American Journal of Plant Sciences, 2012, 3, 988-994
http://dx.doi.org/10.4236/ajps.2012.37117 Published Online July 2012 (http://www.SciRP.org/journal/ajps)

Alkaloids, Limonoids and Phenols from Meliaceae Species Decrease Survival and Performance of *Hypsipyla grandella* Larvae

Julián Pérez-Flores[1*], Sanford D. Eigenbrode[2], Luko Hilje-Quiroz[3]

[1]Colegio de Postgraduados-Campus Tabasco, H. Cárdenas Tabasco, México; [2]PSES Department, University of Idaho, Moscow, USA; [3]Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Turrialba, Costa Rica.
Email: [*]julianflores@colpos.mx

Received May 2nd, 2012; revised May 30th, 2012; accepted June 9th, 2012

ABSTRACT

Meliaceae plants are distinguished by the attack of the shootborer *Hypsipyla grandella* and also for the occurrence of limonoids, alkaloids and phenolic compounds. Such compounds extracted from leaves of Meliaceae species *Cedrela odorata* L., *Swietenia macrophylla* King, *Khaya senegalensis*, *Toona ciliata*, and *C. odorata* grafted onto *T. ciliata* plants, were tested on *C. odorata* leaf disks to determine their effects on survival and performance of *H. grandella* larvae. Larval survival was assessed 2, 10 and 25 days after starting the bioassays. Leaf consumption and weight gain per larva, days to pupa and to adult stages, pupal weight and length, and moth wing appearance were assessed for larval performance. The three compounds from the four Meliaceae species and the grafted combination affected ($P \leq 0.02$) larval leaf consumption, weight gain, time to pupa and to adult stages, wing development and larval survival of *H. grandella*. Pupa weight ($P = 0.78$, $F = 0.72$, d.f. = 18,160) and length ($P = 0.48$, $F = 0.98$, d.f. = 18,160) were similar regardless of the coumpound used. Limonoid reduced larval survival on the three dates of evaluation. Alkaloids decreased leaf consumption, weight gain of larvae and time needed to reach pupa and adult stages. Alkaloids from *T. ciliata* and phenols from *C. odorata* were the best coumpounds to reduce leaf consumption and weight gain. Alkaloids from the grafted plants caused 20% of *H. grandella* adults to form abnormal wings.

Keywords: Leaf Extracts; *Cedrela*; *Swietenia*; *Khaya*; *Toona*; Shootborer

1. Introduction

High-quality timber from Spanish cedar (*Cedrela odorata* L.) and mahogany (*Swietenia macrophylla* King) is of major significance for economies in many neotropical countries [1]. Unfortunately, natural populations of these species are being reduced quickly due to selective harvest [2]. In addition, the mahogany shootborer, *Hypsipyla grandella* (Zeller) (Lepidoptera: Pyralidae), has limited their establishment in commercial plantations in Latin America, as its larva mainly feeds on apical shoots, inducing branching on the trees and rendering the timber unmarketable [3]. Larvae also can feed on fruits, leaves, bark, and root tissues.

In tree tissues internal chemicals may exert their effect in the volatile state causing an insect to avoid the tree completely, or they may deter the insect after it contacts the tree or ingests tissue [4]. Research on the biochemical basis for resistance to *H. grandella* in Meliaceae has been completed on limonoids [5,6], while Gripjma [7] indicated that the biochemical basis for resistance of *Toona ciliata* (Meliaceae) may be due to alkaloids. Further, Newton *et al.* [8] suggested that proantocianydins (*i.e.* phenolic compounds) may reduce susceptibility of *C. odorata* to attack by *H. grandella* larvae.

The family Meliaceae stands out because of the common occurrence of limonoids [9], which possess antifeedant, toxic, or growth-reducing properties to different species of insects [10]. Azadirachtin, the most well-known limonoid [11] was toxic to the Meliaceae´s shootborer *H. grandella* larvae when incorporated in diet mixtures [7]. Such toxic effect plus growth-disruptant activity were reported by Mancebo *et al.* [12] for azadirachtin. Limonoids, however, seemed unrelated to the induced resistance of *C. odorata* grafted onto *T. ciliata* against *H. grandella* larvae [6]; instead, these authors stated that phenols (cycloartanes, catechin and proanthocyanidins) were likely responsible for such resistance, as all of them were absent from *C. odorata* scions or intact (non-grafted) plants but present in *T. ciliata*.

Phenols such as methylcoumarins and the furanocou-

[*]Corresponding author.

marin bergapten have been found in *T. ciliata* [13], proanthocyanidins in *C. odorata* [8], and the flavonoids quercitin and kaenferol in *C. odorata* and *T. ciliata* [14]. Furanocoumarins are potent feeding deterrents to certain insect species [15], and bergapten might promote the resistance of *T. ciliata* against *H. grandella* larvae. Other chemicals found in Meliaceae species which provide resistance against *H. grandella* are alkaloids. Alkaloids are nitrogen compounds that function as plant defenses against herbivores [16] and were detected in ethanolic extracts from *T. ciliata* [17]. These extracts were toxic to and reduced growth on *H. grandella* larvae [18]. For this reason, alkaloid compounds could be responsible for the resistance of *T. ciliata* against *H. grandella* larvae.

