Role of Semiochemicals in Host Finding, Oviposition and Sexual Communication in Guatemalan Potato Moth *Tecia solanivora*

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Doctoral Thesis
Swedish University of Agricultural Sciences
Alnarp 2011

Acta Universitatis agriculturae Sueciae
2011:55

Cover: *Tecia solanivora* on a potato flower
(photo: M.F. Karlsson)

ISSN 1652-6880
ISBN 978-91-576-7599-6

Print: Repro, Alnarp 2011

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Abstract

Semiochemicals are important cues in the interaction between plant and insects and between conspecific insects. Volatile compounds emitted by plants provide herbivorous insects with cues for host finding, selection and discrimination. In moths, female emitted sex pheromones enable conspecific males to find them for mating. This thesis investigated the role of semiochemicals in the behaviour of the Guatemalan potato moth *Tecia solanivora* (Lepidoptera: Gelechiidae), a pest insect of potato.

Identification of odours of foliage, flowers, and tubers of potato, *Solanum tuberosum,* were done with coupled gas chromatography-mass spectrometry and with high-performance liquid chromatography for non-volatile compounds in tubers. Antennal activity of potato volatiles was tested with electroantennographic recordings. Attraction of *T. solanivora* to potato volatile compounds was investigated through olfactometer, wind tunnel and field bioassays. Male behavior towards two different synthetic pheromone blends was similarly tested, to clarify their mode of action in mating disruption management.

Potato emits structure-specific volatile blends that change during the development of the plant. Tuberization stage was the preferred stage for oviposition while foliage released deterrent compounds. A three-component flower-odour mimic attracted males and females, virgin and mated, and enhanced the number of eggs laid. Female oviposition in the soil, during the tuberization stage might be guided by odours from spatially separated flowers, as an indication of suitable host vicinity. Larval survival was low in tubers with high concentrations of glycoalkaloids. This study demonstrates that odours from qualitatively different sites guide female to oviposit on tubers with high suitability for larval performance. Mating disruption was obtained with pheromone-permeated air with the two blends, but the disruption mechanism were different between them.

This first study on chemical communication between *T. solanivora* and its host plant showed that potato volatile compounds are perceived by the moth and act as cues in host location and oviposition. It highlights the possibility of using semiochemicals to manipulate the behavior of the moths and provides a base for further investigation and development of odour-based pest management.

Keywords: Gelechiidae, *Solanum tuberosum*, electrophysiology, mating disruption, chemical analysis, host-plant cues, behaviour, attraction

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Dedication

To the farmers of the world. What would we be without you?

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Karlsson M.F., Birgersson G., Cotes A.M., Bosa C.F., Bengtsson M., Witzgall P. (2009). Plant odor analysis of potato: response of Guatemalan moth to above- and belowground potato volatiles. *Journal of Agricultural Food and Chemistry* 57(13), 5903-5909

II Karlsson Miriam Frida, Göran Birgersson, Peter Witzgall, Jonas D. Stevens-Lekfeldt, Nimal Punyasiri and Marie Bengtsson Guatemalan potato moth *Tecia solanivora* distinguish odour profiles from qualitatively different potatoes *Solanum tuberosum* L. (manuscript)

III Karlsson Miriam Frida, Magali Proffit, and Göran Birgersson. Flower volatiles assist host-plant location in the Guatemalan potato moth *Tecia solanivora* (manuscript)

IV Clavijo Mc Cormick Andrea, Miriam Frida Karlsson, Carlos Felipe Bosa Ochoa, Magali Proffit, Maria Victoria Zuluaga, Alba Marina Cotez Prado, and Peter Witzgall. Mechanisms of mating disruption for two synthetic pheromone blends of the Guatemalan potato moth *Tecia solanivora* (Povolny) (manuscript)

The contribution of Miriam Frida Karlsson to the papers included in this thesis were as follows:

I Carried out odour collection, chemical identification and analysed data. Did part of behavioural assays. Wrote the paper in collaboration with co-authors.

II Carried out chemical identification of volatile compounds. Performed bioassays. Analysed data. Wrote the paper in collaboration with co-authors.

III Carried out olfactory and behavioural assays. Analysed data and wrote the paper in collaboration with co-authors.

IV Performed part of fieldwork and part of wind tunnel assays. Did part of analysis and writing.

Objectives

The objective of this doctoral thesis was to elucidate the role of semiochemicals in the behaviour of the nocturnal Guatemalan potato moth, *Tecia solanivora* toward its host plant and conspecific. The aim was to investigate odour perception, discrimination and behaviour towards different structures and phenological stages of the potato plant, *Solanum tuberosum*, and between qualitatively different potato tubers. Also attempted was the creation of synthetic blends that could mimic odours from potato structures to use them while testing attraction and oviposition-site cues. A further aim was to elucidate how semiochemicals enhance and disrupt the mating behaviour and the mode of action that pheromone blends have in a mating disruption regime. We sought information about the relationship between volatile and non-volatile compounds from potato to elucidate whether odours from potato influence the females choice to oviposit on a specific site. This result would confirm or contradict the preference-performance hypothesis.

Introduction

The potato, a host for insects

The earliest records of potato cultivation trace back to 7000 years ago, around Lake Titicaca, where wild species; the *Solanum brevicaule* complex became the origin of the landraces developed by pre-Columbian farmers (Spooner et al 2005). Some of the worst potato pests also have their origin in this region. Within the Lepidoptera moth family Gelechiidae, a number of species are pests on potato and other Solanaceae plants. The Potato tuber moth *Phthorimaea operculella* (Zeller) is a pest of potatoes that spread from Ecuador in the 1980s to fields and storages in warm and dry areas of the world, such as northern Africa, the Middle East, Central America, and southern Europe (Trivedi and Rajagopal 1992). Also the Andean potato tuber moth, *Symmetrischema tangolias* (*plaesiosema*) (Gyen), has spread from Peru to Ecuador, Bolivia, Venezuela, Colombia, and further to North America and Australia in recent years. Together with *T. solanivora*, these potato tuber moths share the same resource at larval stage; with the difference that the *P. operculella* and *S. tangolias* larvae additionally feed on potato foliage. Another Gelechiidae from the Andes is the tomato moth, *Tuta* (*Phthorimaea*) *absoluta* (Meyrick), (Barrientos 1997) which is also a pest of potato but the damage is restricted to the aboveground part of the plant.

Currently the three tuber-feeding moths *P. operculella, T. solanivora* and *S. tangolias* are sympatric in similar environments only in southern Colombia (A. Lopez-Avila, personal communication) and Ecuador (Dangles et al 2008, Pollet et al 2003). Elsewhere they do not co-occur, probably due to their different physiological response to temperature (Dangles et al 2008). When they co-occur, the three moth species interrelate and their damage together is more severe to the crop than the effects of each pest alone (Danlges et al 2009). Subsequent species take advantage of the entry holes made by the first species and the feeding rate and the survival of the Andean potato tuber moth increase when the tubers are previously infested by *T. solanivora* or *P. operculella* (Mazoyer 2007). However, the three species also compete when larvae feed on the same tuber. For example, both the survival and feeding activity of *P. operculella* decrease in the presence of *T. solanivora* (Danlges et al 2009).

The Andean potato weevil *Premnotrypes vorax* Hustache (Coleoptera: Curculionidae) originates from the same region and is among the most serious pest of potatoes at high altitudes in the Andean region (Alcazar 1997, Heath et al 2001). Its main host plant is potato *Solanum tuberosum* but the weevil is polyphagous and also feeds on other Solanaceae (*e.g. S. nigrum, S. caripense*) and other plants. Crop loss is due to larvae excavation and feeding on tubers in potato fields (Herrera 2002).

Guatemalan potato moth, a pest of potato

As the name implies, the Guatemalan potato moth *Tecia* (*Scrobipalpopsis*) *solanivora* (Povolny) (Lepidoptera: Gelechiidae) was first identified in Guatemala (Gomez de Paz 2008, Niño 2004). In 1956, the moth was described as a pest of potato and has thereafter spread to other potato growing areas in Latin America and beyond. It is suggested that this insect species, before its description as a pest, was feeding on wild native Solanaceous. It might have shifted hosts from plants without tubers, to more abundant tuber forming potato species such as *Solanum tuberosum* and *S. pureja*. Since the early 1970s, the moth has been observed in the countries between Guatemala and Ecuador. In 1973 it was found in Panama (Povolny 1973), and ten years later, it was introduced to Venezuela followed by its first report from potato fields in Colombia in 1985. The introduction is believed to be a result of importation of infested seed potato from Costa Rica into Venezuela. The geographic origin of *T. solanivora* in the Canary Island is not known. Nevertheless, it is thought that the species was introduced into this country through the importation of a bag of infested potatoes from South America (EPPO 2006). Nowadays, the Guatemalan potato moth is a serious pest of potato crops and in storage in all countries where it is present in Central America, northern South America and the Canary Islands (Hilje and Cartin 1990, Torres et al 1997, Pollet 2001, Pollet and Onore 2004).

The potato production in Colombia has the highest demand for insecticides and fungicides compared to other crops in the country. Chemical treatment is also the most common method for direct control of *T. solanivora* (Feola and Binder 2010). Researchers at the Colombian Corporation for Agricultural Research (*Corpoica*) have been studying the Guatemalan potato moth (in

Colombia: “la guata”) since it was introduced in the country. Pest control has been investigated both in the field and in storages environments. A biopesticide formulation was developed using a granulovirus, *Baculovirus phthorimaea*, to control *T. solanivora* in storage facilities for seed potatoes. However, there are no virus-based products registered for use in storages with potato for human consumption (Niño and Notz 2002). The present PhD project was done in collaboration with Colombian researchers at *Corpoica*.

