Effects of Ethanolic Extracts of Argemone ochroleuca (Papaveraceae) on the Food Consumption and Development of Spodoptera frugiperda (Lepidoptera: Noctuidae)

Authors: Martínez, Ana M., Aguado-Pedraza, Aldo J., Viñuela, Elisa, Rodríguez-Enríquez, Christian L., Lobit, Philippe, et al.

Source: Florida Entomologist, 100(2) : 339-345

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.100.0232

Effects of ethanolic extracts of *Argemone ochroleuca* (Papaveraceae) on the food consumption and development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

Ana M. Martínez[1], Aldo J. Aguado-Pedraza[1,2], Elisa Viñuela[3], Christian L. Rodríguez-Enríquez[1], Philippe Lobit[1], Benjamín Gómez[4], and Samuel Pineda[1,]*

Abstract

The effects of 2 concentrations (15 and 30%) of ethanolic *Argemone ochroleuca* Sweet (Papaveraceae) extracts were determined on the feeding behavior and development of 3rd instar larvae of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) through their continuous ingestion of treated maize leaves for 48 h. The extracts were obtained from dried whole plants including stems, leaves, and flowers. Armyworm food consumption was slightly reduced (by 13 to 14%) at both concentrations compared with the controls. Larval growth was significantly affected only at the highest concentration, with a 23% reduction in larval weight after 48 h of feeding on the treated leaves and a 38% reduction after another 48 h of feeding on an untreated artificial diet. By contrast, the duration of the larval and pupal stages was prolonged by 43% (from 13.5 to 19.4 d) and by 8% (from 2.1 to 2.2 d), respectively. Total larval mortality was also higher at the highest concentration (31%) than in controls (10%). During the pupal stage, neither the number of pupae that formed nor their mortality or weight was significantly affected by the treatments. However, both of the bioassay concentrations significantly lengthened the duration of the pupal stage in males, whereas in females, only the higher concentration did. In addition, no effect was observed on the adult emergence, sex ratio, and fecundity. In summary, the primary effect of ethanolic *A. ochroleuca* extracts appears to be a reduction in feeding, which simultaneously slows larval growth and increases mortality.

Key Words: botanical insecticide; fall armyworm; effect on development; feeding behavior

Resumen

En este estudio se determinó, a través de la ingestión continua de hojas tratadas de maíz durante 48 h, el efecto de dos concentraciones (15% y 30%) de extractos etanólicos de *Argemone ochroleuca* Sweet (Papaveraceae) sobre el comportamiento de alimentación y desarrollo de larvas de tercer ínstar del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). Los extractos se obtuvieron de plantas completas secas incluyendo tallos, hojas y flores. El consumo de alimento se redujo ligeramente (en 13 a 14%) en ambas concentraciones comparado con el testigo. El crecimiento fue afectado significativamente solamente en la más alta concentración, con una reducción de 23% del peso larval después de 48 h de alimentación sobre las hojas tratadas y un 38% de reducción después de otras 48 h de alimentación sobre dieta artificial no tratada. En contraste, la duración de los estados de larva y pupa se incrementó en 43% (desde 13.5 a 19.4 d) y 8% (desde 2.1 a 2.2. d), respectivamente. La mortalidad larvaria también fue más alta en la concentración más alta (31%) que en el testigo (10%). Durante el estado de pupa, ni el número de pupas formadas ni su mortalidad o peso fue afectado significativamente por los tratamientos. Sin embargo, ambas concentraciones ensayadas prologaron significativamente la duración del estado de pupas en machos mientras que en hembras este efecto solamente se observó en la concentración más alta. También, ningún efecto se observó en la emergencia de adultos, proporción de sexos y fecundidad. Se concluye que el principal efecto de los extractos etanólicos de *A. ochroleuca* son la reducción en la alimentación, lo cual, al mismo tiempo, prologa el crecimiento larval e incrementa la mortalidad.

Palabras Clave: insecticidas botánicos; gusano cogollero; efectos en el desarrollo; comportamiento de alimentación

The widespread use of synthetic insecticides for controlling insect pests has led to many detrimental effects such as the development of pest resistance, environmental pollution, interference with biological control, undesirable effects on workers, the presence of residues in food if safety periods are not observed, and a loss of vital plant pollinators (Pimentel & Peshin 2014). For these reasons, scientists and growers are continuously seeking new tools that are effective against the target pest, are compatible with integrated pest management prac-

[1]Universidad Michoacana de San Nicolás de Hidalgo, Instituto de Investigaciones Agropecuarias y Forestales, Km. 9.5 Carretera Morelia-Zinapécuaro, 58880 Tarímbaro, Michoacán, Mexico; E-mail: amabel_66@hotmail.com (A. M. M.), saap_3k15@hotmail.com (A. J. A.-P.), chrisluroen@gmail.com (C. L. R.-E.), plobit@gmail.com (P. L.), spineda_us@yahoo.com (S. P.)
[2]Instituto Tecnológico del Valle de Morelia, Km. 6.5 Carretera Morelia-Salamanca, 58100 Los Ángeles, Morelia, Michoacán, Mexico
[3]Protección de Cultivos, Escuela Técnica Superior de Ingeniería Agronómica, Alimentaria y de Biosistemas, E-28040 Madrid, Spain; E-mail: elisa.vinuela@upm.es (E. V.)
[4]Universidad Michoacana de San Nicolás de Hidalgo, Facultad de Medicina Veterinaria y Zootecnia, Km. 9.5 Carretera Morelia-Zinapécuaro, 58880 Tarímbaro, Michoacán, Mexico; E-mail: roschberith@gmail.com (B. G.)
*Corresponding author; E-mail: spineda_us@yahoo.com (S. P.)

tices (Copping & Menn 2000; Garcia et al. 2006), and have a favorable ecotoxicological profile including short persistence in the environment (Isman 2006; Senthil-Nathan 2015).