Therefore, the objective of the research was to detect the effects of alkaloid, limonoid, and phenolic extracts from foliage of susceptible and resistant Meliaceae species, as well as from *C. odorata* grafted onto *T. ciliata* plants, on *H. grandella* larval survival and performance. The hypothesis to test was that the three different compounds from the susceptible and resistant Meliaceae species as well as from the grafted plants affect larval survival and performance of the Meliaceaes shootborer.

2. Materials and Methods

2.1. Plant Material and Extract Preparation

The extracts were prepared in the Animal Nutrition Laboratory at the Tropical Agricultural Research and Higher Education Center (CATIE), in Turrialba, Costa Rica, from the susceptible Meliaceae species *C. odorata* and *S. macrophylla*, the resistant species *Khaya senegalensis* and *T. ciliata* as well as from *C. odorata* grafted onto *T. ciliata* plants. Plants were grown from seeds in a nursery at the Cabiria Experiment Station, within the premises of CATIE.

Fresh leaves and shoots (500 g) from 1-year-old plants were cut into 2 to 5 cm pieces, and then ground in liquid nitrogen to 0.05 mm in a mill (Model 3 Wiley Mill®, Thomas Co., Philadelphia). Later, the ground material was extracted with 2 L 70/30 methanol/water by volume. Extraction was completed at room temperature (20˚C) for 8 d. Each extract was filtered through Whatman paper No. 4, and the extract was concentrated to a small volume (200 mL) by a rotary evaporator (40˚C) (so all methanol was removed). Each concentrate was partitioned among ether and dichloromethane to produce extracts likely to contain predominantly alkaloids, phenols and limonoids, respectively.

Alkaloid and phenolic fractions. These compounds were isolated by the acid-base separation method [19]. Back extraction of the ether extract with 0.5 M HCl removed amine bases such as alkaloids. A second extraction of the remaining ether extract with 0.5 M NaOH removed phenols, which are ionizable at high pH. The basic and phenolic compounds were recovered by adjusting the pH of each extract to the point where the compounds were present in their uncharged forms (alkaloids, pH ≅ 11; phenols, pH ≅ 7).

Limonoid fractions. For the isolation of limonoids 25 mL of crude extract were separated on a silica gel column (400 mm × 8 mm, Silica Gel grade 60, 254 g gravity flow), and then eluted with dichloromethane [20]. This dichloromethane fraction was then concentrated to a small volume (50 mL) by a rotary evaporator and then used in the bioassays.

2.2. Test Insects

Hypsipyla grandella larvae for bioassays were taken from a colony kept at the Entomology Laboratory at CATIE. The colony was established in 1998, and renewed yearly, from field-collected larvae feeding on *C. odorata.* Larvae in the colony are normally fed with tender *C. odorata* leaves from instars I-III, and then placed onto an artificial diet [21] until pupation. Combining leaves and artificial diet ease the management of the colony. Eggs hatch hardly on diet but easily on leaves. Feeding larvae only with leaves is hard since they are so voracious that if they do not have anything to eat they eat each other. On another hand, tender leaves are scarce on dry season. Pupae are moved to a metal-framed cage covered with fine mesh, kept at a greenhouse, where adults emerge, mate, and oviposit. Eggs are collected and taken to the laboratory to sustain the colony.

Instar II larvae (4 - 8 mm length) were selected for bioassay because they are less sensitive to handling than instar I and approximate what would occur in nature regarding initial plant attack by *H. grandella* larvae. Instar II bores into the apical bud only after feeding on tender petioles and foliage [3].

2.3. Bioassays

The bioassay was completed in an environmental chamber Percival I35-L (Boone, Iowa) at 25 C, 80% to 90% RH, and 12:12 L:D, at the Entomology Laboratory at CATIE, from October 15 through December 6, 2005. *Cedrela odorata* leaf disks were taken from plants as the food source for rearing the *H. grandella* colony. Leaf disks were cut from central leaflets, by using a cork borer (2.30 cm diameter). Ten leaf disks per treatment were put on a Petri dish and then sprayed with 12 $\mu L \cdot cm^{-2}$ of each extract added with 0.03% Citowett (BASF, Canada, Inc.) as surfactant agent. The application of extracts to leaf disks was done by using a De Vilbiss 15 sprayer (The De Vilbiss, USA) connected to a vacuum pump (GAST™

DOA-P104-AA, GAST Manufacturing Corp. Benton Harbor, Michigan), with 0.7 $kg \cdot cm^{-2}$ constant pressure. Sprayed leaf disks were allowed to dry and were placed individually, with the abaxial side up, inside a 30-mL glass vial. Then, an instar II *H. grandella* larva was placed onto the leaf disk. Larvae had been deprived of food for 3 h [12]. A wet piece of paper towel was fastened to the lid of each vial to avoid excessive desiccation of leaf disks. The vial was then turned over so the larva was below the leaf disk.