In the field

The Guatemalan potato larvae exclusively feed on potato of different species within the *Solanum* family. In the field, the female moth enters soil crevices to oviposit close to potato tubers. The eggs hatch after approximately one week and the larvae crawl downward in the soil to find the tubers under the potato plant. The larvae feed inside the tubers (Figure 1) where they are significantly protected. Earthing up soil around the plant to cover the tubers limits the larva from reaching the tubers and reduces loss of potato in the field. During the whole development of the four larval stages, the larvae mine inside the potato tuber. The entry hole is inconspicuous but when the forth instar larva leaves the tuber, a 2-3 mm round hole is seen (Figure 1). Larvae thereafter pupate in the top five centimetres of the soil. The pupation last nearly two weeks. From the pupa, a brownish moth ecloses that can live up to twenty days. The size of the female moth is approximately 13 mm long, while the male is slightly smaller (9-10 mm long) (Figure 2).

Figure 1: Damage caused by the Guatemalan potato moth *Tecia solanivora* on potato tubers. Transactions of damaged potatoes with mines of larvae (left) and outside aspect of damaged potatoes after larvae left the tubers to pupate (right).

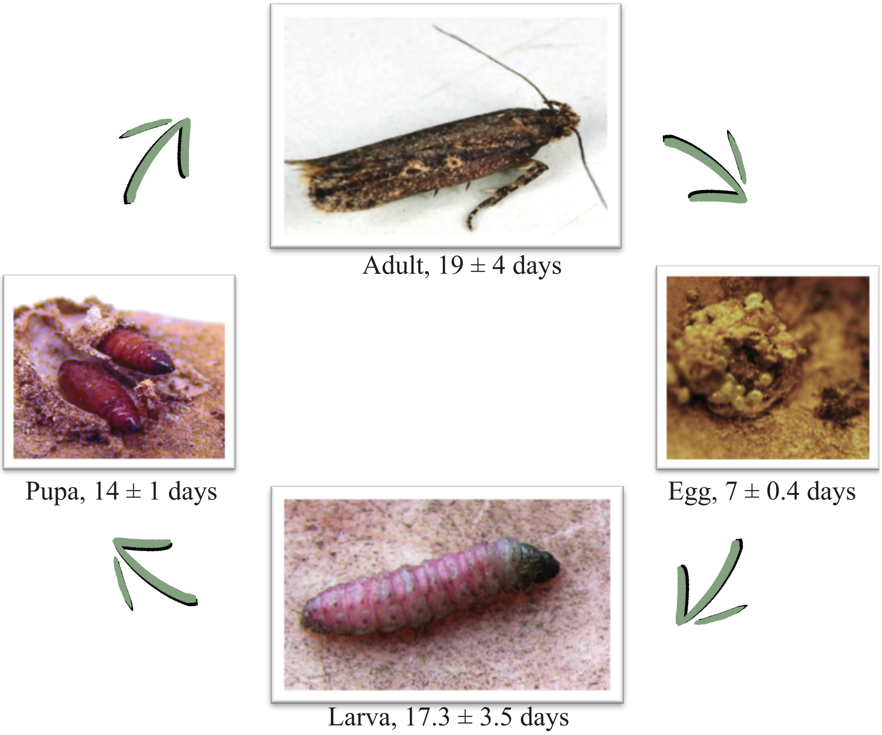

Figure 2: Lifecycle of Guatemalan potato moth *Tecia solanivora* at 20 °C and 85 ± 10 % RH (Torrez et al 1997)- Duration of each stage.

In storage

In potato storages, the female primarily oviposits on potato tubers, preferably on tubers with soil and close to the eyes of the tubers. In piled potato tubers, most females chose to oviposit in the centre and under the heap (Barreto et al. 2003). The larvae leave the tubers to pupate on the surface of the potatoes or adhered to bags and other soft material in the potato storage. Farmers store fresh potatoes to use them as seed potatoes or to strategically sell them later in the season when prices might be higher. Storage rooms in households and small farms in Colombia are often not hermetically closed (Figure 3) and potatoes are vulnerable to attracted females for oviposition and further larval damage. During this storage period, the potatoes are often sprayed with insecticides in an attempt to protect them from *T. solanivora* attack. The advantages of non-hermetical storages, where potatoes are subjected to light, is that the potatoes are slightly protected from attack of the light-evading *T. solanivora*. The disadvantage of the illumination in the storages is the enhanced risk of steroid glycoalkaloids production in the potato tuber, which can have noxious effect on humans (McMillan and Thompson 1979)

Figure 3: Common potato storages in Boyacá, Colombia.

Semiochemicals in insect host-selection and communication

The relationship between plants and herbivorous insects has resulted in a high degree of host-plant specialization. Insects synchronize their life cycle with their host and it is especially true for food specialists (Kooi et al 1991, Schoonhoven et al 2006). Synchronization is achieved when both insects and plants respond to the same environmental changes, such as photoperiod, temperature, and humidity. Furthermore, the synchronization can be achieved when insects respond to signals indicating different phenological stages of the host plant (Proffit et al 2007). The signals transferred to the insect, in the proximity to the plant can be colour, shape, texture and chemical composition. The insect perceives these signals by visual, olfactory, and/or gustatory sensory organs (Blaney et al 1986, Visser 1986, Schoonhoven et al 2006). Plant chemical compounds are important in host-specialization (Bierbaun and Bush

1990, Del Campo 2003). Chemical compounds that act as signals by transferring information between individuals are called semiochemicals. Compound classification within the heterogenic group of semiochemicals is named according to their communication range and the behaviour that the compounds modify. Allelochemicals enable communication between individuals of different species, while pheromones are odours emitted by an individual inducing certain behaviour in another individual of the same species (Nordlund and Lewis 1976). Semiochemicals are sensed by the insects´ olfactory and/or gustatory organs and have often a stronger effect on the behaviour of nocturnal insects than visual or acoustic stimuli (Dobson 2006, Balao et al 2011).

Plant tissue produces numerous compounds, both non-volatile and volatile that influence surrounding organisms (Poeslman et al 2008, Knudsen et al 2006, Kessler and Halitsche 2007). The volatile chemical compounds commonly act within a long distance from the emission source while less volatile compounds act in a close range (Romeis and Zebitz 1996, Judd and Borden 1992). Plants may release hundreds of different compounds but only very few (with few exceptions) trigger a behavioural response in insects (Bruce et al 2005, Tasin et al 2006, Zhang et al 1999). The proportion of compounds in the odour blend is fundamental for behavioural activity, both in host attraction and acceptance by host plant odours (Tasin et al 2007, Najar-Rodriguez et al 2010) and in location of conspecifics with pheromone compounds (Hillbur et al 2002).

Host plant selection by phytophagous insects varies during the day and their lifetime. Factors that alter the selection behaviour are related to the physiological changes in the insects *e.g.* age, sex, circadian rhythm, nutritional stage, egg load, and mating status (Städler 1992, Silvegren et al 2005). Other biotic factors include the host plant characteristics *i.e.* age, phenological stage, nutritional status, and habitat (Ramaswamy 1988, Renwick and Chew 1994). Plant compounds may as well affect the development of an insect such as diapause, morphism, and maturation (Schoonhoven et al 2006 and references therein). Plants can mediate reproductive behaviour and provide signals for recognition and location of mates and larval food plants from a distance (Cardé and Minks 1995, Metcalf and Metcalf 1992, Shonhooven et al 2006, Visser 1986). Sexual behaviours of phytophagous insects and their host plants are also connected in numerous ways (Landolt and Phillips 1997, Cocroft et al 2008). Plants can influence the timing and location of insect reproduction as several species of insects meet and mate on or close to their host plants (Feder et al 1994, Cocroft et al 2008, Prokopy and Owens 1983, Landolt and Phillips 1997, Reddy and Guerrero 2004).

Insect perception of the plant quality is used to discriminate between plant individuals. Chemical compounds can act both as cues for host acceptance and as repellence (Karban 2008, Baldwin et al 2001, Pichersky and Gershenzon 2002, Pichersky et al 2006). Insects generally assess the quality of the oviposition sites by the presence, or absence, of certain chemicals, together with the surface and the visual characteristics of the site (Jallow et al 1999, Simmonds 2001, Gouinguene et al 2005, Masante-Roca et al 2007, Sole et al 2010). The plant compounds that determine larval fitness and survival are most often non-volatile and it is unclear to what extent females, in search of an oviposition site, can detect them either through their olfactory or taste receptors (Simmonds 2001, Calas et al 2007). In spite of this, it is common that specialist insect herbivore preferences for oviposition sites correlate with offspring survival (Jaenike 1978, Mayhew 2001, Rajapakse and Walter 2007, Gripenberg et al 2010). This correlation between maternal choice of host plant and the resulting survival and development of the progeny is often considered in the context of the preference-performance hypothesis, which states that herbivorous insects select to oviposit on the host with the best suitability for their larval offspring (Jaenike, 1978, Valladares and Lawton 1991). This is specially related to insects whose larvae have little or no ability to relocate, and are thus reliant on the host-plant choice of the mother (Heisswolf et al 2005, Staley et al 2009). However, there are also examples of where oviposition choice was done at the expense of the offspring (Scheirs et al 2000, Clark et al 2011). Factors that can influence the female choice, irrespective of offspring development, include presence of parasitoids and predators, which will influence the insects' exposure (Mulatu et al 2006), or preference for host plants with better nutritional status that maximises mother fitness (Scheirs et al 2000).

Purpose and methods

The Guatemalan potato moth, *T. solanivora* is only known as a serious pest of potato. Knowledge of its behaviour in the field and in the potato storages can increase the possibilities to manipulate the innate behaviour, as a tool to reduce crop losses. Knowledge about odour related behaviour of *T. solanivora* could enhance the development of semiochemical based control measures, either to interrupt or to exploit their natural behaviour.

Behaviours that are partly guided by odours include searching for food and oviposition sites, commonly described as host seeking, as well as searching for a mate. Host searching behaviour consists of a series of steps, compiled into a sequence of behaviours. To truly comprehend all steps of this selection behaviour, it is required to look at the all cues used by the insects. In this project, odours cues have almost exclusively been of interest. Olfaction has been separated from contact chemoreception and vision in order to determine the effect of the volatile chemicals on *T. solanivora* behaviour. At a different step in the behavioural sequence, the odour cues might differ. Similarly, different compounds might guide the different purpose for the search. The males searching for a female for mating use environmental cues other than a female searching for oviposition sites.

