Bioactivity of 1-octacosanol from Senna crotalarioides (Fabaceae: Caesalpinioideae) to control Spodoptera frugiperda (Lepidoptera: Noctuidae)

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Abstract

Spodoptera frugiperda J. E. Smith (Lepidoptera: Noctuidae) is a pest native to the Americas that affects a variety of crops. Its control is based on chemical insecticides. However, this practice has been associated with changes in the susceptibility of pests to various insecticides. The use of plant products represents an eco-friendly alternative. The objective of this work was to evaluate the larvicidal activity of the chloroform extract of Senna crotalarioides (Kunth) H.S. Irwin & Barneby (Fabaceae) to control S. frugiperda. The chloroform extract of S. crotalarioides caused significant larval mortality, and reduced pupal weight and adult emergence. The analysis by gas chromatography coupled with mass spectrometry (GC-MS) revealed the presence of 22 compounds in the chloroform extract of S. crotalarioides leaves, with the straight-chain aliphatic fatty alcohol 1-octacosanol as the main component. This study revealed that the leaves of S. crotalarioides synthesize long chain alcohols, which increased the mortality of S. frugiperda in its larval stage, including the pupal stage. The extract also caused a decrease in the S. frugiperda pupal weight. The potential use of the chloroform extract obtained from S. crotalarioides and its principal chemical constituent is proposed as a promising alternative to control S. frugiperda.

Key Words: botanical; fall armyworm; insecticide; management

Resumen

Spodoptera frugiperda J. E. Smith (Lepidoptera: Noctuidae) es una plaga nativa del continente Americano que afecta a muchos cultivos. Para su control, se emplean insecticidas químicos sintéticos. Sin embargo, esta práctica se ha asociado con la generación de resistencia del insecto a estos productos. El uso de productos botánicos representa una alternativa eco amigable. El objetivo de este trabajo fue evaluar la actividad larvicida del extracto clorofórmico de Senna crotalarioides (Kunth) H.S. Irwin & Barneby (Fabaceae) para controlar S. frugiperda. El extracto ocasionó mortalidad significativa de la larva. También se observó reducción de peso pupal y de emergencia de adultos. El análisis mediante cromatografía de gases acoplada a espectrometría de masas (GC-MS) reveló la presencia de 22 compuestos en el extracto clorofórmico de las hojas de S. crotalarioides, siendo el alcohol alifático de cadena lineal 1-octacosanol el componente mayoritario. Este estudio reveló que las hojas de S. crotalarioides sintetizan alcoholes de cadenas largas, los cuales ocasionan un incremento en la mortalidad en el estado larval e incluso en el estado pupal de S. frugiperda. También el extracto indujo la disminución del peso pupal. El uso potencial del extracto clorofórmico obtenido de S. crotalarioides así como de su compuesto mayoritario para controlar S. frugiperda se plantea como alternativas promisorias.

Palabras Clave: botánico; gusano cogollero de maíz; insecticida; manejo

The genus Spodoptera (Lepidoptera: Noctuidae) includes some of the most important insect pests that cause significant yield reductions and economic losses in the American and African continents (Aragón et al. 2011; Igyuve et al. 2018). Spodoptera frugiperda J. E. Smith (Lepidoptera: Noctuidae), commonly known as “gusano cogollero de maíz” (Spanish), fall armyworm, corn leafworm, and southern grassworm, is a highly polyphagous pest that affects more than 180 crops, among which the following stand out for their importance in Western Hemisphere countries: Arachis hypogaea L. (peanut) (Fabaceae), Glycine max L. Merrill (soybean) (Fabaceae), Gossypium hirsutum L. (upland cotton) (Malvaceae), Linum usitatissimum L. (linseed) (Linaceae), Medicago sativa L. (alfalfa) (Fabaceae), Oryza sativa L. (Asian
rice) (Poaceae), *Phaseolus vulgaris* L. (common bean) (Fabaceae), *Saccarum officinarum* L. (sugar cane) (Poaceae), *Solanum lycopersicon* L. (tomato) (Solanaceae), *Solanum tuberosum* L. (potato) (Solanaceae), *Sorghum bicolor* L. Moench (sorghum) (Poaceae), and *Zea mays* L. (maize) (Poaceae) (Hernández-Mendoza et al. 2008; Casmuz et al. 2010). In the case of maize, the larvae of *S. frugiperda* cause damage at all growth stages, including senescence (Rodríguez-del-Bosque et al. 2011). The presence of this indigenous insect in the Americas also has been reported in African cornfields (Goergen et al. 2016). Many yr ago, the control of *Spodoptera* species had been based on the use of conventional synthetic insecticides (approximately 3,000 tons of active ingredient per yr) (Blanco et al. 2014). However, the intense and non-rational use of these products has been associated with a strong selection pressure on insects, genetic variability (Pérez-Zubiri et al. 2016), and the development of insecticide resistance (León-García et al. 2012). This phenomenon limits the success of pest control in many countries. In addition, there is evidence of human intoxication due to exposure to the insecticides used in the management of *Spodoptera* pests (Barrientos-Gutiérrez et al. 2013). Alternative strategies have been proposed to control *S. frugiperda*, including the use of genetically modified crops (Aguirre et al. 2016), natural enemies (Nuñez-Valdez et al. 2008; Ordóñez-García et al. 2015), semiochemicals, and other natural product-based approaches (Guerrero et al. 2014).

