Evaluation of a Synthetic Pheromone Dispensers to Control the Potato Moth, Tecia Solanivora (Lepidoptera: Gelechiidae)

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Abstract

The synthesis of biologically active natural compounds like (E)-3-dodecenyl acetate from the Tecia solanivora Polvony, constitute an important step for the biological control of this pest, especially in undeveloped countries.
The sex pheromone produced by virgin female Tecia solanivora moths contains 98%
(E)-3-dodecenyl acetate and 2% of the Z isomer. An efficient three-step synthesis of (E)-3-dodecenyl acetate and its Z isomer as a byproduct is described. The synthetic route comprises the stereoselective formation of the E3 double bond by a modified Knoevenagel condensation, followed by a reduction of the carboxylic acid and finally the ester formation by acylation of the (E)-3-dodecenol intermediate. A complete structural characterization of the intermediates and final products, the overall yield was 59%, with a stereochemical purity for the target acetate of 98%.
The performance of dispensers loaded with the synthetic sex pheromone of potato moths, Tecia solanivora, was compared with a commercial dispenser under laboratory conditions.
In the field, data recorded included males captured in traps baited with synthetic pheromone. Male T. solanivora attraction to the synthetic pheromone polymeric dispensers was almost completely achieved over the course of 1 month, demonstrating its potential use for the control of this economically important insect pest in Colombia.

Key Words - *Tecia solanivora, synthetic pheromone, polymeric dispensers.*

I. INTRODUCTION

The potato moth, *Tecia solanivora* Polvony (Lepidoptera: Gelechiidae), is a serious pest of potatoes in Central and South America, but also recently introduced to Spain.[1a,b] Behavior-modifying compounds, including sex pheromones, which target the adult life stage, are a promising alternative to insecticides for control of *T. solanivora*. Insect pheromones, especially volatile organic molecules of low molecular weight, are effective attractants that elicit a behavioral response from individuals of the same species.[2,3] Previous studies regarding the communication mechanisms of *T. solanivora* [4,1a,b]have shown that the sexual behavior of adult moths is mediated by pheromone components. Nesbitt *et al*, [4] isolated and identified the sex pheromone of *T. solanivora* as a blend of *(E)*-3-dodecenyl acetate and 2% of the *Z* isomer. The mixture of these compounds caused significant male antennal responses by GC-EAD (coupled gas chromatography-electroantennographic detection) and field trapping studies showed their potential as biological control of *T. solanivora* in potato crops.
In order to develop an integrated pest management protocol, synthetic pheromones are utilized. They are probably the most specific, economical and convenient pest control monitoring tool available today. [5]
Here, we described the synthesis of *(E)*-3-dodecenyl acetate initiate by a modified Knoevenagel condensation through the reaction between commercially available decanal and malonic acid, using dimethylsulfoxide (DMSO) as a solvent. It is widely documented that the reaction of saturated acyclic aldehydes under these conditions leads stereoselectively to *(E)*-3-unsaturated acids (Ragoussis and Ragoussis, 1998; Ragoussis *et al.*, 2004). Next, the carboxylic acid obtained was reduced to the corresponding alcohol employing lithium aluminum hydride ($LiAlH_4$) without affecting either the double bond or its configuration, as previously reported in other reactions using this reducing agent. [6] Finally, the resultant *(E)*-3-alkenol was acetylated in the presence of anhydride acetic to obtain *(E)*-3-dodecenyl acetate as the main product in high stereoselectivity purity and good yield. [7]
Although the synthesis of *(E)*-3-dodecenyl acetate has already been disclosed, here, we describe a modified and improved protocol to obtain this target acetate, providing a highly regioselective product with an overall yield of 59%. A complete characterization of the main product and its intermediates is also provided. Additionally, the synthetic route and experimental conditions yield an important byproduct, the *(Z)*-3-dodecenyl acetate, which is part of the *T. solanivora* sex pheromone.

Applying pheromones under field conditions are difficult due to their chemical volatility. Recently, several polymers nanofibers have been studied[8,9] as dispensers for volatile pheromones. Challenges for pheromone dispensers include protecting components from degradation by environmental factors and the uniform release over time of different types of compounds with varying chemical and physical properties. As this work aims to scale up the final results, several economic factors are taken into consideration. Specifically, the material chosen for the study were two very economical polymers (polyethylene and polypropylene), both produced in Colombia (Thermoplast, Bogotá, Colombia) and a widely available commercial dispenser (polybutadiene) provided by Sigma-Aldrich (Darmstadt, Germany). Polyethylene pheromone dispensers have shown to be effective for 10 weeks in the control of light brown apple moth, *Epiphyas postvittana* (Lepidoptera:Tortricidae) with high activity in mating disruption[10] and for disruption of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), and the oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae).[11]

On the other hand, polypropylene dispensers showed to be less attractive, but with similar efficiency, as pheromones released from polyethylene. [12]

