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# Antifeedant activity and effects of terpenoids on detoxication enzymes of the beet armyworm, *Spodoptera exigua* (Hübner)

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## Abstract

It has been determined the antifeedant activity of a mixture of limonoids 1,7-di-*O*-acetylhavanensin and 3,7-di-*O*-acetylhavanensin (F18), isolated from seeds of *Trichilia havanensis* Jacq. (Meliaceae), and the *neo*-clerodane diterpene scutecyprol A, isolated from *Scutellaria valdiviana* (Clos) Epling (Labiatae), on fifth instar larvae of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Choice and no-choice feeding assays, nutritional tests, and post-treatment studies indicated that scutecyprol A acts as an insect feeding deterrent against *S. exigua*, whereas the antifeedant activity of F18 is likely associated with a toxic mode of action. In addition, it was tested the hypothesis that *S. exigua* larvae will try to metabolize the compounds that are toxic by the induction of their detoxication systems, whereas no such induction happens for those compounds that are deterrents. The mixture of limonoids F18 significantly increased glutathione S-transferases during the treatment and post-treatment periods, whereas esterases were inhibited during the treatment period. On the contrary, scutecyprol A did not have any significant effect on any of the enzymatic processes. Hence, the metabolic response of *S. exigua* larvae to the ingestion of the secondary metabolites tested depends on their mode of action.

**Additional key words**: esterases, glutathione S-transferases, limonoids, *neo*-clerodane diterpenes, nutritional indexes, *Scutellaria, Trichilia*.

## Resumen

### Actividad antialimentaria de terpenoides y efectos sobre las enzimas de destoxificación de la gardama, *Spodoptera exigua* (Hübner)

Se ha determinado la actividad antialimentaria de una mezcla de limonoides 1,7-di-*O*-acetilhavanensina y 3,7-di-*O*-acetilhavanensina (F18), aislados a partir de semillas de *Trichilia havanensis* Jacq. (Meliaceae), y del diterpeno *neo*-clerodano scutecyprol A, procedente de *Scutellaria valdiviana* (Clos) Epling (Labiatae), frente a larvas de 5º estadío de la gardama, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Los resultados de ensayos de preferencia y no preferencia, de ensayos nutricionales y del estudio de los efectos post-ingestivos indican que el scutecyprol A actúa como disuasorio de la alimentación frente a larvas de *S. exigua*, mientras que la actividad antialimentaria de F18 está asociada a un modo de acción tóxico. Además, se ha comprobado la hipótesis de que las larvas de *S. exigua* tratan de metabolizar los compuestos que son tóxicos mediante la inducción de enzimas de destoxificación, mientras que esta inducción no es necesaria para aquellos compuestos que son disuasorios de la alimentación. La mezcla de limonoides F18 incrementó de forma significativa la actividad glutatión S-transferasa durante los periodos de tratamiento y post-tratamiento, mientras que la actividad esterasa se redujo durante el periodo de tratamiento. Por el contrario, el scutecyprol A no tuvo ningún efecto significativo sobre estos procesos enzimáticos. Se puede concluir, pues, que la respuesta metabólica en larvas de *S. exigua* depende del modo de acción del compuesto ingerido.

**Palabras clave adicionales**: diterpenos *neo*-clerodánicos, esterasas, glutatión S-transferasas, índices nutricionales, limonoides, *Scutellaria, Trichilia*.

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## Introduction[1]

Insects are able to metabolize a great variety of exogenous compounds, including secondary metabolites produced by plants (Brattsten, 1979; Ahmad *et al*., 1986). The most common detoxication enzyme systems are esterases, glutathione S-transferases and P450 monooxygenases (Ranson *et al*., 2002; Feyereisen, 2005). It is assumed that the activity of these enzymes in herbivorous insects is induced by the consumption of toxic plant compounds (Snyder and Glendinning, 1996). Detoxication enzymes are energetically costly and, therefore, a common strategy in insects is to produce them proportionally to the amount of xenobiotics encountered (Berenbaum and Zangerl, 1994).