Botanicals have a long history of use. The use of plant derivatives that can act as antifeedants, repellents, and toxicants extends back at least 2 millennia in some countries (Egypt, China, Greece, and India) and more than 150 yr in Europe and North America (El-Wakeil 2013). However, besides pyrethrum, neem, and some plant essential oils, only a few plant-derived substances are available as commercial products in the world (Sola et al. 2014), even though these products are key tools in organic agriculture (DOUE 2016). At present, crude plant materials or extracts and a few commercial formulations produced by farmers and small-scale industries are applied to crops in order to target various pests in Africa, Asia, and Latin American countries (Dubey et al. 2011; Isman & Paluch 2011). In Mexico, botanical pesticides that were extracted from plants of various families [e.g., *Allium sativum* L. (Amaryllidaceae), *Cinnamomum verum* J. S. Presl. (Lauraceae), *Capsicum frutescens* L. (Solanaceae), *Larrea tridentata* (Moç. and Seseé ex DC.) Coville (Zygophyllaceae), *Azadirachta indica* A. Juss (Meliaceae), and *Trichilia havanensis* Jacq. (Meliaceae)] were evaluated for their control of lepidopteran, hemipteran, thysanopteran, and mite pests in horticultural and berry crops (López-Olguín et al. 1999, 2000; A. J. Aguado-Pedraza, Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, personal communication). In recent years, the interest in botanical pesticides has resurged globally due to the risk associated with synthetics, and the voluminous scientific literature describing their effects includes several books (see review articles by Isman 2006; El-Wakeil 2013). Although botanical pesticides make up only approximately 1% of the global insecticide market (Isman & Paluch 2011), they are essential in organic agriculture. These compounds have been reported to be safer for beneficial organisms than conventional products (Croft 1990; Hasseeb et al. 2004).

Plants and herbivorous insects have evolved together since the Carboniferous period (Wink 2003; Abdul Majeed & Abidunnisa 2011). The diversity of plant allelochemicals and their chemical structures is enormous, and to date, more than 100,000 compounds have been isolated, and their roles in preventing insect damage have been elucidated (Koul 2005). In contrast to the mode of action of many synthetic pesticides, only a few of these secondary metabolites target the insect nervous system. Plant allelochemicals act primarily as inhibitors of growth, development, and reproduction; as antifeedants and inhibitors of oviposition; and as repellents (Isman & Paluch 2011). Based on the results of our group, some metabolites from *Cestrum parqui* (Lamarck) L'Héritier (Solanaceae) and *Drimys winteri* J. R. Forster & G. Forster (Winteraceae) offer excellent potential as pesticides due to their antifeedant and toxic activity against key pests from various orders (Zapata et al. 2006, 2009, 2010).

Our study was focused on *Argemone ochroleuca* Sweet (Papaveraceae), a plant that is native to Mexico and known locally as "chicalote," which is widely distributed from the southern United States across Mexico to Central and South America, the Caribbean, Australia (Heard & Segura 2012; Brahmachari et al. 2013), India (Sharma et al. 2010; Patel 2013) and Africa (Ibrahim & Ibrahim 2009). This species is an annual prickly herb (growing up to 1 m high) that grows year-round and can be distinguished from other species in the genus by the presence of yellow sap and pale yellow petals (Heard & Segura 2012; Patel 2013). The aerial parts of *A. ochroleuca* have been used in traditional human medicine for mitigation of some illnesses such as skin diseases (warts and spots), insomnia, nervous diseases, eye infections, coughs, microbial infections, and rage (Rzedowski 1991; Argueta & Cano 1994). Fatty acids (Fletcher et al. 1993), flavonoids (Bhardwaj et al. 1982; Saleh et al. 1987; Chang et al. 2003), and essential oils (Rzedowski 1991) were isolated from *A. ochroleuca*, and a great variety of alkaloids (berberine, palmatine, protopine, sarguinarine, optisine, chelerythrine, atropine, dihydrosanguinarine, dihydro-chelerythrine, α-allacriptopine, heleritrine, queilantifoline, scouletrine, reticuline, and copsitine) were identified (Israilov et al. 1986; Takken et al. 1993; Espinosa & Sarukhán 1997). It is believed that the high contents of these components protect this species from herbivorous insects. Nevertheless, the insecticidal activity of *A. ochroleuca* is not well documented. Alkaloids from the *Argemone* genus possess a wide variety of biological activities, including pharmacological, cytotoxic, antibacterial, antifungal, and antifeedant properties (Schmeller et al. 1997; Chang et al. 2003; Alamri & Moustafa 2010; Brahmachari et al. 2013).

Even though the *Argemone* genus includes 30 species (Karlsson et al. 2003), most of the information that is available in the literature concerning their medicinal and insecticidal properties has been generated from research on the most abundant species, *Argemone mexicana* L. (Papaveraceae). Various plant parts of this species (the roots, branches, stems, leaves, flowers, seed extracts, and oils) possess insecticidal, repellent, nematicidal, and bactericidal effects, and they were tested against a wide range of pests of agricultural importance (Prakash & Rao 1997). Crude *A. mexicana* extracts were demonstrated to have ovicidal and larvicidal activity against lepidopteran, dipteran, coleopteran, and hemipteran pests (Cepero 1994; Zambare et al. 2012; Kangade & Zambare 2013). These extracts have a wide range of sublethal effects including growth delay (Rao & Chitra 2000), reduced fecundity and fertility, molting disorders, morphogenetic defects (Malarvannan et al. 2008; Kangade & Zambare 2013), and repellency (Abdul Majeed & Abidunnisa 2011).

The only documented case of biological activity of *A. ochroleuca* on insects is from Bakhashwain & Alqurashi (2010), who found repellent and insecticidal effects of extracts from leaves of this species on the flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Therefore, to help better understand the biological activity of *A. ochroleuca*, we report the lethal and sublethal effects of ethanolic extracts from a mix of dried stems, leaves, and flowers of this species on 3rd instar larvae of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), which is the most serious maize pest in Latin America.