In my attempt to find out how potato compounds and sex pheromones are related to the Guatemalan potato moth, the following methods were used.

- Bioassay to confirm that the Guatemalan potato moth is attracted to its only host potato, *Solanum* ssp., was done in collaboration with *Corpoica,* Colombia.
- Oviposition bioassays to observe preferences of potato plant in selection of oviposition site, as well as oviposition selection between healthy and stressed potato tubers.

- Collection of headspace from potato structures; foliage, tuber and flower together with headspace collections from healthy and stressed potato tubers.
- Chemical identification of volatile compounds from potato, with coupled gas chromatography-mass spectrometry analysis (GC-MS).
- Chemical identification of non-volatile compounds from healthy and stressed potato tubers, with high-performance liquid chromatography (HPLC) and HPLC-MS-MS methods (done in collaboration with the department of Agriculture at SLU, Alnarp and Department of Integrated Pest Management, Aarhus University, Denmark).
- Electroantennographic recordings both EAG and GC-EAD, were used to test which compounds are perceived by the antenna of *T. solanivora.*
- Olfactometer bioassays with synthetic chemical compounds, identified from potato plants.
- Oviposition bioassays with synthetic chemical compounds.
- Field experiments to evaluate mating disruption mechanism with pheromones blends.
- Wind-tunnel assays to study attraction and mating disruption mechanisms, with calling females and synthetic pheromone.
- Trapping assays with synthetic compounds identified from potato and from *T. solanivora*.

Results from these experiments are compiled in four papers, referred to as I, II, III, and IV

Synchronised rhythms

Potato phenology

The development of a potato plant can be divided into several phenological stages; sprout development, vegetative growth, development of tubers, growth of reproduction parts and senescence (Valbuena 2000, Struik 2007). To elucidate how the odour profiles change during the potato development, headspace collections were made on foliage during the sprouting, tuberization and flowering stages. Comparison between the stages showed that most compounds were present in the foliage headspace in all three phenological stages, although the release rates of several sesquiterpenes increased significantly between sprouting and tuberization (I; Figure 4). Potato plants in flowering stage released large amounts of methyl phenylacetate (I).

When mated *T. solanivora* females were released into a mesh house and could choose between potato plants in different phenological stages, significantly more eggs were laid near flowering plants than near younger, pre-flowering plants (I). These results are in accordance with what is seen in the potato field, where the number of moths increases during the flowering stage (Soriano 2000, Barreto et al 2003, Sánchez 2003) and the main infestation occurs after tuber formation and peak flowering (Torres et al 1997). The potato flowering stage is additionally correlated to the tuber formation of the potato plant (Valbuena 2000, Struik 2007). Since *T. solanivora* larvae only feed on tubers, the female choice of oviposition sites (under potato plants with formed potato tubers (I)) correlates well with the suitability of the larvae, with the enhanced food source. Such attraction to plant parts related to specific phenological stage offers reproduction potential (Masante-Roca et al 2007).

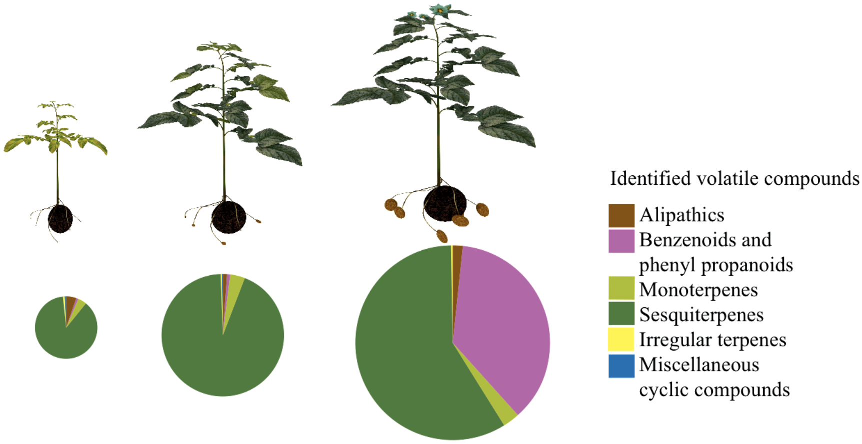

Figure 4:. Headspace composition of potato *Solanum tuberosum* in different phenological stages; sprout, tuberization and flowering.

The daily rhythm

The Guatemalan potato moth is a nocturnal insect with high activity during dusk and dawn. However, this fact are based on male trap caches in the field with pheromone-baited traps (Corredor and Florez 2003). The behaviour of female moths is poorly studied. This is probably due to the difficulties in observing females in the field as they hide under, and inside, the foliage of the potato. In this study we observed that females seek to stay in dark places, preferably under something or in a protected corner. Females hide during the day and oviposit during the night. In laboratory experiments, *T. solanivora* oviposited from the first scotophase after mating and during several subsequent days (Figure 5) (unpublished data, Torres et al 1997). Olfactometer assays were done during the entire scotophase and no significant difference in responsiveness during these hours was observed (III).

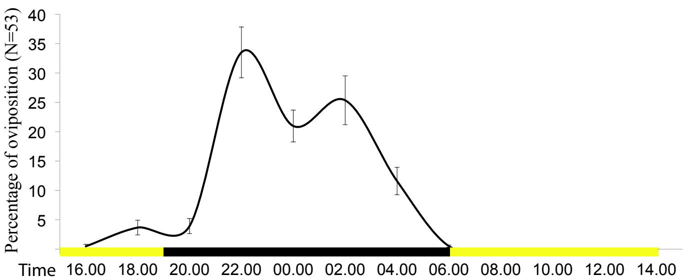

Figure 5: Percent of total eggs laid during the day by Guatemalan potato moth *Tecia solanivora.*

Females are only visible when they call, usually early in the morning. Just before sunrise, dawn, or when turning on the light, females can be seen on potato foliage, where they characteristically bend the abdomen upwards and emit the sex pheromone from the gland, in the tip of the abdomen (Figure 6). When males sense the pheromone, they activate, wing fan and move intensely, searching for the female. The number of *T. solanivora* males that get activated with pheromone at other times of the day than in the morning is very low. Under natural lighting conditions, approximately ten percent of the insects will mate even during dusk, but this is not seen in laboratory where lights turn off abruptly.

Figure 6: Guatemalan potato moth *Tecia solanivora* female calling in the morning.

Potato odour

Distinct smell of different potato structures

Each potato structure; foliage, flower, and tuber have a distinct odour profiles (I). It was previously known that the volatile emission from intact potato foliage is a blend of terpenoids, especially sesquiterpene hydrocarbons and fatty acid derivatives such as aldehydes and alcohols (Visser et al 1979, Weissbecker et al 2000, Szafranek et al 2005). Results from our study showed that potato foliage released 32 sesquiterpenes, accounting for approximately 90% of the volatiles released. The most abundant sesquiterpenes were β-caryophyllene, germacrene D, *E,E*-α-farnesene, kunzeaol, and germacrene-D-4-ol (figure 1 in I). Tuber headspace can be distinguished from foliage headspace by the trace amounts of sesquiterpenes. Tubers released predominantly aliphatic alcohols, aldehydes and ketones together with several benzenoids and monoterpenes. Disregarding different potato varieties, identified compounds are in agreement with previous studies (Maga 1994, Dresow and Böhm 2009 and ref herein). The main components of the flower headspace that differentiated the flower odour from foliage were methyl phenylacetate 2-phenylethanol and farnesol (I).

Antennal perception of potato odours

Combined gas chromatography and electroantennal detection analysis with potato headspace from foliage, tubers and flowers revealed 21 compounds in potato odour that are antennal active for *T. solanivora* (I; Figure 7). These antennal active compounds were tested in a dose vs. antennal response function with electroantennographic recording technique over four orders of magnitude. Out of the 21 tested compounds (aliphatics, bezenoids and sesquiterpenes), fourteen compounds elicited a significant enhanced antennal response caused by the level of exposure but several sesquiterpenes did not (III).

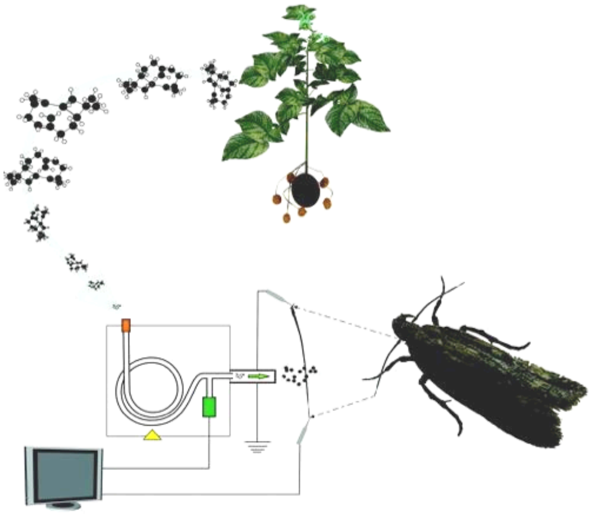

Figure7: Electroantennographic recordings with potato headspace collection injected in a gas chromatograph and delivered to the antenna of *Tecia solanivora.*

Attraction and oviposition guided by potato odour

The Guatemalan potato moth has the capacity to perceive several compounds from the potato. We also tested and showed that *T. solanivora* can distinguish by the odours, between the different potato structures. The role of these chemical signals in attraction and oviposition of the moth was tested.