Terpenoids are the most diverse group of plant allelochemicals, some of them showing biological activities against some economically important pests (Pickett, 1991; Mizutani, 1999). The plant family Meliaceae has been the subject of search as one of the most promising sources of terpenoids with insect control properties, such as azadirachtin, a limonoid (tetranortriterpene) obtained from the seed kernels of the neem tree *Azadirachta indica* A. Juss. (Meliaceae) (Mordue and Blackwell, 1993). Likewise, López-Olguín (1998) showed that limonids isolated from the seed kernels of *Trichilia havanesis* Jacq. (Meliaceae), exhibit strong antifeedant activity against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) and the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Another abundant source of this kind of compounds are the plants belonging to the genus *Scutellaria* (family Labiatae), from which several *neo*-clerodanes with insect antifeedant activity have been isolated (Cole *et al*., 1990; Rodríguez *et al*., 1993; Caballero, 2004).

The antifeedant activity of limonoids and *neo*-clerodanes appears to be due to their effects on chemosensory mouthparts (deterrent activity), but also through their post-ingestive toxic effects (Mordue and Blackwell, 1993). It is expected that insects will try to eliminate the compounds that are toxic by the induction of their detoxication systems, whereas no such induction is necessary for those compounds that are deterrents. However, very few studies have tested experimentally this hypothesis (Wheeler *et al*., 2001; Weimin *et al*., 2003). Ortego *et al*. (1999) concluded that the effects of terpenoids on digestive detoxication enzymes in the larval midgut of *L. decemlineata* reflect their mode of action, being altered (induced or inhibited) by toxic compounds, whereas no effects appeared with deterrent compounds.

The present paper reports the antifeedant activity of F18, a mixture of limonids (1,7-di-*O*-acetylhavanensin and 3,7-di-*O*-acetylhavanensin), isolated from *T. havanensis* and the *neo*-clerodane diterpene scutecyprol A, isolated from *Scutellaria valdiviana* (Clos) Epling (Labiatae), on larvae of *S. exigua*. In addition, nutritional tests and enzymatic assays were performed to test if the enzymatic response depends on the mode of action.

## Materials and Methods

### Insect rearing and plants

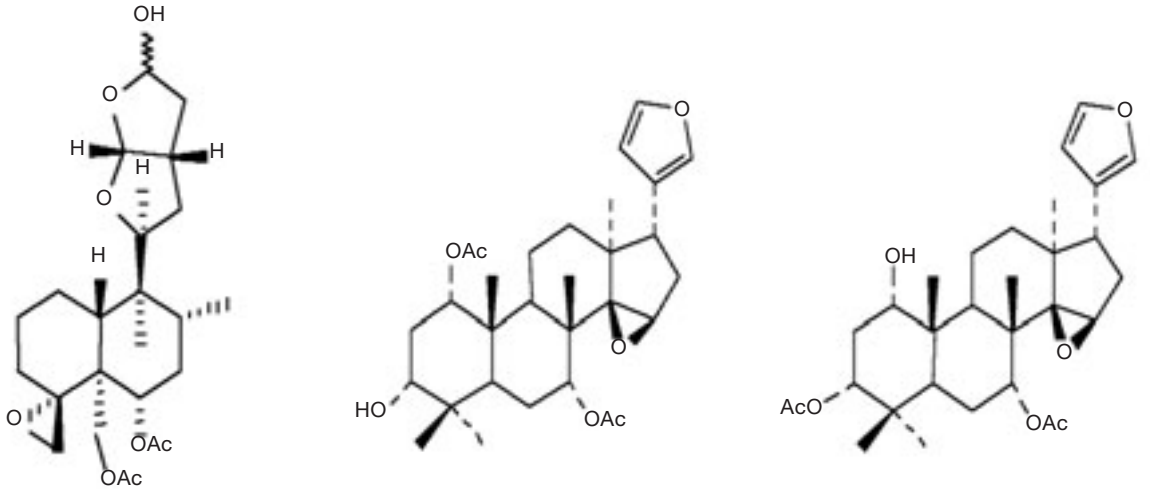
A colony of *S. exigua* was established with eggs provided by N. J. de Boer (Univ. Leiden, the Netherlands). The larvae were reared on a semi-artificial diet adapted from Poitout and Bues (1970) by the addition of 0.63% (w/w) Wesson's salt mixture, at 25 ± 2°C, > 75% RH and 16:8 (L:D) h photoperiod in an environmental chamber.