The genus *Senna* Mill. (Fabaceae: Caesalpinioideae) comprises more than 350 species (http://www.theplantlist.org) of herbs, shrubs, woody climbers, and tree species, unarmed or armed, distributed in a wide range of zones, with different climates and latitudes (Marazzi et al. 2006). Some species of the genus *Senna* are used as foods, ornamental plants, or with medicinal purposes (Mazzi & Soliman 2010), while others have become invasive species (Richardson & Rejmánek 2006). Some species of the genus *Senna* are used as foods, ornamental plants, or with medicinal purposes (Mazzi & Soliman 2010), while others have become invasive species (Richardson & Rejmánek 2006). Some species of the genus *Senna* are used as foods, ornamental plants, or with medicinal purposes (Mazzi & Soliman 2010), while others have become invasive species (Richardson & Rejmánek 2006).

**Materials and Methods**

All reagents used in this study were of analytical grade and commercially available. Agar, brewer’s yeast, L-ascorbic acid, and neomycin sulfate were purchased from Fisher Scientific (Thermo-Fisher Scientific, Waltham, Massachusetts, USA); acetone, ethanol, formaldehyde, methyl p-hydroxybenzoate, 1-octacosanol were from Sigma-Aldrich (St. Louis, Missouri, USA).

**PLANT MATERIAL**

The aerial parts (leaves, stems, buds, pods, and seeds) of *S. crotalariaoides* were collected in Sep 2017, from 10 random specimens in the locality of Comadres (22.616666°N, 100.4000000°W, 1640 masl), a municipality of Guadalcazar (state of San Luis Potosí, Mexico). The specimens of the plant were authenticated, based on macro- and microscopic features of the plant: texture, shape, apex and leaf margin, pods, seeds, stems, and floral structure, by the Biólogo José García-Pérez, Instituto de Investigación en Zonas Desérticas, Universidad Autónoma de San Luis Potosí, San Luis Potosí, state of San Luis Potosí, Mexico. A voucher herbarium is preserved in the collection of the Isidro Palacios Plant Herbarium at the Universidad Autónoma de San Luis Potosí with the code number SPLM43012. The leaves were separated and dried in the shade at 27 ± 2 °C, for 15 d. Subsequently, the dry plant material was milled in a Thomas Model 4 Wiley™ mill to 1.0 mm particle size (Thomas Scientific, Swedesboro, New Jersey, USA). The ground material was placed in a 1 L flask with 500 mL of chloroform, and extracted under reflux conditions at 50 °C. Then, the supernatant was filtered under vacuum through a Büchner funnel (Corning Inc., Corning, New York, USA), and the solvent evaporated until dry under reduced pressure using a BUCHI R-210 rotary evaporator (Büchi, Flawil, Switzerland) to obtain the crude extract.

*SPODOPTERA FRUGIPERDA* TEST INSECTS AND DIET

The experiments were carried out in the Laboratorio de Com- puestos Naturales Insecticidas, Universidad Autónoma de Querétaro, Querétaro, state of Querétaro, Mexico. Larvae of *S. frugiperda* obtained from the University of Querétaro were used throughout the study. The insects had been reared in the laboratory since 2012. Periodically, the population is replaced to avoid inbreeding. The artificial diet used to establish a laboratory population consisted of a mixture prepared with 30 g common bean grains and 90 g maize grains (ground in a Thomas Model 4 Wiley™ mill to 1.0 mm particle size), 20 g brewer’s yeast, 10 g vitamin mix Lepidoptera # 722 (calcium pantothenate, crystalline biotin, folic acid, niacin, pyridoxine HCl, riboflavin, sucrose, thiamine HCl, vitamin B12, 1% mannitol) (Bio Serv, Flemington, New Jersey, USA), 17 mL of a 10% w/v ethanol solution of L-aspartic acid, 2.5 mL formaldehyde, 1.7 g methyl p-hydroxybenzoate, and 0.6 g neomycin sulfate, mixed in 800 mL of boiled agar solution (12.5 g per L). The larval diet was deposited, the larva was fed with artificial diet (2–3 g cube), and replaced by a new one each wk until the larva completed its pupal stage. Larvae were maintained at 27 ± 2 °C, 70 ± 5% relative humidity (RH), and a 14:10 h (L:D) photoperiod, in a climatic chamber with a timer, and revised every third d. When molted to pupae after 24 h, the insects were collected and transferred to another plastic container. The plastic containers were closed with the lid to avoid contamination, and to prevent pupae from escaping until adult emergence. Twenty pupae were placed in each container.

**INSECTICIDAL ACTIVITY**

Insect culturing and bioassays were run in the same experimental conditions, in a room kept at 27 ± 2 °C, 70 ± 5% RH, and a 14:10 h (L:D) photoperiod. Preliminary screening was performed by testing 5 logarithmic concentrations ranging from 0.5 to 5,000 ppm. For the final bioassay, the concentrations evaluated were 5,000, 4,000, 2,000, 1,000, and 500 ppm. Polyvinylpyrrolidone was used as co-solvent of distilled water to prepare all chloroform extracts of *S. crotalariaoides*. The extracts were mixed with the larval diet ingredients during prepa-
ration. The extract was added when the temperature of the agar solution cooled to hand hot temperature (about 45 °C). Control larval diet was prepared by adding the same volume of distilled water and polyvinylpyrrolidone to the artificial diet. A 2 to 3 g cube of artificial larval diet was placed in each container. The artificial diet was replaced by a new one each wk. Bioassays were carried out using 20 second instars for each concentration and for the control, divided into 5 experimental units with 4 larvae each, selected randomly, distributed in 20 plastic containers with a larva each. The containers were covered with plastic lids and stacked close to each other. The larvae were maintained inside the same plastic containers at 27 ± 2 °C, 70 ± 5% RH, and a 14:10 h (L:D) photoperiod until reaching the pupal stage. The pupae were weighed 24 h after pupation (mg), and then each pupa was moved to another plastic container (3 × 3 × 3.8 cm) to allow the development of adults. The insecticidal and insectistatic parameters evaluated were mortality (%) of larval and pupal stages and cumulated, and duration from larva to adult (d). The median lethal concentration (LC50) of the larval population of S. frugiperda was calculated by using data from the total larval period.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

