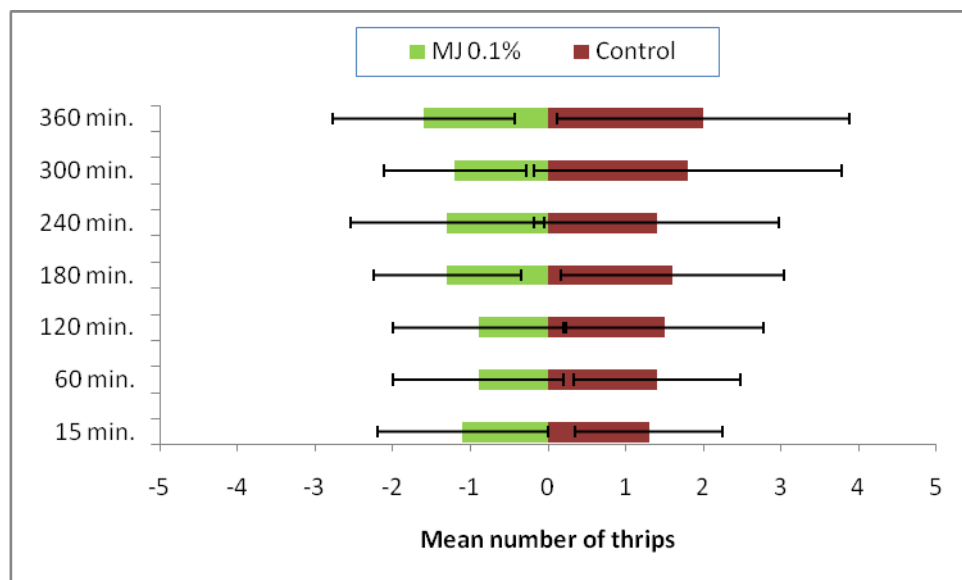


Fig. 13: Settling preference of *T.tabaci* for leek leaf discs treated with methyl jasmonate 0.1% or control substance within a 360 min period (mean number of thrips settled  $\pm$  s.e.).

There were no significant preferences of thrips for leaf discs treated with methyl jasmonate at 1 % concentration or the control leaf discs (Fig. 14).

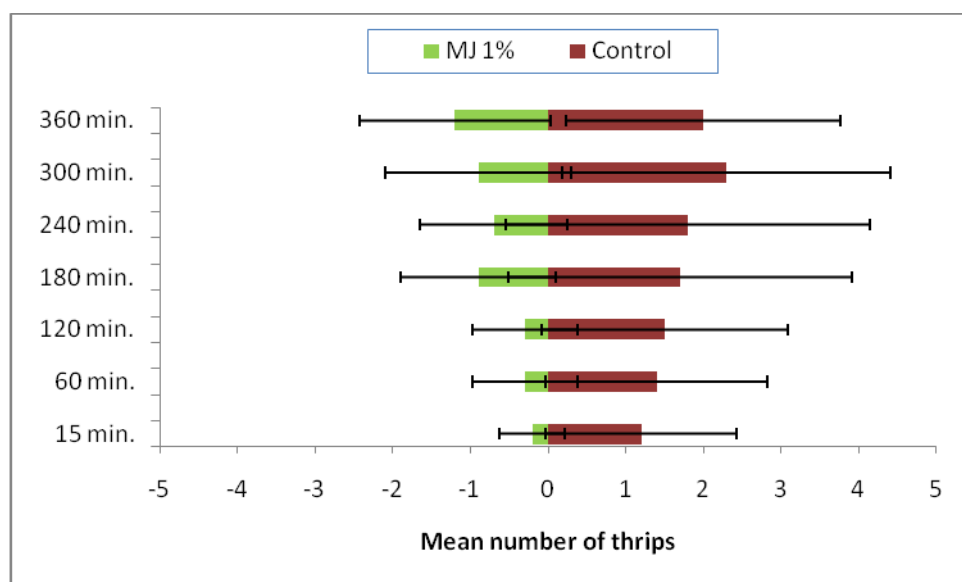


Fig. 14: Settling preference of *T.tabaci* for leek leaf discs treated with methyl jasmonate 1% or control substance within a 360 min period (mean number of thrips settled  $\pm$  s.e.).

The lowest concentration of the test substance methyl salicylate (0.1 %) had no significant deterrent effect on thrips (Fig. 15).



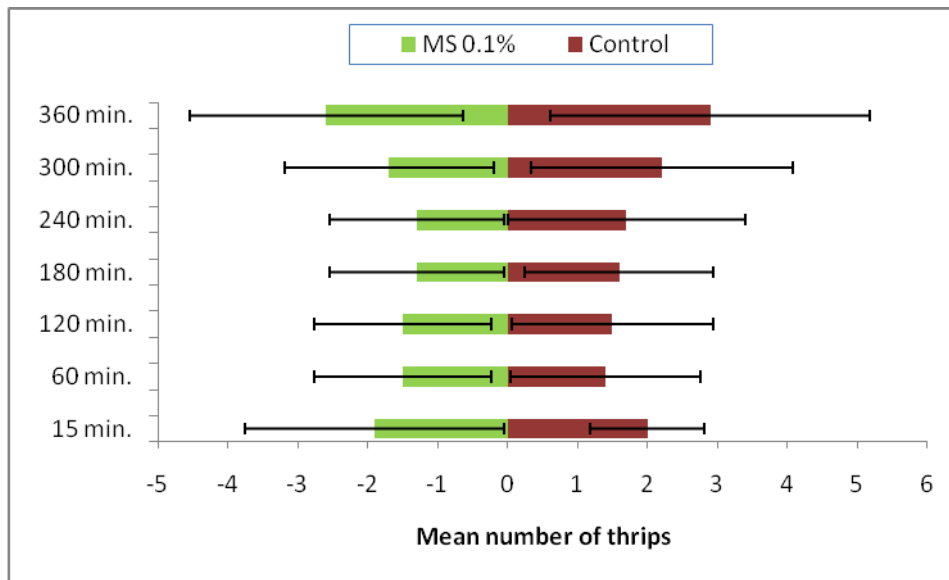


Fig. 15: Settling preference of *T. tabaci* for leek leaf discs treated with methyl salicylate 0.1% or control substance within a 360 min period (mean number of thrips settled  $\pm$  s.e.).

The test substance methyl salicylate at 1% concentration had a significant deterrent effect on thrips after 15, 180 and 300 minutes (Fig. 16).