Previous studies made in olfactometer revealed that virgin females are more attracted to flowers of potato, *S. tuberosum*, compared to other potato plant structures (Bosa 2011). In comparable olfactometer bioassays, mated females of *T. solanivora* elicited the highest attraction behaviour to flowers of the potato *S. pureja* (Rincón et al 2007). Odour mimics of the potato structures (foliage, flowers and tubers) were created based on the identified potato compounds and their emission from he plant. The synthetic potato mimics´ blends were composed exclusivly with compounds that gave a significant antennal response that was dose dependent in *T. solanivora* (table 1 in III). This reduced the number of compounds in each blend to fourteen compounds in total. In dual-choice bioassays, the synthetic potato blends were tested against a control of solvent (hexane). Adults of *T. solanivora* were attracted to the synthetic floral blend when tested in the olfactometer assay. Approximately 70 % of the insects, males and females, both mated and virgin, showed attraction toward the synthetic flower mimic blend. The flower blend mimic

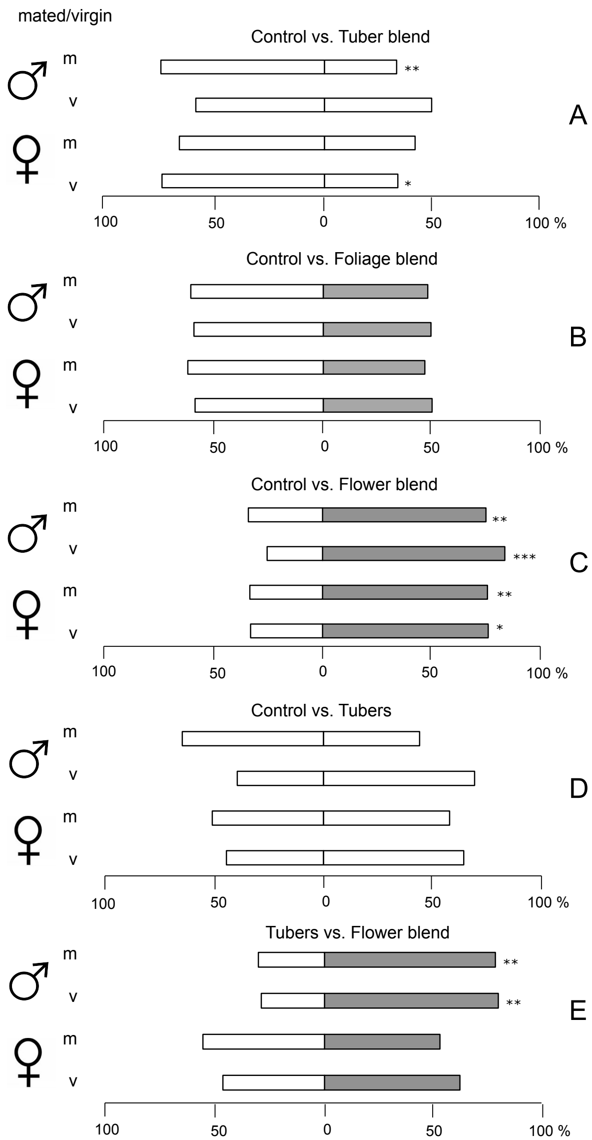

Figure 8: Response of male and females, virgin and mated, in Y-tube experiments when presented with odour blends vs. control (A-D) A) Tuber blend B) Foliage blend C) Flower blend D) Tuber E) Tuber vs. Flower blend (III).

consisted of methyl phenyl-acetate, 2-phenylethanol and phenylacetaldehyde, which are chemically similar (III). The foliage and tuber blends did not attract the moth. On the contrary, mated males and virgin female moths avoided the tuber blend in a dual choice with a control (III; Figure 8).

In dual-choice assays with potato tubers and control, no directional trends in the olfactometer for any of the tested adult moth groups were found. It is good to note, however, that 70 % of the mated females choose potato tubers. Mated females were attracted to flower blend when presented in a dual choice with the control but did not show a significant preference to flower blend when it was presented in a dual choice with tubers. However, virgin and mated males were significantly more attracted to the synthetic floral blend than to the potato tubers (III; Figure 8). The behavioural effect of the combination of odours led us to conclude that for the mated female, a combination of potato plant organs; the flowers and the tubers, is more attractive any of the odours from potato structure alone (III).

Furthermore, the smell of the potato guides the female to select an oviposition site. In a bioassay with potted potato plants, foliage was avoided. Eggs were deposited only on soil, close to the stems of potato plants, but not directly on stems or leaves. Likewise were shown in a dual choice ovipositions assay with and without potato foliage odour. Most eggs were laid on the control site, without potato foliage odour (I).

Foliage emits vast amounts of sesquiterpenes and *T. solanivora* perceives these compounds (I). At the same time, the female moths both avoid to ovipositing on the structure emitting the compounds (I) and are not attracted to a synthetic blend that mimics foliage (III). The ability to sense these compounds, might give an indication of that the insects use them to orientate. However, the function of the foliage compounds, for *T. solanivora* is still unclear but can related to the female moths ability to select a suitable oviposition site (II).

Flower smell attractants

Flower odours are commonly emitted in larger amounts than foliage odours (Knudsen et al 2006) and have probably evolved to enhance the attraction of pollinators (Raguso 2004). Most flowers in the genus *Solanum*, such as the potato flowers, are conspicuous and sweet-scented (Figure 9) but they do not produce nectar (Buchmann and Cane 1989, Nicolson et al 2007). Pollen is the sole reward for bees visiting *Solanum* nectarless flowers (Buchmann and Cane 1989) and potato flowers are exclusively pollinated by bumbles bees (Simmonds 1976). Nevertheless, *T. solanivora* females are more attracted to flowers than to potato tubers and foliage (I, III, Bosa et al 2011, Rincón et al 2007). However, it cannot be excluded that the attraction to potato flower mimics is related to nectar or pollen reward since flowers from several species emit the same compounds (Andersson et al 2002) and adult *T. solanivora* might feed on other flowers. It is still unknown if *T. solanivora* feed as adults, though it is likely to occur as adults feed on honey water. This is to be verified in the field.

Figure 9: *Flower of Solanum tubersum, S. ruiz-ceballosii, S. pureja, S. demissum*

Significantly more eggs were deposited when the three-component flower blend was present than in an air stream of solvent (hexane). Flower odour is therefore a positive signal for the female in selection and acceptance of ovipositon site. It is likely that flowers are not attractive *per se*, but the flower odours act as an indicator of a suitable phenological stage, *i.e.* the formation of tubers.

Also the males were attracted to flower compounds, in the olfactometer assays done during the scotophase. Moth mobility during the night and the attraction to floral compounds is intriguing, since potato flowers close up during the night. It is not until the sun rises that the flowers open again, concurrently with the onset of female calling. We therefore postulate that flower odour can act in combination with the female sex pheromone emitted at sunrise. Increased pheromone production and emission as well as earlier and more long-lasting calling behaviour are reported in host plant presence (Feder et al 1994, Landolt and Philips 1997, Sadek and Andersson 2007, Raguso 2008). In addition, a combination of pheromone components and plant compounds, such as floral scent, can enhance the attraction in phytophagous insect species (Reddy and Guerrero 2004). Incorporation of plant and plant habitat cues while searching for mates significantly influence the evolution of phytophagous insects (Feder 1998, Drès and Mallet 2002, Cocroft et al 2008).

We hypothesize that adult *T. solanivora,* of different sex and mating stage, are attracted to their host plant by similar blends of compounds. Therefore, virgin moths orient towards potato for mating, while mated females search for an oviposition site.

Female choice and larval survival

While exploring the landscape of integrated sensory signals both attractant and deterrent chemical compounds are considered before acceptance of a host (Pichersky and Gershenzon 2002, Haribal and Feeny 2003). To assess whether the Guatemalan potato moth distinguishes olfactory cues from qualitatively different potato tubers, headspace collections were made from light-treated tubers, larval-damaged tubers and from non-treated tubers *i.e.* healthy tubers. Female oviposition choices were then compared to larval survival for the different treatments. The results elicit a reflection upon the relation between compounds in the plants' biosynthetic pathways and whether there is a common biosynthetic pathway between plant-produced volatile and non-volatile compounds (II).

Potato odour

Plants generally change in their nutritive values, along with changes in volatilized and non-volatile plant compound, during the growing season and while exposed to stress. To test whether stressed potato changed odour profile compared to non-treated tubers, headspace collections from tubers were done from tubers in two light regimes, as well as from larval damaged tubers. The light treated tubers were kept in light two weeks before the onset of the experiment and the damaged tubers were subjected to larval feeding. Odour profiles from the different treatments were significantly different. Stressors, such as light exposure and damage, cause changes in potato odour profiles compared to healthy tubers (II). Divergences were due both to the different compounds in each profile and also the proportion between the compounds in common. Analysis shows that some compounds are the most determinant for the differences between the treatments. The odour collections from healthy potato tubers mainly contained aliphatic alcohols, aldehydes as well as the ketone sulcatone, in accordance with the results in Paper I. Larval damaged tuber odour profile differed from healthy tubers mainly by the proportional increase of two sesquiterpenes and two aldehydes. Compounds identified only in damaged tubers were the aliphatic heptanal, the monoterpenes 3-carene and verbenone, three sesquiterpene hydrocarbons, and the aromatic styrene (II). This study corroborate the work by Lacy Costello et al (2001) who identified benzothiazole, 2-ethyl-1-hexanol and hexanal in large amounts as well as styrene, butanal, copaene, and verbenone in fungal infected potato. Larval damage might have caused secondary fungal infection, which might be the reason for the similarity in headspace compounds. Light treated tubers emitted isopropyl myristate and decanal in higher proportion than healthy tubers and lower proportion of *E,E*-α-farnesene, phenylacetaldehyde, benzothiazole, heptadecane and octadecane (II).

