Toxicity and Antifeedant Activity of Selected Plant Extracts Against Larval Obliquebanded Leafroller, *Choristoneura rosaceana* (Harris)

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Abstract. Several plant extracts were evaluated against obliquebanded leafroller larvae to determine potential toxicity and antifeedant effects. Two of the plant extracts exhibited contact toxicity; the LC_{50} value of *Humulus lupulus* extract against 3rd instar larvae was 24.5 µg / insect. In a no choice context, larval survivorship was reduced 23 - 57 % after feeding on a meridic pinto bean diet treated with the extracts. Furthermore, average female and male pupal weight was reduced for leafrollers that fed on diet treated with each of the individually-tested extracts at a 4 % concentration (w/w). Incorporation of *Bifora radians* and *H. lupulus* into diet deterred larval feeding at a 1 % concentration by weight. *H. lupulus* and *Arctium lappa* extracts were deleterious to larvae in each of the bioassay methods conducted, exhibiting toxic, developmental, and antifeedant effects. These two extracts may be promising candidates for further development as botanical insecticides that could potentially substitute broad-spectrum synthetic neurotoxins for leafroller control.

INTRODUCTION

The Obliquebanded leafroller, *Choristoneura rosaceana* (Harris), is a tortricid moth with a wide host range including woody plants in the Rosaceae as well as *Ulmus, Populus, Quercus, Betula*, and *Tilia* [1]. Depending on the geographic region and climate, there are one or two generations of adults per year with an overwintering second or third-instar larval generation [2]. The polyphagous larvae are primarily foliage feeders, but are also known to feed on and damage apple fruit [3].

Obliquebanded leafrollers are major pests in apple growing regions throughout the U.S.A. and Canada [4]. Due to azinphos-methyl resistance, population densities of this pest have increased in commercial apple growing regions in the Eastern United States [5]. Resistance to organophophates is also becoming prevalent in Canada [6, 7]. Physiological resistance to other broad-spectrum insecticides and newer chemistries such as insect growth regulators has been also identified for several compounds including chlorpyrifos, esfenvalerate, and tebufenozide [4, 8].

Botanical insecticides such as azadirachtin are often effective alternatives to organophosphates or other neurotoxins for pest control due to multiple modes of action. These include toxicity, antifeedant and anti-oviposition effects [9,10]. Natural products containing secondary plant compounds such as terpenes, steroids, alkaloids, phenolics and cardiac glycosides [11] affect insect behavior and are toxic in some cases [12-18]. Identification of plant extracts that exhibit the

above-described deleterious effects on pest insect physiology and behavior represents a potential alternative strategy for development of biorational controls that could replace synthetic neurotoxins. Our intent was to identify potential botanical insecticides that may prove to be effective alternatives for controlling obliquebanded leafroller.

In the current investigation, we tested the effects of several plant extracts, known to produce secondary compounds such as monoterpenes, sesquiterpenes and triterpenes [19-22] on obliquebanded leafroller larvae. These extracts were also chosen based on their previously-documented insecticidal activity [17,18,23]. The specific objectives of this study were to determine whether the selected plant extracts: 1) exhibit contact and/or ingestion toxicity to obliquebanded leafroller larvae, 2) affect pupal weight following ingestion by larvae, and/or 3) deter larval feeding.

MATERIALS AND METHODS

Insect Culture

Obliquebanded leafrollers were drawn from a six-year-old laboratory colony originally collected from unsprayed apple orchards in Southwestern Michigan, U.S.A. Moths were collected from the field as 1st and 2nd generation pupae and reared continuously without diapause. Insects were reared at 24°C and 60 % RH on a meridic pinto bean-based diet [24] under a 16:8 (L:D) photoperiod.

