

Pheromone-mediated Communication
Disruption in Guatemalan Potato Moth,
Tecia solanivora Povolny

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Abstract

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The Guatemalan potato moth, *Tecia (Scrobipalopsis) solanivora*, is a serious potato pest in Central America and adjacent South American countries. Recently, it has been introduced to the Canary Islands, Spain. Insecticide treatments are not sufficiently effective against eggs and larvae of Guatemalan potato moth, which are protected from sprays in soil crevices and in galleries inside potato tubers, respectively. Deregulation of insecticides and restrictions in the use of the few available insecticides, parallel to a public demand for reduction of pesticide use in potato production, have increased the interest in biological control techniques.

Behaviour-modifying chemicals, including sex pheromones, which target the adult life stage, are a particularly promising alternative to insecticides for control of Guatemalan potato moth. The aim of this research was to study *T. solanivora* sex pheromone and to investigate the feasibility of pheromone-mediated mating disruption as environmentally safe control technique.

Chemical analysis of female pheromone gland extracts confirmed two previously identified compounds (*E*)-3-dodecenyl acetate (*E*3-12Ac) and (*Z*)-3-dodecenyl acetate (*Z*3-12Ac), plus an additional, saturated compound, dodecyl acetate (12Ac). These three compounds elicited significant male antennal responses by GC-EAD. Field trapping studies showed that a 100:1:20-blend of these compounds formulated at 1000 µg on rubber septa, captured more males than the main compound alone. This trap lure can accordingly be used in field traps for detection and population monitoring. During two years, potato fields were treated with a 100:56:100-blend of the three pheromone compounds formulated in polyethylene tube dispensers. Male attraction to *E*3-12Ac and *Z*3-12Ac is optimal at a 100:1 blend ratio, and only few males were attracted to traps baited with a 100:50-blend. Mating disruption pheromone dispensers, containing these two compounds in a 100:56 ratio, did not attract males in the wind tunnel or in the field. The application rate of the 100:56:100-blend of the three pheromone compounds was 28 g/ha in the first, and 86 g/ha in the second year. In both years, *T. solanivora* male attraction to synthetic pheromone traps were almost completely suppressed, indicating that sexual communication was disrupted. During the second year, additional tests confirmed that the pheromone treatment prevented mate-finding and mating. Attraction to traps baited with live calling females was reduced by 89% in the pheromone treatment, during two months. In addition, matings in small field cages were significantly reduced. Visual observations showed that few males were observed in the pheromone-treated, as compared to the control field. At harvest, potato infestation was significantly lower in the treated field, as compared to control. Future studies will aim at the optimization of dispenser formulation, and area-wide implementation of the mating disruption technique for control of Guatemalan potato moth.

Keywords: Sex pheromone, Guatemalan potato moth, *Tecia (Scrobipalopsis) solanivora*, potato - *Solanum tuberosum*, chemical analysis, electrophysiology, wind tunnel, field trapping, mating disruption.

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Contents

Introduction, 7

Guatemalan potato moth, 7

Life cycle

Current management

Mating disruption, 9

Objectives, 10

Methods, 11

Laboratory studies, 11

Pheromone gland extraction

Chemical analysis

Antennal recordings

Wind tunnel tests

Field studies, 13

Field trapping

Behavioural effect of isomers

Orientation disruption

Results, 15

Sex pheromone of Guatemalan potato moth (Paper I), 15

Development of a monitoring lure (Paper I), 15

Wind tunnel

Field trapping

Pheromone-mediated communication disruption (Paper I and II), 16

Effect of isomer blend ratio on male attraction

Communication disruption

Traps baited with females

Mating of caged females

Behavioural observations

Potato infestation

Conclusions and Outlook, 21

References, 22

Acknowledgements, 24

Appendix

Papers I-II

This thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Bosa, C.F., Cotes, A.M., Fukumoto, T., Bengtsson, M., and Witzgall, P., 2005. Pheromone-mediated communication disruption in Guatemalan potato moth, *Tecia solanivora*. *Entomologia Experimentalis et Applicata* 114, 137-142.