Oviposition response

Our oviposition assays indicate that the female choice of oviposition site is guided with olfactory cues. In a dual choice oviposition bioassay, the female laid more eggs towards the healthy potato tubers side compared to the side with light or larval damaged tubers (II; Figure 10). Similarly, the closely related Potato tuber moth, *P. operculella* prefers to oviposit on undamaged tubers as compared to damaged potato tubers (Arab et al 2007). An antivideponent response was caused by the aldehydes heptanal, octanal, and nonanal in oviposition assays with *P. operculella* (De Cristofaro et al 2007). Since these compounds increased in proportion when tubers were damaged and *T. solanivora* rejected ovipostion close to the odour of damaged tubers, it is likely that compounds within the aldehyde and sesquiterpene groups, generate deterrence behaviour in the moth, especially during oviposition acceptance (II). Gómez and Poveda (2009) showed a synergistic effect on *T. solanivora* oviposition with the combined use of stimuli that alone did not have a significant effect. Garlic-pepper extract, which slightly reduced oviposition and the potato variety Roja Nariño, which slightly stimulated oviposition, had a synergistic effect and reduced crop loss in the fields attacked by the Guatemalan potato moth. These results confirm that phytophagous insect specialists do not choose host plants solely based on attractants but also based upon avoidance cues, agreeing with several previous studies (Feder and Forbes 2007, Pichersky and Gershenzon 2002, Haribal and Feeny 2003).

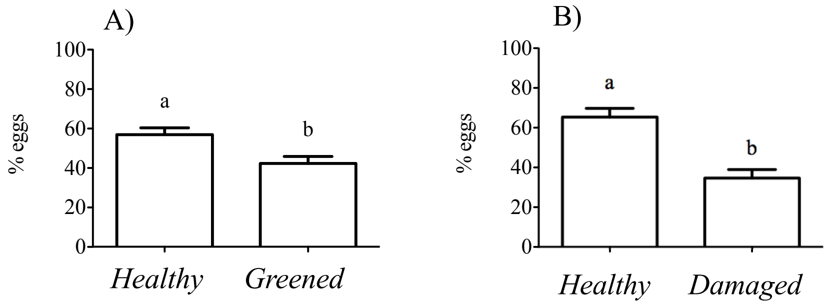

Figure 10: Female choice for oviposition in tube with no physical contact with odour source A) healthy tubers vs. greened tubers B) healthy tubers vs damaged tubers. Bars show mean number of eggs per female and standard error.

Larval survival

In parallel to the odour collections, larval survival rate was examined in three different potato cultivars and in tubers with two different light treatment regimes. Compounds that have a documented negative effect in Lepidoptera larval fitness and survival in potato are steroid glycoalkaloids, sesquiterpenes, and phenols. Their interactions with insects cause toxicity, repellence, and/or feeding modification (Jadhav et al 1991, Simmonds 2001, Friedman 2006, Treutter 2006). In tubers subjected to light and larval damages, the concentration of steroid glycoalkaloids compounds is enhanced, although the change differed between the varieties (II, Percival 1999). Larval performance was negatively correlated to the content of potato tuber steroid glycoalkaloids, specifically α-solanine and α-chaconine (II). Larvae of *T. solanivora* feed exclusively on potato tubers and hence tolerate low concentrations of steroid glycoalkaloid compounds. However, with a concentration above 300 $\mu g\ ml^{-1}$ of α-solanine, and α-chaconine, the survival is reduced to approximately 35 % (II). Stress, such as light exposure, generally enhances the phenol content in tubers as well (Griffiths et al 1995, Andre et al 2009). However, in our study, polyphenols were found in low concentrations and are concluded to not influence the survival rate of the larvae. These results give a good indication that steroid glycoalkaloid content in the tubers are involved in larval mortality (II).

The Potato tuber moth, *P. operculella,* and the Andean potato tuber, *S. tangolias,* moth are closely related species to *T. solanivora*, and their larvae feed similarly on potato tubers. In addition the larvae feed on potato leaves and stems (Palacios et al 1999, Rondon 2010). However, they are different not only in where the larvae feed on the plant, but also where they feed within the tuber. *Phthorimaea operculella* feed more peripherally than *T. solanivora* and *S. tangolias,* which mine in the whole tuber. Both leaves and peel contain much higher concentrations of steroid glycoalkaloids than the flesh of the tubers (Friedman and McDonald 1997). This feeding divergence might be due to a lower tolerance to steroid glycoalkaloids in *T. solanivora* or might as well be a resource partioning between closely related species, by choosing different plant parts (Benson 1978). Dangles et al (2009) found that potato damage was increased with a co-occurrence of Gelechiidae species in the same potato tuber. The probability for a similar situation in the field is unlikely, considering that both *T. solanivora* (II) and *P. operculella* (De Cristofaro et al 2007) avoid ovipostion on damaged potato.

Preference performance hypothesis

Oviposition assays demonstrate that volatile compounds from stressed tubers (light and/or larval damaged) guide the female moth to reject oviposition on tubers with low quality for the larvae. Since sesquiterpenes and steroid glycoalkaloids have the same key precursor and are produced in potato in response to stress (Desjardins et al 1995, Mathews et al 2005, Machado et al 2007). We assume that the Guatemalan potato moth has adapted its behavioural response to potato volatiles, enabling them to locate suitable hosts. Therefore, we propose that the volatiles that mediate oviposition behaviour correlate with biosynthetically related, non-volatile compounds, such as steroidal glycoalkaloids, which impact larval survival. In addition, we conclude that the oviposition response and larval survival of Guatemalan potato moth on healthy vs. stressed tubers supports the preference performance hypothesis for insect herbivores. Lepidopteran larvae are often immobile and their survival rate is to a large extent dependent upon where the eggs are deposited (Jaenike 1978). Neonate Lepidoptera larvae are small and are generally not able to move long distances. Guatemalan potato moth larvae do not have the capacity to completely choose their feeding site. The host-choice is subsequently mainly made by the adult moths and specifically, the female moth, by choosing the oviposition site. When the eggs are oviposited on the soil, the larvae eclose and crawl through the soil downwards by geotaxis until finding a potato tuber (Lopez-Ávila, personal communication).

Sex pheromones

Like most Lepidoptera, *T. solanivora* female use sex pheromones to attract male moths for mating. Three compounds of the female sex pheromone of *T. solanivora* are identified from the female gland, *E*-3-dodecenyl acetate (E3-12:Ac), *Z*-3-dodecenyl acetate (Z3-12:Ac), and dodecyl acetate (12:Ac) in a proportion of 100:1:10, respectively (Nesbitt 1985, Bosa et al 2005). Additionally, two yet unidentified compounds are found in the gland and might be a part in the pheromone blend composition. The content per female gland of the main compound E3-12:Ac is 5.6 ± 0.48 ng (unpublished data).

Attractive synthetic pheromone blends are used to trap insects for monitoring and/or control purposes (McNeil 1991). A lack of just one compound can significantly reduce the attraction (Coracini et al 2001, Hillbur et al 2002). An increased proportion of Z3-12:Ac and 12:Ac relative to the main compound in the Guatemalan potato moth pheromone blend decreases the attraction to the blend (Bosa et al 2006, IV).

To further test the sensitivity to the changed ratio of the pheromone compounds, a wind tunnel assay was performed. Females were placed in the upwind end of the wind tunnel and when dimmed light was turned on, the female started to call i.e. to emit pheromones. Male moths were placed in the downwind end of the wind tunnel and the male behaviour was observed. Comparison was made with two synthetic pheromone blends. One blend had a ratio of E3-12:Ac : Z3-12:Ac : 12:Ac, similar to the natural one, 10 : 1 : 10. The other blend had a similar mixture of the three pheromone components 100 : 6 : 45. The aim of this assay was to elucidate how this relatively small enhancement of the two components Z3-12:Ac and 12:Ac affect the male behaviour towards the pheromone source in the wind tunnel, or if the synthetic blends act in a similar mode with increased release rates. The two synthetic blends gave comparable behavioural results, both in oriented flight and landing rates, when tested in amounts similar to what is emitted by one calling female. However, with an increasing release rate of the two syntetic pheromone blends, similar to levels produced by 100 calling females, the attraction measured as landing in the opposite side of the wind tunnel, was reduced (unpublished data).

The reduction of oriented flight and landing with higher release rates are comparable to what is seen in assays with polyethylene tube dispensers (Shin-Etsu Chemical Co., Tokyo, Japan) loaded with pheromone component mixture 100 : 6 : 45, hereafter called *female blend* and with the compound ratio 100 : 50 : 100, entitled *off blend.* The dispensers released 0.1 mg/day of the main compound or 4000 ng h^{-1}, which is comparable to approximately 500 females calling. With both *female blend* and *off blend,* only 4 % of the males arrived at the end of the wind tunnel, and only 20 % performed oriented flight (IV), showing that there is a limitation of attraction related to amount of pheromone. This disruption of male attraction toward female sex pheromone in high doses is used as a control method for insect pest in the field. By permeating the air in the field, the attraction towards calling females is decrease, or even ceased. The olfactory communication and subsequently mate-finding can thereby be prevented (Ridgway et al 1990).

Calling females, placed in a wind tunnel together with either of the two dispensers (*female blend* and *off blend*) resulted in a reduction from 60% oriented flight towards calling females alone, to 20% with calling females in combination with a plume of synthetic pheromone blend. In accordance, landing was reduced from 40% to 4%. This indicates that mating will be disrupted if the air is permeated with the synthetic pheromone blend of the compounds of E3-12:Ac : Z3-12:Ac : 12:Ac in either a proportions of 100 : 6 : 45 or 100 : 50 : 100 respectively (IV).

The mode of action in mating disruption

The mating disruption method was shown to be useful for control of Guatemalan potato moth in large potato fields, when using the *Off blend* mixture of pheromone components (Bosa et al 2005). Several olfactory and behavioural actions explain the mechanisms in mating disruption, generally classified as either competitive *i.e.* false trail following, or non-competitive *i.e.* camouflage, sensory fatigue, sensory unbalance, antagonism, etc. These mechanisms may act individually or in combination, as well as vary in importance according to the dispenser type used and the target species (Stelinski 2007, Suckling 2000). Therefore, their elucidation plays a crucial role in the choice of an adequate mating disruption strategy, creating knowledge of the distribution and the proportion of compounds (Cardé and Minks, 1995, Sanders 1997, Howse et al 1998, Welter et al 2006, Stelinski 2007) as well as how to reduce risk of resistance (Muchizuki et al 2008).