Materials and Methods

INSECT REARING

The insects used in these tests came from an *S. frugiperda* colony that was maintained at the Instituto de Investigaciones Agropecuarias y Forestales (IIAF) of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH) (Tarímbaro, Michoacán, Mexico) and had no history of insecticide exposure. The colony was refreshed annually with the introduction of wild stock (120 to 149 pupae each yr) that were collected from a maize field at El Trébol, within a 9.5 km radius of the town of Morelia, Michoacán, Mexico, to help maintain the genetic variability. The larvae were reared on an artificial diet (Poitout & Bues 1974), and the adults were fed with a 15% honey solution. Brown paper was provided as an oviposition substrate and replaced as needed. Insect rearing and bioassays were performed at 25 ± 2 °C with 75 ± 5% RH and a photoperiod of 16:8 h L:D.

EXTRACT PREPARATION

Six *A. ochroleuca* flowering plants (approx. 0.80 m in height) were collected in Feb 2013 at the campus of the Universidad Tecnológica de Morelia (19.7494171°N, 101.1688899°W) in Morelia, Michoacán,

Mexico. Specimen identification was conducted through comparisons with a voucher specimen (No. 46485) that was deposited in the Herbarium of the Faculty of Biology at the UMSNH. Fresh materials including stems, leaves, and flowers were washed with distilled water to remove any debris and dried in the shade at room temperature for 1 wk. All the dried material was chopped, ground in a porcelain mortar, and extracted with 500 mL of 96% ethanol (J. T. Baker, Xalostoc, Estado de México, Mexico). This mixture was maintained under laboratory conditions at approximately 25 °C for 5 d and gently shaken at 24 h intervals. Finally, it was filtered (paper Whatman® No. 1, Whatman International Ltd., Maidstone, United Kingdom), and the solvent was evaporated using a stirring hot plate (Cimarec®, Dubuque, Iowa) at 60 to 65 °C until a concentrated extract was obtained. As reported by Al-Hayyan (2006), the alkaloids in *A. ochroleuca* had good extractability in organic solvents (ethanol, dichloromethane, acetone, petroleum ether, chloroform, methanol, and ethyl acetate), and thus we decided to use ethanolic extracts. Dilutions of 15 and 30% (vol/vol) were prepared by diluting 2.25 mL or 4.5 mL of concentrated extract in water up to 15 mL.

PLANT MATERIAL

Maize seeds (*Zea mays* L.; Poaceae) of cv. HY-311 were allowed to germinate for 3 d on damp filter paper and then planted individually in a polystyrene container (11 cm diameter × 17 cm height) filled with a mixture of volcanic gravel known locally as "tezontle" and humus-rich soil (1:1). All of the plants were watered at 3 d intervals and fertilized once a week with approximately 0.1 g per plant of a commercial granular fertilizer (NPK, 14-20-7 with micronutrients; Industrias Agrícolas Unidas, Zamora, Mexico). Maize plants were maintained in a ventilated heated greenhouse located on campus at the IIAF-UMSNH.

EFFECT ON FOOD CONSUMPTION

Newly molted (<8 h old) 3rd instars of *S. frugiperda* were fed with maize leaf pieces (20 mm × 15 mm), which were obtained from expanding true maize leaves that had been surface sterilized with 0.01% (wt/vol) sodium hypochlorite followed by 2 washes with sterile distilled water.

Maize leaf pieces were dipped in 15 mL of 15 and 30% *A. ochroleuca* solutions for 5 s each. To enhance the wetting of the maize leaf pieces, the surfactant sodium dodecyl sulfate was used at 0.01% (wt/vol). Control maize leaf pieces were dipped in distilled water plus surfactant only. Treated maize leaf pieces were allowed to dry for 1 h and then individually placed inside the cylindrical wells (each of which had a 22.1 mm bottom diameter) of 12-well Costar® tissue culture plates (Corning®, New York, New York) containing 1 mL of 1.5% (wt/vol) agar to prevent desiccation. The larvae were starved for 5 h to induce a high feeding rate before they were placed individually in the wells, and an experimental unit consisted of a group of 12 larvae in a culture plate. In each group, 1 of the treatments was applied: 15 replicates were treated with the 15% ethanolic extract, 18 replicates with the 30% ethanolic extract, and 18 with the control. . The maize leaf pieces were removed after 48 h, and food consumption was measured. The larvae were allowed to feed on the maize leaf pieces for only 48 h to test whether their development was affected once the extract under investigation was removed, as in *Spodoptera litura* F. (Lepidoptera: Noctuidae) (Arivoli & Tennyson 2013).

Food consumption was determined on each maize leaf piece on which *S. frugiperda* 3rd instars had fed. Images of these maize leaf pieces were obtained using an HP Scanjet 3770 scanner. They were segmented using free GIMP v.2.6.6 software (GNU Image Manipulation Program, http://www.gimp.org) to distinguish between intact and consumed areas, and the resulting images were analyzed using UTHSCSA Image Tool v.3.0.25 (Wilcox et al. 2002). The food consumption of *S. frugiperda* 3rd instars was expressed as the average percentage of maize leaf piece area consumed per larva, and it accounted for the number of larvae that were alive at that time, which was calculated as:

$$\% \text{ of leaf consumed} = CAL \times 100/TAL$$

where CAL is the consumed area of the maize leaf piece and TAL is the total area of the maize leaf piece.

EFFECT ON DEVELOPMENT

Surviving larvae from the food consumption assay were transferred to a new plate in which each well contained approximately 8 g of untreated semi-synthetic diet. The larvae were checked at 24 h intervals for pupation or until death occurred, and they were considered dead when no movement was observed after a light touch with a brush. The individual weights of a minimum of 20 and a maximum of 64 larvae per extract concentration and control were determined after they were transferred to the untreated semi-synthetic diet and 48 h later. The larval and prepupal durations were also determined. The development of surviving individuals was tracked, and the sublethal effects on pupae (the formation, duration, mortality, and weight percentages) were recorded. To determine pupal weights, 52 male and 52 female pupae per treatment were individually weighed 3 d after pupation.

The pupae were placed individually in the wells of tissue culture plates as described for the consumption experiment and were examined daily for adult emergence. They were considered dead if any adult had not emerged after 14 d. The emergence, sex ratio, and fecundity also were assessed. The sex ratio was evaluated as the number of males divided by the number of females + males. To study fecundity, between 7 and 16 couples per treatment (<48 h old) were individually placed in oviposition containers (7.5 cm in diameter × 5 cm in height) lined with brown paper (replaced at 2 d intervals) and provided with a 15% honey solution. The cumulative number of eggs per female was recorded until death to compare the fecundity among treatments.