II. Bosa, C.F., Cotes, A.M., Osorio, P., Fukumoto, T., Bengtsson, M., and Witzgall, P., 2005. Orientation disruption of the Guatemalan potato moth *Tecia solanivora* (Lepidoptera: Gelechiidae) by sex pheromone: flight tunnel and field studies. Submitted.

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Introduction

Guatemalan potato moth

The Guatemalan potato moth *Tecia (Scrobipalopsis) solanivora* (Povolny, 1973) was introduced to South America by imported potato tubers from Costa Rica, Central America, first to Venezuela in 1983, to Colombia in 1985, and to Ecuador in 1998 (Pollet, 2001; Pollet et al. 2003) (Figure 1). Recently this pest has also been detected in the Canary Islands in Spain (Pemberton, 2001; Barragán et al. 2004).



Figure 1. Guatemalan potato moth, female (left) and male (right) (Photo by Felipe Bosa).

Larvae of *T. solanivora* damage potato tubers both in the field and in storage facilities. The larvae bore into the potatoes, producing conspicuous galleries and destroying the entire tuber (Figure 2). Infestation by this insect pest may compromise 50% or up to 90% of the tuber production at harvest. Chemical insecticides are inadequate for control of *T. solanivora*, and continuous spraying with all available compounds has significant impact on the environment and human health (Hilje-Quir, 1994). Losses of up to 150 million dollars/year in Central and South America demand the development of more efficient and sustainable control methods (Hilje and Cartin, 1990).



Figure 2. Potato infested with Guatemalan potato moth, *Tecia solanivora* (Photo by Felipe Bosa).

Life cycle

Egg: Gravid females oviposit on the base of potato stems, preferably underground, in soil crevices and on tubers that have not been well covered with soil. Eggs are semi-spherical and ca. 0.5 mm in diameter. In the beginning they are white, but turn brown shortly before hatching (Torres et al. 1997). **Larva:** The larval stage comprises four instars, which develop inside the potatoes. First instar larvae migrate towards the potato tubers and produce galleries under the epidermis. Larvae are about 1.3 mm long, they are transparent white with a dark brown head. The second instar exhibits a creamy white color, making superficial galleries under the tuber surface. During the third instar, larvae exhibit a greenish cream color and they start to build deeper galleries. At the fourth instar, larvae exhibit greenish color on the ventral area, pink color on the dorsal area, and they are 14 to 16 mm long. Mature larvae abandon tubers to pupate (Torres et al. 1997). *T. solanivora* larvae have black spots in a trapezoidal shape on the lateral area of each thoracic and abdominal segment, which represent an important morphologic characteristic for differentiation from the potato moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) (Salazar and Torres, 1986). **Pupa:** Larvae produce a cocoon using particles of soil and fibers. Cocoons are brown, the pupal stage lasts 15-18 days. There is no diapause reported, neither in the larval nor in the pupal stage. **Adult:** There is a marked sexual dimorphism in male and female moths (Figure 1). Females are ca. 12 mm long and 3.4 mm wide, while males are 9.7 mm long and 2.9 mm wide. The female forewings show a pattern of longitudinal bands from the apex to the end, a female is clearly distinguished by its bulky abdomen, unlike the slim abdomen in

males. Adults exhibit a negative phototropism and remain hidden under potato foliage, or in soil crevices during day time. Matings may take place on the day after emergence, and females lay 156 - 250 eggs, during ca. 10 days (Notz, 2002).

Current management

The hidden lifestyle of adult moths, together with oviposition on potato stems in the soil and larval feeding inside potato tubers render control of Guatemalan potato moth with insecticides difficult (Inoue et al. 1994, Notz, 2002). One larva is enough to destroy a potato tuber. This pest is active during the whole year under tropical conditions, population growth is favored by continuous potato production all year round. Populations are highest in the dry season, when *T. solanivora* can destroy the entire crop. Generally, 12 – 24 sprays per potato crop are used to keep population densities down (Hilje-Quir, 1994). In addition to secondary problems such as insecticide resistance, destruction of natural enemies and non-target organisms, there is considerable accumulation of pesticide residues in soil and ground water.