Samples of the S. crotalarioides extract were dissolved in distilled water. The gas chromatography-mass spectrometry analysis was performed using an Agilent 5973 inert Gas Chromatograph/Mass Spectrometer (Agilent Technologies Inc., Santa Clara, California, USA) equipped with an Agilent HP-5MS fused-silica capillary column (length 30 m; inner diameter 0.250 mm; film thickness 0.25 µm), coated with 5% phenyl-methylsiloxane, at 250 °C. Pure helium was used as a carrier gas with a flow rate of 1 mL per min. Split ratio was 2:1. The column temperature was initially 50 °C (for 3 min) and was gradually increased to 240 °C, at 3 °C min−1; this temperature was held for 2 min. The injector temperature was 250 °C and 1 L of samples was injected twice. The spectra were collected at 71 eV ionization voltages, and the analyzed mass range was 15 to 600 m/z. The identification of the components was confirmed by comparison of the retention indices with those of authentic compounds using the Kovats index (Kovats 1958), based on n-alkanes C6 to C26, and by comparison of their retention times with those of WILEY 09 and NIST 11 mass spectral database. The relative percentage of the individual components in the crude extract from leaves of S. crotalarioides was expressed as percentage based on the peak areas obtained.

STATISTICAL ANALYSIS

Data are expressed as the means ± standard errors of the mean for 4 replicates. Results were excluded from analysis if the mortality rate in the control samples was above 20%. In addition, if the percentage of larvae killed during each time interval in the control samples ranged between 5 and 20%, the mortality of treated samples was corrected using Abbott’s formula (Abbott 1925).

\[
\text{Mortality} = \frac{(x - y)}{y} \times 100
\]

where x = percentage mortality in the treated sample, and y = percentage mortality in the control.

The SYSTAT (vers. 9) analysis program (SYSTAT Software Inc., San Jose, California, USA) (Stein et al. 1997) was used to fit treatment concentration–response, and for calculating the LC50, lower and upper fiducial limits, and chi-square values by Probit analysis. Accumulated mortality at each concentration was expressed as the sum of the percentage of larval mortality plus the percentage of pupal mortality. The differences in the mean values were evaluated by analysis of variance (ANOVA). The Tukey test was used for all pair-wise multiple comparisons of groups.

ChemBioDraw Ultra 13 molecule editor (PerkinElmer, Waltham, Massachusetts, USA) was used for drawing chemical structures.

**Results**

### INSECTICIDAL AND JUVENOMIMETIC ACTIVITIES OF SENNA CROTALARIOIDES

The chloroform extract of S. crotalarioides caused an increase in S. frugiperda mortality (P < 0.001) during the development of the insects (Table 1). Comparison of the mean mortality at each concentration with the 0 ppm concentration (control) showed a concentration-dependent effect (Fig. 1).

Higher rates of mortality were obtained when higher concentrations of the chloroform extract were used. From 1,000 ppm and above, the chloroform extract of S. crotalarioides significantly affected S. frugiperda larvae, hindering their pupation. At a concentration of 2,000 ppm of chloroform extract, 45% of the larvae completed the larval stage, but only 30% were able to pupate and develop into adults. The chloroform extract of S. crotalarioides at 4,000 ppm caused 100% accumulated mortality. At the pupal stage, a concentration-dependent effect could not be observed.

The exposure to the chloroform extract of S. crotalarioides extends the duration of the larval stage of S. frugiperda, including the prepupal period. In particular, larvae exposed to 2,000, 4,000, and 5,000 ppm of chloroform extracts of S. crotalarioides took longer to reach the pupal stage compared to the control insects (Table 2). In the pupal stage, the most marked effects were observed when the larvae were exposed to

### Table 1. Insecticidal activities of the chloroform extract of Senna crotalarioides leaves to control Spodoptera frugiperda.

| Concentration (ppm) | Mortality (%) |
|---------------------|---------------|
|                     | Larvae        | Pupae         | Cumulative |
|---------------------|---------------|---------------|------------|
| 5,000               | 90.0 ± 6.9 A  | 10.0 ± 6.9 A  | 100.0 ± 0.0 A |
| 4,000               | 80.0 ± 9.2 A  | 20.0 ± 9.2 A  | 100.0 ± 0.0 A |
| 2,000               | 70.0 ± 10.5 AB| 15.0 ± 8.2 A  | 85.0 ± 8.2 A  |
| 1,000               | 55.0 ± 11.4 AB| 15.0 ± 8.2 A  | 70.0 ± 10.5 A  |
| 500                 | 35.0 ± 10.9 BC| 0.0 ± 0.0 A   | 35.0 ± 10.9 B |
| Control             | 10.0 ± 6.9 C  | 0.0 ± 0.0 A   | 10.0 ± 6.98   |

LC50 [LFL-UFL] 1001.1 [463.2–1410.1 ppm]* 872.7 [689.8–1027.4 ppm]*

Results are the mean value based on 4 determinations ± standard error of the mean. Means within a column not labeled by the same letter are different.