Plant Materials

The plant materials used in this study are given in Table **1**. The detailed experimental protocol for preparing plant extracts was described by Gökçe *et al*. [25]. All plant materials were collected during spring and summer seasons of 2002-2005. Dried and ground plant materials were treated with methanol for 24 h. Thereafter, the suspensions were

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Table 1. **Plant Material Used to Prepare Extracts for Assaying Toxicity and Antifeedant Bioactivities against 3rd instar Oblique-banded Leafroller Larvae. All Collections Were Made in Tokat, Turkey, 2002-2005**

Family Name	Scientific Name	Tissue Used	Month of Collection
Apiaceae	*Bifora radians* M Bieb	Leaves	April
Asteraceae	*Arctium lappa* L.	Leaves	June
Asteraceae	*Xanthium strumarium* L.	Fruit	August
Canabinaceae	*Humulus lupulus* L.	Flower bud	September
Scrophyllaceae	*Verbascum songaricum* Schrenk ex Fisch & Mey	Leaves	July

sieved through cheese cloth and excess methanol was removed using a rotary evaporator (RV 05 Basic 1B IKA Group, Wilmington, NC, U.S.A.) at 32 ± 2 °C. Residues of each plant species were diluted with sufficient HPLC grade acetone (Sigma-Aldrich, Milwaukee, WI, U.S.A.) to give 40 % (w/w) stock solutions.

Contact Toxicity of Plant Extracts

This experiment tested the hypothesis that topically-applied plant extract solutions exhibit contact toxicity to obliquebanded leafroller larvae. Toxicity of the plant extracts was tested using 3rd instar larvae. For each replicate, 10 larvae were transferred to a Whatman No. 1 filter paper disc in a 90 mm disposable Petri dish. Three replicate Petri-dishes of 10 larvae were treated with each plant extract. Each larva was treated topically with 20 μg of plant extract in 1 μl of acetone using a 50 μl Hamilton syringe. In the control treatment, larvae were treated with 1 μl of acetone. After application of treatments, the larvae were allowed to dry for 10 min at 25 ± 2 °C and were subsequently transferred individually into 240 ml soufflé cups loaded with 3.0 g of the meridic pinto bean diet described above. Following treatment application, larvae were maintained at 25 ± 2 °C and on a 16:8 (L:D) photocycle for 48 h, after which mortality was assessed. The experiment was repeated on three different days, which were regarded as blocks. Plant extract stock suspensions described above were prepared immediately prior to each block of assays.

Toxicity of *H. lupulus* Extract

Additional toxicity studies were conducted using *H. lupulus* extract based on its substantial toxic effects on obliquebanded leafroller larvae in the initial screening assay (see Results). For each replicate, 10 larvae were placed on Whatman # 1 filter paper in a 90 mm Petri dish. The dosages of *H. lupulus* compared were 0.3, 1.5, 3.0, 15.0 and 30.0 μg of plant extract / insect. Insects were treated by applying 1 μl of HPLC grade acetone solution of plant extract to the thorax using a Hamilton syringe. Control insects (N = 10 / replicate) were treated with 1 μl of HPLC grade acetone. Following application of treatments, insects were incubated as described above and mortalities were assessed after 48 h. Each treatment was replicated three times per trial and three trials (blocks) were completed on separate days for a total of 9 Petri-dishes of 10 larvae per dose.

Ingestion Toxicity and Developmental Effects of Plant Extracts

This experiment tested the hypothesis that larval feeding on diet treated with plant extracts in a no choice context af-

fects larval survival and pupal weight. Ten, 20.0 or 40.0 mg of each plant extract in 100 μl of HPLC grade acetone was incorporated per g of freshly prepared pinto bean diet (described above) to give 1, 2 or 4 % (w/w) concentrations. Each plant extract diet treatment was formulated separately. For the control treatment, 100 μl of HPLC grade acetone was added to the diet. Three g of each diet treatment was transferred into 240 ml soufflé cups. To avoid cannibalism, one freshly-moulted 3rd instar larva was transferred into each cup. The larvae were incubated at 25 ± 2 ºC and on a 16:8 (L:D) photocycle for 14 d. Larvae were checked daily until all had pupated. Pupation was considered complete following melanization. The pupae were sexed using a dissection microscope and pupal weight was recorded using a bench balance (Sartoroius, CP124 S, Elk Grove, IL, U.S.A.). The experiment was repeated on three different days (blocks) and 30 larvae were exposed to each plant extract or control treatment per block.