Sugar beet plants, *Beta vulgaris* L. cv. Roberta (Chenopodiaceae) were grown at 25 ± 1°C, > 75% RH and 16:8 (L:D) h photoperiod in a Conviron S10H growth chamber.

### Chemicals and equipment

A mixture (4:1) of 1,7-di-*O*-acetylhavanensin and 3,7-di-*O*-acetylhavanensin (F18) (Fig. 1) was isolated from the seed kernels of *T. havanesis* (López-Olguín, 1998). The *neo*-clerodane diterpenoid scutecyprol A (Fig. 1) was obtained from *S. valdiviana* (Bruno *et al.,* 1996). All enzymatic substrates were supplied by Sigma

[1] Abbreviations used: AI (antifeedant index), C (consumption, in mg of dry weight, of control disks), CDNB (1-chloro-2,4-dinitrobenzene), Cp (consumption during the assay), F18 (limonoids mix of 1,7-di-*O*-acetilhavanensina and 3,7-di-*O*-acetilhavanensina), GST (glutathione S-transferase), NA (naphthyl acetate), NADP (nicotinamide adenine dinucleotide phosphate), NADPH (reduced nicotinamide adenine dinucleotide phosphate), ppm (parts per million), RCR (relative consumption rate), RGR (relative growth rate), RH (relative humidity), t (duration of the assay), SE (standard error), T (consumption, in mg of dry weight, of treated disks), Wi (initial larval weight), ΔW (increment of larval weight).




**Scutecyprol A** **1,7-di-*O*-acetyl-havanensin** **3,7-di-*O*-acetyl-havanensin**

**Figure 1.** Chemical structure of compounds tested against *Spodoptera exigua* larvae.

Chemical Co (St. Louis, MO). Spectrophotometric measurements were made using a Hitachi U-2000 spectrophotometer.

## Choice and no-choice feeding assays

The arena for the assays consisted of plastic Petri dishes (15 × 90 mm), coated on their bottom half with a 2.5% agar solution, as previously described (Ortego *et al*., 1995). Sugar beet leaf disks (1.77 $cm^2$) were treated, using a micropipette, on the upper surface with 20 µL of an acetone (analytical grade) solution containing the test compound (1,000 ppm with respect to the average weight of the leaf disks) or the solvent carrier alone (as control disks). After complete evaporation of the solvent, newly emerged fifth instar larvae (less than 24 h old and starved for 6 h) were individually placed in each dish in a Sanyo MLR-350T growth chamber at 26 ± 0.5°C and 85 ± 10% RH, where they were allowed to feed.

In the choice assay, three treated and three control disks were alternatively arranged in each arena. For the no-choice assays, six treated or control disks were used. Feeding was terminated after the consumption of 50% of the disks in the choice assay, and after the consumption of about 75% of the control disks in the no-choice assay. Ten replicates per treatment were used in both assays. The Antifeedant Index (AI) was calculated in the no- choice assay by the equation $[(C - T)/C] \times 100\%$ (Bentley *et al*., 1984) and in the choice assay by the equation $[(C - T)/(C + T)] \times 100\%$ (Simmonds *et al*., 1989), where C and T represent the consumption (in mg of dry weight) of control and treated disks, respectively. For each assay, initial dry weight of the leaf disks was estimated using 60 leaf disks to calculate the ratio of fresh to dry weight. At the end of the experiment, the uneaten leaf disks were oven dried at 60°C for 2 d and weighted.

## Nutritional indexes

The arena for the assays consisted of the plastic Petri dishes (15 × 90 mm), coated on their bottom half with a 2.5% agar solution, described above. Twenty newly emerged fifth instar larvae were individually fed over 12 h on eight sugar beet leaf disks treated with 0, 100, 300 or 1,000 ppm of F18 or scutecyprol A. At the end of this period, 10 larvae per treatment were allowed to continue feeding on eight untreated sugar beet leaf disks for another 12 h. Nutritional indexes were calculated for the 12 h that the larvae were feeding on treated leaf disks (treatment period), and for the following 12 h that the larvae were feeding on untreated leaf disks (post-treatment period). In each assay, a feeding deterrent simulation assay was run simultaneously to distinguish between deterrent and toxic effects. In this case, 20 larvae were starved during the 12 h of the treatment period, and allowed to feed on eight untreated sugar beet leaf disks during the 12 h of the post-treatment period. The Relative Consumption Rate (RCR = Cp/Wixt) and the Relative Growth Rate (RGR = ΔW/Wixt) were calculated according to Farrar *et al*. (1989); where Cp and ΔW represent, respectively, the consumption