DATA ANALYSES

Data on the food consumption, larval weight, duration of the larval and prepupal stages, larval and pupal mortality, pupae formation, adult emergence, and fecundity were subjected to an analysis of variance (ANOVA) followed by least significant difference (LSD) multiple range tests ($P < 0.05$) to separate the means with the Statgraphics graphic software system (STSC Inc., Rockville, Maryland). In cases in which the ANOVA assumptions were violated and could not be met by transformation, a non-parametric Kruskal–Wallis test was applied.

The pupal weights were analyzed using the general linear models (GLM) procedure, with LSD for means separation (SAS/STAT® software version 8.1; SAS Institute, Carry, North Carolina). For the sex ratio, a binomial distribution by sex was used.

Results

FOOD CONSUMPTION

When *S. frugiperda* 3rd instar larvae were fed with maize leaves that had been treated with the 2 concentrations of *A. ochroleuca* ethanolic extracts for 48 h, a slight but significant antifeedant activity (which was estimated as the percentage of leaf damage per larva) was detected for both bioassay concentrations (19.14 ± 0.81 and 19.38 ± 0.90% in 15 and 30% concentrations, respectively, compared with 22.26 ± 0.90% in the controls) (Table 1).

Table 1. Effects of feeding *Argemone ochroleuca* ethanolic extracts for 48 h on the food consumption, mean weight, duration, and larval mortality (± SE) of *Spodoptera frugiperda* 3rd instar larvae.

A. ochroleuca extract concentration (%)	Larval weight (g)			Duration (d)		
	% food consumption per larva (*n*)[a]	After 48 h feeding on treated maize leaves (*n*)[b]	After additional 48 h feeding on untreated artificial diet (*n*)[c]	Larval stage (*n*)[d]	Prepupal stage (*n*)[e]	Larval mortality from treatment day to prepupation (%)[f]
Control	22.26 ± 0.90 (148) a	4.53 ± 0.21 (59) a	13.80 ± 0.55 (53) a	13.52 ± 0.06 (189) a	2.09 ± 0.03 (183) a	10.25 ± 2.56 a
15	19.14 ± 0.81 (145) b	4.48 ± 0.20 (61) a	12.97 ± 0.65 (44) a	14.24 ± 0.81 (158) b	2.08 ± 0.03 (152) a	10.94 ± 2.44 a
30	19.38 ± 0.90 (135) b	3.48 ± 0.16 (64) b	8.56 ± 0.43 (20) b	19.38 ± 0.90 (133) c	2.25 ± 0.06 (117) b	31.01 ± 2.18 b

Within the same column, data (mean ± SE) followed by the same letter are not significantly different ([a,b,c]LSD mean separation, $P > 0.05$; [d,e]Kruskal–Wallis, $P = 0.05$).
[a] $F = 4.01$; df = 2,425; $P = 0.018$; *n* = number of living larvae.
[b] $F = 9.36$; df = 2,181; $P = 0.0001$; *n* = larvae were weighed individually.
[c] $K = 26.82$; $P < 0.0001$; *n* = larvae were weighed individually.
[d] $K = 309.93$; $P < 0.0001$.
[e] $K = 11.76$; $P = 0.002$.
[f] $F = 24.79$; df = 2,48; $P < 0.0001$.

Immediately after the larvae were fed with treated maize leaves for 48 h, we detected a significantly lower larval weight compared with that of the control larvae when the larvae fed on 30% *A. ochroleuca* extract (3.48 ± 0.16 versus 4.53 ± 0.21 g); the weight difference remained after 48 h of feeding on an untreated artificial diet (8.56 ± 0.43 versus 13.80 ± 0.55 g) (Table 1).

EFFECTS ON DEVELOPMENT

The 2 ethanolic extracts (15 and 30%) of *A. ochroleuca* had direct and indirect effects on *S. frugiperda* larvae. The molting process was impaired and several morphological alterations were observed; namely, we found double head capsule formation, extrusion of the hindgut, loss of hemolymph, an inability to shed the old cuticle, and cuticular blackening. In addition, shortly after treatment, some affected larvae exhibited tremors followed by paralysis and death.

Ethanolic *A. ochroleuca* extracts caused a delay in the development of larvae, prepupae, and pupae of *S. frugiperda* when the 3rd instars ingested the extract. At both concentrations (15 and 30%), we found a significantly lengthened duration of the larval stage (14.24 ± 0.81 and 19.38 ± 0.90 d, respectively) compared with controls (13.52 ± 0.06 d) (Table 1). The duration of the prepupal stage was rather similar in insects that were given treated and untreated leaves, even though a significant effect was detected at the 30% concentration (2.25 ± 0.06 d compared with 2.09 ± 0.03 d in controls) (Table 1).

The ingestion of maize leaves that were treated with 30% *A. ochroleuca* extract caused significant direct mortality (31.01 ± 2.18%) in *S. frugiperda* 3rd instar larvae until just before pupation compared with controls (10.25 ± 2.56%) (Table 1).

The percentages of pupae that formed were not affected by the treatments, and between 93.3 ± 3.96 and 96.4 ± 1.39% of the larvae molted successfully into pupae (Table 2). By contrast, feeding on treated leaves at both concentrations significantly lengthened the duration of the pupal stage in males, which lived up to 1.37 d longer than the controls (11.50 ± 0.21 d). In females, the effect was detected only at the 30% concentration, and they lived 1.04 d longer (11.08 ± 0.22 d) (Table 2).

Ethanolic extracts of *A. ochroleuca* did not affect pupal mortality (13.51 ± 3.39 to 19.94 ± 3.88%), pupal weight (217.05 ± 3.44 to 222.27 ± 3.47 g in males and 215.63 ± 3.89 to 225.06 ± 3.88 g in females) (Table 2), adult emergence (72.12 ± 6.12 to 81.38 ± 38%), the sex ratio (48 to 57% males), or the cumulative number of eggs laid per female during its lifetime (902.33 ± 62.49 to 1,012 ± 96.52) (Table 3).