Feeding Deterrence Assay

This experiment tested the hypothesis that larval feeding is deterred by plant extracts incorporated into the pinto bean diet in a choice context. Ten or 40.0 mg of each plant extract in 100 μl of HPLC grade acetone was incorporated per g of freshly prepared diet to give 1 or 4 % (w/w) concentrations of each extract in diet. For the control treatment, 100 μl of HPLC grade acetone was added to the diet. For choice tests, larvae were presented with 1.0000 ± 0.0008 g of diet treated with a plant extract versus an equal amount of solvent-treated (control) diet in 50 mm round Petri dishes. Each plant extract and concentration treatment combination was tested individually versus the control. Larvae were inserted into Petri dishes individually on a piece of Whatman # 1 filter paper (1 x 1 cm) and placed centrally on portions of both treated and control diet pieces. Larvae were incubated for 72 h at 25 ± 2 ºC and on a 16:8 (L:D) photocycle. After incubation, the site of larval feeding was determined and scored as feeding on either extract-treated or control portions of the diet. Thereafter, larvae and visible frass were removed from diet with soft forceps and both extract-treated and control portions of the diet were weighed as described above. Remaining weights of treated and control portions of the diet were used to calculate an antifeedant index (AFI) modified from Isman *et al.* [26]. AFI = (C − T) / (C + T), where C and T are the weights of control and plant extract-treated diet consumed. Forty larvae were exposed to each plant extract or control treatment combination at both concentrations tested. Weight loss of diet due to water evaporation was quantified by establishing two positive control treatments of 1.0000 ± 0.0008 g of diet treated with a plant extract or solvent (N =
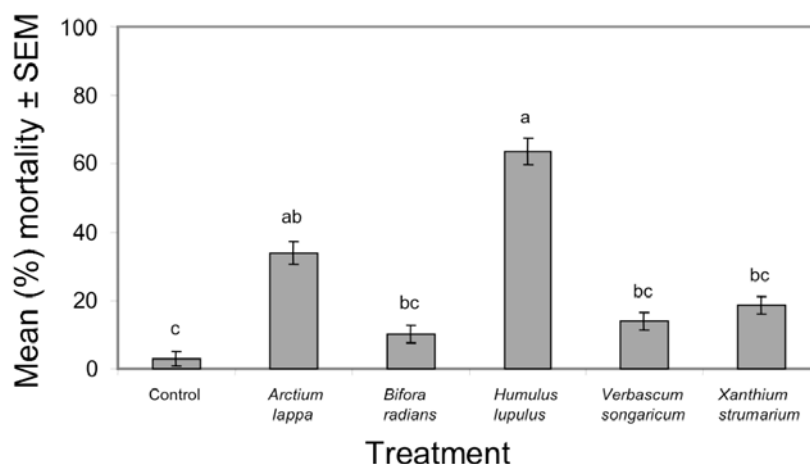
Fig. (1). Contact toxicity of selected plant-extract solutions [20 µg plant extract / insect)] to 3rd instar obliquebanded leafroller larvae. Means indicated by different letters are significantly different (P < 0.05) according to Tukey's test.

20). These were incubated and weighed in a manner identical to that described above.