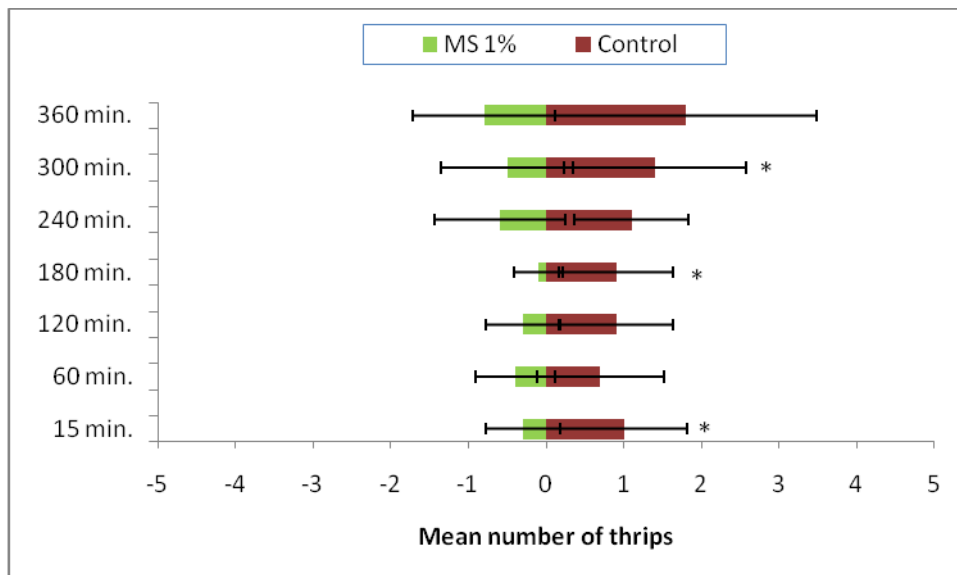


Fig. 16: Settling preference of *T. tabaci* for leek leaf discs treated with methyl salicylate 1% or control substance within a 360 min period (mean number of thrips settled  $\pm$  s.e.). Means are significantly different at  $P \leq 0.05$  (\*).

#### 4.2.2 Feeding Damage

The area of feeding damage caused by *T.tabaci* on the leaf discs treated with the control substance was significantly higher than on the water-treated leaf discs (Fig. 17).

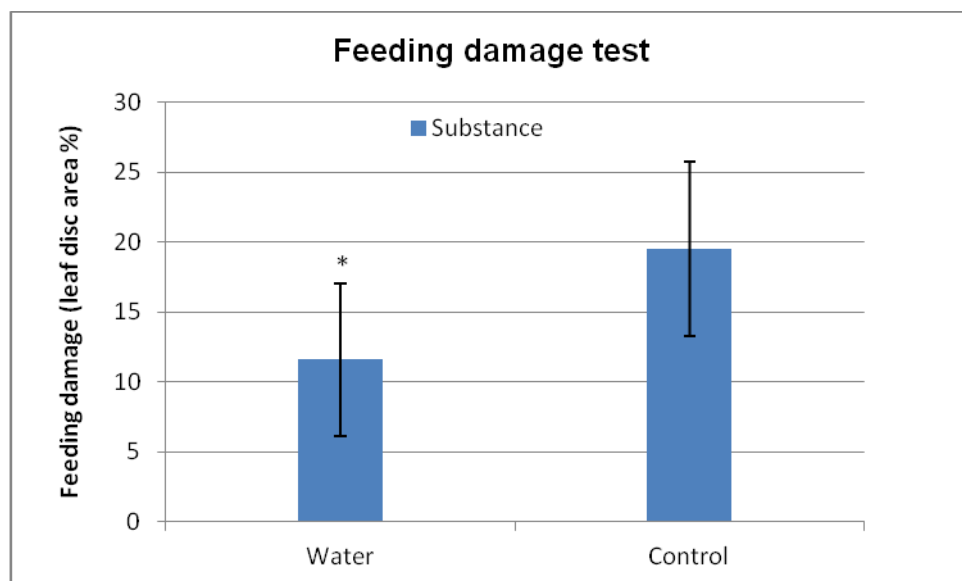


Fig. 17: Feeding damage (mean percentage of damaged leaf area  $\pm$  s.e.) on treated or untreated leek leaf discs caused by *T.tabaci* after 24 hours. Means are significantly different at  $P \leq 0.05$  (\*).

Application of the test substance *cis*-jasmone showed a deterrent effect on the feeding behaviour of *T.tabaci*. The percentage of damaged leaf area caused by thrips feeding was significantly lower on the leaf discs treated with *cis*-jasmone at 0.1% or 1% concentration than on the control leaf discs (Fig. 18).

The treatment of the leaf discs with methyl jasmonate at 0.1% or 1% concentration had no significant deterrent effect on the feeding behaviour of *T.tabaci* (Fig. 18).

No significant reduction of the feeding damage caused by *T.tabaci* occurred after application of the test substance methyl salicylate at 0.1 % or 1% concentration (Fig. 18).

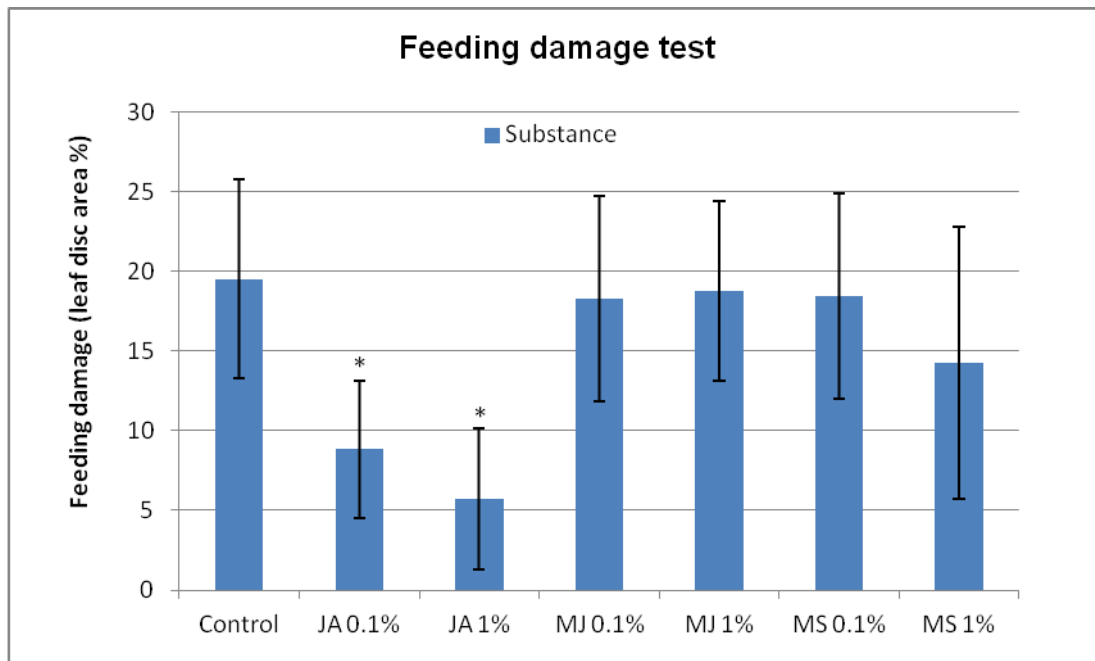


Fig. 18: Feeding damage (mean percentage of damaged leaf area  $\pm$  s.e.) on treated or untreated leek leaf discs caused by *T.tabaci* after 24 hours. Means are significantly different at  $P \leq 0.05$  (\*).

#### 4.2.3 Oviposition Rate Test

In this no-choice experiment neither water nor the control substance applied on the leaf discs had a significant effect on the oviposition activity of *T.tabaci* (Fig. 19).