2.4. Assessments

All of the larvae were weighed before and 2 d after starting the bioassay and then an average weight gain per larva per treatment was calculated by subtracting initial from final weight. Also after 2 d, larval survival was assessed and leaf consumption estimated. To estimate leaf consumption, the disk was glued to a transparent film and then overlayed onto graph paper (1 mm^2 grid size) to count the leaf area eaten. Living larvae were individually transferred by means of a thin paintbrush into a vial containing ca. 6 mL of artificial diet [21], and then reared until adult emergence. Larval survival at 2, 10 and 25 d after starting bioassay, time (days) to achieve the pupa and adult stages, pupal weight (mg) and length (mm), and wing shape were all determined for these larvae.

Larval and pupal mortality were recorded as 0 and 1 for dead and live larvae, respectively, since just one larva was used per leaf disk. Larvae were classified as dead if they were immobile or blackened. Pupae were classified as dead if they failed to emerge after 45 days or if they appeared blackened or shriveled [12]. Data for pupa were determined 1 day after pupation. On the day of adult emergence, wing shape was recorded after wing expansion and drying were completed. Normal and abnormal wings were recorded as 0 and 1, respectively. Wings were considered normal when both forewings were similar in length and covered the whole abdomen longitudenally [22]. Abnormalities included absence or rudimenttary forewings or shortened forewings exposing the abdomen. To exclude possible effects of the artificial diet alone on these characteristics, 100 pupas were selected at random from the colony and reared to adults. None of these insects had abnormal wings.

2.5. Experimental Procedure and Statistical Analysis

The bioassay was replicated three times. Each bioassay consisted of 19 treatments: five limonoid, alkaloid and phenolic extracts with each one from the four Meliaceae species and the grafted plants, dichloromethane and ether solvents as well as larvae without leaf disk as relative controls, and distilled water as absolute control. The experimental unit was a leaf disk with one instar II *H. grandella* larva in a capped vial. The vials were arranged in a completely randomized design with a factorial arrangement of treatments. The factors were the extract (limonoids, alkaloids and phenols) and the source of extract (*C. odorata*, *S. macrophylla*, *K. senegalensis*, *T. ciliata*, and the grafted combination *C. odorata* onto *T. ciliata*). Each treatment was replicated 10 times.

Data were examined for compliance of assumptions required for analysis of variance (ANOVA). If necessary, data were transformed by Y = sqrt (Y + 0.5) to meet these assumptions. ANOVA was completed using the general lineal model (GLM) procedure [23]. Orthogonal contrasts were used to test the effect of alkaloids, limonoids and phenols on larval survival and performance. The contrasts were as follows: 1) alkaloids versus limonoids, 2) alkaloids versus phenols, and 3) limonoids versus phenols.

3. Results and Discussion

The three kind of compounds from leaves of the four Meliaceae species and the grafted combination affected ($P \leq 0.02$) larval leaf consumption, weight gain, time to pupa and to adult stages, wing development and larval survival of *H. grandella* (**Table 1**). Pupa weight ($P = 0.78$, $F = 0.72$, d.f. = 18,160) and length ($P = 0.48$, $F = 0.98$, d.f. = 18,160) were similar regardless of the extract used.

3.1. Larval Survival

Alkaloids, limonoids and phenols affected larval survival differently (**Table 1**) at 2, 10 and 25 days after starting bioassay. Fewer larvae reared on disks sprayed with limonoids survived compared to larvae reared on disks sprayed with alkaloids or phenols similar to the neem-derived Azadirachtin [16]. These authors reported that small amounts of ingested *C. odorata* leaf disks dipped in Azadirachtin 10% were enough to kill 100% of larvae in a 24 h period of exposure.

Reduction of larval survival at 2 d after starting bioassay was most notable for limonoid extracts from *K. senegalensis*, compared to alkaloids from *C. odorata* or from the grafted plants and phenols from *K. senegalensis* or from *T. ciliata*. A similar trend on larval survival was observed at 10 and 25 d after starting bioassay; larval survival was reduced by limonoids from *S. macrophylla* and *K. senegalensis* compared to alkaloids from *S. macrophylla* or from the grafted plants and also compared to phenols from *K. segalensis* or from *T. ciliata*; such reduction on larval survival by limonods was only comparable to this of larvae without leaf disk (**Figure 1**). These results could indicate toxicity of limonoids from

Table 1. Probability values for orthogonal contrasts and ANOVA for several variables assessed in a bioassay with *Hypsipyla grandella* larvae on *Cedrela odorata* foliar disks treated with alkaloids, limonoids and phenols extracts from Meliaceae species and a graft combination.