To contrast the previously tested *off blend* with the *female blend*, wind tunnel assays were done as well as mesh house and field tests. The purpose was to compare the two blends and elucidate how they disrupt mating. Results showed that both blends significantly reduced all responses involved in the flight sequence in the wind tunnel, inhibited attraction towards female-baited traps in mesh house and monitoring traps in the field, and prevented mating under mesh house conditions. Differences found among blends suggest different modes of action. The *female blend* possibly acts through camouflage and/or sensory fatigue while the *off blend* involves sensory imbalance as a mating disruption mechanism (IV).

Semiochemicals control *Tecia solanivora*?

Trapping of insects

Chemical ecology research was born through agriculture and forestry, partly due to the need to reduce insect pest damage in the crops. The study of insects and their olfactory-dependent behaviour has led to the development of cropping management tools and synthetically-produced compounds, used to manipulate the behaviour of the insects (Foster and Harris 1997, Suckling 2000, Norin 2007)

Insect attraction behaviour towards sex and aggregation pheromones, is exploited in crop protection by trapping insects with pheromone-baited traps (Trematerra and Battaini 1987, Sternlicht et al 1990). Traps baited with host odours are now in development but few are in use (Meagher and Landolt 2010, Socorro 2010). Trapping insects might be done either to monitor the insect population or trap numerous insects to control the population size, *i.e.* mass trapping. The release rate and composition of the chemical compounds must be adjusted so that the sampling rate of the traps is appropriate to the requirements for the protection of the crop (Weatherson and Minks 1995).

Pheromones are species-specific and generally the most attractive pheromone blend is a mixture of compounds in a proportion similar to that emitted by the insect itself but this might not be required for trapping purposes (Greenway and Wall 1981, Renou and Guerrero 2000). Sex pheromone of the Guatemalan potato moth has been used for monitoring presence of the insects in potato fields in Colombia (López-Ávila and Triana 2004). Several farmers have also used the traps to control the population in the field (personal observation). *Tecia solanivora* pheromone traps attract only male moths. We have also observed that the male moth is more mobile than females (II) and it is likely that males are easier than females to trap in the field with volatile semiochemicals. Efficiency of reducing male populations require a higher number of moths trapped, compared to the number of females needed to be trapped for the same population reduction. As female oviposit approximately 200 eggs and males mate approximately five times, the efficiency is higher with mass-trapping of females. By an addition of host plant compounds to pheromone-baited traps the attraction of males can increase but might also attract females (Ladd et al 1981, Kuhar et al 2006, Dai et al 2008). Compounds that function as a signal for egg-laying females formulated in lures for mass-trapping might also be a method to control females in a population (Oehlschlager et al 2002, Cork et al 2003, Anfora et al 2009). Also aggregation pheromones (Bengtsson et al 2010), or food baits (Barry and Polavarapu 2004, Ekesi et al 2007), can be used to attract females.

We have found that the Guatemalan potato moth is attracted by floral odours (I, III, Bosa el al 2011) and that females oviposit more in presence of flower odour. However, *T. solanivora* are not seen in the flowers and the attractive odour from flowers is spatially separated from the site where the female oviposit, mates and arrests. Trapping assays has been done with flower compounds but a very low percentage are trapped, especially females. Similar results were found with the sole compound methyl phenylacetate, where almost exclusively males were trapped (Bosa et al 2011). Bioassays in the laboratory did not attract over a long range and/or were not effective enough to trap the insects. This system both resembles, and contrasts to, the situation where the palm tree leaves emit odours as signals to advertise the presence and location of nectar-rich flowers (Dufaÿ et al 2003). Thus, it has not been possible to trap females, nor to show an improved male attraction to pheromone baits boosted with host-plant compounds. Preliminary data on on-going field tests may confirm these results.

It has not yet been fully concluded which cues are used by the *T. solanivora* to accept to oviposit beside a potato plant, or to oviposit on the surface of a tuber in potato storages. The flower smell might be the signal to search for an oviposition site, but additional compounds are needed to accept and to actually reach the soil to oviposit. There are indications that compounds emitted from the potato guides the female to accept healthy tubers for oviposition, while other compounds cause rejection.

It is suggested that oviposition assays should be created in the field, since this might be useful to reveal which semiochemicals attract and arrest the female. The prospect of using host plant attractants to manipulate *T. solanivora* females is far from being employed. Host-plant blends are more complex than pheromone blends, and they contain compounds with different chemical characteristics and volatility. Therefore, formulation and proportional release are problematic.

Mating disruption

Mating disruption is caused when sources of synthetic pheromone are introduced into the environment preventing males from locating calling females. For successful disruption the air must be permeated with the disrupting compound in sufficient concentration throughout the mating period (Thorpe et al 2006). An estimation of mating disruption success is when the number of trap catches in the treated area is reduced with 90-95 % compared to the control field. In this situation the population will have reduced and damaged in the crop is significantly lower with the mating disruption management (Baker, personal communication, Kehat et al 1999). Our field experiments show a significant reduction of the trap catches, with around 90%, in the *Off blend* treated area (IV). Nevertheless, the data on larval feeding on the potato do not show a reduced damage in the treated areas compared to the control plot, mostly due to a low over-all damage.

The potential for an effective use of mating disruption management for control of Guatemalan potato moth in the study area is not evident. The sizes of the potato fields in the region are often small, less than one hectare, and there is a risk that moth can enter from outside or fly into the plot and mate. Mating disruption technique in storage facilities, which are not hermetically closed, is not feasible. To saturate the atmosphere with synthetic pheromone in a small area, open to closed-by potato field is not achievable. Neither can immigration of mated females to the storage be avoided. Mating disruption is therefore not an efficient method in such storage facilities.

Release in the field

The use of semiochemicals with different volatility and stability require a devise that can release at constant rate during an efficient time period (Camps et al 1988). During this thesis work, behavioural assays with volatile synthetic potato compounds have been done with known and continuous release rates of the compounds. Examined release of different solvents at a specific temperature made it possible to use release rates of the compounds set by the evaporation rate of the carrier solvent hexane. The compounds were released through a cotton wick inserted in a Teflon tube (⃠2 mm). The cotton wick was soaked in the solvent dilution treatments mixed in a vial, in the quantified amounts released from the potato plant (III, Figure 11). For laboratory and field research, these “dispensers” are useful but commercially they might be impractical.

The attractive flower blend used in the olfactometer assays were not reproduced in trap catches in laboratory and field assays. Some explanations are discussed above, although additional reasons might be trap placement, and trap design; colour or structure. The traps used were white delta traps with a sticky paper or white/red water-traps.

Figure 11: Device for release of volatile compounds diluted in solvent.

CONCLUSION

This first study on the attraction of Guatemalan potato moth *Tecia solanivora* to its host plant reveals that volatile signals from potato, *Solanum tuberosum*, play a role in host finding and oviposition choice of the moth. Chemical analysis, antennal recordings, and behavioural studies have been employed to study volatiles that initiate female attraction toward larval food plants, attract males and females to mating site, and disrupt male sexual behaviour. This thesis presents evidence that volatile compounds from potatoes are perceived by the olfactory system in the moth. Results confirm the role of semiochemicals in plant-insect and insect-insect relationships, presenting the chemicals encoding for some of these interactions.

Behavioural-mediating chemical compounds have been found both for male and female *T. solanivora.* Even thought *T. solanivora* exploits the potato tubers, the flower is the only structure of potato attractive for the moths. In potato plant, the presence of flower above ground is related to the presence of large tubers below ground, thus the moth probably uses this signal as an indicator of a host-suitable stage for larval development. The most attractive blend was a flower blend mimic consisting of methyl phenylacetate, 2-phenylethanol and phenylacetaldehyde. Enhanced oviposition in the presence of the flower mimic indicates that mated females distinguish odour profiles and uses odour to choose oviposition site. The divergence between the plant structures that are behaviourally exploited by the insect, and the plant part with an attractive odour emission is one example where spatially separated plant structures indicate the presence of a suitable host. This spatial divergence might explain unattainable trap catches with the flower mimic.

The combination of behavioral response towards both attractants and deterrent structures and compounds was used to entail a broader understanding of olfactory cue dependence. The chemical compounds causing deterrence and ovipostion avoidance produced from the potato foliage and stressed tubers were in part aldehydes and sesquiterpenes of which some are also presente in non-stressed tubers. During the tuberization stage, the flowers and tubers are present at the same time and this stage was preferred for oviposition. In dual choice assay between tubers and flowers males were more attracted to flower odour mimic while females were equally attracted to both odours. There was a tendency that the combination of the odours enhanced the motivation to choose when both flowers and tubers were present but could not be statistically confirmed. The right combination and ratios of compounds needed to trap *T. solanivora* have yet to be identified, the answer might well be a combined blend with flower and tuber compounds. The compounds perceived by *T. solanivora* antenna and the behaviorally active compounds should be considered altogether to fully explain the role of host-plant odours for the insect.

Male attraction to calling females and mating success was reduced with both proportionally different three-component pheromone dispensers. Also trap catches with monitoring lures were reduced. These results in combination with results from trap catches with the dispensers and observations in wind tunnel bioassays confirm the mode of action for the two dispensers in a mating disruption regime. We deduced that the *off blend* acts with sensory imbalance mechanisms while the *female blend* possibly acts through camouflage as a long distance mechanism and sensory fatigue is more likey to act in proximaty to the pheromone-releasing dispensers.

Aside from olfaction, other sensory cues are most likely involved in orientation and acceptance of potato as host plants for the Guatemalan potato moth. Vision and mechanosensory cues are probably part of the behavioral cues leading the moth to oviposit, mate, and communicate. Development of laboratory and field experiments to evaluate male and female behavior is required for a more complete understanding of the role of semiochemicals in Guatemalan potato moth behavior. Studies of synergism between pheromones and plant compounds might enhance the potential for mass trapping of males. Further research on blend composition to create source contact will improve the knowledge of possible usage of semiochemicals in control of the Guatemalan potato moth.