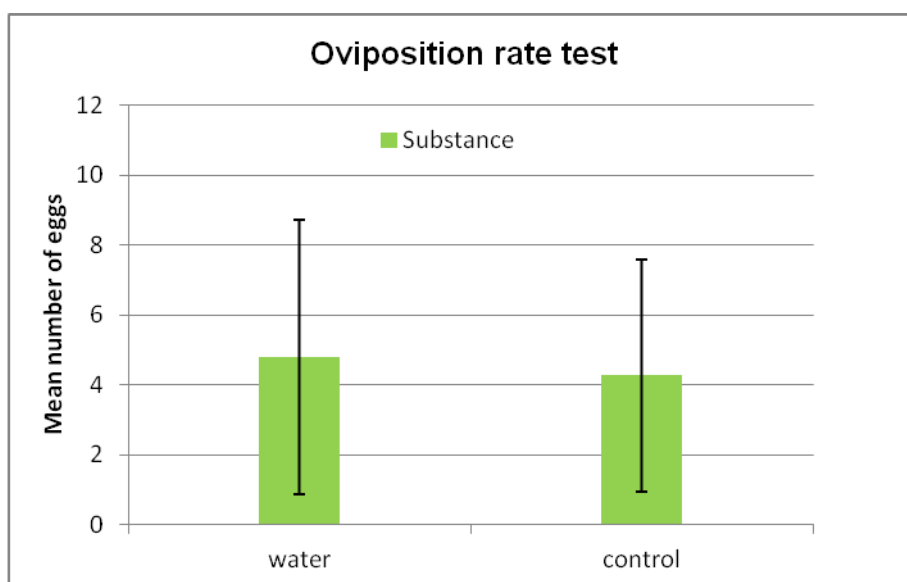


Fig. 19: Mean number ( $\pm$  s.e.) of eggs per *T.tabaci* female counted on treated or untreated leek leaf discs.

Generally, the treatment of leaf discs with test substances had no significant deterrent effect on the oviposition behaviour of *T.tabaci* (Fig. 20).

Application of methyl salicylate at 0.1 % concentration showed significantly higher oviposition rate of thrips on the leaf discs. On the contrary, the treatment with methyl salicylate at 1 % concentration had no significant effect on the oviposition activity of thrips (Fig. 20).

The lowest number of eggs was counted on the leaf discs treated with *cis*-jasmonone at 0.1 % or 1 % concentration, although this effect was not significant (Fig. 20).

The treatment of the leaf discs with methyl jasmonate at 0.1% or 1% concentration had no significant deterrent effect on the oviposition behaviour of *T.tabaci* (Fig. 20).

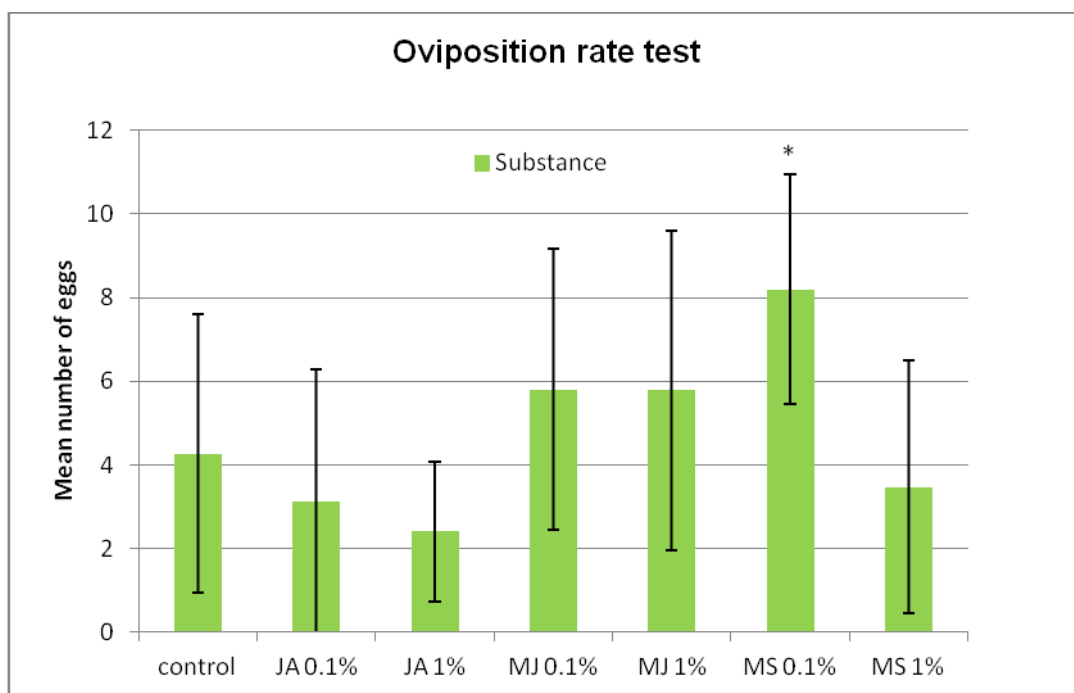


Fig. 20: Mean number ( $\pm$  s.e.) of eggs per *T.tabaci* female counted on treated or untreated leek leaf discs. Means are significantly different at  $P \leq 0.05$  (\*).

## 5 Discussion

Plants produce natural semiochemicals known as herbivore-induced plant volatiles (HIPVs) in response to herbivore attack (Moraes et al., 2009; Simpson et al., 2011). Herbivory may induce plant defences that promote the production of plant volatiles that directly deter herbivores (Howe and Schaller, 2008) or attract natural enemies of herbivores, indirectly protecting the plant via tritrophic interactions (Bruinsma and Dicke, 2008; Qualley and Dudareva, 2008). In this respect, the deployment of synthetic HIPV in crops could force up the biological control of pests (Simpson et al., 2011).

Amongst the herbivore-induced plant volatiles the important three are *cis*-jasmonone (Birkett et al., 2000), methyl jasmonate (Bruinsma and Dicke, 2008) and methyl salicylate (Turlings and Ton, 2006).

*Cis*-jasmonone is emitted naturally from plants after insect damage and can trigger defensive responses via airborne transport (Heil, 2008). It is a catabolite of the stress produced jasmonic acid (Bruce et al., 2003; Bruinsma and Dicke, 2008). Jasmonic acid or its metabolites act as phloem-mobile signals and induce the expression of defence genes in plant tissue (Howe and Schaller, 2008).

Bruce et al. (2008) reported that cotton leaves damaged by *Spodoptera exigua* larvae, cotton buds damaged by *Helicoverpa zea* larvae, *Nicotiana* damaged by *Manduca sexta* larvae and maize plants damaged by *Spodoptera littoralis* emitted *cis*-jasmonone.

*Cis*-jasmonone is well known as an attractant of pollinating insects. It also has been shown to attract the phytophagous Japanese beetle, *Popillia japonica* in field trapping trials (Birkett et al., 2000).

*Cis*-Jasmonone was found to have a repellent effect on the damson-hop aphid *Phorodon humuli* and to act as an attractant on an aphid antagonist, the seven-spot ladybird *Coccinella septempunctata* (Pickett et al., 2006), as well as on the stink bug egg parasitoid *Telenomus podisi* (Moraes et al., 2009). According to Birkett et al. (2000) the attractiveness of *cis*-jasmonone to *Coccinella septempunctata* and the parasitoid *Aphidius ervi* would be expected because stress-related compounds help them to locate herbivores on host plants. Therefore at the third trophic level *cis*-jasmonone can indicate the presence of a herbivorous host.

The present study is the first in which effects of *cis*-jasmonone were tested on *T.tabaci*. Individuals of *T.tabaci* showed no significant olfactory responses to *cis*-jasmonone odour at concentrations ranging from 0.1 % to 10 %. Although there was a non-significant tendency that more thrips chose *cis*-jasmonone odour at 0.1 % concentration. More thrips also chose *cis*-

jasmane odour at the higher concentrations (1% or 10 %), although differences were not significant.