Discussion

Nine out of 29 living insect orders feed on the tissues of higher plants, which have developed several mechanisms to prevent damage (Panda & Khush 1995). Phytochemicals produced by secondary plant metabolism are the major measures against herbivory, and the pattern of distribution varies considerably among families (Koul 2005). Alkaloids, a heterogeneous group with one or more nitrogen atoms in a cyclic system, are present in many plants, and they have been evaluated widely because of their toxicity and phagodeterrent effects against various species (Levin 1976). The genus *Argemone* is very rich in alkaloids, which have both pharmaceutical and pest control properties. This genus is used to treat various human ailments because of its antimicrobial, antiparasitic, antimalarial, cytotoxic, and neurological properties (Rubio-Pina & Vazquez-Flota 2013). Additionally, it plays an important role in pest control. Its aqueous and organic extracts help to lessen crop damage due to the presence of sanguinarine, berberine,

Table 2. Effects (mean ± SE) of feeding *Argemone ochroleuca* ethanolic extracts on development parameters when 3rd instar *Spodoptera frugiperda* larvae were fed with maize leaves that had been treated with extracts for 48 h.

A. ochroleuca extract concentration (%)	Formation of pupae (%)[a,b]	Duration of pupal stage (d)[c]		Pupal mortality (%)[d]	Pupal weight (g)[e]	
		Male (*n*)	Female (*n*)		Male	Female
Control	93.3 ± 3.96 a	11.50 ± 0.21 (60) a	111.08 ± 0.22 (57) a	19.94 ± 3.88 a	222.27 ± 3.47 a	225.06 ± 3.88 a
15	94.2 ± 2.46 a	12.57 ± 0.23 (52) b	11.50 ± 0.21 (60) a	17.87 ± 4.10 a	221.30 ± 3.55 a	215.63 ± 3.89 a
30	96.4 ± 1.39 a	12.88 ± 0.21 (59) b	12.12 ± 0.22 (55) b	13.51 ± 3.39 a	217.05 ± 3.44 a	216.75 ± 3.84 a

For each variable measured, means followed by the same lowercase letter are not significantly different between treatments (LSD mean separation, $P > 0.05$); n = number of specimens whose development was followed.

[a]Pupal formation was calculated based on the total number of living larvae.

[b]$F = 0.33$; df = 2,50; $P = 0.72$.

[c]$F = 9.95$; df = 5,337; $P < 0.0001$.

[d]$F = 0.79$; df = 2,50; $P = 0.46$.

[e]$F = 1.61$; df = 5,306; $P = 0.15$.

and palmatine, among other compounds, which have antifeedant and repellent effects on a wide range of pests (Schmeller et al. 1997; Malikova et al. 2006). In this respect, high concentrations (200 to 800 mg of active ingredient per L) of ethanolic extracts from our *A. ochroleuca* study plant caused 50 to 60% repellency in *T. castaneum* (Bakhashwain & Alqurashi 2010). In our present study, 3rd instar *S. frugiperda* larvae that were fed with maize leaves treated with 2 concentrations of *A. ochroleuca* ethanolic extracts (15 and 30%) had a slight but significant antifeedant activity. This finding could explain why these larvae weighed less than controls after 48 h of consumption, at least in the case of the high concentration.

In other studies, ultra-low concentrations (0.5 to 0.80 g/cm^2) of aqueous crude extracts from leaves of *A. mexicana* caused 80 to 96% repellency of adults of *T. castaneum* and the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) at 1 h after application (Abdul Majeed & Abidunnisa 2011). In addition, the crude extract from leaves of this species had an antifeedant effect on the larvae of *Crocidolomia binotalis* Zeller (Lepidoptera: Crambidae) and *S. litura* (Bosch 2007).

For a given plant and insect, the effects of plant extracts are dependent on factors that are linked to the plant (the harvest season, part used, type of extract, and concentration), the insect (development stage and age), and the application method (Jenkins et al. 2003; Malarvannan et al. 2008; Arivoli & Tennyson 2013; Kangade & Zambare 2013). The type of organic solvent that is used to extract the metabolites is very crucial; the same concentrations (≤2 mL/kg) of *A. mexicana* leaf extracts caused different mortality rates in *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) 4th instar larvae when they were extracted in different solvents: the methanolic 10 to 20% extracts, the acetonic 10 to 70% extracts, the ethanolic 20 to 90% extracts, and the chloroformic 10 to 100% extracts (Kangade & Zambare 2013). Ethanolic extracts seem to act very quickly, which is a very desirable quality for halting pest damage as soon as possible.

A high concentration of ethanolic extracts from *A. ochroleuca* leaves (200 to 800 mg active ingredient per L) caused 43 to 78% mortality in both 3rd instars and adults of *T. castaneum* 6 d after treatment (Bakhashwain & Alqurashi 2010). In the present study, the ingestion of maize leaves that were treated with 30% *A. ochroleuca* ethanolic extracts caused 31% mortality in *S. frugiperda* 3rd instar larvae until just before pupation. Vidal et al. (2009) reported that lower concentration (20 to 154 mg/L) extracts from leaves of *Argemone subfusiformis* Ownbey (Papaveraceae) caused high mortality (90 to 100%) in the 4th instars of yellow fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae), at 24 h post treatment. A field application of a 50% concentration of extracts from leaves of *A. mexicana* caused 90% mortality in adults of the stink bug *Oebalus insularis* (Stål) (Hemiptera: Pentatomidae) at 48 h after application (Cepero 1994). Some species, including larvae and pupae of the mosquito *A. aegypti*, are very sensitive to certain plant extracts, such as the ethanolic extracts from leaves of *A. subfusiformis.* The median lethal concentration for *A. aegypti* pupae at 48 h was only 9 mg/L (Vidal et al. 2009).