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ACKNOWLEDGEMENT

Nobody ever promised it should be easy but I was promised to learn a lot. It has truly been so. I would like to thank everybody who has in some way helped me to write this thesis.

Först ett stort Tack till *Göran* för din stora entusiasm, din kunskap och förmåga att organisera. Jag är oerhört tacksam, mer än jag kan beskriva. *Magali,* ¿qué hubiera hecho yo sin ti? Me has enseñado tanto de ciencia y de estadística. Gracias por esto y por mucho más. Ett stort tack till *Ylva* som alltid var positiv och gav mig motivation att fortsätta. Tack för möjligheten att resa till Etiopen.

Peter, tack för att du föreslog att doktorera; att du trodde på mig. Så långt gick det bra. Tack *Marie* för att du gillar kemi och undervisning, det smittade av sig.

Por supuesto, muchísimas gracias a *Felipe Bosa* que siempre está allí, siempre luchando y ganando! Gracias *Andrea* por tu empeño, tu cariño y tu locura!

Thanks to all the people in Alnarp, former and present colleagues who taught me so much and who chaired with me the everyday work. Special thanks to the ”Monday morning meeting group”, thanks *Saveer* and *Paul* for computer help and always happy smiles. Tack till *Will* för bastukvällarna och för att ha gjort språkgranskning av min avhandling. Thanks *Eduardo* for reading thesis y gracias *Marcelo* por siempre ayudarme con el español. Tack *Anna* för stöd och förebild. Gracias *Isabell* por compartir las penas y la alegría, te deseo todo lo mejor. Tack *Gunda* och *Owe* för härliga middagar, Grazie *Irene* for great chats, hospitality and talks about the future. Thanks *Ngwabi* for being a wonderful friend. Thanks to *Jonas, Sebastian, Futini, Patrik, Tomas, Elin, Maimoun* for nice company in office and in lab, specially late at night…

Quisiera agradecer a todos en *Corpoica* que siempre me ayudaron cuando quería saber, necesitaba tener o simplemente quería estar. Gracias *Alba Marina* por haber empezado la colaboración con SLU. Creo que no hay personas que sabe más de *Tecia* que *Aristóbulo* y *Nancy*. Gracias *Aris* por las charlas y el café y a *Nancy* que tomó su tiempo a ir conmigo al campo. *Sandra* y *Felipe*, gracias por su ayuda y compañía. Les deseo mucha suerte en sus doctorados. Gracias *Claudia* y *Jorge.*

Tak til *Inge* og *Jonas* og den dejlige arbejdsgruppe af Flakkebjerg i Danmark.

Erokamano ahinya *Aman* kuom gik moko duto.

Tack mina vänner och kollektivkompisar för all god mat, bastukvällar, fiolspel, dans, telefonsamtal och annat som gett mig energi att jobba. Tack *Wero, Lena, Tora, Marcus, Christina, Ida, Camilla, Krister, Fares, André, Ingela, Magnus, Pia, Maria, Alex, Oscar, Elisabeth, Erik, Marie, Pontus, Suzan, Åsa, Ethel* och alla andra som jag för tillfället glömt.

Tack till *syster, bröder, fastrar, mostrar, farbröder, kusiner, far*- och *morföräldrar* – jag längtar till att träffa er! Tack mina kära föräldrar för att ni alltid funnits där. Tack för de små paketen *mamma* och tack *pappa* för att du lärt mig kämpa. Jag hade inte varit här utan er. TACK!

Dofters inverkan på värdväxtval, äggläggning och parning hos den guatemalanska potatismalen *Tecia solanivora*

Dofter, d.v.s. blandningar av flyktiga substanser från såväl växter som insekter, har betydelse för insekters förmåga att orientera sig i landskapet. Dessa substanser utsöndras av växter och insekter och är specifika för varje art. Till följd av att insekter kan uppfatta dessa dofter kan de urskilja en växt från en annan men också avgöra egenskaper och utvecklingsstadier hos växten. Dofter som fungerar inom och mellan arter kallas feromoner och utgörs av specifika blandningar av substanser för varje art. Sexferomoner är substanser som främst honor avger för att locka till sig hanar för parning.

I norra Sydamerika och Latinamerika är den guatemalanska potatismalen, *Tecia solanivora*, (Lepidoptera: Gelichiidae) en skadegörare i potatis, *Solanum* spp. Larven äter av knölar både i fält och i lagerlokaler. Som ett led i forskningen om doftbaserade bekämpnings-metoder, har jag i det här doktorandarbetet undersökt vilka dofter som påverkar den guatemalanska potatismalens beteende. Substanser som avges från potatis vid olika utvecklingsstadier och från olika växtdelar har identifierats. Dessa substanser har sedan relaterats både till honans beteende vid äggläggning samt till honors och hanars sök av värdväxt. Vidare testades olika blandningar av feromonsubstanser för att förstå hur mängdförhållanden mellan substanser i feromonet påverkar hanarna. Både forskningen om potatis-dofter och malferomoner ligger till grund för ökad förståelse för malens doftrelaterade beteende för att i en förlängning av projektet skapa nya bekämpningsmetoder och reducera skörde-bortfall.

Guatemalansk potatismalshona

Metoder för att undersöka doft-relaterat beteende

För att kunna avgöra vilka dofter som *T. solanivora* kan detektera med antennerna gjordes elektroantennografiska studier med potatis i olika utvecklingsstadier. Dofter från potatisknölar, blast respektive blommor identifierades liksom substanser, både flyktiga och icke-flyktiga, i larvskadade och ljusbehandlade knölar. Attraktion till syntetiska substansblandningar testades i en Y-formad "arena", en s.k. olfactometer, där insekternas beteende registrerades när de blev presenterade för olika dofter i en luftström.

Äggläggningsförsök gjordes med potatisplantor i olika stadier, med blast, larvskadade, ljusexponerade eller obehandlade knölar, samt med syntetiska substanser. Larvers överlevnad mättes på tre potatissorter som exponerats för ljus eller inte och som bl.a. innehöll olika höga halter av steroidalkaloiderna α-solanin och α-chaconin.

I låga doser kan det syntetiska feromonet locka hanar, och detta används redan med fällor i prognos- eller bekämpningssyfte. Ytterligare användning av syntetiska feromoner i bekämpning av skadeinsekter sker genom att släppa ut relativt höga halter som stör kommunikationen mellan honor och hanar vilket förhindrar parningen.

Hanars naturliga beteende gentemot lockande honor som avger feromoner är att vifta med vingarna, flyga mot honan och para sig. Detta beteende jämfördes med lockade honor i kombination med höga nivåer av syntetiska feromoner, samt med enbart syntetferomoner. Två proportionella blandningar av potatismalens feromonkomponenter testades i fältförsök, i semi-fältförsök, och i vindtunnel. Med dessa försök ville vi undersöka hur det sexuella beteendet förändras och förhindras. En av de rådande teorierna är att hanen uppfattar de höga syntetiska feromonhalterna som att väldigt många honor lockar samtidigt vilket därmed tävlar med det naturliga feromonet och minskar hanens chans att hitta till honan. En annan teori är att hanens luktsinne påverkas så att honans doft inte längre kan uppfattas eller att hanen blir okänslig för någon av substanserna i feromonblandningarna. För att avgöra hur höga halter av feromon påverkar luktsinnet hos hanar placerades de i lådor med höga koncentrationer av de två feromonblandningarna. Därefter observerades deras sexuella beteende gentemot lockande honor respektive mot starka syntetiska feromonblandningar.

Resultat av hur potatisdofter och sexferomoner påverkar beteendet hos den guatemalanska potatismalen

Resultaten från mina försök visade att potatis avger specifika substanser från de olika växtdelarna och att den guatemalanska potatismalen kan detektera ett tjugotal av dessa ämnen. En syntetisk blomdoft attraherade både hanar och honor, såväl oparade som parade. När honor var placerade i en luftström av samma syntetiska blomdoft lade de fler ägg än när de var utan doft. Knölar attraherade inte malen och den syntetiska knöldoftblandningen var frånstötande. Inte heller den syntetiska blast-doften attraherade. Malen valde dessutom att lägga fler ägg på jord än på blad.

Larvers överlevnad visade sig vara negativt påverkad av höga halter av α-solanin- och α-chaconin, substanser som ökade vid ljusexponering och då larver ätit på knölen. Dofter från oskadade knölar skiljde sig från ljusexponerade och larvskadade knölar. Resultaten visade att honan använde doften av knölarna för att välja äggläggningsplats. Fler ägg blev lagda vid de obehandlade knölarna än vid de stressade. Denna överensstämmelse mellan larvens överlevnadsgrad och honans äggläggningsval bekräftar teorin om samspelet mellan insekter och växter, mellan honans värdväxtval och larvers framgång på värdväxten. Honans val av äggläggningsplats är till stor del avgörande för hur larvens överlevnadsbetingelser kommer att se ut eftersom de inte kan röra sig långa sträckor och överlever inte länge utan föda.

Feromonförsöken visade att höga halter av feromoner gjorde att hanarna inte hittade till de lockande honorna i vindtunneln och att antalet parade honor minskade i semi-fältförsöken. Försöken visade även att blandningarna med feromonsubstanser i olika proportion påverkade antalet fångade insekter. En timme efter att ha befunnit sig i höga doser av de två syntetiska blandningarna, minskade hanarnas attraktion till lockande honor medan beteendet efter 24 timmar liknade det ursprungliga. De två feromonblandningarna påverkade hanarna annorlunda vilket kan få effekter vid användning av denna bekämpningsmetod i fält.

Slutsatser om potatisblommans doftsignaler

Ökad äggläggning och attraktion till blomdoft hos både honor och hanar tyder på att blommorna avger signaler som den guatemalanska potatismalen använder för att hitta sin värdväxt. Blomdoftsignaler är förmodligen inte relaterat till attraktion till födosök i potatisblomman eftersom dessa blommor inte producerar någon nektar. Hanars attraktion till blomdoft kan eventuellt korreleras med en ökad chans att hitta en partner vid potatisplantan eftersom potatisblommor öppnar sig samtidigt som honan lockar. Parade honor använder sannolikt blomdoft som signaler om potatisplantans utveckling, eftersom potatisplantan blommar och sätter knöl samtidigt. Larvens överlevnadsbetingelser är därmed högst då potatisplantan blommar.