*LC50 values and 95% fiducial limits [in brackets] were determined through Probit analyses of the percent of dead larvae results corrected using Abbott’s formula.
4,000 and 5,000 ppm of the extract, observing statistically significant differences when comparing the duration of the stages in relation to the control. On the other hand, exposure of larvae to the chloroform extract at concentrations higher than 1,000 ppm reduced the body weight of the pupae.

**GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY ANALYSIS OF THE CHLOROFORM EXTRACT OF SENNA CROTALARIOIDES**

A total of 22 compounds were identified in the chloroform extract of *S. crotalarioides* leaves using gas chromatography-mass spectrometry, by comparing their retention indices and mass spectra fragmentation patterns with the reference spectra of the National Institute of Standards and Technology library. Among these compounds were fatty acids, terpenes, aldehydes, esters, and primary aliphatic alcohols, which constitute 99.2% of the chemical components present in the extract. The retention times, peak areas (%), and retention indexes of these compounds are presented in Table 3.

**Table 2. Insecticidal activities of the chloroform extract of Senna crotalarioides leaves to control Spodoptera frugiperda.**

| Concentration (ppm) | Larva | Pupa | Pupal weight (mg) |
|---------------------|-------|------|-------------------|
| 5,000               | 56.5 ± 4.5 A | ND   | 98.0 ± 3.0 C |
| 4,000               | 50.0 ± 1.2 A  | ND   | 105.8 ± 7.7 C |
| 2,000               | 31.3 ± 2.4 B  | 13.7 ± 0.3 A | 140.2 ± 10.8 BC |
| 1,000               | 29.4 ± 0.7 B  | 11.0 ± 0.4 B | 164.5 ± 11.9 B |
| 500                 | 23.7 ± 1.1 C  | 9.8 ± 0.2 C | 207.5 ± 11.2 A |
| Control             | 21.8 ± 0.7 C  | 9.6 ± 0.2 C | 229.8 ± 4.6 A |

Results are the mean value based on 4 determinations ± standard error of the mean. Groups with the same letter are not significantly different at \( P < 0.001 \); ND = No data because no adult emerged.

**INSECTICIDAL AND JUVENOMIMETIC ACTIVITIES OF 1-OCTACOSANOL**

The 1-octacosanol caused an increase in *S. frugiperda* mortality (\( P < 0.001 \)) during the development of the insects (Table 4). Higher rates of mortality were obtained when higher 1-octacosanol concentrations were used. From 1,000 ppm and above, 1-octacosanol significantly affected *S. frugiperda* larvae, hindering their pupation. At a concentration of 1,000 ppm of 1-octacosanol, 35% of the larvae completed the larval stage, but only 15% were able to pupate and develop into adults. At the pupal stage, a dose-dependent effect could not be observed, starting with the lowest concentration tested.

The exposure to 1-octacosanol extends the duration of the larval stage of *S. frugiperda*. In particular, larvae exposed to 400, 600, and 1,000 ppm of 1-octacosanol took longer to reach the pupal stage compared to the control insects of the corresponding treatment (Table 5). In the pupal stage, the most marked effects were observed when the larvae were exposed to 1,000 ppm 1-octacosanol, observing statistically significant differences when comparing the duration of the stage in relation to the control (\( P < 0.001 \)). On the other hand, the exposure of the larvae to 1-octacosanol at concentrations higher than 600 ppm reduced the body weight of the pupae.

**Discussion**

The insecticidal effect of various *Senna* species has been evaluated to control coleopterans that infest stored grains, and that produce important economic losses and affect their quality and safety. The n-hexane extract of the pods of *Senna italica* Mill. (Fabaceae) caused 100% mortality in adults of *Callosbruchus analis* F. (Coleoptera: Chrysomelidae) (Yagi et al. 2013). The leaf extract from *Senna obtusifolia* (L.) H.S. Irwin & Barneby (Fabaceae) showed repellency activity of class II (between 20.1–40%) to control adults of *Sitophilus zeamais* (Motschulsky) (Coleoptera: Dryophthoridae) (de Souza Tavares et al. 2014). For its part, the ethyl acetate extract of the seeds (EtOAc) and secondary metabolites of *Senna tora* (L.) Roxb. (Fabaceae) (= *Cassia tora*) showed 60% repellency activity against *Sitophilus oryzae* L., the most important toxic action was observed on larvae. The most important toxic action was observed on larvae. The most important toxic action was observed on larvae.
Table 3. Chemical composition of the chloroform extract of Senna crotalarioides leaves.