The study results of El-Sayed et al. (2009) demonstrated that *cis*-jasmane acted as an attractant on *Thrips obscuratus*. In comparison with two other thrips attractants (ethyl nicotinate and *p*-anisaldehyde) *cis*-jasmane showed the next highest trap catch of *T. obscuratus* after ethyl nicotinate. It was also observed a smaller number of *T. tabaci* caught in traps baited with *cis*-jasmane in the peach orchard.

In contrast to the olfactory responses, upon direct contact with a *cis*-jasmane-treated plant surface, *T. tabaci* showed a significantly lower settling preference on the leaf discs treated with *cis*-jasmane at 1 % concentration after 2 hours. The feeding damage on leaf discs treated with *cis*-jasmane at 0.1 % and 1 % concentration was also significantly reduced. The lowest damaged area caused by thrips feeding was observed at concentration of 1 %. In the present study the application with *cis*-jasmane had no clear effect on the oviposition activity of *T. tabaci*. Although the lowest non-significant number of eggs was counted on the leaf discs treated with *cis*-jasmane at 0.1 % and 1 % concentration.

Wheat plants sprayed with *cis*-jasmane were less attractive to the grain aphids *Sitobion avenae* but more attractive to their parasitoids in laboratory bioassays. In the field, similar treated plants had lower aphid infestation (Bruce et al., 2003). In another study (with *Arabidopsis* plants), the specialist aphid *Lipaphis erysimi* showed significant attraction to volatiles of *cis*-jasmane-induced plants, suggesting that the specialist insects use different semiochemicals for host plant recognition. In contrast to *L. erysimi* the generalist aphid *Myzus persicae* was significantly repelled by the volatiles of the induced plants (Bruce et al., 2008).

Bruce et al. (2008) suggested that *cis*-jasmane is well suited for use as an artificial inducing agent. *Cis*-jasmane induction would offer the possibility to initiate plant defence against herbivores based on volatile chemical signals without unduly influencing other important plant physiological processes.

The most extensively tested elicitor is methyl jasmonate which is methyl ester of the phytohormone jasmonic acid. This elicitor was shown to play an important role in induced defence in many plant species against a wide range of herbivores (Bruinsma and Dicke, 2008). Jasmonates have a broad spectrum of plant physiological activities, ranging from seed germination to senescence. They also serve important roles as signalling molecules in plant defence, particularly defence against insect herbivores and pathogens (Schaller and Stintzi, 2008). According to Simpson et al. (2011) methyl jasmonate can activate jasmonic acid-dependent defence reactions in neighbouring plants or other parts of the same plant. Methyl jasmonate-induced plants can attract carnivorous arthropods (Bruinsma and Dicke, 2008). In contrast to *cis*-jasmane the treatment with methyl jasmonate can derogate the

development processes of the plant. According to Steppuhn and Baldwin (2008) treatment with methyl jasmonate can inhibit the gene transcription of proteins essential for growth, for example, RubisCO and chlorophyll a/b-binding proteins, and as a consequence photosynthesis is reduced. Sampedro et al. (2010) reported about a significant height growth reduction of *Pinus pinaster* after methyl jasmonate induction compared to control plants.

The proliferation of female spider mite (polyphagous pest *Tetranychus urticae*) on the plants treated with methyl jasmonate was lower than proliferation on control plants (Rohwer and Erwin, 2010). Sampedro et al. (2011) reported that methyl jasmonate induction was effective against the pine weevil *Hylobius abietis*, as induced seedlings were 21 % less damaged than control plants.

In the experiment, performed by Simpson et al. (2011), methyl jasmonate and methyl salicylate mixed with a vegetable oil were tested in three concentrations (0.5 %, 1 % or 2 %) sprayed onto wine grape, broccoli and sweet corn plants. Mainly herbivorous thrips species were attracted to compounds at 0.5 % and 1 %. Their abundance was significantly lower near plants treated with the compounds at 2 % compared with a 0.5 % concentration.

In the present study *T.tabaci* showed no significant olfactory responses to methyl jasmonate at all tested concentrations. In the olfactometer study, conducted by Bruhin (2009), *Frankliniella occidentalis* also showed no significant responses to methyl jasmonate at 0.1 %, 1 % or 10 %. *T.tabaci* individuals showed no significant deterrent responses to methyl jasmonate at 0.1 % or 1 % in the settling preference, feeding damage and oviposition rate bioassays of the present study. These results are in contrast to the findings of Bruhin (2009), who reported that *F.occidentalis* thrips showed low settling and oviposition rates on chrysanthemum leaf discs treated with methyl jasmonate.

Pickett et al. (2006) identified methyl salicylate as a stress-related plant semiochemical, to which the most insects show strong electrophysiological responses. Blande et al. (2010) reported that methyl salicylate is the most distinctive indicator of aphid feeding in the induced VOC blend of the tree species silver birch and black alder, which is in agreement with Zhu and Park (2005), who found methyl salicylate emissions to be a good indicator of aphid feeding on soybean leaves. According to Simpson et al. (2011) methyl salicylate can activate jasmonic acid-dependent defence reactions in neighbouring plants or in other parts of the same plant. Moraes et al (2009) reported about a significantly higher amount of methyl salicylate released by soybean plants 96 h after treatment with *cis*-jasmane.

Furthermore, synthetic methyl salicylate attracted carnivore species, such as predatory mites, lacewings, and mirid bugs, but not the parasitoid wasp *Diadegma semiclausum* (Snoeren et al., 2010). Methyl salicylate deterred females of *Frankliniella occidentalis* from

feeding and egg-laying (Koschier et al., 2007). Blande et al. (2010) reported about aphid-repellent qualities of methyl salicylate.

The results of this study demonstrated that methyl salicylate at 1 % concentration significantly reduced settling preference on the treated leaf discs, which is similar to the results of the settling bioassay with *Frankliniella occidentalis* (Koschier et al., 2007). On the contrary, methyl salicylate at 0.1 % concentration had no significant effects. No significant reduction of the feeding damage caused by *T.tabaci* occurred after application of methyl salicylate. The treatment with methyl salicylate (only at 1 %) significantly reduced the damaged area caused by feeding of *F.occidentalis* (Koschier et al., 2007). In the olfactometer bioassay *T.tabaci* showed no significant olfactory responses to methyl salicylate odour at all tested concentrations. Methyl salicylate at 0.1 % concentration, but not at 1 % concentration, incited *T.tabaci* to lay significantly more eggs on the treated leaf discs. In the study of Koschier et al. (2007) an application of methyl salicylate at 1 % concentration, but not at 0.1 % concentration, on bean and cucumber leaf discs significantly prevented *F.occidentalis* from oviposition. This can confirm the assumption that the thrips responses depend on substances concentration. Previous studies on *F.occidentalis* indicated that the plant odour concentration is crucial for its attractiveness or repellence (Koschier et al., 2000; Koschier et al., 2002; Bruhin 2009). According to Qualley and Dudareva (2008) methyl salicylate attracted predatory mites in a dose-dependent manner.