Contrast	Leaf consumption (mm^2)	Weight gain (mg)	Days to		Normal wings	Larval survival		
			Pupa	Adult		2 DAS	10 DAS	25 DAS
					Probabilities			
Alkaloids vs limonoids	0.71	<0.0001	0.59	0.34	0.02	0.39	0.09	0.84
Alkaloids vs phenols	0.90	<0.0001	0.44	0.46	0.06	0.13	0.41	0.07
Limonoids vs phenols	0.80	0.91	0.19	0.84	0.67	0.02	0.01	0.05
ANOVA								
P	<0.0001	<0.0001	0.007	0.001	0.0003	<0.0001	0.0008	0.02
F	3.15	97.94	2.12	2.51	2.81	4.94	2.58	1.86
d.f.	17,162	18,171	18,161	18,154	18,153	18,171	18,170	18,170

Abbreviations: DAS, days after starting the bioassay. Data are from three bioassays combined into one data set analysis.

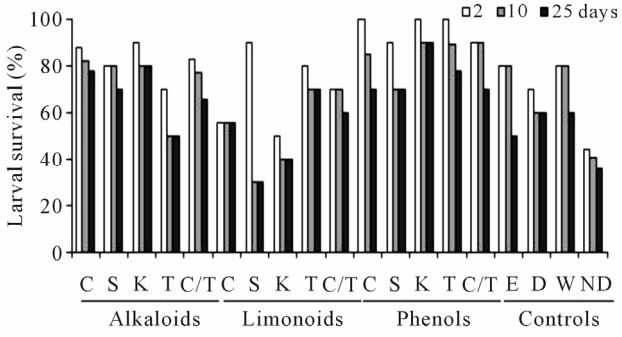

Figure 1. Survival of instar II *H. grandella* larvae exposed for 2 d to *C. odorata* leaf disks treated with alkaloids, limonoids, and phenols from four Meliaceae species and a graft combination. Data were taken 2, 10 and 25 d after starting bioassay. C = *C. odorata*, S = *S. macrophylla*, K = *K. senegalensis*, T = *T. ciliata*, C/T = *C. odorata* grafted onto *T. ciliata*, E = Ether, D = Dichloromethane, W = Water. Data are means (n = 30) from three bioassays.

K. senegalensis or *S. Macrophylla* against *H. grandella* larvae. Chronic toxicity on *Spodopthera littoralis* (Lepidoptera: Pyralidae) larvae was caused by extracts from *Reynoutria* sp. (Polygonaceae) plants containing phenollic compounds [20]. Althought *S. littoralis* and *H. grandella* belong to the same family, they are different genera. Also, the difference between our results and those of Pavela *et al*. [24] can be attributed to the kind of phenols contained on *Reynoutria* and Meliaceae species.

The lowest larval survival scored (30%) was caused for limonoids from *S. macrophylla* (**Figure 1**), but this results differed from those of Pérez *et al*. [25] who found that besides causing deterrence, crude extracts from Meliaceae species, specifically the extracts from *T. ciliata* species, decreased up to 0% *H. grandella* larval survival. Such difference seems consistent with a probable sinergestic effect of multiple defenses [26]. Anyway, limonoids and phenols differed only for larval survival but not for larval performance variables (**Table 1**).

3.2. Larval Performance

Consumed leaf area and weight gain. These variables had a direct relation when leaf disks were sprayed with limonoid extracts (**Figure 2**). Although limonoids seemed to have a phagoestimulatory effect on *H. grandella* larvae, larvae weight gain was lower than the expected, or larvae died after leaf consumption. Limonoids may be found in all tissues on plants, but different plant organs may produce different kinds of limonoids [10] with different action on plant protection.

The alkaloid and phenol effects depended on the plant source for leaf consumption. The alkaloids from grafted plants reduced *H. grandella* leaf consumption compared to alkaloids from *C. odorata* or limonoids and phenols from *S. macrophylla* and also compared to the control water. In contrast, limonoid from *K. senegalensis* and *C. odorata* differed to limonoids from *S. macrophylla*, *T. ciliata* and the graft combination in their effect on leaf consumption. Phenols from *C. odorata* and *T. ciliata* reduced leaf consumption compared to those of the other two species and the grafted plants (**Figure 2(a)**).

Larval weight gain differed among larvae fed with alkaloids. The alkaloid fraction effect on weight gain was more notable between *S. macrophylla* and grafted plants. From grafted plants alkaloid fractions reduced weight gain more than phenols or limonoids (**Figure 2(b)**). Except for phenols from *C. odorata* or *T. ciliata* which reduced weight gain, the other phenols were similar each other and also were similar to the control water to allow weight gain of *H. grandella* larvae. Limonoids from

Cedrela spp. mixed in an artificial diet showed a postdigestive toxicity for *Spodoptera frugiperda* neonates, reducing growth and causing significant mortality after rearing [20].