Pest control by techniques that involve male or female attraction invariably require a few different pheromones and are potentially cost-effective, regardless of the dispense material used. Also, most controlled release formulations have first-order release kinetics whereby release is proportional to the amount of pheromone present. [13] However, one of the limitations of solid matrix dispensers is the difficulty in maintaining a constant release rate (i.e. zero-order release kinetics). Kruger and Tolmay (2002) noted that release rates from dispensers are heavily dependent on the diffusion speed of the compound through the matrix and the evaporation rate of the compound into the air. Diffusion speed is influenced by a variety of characteristics associated with the dispenser, including type, size, and shape of the matrix, along with the distribution of the semiochemical in the matrix. [14] In this way, polymers have been widely used for the controlled release of sex pheromones, such is the case of polyvinylchloride (PVC)-resins, modified to optimize release rate characteristics of several pheromone components, achieving a high level of protection of chemically labile compounds of the yellow rice stem borer *Scirpophaga incertulas* (Lepidoptera, Pyralidae). [13] Attygalle *et al*[15] used a rubber septa as a dispenser of a sex pheromone of the tomato pest *Scrobipalpuloides absoluta* (Lepidoptera: Gelechiidae). Also, in 2015 polymers as dispensers were used for controlled liberation of pheromones. Test results demonstrated that release rates of the three semiochemicals at the linear fall stage increased exponentially as ambient temperature increased, consequently those rates were not apparently affected by relative humidity. [14]

Semiochemical-based monitoring and mass trapping are potential strategies for the management of *T. solanivora*. In this study, a female specific sexual pheromone released by the Guatemalan potato moth was isolated, identified and synthetized. Its attractiveness on impregnated polymeric dispensers was evaluated in the laboratory and confirmed in a field test. This study is a first step towards the development of synthetic pheromone dispensers to control the potato moth, *Tecia solanivora* in Colombia.

II. MATERIALS AND METHODS

A. Pheromone gland extraction and chemical analysis

Tecia solanivora insects were mass-reared on fresh potatoes at 20–25°C, under a photoperiod of L12:D12. Then pupae were separated by sex dimorphism i) according to their number of abdominal segments: four segments are visible in females, and five in males; and ii) through the location of their genital opening. [16,1a]Adult insects were kept in inverted plastic containers, where oviposited eggs were collected and brought to fresh potatoes. After this, pheromone glands were dissected from the abdominal tips of

2–3-day-old calling virgin females during the first 3 h of the scotophase and extracted in batches of 24 glands in 50 µL of hexane and dichloromethane HPLC grade (Sigma-Aldrich, Darmstadt, Germany), separately.

Compounds in the gland extracts were identified by gas chromatography – mass spectrometry (GC-MS) analyses. 1 µL of the extracts was injected in an HP 6890 Series GC coupled to an HP 7973 mass selective detector (Hewlett Packard, Palo Alto, USA) equipped with one capillary column, ZB-5 (30 m × 0.25 mm i.d., 0.25 µm film thickness; Zebron, Phenomenex, Torrance, USA). The column oven was programmed from 50 °C for 1 min, then up to 250 °C at 7 °C min^{-1} and maintained at that temperature for 10 min. The injector temperature was fixed at 250 °C, using helium as carrier gas at 1.5 mL min^{-1}. The injection port was operated in splitless mode.

Linear retention indices (LRI) were calculated according to the Kovats method, using a mixture of *n*-alkanes as external references. Mass spectral identification was completed via comparison with commercial reference standards and spectra from commercial mass spectral databases (Wiley 9th Edition/NIST 2008). MS-data acquired using electron ionization (EI) were recorded in a mass range of 30–650 u, with electron energy of 70 eV, and processed by HP Chemstation software.

The commercial standard of *(E)*-3-dodecenyl acetate was purchased from Chemtica International S.A (Santo Domingo, Heredia, Costa Rica). The

compound was extracted from a rubber septum by dynamic headspace (DHS) collection, within an individual all-glass aeration chamber (33 cm high × 4 cm outlet diameter). The released volatile was collected continuously for 2 days, and trapped on glass columns (10 cm high × 0.5 cm i.d.) packed with 0.5 g of Hayesep D80/100 (DVB, Supelco, Darmstadt, Germany). Before use, the packed column was washed with HPLC grade hexane (Merck, Darmstadt, Germany). Then charcoal-filtered humidified air was pushed through the aeration system (1.0 L min^{-1} per chamber). The adsorbed volatile compound was eluted using 300 µL of HPLC grade hexane (Merck, Darmstadt, Germany). The extract was concentrated to approximately 50 µL under a stream of nitrogen in a clean conical-bottom vial.