and the increment of larval weight during the assay (in mg of dry weight), Wi the larval weight at the beginning of the assay (in mg of dry weight), and t the duration of the assay (in days). Larval dry weights at the beginning and the end of the assay were estimated by the regression equation: dry weight = 3.28 + 0.09 fresh weight; calculated with fifth instar larvae from this species. The dry weight of the leaf disks was obtained as explained before.

### Enzyme assays

At the end of the treatment and post-treatment periods, 10 larvae were dissected and the midgut homogenized in 500 µL of 0.15 M NaCl and centrifuged at 10,000 g for 5 min at room temperature. The supernatants were frozen individually to provide 10 samples of each treatment for enzymatic activity assays.

Glutathione S-transferase activity was measured in 1 mL of reaction mixture containing 30 µL of midgut extract, 1 mM CDNB (1-chloro-2,4-dinitrobenzene), 5 mM reduced glutathione, and 0.1 M Tris buffer pH 8.5. The increment in absorbance at 340 nm was recorded during 2 min at 30°C to determine the nmol substrate conjugated per min per mg protein, using a molar extinction coefficient of 9.6 $mM^{-1}$ $cm^{-1}$ (Habig *et al*., 1974).

Esterase activity was determined using 1-NA (1-naphthyl acetate) as substrate based on the procedure described by Gomori (1953). The reaction was started by the addition of 20 µL of 1/50 diluted midgut extract to a total 1 mL of reaction mixture containing 0.25 mM 1-NA in 0.1 M Tris buffer pH 7.0. The mixture was incubated at 30°C for 1 h, and the reaction was terminated by the addition of 500 µL of an aqueous solution containing 0.4 mg Fast blue B salt and 15 mg sodium dodecyl sulfate $mL^{-1}$. The absorbance was determined at 600 nm and the activity expressed as nmol substrate hydrolyzed $min^{-1}$ $mg^{-1}$ protein, using 1-naphthol as standard.

P450 monooxygenases were assayed by the reduction of cytochrome c by NADPH-cytochrome P-450 reductases according to Masters *et al*. (1965). The 1 mL of incubation mixture contained 15 µL of midgut extract, 50 µM cytochrome c, NADPH-generating system (0.5 mM NADP, 2.5 mM glucose-6-phosphate and 0.25 units of glucose-6-phosphate dehydrogenase), and 0.1 M Glycine-NaOH buffer pH 9.0. The increment in absorbance at 550 nm was recorded during 5 min at 30°C to determine the nmol substrate reduced $min^{-1}$ $mg^{-1}$ protein, using a molar extinction coefficient of 27.6 $mM^{-1}$ $cm^{-1}$ for the reduced form of cytochrome c.

Total protein in the midgut extracts was determined according to the method of Bradford (1976) using bovine serum albumin as the standard.

### Statistical analysis

Significant differences between the consumption of control and treated leaf disks were determined using the Student's t test in no-choice assays and by the Wilcoxon's matched pair test in choice assays. Nutritional indexes and enzymatic activities were compared with respect to the control by the Dunnet two-tailed test, using initial larval weight or protein content as covariate, respectively (Ortego *et al*., 1999).

## Results

### Choice and no-choice feeding assays

The compounds tested affected the feeding behaviour of *S. exigua* larvae, showing some degree of antifeedant activity. The term «antifeedant» is used here as the capacity to reduce feeding, independently of a deterrent (antixenosis) or toxic (antibiosis) mode of action. Scutecyprol A significantly reduced feeding at 1,000 ppm in both, choice and no-choice assays (Table 1). The mixture of limonoids F18 caused a significant feeding reduction in the no-choice assay, indicating a potent antifeedant activity (Table 1). On the contrary, consumption of F18 treated disks was not significantly reduced in the choice test, indicating low or a lack of discrimination between treated and control disks (Table 1).