Finally it has to be noted that in this study the control substance had a significant deterrent effect on the settling preference of *T.tabaci* after four hours and for the rest of the observation period. A similar deterrent effect (only after two and six hours) was observed by Bruhin (2009) in the settling preference test with *Frankliniella occidentalis*. In the oviposition rate bioassay neither water nor the control substance applied on the leaf discs had a significant effect on the oviposition activity of *T.tabaci*. On the contrary, the area of feeding damage caused by *T.tabaci* on the leaf discs treated with the control substance was significantly higher than on the water-treated leaf discs. In contrast, no such deterrent effects of water were observed in the feeding damage test with *F.occidentalis* (Bruhin, 2009). This effect might occur because of the position of the Petri dishes with thrips in the climate chamber. The non-homogeneous distribution of luminous intensity might affect the feeding behaviour of *T.tabaci*.



In conclusion, the results of the present study indicate the potential for *cis*-jasmonone or methyl salicylate as deterrents to settling and feeding of *T.tabaci* on leek plants. The application of *cis*-jasmonone prevented the settling as well as the feeding of thrips. Also the treatment with methyl salicylate had a significant deterrent effect on the settling preference of *T.tabaci*.

Further studies under the field or greenhouse conditions are needed to investigate the defence potential of *cis*-jasmonone or methyl salicylate against the herbivore attack of *T.tabaci* for using in the sustainable pest management as well as to determine how would the natural enemies of *T.tabaci* response to these substances. If effective in the field or greenhouse, these volatiles could be important components of an integrated pest management program against *T.tabaci* reducing insecticide applications.

## 6 Summary

In this study the effects of three plant volatiles *cis*-jasmone, methyl jasmonate and methyl salicylate on behavioural responses of the onion thrips *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) were evaluated. The substances were tested at a range of concentrations (0.1 % - 10 %) depending on the bioassay.

The olfactory responses of *T.tabaci* were tested using a glass Y-tube olfactometer with the test substance odours at 0.1 %, 1 % or 10 % concentration.

*T.tabaci* showed no significant repellent olfactory responses to all test substance odours.

The contact chemoreceptory responses of *T.tabaci* were tested on the leek (*Allium ampeloprasum* var. *porrum*) leaf discs treated with the test substances at 0.1 % or 1 % concentration. The settling preference (a choice test) of *T.tabaci* was tested within a 6-hour period. The oviposition rate and the feeding damage were observed in no-choice tests after a 24-hour period. For the oviposition rate bioassay synchronized females (at the same age) of *T.tabaci* were used.

The results of the study indicate that the application of *cis*-jasmone at 1 % concentration after 2 hours had a significant deterrent effect on the settling preference of *T.tabaci*. The feeding damage was significantly reduced when *cis*-jasmone at 0.1 % or 1 % concentration was applied on the leek leaf discs. The oviposition rate was not significantly affected by the treatment with *cis*-jasmone.

The application of methyl salicylate at 1 % concentration after 15, 180 and 300 minutes had a significant deterrent effect on the settling preference of *T.tabaci*. The oviposition rate was influenced by the treatment with methyl salicylate at 0.1 %. The feeding damage was not significantly reduced by the treatment with methyl salicylate.

*T.tabaci* individuals showed no significant deterrent responses to methyl jasmonate at 0.1 % or 1 % in the settling preference, feeding damage and oviposition rate bioassays.

The results obtained in the present study were discussed with the results from similar studies. Further field-based studies are needed to investigate the defence potential of *cis*-jasmone or methyl salicylate against *T.tabaci*.

## 7 References

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## 8 Annex Data Tables

Olfactometer Test Protocol								
Thrips species: <i>Thrips tabaci</i>								
Test substance: <i>Cis</i> -Jasmone (JA)			Concentration: <b>0,1 %</b>			Amount: 1 µl		
Control substance: Paraffin oil								
Series:		1		2		3		
Nr.		JA	Control	JA	Control	JA	Control	
1		x			x	x		
2		x		x			x	
3		x		x		x		
4			x		x		x	
5		x		x		x		
6		x		x		x		
7		x		x			x	
8			x		x	x		
9			x		x	x		
10		x		x			x	
11			x		x		x	
12		x		x		x		
13		x			x		x	
14		x		x			x	
15		x			x	x		
16		x		x			x	
17		x		x		x		
18			x		x		x	
19		x		x		x		
20		x		x		x		
Total:		15	5	12	8	11	9	
Percentage:		75	25	60	40	55	45	
						JA	Control	
Total:						38	22	



Olfactometer Test Protocol									
Thrips species: <i>Thrips tabaci</i>									
Test substance: <i>Cis</i> -Jasmone (JA)				Concentration: <b>1 %</b>			Amount: 1 µl		
Control substance: Paraffin oil									
Series:		1		2		3			
Nr.		JA	Control	JA	Control	JA	Control		
1			x	x			x		
2		x			x	x			
3		x		x		x			
4			x	x		x			
5			x	x			x		
6			x		x	x			
7		x		x			x		
8		x			x		x		
9		x			x	x			
10			x	x		x			
11		x			x		x		
12		x		x			x		
13		x			x	x			
14		x		x		x			
15			x	x		x			
16		x		x			x		
17		x		x			x		
18		x		x			x		
19		x			x	x			
20			x	x			x		
Total:		13	7	13	7	10	10		
Percentage:		65	35	65	35	50	50		
							JA	Control	
Total:							36	24	

Olfactometer Test Protocol								
Thrips species: <i>Thrips tabaci</i>								
Test substance: <i>Cis</i> -Jasmone (JA)				Concentration: <b>10 %</b>		Amount: 1 µl		
Control substance: Paraffin oil								
Series:		1		2		3		
Nr.		JA	Control	JA	Control	JA	Control	
1		x		x		x		
2		x		x			x	
3			x	x		x		
4		x			x	x		
5		x			x	x		
6		x			x	x		
7			x		x		x	
8			x	x			x	
9		x			x	x		
10			x	x		x		
11			x		x		x	
12		x		x		x		
13			x		x		x	
14		x		x		x		
15		x			x		x	
16			x	x			x	
17		x		x		x		
18			x	x			x	
19			x		x	x		
20		x		x		x		
Total:		11	9	11	9	12	8	
Percentage:		55	45	55	45	60	40	
						JA	Control	
						34	26	

Olfactometer Test Protocol									
Thrips species: <i>Thrips tabaci</i>									
Test substance: Methyl jasmonate (MJ)				Concentration: <b>0,1 %</b>		Amount: 1 µl			
Control substance: Paraffin oil									
Series:		1		2		3			
Nr.		MJ	Control		MJ	Control		MJ	Control
1		x			x			x	
2			x		x			x	
3		x			x			x	
4		x				x			x
5			x		x				x
6			x		x				x
7			x			x			x
8			x			x		x	
9		x				x		x	
10			x		x			x	
11			x			x		x	
12		x			x				x
13		x				x			x
14			x		x			x	
15		x				x		x	
16		x			x			x	
17			x		x				x
18		x				x		x	
19			x		x				x
20		x				x			x
Total:		10	10		11	9		11	9
Percentage:		50	50		55	45		55	45
							MJ	Control	
Total:							32	28	

Olfactometer Test Protocol									
Thrips species: <i>Thrips tabaci</i>									
Test substance: Methyl jasmonate (MJ)			Concentration: <b>1 %</b>			Amount: 1 µl			
Control substance: Paraffin oil									
Series:		1		2		3			
Nr.		MJ	Control		MJ	Control		MJ	Control
1			x			x			x
2		x			x			x	
3			x			x		x	
4		x			x				x
5			x			x		x	
6		x			x			x	
7			x		x				x
8			x			x			x
9			x		x				x
10		x			x				x
11		x			x				x
12		x				x			x
13		x				x			x
14		x				x		x	
15			x			x		x	
16			x		x				x
17		x			x				x
18		x				x			x
19			x			x		x	
20		x			x				x
Total:		11	9		10	10		7	13
Percentage:		55	45		50	50		35	65
								MJ	Control
						Total:		28	32