B. Pheromone synthesis

The synthesis of *(E)*-3-dodecenyl acetate (Fig. 1) is initiated by a modified Knoevenagel condensation through the reaction between commercially available decanal (**1**) and malonic acid, using dimethylsulfoxide (DMSO) as solvent. It is widely documented that the reaction of saturated acyclic aldehydes under these conditions leads stereoselectively to the *(E)*-3-unsaturated acids. [17,18]Following this, the carboxylic acid obtained (**2**) was reduced to the corresponding alcohol (**3**) employing lithium aluminum hydride ($LiAlH_4$) without affecting either the double bond or its configuration, as previously reported in other reactions using this reducing agent.[6] Finally, the resultant *(E)*-3-alkenol was acetylated in the presence of anhydride acetic to obtain

(E)-3-dodecenyl acetate as the main product, in high stereoselective purity and good yield.[7] Although the synthesis of *(E)*-3-dodecenyl acetate has been published previously,[6] in this paper, a modified and improved protocol to obtain this acetate is described, providing a highly regioselective product with an overall yield of 59%. A complete characterization of the main product and its intermediates are also supplied. Additionally, the synthesis route and experimental conditions make possible the formation of a very important byproduct, the *(Z)*-3-dodecenyl acetate, which in order to commercialize a biological trap for *T. solanivora* in potato crops, facilitates the real world applicability of the sex pheromone.

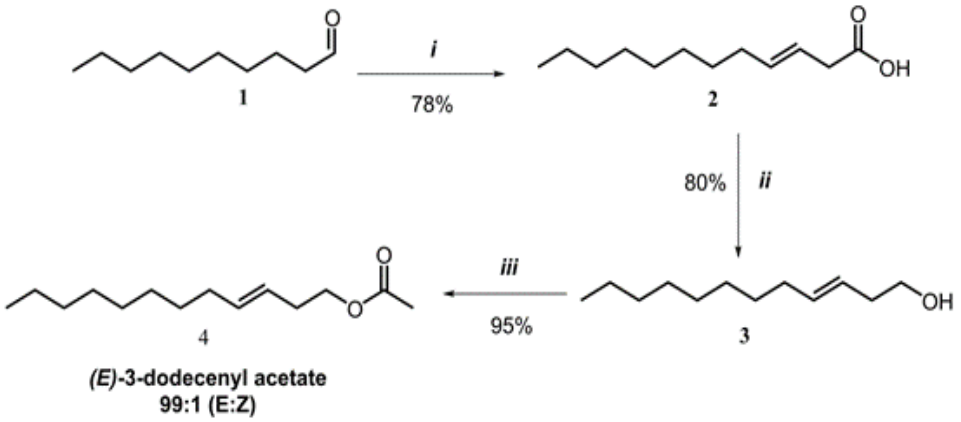

Figure 1. General scheme for the synthesis of biologically active natural compounds of Tecia solanivora Polvony. Reagents and conditions: i) Malonic acid, piperidinium acetate, DMSO, 85ºC, 5h. ii) LiAlH4, THF, 5ºC, 2 h; iii) a. Acetic anhydride, pyridine, 5ºC, 1h.

General spectroscopic data were obtained on the following instruments: NMR spectra were recorded at room temperature on a Bruker Avance 9.4 Tesla (^1H: 400 MHz, ^{13}C: 101 MHz) spectrometer. The chemical shifts were reported in δ [ppm] relative to tetramethylsilane as external standard and using abbreviations for the characterization of the signals: s=singlet, d=doublet, t=triplet, m=multiplet, dd=doublet of doublets, q=quartet. FT-IR spectra were recorded with an FT-IR spectrometer (Prestige 21 Shimadzu). Gas chromatography-mass spectrometry (GC-MS) analyses were carried out with a Thermo scientific ITQ 900 trace GC ultra, equipped with a capillary column Mega-5MS; helium at 1.0 mL/min as carrier gas; injector at 250 °C, and the oven with a ramp of 50 °C for 1 min followed by an increase at 15 °C/min until 250ºC, and kept for 10 min. Commercial reagents were used as provided and solvents (analytical grade)

were used for reaction protocols and dried with reported procedures when necessary.

***(E)*-3-dodecenoic acid** (**2**): In a round-bottom flask, equipped with a condenser and a bubbler, a solution of malonic acid (1 mol) and piperidinium acetate (piperidine and acetic acid, 0.01 mmol) in DMSO (60 mL) was stirred at room temperature for 15 min, and then decanal (0.5 mol) was added at once. The stirring was continued for 30 min, then the reaction mixture was gently heated to 85 °C, showing the evolution of carbon dioxide (for approximately 3 h), after which the temperature was maintained for one extra hour. After cooling to room temperature, the reaction mixture was poured into cold water (150 mL) and