Insufficient knowledge of *T. solanivora* biology and life cycle adds to the difficulty to design appropriate management strategies. There are some agronomical measures which can help to reduce damage. However, these are not sufficient for population control. Irrigation shortly before onset of tuber production, and removal of all infested potatoes after harvest are nonetheless important measures. Another factor which renders control of Guatemalan potato moth more difficult is that there are virtually no natural enemies in areas, where this insect has been newly introduced. Traps baited with synthetic pheromone are used for detection and monitoring (Salazar and Torres, 1986; Fernández, 1996).

Mating disruption

Insects use sex pheromones to communicate for mating. Pheromones elicit strong behavioural reactions at minute amounts. By permeating the atmosphere with synthetic pheromones, olfactory communication and mate-finding can be prevented. Sex pheromone blends of moths are species-specific, non-toxic, and control by mating disruption will therefore only affect the pest species. Beneficial predators, and parasitoids are left unharmed.

The mating disruption technique is based on the knowledge of the chemical composition of the sex pheromone of the species to be controlled. It also relies on a homogenous dispersal of synthetic pheromone in the crop during the insect's entire flight period.

Mating disruption dispensers, therefore, must have a sufficient release of pheromone over at least several weeks (Witzgall and Arn, 1997). Last not least, the cost of the pheromone treatment has to be competitive with conventional pest control.

As the use of insecticides is increasingly restricted, growers depend on the development of new, non-toxic techniques. Mating disruption is commercially available for control of more than a dozen moth species and is used world-wide on approximately 200.000 ha (Ridgway et al. 1990). However, mating disruption in *T. solanivora* has not been studied.

Aims of the present study

This study was done to develop mating disruption as a reliable and environmentally sustainable strategy for control of Guatemalan potato moth. I have investigated *T. solanivora* sex pheromone compounds by chemical analysis, wind tunnel experiments and field tests. Subsequently, synthetic pheromone was formulated in dispensers for field trials, concerning the development of a trap lure and pheromone-mediated communication disruption for population control of Guatemalan potato moth.

Methods

The following methods were used to study the sexual chemical communication and mating disruption in *T. solanivora*.

Laboratory studies

Pheromone gland extraction

Insects were mass-reared on fresh potatoes at 18-22°C, under a controlled photoperiod. Pupae were separated by sex according to their number of abdominal segments. Adult insects were kept in 33 x 33 x 33 cm glass cages. Female moths were observed to start shortly releasing sex pheromone after the beginning of the photophase. Pheromone glands were extracted from the abdominal tips of 2-3-day-old calling virgin females in batches of 30-50 glands (n=5) in 7 µl of redistilled heptane, and during the first 30-45 minutes of calling. Gland extracts were then used for chemical and electrophysiological analysis.

Chemical analysis

Compounds in gland extracts were identified using a Hewlett Packard 5970B (Hewlett-Packard, Palo Alto, CA) mass spectrometer (MS) with electron impact ionization (70 eV), interfaced with a Hewlett Packard 5890 gas chromatograph (GC), equipped with a polar DB-WAX column (30 m x 0.25 mm; J&W Scientific, Folsom, CA, USA). Retention times of the identified gland compounds were compared with synthetic compounds on a Hewlett Packard 5890 GC, with flame ionization detection, on a DB-WAX column, and on a non-polar SE-54 column (25 m x 0.32 mm; Kupper & Co., Bonaduz, Switzerland). The columns were programmed from 80°C (2 min hold) at 10°C/min to 220°C (10 min hold).

Antennal recordings

The male response to sex pheromone compounds from female gland extracts was studied using an antennae as sensor. The recorded signal obtained from the entire antenna is termed electroantennogram (EAG). For EAG recordings, an excised cut antenna is suspended between two electrodes connected to an amplifier. When a relevant odour stimulus is applied, a change in potential (depolarisation) occurs over the antenna, which is measured and recorded. The antennal response is dose-dependent, and the EAG amplitude increases with the stimulus amount.