The morphological alterations detected in larvae in our assay during the molting process (e.g., the double head capsule, extrusion of hindgut, inability to shed the old cuticle, etc.) after the application of both ethanolic extract concentrations (15 and 30%) from *A. ochroleuca* are comparable to the development-disrupting action caused by ecdysone agonist compounds (Dhadialla et al. 2005; Pineda et al. 2006). The tremors and paralysis of larvae after treatment are indicative of a neurotoxic mode of action that could be attributed, at least partially, to the presence of sanguinarine because Schmeller et al. (1997) reported that this alkaloid inhibits the activity of the acetylcholinesterase enzyme due to its structural similarity to acetylcholine. Alterations in the regular pattern of movement (circular as opposed to zigzag in control

Table 3. Effects (mean ± SE) of *Argemone ochroleuca* extracts on *Spodoptera frugiperda* adult parameters when 3rd instar larvae were fed 2 concentrations for 48 h.

A. ochroleuca extract concentration (%)	Adult emergence (%)[a]	Sex ratio (% males)[b]	Fecundity (eggs per female)[c]
Control	81.38 ± 4.13 a	48 a	973 ± 61.0 a
15	73.33 ± 4.13 a	52 a	902 ± 62.5 a
30	72.12 ± 6.12 a	57 a	1,012 ± 96.5 a

For each variable measured, means followed by the same lowercase letter are not significantly different between treatments (LSD mean separation, $P > 0.05$).
[a] $F = 1.14$; df = 2,50; $P = 0.33$.
[b] $F = 0.80$; df = 2,257; $P = 0.45$.
[c] $F = 0.60$; df = 2,29; $P = 0.56$.

units) have also been described in *A. aegypti* 2nd instars after exposure to petroleum ether extracts of seeds from *A. mexicana* (Sakthivadivel & Thilagavathy 2003).

Delays in the development of *S. frugiperda* larvae, prepupae, and pupae were observed in our assays when the 3rd instars ingested the *A. ochroleuca* extracts. Such delays were also observed by Malarvannan et al. (2008) in *S. litura* after consuming *A. mexicana* leaf extracts. The occurrence of extra molts could have accounted for the results, and additional studies are under way to examine this possibility. The prolonged duration of the larval stage is a very undesirable effect, because larvae feed continuously for a longer period and more severe crop damage can be recorded. However, because the larval food consumption in both treatments was slightly less and the larvae weighed less than in the control, we can conclude that their fitness was impaired and the success of future generations could be compromised, as observed in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (Rodríguez-Enríquez et al. 2010) and *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (Pineda et al. 2006) when they were treated with methoxyfenozide, an insect growth regulator insecticide.

In our study, no long-term effects were detected because formation of pupae, pupal mortality, the sex ratio, and adult emergence of *S. frugiperda* were similar in the treated and control units after consumption of *A. ochroleuca* extracts. By contrast, under laboratory conditions when *C. cephalonica* larvae were fed with rice grains coated with diverse types of organic extracts from *A. mexicana* at concentrations of 1.5 and 2.0 mL/kg, the pupal mortality reached up to 40% (Kangade & Zambare 2013). The longer time period over which the extracts were continuously administered to *C. cephalonica* larvae (from 4th instar until pupation) compared with that of our study (48 h of feeding) could have accounted for this result as well as the accumulation and persistence of extracts in the larval tissue until pupal molt.

The pupal weight was not modified in our assays after feeding the larvae with extracts for a short period (48 h), but if the ingestion period is expanded, there could be effects. Malarvannan et al. (2008) reported a 20 to 37% weight reduction in *S. litura* pupae after the larvae during all 4 instars (4 d according to the conditions [22 ± 2 °C and 70 to 75% RH] at which the bioassays were performed by these authors) ingested extracts consisting of 10% aqueous, ether petroleum, and hexane *A. mexicana* leaf extracts.

There is not much information available on the effects of *Argemone* extracts on reproduction. A reduction of 50 to 100% in adult fecundity of *S. litura* has been reported after ad libitum feeding over the life span on diverse types of *A. mexicana* extracts (Malarvannan et al. 2008). However, after a short larval feeding period of 48 h in our study, this parameter was not affected.

In conclusion, when 3rd instar *S. frugiperda* larvae fed on ethanolic extracts from a mixture of dried stems, leaves, and flowers of *A. ochroleuca* for a short period (48 h) at the highest tested concentration (30%), we detected some lethal and sublethal effects in larvae. However, the treatment only prolonged the duration of the pupal stage and did not affect the adults (in terms of their emergence, sex ratio, and fecundity). We also found an increase in the duration of the larval stages and a lengthening of the feeding period on the crop. The magnitude of the reduction of feeding and the increase in larval mortality was low, which may be attributed to the short exposure time used in this study. More conclusive results may be observed in studies with younger larvae or longer feeding periods. However, several symptoms of molting disruption were observed, suggesting that *A. ochroleuca* ethanolic extracts may contain some ecdysone agonist compounds. Further studies should be conducted to isolate, assay, and identify these compounds.

Acknowledgments

This research was supported by the Coordinación de la Investigación Científica, Universidad Michoacana de San Nicolás de Hidalgo, and it forms part of the undergraduate thesis of A.-J. A.-P. We thank Javier Madrigal (Faculty of Biology, UMSNH) for his kind help with the plant identification. The manuscript was improved with comments from Trevor Williams (Instituto de Ecología A. C., Xalapa, Veracruz, Mexico). We are grateful to American Journal Experts for reviewing the English style.

References Cited

Al-Hayyan AM. 2006. Study of the alkaloids of *Argemone ochroleuca* growing in Saudi Arabia. M.S. thesis, College of Pharmacy, King Saud University, Saudi Arabia.

Alamri SA, Moustafa MF. 2010. Antibacterial activity of the latex of *Argemone ochroleuca* Sweet. Saudi Medical Journal 31*:* 1207–1210.

Argueta VA, Cano AJ. 1994. Plant Atlas of the Traditional Mexican Medicine. Instituto Nacional Indigenista, Distrito Federal, Mexico. [In Spanish]

Arivoli S, Tennyson S. 2013. Antifeedant activity, developmental indices and morphogenetic variations of plant extracts against *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae). Journal of Entomology and Zoology Studies 1: 87–96.

Bakhashwain A, Alqurashi AD. 2010. Repellent and insecticidal effects of some plant extracts on flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). Alexandria Science Exchange Journal 31: 248–254.