extracted with diethyl ether (3x50 mL). The combined extracts were washed with water (50 mL) and dried over anhydrous Na_2SO_4, and the solvent was removed under vacuum. The desired intermediate was obtained as a white solid, with a mp: 32-35ºC. (78% yield, 98% purity by GC). ^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm]: 9.35 (s, 1H, COOH), 5.56 (dd, 1H, *J*=14.1, *J*=7.5 Hz, CH), 5.46 (dd, 1H, *J*=14, *J*=6.6 Hz, CH), 3.07 (d, 2H, *J*= 6.5 Hz, CH_2), 2.03 (q, 2H, *J*=6,7 Hz, CH_2), 1.18 (m, 12H, CH_2), 0.87 (t, 3H, *J*=6.8 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 101 MHz) δ [ppm]: 178.2 (C=O); 135.6 (C=C); 120.6 (C=C); 37.7 (CH_2); 32.4 (CH_2); 31.8 (CH_2); 29.4 (CH_2); 29.2 (CH_2); 29.1 (CH_2); 29.1 (CH_2); 22.6 (CH_2); 14.1 (CH_3). FT-IR (KBr window, cm^{-1}): 3300 (OH), 2854 (CH), 1710 (C=O), 1290 (C-O), 966 (H-C=C-H), 721 (-$(CH_2)_n$-). Anal. calcd. for $C_{12}H_{22}O_2$: C, 72.64; H, 11.18; O, 16.14. Found: C, 74.81; H, 11.31; O, 13.88.

***(E)*-3-dodecenol** (**3**): *(E)*-3-dodecenoic acid **2** (1 mmol) dissolved in THF (5 mL) was added dropwise into a cold (5ºC) solution of $LiAlH_4$ (2.4 mmol) in THF (5 mL) under nitrogen. Stirring was continued for 1 hour at the same temperature, and then, the solution was left overnight at room temperature. Finally, the reaction mixture was hydrolyzed by dropwise addition of ice cold 5% HCl (10 mL) under inert atmosphere, and then diluted with water and extracted with diethyl ether (3x10 mL). The organic phase was dried over anhydrous Na_2SO_4, and the solvent was evaporated under vacuum. The desired intermediate was obtained as a light-yellow oil (0.600 g, 80% yield, 95% purity by GC). ^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm]: 5.56 (dd, 1H, *J*= 14.6, *J*=7.8 Hz, C=C), 5.43 (dd, 1H, *J*= 14.5, *J*=7.1 Hz, C=C), 3.55 (t, 2H, *J*= 6.3 Hz, CH_2-OH), 2.23 (d, 2H, *J*= 6.5 Hz, CH_2), 2.02 (d, 2H, *J*= 6.9 Hz, CH_2), 1.31 (d, 12H, *J*= 13.6 Hz, CH_2), 0.92 (t, 3H, *J*= 6.7 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 101 MHz) δ [ppm]: 132,4 (C=C); 126.0 (C=C); 61.6 (C-OH); 35.6 (CH_2); 32.3 (CH_2); 31.6 (CH_2); 29.2 (CH_2); 29.1 (CH_2); 29.0 (CH_2); 28.8 (CH_2); 22.3 (CH_2); 13.0 (CH_3). FT-IR (KBr window, cm^{-1}): v 3342 (OH), 2922 (CH), 1465 (H-C=C-H), 1047 (CH_2-OH), 968 (H-C=C-H), 721 (-$(CH_2)_n$-). Anal. Calcd. for $C_{12}H_{24}O$: C, 78.20; H, 13.12; O, 8.68. Found: C, 79.29; H, 12.73; O, 8.00.

***(E)*-3-dodecenyl acetate** (**4**): To a solution of acetic anhydride (8.4 mmol) in pyridine at 0-4 °C, *(E)*-3-dodecenol **3** (1 mmol) was added under stirring. The reaction was maintained at the same temperature for 1 hour and then brought to room temperature. When the reaction was finished (5 h), the reaction mixture was poured in to cold water (20 mL) and extracted with diethyl ether (2×10 mL). The organic phase was washed with dilute 5% HCl, then with saturated $NaHCO_3$ and finally dried over anhydrous Na_2SO_4. The solvent was removed under vacuum to give the target compound **4** as a yellow oil (504 mg, 95% yield, 97% purity by GC, isomer ratio E/Z: 99/1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm]: 5.53 (dd, 1H, *J*= 14.7, 6.6 Hz, C=C), 5.37 (dd, 1H, *J*= 14.6, 6.7 Hz, C=C), 4.08 (t, 2H, *J*= 6.9 Hz, CH_2-O-), 2.32 (q, 2H, *J*= 6.8 Hz, CH_2), 2.06 (s, 3H, CH_3-C=O), 2.00 (q, 2H, *J*= 6.7 Hz, CH_2), 1.28 (s, 12H, CH_2), 0.89 (t, 3H, *J*= 6.7 Hz, CH_3). ^{13}C NMR ($CDCl_3$, 101 MHz) δ [ppm]: 171.1 (C=O), 133.6 (C=C), 124.9 (C=C), 64.1 (CH_2-O-), 32.6 (CH_2), 31.9 (CH_2), 31.8 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 22.6 (CH_2), 20.9 (CH_3), 14.1 (CH_3). FT-IR (KBr window, cm^{-1}): v 3469 (OH), 2924 (CH), 1741 (C=O), 1460 (H-C=C-H), 1363 (CH_3C=O), 1033 (CH_2-O-C=O), 968 (H-C=C-H), 750 (-$(CH_2)_n$-). MS *m/z* (%): 166, 95, 81, 67, 55, 43. Anal. Calcd. for $C_{14}H_{26}O_2$: C, 74.29; H, 11.58; O, 14.14. Found: C, 73.47; H, 11.03 ; O, 15.53.