An extended application of the EAG technique is to use the antennal detector in parallel with a conventional flame ionisation detector (FID) of a gas chromatograph (GC). Splitting the outlet of the gas chromatograph between an antenna and the flame ionisation detector allows a simultaneous recording of both chemical and physiological information. Coupled gas chromatographic-electroantennographic detection (GC-EAD), provides information about the antennal response to each compound in a pheromone extract. One can thus distinguish potentially bioactive compounds and to identify them (Arn et al. 1975).

Freshly prepared extracts from five female glands were analyzed by coupled gas chromatography and electroantennographic detection (GC-EAD) ($n = 3$), on a Hewlett Packard 6890 GC with a 30-m, 0.25-mm ID, HP-INNOWAX capillary column, interfaced with an electroantennogram apparatus (Syntech, Hilversum, The Netherlands). One arm of the split column led into a glass tube (ID 8 mm), with a charcoal-filtered and humidified air stream (0.5 l/min). Two-day-old males were used for electrophysiological recordings. Male antennae were placed at 0.5 cm from the end of this glass tube, 30 cm from the EAD-outlet of the GC. The antennae were cut at the base, the antennal tip was left intact, and they were mounted between two glass pipette electrodes containing Beadle-Ephrussi Ringer solution (Beadle and Ephrussi, 1936). The GC was operated in splitless injection mode, the oven was programmed from 50°C (2-min hold), at 10°C/min to 230°C. Injector and EAD-outlet temperature was 220°C, the split ratio between FID and EAD was ~ 1:1.

Wind tunnel tests

Electrophysiological recordings offer information about the antennal detection of pheromone compounds. However, bioassays are required to reveal the behavioural role of the compounds showing antennal activity. Male attraction to the synthetic compounds identified in the pheromone gland extracts was tested in a wind tunnel similar to the one described by Witzgall et al. (2001). The wind tunnel was lit diffusely from behind and above at 6-12 lux. The wind speed was 30 cm s⁻¹, and air temperature ranged from 18-21°C.

Synthetic compounds were formulated in various blends on red rubber septa for wind tunnel tests. Single two-day-old virgin males were transferred to glass tubes (2.5 x 15 cm long) covered with gauze at both ends, 5 min before the tests. Males were placed individually into the wind tunnel at the beginning of the photophase, and the following male behaviours were recorded: activation, taking flight, upwind flight over 100 cm towards the source, landing on the stimulus source, and wing-fanning at the source. Calling females

were always used as a reference pheromone source and were held individually in glass tubes (2.5 x 5 cm), covered with gauze at both ends.

Polyethylene tubes formulated with pheromone were used for mating disruption in the field (see below). Attraction of males to these mating dispensers, alone and in combination with calling females was tested in the wind tunnel.

Field studies

Field trapping

Field trapping tests with synthetic sex pheromone compounds were done to verify the results obtained in the wind tunnel and to optimize an attractant blend for male monitoring. Synthetic lures were prepared by formulating compounds on red rubber septa (Phero.Net AB). Tetra traps (Figure 3; Arn et al. 1979) with these rubber septa baited were placed at 40 cm above ground in potato fields at Mosquera, Colombia, using a randomized block design. Male captures were checked once a week.



Figure 3. Trap baited with a rubber septum.

Behavioural effect of isomers

Different blends of the geometrical isomers (*E*)-3-dodecenyl acetate (*E*3-12Ac) and (*Z*)-3-dodecenyl acetate (*Z*3-12Ac) were tested at two dosages for male field attraction at Mosquera, Colombia.

Orientation disruption

A 100:56:100-blend of *E*3-12Ac, *Z*3-12Ac and dodecyl acetate (12Ac) was formulated at 70 mg in 20-cm high-density polyethylene tube dispensers (Shin-Etsu Chemical Co., Tokyo). Antioxidants and UV-absorbers were added at 2%. The chemical purity of the isomer blend of Δ 3-12Ac was 93.6%, and to 12Ac was 98.8% pure.

Field tests were done at Mosquera, Colombia. During the first year, a 2.5-ha potato field was treated in December with 400 dispenser/ha (28 g active ingredient/ha). Dispensers were twisted around stems at 5 cm from the ground. Orientation communication disruption was assessed by pheromone traps, baited with an optimized 3-component blend (100:1:20- μ g of *E3-12Ac*, *Z3-12Ac* and *12Ac*) as compared to an untreated field. Traps were checked once a week during two months.