Jag har kunnat visa att *T. solanivora* kan skilja mellan variationer i dofter och att de kan skilja på doften hos olika växtdelar av en potatisplanta. Dessutom påverkar doften honans val av äggläggningsplats, vilket ger ytterligare svar på vilka substanser som lockar den guatemalanska potatismalen till potatis. Dofters specifika blandning av substanser, både från växter och insekter är avgörande kännetecken för potatismalen då den skall hitta sin värdväxt och sin partner. Genom att manipulera insekters doftrelaterade beteende med dessa dofter kan nya bekämpningsmetoder skapas. Detta gör att studier som denna, som relaterar dofter till beteende, kan leda vidare mot doftbaserad kontroll av den guatemalanska potatismalen.

El papel de los semioquímicos en la búsqueda de hospedador, oviposición y comunicación sexual en la polilla guatemalteca de la papa *Tecia solanivora*

Los semioquímicos son normalmente mezclas de compuestos que median procesos interespecíficas (aleloquímicos) o intraespecífica (feromonas) entre individuos. Estas señales químicas afectan la conducta de los insectos, sea su orientación hacia el hospedador, atracción, repelencia, oviposición o cópula. En interacciones entre insectos y plantas, estos compuestos juegan un rol fundamental para la localización de plantas hospedadoras y para la distinción entre plantas, sus características y nivel de desarrollo. Las feromonas sexuales son mezclas de compuestos que, en su mayoría, son emitidas por hembras para atraer machos para aparearse. Las sustancias emitidas por las plantas e insectos son especie-específicas.

La polilla guatemalteca de la papa, *Tecia solanivora* (Lepidóptera: Gelechiidae) es una plaga en *Solanum* spp. en el norte de América Latina. Las larvas de estos insectos se alimentan de los tubérculos en el campo y en los lugares de almacenamiento. Basado en la importancia de esta plaga, la presente tesis de doctorado forma parte de la investigación de posibles métodos de control mediante el uso de compuestos volátiles. El trabajo estudia el comportamiento de la polilla en relación al su uso de olores para reconocimiento de su planta hospedadora y de sus conspecíficos.

Las respuestas de los machos frente a hembras que emiten feromonas, incluye una secuencia de comportamientos: aleteo, vuelo hacia la hembra y subsecuente apareamiento. Formulaciones sintéticas de feromonas atrae a los machos cuando son emitidas en concentraciones bajas. Esto puede ser aplicado en trampas con el propósito de monitorear o controlar la plaga. El empleo de altas concentraciones de formulaciones sintética de feromonas interrumpe la comunicación entre machos y hembras, lo que impide el apareamiento. Ambos utilidades de feromonas son importantes en el manejo de plagas. El uso de compuestos volátiles de plantas hospedadoras está incrementando en importancia en el manejo de plagas.

Métodos para investigar comportamientos mediados por claves olfativas

El trabajo consistió en la identificación y uso de compuestos emitidos por plantas de papa en diferentes niveles de desarrollo y por las diferentes partes de la planta. Estos compuestos han sido relacionados, tanto con el proceso de oviposición, cuanto con la búsqueda de la planta hospedadora por parte de la hembra y el macho. Así mismo, se evaluó dos formulaciones de componentes de la feromona para estudiar cómo afectan a los machos diferentes proporciones relativas de los componentes de la feromona. Los estudios sobre olores de la papa y feromonas de la polilla tienen como fin de incrementar la comprensión de su comportamiento, para permitir un futuro desarrollo de métodos de control de la polilla guatemalteca de papa.

Con el propósito de determinar cuales sustancias eran detectadas por las antenas de *T. solanivora* se realizó estudios de electroantenografía. Se empleó compuestos emitidos por la papa en sus diferentes etapas fenológicas y compuestos emitidos por tubérculos, hojas o flores de las plantas de papa. Además sustancias volátiles, así como no-volátiles, fueron estudiadas en tubérculos de papa afectados por la larva de *T. solanivora* y expuestos a luz.

Los compuestos identificados fueron seguidamente evaluados en su capacidad de modificar el comportamiento de la polilla. La atracción inducida por mezclas sintéticas de estos compuestos fue evaluada en un olfatómetro en tubo-Y, donde se registró el comportamiento de los hembras y machos presentándoles diferentes formulaciones de compuestos en un corriente de aire. Fueron realizados ensayos de oviposición para determinar qué sustancias son utilizadas por las hembras para la elección de sitios de oviposición. Como fuente de volátiles se empleó plantas de papa en distintas etapas fenológicas, tubérculos afectados por larvas de *T. solanivora* o expuestos a luz. Similarmente, en los ensayos de oviposición se empleó imitaciones versiones sintéticas, de las sustancias previamente identificadas en las plantas de papa. Adicionalmente, en otros ensayos fue evaluada la supervivencia de larvas de esta polilla en tubérculos de distintas cualidades. Para eso se empleó tres variedades de papa, en dos tratamientos de iluminación diferentes que presentan diferentes contenidos de compuestos glicoalcaloides como la α-solanina y α-chaconina.

El objetivo de los experimentos de feromona sexual fue de caracterizar el mecanismo por el cual se promueve la disrupción de copula. El comportamiento de los machos hacia fuentes de feromona fue comparado en varios situaciones: con hembras llamando, hembras llamando en combinación con altos niveles de feromona sintética, o con feromona sintética únicamente. Los mismos dos mezclas con distinta proporción relativa de componentes de la feromona fueron probados en ensayos de campo, semi-campo y túnel de viento. Además se realizó ensayos que consistieron en colocar machos en jaulas con altas concentraciones de las dos mezclas de feromonas para observar el comportamiento (1 y 24 h después) hacia hembras llamando y hacia las mezclas altas de feromona sintética.

Resultados del estudio

Los resultados de los estudios de electro-antenografia y cromatografía de gases indicaron que las diferentes partes las plantas de papa emiten sustancias específicas y que la polilla puede detectar al menos veinte de estos compuestos. Cuando se evaluó una versión sintética del olor de flor fueron atraídos machos y hembras, tanto vírgenes como copulados. Cuando las hembras fueron colocadas en una corriente de aire llevando el mismo olor floral, hubo mayor oviposición que en su ausencia. No hubo atracción cuando tubérculos u olor sintético de tubérculos de papa fueron utilizados. Un resultado semejante fue observado cuando fue usado olor sintético de follaje. Se observó también que las polillas prefirieron oviponer en el suelo, antes que en el follaje o cerca de fuentes de olor de follaje.

Cuando larvas de *T. solanivora* fueron sometidas a altos niveles de α-solanina y α-chaconina, su sobrevivencia fue afectada negativamente. El contenido de dichas sustancias además incrementó en los tubérculos al ser sometidas a luz y al larvas alimentándose de los tubérculos. Los resultados indicaron que las hembras utilizan el olor de los tubérculos para seleccionar los sitios de oviposición". Asimismo, se pudo observar que las hembras distinguieron tubérculos dañados expuestos a luz o afectadas por larvas. Por lo tanto el hecho de haber una mayor supervivencia de las larvas en los mismos sitios preferidos por la hembras para oviponer, es consistente con las suposiciones de la teoría del forrajero óptimo. La elección materna de sitios de oviposición determina la probabilidad de la descendencia de encontrar alimento adecuado, dado que las larvas no pueden recorrer largas distancias y no sobreviven por mucho tiempo sin alimentarse.

Cuando se realizaron pruebas con altos niveles de feromona, tanto en túnel de viento, como en semi-campo y campo, se observó que el vuelo hacia la hembra, el encuentro y la cópula de los machos hacia las hembras disminuyó. Por otro lado, se observó que los mecanismos de la disrupción de cópula actúa diferente con los dos mezclas de feromona. Las mecanismos sugeridas para las mezclas fueron de competencia y de no-competencia. El mecanismo de competencia ocurre cuando los machos siguen las plumas de feromona sintética como falsas hembras en comportamiento de llamado. La teoría de no-competencia se basa en que las altas dosis de componentes de la feromona afectan el olfato de los machos, creando una percepción de los compuestos incorrecta o desequilibrada. Esto hace que el macho no percibe los señales emitidos de las hembras. Nosotros proponemos que los machos fueron afectados por las mezclas de feromonas de diferentes maneras, lo que puede tener efectos cuando se utiliza el método de disrupción de copula en el manejo de la polilla.

Conclusiones sobre las señales del olor

La atracción tanto de hembras como de machos frente a olor de flores de *S. tuberosum*, así como el aumento de la oviposición, sugiere que las flores emiten señales que permiten que la polilla guatemalteca localice a su planta hospedadora. Es probable que este olor no sea utilizado como una señal de fuente de alimentación, ya que la flor de papa carece de néctar. Es también probable que la respuesta frente a este olor esté altamente relacionada con el hecho de buscar un conspecífico para la cópula, debido a que la planta de papa florece en la mañana, momento en que normalmente ocurre la cópula en estas polillas. Asimismo, estos olores representarían un señal de la disponibilidad de alimento para las larvas y, por lo tanto, de un potencial aumento de su probabilidad de sobrevivencia, ya que el periodo de floración ocurre simultáneamente con el de tuberización.

Este trabajo confirma que *T. solanivora* es capaz de diferenciar olores provenientes de diferentes partes de la planta de papa. La especificidad de los olores emitidos por plantas e insectos es fundamental para que estos encuentren su planta hospedadora o un conspecífico. Al poder manipular los comportamientos controlados por estos olores, será posible desarrollar nuevas estrategias de manejo de esta plaga. Por lo tanto, estudios como el presente podrán permitir el control de la polilla guatemalteca basado en el uso de semioquímicos.