Data Analyses

For the initial screening bioassay, data were corrected for mortality in the controls using Abbott's formula [27] and then normalized using an arcsine transformation [28]. Transformed data were submitted to a randomized complete block analysis of variance (ANOVA) (P ≤ 0.05) and differences between treatments were compared using Tukey's test (P ≤ 0.05). Obliquebanded leafroller larval mortality data obtained from the dose-mortality bioassay utilizing *H. lupulus* were also corrected for control mortality with Abbott's formula. The corrected mortality data were analyzed using POLO-PC [29] to estimate lethal concentration 10, 50, and 90 values (LC$_{10, 50, and 90}$) and the regression line slope. For the ingestion toxicity experiment, data were corrected with Abbott's formula and then normalized using an arcsine transformation [28]. Transformed data were submitted to a randomized complete block ANOVA (P ≤ 0.05) and differences between treatments were tested using Tukey's test (P ≤ 0.05). Pupal weights of male and female leafrollers were analyzed separately and submitted to a randomized complete block ANOVA (P ≤ 0.05) followed by Tukey's test (P ≤ 0.05). For the feeding choice assay, the numbers of larvae feeding on extract-treated versus control portions of the diet were compared by χ^2 tests. All statistical analyses were carried out using MINITAB Release 14 [30].

RESULTS

Contact Toxicity of Plant Extracts

Topically-applied *H. lupulus* induced the highest mortality of obliquebanded leafroller larvae (Fig. **1**). Mortality of 3rd instar obliquebanded leafrollers was significantly (F = 9.8; d.f. = 5,12; P < 0.05) greater for larvae treated with *H. lupulus* and *A. lappa* plant extracts compared with the solvent control (Fig. **1**). There was no significant effect (P > 0.05) of topical treatment with *B. radians*, *V. songaricum*, and *X. strumarium* extract solutions compared with the solvent control (Fig. **1**).

Toxicity of *H. lupulus* Extract

The slope and intercepts of the dosage-mortality relationship for obliquebanded leafroller larvae treated with *H. lupulus* extract were 0.93 ± 0.15 and 0.02 ± 0.02, respectively. The LC$_{10, 50, and 90}$ values (Fiducial Limits %) were 1.03 (0.16 - 2.42), 24.52 (14.08 - 60.32), and 581.53 (164.20 to 11,724.49) µg / insect, respectively.

Ingestion Toxicity and Developmental Effects of Plant Extracts

There was no significant (F = 1.3; d.f . = 5,12; P = 0.32) effect of any of the plant extracts on survivorship of feeding larvae at the 1 % concentration (Table **2**). Significantly fewer (F's = 7.1 and 4.8, d.f. = 5,12, P < 0.05) obliquebanded leafroller larvae survived to pupation at the 2 and 4 % concen-

Table 2.   Average Survivorship ± S.E. of Obliquebanded Leafroller Larvae Reared on Pinto Bean Diet Containing Various Concentrations of the Plant Extracts

Plant Species	Larval Survival (%) at Various Concentrations of Plant Extract in Diet (w/w)		
	1 %	2 %	4 %
Arctium lappa	98.85 ± 1.96 a[a]	90.76 ± 4.03 ab	80.68 ± 0.95 ab
Bifora radians	98.85 ± 1.96 a	86.98 ± 0.38 b	58.04 ± 8.46 b
Humulus lupulus	93.10 ± 3.19 a	73.50 ± 0.25 b	43.16 ± 0.67 b
Verbascum songaricum	95.46 ± 1.96 a	76.82 ± 0.25 b	49.99 ± 0.93 b
Xanthium strumarium	100 ± 0.00 a	86.98 ± 0.38 b	67.08 ± 3.19 b
Solvent control	100 ± 0.00 a	100 ± 0.00 a	98.85 ± 1.96 a

[a]Means in a column followed by the same letter are not significantly different (ANOVA, *P* < 0.05) followed by Tukey's mean separation (*P* < 0.05).