**Table 1.** Effect of scutecyprol A and a mixture of 1,7-di-*O*-acetylhavanensin and 3,7-di-*O*-acetylhavanensin (F18) on the feeding behaviour of fifth instar larvae of *Spodoptera exigua*

| Compound (1,000 ppm) | No-choice bioassay AI[a] | Choice bioassay AI[b] |
|---|---|---|
| Scutecyprol A | 48.4 ± 11.5* | 91.4 ± 5.6* |
| F18 | 87.7 ± 2.5* | 21.3 ± 16.4 |

[a] Average antifeedant index $[(C-T)/C]\times 100\% \pm$ SE ($n = 10$). * Significant differences between the consumption of control (C) and treated (T) sugar beet leaf disks (Student's t test, $P \leq 0.05$).[b] Average antifeedant index $[(C-T)/C+T)]\times 100\% \pm$ SE ($n = 10$). * Significant differences between the consumption of control (C) and treated (T) sugar beet leaf disks within each arena (Wilcoxon's matched pair test, $P \leq 0.05$).

## Nutritional indexes and detoxication enzyme activities

The effect of F18 and scutecyprol A on feeding behaviour was further studied with the help of nutritional indexes. Feeding during 12 h on sugar beet leaf disks treated with F18 significantly decreased consumption (RCR) and larval growth (RGR) at all concentrations tested (Table 2A). When these larvae were transferred to untreated leaf disks for another 12 h they were not able to recover, whereas larvae that have been starved during the treatment period reached the usual nutritional indexes once they started eating (Table 2A). Similar reductions in RCR and RGR were obtained in larvae fed on scutecyprol A treated disks during the treatment period, but they and those from the starved

**Table 2.** Nutritional indexes and detoxication enzyme activities of fifth-instar larvae of *Spodoptera exigua* fed for 12 h on sugar beet leaf disks treated with A) a mixture of 1,7-di-*O*-acetylhavanensin and 3,7-di-*O*-acetylhavanensin (F18) (treatment period) or B) scutecyprol A (treatment period) and subsequently on untreated sugar beet leaf disks for another 12 h (post-treatment period)

| Concentration (ppm) | Nutritional indexes[a] | | Enzyme activities[b] | | |
|---|---|---|---|---|---|
| | RCR | RGR | GST (CDNB) | Esterase (1-NA) | P450 monooxygenase (cytochrome c) |
| **A) Treated with a mixture of 1,7-di-*O*-acetylhavanensin and 3,7-di-*O*-acetylhavanensin (F18)** | | | | | |
| *Treatment* | | | | | |
| Control | 2.36 ± 0.05 | 1.06 ± 0.03 | 293± 65 | 867± 84 | 73 ± 7 |
| 100 | 0.69 ± 0.03* | 0.21 ± 0.01* | 392 ± 67 | 702 ± 120 | 73 ± 14 |
| 300 | 0.49 ± 0.03* | 0.09 ± 0.01* | 545 ± 96* | 546 ± 159 | 72 ± 15 |
| 1,000 | 0.25 ± 0.02* | 0.03 ± 0.01* | 570 ± 85* | 492 ± 63* | 97 ± 16 |
| Starved | 0.00 ± 0.00* | –0.05 ± 0.01* | 334 ± 57 | 915 ± 126 | 101 ± 9 |
| *Post-treatment* | | | | | |
| Control | 2.53 ± 0.12 | 0.87 ± 0.08 | 312± 55 | 537± 84 | 62± 11 |
| 100 | 1.42 ± 0.11* | 0.44 ± 0.05* | 385 ± 56 | 480 ± 36 | 55 ± 7 |
| 300 | 1.29 ± 0.10* | 0.39 ± 0.04* | 387 ± 50 | 510 ± 42 | 74 ± 23 |
| 1,000 | 0.97 ± 0.09* | 0.26 ± 0.04* | 485 ± 57* | 555 ± 72 | 62 ± 7 |
| Starved | 2.27 ± 0.10 | 1.03 ± 0.08 | 299 ± 20 | 552 ± 63 | 91 ± 9 |
| **B) Treated with scutecyprol A** | | | | | |
| *Treatment* | | | | | |
| Control | 2.20 ± 0.12 | 0.75 ± 0.04 | 258 ± 23 | 669 ± 61 | 59 ± 7 |
| 100 | 1.53 ± 0.15* | 0.53 ± 0.05* | 254 ± 17 | 651 ± 120 | 83 ± 9 |
| 300 | 0.34 ± 0.06* | 0.10 ± 0.04* | 346 ± 33 | 643 ± 83 | 52 ± 12 |
| 1,000 | 0.23 ± 0.07* | 0.09 ± 0.07* | 355 ± 35 | 407 ± 69 | 47 ± 15 |
| Starved | 0.00 ± 0.00* | –0.24 ± 0.02* | 365 ± 30 | 854 ± 68 | 52 ± 11 |
| *Post-treatment* | | | | | |
| Control | 1.57 ± 0.09 | 0.69 ± 0.05 | 285 ± 16 | 673 ± 49 | 73 ± 10 |
| 100 | 1.57 ± 0.21 | 0.66 ± 0.06 | 306 ± 22 | 759 ± 47 | 77 ± 8 |
| 300 | 1.71 ± 0.25 | 0.67 ± 0.07 | 270 ± 31 | 956 ± 219 | 92 ± 9 |
| 1,000 | 1.44 ± 0.13 | 0.75 ± 0.13 | 302 ± 37 | 957 ± 106 | 103 ± 19 |
| Starved | 1.91 ± 0.16 | 0.85 ± 0.06 | 342 ± 18 | 921 ± 186 | 73 ± 12 |