| No | Retention time | Name of compound | Total % | Kovats index (KI)* |
|----|----------------|------------------|---------|-------------------|
|    |                |                  |         | Experimental      | Literature |
| 1  | 13.523         | Neophytadiene    | 1.551   | 1,774             | 1,827†   |
| 2  | 13.637         | Myristic acid    | 0.360   | 1,788             | 1,778*   |
| 3  | 13.961         | 3,7,11,15-tetramethyl-2-hexadecen-1-ol | 0.418 | 2,045             | 2,114*   |
| 4  | 15.587         | Palmitic acid    | 5.281   | 1,987             | 1,955.4  |
| 5  | 16.759         | Phytol          | 0.360   | 2,206             | 2,099.1† |
| 6  | 17.087         | (9,12,12)-Octadecadienonic acid | 0.140 | 2,202             | 2,150.5† |
| 7  | 17.125         | (9E)-Octadecenonic acid | 0.075 | 2,194             | 2,223*   |
| 8  | 17.167         | α-Linolenic acid, trimethylsilyl ester | 0.369 | 2,210             | 2,222*   |
| 9  | 17.333         | Stearic acid, trimethylsilyl ester | 0.694 | 2,186             | 2,245‡   |
| 10 | 20.865         | Heptacosane      | 0.279   | 2,705             | 2,700†   |
| 11 | 21.874         | Squalene         | 0.815   | 2,914             | 2,814*   |
| 12 | 22.256         | Tetratetracontane | 0.589 | 4,395             | 4,400*   |
| 13 | 22.575         | 1-Hexacosanol    | 1.638   | 2,890             | 2,935*   |
| 14 | 23.296         | Octacosan        | 5.209   | 2,993             | 3,016†   |
| 15 | 24.013         | 1-Octacosanol    | 63.245  | 3,089             | 3,133*   |
| 16 | 24.194         | α-tocopherol     | 0.533   | 3,226             | 3,131*   |
| 17 | 24.724         | Trimethylsilyl octacosanoate | 0.475 | 3,180             | 3,229*   |
| 18 | 24.848         | Triacantanol     | 0.857   | 3,192             | 3,180*   |
| 19 | 24.848         | Stigmasterol     | 1.045   | 2,797             | 3,269*   |
| 20 | 25.706         | 1-Triacantanol   | 9.472   | 3,287             | 3,330*   |
| 21 | 26.225         | β-Sitosterol     | 4.549   | 2,789             | 3,332*   |
| 22 | 26.871         | Stigmast-5-ene, 3 β -(trimethylsiloxy)-, (24S)- | 1.095 | 2,789             | 3,348** |

Results are mean of 3 replicates calculated from gas chromatography-mass spectrometry areas; Kovats (1958), Bicchi et al. (1997), Babushok et al. (2011), Li et al. (1998), Khannoon et al. (2011), Ivanov et al (2018), Naemi et al. (2014), Mizuno et al. (2018), Radulovic et al. (2014), Tokuda et al. (1988), Harris et al. (2012), Isidorov et al. (2008).

Table 4. Insecticidal activities of 1-octacosanol to control Spodoptera frugiperda.

| Concentration (ppm) | Mortality (%) | Cumulative mortality |
|---------------------|---------------|---------------------|
|                     | Larvae        | Pupae               |                     |
| 1,000               | 65.0 ± 10.9 A | 20.0 ± 9.2 A        | 85.0 ± 8.2 A        |
| 600                 | 40.0 ± 11.2 AB| 25.0 ± 9.9 A        | 65.0 ± 10.9 AB      |
| 400                 | 35.0 ± 10.9 AB| 15.0 ± 8.2 A        | 50.0 ± 11.5 ABC     |
| 120                 | 25.0 ± 9.9 AB | 15.0 ± 8.2 A        | 40.0 ± 11. BC       |
| 80                  | 20.0 ± 9.9 B  | 15.0 ± 8.2 A        | 35.0 ± 10.9 BC      |
| Control             | 10.0 ± 6.9 B  | 10.0 ± 6.9 A        | 20.0 ± 9.2 C        |
| LC50 [UFL-LFL]      | 832.2 [732.3–973.9 ppm]* | 434.4 [362.0–508.2 ppm]* |

Results are the mean value based on 4 determinations ± standard error of the mean. Means within a column not labeled by the same letter are different. LC50 values and 95% fiducial limits [in brackets] were determined through Probit analyses of the percent of dead larvae results corrected using Abbott’s formula.

Table 5. Insectistatic activities of 1-octacosanol against Spodoptera frugiperda.

| Concentration (ppm) | Duration stage (d) | Pupal weight (mg) |
|---------------------|-------------------|-------------------|
|                     | Larval Pupal      |                   |
| 1,000               | 37.0 ± 1.2 A      | 17.5 ± 0.50 A     | 170.3 ± 10.0 C     |
| 600                 | 37.9 ± 1.63 A     | 15.0 ± 0.63 AB    | 190.6 ± 7.2 BC     |
| 400                 | 31.3 ± 0.70 B     | 14.1 ± 0.55 AB    | 210.5 ± 8.3 AB     |
| 120                 | 28.6 ± 0.83 BC    | 14.8 ± 0.68 AB    | 221.0 ± 6.4 A      |
| 80                  | 27.8 ± 0.40 C     | 13.5 ± 0.33 AB    | 219.0 ± 5.4 A      |
| Control             | 23.5 ± 0.54 D     | 12.2 ± 0.78 B     | 225.9 ± 5.8 A      |

Results are the mean value based on 4 determinations ± standard error of the mean. Different letters indicate significant differences between groups; p < 0.001.
stage of *Spodoptera frugiperda* is the stage that causes the greatest damage to crops (Tavares et al. 2013). On the other hand, the exposure to the chloroform extract of the leaves of *S. crotalarioides* extends the duration of the larval stage. This response has been described as a compensatory action for the larvae to recover when feeding on a low-quality host and still be able to pupate and achieve a greater weight (Silva et al. 2017).

Analysis by gas chromatography-mass spectrometry allowed the identification of various chemical compounds within the chloroform extract of the leaves of *S. crotalarioides*. Major chemical constituents were 1-octacosanol (*C_{28}H_{55}O*) (63.245%, Rt 23.296 min), a primary 28 carbon atom saturated alcohol (Fig. 2), followed by 1-triacontanol (*C_{30}H_{63}O*) (9.472%, Rt 25.706 min), palmitic acid (*C_{16}H_{31}COOH*) (5.281%, Rt 15.587 min), and octacosanal (5.209%, Rt 23.296 min). Other identified components appeared at a proportion of less than 5%. The lowest percentage content of peak area (0.075%) was for (9E)-octadecenoic acid (Rt 17.125 min). Some components identified by gas chromatography-mass spectrometry in *S. crotalarioides* chloroform extract, such as phytol, tetracotane, squalene, α-tocopherol, triacontanol, octadecadecanoic acid, and stigmasterol, also have been identified in the species *S. italic* and *Senna* spp. (Gololo et al. 2016; Silva et al. 2016; Madkour et al. 2017).