Although phenols differed from alkaloids on weight gain (**Table 1**), phenols from *C. odorata* and alkaloids from the grafted plants reduced the leaf consumption by *H. grandella* to the same level (**Figure 2(a)**). Such results caused by alkaloids from the grafted plants agree with the hypothesis suggested by Grijpma [7] about the transfer of alkaloids from *T. ciliata* to *C. odorata* to confer resistance in this susceptible scion.

Time to pupa and to adult stages. All of the tested compounds from *S. macrophylla*, as well as limonoids from the grafted plants or phenols from *T. ciliata*, reduced the time to pupation by 2 and 4 d compared to water and no-disk controls, respectively; but their effect was similar to the other controls (**Figure 3(a)**). All of the compounds that reduced the time to pupation, also reduced the time to the adult stage by a difference of 5 d compared to the no-disk control, but their effect was again similar to all the other controls and extracts from the various species (**Figure 3(b)**).

Alkaloids from grafted plants and limonoid from *C. odorata* or from *K. senegalensis* seemed to delay pupation compared to the other fractions (**Figure 3(a)**). Such treatments and the phenols from *C. odorata* also seemed to delay adulthood compared to the controls ether and dichloromethane (**Figure 3(b)**). However, *H. grandella* larval development last about 30 d and pupation about 10 to 12 d [27], therefore the three evaluated compounds reduced time to pupa and to adult stage. Pérez *et al*. [25] cited that *H. grandella* larvae fed leaf disks from *K. senegalensis* grafted onto *S. macrophylla* extended by 10 d both time to reach pupa and to adult stages (34.3 ± 3.8 d vs. 24.3 ± 0.5 d; and 45.2 ± 2.8 vs. 35.2 ± 1.3 d, respectively) compared to larvae fed *C. odorata* leaf disks. Also, these authors cited that crude extracs from grafted

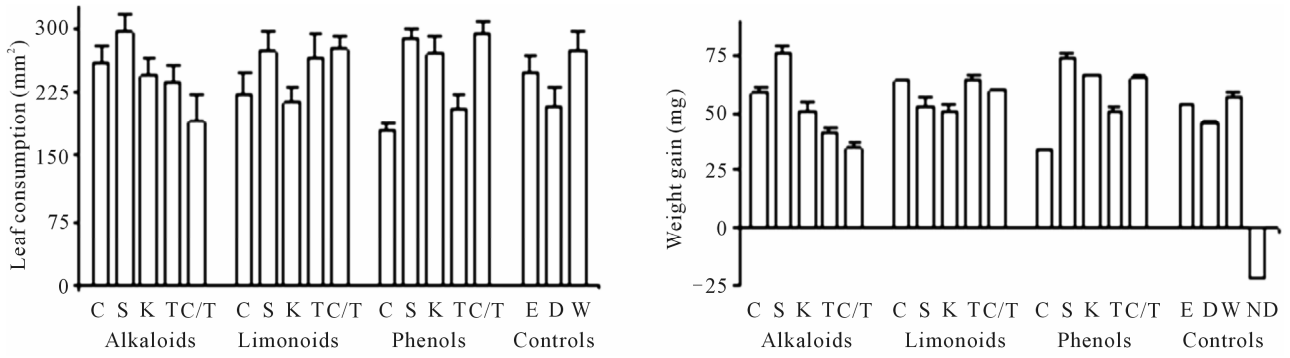

Figure 2. Leaf consumption (a) and weight gain (b) for instar II *Hypsipyla grandella* larva reared on *Cedrela odorata* leaf disks treated with alkaloids, limonoids and phenols from four Meliaceae species and a graft combination. C = *C. odorata*, S = *S. macrophylla*, K = *K. senegalensis*, T = *T. ciliata*, C/T = *C. odorata* grafted onto *T. ciliata*, E = Ether, D = Dichloromethane, W = Water, ND = Larva without leaf disk. Data were taken 2 d after starting bioassay. Data are means (±SE, n = 30) from three bioassays.