Evaluation of pheromone release from dispensers

Pheromone dispensers used in the evaluation (Table 1) were selected according to both commercial availability and price. Polymers such as polypropylene and polyethylene are commonly used in many fields, and are also the only polymers produced in Colombia. Looking forward, the process described here will be scaled so that Colombian farmers can replace the pesticides they normally use for this environmentally friendly alternative. The commercial septum Chemtica, used in the trial as a comparison standard, is commercialized in Colombia, but it is expensive and is not easily accessible to farmers who grow potatoes in Colombia. In addition, several reports in literature show the use of polypropylene and polyethylene as pheromone dispensers. [13,14,15]

Table 1. Pheromone dispensers used in the evaluation

No	Identification	Description	Provider
1	PP	Polypropylene sheet	Plásticos Thermoplast Ltda
2	PE	Polyethylene sheet	Plásticos Thermoplast Ltda
3	PB red	Polybutadiene red septum	Sigma-Aldrich
4	Commercial	Commercial septum	Chemtica International S.A

Delta traps (P018-Trap, Chemtica International, S.A, Santo Domingo, Heredia, Costa Rica) were baited with a synthetic pheromone consisting of a polypropylene and polyethylene sheets (25 mm x 25 mm x 5 mm thick, Plásticos Thermoplast Ltda, Bogotá, Colombia) and Polybutadiene septum impregnated for 24 hours with 10 mg of a 98:2 blend of synthesized *(E)*-3-dodecenyl acetate and *(Z)*-3-dodecenyl acetate prepared in dichloromethane[15] and exposed to air in the laboratory at environmental conditions (20 ± 2 °C, 80 ± 10% RH) throughout the duration of the experiment. Compounds impregnated

were at least 98% chemically and isommerically pure by gas chromatography (Week 0, Table 3). The effectiveness of these dispensers was compared with a commercial product: septum P176 lure (Chemtica International S.A) loaded with 1000 g/Kg of *(E)*-3-dodecenyl acetate.

The release efficiency was measured for 12 weeks, based on the area of the chromatographic peak obtained. Each week the dispensers were closed in 30-mL glass flasks in a room free from odorants. The compounds released by synthetic pheromone dispensers were collected in the laboratory by using headspace-solid phase microextraction (HS-SPME) in static air. [19] The volatile compounds were collected using a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 µm thickness, Supelco, Bellefonte, PA, USA) for 36 min. Analysis of background volatiles was performed with the SPME fiber in the flask without dispensers.

Volatile compounds collected by HS-SPME were desorbed in a gas chromatograph 7820A system GC-FID (Agilent Technologies, USA), equipped with a capillary column HP-5 (30 m × 0.32 mm i.d., 0.25 µm film thickness; Agilent J&W Scientific, Chromatography-Handel Müller, Fridolfing, Germany). Desorption time was set at 5 min. The column oven was programmed from 50 °C for 1 min, then raised to 250 °C at 15 °C min^{-1}, maintaining the final temperature for 10 min. The injector temperature was fixed at 250 °C, using helium as carrier gas at 1.5 mL min^{-1}. The injection port was operated in splitless mode.

C. Field bioassays

Synthetic samples of the acetate blend (98:2) were applied using dicloromethane (10 ppm) to polymeric dispensers PP sheet and PB red septum, which were used as baits in the field test. This selection was done based on laboratory dispensers released rates (since the PB red and PB white samples presented the same behavior, for easiness only PB red was studied on field) and taking into account that the polyethylene sample could not be fully characterized since it contains several unknown additives, which are in the process of being identified.

The test was performed in a potato crop located at Tunja, Boyacá, Colombia (5 45'21" N, - 73 41' 9" W) on infested plants with *Tecia solanivora*. It was performed between February and March of 2018. Delta traps with pheromone dispensers were suspended 30 cm above ground with wood sticks, and 50 m apart in an 8 ha potato field. Also, two control field traps were used, one delta trap without a dispenser and another one with a commercial dispenser. In the field bioassay, traps were inspected every day for 35 days and moths captured were counted and removed.

Statistical analysis

The number of males attracted and captured in field traps was submitted to an analysis of variance (ANOVA), followed by Tukey's test. The significance level was set to 0.05.