Exposure to 1-octacosanol, the major component of the chloroform extract of *S. crotalarioides*, increased mortality of the *Spodoptera frugiperda* in the larval stage, including the pupal stage. Also, this C28-chain alcohol caused a decrease in the body weight of *Spodoptera frugiperda* pupae. Although there are no data on the insecticidal activity of 1-octacosanol, previous studies indicate that some long-chain alcohols deploy antifeedant activities (Ganassi et al. 2016; Aznar-Fernández et al. 2018), besides ovicidal and larvicidal activities (Sinniah 1983). The second most abundant compound in the extract (triaconanol) is a plant growth regulator that partially reverses the jasmonic acid-induced proteinase inhibition (Ramanaray & Swamy 2004). On the other hand, it has been suggested that fatty acids, such as palmitic acid, possess insecticidal activity and inhibit the growth of the related species *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) (Farag et al. 2011). Finally, the C28-aldehyde, octacosanal, suppresses aggressiveness in some insects (Mizuno et al. 2018). The obtained results suggest that the major compounds identified in the chloroform extract of *S. crotalarioides* contribute significantly to the larvicidal and pupicidal activities.

The chloroform extract of *S. crotalarioides* caused significant larval mortality and reduction of the pupal weight and adult emergence in *S. frugiperda*. Chromatographic analysis using gas chromatography-mass spectrometry revealed that the leaves of *S. crotalarioides* synthesize long chain alkanes that increase the mortality of the *Spodoptera frugiperda* larval stage, including the pupal stage. The insecticidal and insecticstatic evaluation of 1-octacosanol, as the major component of *S. crotalarioides* chloroform extract, is presented for the first time. These results can serve as a starting point for the development of botanical insecticides based on *S. crotalarioides* leaf extracts to be used in integrated pest management, and to reduce the use of synthetic pesticides and their negative effects on the environment.

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**Fig. 2.** Structure of the major compounds identified in the chloroform extract of *Senna crotalarioides*: (a) 1-octacosanol, (b) triacontanol, (c) octacosanal, and (d) palmitic acid.

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The authors declare no potential conflicts of interest with respect to the research, authorship, and publication of this article. All mandatory laboratory health and safety procedures were followed at all times during the experiment. The handling of the insects was conducted following the “Recommendations concerning insect handling and insect allergies,” https://www.biology.lu.se/internal/employment/work-environment/insect-handling.

### References Cited

Abbott WS. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265–267.

Aguirre LA, Hernández-Juárez A, Flores M, Cerna E, Landeros J, Frías GA, Harris MK. 2016. Evaluation of foliar damage by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to genetically modified corn (Poales: Poaceae) in Mexico. Florida Entomologist 99: 276–280.

Aragón GA, Damían HMA, Huerta LM, Sáenz-Cabezón FJ, Pérez-Moreno I, Marco-Mancebéon V, López-Olguín JF. 2011. Insect occurrence and losses due to phytophagous species in the amaranth *Amaranthus hypochondriacus* L. crop in Puebla, Mexico. African Journal of Agricultural Research 6: 5924–5929.

Aznar-Fernández T, Jimmín A, Masí M, Rubiales D, Evidente A. 2018. Antifeedant activity of long-chain alcohols, and fungal and plant metabolites against pea aphid (*Acyrthosiphon pisum*) as potential biocontrol strategy. Natural Product Research 33: 2471-2479.

Babushok VI, Linstrum PJ, Zenkevich IG. 2011. Retention indices for frequently reported compounds of plant essential oils. Journal of Physical and Chemical Reference Data 40: 043101-1–043101-47.

Barrientos-Gutiérrez JE, Huerta-de la Peña A, Escobedo-Garredo JS, López-Olguín JF. 2013. Conventional management of *Spodoptera exigua* in crops of the municipality of Los Reyes de Júarez, Puebla. Revista Mexicana de Ciencias Agrícolas 4: 1197–1208.

Bichi C, Fresia M, Rubilio P, Monti D, Franz C, Goehler I. 1997. Constituents of *Tagetes lucida* Cav. ssp. Lucida essential oil. Flavour and Fragrance Journal 12: 47–52.

Blanco CA, Pelegaude JN, Nava-Camberos U, Lugo-Barrera D, Vega-Aquino P, Coello J, Terán-Vargas AP, Vargas-Canuls J. 2014. Maize pests in Mexico and challenges for the adoption of integrated pest management programs. Journal of Integrated Pest Management 5: 3–9.

Branco A, Pinto AC, Schripsema J, Braz-Filho R. 2011. Antiaquaphiones from the bark of *Senna macranthera*. Anais da Academia Brasileira de Ciências 83: 1159–1164.

Capataz-Tafur J, Orozco SF, Vergara RR, Hoyos SR. 2007. Antifeedant effect from extracts and cellular suspensions of *Azadirachta indica* against *Spodoptera frugiperda* j. E. Smith in greenhouse conditions. Revista Facultad Nacional de Agronomía, Medellin 60: 3703–3715.