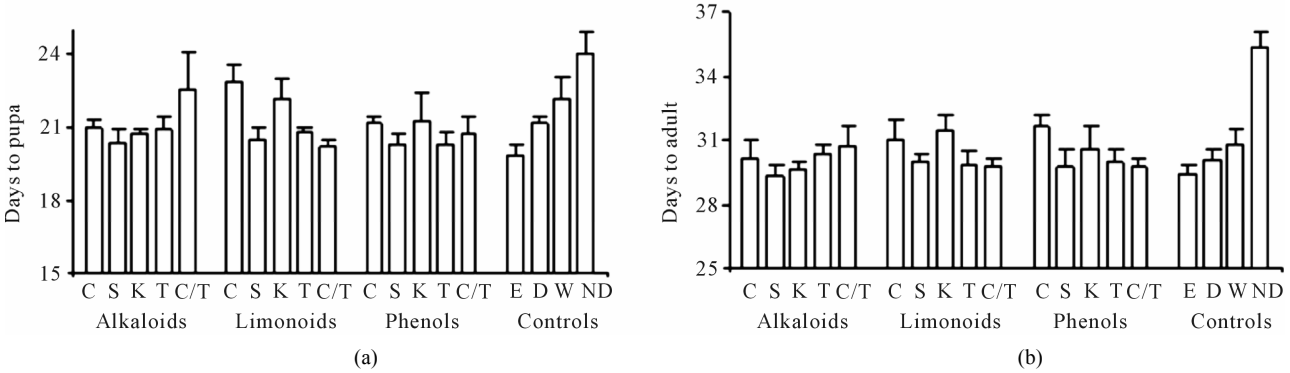

Figure 3. Time to pupa (a) and to adult (b) stages of *Hypsipyla grandella* larvae reared on *C. odorata* leaf disks treated with alkaloids, limonoids, and phenols from four Meliaceae species and a graft combination. C = *C. odorata*, S = *S. macrophylla*, K = *K. senegalensis*, T = *T. ciliata*, C/T = *C. odorata* grafted onto *T. ciliata*, E = Ether, D = Dichloromethane, W = Water, ND = Larva without leaf disk. Data are means (±SE, n = 30) from three bioassays.

and intact Meliaceae plants affected ($P \leq 0.05$) both variables. Pavela *et al*. [24] observed a larval period increased on neonate larvae of *Spodoptera littoralis* by feeding them with diet containing different concentrations of crude extracts of *Reynoutria* sp. (Polygonaceae) which contained phenolic compounds.

Moth wing appearance. Development of wings was affected ($P = 0.0003$, $F = 2.81$, d.f. = 18,153) by the tested compounds. Alkaloids from grafted plants and phenols from *T. ciliata* caused 20% and 4% of adults with abnormal wing shape, respectively. All larvae fed leaf disks treated with any other compound got from any other plant or the controls formed normal wings when they developed into adult moths. These results are consistent with those of Pérez *et al*. [25] who reported abnormal wings formed on *H. grandella* larvae fed leaf disks from *K. senegalensis*, *K. senegalensis* grafted onto *S. macrophylla*, *C. odorata* grafted onto *K. senegalensis*, or from *S. macrophylla* grafted onto *K. senegalensis*. The same authors reported abnormal wings developed on one adult fed on *C. odorata* leaf sprayed with crude foliar extract from *C. odorata* grafted onto *T. ciliata*. Development disruption was reported for *Locusta migratoria* due to limonoid Azadirachtin at 1 - 10 ppm in artificial diets [28].

Meliaceae plants stands out by the occurrence of limonoids [9], but other secondary compounds such as flavonoids, alkaloids, terpenes and antraquinones have been isolated from *Toona sinensis* leaves [29] and recently 12 phenolic compounds have been identified in this species [30]. The primary selective advantage of the production of secondary compounds on plants is protection against insect herbivory. In this way, limonoids Cedrelone and Anthotecol from *Toona* and *Khaya* spp. showed potent growth reducing activity to *Spodopthera frugiperda*, *Heliothis zea*, *Pectinophora gossypiela* and *Ostrinia nubilalis* larvae [10]. Also from the same species, limonoids Bussein and Anthotecol inhibited ecdysis on *O. nubilalis*.

Taking into account that an efficient control for *H. grandella* is currently lacking due to the low damage threshold of one larva per plant [31], the extracts represent potentially useful raw material for developing microinjections or implants into tree stems as slow-release formulas, increasing their persistence. A further step would be to identify the specific alkaloid, limonoid, and phenol that act against *H. grandella* in order to synthesize, combine, and incorporate them in commercial products. Such products could be deployed to protect *Cedrela* spp. and *Swietenia* spp. trees for 5 to 8 years (critical period to *H. grandella*), which is the time required to achieve a commercial trunk for these species, depending on the site where they grow [32].

4. Conclusion

This study demonstrates that survival of *Hypsipyla grandella* larvae is affected by limonoids, specifically those extracted from *S. macrophylla* and *K. senegalensis*. Alkaloids reduced leaf consumption and weight gain per larva compared to limonoids and phenols and those extracted from *S. macrophylla* were the best to reduce time to pupa and to adult stages compared to the starved control. Alkaloids from *C. odorata* grafted onto *T. ciliata* and phenols from *T. ciliata* caused abnormal wing shape on *H. grandella* moths. Therefore, our hypothesis that alkaloids, limonoids and phenols from the susceptible and resistant Meliaceae species, as well as from the grafted plants, affect larval survival and performance of the Meliaceae's shootborer was accepted.

5. Acknowledgements

J. P. F. thanks to CONACYT and the Colegio de Postgraduados (México), and the Department of Plant, Soil and Entomological Sciences at the University of Idaho (USA) for providing founds for this research.