III. RESULTS AND DISCUSSION

A. Chemical identification of the sex pheromone

As expected, the glands of calling *T. solanivora* females contain the volatile organic compound *(E)*-3-dodecenyl acetate. The compound was identified according to comparison of the retention time [KI 1593/ZB-5] of the product extracted with the commercial standard, and its mass spectra. For the main compound, the mass spectrum showed a molecular ion at *m/z* 166 and, a base peak at *m/z* 43 (Fig. 2). The mass spectrum of the main volatile extracted with hexane and dichloromethane was the same.

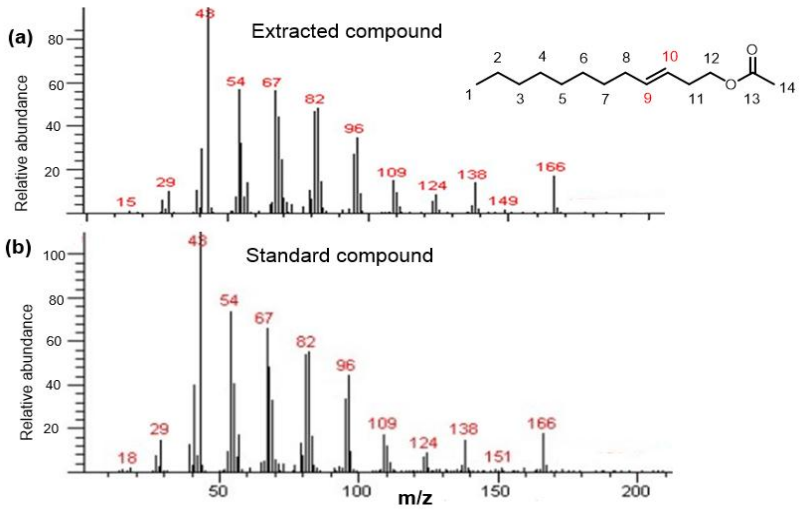

Figure 2. Mass spectrum comparison of the extracted compound (a) and commercial standard of (E)-3-dodecenyl acetate (b) in hexane.

B. Pheromone synthesis

The main sex pheromone component of potato moth, *(E)*-3-dodecenyl acetate, was synthesized by a stereoselective route, as shown in Fig. 1. The methodology used comprises the following three steps: First, the modified Knoevenagel condensation of decanal with malonic acid in the presence of *in situ* piperidinium acetate, to yield the β,γ-unsaturated acid. This corresponds to the key step for the stereoselective and regioselective synthesis of the target pheromone component.

Regarding this first step, the reaction between decanal and malonic acid, it is important to highlight that the product obtained was a white solid although the state of the art described the compound as a yellow liquid. Nevertheless, the authors of this prior reports did not reveal much spectroscopic evidence about the characterization of *(E)*-3-dodecenoic acid. [1b]

In the present work, each reaction intermediate was completely characterized before obtaining the target pheromone acetate (see supplementary material).

The second step uses $LiAlH_4$ in THF as solvent instead of the previous described diethyl ether, [6] to reduce the carboxylic acid without affecting its β,γ-unsaturation. The final step requires an acetylation of the unsaturated alcohol with acetic anhydride in pyridine to obtain with high yield and purity (95 % and 97% respectively) the target acetate **4**. The stereoselectivity of this methodology to yield **4** was unambiguously confirmed by ^{1}H NMR, which shows the presence of a coupling constant of 14.7 Hz at 5.53 ppm and one more of 14.6 Hz at 5.37 ppm, revealing two *trans* olefinic protons at C10 and C9, respectively (Fig. 2).

C. Dispensers release rates

Positive detection of the compound by GC indicated that the dispensers indeed release the compound (Table 2). From these results, it can be confirmed that the compound is released in the laboratory at least during the time of the life cycle of the insect which can last from 35-50 days and represents about 7-10 generations per year. [20]

The values were normalized by dividing the logarithm values of the areas for the chromatographic peaks obtained for each polymer by the highest value in each series (Fig. 3).