Casmuz A, Juárez ML, Socías MG, Murúa GA, Prieto S, Medina S, Evert E, Babushok VI, Linstrom PJ, Zenkevich IG. 2011. Retention indices for frequently used plant and plant-derived components. Zeitschrift für Naturforschung C 66: 129–135.

Cav. ssp. Lucida essential oil. Flavour and Fragrance Journal 4: 1197–1208.

Ciencias Agrícolas 4: 1197–1208.

Coello J, Terán-Vargas AP, Vargas-Canuls J. 2014. Maize pests in Mexico and challenges for the adoption of integrated pest management programs. Journal of Integrated Pest Management 5: 3–9.

de Souza Tavares W, Faroni LRDA, Ribeiro RC, Fouad HA, de Sousa Freitas S, Coello J, Terán-Vargas AP, Vargas-Canuls J. 2014. Maize pests in Mexico and challenges for the adoption of integrated pest management programs. Journal of Integrated Pest Management 3: 9–11.

Estrada CE, Yen MC, Delgado SA, Villarreal QJA. 2004. Leguminosas del centro de México. Anales del Instituto de Biología, Universidad Nacional Autónoma de México. Journal of Economic Entomology 18: 265–267.

Evidente A. 2018. Antifeedant activity of long-chain alcohols, and fungal and plant metabolites against pea aphid (*Acyrthosiphon pisum*) as potential biocontrol strategy. Natural Product Research 33: 2471-2479.

Essiet UA, Bassey IE. 2013. Comparative phytochemical screening and nutritional potentials of the flowers (petals) of *Senna alata* (L.) Roxb., *Senna hirsuta* (L.) Irwin and Barneby, and *Senna obtusifolia* (L.) Irwin and Barneby (Fabaceae). Journal of Applied Pharmaceutical Sciences 3: 97–101.

Estrada CE, Yen MC, Delgado SA, Villarreal QJA. 2004. Leguminosas del centro de México. Anales del Instituto de Biología, Universidad Nacional Autónoma de México. Journal of Economic Entomology 18: 265–267.

Farah M, Ahmed MH, Youssef H, Abdel-Rahman AH. 2011. Repellent and insecticidal activities of *Melia azedarach* L. against cotton leafworm, *Spodoptera littoralis* (Boisd.). Zeitschrift für Naturforschung C 66: 129–135.

Fürstenberg-Hägg J, Zagrobelny M, Bak S. 2013. Plant defense against insect herbivores. International Journal of Molecular Science 14: 10242–10297.
Zavala-Sánchez et al.: Activity of 1-octacosanol against fall armyworm

Ganassi S, Grazioso P, De Cristofaro A, Fiorentini F, Sabatini MA, Evidente A, Altomare C. 2016. Long chain alcohols produced by Trichoderma citrinoviride have phagodeterrent activity against the bird cherry-oat aphid Rhopalosiphum padi. Frontiers in Microbiology 7: 1–13.

Goergen G, Kumar PL, Sankung SB, Togola A, Tamo M. 2016. First report of outbreaks of the fall armyworm Spodoptera frugiperda (JE Smith) (Lepidoptera Noctuidae), a new alien invasive pest in West and Central Africa. PLoS ONE 11: e0165632. doi: 10.1371/journal.pone.0165632

Goloso SS, Mapfumai NS, Sethoga LS, Olivier MT, Shai LJ, Mogale MA. 2016. Identification of phytochemical constituents within the n-hexane leaf extract of Sennta italica (Mill) using gas chromatography-mass spectrometry (GC-MS) analysis. Journal of Pharmaceutical Science Research 5: 1141–1143.

Graham JG, Zhang H, Pendlain SL, Santansiero BD, Mesecar AD, Cables F, Farnsworth NR. 2004. Antimycobacterial naphthopyrones from Sennea obliqua. Journal of Natural Products 67: 225–227.

Guerrero A, Malo EA, Coll J, Quero C. 2014. Semiochemical and natural product-based approaches to control Spodoptera spp. (Lepidoptera: Noctuidae). Journal of Pest Science 87: 231–247.

Harris RL, Davies NW, Nicol SC. 2012. Chemical composition of odorous secretions in the lacertid lizard Acanthodactylus boskianus. Biochemical Systematics and Ecology 39: 72–81.

Hernández-Mendoza JL, López-Barbosa EC, Garza-González E, Mayek-Pérez N. 2008. Spatial distribution of Spodoptera frugiperda (Lepidoptera: Noctuidae) in maize landraces grown in Colima, Mexico. International Journal of Tropical Insect Science 28: 126–129.

Igyuve TM, Ojo GOS, Ugbaa MS, Ochigbo AE. 2018. Fall army worm (Spodoptera frugiperda (J. E. Smith)) in northeastern Mexico. Southwestern Entomologist 36: 377–379.

Isidorov VA, Lech P, Zolciak A, Rusak M, Szczepaniak L. 2008. Gas chromatographic mass spectrometric investigation of metabolites from the needles and roots of pine seedlings at early stages of pathogenic fungi Armillaria ostoyae attack. Trees 22: 531–542.

Ivanov S, Dinceva I, Badjakov I, Petkova N, Denev P, Pavlov A. 2018. GC-MS analysis of unsporal fraction from Ficus carica L. (fig) leaves. International Food Research Journal 25: 282–286.

Khannaon ER, Flachsbarth B, El-Gendy A, Mazik K, Hardege JD, Schulz S. 2011. New pharmaceutical activities of natural products: new esters of long-chain alcohols from the essential oil and anti trichomonas activity of leaf, stem, and flower of Rheum ribes L. extracts. Avicenna Journal of Phytomedicine 4: 191–199.