REFERENCES

[1] A. C. Newton, P. Baker, S. Ramnarine, J. F. Mesén and R. R. B. Leakey, "The Mahogany Shoot Borer: Prospects for Control," *Forest Ecology Management*, Vol. 57, No. 1-4, 1993, pp. 301-328. doi:10.1016/0378-1127(93)90179-Q

[2] P. D. Albert, A. A. López, T. M. Rodríguez and R. M. Duarte, "Recursos Fitogenéticos Forestales, I. Familia Meliaceae," *Fontqueria*, Vol. 42, 1995, pp. 329-351.

[3] P. Grijpma, "Studies on the Shoot-borer *Hypsipyla grandella* (Zeller) (Lepidoptera, Pyralidae). V. Observation on Rearing Technique and on Host Selection Behavior of Adults in Captivity," *Turrialba*, Vol. 21, 1971, pp. 202-213.

[4] S. A. Cunningham and R. B. Floyd, "Leaf Compositional Differences Predict Variation in *Hypsipyla robusta* Damage to *Toona ciliata* in Field Trials," *Canadian Journal of Forest Research*, Vol. 34, No. 3, 2004, pp. 642-648. doi:10.1139/x03-228

[5] M. F. Das G. F. Da Silva, S. M. M. Agostinho, J. R. De Paula, J. O. Neto, I. Castro-Gamboa, R. E. Filho, J. B. Fernandes and P. C. Vieira, "Chemistry of *Toona ciliata* and *Cedrela odorata* Graft (Meliaceae): Chemosystematic and Ecological Significance," *Pure and Applied Chemistry*, Vol. 71, No. 6, 1999, pp. 1083-1087. doi:10.1351/pac199971061083

[6] J. R. De Paula, I. J. C. Vieira, M. F. Das G. F. Da Silva, F. E. Rodriguez, J. B. Fernandes, P. C. Vieira, A. L. Pinheiro and E. F. Vilela, "Sesquiterpenes, Triterpenoids, Limonoids and Flavonoids of *C. odorata* Graft and Speculations on the Induced Resistance against *Hypsipyla grandella*," *Phytochemistry*, Vol. 44, No. 8, 1997, pp. 1449-1454. doi:10.1016/S0031-9422(96)00747-9

[7] P. Grijpma, "Resistance of Meliaceae against the Shoot Borer *Hypsipyla grandella* with Particular Reference to *Toona ciliata* M.J. Roem. var. *australis* (F. & M.) C.D.C.," In: J. Burley and B. T. Styles, Eds., *Tropical Trees*: *Variation*, *Breeding and Conservation*, Academic Press, London, England, 1976, pp. 69-78.

[8] A. C. Newton, A. D. Watt, F. López, J. F. Cornelius, J. F. Mesén and E. A. Corea, "Genetic Variation in Host Susceptibility to Attack by the Mahogany Shoot Borer, *Hypsipyla grandella* (Zeller)," *Agricultural and Forest Entomology*, Vol. 1, 1999, pp. 11-18. doi:10.1046/j.1461-9563.1999.00002.x

[9] D. H. A. Taylor, "Chemotaxonomy: the Occurrence of Limonoids in the Meliaceae," In: T. D. Pennington, Ed., *Flora Neotropica*, The New York Botanical Garden, 1981, pp. 450-458.

[10] D. E. Champagne, O. Koul, M. B. Isman, G. G. E. Scuder, and G. H. N. Towers, "Biological Activity of Limonoids from the Rutales," *Phytochemistry*, Vol. 31, No. 2, 1992, pp. 377-394. doi:10.1016/0031-9422(92)90003-9

[11] R. C. Saxena, "Insecticides from Neem," In: J. T. Arnason, B. J. R. Philogéne and P. Morand, Eds., *Insecticides of Plant Origin*, American Chemical Society, Washington DC, 1989, pp. 110-135. doi:10.1021/bk-1989-0387.ch009

[12] F. Mancebo, L. Hilje, G. A. Mora and R. Salazar, "Biological Activity of Two Neem (*Azadirachta indica* A. Juss., Meliaceae) Products on *Hypsipyla grandella* (Lepidoptera: Pyralidae) Larvae," *Crop Protection*, Vol. 21, No. 2, 2002, pp. 107-112. doi:10.1016/S0261-2194(01)00069-2

[13] R. Chowdhury, "5-Methylcoumarins from *Toona ciliata* Stem Bark and Their Chemotaxonomic Significance," *Biochemical Systematics and Ecology*, Vol. 32, 2004, pp. 103-105. doi:10.1016/S0305-1978(03)00182-0

[14] A. T. Chatterjee, T. Chakraborthy and S. Chandrasekharan, "Chemical Investigation of *Cedrela toona*," *Phytochemistry*, Vol. 10, 1971, pp. 2533-2535. doi:10.1016/S0031-9422(00)89902-1

[15] L. M. Schoonhoven, "Secondary Plant Substances and Insects," *Recent Advances in Phytochemistry*, Vol. 4, 1972, pp. 197-242.