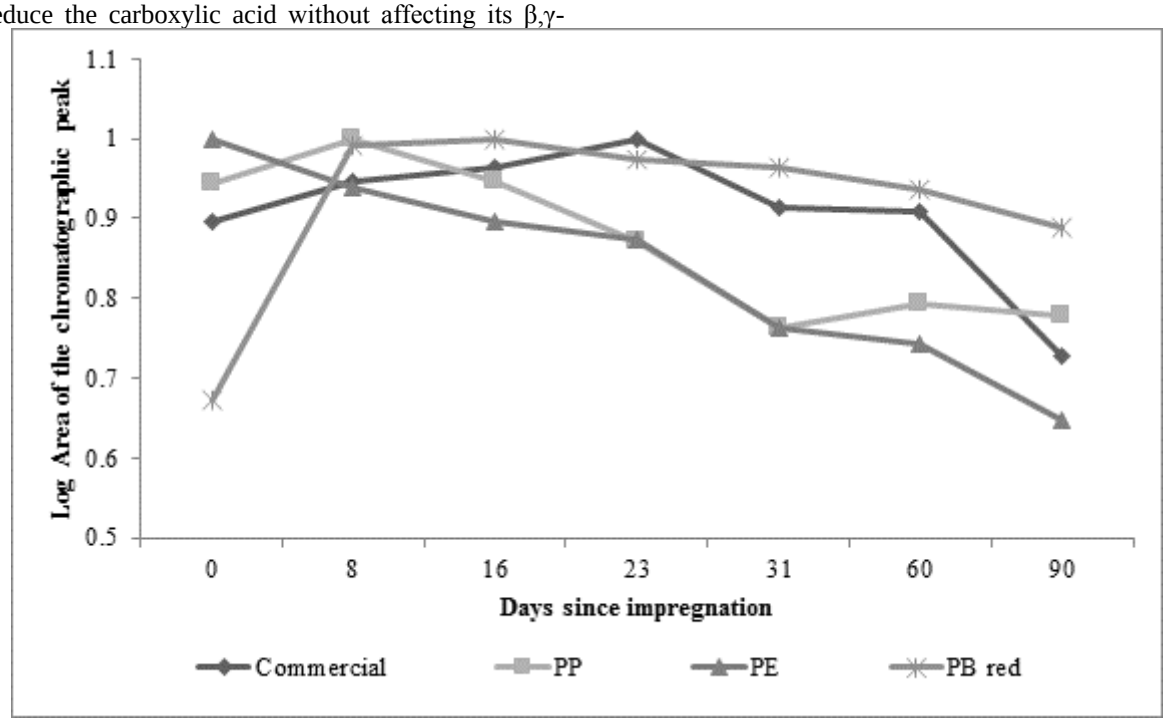

Figure 3. Log area of the chromatographic peak for the evaluated dispensers in 90 days.

D. Variation of the ratio of E/Z isomers

Initially, according to the GC-MS analysis, the synthesized 3-dodecenyl acetate (**4**) had 98% of the compound in the *E* configuration and 2% of *Z* compound. Each week the respective areas of the peaks for those two compounds were obtained (Table 3), these values were divided between the total sum of the areas and the ratio between the isomers for each dispenser were obtained. [21]

$$Percent\ E = \frac{Area\ compound\ E}{Total\ area\ (E+Z)} * 100$$

Table 2 Variation of the ratio of *E/Z* isomers

Dispenser /Week	Percent of *E* / *Z* isomers						
	0	1	2	3	4	8	12
Commercial	ND*	ND*	ND*	ND*	ND*	ND*	ND*

PP	99:1	99:1	99:1	99:1	99:1	99:1	94:6
PE	99:1	98:2	98:2	97:3	98:2	98:2	98:2
PB white	ND*	99:1	99:1	99:1	98:2	98:2	97:3
PB red	ND*	99:1	98:2	98:2	98:2	98:2	98:2

* ND: Z isomer No detectable

GC-MS results show a 98:2 ratio for E: Z isomers at the time of synthesis and differ from those observed for dispensers. This can be explained in terms of polymer-additive interactions; some reports have shown that the transport or diffusivity of an additive (small molecules) through a polymer depends on several factors such as pressure, temperature, concentration gradient or effect of an external field. In addition, this diffusivity can vary for different polymer-additive systems, depending on factors such as chemical structure, polarity and size, among others. The latter suggests that (E) -3-dodecenyl acetate and (Z) -3-dodecenyl acetate may interact at the molecular level in different ways with the polymer chains of the diffuser, leading to different diffusion rates that, interestingly, are also affected by the time the diffuser remains in the field. [22,23]

E. Field trapping test

All dispensers significantly increased male captures in lure baited traps over the entire time compared with the untreated control (Fig. 3). No significant differences were found among dispensers for the cumulative captures of males in virgin-female-baited traps during either moth flight period. The data obtained show a good efficacy of the polymeric dispensers in the control of the *T. solanivora*, comparable with the commercial dispenser. Using the synthetic pheromone indeed allowed the capture of moth males in traps for monitoring, which could produce a suppression of the potatoes damage and a decrease in the number of insecticide applications.

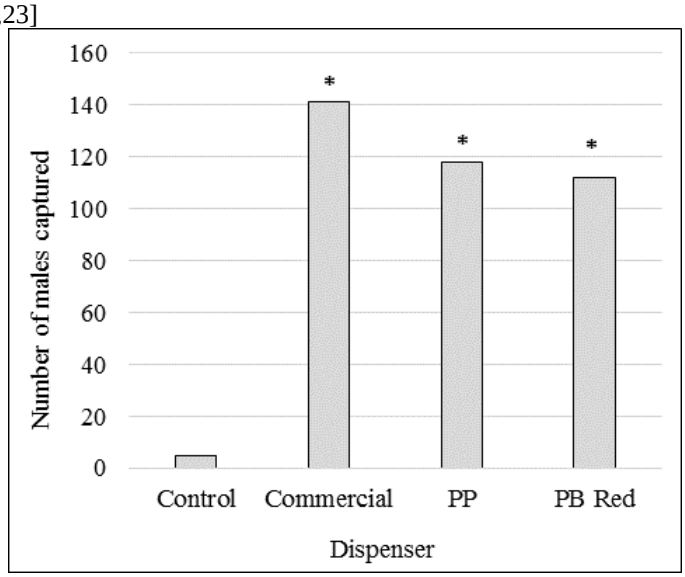

Figure 4. Number of males of *T. solanivora* captured during 35 days in the potato crops. Polypropylene (PP), and polybutadiene (PB). Asterisks denote statistical differences between treatment and control.