During the second year, a 3-ha potato field was treated with the dispensers in June, before flowering. The main growing season of potato in Colombia normally lasts from March to September. Dispensers were applied at a density of 1227 dispenser/ha (application rate of 86 g active ingredient/ha). A 1-ha control field was 1 km away. The effect of the pheromone treatment was determined by captures of males in pheromone traps (1000:10:200- μ g of *E3-12Ac*, *Z3-12Ac* and *12Ac*), in traps baited with live calling females, matings of females and males confined in 100 x 43 x 43 cm field cages, potato infestation at harvest, and visual observations of male moth behaviour.

Results

Sex pheromone of Guatemalan potato moth (Paper I)

The sex pheromone of *T. solanivora* has been reported to contain two compounds, the main compound (*E*)-3-dodecenyl acetate (*E*3-12Ac) and its geometrical isomer (*Z*)-3-dodecenyl acetate (*Z*3-12Ac) (Nesbitt et al. 1985).

Chemical analysis of pheromone gland extract by GC-MS, showed the presence of an additional pheromone-related compound, dodecyl acetate (12Ac). The compounds were identified according to their retention times on different GC columns, and according to their mass spectra, in comparison with standards. Glands of calling females contained on average 0.05 ± 0.01 ng of the *E*3-12Ac, $2.1 \pm 4\%$ of the *Z*-isomer, and $8 \pm 4.3\%$ of the saturated 12Ac. In GC-EAD recordings, the male antennae responded to peaks matching the retention times of 12Ac and the isomers of Δ 3-12Ac.

Development of a monitoring lure (Paper I)

Wind tunnel

In 2003, behavioural assays were conducted in the wind tunnel. At a dose of 1 μ g and 10 μ g of the main compound *E*3-12Ac on rubber septa, the number of males landing at the source was not affected by the addition of 1% or 5% of the *Z*-isomer. The saturated 12Ac added at 100% to 1 μ g of *E*3-12Ac, with or without 5% *Z*3-12Ac, had no effect on male attraction. Significantly more males flew upwind and landed on septa containing 10 μ g, as compared to 1 μ g of the main compound. Male landings at calling females (control), and at 10 μ g *E*3-12Ac, with or without 1% or 5% addition of *Z*3-12Ac, were not significantly different.

Field trapping

The effect of the minor compounds identified from female glands on male trap captures was studied by field trapping tests. At the lowest dose of the main compound *E*3-12Ac (10 μ g), the addition of the *Z*-isomer and/or the saturated 12Ac at 20% or 100% had no significant effect on trap capture. However, most males were attracted to lures containing *E*3-12Ac, 1% *Z*-isomer and 20% 12Ac (Table 1).

Table 1. Field attraction of Guatemalan potato moth males, *T. solanivora*, to traps baited with compounds identified from female pheromone glands (n = 10 replicates per blend). Number of males within one test followed by similar letters are not significantly different at P=0.05 (ANOVA, followed by Tukey's test).

Compounds	(µg)							
E3-12Ac	10	10	10	10	10	10	10	10
Z3-12Ac		0.1	0.5			0.1	0.5	0.5
12Ac				2	10	2	2	10
Males/trap								
I ^a	12 ab	-c	9 ab	27 a	25 a	-c	6 b	5 b
II ^b	163 a	169 a	157 a	196 a	-c	203 a	-c	-c

^a Colombia, November 2003 to January 2004.

^b Colombia, March to April 2004.

^c Blend not included in test.

At the highest dose tested (1000 µg), the main compound E3-12Ac attracted more males than at 100 µg (Table 2). Male captures with the 100:1:20-blend of E3-12Ac, Z3-12Ac and 12Ac were significantly different from the main compound only at 1000 µg, and not at 10 or 100 µg (Tables 1 and 2). The effect of the minor compounds became accordingly more important at the highest dose of the main compound. Most males were captured with the 100:1:20-ratio of E3-12Ac, Z3-12Ac and 12Ac, both at 100 and 1000 µg (Table 2).