[16] L. H. Caporale, "Chemical Ecology: A View from the Pharmaceutical Industry," *Proceedings of the National Academy of Sciences USA*, Vol. 92, 1995, pp. 75-82. doi:10.1073/pnas.92.1.75

[17] S. Smolenski, H. Silinis and M. Farnworth, "Alkaloid Screening IV," *Lloydia*, Vol. 37, No. 1, 1974, pp. 30-61.

[18] P. Grijpma and S. C. Roberts, "Biological and Chemical Screening for the Basis of Resistance of *Toona ciliata* M.J. Roem. var. *australis* (F. & M.) C.D.C.," *Turrialba*, Vol. 25, No. 2, 1975, pp. 152-159.

[19] J. G. Millar and J. J. Sims, "Preparation, Cleanup, and Preliminary Fractionation of Extracts," In: J. G. Millar and K. F. Haynes, Eds., *Methods in Chemical Ecology*, *Chemical Methods*, Vol. 1, Kluwer Academic Publishers, Boston, 1998, pp. 1-31.

[20] C. L. Céspedes, J. S. Calderón, L. Lina and E. Aranda, "Growth Inhibitory Effects on Fall Armyworm *Spodoptera frugiperda* of Some Limonoids Isolated from *Cedrela* spp. (Meliaceae)," *Journal of Agricultural and Food Chemistry*, Vol. 48, No. 5, 2000, pp. 1903-1908. doi:10.1021/jf990443q

[21] C. Vargas, P. J. Shannon, R. Taveras, F. Soto and L. Hilje, "Un Nuevo Método Para la Cría Masiva de *Hypsipyla grandella*," Hoja Técnica No. 39, *Manejo Integrado de Plagas* (*Costa Rica*), Vol. 62, 2001, pp. I-IV.

[22] D. Sliwa and V. O. Becker, "Studies on the Shootborer *Hypsipyla grandella* (Zeller) (Lep., Pyralidae). XXI. Observations on Emergence and Mating of Adults in Captivity," *Turrialba*, Vol. 23, 1973, pp. 351-356.

[23] SAS Institute, "SAS User's Guide: Statistical Analysis System," Version 8.2, SAS Institute Inc., Cary, 2001.

[24] R. Pavela, N. Vrchová and B. Šerá, "Growth Inhibitory Effect of Extracts from *Reynoutria* sp. Plants Against *Spodoptera littoralis* Larvae," *Agrociencia*, Vol. 42, No. 5, 2008, pp. 573-584.

[25] J. Pérez, S. Eigenbrode, L. Hilje, R. Tripepi, M. E. Aguilar and F. Mesén, "Leaves from Grafted Meliaceae Species Affect Survival and Performance of *Hypsipyla grandella* (Zeller) (Lepidoptera: Pyralidae) Larvae," *Journal of Pest Sciences*, Vol. 83, No. 2, 2010, pp. 95-104. doi:10.1007/s10340-009-0275-8

[26] W. T. Warthen, "Insect Feeding Deterrents (1976-1980)," In: E. Morgan and N. Mandava, Eds., *CRC Handbook of Natural Pesticides*, CRC Press, Boca Raton, 1990, pp. 23-24.

[27] R. Taveras, L. Hilje and M. Carballo, "Development of *Hypsipyla grandella* (Zeller) (Lepidoptera: Pyralidae) in Response to Constant Temperatures," *Neotropical Entomology*, Vol. 33, No. 1, 2004, pp. 1-6. doi:10.1590/S1519-566X2004000100002

[28] H. Rembole and K. P. Suber, "Inhibition of Oogenesis and Ovarian Ecdysteroid Synthesis by Azadirachtin in *Locusta migratoria*," *Zeitschrift für Naturforschung*, Vol. 36, 1981, pp. 466-469.

[29] X. D. Luo, S. H. Wu, Y. B. Ma and D. G. Wu, "Studies on Chemical Constituents of *Toona sinensis*," *Chinese Traditional and Herbal Drugs*, Vol. 32, No. 5, 2001, pp. 390-391.

[30] K. J. Wang, Ch. R. Yang and Y. Zhang, "Phenolic Antioxidants from Chinese Toon (Fresh Young Leaves and Shoots of *Toona sinensis*)," *Food Chemistry*, Vol. 101, No. 1, 2007, pp. 365-371. doi:10.1016/j.foodchem.2006.01.044

[31] L. Hilje and J. Cornelius, "Es Inmanejable *Hypsipyla grandella* Como Plaga Forestal? Hoja Técnica No. 38," *Manejo Integrado de Plagas* (*Costa Rica*), Vol. 61, 2001, pp. I-IV.

[32] D. Cibrián, J. T. Méndez, R. Campos, H. O. Yates III and J. E. Flores, "Insectos Forestales de México," Universidad Autónoma Chapingo—Comisión Forestal de América del Norte (COFAN), Publ. No. 6, 1995, 453 p.