Bhardwaj DK, Bisht MS, Jain RK, Munjal A. 1982. Phenolics from the seeds of *Argemone mexicana*. Phytochemistry 21: 2154–2156.

Bosch CH. 2007. *Argemone mexicana* L. *In* Schmelzer GH, Gurib-Fakim A [eds.], Prota 11(1): Medicinal plants/Plantes médicinales 1. [CD-Rom]. PROTA, Wageningen, Netherlands.

Brahmachari G, Gorai D, Roy R. 2013. *Argemone mexicana*: chemical and pharmacological aspects. Revista Brasileira de Farmacognosia 23: 559–575.

Cepero J. 1994. Preliminary study on the insecticidal effect of an botanical extract of *Argemone mexicana* L. on *Oebalus insulares* Stål (Heteroptera: Pentatomidae). VII Jornada Científica INIFAT-MINAG. Santiago de Las Vegas. La Habana, Cuba. [In Spanish]

Chang YC, Hsieh PW, Chang FR, Wu RR, Liaw CC, Lee KH, Wu YC. 2003. Two new protopines argemexicaines A and B and the anti-HIV alkaloid 6-acetonyldihydrochelerythrine from Formosan *Argemone mexicana.* Planta Medica 69: 148–152.

Copping LG, Menn JJ. 2000. Biopesticides: a review of their action, applications and efficacy. Pest Management Science 56: 651–676.

Croft BA. 1990. Arthropod Biological Control Agents and Pesticides. John Wiley & Sons, New York, New York.

Dhadialla TS, Retnakaran A, Smagghe G. 2005. Insect growth- and development-disrupting insecticides, pp. 55–115 *In* Gilbert LI, Iatrou K, Gill SS [eds.], Comprehensive Insect Molecular Science, Volume 6 : Control. Elsevier, Oxford, United Kingdom.

DOUE (Diario Oficial de la Unión Europea). 2016, Reglamento de ejecución (UE) 2016/673 de la comisión de 29 de abril de 2016, http://www.magrama.gob.es/es/alimentacion/temas/la-agricultura-ecologica/r2016673_tcm7-420887.pdf (last accessed 25 Nov 2016).

Dubey NK, Shukla R, Kumar A, Singh P, Prakash B. 2011. Global scenario on the application of natural products in integrated pest management programmes, pp. 1–20 *In* Dubey NK [ed.], Natural Products in Plant Pest Management. CABI, Preston, United Kingdom.

El-Wakeil NE. 2013. Botanical pesticides and their mode of action. Gesunde Pflanzen 65: 125–149.

Espinosa FJ, Sarukhán J. 1997. Manual of Weed of Mexican Valley. Keys, Descriptions and Illustrations. Universidad Nacional Autónoma de México y Fondo de Cultura Económica. Distrito Federal, Mexico. [In Spanish]

Fletcher M, Takken G, Blaney B, Alberts V. 1993. Isoquinoline alkaloids and keto-fatty acids of *Argemone ochroleuca* and *A. mexicana* (Mexican poppy) seed. I. An assay method and factors affecting their concentration. Australian Journal of Agricultural Research 44: 265–275.

Garcia JF, Grisoto E, Vendramim JD, Botelho PSM. 2006. Bioactivity of neem, *Azadirachta indica*, against spittlebug *Mahanarva fimbriolata* (Hemiptera: Cercopidae) on sugarcane. Journal of Economic Entomology 99: 2010–2014.

Hasseeb M, Liu T-X, Jones WA. 2004. Effects of selected insecticides on *Cotesia plutellae* endoparasitoid of *Plutella xylostella*. BioControl 49: 33–46.

Heard AT, Segura R. 2012. *Argemone mexicana* L. and *Argemone ochroleuca* Sweet – Mexican poppy, pp. 65–72 *In* Mic J, McFadyen R, Cullen J. [eds.], Biological Control of Weeds in Australia. CSIRO Publishing, Victoria, Australia.

Ibrahim HA, Ibrahim H. 2009. Phytochemical screening and toxicity evaluation on the leaves of *Argemone mexicana* Linn. (Papaveraceae). International Journal of Applied Sciences 3: 39–43.

Isman MB. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology 51: 45–66.

Isman MB, Paluch G. 2011. Needles in the haystack: exploring chemical diversity of botanical insecticides, pp. 248–265 *In* López O, Fernández-Bolaños JG [eds.], Green Trends in Insect Control. RSC Publishing, Cambridge, United Kingdom.

Israilov I, Chelombit'ko V, Nazarova LE 1986. *Argemone* alkaloids. Him Prir Soedin 6: 798–799.

Jenkins DA, Dunkel FV, Gamby KT. 2003. Storage temperature of neem kernel extract: differential effects on oviposition deterrency and larval toxicity of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Environmental Entomology 32: 1283–1289.

Kangade YP, Zambare SP. 2013. Effect of extracts of *Argemone mexicana* leaves on the development of *Corcyra cephalonica* (Stainton) for the protection of the stored grains. Indian Journal of Applied Research 3: 20–22.

Karlsson LM, Tamado T, Milberg PER. 2003. Seed dormancy pattern of the annuals *Argemone ochroleuca* and *A. mexicana* (Papaveraceae*).* Flora - Morphology, Distribution, Functional Ecology of Plants 198: 329–339.

Koul O. 2005. Insect Antifeedants. CRC Press, Boca Raton, Florida.

Levin DA. 1976. Alkaloid-bearing plants: an ecogeographic perspective. The American Naturalist 110: 261–284.

López-Olguín JF, Aragón G, Viñuela E, Castañera. 1999. *Trichilia havanensis:* a plant with high potential for use in pest control, pp 189–198 *In* Proceedings of 4th International Symposium and 5th National Meeting on Sustainable Agriculture. Morelia, Michoacán, Mexico, 30 Nov to 2 Dec 2016.

López-Olguín JF, De La Torre MC, Aragón G, Viñuela E, Castañera. 2000. Antifeedant effect of extracts of *Trichilia havanensis* on larvae of *Leptinotarsa decemlineata*, pp 45–59 *In* Proceedings of the 5th National Symposium on Vegetable and Mineral Substances in Pest Control. Acapulco, Mexico, 13 Jun 2000. [In Spanish]

Abdul Majeed S, Abidunnisa T. 2011. Study on repellent activity of *Argemone mexicana* on *Tribolium castaneum* and *Sitophilus oryzae*. International Journal of Pharmaceutical Research and Development 3: 206–211.