Olfactometer Test Protocol									
Thrips species: <i>Thrips tabaci</i>									
Test substance: Methyl jasmonate (MJ)				Concentration: <b>10 %</b>		Amount: 1 µl			
Control substance: Paraffin oil									
Series:		1		2		3			
Nr.		MJ	Control		MJ	Control		MJ	Control
1			x		x			x	
2			x			x		x	
3			x			x			x
4			x			x			x
5		x				x			x
6		x			x			x	
7			x			x			x
8			x			x		x	
9			x		x				x
10			x			x		x	
11			x		x				x
12			x		x			x	
13		x				x			x
14			x			x			x
15		x			x			x	
16			x			x		x	
17		x				x			x
18			x			x			x
19		x				x		x	
20			x		x				x
Total:		6	14		7	13		9	11
Percentage:		30	70		35	65		45	55
							MJ	Control	
Total:							22	38	

Olfactometer Test Protocol								
Thrips species: <i>Thrips tabaci</i>								
Test substance: Methyl salicylate (MS)				Concentration: <b>0,1 %</b>		Amount: 1 µl		
Control substance: Paraffin oil								
Series:		1		2		3		
Nr.		MS	Control	MS	Control	MS	Control	
1		x		x		x		
2		x			x		x	
3		x			x	x		
4		x			x		x	
5			x		x		x	
6		x		x		x		
7		x		x		x		
8			x		x	x		
9			x	x			x	
10		x		x		x		
11			x		x	x		
12		x		x		x		
13		x			x		x	
14			x	x		x		
15		x			x	x		
16		x			x		x	
17		x		x		x		
18		x			x		x	
19			x	x			x	
20		x		x		x		
Total:		14	6	10	10	12	8	
Percentage:		70	30	50	50	60	40	
						MS	Control	
					Total:	36	24	

Olfactometer Test Protocol								
Thrips species: <i>Thrips tabaci</i>								
Test substance: Methyl salicylate (MS)				Concentration: <b>1 %</b>			Amount: 1 µl	
Control substance: Paraffin oil								
Series:		1		2		3		
Nr.		MS	Control	MS	Control	MS	Control	
1			x		x	x		
2		x			x	x		
3		x			x		x	
4		x			x	x		
5		x			x		x	
6		x		x			x	
7		x			x	x		
8			x	x		x		
9		x		x			x	
10			x	x		x		
11		x		x			x	
12			x		x	x		
13		x		x			x	
14		x		x		x		
15		x		x			x	
16		x		x		x		
17		x			x	x		
18		x		x		x		
19			x		x		x	
20			x	x		x		
Total:		14	6	11	9	12	8	
Percentage:		70	30	55	45	60	40	
						MS	Control	
Total:						37	23	

Olfactometer Test Protocol								
Thrips species: <i>Thrips tabaci</i>								
Test substance: Methyl salicylate (MS)				Concentration: <b>10 %</b>			Amount: 1 µl	
Control substance: Paraffin oil								
Series:		1		2		3		
Nr.		MS	Control	MS	Control	MS	Control	
1		x			x	x		
2		x		x		x		
3			x	x			x	
4		x			x		x	
5			x	x			x	
6			x		x	x		
7			x		x	x		
8		x		x			x	
9			x	x			x	
10		x			x	x		
11			x		x	x		
12		x		x			x	
13		x		x		x		
14			x	x			x	
15		x		x		x		
16		x		x		x		
17			x	x		x		
18			x	x			x	
19		x		x		x		
20			x	x		x		
Total:		10	10	14	6	12	8	
Percentage:		50	50	70	30	60	40	
						MS	Control	
Total:						36	24	



Settling Preference								
Species	Thrips tabaci				Plant	leek (Allium porrum L.)		
Treatment	cis -Jasmone 0,1 %				Nr. individuals / unit		10	
15 min								
rep.	treat	control	elsewhere					
1	1	1	8					
2	1	1	8					
3	0	1	9					
4	1	1	8					
5	2	1	7					
60 min					240 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	1	9		1	0	1	9
2	2	1	7		2	2	1	7
3	0	1	9		3	0	0	10
4	1	2	7		4	1	2	7
5	2	1	7		5	2	0	8
120 min.					300 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	1	9		1	0	1	9
2	2	1	7		2	2	2	6
3	0	0	10		3	0	0	10
4	1	2	7		4	2	2	6
5	2	0	8		5	2	1	7
180 min.					360 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	1	9		1	1	1	8
2	2	1	7		2	1	3	6
3	0	0	10		3	1	0	9
4	1	2	7		4	2	2	6
5	2	0	8		5	2	1	7

Settling Preference								
Species	Thrips tabaci				Plant	leek (Allium porrum L.)		
Treatment	cis -Jasmone 0,1 %				Nr. individuals / unit		10	
15 min								
rep.	treat	control	elsewhere					
1	0	1	9					
2	2	0	8					
3	2	5	3					
4	0	3	7					
5	2	2	6					
60 min					240 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	1	9		1	1	1	8
2	0	0	10		2	2	0	8
3	2	5	3		3	0	5	5
4	0	3	7		4	0	4	6
5	2	1	7		5	4	1	5
120 min.					300 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	0	10		1	3	1	6
2	1	0	9		2	5	0	5
3	1	5	4		3	0	6	4
4	0	3	7		4	2	5	3
5	4	1	5		5	3	1	6
180 min.					360 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	0	10		1	4	1	5
2	2	0	8		2	6	2	2
3	0	5	5		3	0	9	1
4	0	3	7		4	2	5	3
5	4	1	5		5	3	2	5

Settling Preference								
Species	Thrips tabaci				Plant	leek (Allium porrum L.)		
Treatment	cis -Jasmone 1 %				Nr. individuals / unit		10	
15 min								
rep.	treat	control	elsewhere					
1	0	4	6					
2	0	4	6					
3	0	5	5					
4	0	3	7					
5	2	0	8					
60 min					240 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	5	5		1	0	6	4
2	0	4	6		2	0	4	6
3	0	4	6		3	0	4	6
4	0	4	6		4	0	3	7
5	2	3	5		5	1	3	6
120 min.					300 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	5	5		1	0	6	4
2	0	4	6		2	0	4	6
3	0	4	6		3	0	6	4
4	0	4	6		4	0	5	5
5	2	3	5		5	1	3	6
180 min.					360 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	5	5		1	0	7	3
2	0	4	6		2	0	5	5
3	0	4	6		3	1	7	2
4	0	4	6		4	0	6	4
5	1	3	6		5	2	3	5