Table 3.   **Average Pupal Weight of Obliquebanded Leafrollers Reared on Pinto Bean Diet Containing Various Concentrations of Plant Extracts**

Plant Species	Average Pupal Weight ± SE (mg) at Various Concentrations of Plant Extract in Diet (w/w)					
	Females			Males		
	1 %	2 %	4 %	1 %	2 %	4 %
Arctium lappa	85.17 ± 2.65 a [a]	80.37 ± 4.35 ab	61.03 ± 5.95 b	53.16 ± 5.90 a	49.23 ± 5.11 ab	44.93 ± 6.75 b
Bifora radians	83.83 ± 6.32 a	67.50 ± 6.88 ab	47.40 ± 8.46 cd	52.93 ± 5.66 a	44.73 ± 8.37 ab	34.50 ± 6.88 c
Humulus lupulus	79.20 ± 1.85 a	62.27 ± 3.50 b	41.80 ± 6.68 d	49.43 ± 7.24 a	43.57 ± 8.58 ab	30.07 ± 6.04 d
Verbascum songaricum	79.13 ± 1.93 a	56.40 ± 2.40 b	45.40 ± 5.19 cd	50.10 ± 6.86 a	40.23 ± 7.90 b	31.17 ± 5.21 cd
Xanthium strumarium	98.87 ± 7.06 a	63.97 ± 3.15 b	56.40 ± 6.40 bc	53.36 ± 8.32 a	43.13 ± 8.50 ab	36.33 ± 5.03 c
Solvent control	97.53 ± 2.96 a	96.97 ± 6.85 a	98.85 ± 7.97 a	53.53 ± 9.58 a	53.93 ± 6.66 a	54.10 ± 7.87 a

[a]Means in a column followed by the same letter are not significantly different (ANOVA, $P < 0.05$) followed by Tukey's mean separation ($P < 0.05$).

trations for every plant extract treatment except *A. lappa* (Table **2**). Larval mortality appeared to increase in a dosage dependent manner (Table **2**).

Feeding by obliquebanded leafroller larvae on the diet containing 1 % plant extracts (by weight) did not significantly affect pupal weight for both sexes (F's = 0.7 and 3.1; d.f. = 5,12; P > 0.05 for males and females, respectively) (Table **3**). Increasing the concentration of plant extract in diet caused a reduction in pupal weight. Female pupal weight was significantly lower (F = 5.2; d.f. = 5, 12; P < 0.05) for larvae feeding on the 2 % concentration of *H. lupulus*, *V. songaricum*, and *X. strumarium*, while male pupal weight was significantly lower (F = 4.7; d.f. = 5,12; P < 0.05) only for *V. songaricum* at that concentration (Table **3**). At the highest concentration tested (4 % by weight), male and female pupal weight was significantly reduced (F = 141. 7 and 75.8, respectively, d.f. = 5,12; P < 0.05) for each plant extract tested (Table **3**). At the 4 % concentration, *B. radians*, *H. lupulus*, *V. songericum*, and *X. strumarium* had the greatest effect on male and female pupal weight.

Feeding Deterrence Assay

At the 1 % concentration, significantly fewer (P < 0.05) larvae were founding feeding on *B. radians* compared with the solvent control; there was no significant (P > 0.05) effect for the other plant extracts tested (Table **4**). However, sig-

nificantly fewer (P < 0.05) larvae were found feeding on *B. radians* and *H. lupulus* –treated diet compared with the solvent control at the 4 % concentration (Table **4**).

At the 1 % concentration, *B. radians* and *H. lupulus* showed evidence of feeding inhibition on the pinto bean diet with AFI's of 0.56 and 0.27, respectively (Fig. **2A**). There was a high level of feeding inhibition for *B. radians*, *H. lupulus* and *A. lappa*—treated diet at the 4 % concentration with AFI's ranging from 0.69 to 0.85 (Fig. **2B**). There was no apparent antifeedant effect of *V. songaricum* or *X. strumarium* at either concentration tested (AFI's ≈ 0) (Fig. **2A**, **B**).