Table 2. Field attraction of Guatemalan potato moth males, *T. solanivora* (n = 10 replicates per blend). Numbers followed by different letters are significantly different at P=0.05 (ANOVA, followed by Tukey's test).

Compounds	(µg)					
E3-12Ac	100	100	100	1000	1000	1000
Z3-12Ac		1	1		10	10
12Ac			20			200
Males/trap ^a	175 c	176 c	214 c	244 b	325 ab	357 a

^a Colombia, March to April 2004.

Pheromone-mediated communication disruption (Paper I and II)

Effect of isomer blend ratio on male attraction

Traps baited with a blend of 10 µg E3-12Ac and 1% of the Z-isomer, or 10 µg of the E-isomer alone captured more males than the 10:5-blend. At a dose of 1000 µg of the main compound, differences were no longer significant, although, the lowest number of males were captured with the 100:50%-blend (Figure 4). The Z-isomer had accordingly an antagonist effect at the higher proportion.

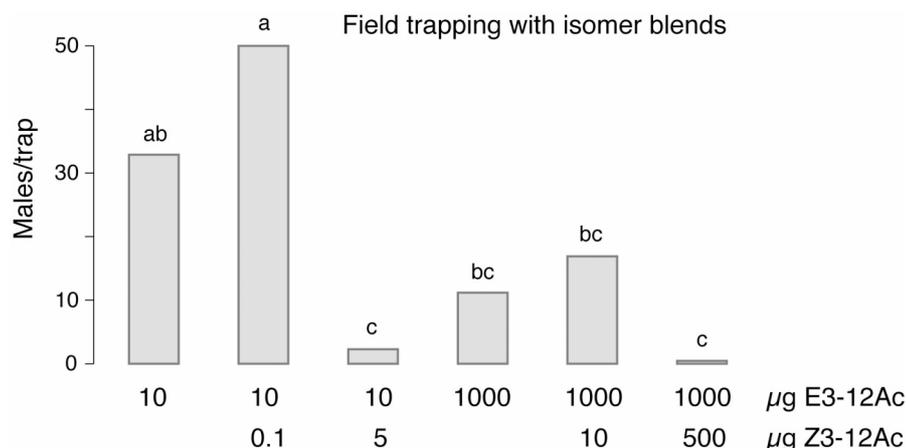


Figure 4. Field attraction of Guatemalan potato moth males, *T. solanivora*, to rubber septa baited with the isomer blends of $\Delta 3$ -12Ac. Columns followed by different letters are significantly different at $P < 0.05$, (ANOVA, followed by Tukey's test, $F = 8.64$; $df = 35$; $P < 0.0001$, $n = 10$ traps per blend). Field test at Mosquera (Colombia), May to July 2005.

Technical synthesis leads to a 100:56-blend of *E* and *Z*3-12Ac, which is formulated together with 12Ac, at 70 mg in polyethylene tube dispensers used for mating disruption. In a wind tunnel experiment, these dispensers did not attract males, compared to 50% males landing at calling females (control). When one such dispenser was placed at 25 cm from 10 calling females, male attraction to females was inhibited. The mating disruption dispenser efficiently masked the female pheromone plume (Table 3).

Table 3. Wind tunnel attraction of *T. solanivora* males to different pheromone sources ($n = 80$ individuals per source).

Pheromone sources	One tube dispenser	Dispenser plus calling females ^a	Calling females ^a
Behaviour	Percentage of males ($n=80$) ^b		
Activation	41 ± 4.8 b	49 ± 0.5 b	85 ± 1.7 a
Taking flight	34 ± 4.6 b	42 ± 1.3 b	83 ± 1.6 a
Upwind flight ^c	0 b	0 b	60 ± 4.2 a
Landing	0 b	0 b	50 ± 3.4 a
Wing-fanning	0 b	0 b	45 ± 5 a

^a Ten calling females were used as a reference source of pheromone.

^b Percentages within one behavioural category followed by different letters are significantly different at $P < 0.05$, (ANOVA, followed by Tukey's test). For activation: $F=10.4$, $P=0.0045$, $df=11$; for taking flight: $F=13.01$, $P=0.0022$, $df=11$.