Settling Preference								
Species	Thrips tabaci				Plant	leek (Allium porrum L.)		
Treatment	cis -Jasmone 1 %				Nr. individuals / unit		10	
15 min								
rep.	treat	control	elsewhere					
1	1	1	8					
2	0	3	7					
3	3	0	7					
4	0	0	10					
5	1	1	8					
60 min					240 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	1	1	8		1	1	1	8
2	0	4	6		2	0	3	7
3	3	0	7		3	2	1	7
4	1	0	9		4	1	1	8
5	1	1	8		5	1	2	7
120 min.					300 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	1	1	8		1	1	1	8
2	0	4	6		2	0	3	7
3	2	0	8		3	2	1	7
4	1	0	9		4	0	2	8
5	1	2	7		5	1	2	7
180 min.					360 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	1	1	8		1	0	3	7
2	0	3	7		2	0	3	7
3	2	0	8		3	3	2	5
4	1	1	8		4	1	2	7
5	1	2	7		5	1	2	7

Settling Preference								
Species	Thrips tabaci				Plant	leek (Allium porrum L.)		
Treatment	Methyl jasmonate 0.1%				Nr. individuals / unit		10	
15 min								
rep.	treat	control	elsewhere					
1	0	1	9					
2	0	1	9					
3	0	2	8					
4	0	0	10					
5	1	0	9					
60 min					240 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	1	9		1	0	0	10
2	0	0	10		2	0	1	9
3	0	3	7		3	2	0	8
4	0	1	9		4	0	0	10
5	0	0	10		5	1	0	9
120 min.					300 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	1	9		1	0	0	10
2	0	0	10		2	0	0	10
3	1	3	6		3	1	1	8
4	0	0	10		4	1	0	9
5	0	0	10		5	3	0	7
180 min.					360 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	2	1	7		1	0	1	9
2	0	1	9		2	1	0	9
3	2	1	7		3	0	1	9
4	0	0	10		4	1	1	8
5	1	0	9		5	3	0	7

Settling Preference									
Species	Thrips tabaci				Plant	leek (Allium porrum L.)			
Treatment	Methyl jasmonate 0.1%				Nr. individuals / unit		10		
15 min									
rep.	treat	control	elsewhere						
1	2	1	7						
2	2	2	6						
3	2	1	7						
4	1	2	7						
5	3	3	4						
60 min					240 min.				
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere	
1	2	1	7		1	2	2	6	
2	2	1	7		2	2	1	7	
3	1	2	7		3	0	4	6	
4	1	2	7		4	3	2	5	
5	3	3	4		5	3	4	3	
120 min.					300 min.				
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere	
1	2	2	6		1	2	3	5	
2	2	1	7		2	1	2	7	
3	0	3	7		3	1	5	4	
4	1	2	7		4	1	2	7	
5	3	3	4		5	2	5	3	
180 min.					360 min.				
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere	
1	2	2	6		1	3	3	4	
2	2	1	7		2	3	1	6	
3	0	4	6		3	1	5	4	
4	2	2	6		4	2	3	5	
5	2	4	4		5	2	5	3	

Settling Preference								
Species	Thrips tabaci				Plant	leek (Allium porrum L.)		
Treatment	Methyl jasmonate 1 %				Nr. individuals / unit		10	
15 min								
rep.	treat	control	elsewhere					
1	0	1	9					
2	0	1	9					
3	0	0	10					
4	0	1	9					
5	0	2	8					
60 min					240 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	0	10		1	0	0	10
2	0	1	9		2	0	2	8
3	0	1	9		3	2	0	8
4	0	3	7		4	2	6	2
5	0	1	9		5	0	0	10
120 min.					300 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	0	10		1	0	1	9
2	0	1	9		2	0	2	8
3	0	1	9		3	2	1	7
4	0	4	6		4	2	6	2
5	0	1	9		5	0	0	10
180 min.					360 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	0	10		1	0	0	10
2	0	2	8		2	0	2	8
3	1	0	9		3	2	1	7
4	3	6	1		4	3	3	4
5	0	0	10		5	1	0	9

Settling Preference								
Species	Thrips tabaci				Plant	leek (Allium porrum L.)		
Treatment	Methyl jasmonate 1 %				Nr. individuals / unit		10	
15 min								
rep.	treat	control	elsewhere					
1	1	0	9					
2	0	2	8					
3	0	1	9					
4	0	4	6					
5	1	0	9					
60 min					240 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	2	0	8		1	2	0	8
2	1	3	6		2	0	4	6
3	0	1	9		3	0	1	9
4	0	4	6		4	1	5	4
5	0	0	10		5	0	0	10
120 min.					300 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	2	0	8		1	3	0	7
2	1	3	6		2	2	4	4
3	0	1	9		3	0	1	9
4	0	4	6		4	0	5	5
5	0	0	10		5	0	3	7
180 min.					360 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	2	0	8		1	3	0	7
2	0	4	6		2	1	4	5
3	1	1	8		3	2	3	5
4	1	4	5		4	0	5	5
5	1	0	9		5	0	2	8



Settling Preference								
Species	Thrips tabaci				Plant	leek (Allium porrum L.)		
Treatment	Methyl salicylate 0.1%				Nr. individuals / unit		10	
15 min								
rep.	treat	control	elsewhere					
1	0	3	7					
2	4	2	4					
3	0	2	8					
4	2	3	5					
5	1	1	8					
60 min					240 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	1	3	6		1	0	2	8
2	2	1	7		2	2	0	8
3	0	2	8		3	0	2	8
4	3	4	3		4	2	6	2
5	0	1	9		5	1	1	8
120 min.					300 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	1	2	7		1	0	1	9
2	2	1	7		2	3	2	5
3	0	2	8		3	0	4	6
4	3	5	2		4	1	6	3
5	0	1	9		5	3	0	7
180 min.					360 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	1	2	7		1	1	4	5
2	2	1	7		2	4	2	4
3	0	2	8		3	0	7	3
4	2	5	3		4	2	6	2
5	0	1	9		5	4	0	6

Settling Preference								
Species	Thrips tabaci				Plant	leek (Allium porrum L.)		
Treatment	Methyl salicylate 0.1%				Nr. individuals / unit		10	
15 min								
rep.	treat	control	elsewhere					
1	5	1	4					
2	4	2	4					
3	0	2	8					
4	2	3	5					
5	1	1	8					
60 min					240 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	3	1	6		1	3	1	6
2	1	0	9		2	0	2	8
3	3	0	7		3	3	0	7
4	0	2	8		4	0	2	8
5	2	0	8		5	2	1	7
120 min.					300 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	3	1	6		1	4	3	3
2	1	0	9		2	0	2	8
3	3	0	7		3	3	0	7
4	0	2	8		4	1	3	6
5	2	1	7		5	2	1	7
180 min.					360 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	3	1	6		1	6	2	2
2	0	1	9		2	0	2	8
3	3	0	7		3	3	0	7
4	0	2	8		4	4	3	3
5	2	1	7		5	2	3	5

Settling Preference								
Species	Thrips tabaci				Plant	leek (Allium porrum L.)		
Treatment	Methyl salicylate 1%				Nr. individuals / unit		10	
15 min								
rep.	treat	control	elsewhere					
1	0	1	9					
2	1	2	7					
3	1	1	8					
4	0	0	10					
5	0	1	9					
60 min					240 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	1	9		1	0	1	9
2	1	0	9		2	2	1	7
3	1	0	9		3	1	1	8
4	0	0	10		4	0	0	10
5	1	1	8		5	0	1	9
120 min.					300 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	1	9		1	0	1	9
2	1	0	9		2	2	1	7
3	0	1	9		3	0	1	9
4	0	0	10		4	0	0	10
5	1	1	8		5	0	2	8
180 min.					360 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	1	9		1	0	2	8
2	0	0	10		2	2	1	7
3	0	1	9		3	0	1	9
4	0	0	10		4	1	0	9
5	0	1	9		5	2	2	6