DISCUSSION

Discovery of novel toxins and/or antifeedants from plant extracts has been recently emphasized as a potential method for the development of "ecologically safe pesticides" [31]. There is ample precedent for screening crude plant extracts for biological activity as botanical insecticides against Lepidoptera. For example, crude methanolic extracts of *Trichilia americana* and ethanolic extracts of *Annona squamosa* seeds reduced pupal weight and exhibited toxic activity against the Asian armyworm, *Spodoptera litura* (Fabr.) [31,32]. In addition, ethanolic seed extracts of *A. squamosa* and *A. muricata* reduced larval growth of *S. litura* and the cabbage looper, *Trichoplusia ni* (Hbn.) [33], while acetonic seed extracts of *A. squamosa* showed insecticidal activity against the cabbage

Table 4.   **Deterrence of 3rd Instar Obliquebanded Leafroller Larval Feeding by Plant Extracts Incorporated into Pinto Bean Diet**

Plant Species	Proportion of Larvae Feeding on Extract-Treated Versus Control Diet					
	1 % Extract in Diet (w/w)			4 % Extract in Diet (w/w)		
	Treatment	Control	χ^2	Treatment	Control	χ^2
Arctium lappa	0.45 a [a]	0.55 a	0.4	0.35 a	0.65 a	4.9
Bifora radians	0.28 a	0.72 b	8.1	0.20 a	0.80 b	14.4
Humulus lupulus	0.35 a	0.65 a	3.6	0.25 a	0.75 b	10.0
Verbascum songaricum	0.48 a	0.52 a	0.1	0.43 a	0.57 a	0.9
Xanthium strumarium	0.55 a	0.45 a	0.4	0.42 a	0.58 a	0.4

[a]Values in a row followed by the same letter are not significantly different ($P < 0.05$).

head caterpillar, *Crocidolomia binotalis* Zeller [34]. Furthermore, crude extracts of *Melia volkensii* have been shown to inhibit larval growth of *T. ni* and the armyworm, *Pseudaletia unipunctata* (Haworth) [35].

(A) 1 % extract in diet (w/w)

(B) 4 % extract in diet (w/w)
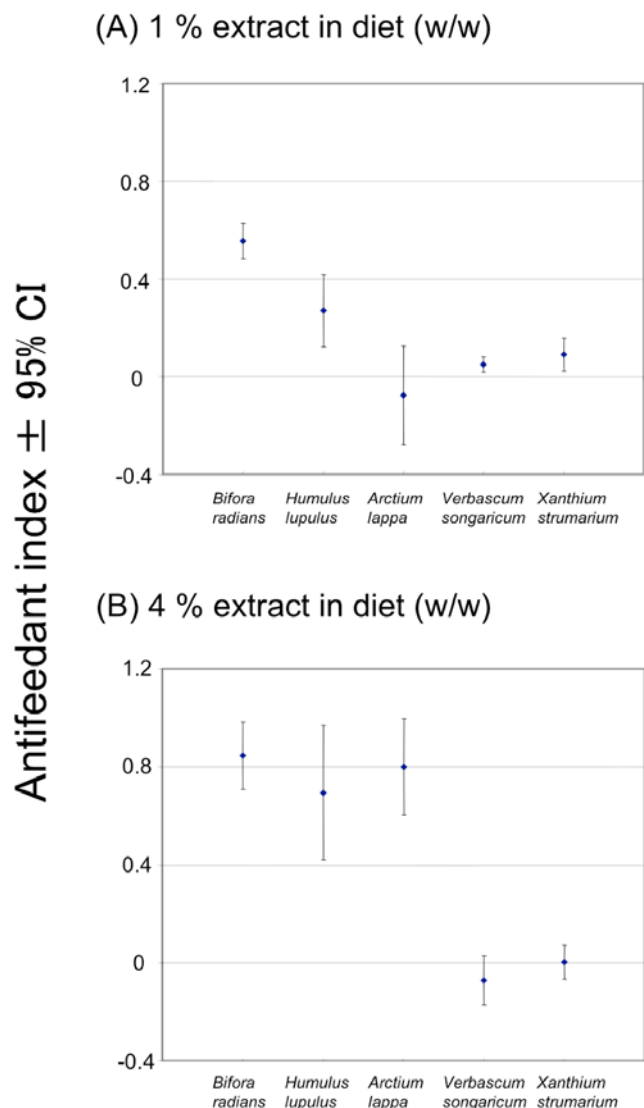
Plant-extract treatment

Fig. (2). Antifeedant effects of plant extracts incorporated into pinto bean diet at 1 % (**A**) and 4 % (**B**) concentrations (w/w) on 3rd instar obliquebanded leafroller larvae. AFI = (C – T) / (C + T), where C and T are the weights of control and plant extract-treated diet consumed.