^c Upwind flight over 100 cm towards the source.

Communication disruption

In 2004, a 2.5-h potato field was treated with the 100:56:100-blend of E3-12Ac, Z3-12Ac, and 12Ac at an application rate of 28 g ha⁻¹ (400 dispenser/ha). Male *T. solanivora* attraction to synthetic pheromone traps was almost completely suppressed for 2 months, 1.3 and 5.3 males/trap were captured in January and February, respectively. In comparison, traps captured 233 and 323 males/trap in the control field, during these two months. However, a question which remained to be answered was whether sexual communication disruption and matings were also disrupted in *T. solanivora*, in addition to male orientation to the synthetic pheromone traps.

In 2005, a 3-ha potato field was treated with the disruptant blend on 4 June at an application rate of 86 g ha⁻¹ (1227 dispenser/ha). In the pheromone-treated field, male captures were significantly reduced (94% catch reduction) as compared to 1-ha control field (Figure 5). Shortly before harvest (10 weeks after treatment), captures started to increase in treated and control field (4.12 and 17.7 males per trap, respectively) (Figure 5). Increasing male captures in the pheromone-treated field probably indicate that the dispensers may have been depleted after 3 months of field exposure.

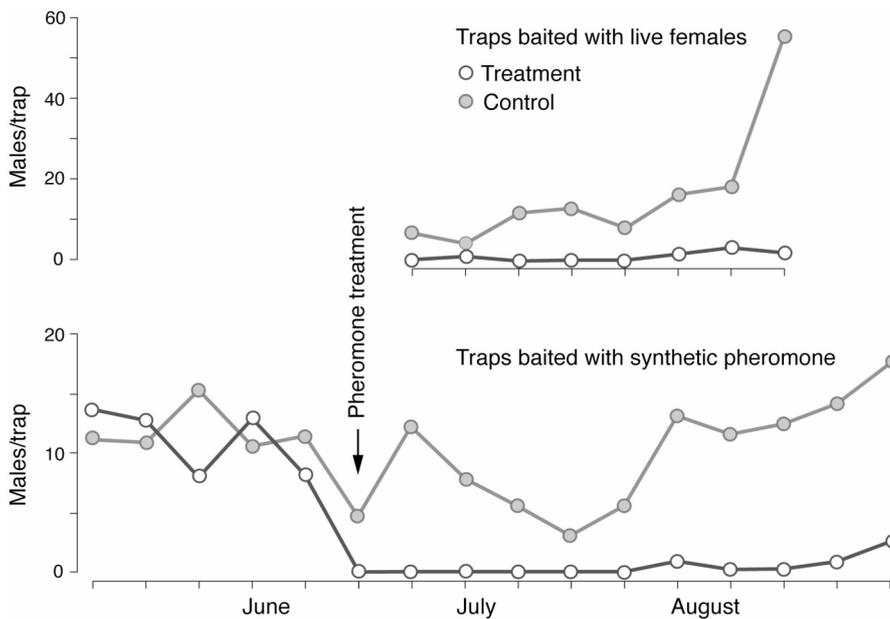


Figure 5. Field male attraction of GPM *T. solanivora* to synthetic pheromone and live calling female traps in a pheromone-treated and control field, 2005. Significant differences were determined between the treatment and control field for both trials at $P < 0.05$, (ANOVA, followed by Kruskal-Wallis' test). For synthetic pheromone traps: before pheromone treatment $F = 0.01$, $P = 0.9267$, $df = 31$; after treatment $F = 47.91$, $P < 0.0001$, $df = 21$. For live female traps: $F = 33.89$, $P < 0.0001$, $df = 15$.

Traps baited with females. Communication disruption was also assessed by placing traps with live virgin females in the field. In the pheromone-treated field, significantly fewer males were captured a total of 13.6 males/trap (89% catch reduction), in comparison with the control field, 130.5 males/trap. At the end of August, 3 and 56 males per trap were captured in the treated and control field, respectively (Figure 5). Some males were capable of locating calling females in traps, showing that sexual communication was not totally suppressed by the pheromone treatment.