Malarvannan S, Giridharan R, Sekar S, Prabavathy VR, Nair S. 2008. Bioefficacy of crude and fractions of *Argemone mexicana* against tobacco caterpillar, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). Journal of Biopesticides 1: 55–62.

Malikova J, Zdarilova A, Hlobilkova A. 2006. Effects of sanguinarine and chelerythrine on the cell cycle and apoptosis. Biomedical Papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia 150: 5–12.

Panda N, Khush GS.1995. Host Plant Resistance to Insects. CAB International, Wallingford, United Kingdom.

Patel PK. 2013. *Argemone ochroleuca* (Papaveraceae) naturalized in Dahod District, Gujarat, India. Phytoneuron 52: 1–5.

Pimentel D, Peshin R. [eds.]. 2014. Integrated Pest Management. Pesticide Problems, Volume 3. Springer, Dordrecht, Netherlands.

Pineda S, Smagghe G, Schneider MI, Del Estal P, Viñuela E, Martínez AM, Budia F. 2006. Toxicity and pharmacokinetics of spinosad and methoxyfenozide to *Spodoptera littoralis* (Lepidoptera: Noctuidae). Environmental Entomology 35: 856–864.

Poitout S, Bues R. 1974. Elevage de chenilles de vingt-huit espèces de Lépidoptères Noctuidae et de deux espèces d'Arctiidae sur milieu artificiel simple. Particularités de l'élevage selon les espèces. Annales de Zoologie Ecologie Animale 6: 341–411.

Prakash A, Rao J. 1997. Botanical Pesticides in Agriculture. CRC Press, Boca Raton, Florida.

Rao SRK, Chitra KC. 2000. Effect of plant extract on larval weigh and duration of *Spodoptera litura* Fabr. Journal of Applied Zoological Research 11: 98–100.

Rodríguez-Enríquez CL, Pineda S, Figueroa JI, Schneider MI, Martínez AM. 2010. Toxicity and sublethal effects of methoxyfenozide on *Spodoptera exigua* (Lepidoptera: Noctuidae). Journal of Economic Entomology 103: 662–667.

Rubio-Pina J, Vazquez-Flota F. 2013. Pharmaceutical applications of the benzylisoquinoline alkaloids from *Argemone mexicana* L. Current Topics in Medicinal Chemistry 13: 2200–2207.

Rzedowski GC. 1991. Flora del Bajío y de regiones adyacentes: "Papaveraceae" / por Graciela Calderon de Rzedowski. Fasc. 1. Instituto de Ecologia, A. C. Centro Regional del Bajío. Pátzcuaro, Michoacán, Mexico.

Sakthivadivel M, Thilagavathy D. 2003. Larvicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from *Argemone mexicana* L. seed. Bioresource Technology 89: 213–216.

Saleh NAM, Maksoud SA, El-hadidi MN, Amer WMM. 1987. A comparative study of flavonoids in some members of the Papaveraceae. Biochemical Systematics and Ecology 15: 673–675.

Schmeller T, Latz-Brüning B, Wink M. 1997. Biochemical activities of berberine, palmatine and sanguinarine mediating chemical defence against microorganisms and herbivores. Phytochemistry 44: 257–266.

Senthil-Nathan S. 2015. A review of biopesticides and their mode of action against insect pests, pp. 49–63 *In* Thangavel P, Sridevi G [eds.], Environmental Sustainability. Role of Green Technologies. Springer, Salem, India.

Sharma S, Sharma MC, Kohli DV. 2010. Pharmacological screening effect of ethanolic and methanolic extract of fruits of medicinally leaves. Digest Journal of Nanomaterials and Biostructures 5: 229–232.

Sola P, Mvumi BM, Ogendo JO, Mponda O, Kamanula JF, Nyirenda SP, Belmain SR, Stevenson PC. 2014. Botanical pesticide production, trade and regulatory mechanisms in sub-Saharan Africa: making a case for plant-based pesticidal products. Food Security 6: 369–384.

Takken G, Fletcher MT, Blaney B. 1993. Isoquinoline alkaloids and keto-fatty acids of *Argemone ochroleuca* and *A. mexicana* (Mexican poppy) seed. II. Concentrations tolerated by pigs. Australian Journal of Agricultural Research 44: 277–285.

Vidal J, Carbajal A, Sisniegas M, Bobadilla M. 2009. Toxic effect of *Argemone subfusiformis* Ownb. and *Tagetes patula* Link on larvae of the 4th instar and pupae of *Aedes aegypti* L. Revista Peruana de Biología 15: 103–110. [In Spanish]

Wilcox CD, Brent S, Dove W, McDavid D, Greer DB. 2002. UTHSCA Image Tool Version 3.0. University of Texas Health Science Center, San Antonio, Texas.

Wink M. 2003. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64: 3–19.

Zambare SP, Kangade YP, Sharma CT, Mali KH. 2012. Ovicidal effect of four plant extracts on the eggs of *Corcyra cephalonica* (Stainton). International Multidisciplinary Research Journal 5: 26–28.

Zapata N, Budia F, Viñuela E, Medina P. 2006. Insecticidal effects of various concentrations of selected extractions of *Cestrum parqui* on adult and immature *Ceratitis capitata*. Journal of Economic Entomology 99: 359–365.

Zapata N, Budia F, Viñuela E, Medina P. 2009. Antifeedant and growth inhibitory effects of extracts and drimanes of *Drimys winteri* stem bark against *Spodoptera littoralis* (Lep., Noctuidae). Industrial Crops and Products 30: 119–125.

Zapata N, Vargas M, Medina P, Viñuela E, Rodríguez B, Fereres A. 2010. The activity of a selected extract of *Drimys winteri* bark and polygodial on settling and probing behavior of the lettuce aphid *Nasonovia ribisnigri*. Phytoparasitica 38: 191–199.