Núñez-Valdez ME, Calderón MA, Aranda E, Hernández L, Ramírez-Gama RM, Lina L, Rodríguez-Segura Z, Gutiérrez MC, Villalobos FJ. 2008. Identification of a putative Mexican strain of Serratia entomophila pathogenic against root-damaging larvae of Scarabaeidae (Coleoptera). Applied Environmental Microbiology 74: 802–810.

Oluwole SO, Adelowe EF, Odelede KA. 2016. Mass spectrometric and phytocidal screening of phenolic compounds in the leaf extract of Seneca alata (L.) Roxb. (Fabales: Fabaceae). Brazilian Journal of Biological Sciences 3: 209–219.

Ordóñez-García M, Ríos-Velasco C, Berlanga-Reyes DI, Acosta-Muñoz CH, Salas-Marina MA, Cambero-Campos OJ. 2015. Occurrence of natural enemies of Spodoptera frugiperda (Lepidoptera: Noctuidae) in Chihuahua, Mexico. Florida Entomologist 98: 843–847.

Parolin P. 2005. Senna reticulata (Willd.) H. S. Irwin & Barneby (Fabaceae) as “pasture killer” (“matapasto”) pioneer tree in Amazonian floodplains. Ecologia Aplicada 4: 41–46.

Pérez-Zubiri JR, Cerna-Chávez A, Aguirre-Uribe LA, Landeros-Flores J, Harris MK, Rodríguez-Herrera R. 2016. Population variability of Spodoptera frugiperda (Lepidoptera: Noctuidae) in maize (Poales: Poaceae) associated with the use of agrochemical insecticides. Fierro Entomologist 99: 329–331.

Quintana-López CM, Ramos-López MA, Figueroa-Brito R, Bah MM, Rico-Rodríguez MA, Pacheco-Aguila JR. 2016. Insecticidal and insecticide activity of Senna crotalariaeoides (Fabaceae) on Spodoptera frugiperda (Lepidoptera: Noctuidae). Entomologia Mexicana 3: 171–176.

Radulovic NS, Mladenovic MZ, Stojanovic-Radz CI. 2014. Synthesis of small libraries of natural products: new esters of long-chain alcohols from the essential oil of Scandix pecten-veneris L. (Apiaceae). Flavour and Fragrance Journal 29: 255–266.

Ramanarayan K, Swamy GS. 2004. Triacetonol negatively modulates the jasmonic acid-stimulated proteinase inhibitors in tomato (lycopersicon esculentum). Journal of Plant Physiology 161: 489–492.

Richardson DM, Rejmánek M. 2011. Trees and shrubs as invasive alien species—a global review. Diversity and Distributions 17: 788–809.

Rodríguez-del-Bosque LA, Rosales-Robles E, Reyes-Rosas MA. 2011. Unusual damage to maize shanks and cobs by Spodoptera frugiperda (Lepidoptera: Noctuidae) in northeastern Mexico. Southwestern Entomologist 36: 377–379.

Silva DM, Freitas BA, dos Santos SC, Oliveira JNP, Neves DMC. 2013. Biology and nutrition of Spodoptera frugiperda (Lepidoptera: Noctuidae) fed on different food sources. Scientia Agraria 18: 17–31.

Silva JG, Silva AA, Coutinho ID, Pessoa CO, Cavalheiro AJ, Silva MG. 2016. Chemical composition of essential uents isolated from flowers and fruits of Cassia obtusifolia. Journal of Agriculture Food Chemistry 50: 5042–5047.

Rodríguez-Segura Z, Gutiérrez MC, Villalobos FJ. 2008. Identification of a putative Mexican strain of Serratia entomophila pathogenic against root-damaging larvae of Scarabaeidae (Coleoptera). Applied Environmental Microbiology 74: 802–810.

Singhurst JR, Mink JN, Holmes WC. 2013. Association of natural products isolated from flowers and fruits of Cassia occidentalis with the volatile components in goat feces. Chinese Journal of Analytical Chemistry 27: 279–287.

Stein PG, Matey JR, Pitts K. 1997. A review of statistical software for the Apple Macintosh. The American Statistician 51: 67–82.

Tavares W de S, Grazziotti GH, de Souza Junior AA, Freitas S de S, Consolario HN, Ribeiro PE de A, Zanuncio JC. 2013. Screening of extracts of leaves and stems of Paeonia officinalis (Rubiaceae) against Staphylocus zeamais (Coleoptera: Curculionidae) and Spodoptera frugiperda (Lepidoptera: Noctuidae) for maize protection. Journal of Food Protection 76: 1892–1901.

Takada H, Saito H, Kuma M, Takano S. 1988. Automated analysis of various compounds with a wide range of boiling points by capillary gas chromatography based on retention indices. Journal of Chromatography 454: 199–209.

Tavares W de S, Grazziotti GH, de Souza Junior AA, Freitas S de S, Consolario HN, Ribeiro PE de A, Zanuncio JC. 2013. Screening of extracts of leaves and stems of Paeonia officinalis (Rubiaceae) against Staphylococcus zeamais (Coleoptera: Curculionidae) and Spodoptera frugiperda (Lepidoptera: Noctuidae) for maize protection. Journal of Food Protection 76: 1892–1901.

Takada H, Saito H, Kuma M, Takano S. 1988. Automated analysis of various compounds with a wide range of boiling points by capillary gas chromatography based on retention indices. Journal of Chromatography 454: 199–209.