Settling Preference								
Species	Thrips tabaci				Plant	leek (Allium porrum L.)		
Treatment	Methyl salicylate 1%				Nr. individuals / unit		10	
15 min								
rep.	treat	control	elsewhere					
1	0	2	8					
2	0	1	9					
3	0	0	10					
4	0	0	10					
5	1	2	7					
60 min					240 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	2	8		1	0	2	8
2	0	1	9		2	1	2	7
3	0	0	10		3	0	1	9
4	0	0	10		4	0	0	10
5	1	2	7		5	2	2	6
120 min.					300 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	2	8		1	0	2	8
2	0	1	9		2	1	2	7
3	0	1	9		3	0	1	9
4	0	0	10		4	0	0	10
5	1	2	7		5	2	4	4
180 min.					360 min.			
rep.	treat	control	elsewhere		rep.	treat	control	elsewhere
1	0	2	8		1	0	2	8
2	0	1	9		2	1	2	7
3	0	1	9		3	0	2	8
4	0	0	10		4	0	0	10
5	1	2	7		5	2	6	2

<b>Settling Preference</b>								
<b>Species</b>	<i>Thrips tabaci</i>			<b>Plant</b>	leek ( <i>Allium porrum</i> L.)			
<b>Treatment</b>	<b>Control substance</b>			<b>Nr. individuals / unit</b>	10			
<b>15 min</b>								
rep.	treat	control	elsewhere					
1	0	0	10					
2	3	0	7					
3	0	1	9					
4	3	1	6					
5	1	2	7					
<b>60 min</b>				<b>240 min.</b>				
rep.	treat	control	elsewhere	rep.	treat	control	elsewhere	
1	1	1	8	1	1	1	8	
2	4	0	6	2	3	1	6	
3	0	1	9	3	0	1	9	
4	4	1	5	4	6	0	4	
5	1	2	7	5	1	4	5	
<b>120 min.</b>				<b>300 min.</b>				
rep.	treat	control	elsewhere	rep.	treat	control	elsewhere	
1	1	0	9	1	2	2	6	
2	4	0	6	2	3	1	6	
3	0	1	9	3	0	2	8	
4	5	0	5	4	6	0	4	
5	1	2	7	5	1	5	4	
<b>180 min.</b>				<b>360 min.</b>				
rep.	treat	control	elsewhere	rep.	treat	control	elsewhere	
1	1	0	9	1	3	3	4	
2	4	0	6	2	4	1	5	
3	0	1	9	3	0	4	6	
4	5	0	5	4	6	0	4	
5	1	3	6	5	2	5	3	

<b>Settling Preference</b>								
<b>Species</b>	<i>Thrips tabaci</i>			<b>Plant</b>	leek ( <i>Allium porrum</i> L.)			
<b>Treatment</b>	<b>Control substance</b>			<b>Nr. individuals / unit</b>	10			
<b>15 min</b>								
rep.	treat	control	elsewhere					
1	2	2	6					
2	3	1	6					
3	5	1	4					
4	7	0	3					
5	4	0	6					
<b>60 min</b>				<b>240 min.</b>				
rep.	treat	control	elsewhere	rep.	treat	control	elsewhere	
1	2	2	6	1	3	2	5	
2	3	1	6	2	2	0	8	
3	5	1	4	3	5	2	3	
4	7	0	3	4	7	0	3	
5	4	0	6	5	5	0	5	
<b>120 min.</b>				<b>300 min.</b>				
rep.	treat	control	elsewhere	rep.	treat	control	elsewhere	
1	2	2	6	1	3	2	5	
2	2	0	8	2	3	0	7	
3	5	2	3	3	5	2	3	
4	7	0	3	4	8	0	2	
5	5	0	5	5	5	0	5	
<b>180 min.</b>				<b>360 min.</b>				
rep.	treat	control	elsewhere	rep.	treat	control	elsewhere	
1	2	2	6	1	6	2	2	
2	2	0	8	2	3	0	7	
3	5	2	3	3	5	2	3	
4	7	0	3	4	8	1	1	
5	6	0	4	5	5	2	3	

### Oviposition Rate Bioassay

Series	water	control	MS 0,1%	MS 1%	MJ 0,1%	MJ 1%	CJ 0,1%	CJ 1%
1	7	1	10	4	9	6	7	3
2	2	0	12	3	7	5	4	3
3	5	4	7	8	8	6	2	1
4	3	5	11	8	9	0	7	2
5	7	7	8	1	9	3	8	6
6	9	7	11	6	6	0	7	1
7	0	6	2	8	10	5	1	4
8	13	10	10	2	6	6	0	1
9	6	6	9	0	8	7	0	4
10	8	0	10	4	1	6	0	3
11	0	5	5	0	0	0	6	4
12	5	1	9	0	5	5	0	0
13	0	3	6	5	3	12	1	0
14	0	9	5	0	0	9	0	2
15	7	0	8	3	6	12	4	2
16						10		
17						2		
18						10		
mean	4,80	4,27	8,20	3,47	5,80	5,78	3,13	2,40
stdev	3,91	3,33	2,76	3,02	3,36	3,83	3,14	1,68
s.e.	0,78	0,67	0,55	0,60	0,67	0,77	0,63	0,34

Feeding Damaged Area on Leek Leaf Discs								
	Water	Control	JA 0.1%	JA 1%	MS 0.1%	MS 1%	MJ 0.1%	MJ 1%
Leaf disc number	damage area (%)	damage area (%)	damage area (%)	damage area (%)	damage area (%)	damage area (%)	damage area (%)	damage area (%)
1	11,61	19,50	8,82	5,70	18,44	14,26	18,29	18,77
2	15,92	20,90	14,93	0,00	3,98	3,23	19,40	8,96
3	19,65	19,65	2,49	1,99	25,37	6,47	18,91	22,89
4	16,67	12,44	10,45	5,72	11,69	7,21	19,15	25,37
5	0,00	23,13	2,74	5,72	23,13	9,45	9,95	18,91
6	15,67	23,13	5,72	0,50	13,43	0,00	8,96	13,68
7	15,42	1,00	4,73	3,73	21,64	15,42	12,19	16,92
8	10,70	17,91	7,46	15,92	20,15	20,65	25,62	26,87
9	4,23	24,13	11,69	9,95	20,65	11,19	26,12	17,91
10	8,46	19,90	8,21	6,47	24,13	21,39	25,62	12,94
11	11,69	22,14	4,48	4,48	18,41	7,21	18,66	16,17
12	6,97	7,71	8,46	0,50	17,66	21,39	16,67	27,86
13	5,97	18,91	17,41	9,20	15,67	28,61	24,88	25,37
14	14,43	15,67	16,92	3,48	24,88	21,14	19,65	20,65
15	14,43	25,87	6,97	7,46	16,67	24,63	5,97	11,44
16	13,93	21,64	12,19	10,45	28,11	15,92	22,64	16,17
17		20,15	6,47		9,45			15,42
18		21,89	8,46					21,64
19		25,12	8,96					
20		23,88	8,96					
21		24,88						
mean	11,61	19,50	8,82	5,70	18,44	14,26	18,29	18,77
stdev	5,44	6,23	4,30	4,41	6,47	8,56	6,46	5,61
s.e.	1,09	1,25	0,86	0,88	1,29	1,71	1,29	1,12