Mating of caged females. Disruption of short-range communication was tested with caged females and males. Dissection of females after 4 days of field exposure for presence of spermatophores, indicated a significant disruption of mating, even at a short range in the pheromone-treated field 23% of mated females as compared to 70% in the control, 1 couple per cage, n=30 couples per field (See Paper II for details).

Behavioural observations. Visual observations were done to compare male flight behaviour in control and pheromone-treated field. Males of Guatemalan potato moth become active at sunrise, which facilitates behavioural observations. Flying males were seen between 5:00 to 6:15 a.m. While light intensity increases male flight activity ceases at ca. 6:15 am. In the control field, males were seen flying actively around pheromone traps (17.4 males per trap), and fewer males were seen close to live female traps (2.2). In the pheromone-treated field fewer males (0.4 and 0.2 males/trap) were observed to be attracted towards female, and to synthetic traps. This observation is in line with/confirms the strong reduction in trap catch (Figure 5).

The onset of female calling behaviour is triggered by the onset of the photophase. Females in traps were calling, releasing pheromone during up to 45 min. Males were not seen to fly towards disruptant dispensers, neither in the treatment nor in the control field.

Potato infestation. Mating disruption was also assessed by crop damage. A first sample in July showed that 14% of the tubers were damaged in the treatment, in comparison with 8% in the control field, which was not significantly different (Table 4). This was probably due to late dispenser application. Dispensers were applied before flowering, which coincides with tuber initiation, and first-instar larvae could probably feed on small tubers. The second sample in August, showed a lower infestation rate in the treatment, as compared to the control. At the third sample in September, differences between the treated and control field were significant. In addition, the potato yield was higher in the pheromone-treated than in the control field (Table 4).

Table 4. Percentage of infested tubers by *T. solanivora* larvae in the pheromone-treated and control field during three sampling periods.

Infestation (%)	July ^a (Mean ± SD)	August ^b (Mean ± SD)	September ^c (Mean ± SD)	Tubers yield (kg ha ⁻¹)
3-ha Treated	14 ± 19 a	4 ± 7 b	9 ± 15 b	9660
1-ha Control	8 ± 9 a	13 ± 9 a	19 ± 19 a	7552

Means within a column followed by the same letter are not significantly different from each other, $P < 0.05$, (ANOVA, followed by Tukey's test).

^a $F = 1.31$; $df = 23$; $P = 0.2643$ ($n = 12$ samples per field).

^b $F = 11.59$; $df = 55$; $P = 0.0013$; ($n = 28$ samples per field).

^c $F = 6.22$; $df = 74$; $P = 0.0149$ ($n = 45$ samples in the 3-ha field, and $n = 30$ samples in the 1-ha field).

The pheromone treatment led accordingly to a significant reduction of potato damage. The infestation rate is related to the number of males trapped (Figure 5).

Conclusions and outlook

The sex pheromone of Guatemalan potato moth is a blend of (*E*)-3-dodecenyl acetate, (*Z*)-3-dodecenyl acetate and dodecyl acetate. A 100:1:20-blend formulated on rubber septa is a reliable field lure, which can be used for detection and population monitoring.

The *Z*-isomer had an antagonist effect on male attraction at an elevated proportion (100:50-blend). However, the behavioural mode of action of the *Z*-isomer needs to be further investigated, especially with respect to mating disruption treatments.

A 100:56:100-blend of the three pheromone compounds in potato fields, reduced male attraction to live calling females. Moreover, the pheromone treatment decreased infestation rates by Guatemalan potato moth. However, the mechanisms underlying short-range communication in pheromone-treated potato fields are not fully understood.

Disruption of mating with/by pheromones has not been reported earlier for *T. solanivora*. The species-specific and non-toxic characteristics of semiochemicals, make them suitable tools in integrated pest management being successfully applied against many species (Ridway et al. 1990; Cardé and Minks, 1995). Nevertheless, sex pheromone only permit manipulation of males, and secondary plant metabolites can affect the behaviour of both males and females (Tasin, 2005). Therefore, complementary knowledge about how plant compounds affect insect behaviour may lead to additional strategies for the control of this serious potato pest.

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