The glands of calling virgin *Tecia solanivora* females contained the compound *(E)*-3-dodecenyl acetate as a main component. Since the available amounts of natural product were too small, structure assignments were based on mass spectra and comparison of gas-chromatography retention time with the commercial standard. This corroborates the identification by Nesbitt el at. (1985). [4]

(E)-3-dodecenyl acetate **4** (Fig. 1), the main pheromone component of the potato moth *Tecia solanivora* Polvony (Lepidoptera: Gelechiidae), was synthesized in a three-step, experimentally-simple reaction with an overall yield of 59% from commercially available decanal. Even though the first step shows the lowest yield (78%) of the total synthetic route, it is where the specific and desired β,γ-unsaturation is reached with an appreciable purity (99% by GC). The above-mentioned modification of the solvent on step 2 improves the yield up to 80%, compared to the one previously reported using diethyl ether as solvent. Moreover, the importance of the solvent modification should not be underestimated since the use of THF instead of diethyl ether as the reaction media increases the solubility of the reducing agent, and furthermore reduces the prior and tedious drying and handling conditions required for the ether. Now, regarding the 97% purity of the synthesized acetate determined by GC, it is remarkable that in the chromatogram just next to the peak corresponding to the *(E)* acetate, another peak is observed. In the gas chromatogram, we corroborated that the major compound (98%) corresponds to the acetate in the *(E)*

configuration and the minor compound (2%) corresponds to the acetate in the *(Z)* configuration. (See supplementary material).

The proposed method is easily scalable and opens the possibility of using the synthesized compound on a large scale, making the resulting *E-Z* acetate mixture a very attractive component for the environmental control of insect pests. The 98:2 blend of the isomers used in this study is the result of a first attempt to achieve an inexpensive synthetic route of both compounds. According to previous reports, the major active component in *T. solanivora* moths has been shown to be *(E)*-3-dodecenyl acetate with approximately 2% to the *Z* isomer. [4]

As seen in Table 2, the commercial septum impregnated with the compound had a constant behavior after 12 weeks of exposure in the laboratory. Contrarily, polypropylene (PP) has a significant drop in terms of the release of the compound after 30 days (4 weeks), but thereafter remains constant. On the other hand, the polyethylene (PE) dispenser constantly decreases the amount of compound released without a plateau as presented by PP. Interestingly, the release behavior of the white (PB white) and red polybutadiene (PB red) septa is very similar between them, but totally different to the other polymer dispensers studied; during the first eight days of being exposed to the atmosphere, the release of the PB dispensers increase abruptly and after that time the release remains constant.

The variation in the ratio of the *E/Z* isomers remains constant at 98:2 throughout the first eight weeks, but after this time, we find values of 94:6 and 97:3, as shown in Table 2. According to the results obtained in the field, it is suggested that the attraction is not affected by the isomeric ratio values. Further experimentation is needed to confirm this assumption, because this result is contrary to that of Nesbitt el at.,[4] who showed that the addition of 1% or 2% of the *Z* isomer increased catches, while the addition of 5% of the *Z* isomer reduced catches.

High emission dispensers have been developed to emit larger quantities of pheromone and use fewer dispensers per hectare to cut down on labor costs.[24] In the field, synthetic *(E)*-3-dodecenyl acetate was shown to be highly attractive to male *T. solanivora* moths, and traps baited with this compound dispensed from polypropylene sheet (PP) and polybutadiene septum (PB red) captured moths as efficiently as commercial dispensers. Both dispensers remained attractive to *T. solanivora* male moths for over 30 days in the field, although the final level of attractiveness was not compared with that the fresh dispensers. Longevity for over 17 weeks in the field, has been reported for polyethylene vials impregnated with 1 mg of synthetic pheromone remained attractive to *T. solanivora* male moths[4] and for rubber septa impregnated with the synthetic *P. operculella* pheromone by Raman. [25]

Field trials carried out from February and March of 2018 confirmed the efficacy of the synthetic pheromone and the successful male captured in the potato crops demonstrated the potential of the polymeric dispensers tested for control of the Guatemalan potato moth *T. solanivora*. It is believed that the polymeric dispensers could help to the expansion of mass trapping, with beneficial effects on the potatoes production and on the environment.

Supplementary material

The supplementary material for this article can be found in a separate file.

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