Our results demonstrated that two of the plant extracts evaluated (*A. lappa* and *H. lupulus*) exhibited contact toxicity to obliquebanded leafroller larvae while four of the extracts decreased larval survivorship and final pupal weight following ingestion (Table **2**). *H. lupulus* yielded the highest contact toxicity with an LC_{50} of 24.5 µg / larva. These results are in agreement with previous studies showing pronounced contact toxicity of *H. lupulus* to various development stages of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) [17,18]. The contact and ingestion toxicities induced by the

tested plant extracts, particularly *H. lupulus*, suggest the potential for their future use against obliquebanded leafroller as botanical insecticides. Further testing of biological activity on other related pest species such as redbanded leafroller, *Argyrotaenia velutinana* (Walker), and Pandemis leafroller, *Pandemis pyrusana* Kearfott, is warranted.

In addition to toxicity *via* contact or ingestion, plant extracts and allelochemicals have been screened for activity as insect antifeedants [36-38]. In some instances, the bioactivity of crude plant extracts on insects is comprised of both toxic and antifeedant effects [32,35]. Azadirachtin, for example, derived from the neem tree (*Azadirachta indica*), is both a toxicant and antifeedant and has been one of the most widely tested and successfully implemented botanical insecticides over the past two decades [39-41]. In the current study, *H. lupulus* and *A. lappa* exhibited antifeedant activity on obliquebanded leafroller larvae in addition to contact toxicity, while *B. radians* was an antifeedant and exhibited toxic effects when ingested. *H. lupulus*, *A. lappa*, and *B. radians* are also known to deter feeding of larval *L. decemlineata* [23]. Our results suggest that potential future application of these extracts or their active components for leafroller control may exploit more than one mode of action. However, future experiments should focus on determining whether prolonged exposure of obliquebanded leafroller larvae to these plant extracts decreases antifeedant effects over time due to habituation of response [42].

H. lupulus contains alpha and beta acids, prenylflavanoids, and proanthocyanidins [43,44]. The beta acid derivative of *H. lupulus* repels both chewing and sucking insect pests of plants [45] and the two-spotted spider mite, *Tetranychus urticae* Koch [46,47]. Future studies will need to be conducted to determine whether the biological activity of *H. lupulus* against obliquebanded leafroller larvae is mediated by the beta acid component. Furthermore, identification of the bioactive components of *A. lappa* and *B. radians* may allow development of botanical insecticides with greater potency than the crude plant extracts evaluated here.

Akhtar and Isman [35] stressed the need for conducting multiple types of bioassays when screening potential botanical insecticides given that effects such as growth inhibition may not always be correlated with other consequences such as feeding deterrence. Therefore, in the current investigation, we evaluated multiple types of effects including: 1) toxicity following topical application or ingestion, 2) effects of ingestion on pupal weight, and 3) feeding deterrence. Two of the extracts tested (*H. lupulus* and *A. lappa*) induced deleterious effects for each bioassay method and thus may be the most promising candidates of those screened here for further development as botanical insecticides against obliquebanded leafroller and potentially other Lepidopteran pests. The next step toward developing these extracts as useful insecticides will focus on field testing to determine whether: 1) the toxic and antifeedant effects described here impact crop injury levels, 2) the efficacy of extracts is affected by prolonged UV and/or rain exposure, and 3) beneficial insects are affected.

ACKNOWLEDGEMENTS

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