[a] Relative Consumption Rate (RCR = Cp/Wixt) and Relative Growth Rate (RGR = ΔW/Wixt), where Cp and ΔW represent the consumption and the increment of larval weight during the assay (in mg of dry weight), Wi the larval weight at the beginning of the assay (in mg of dry weight), and t the duration of the assay (in days). Values are the mean ± SE (n = 20 for treatment period and 10 for post-treatment). [b] Specific activities as nmoles of substrate hydrolyzed (esterase), conjugated (glutathione S-transferase = GST) or reduced (P450 monooxygenases) $min^{-1}$ $mg^{-1}$ protein. Values are the mean ± SE (n = 10). * Significantly different from control (Dunnett two-tailed test $P \leq 0.05$). Means were compared by analysis of covariance using as covariate initial larval weight (nutritional indexes) or protein content (enzyme activities).

group recovered during the post-treatment period (Table 2B).

Ingestion of F18-treated sugar beet disks significantly reduced esterase activity and increased glutathione S-transferase activity at 1,000 ppm during the treatment period, whereas P450 monooxygenase activities was not affected (Table 2A). At 300 ppm, only glutathione S-transferase activity was significantly increased. At the end of the post-treatment period, all enzymatic activities were not significantly different from controls, except for an increase in glutathione S-transferase activity at 1,000 ppm (Table 2A). All enzymatic activities remained unaltered during the treatment and post-treatment periods for larvae feeding on sugar beet leaf disks treated with scutecyprol A (Table 2B). No differences between starved and control larvae were found for detoxication enzyme activities during the treatment and post-treatment periods (Table 2A and 2B).

## Discussion

Choice and no-choice feeding assays, nutritional tests and post-treatment studies indicated that scutecyprol A acts as an insect feeding deterrent against fifth instar larvae of *S. exigua*, whereas the antifeedant activity of the mixture of limonoids F18 is likely associated with a toxic mode of action. Toxic and deterrent modes of action have been suggested as responsible for the antifeedant activity of limonoids and *neo*-clerodanes on different species (Blaney *et al*., 1988; Ascher, 1993). Thus, scutecyprol A has been previously reported as a potent antifeedant when tested in choice assays against *Spodoptera littoralis* (Boisduval) (Rosselli *et al.,* 2004). Likewise, López-Olguín (1998) showed that F18 has toxic effects on *L. decemlineata*.

According to their mode of action, it is expected that the toxic mixture of limonoids F18 should display post-ingestive effects on some biochemical process, whereas no such effects should appear with the deterrent scutecyprol A (Ortego *et al*., 1999). The results of this study showed that the effects of these compounds on the enzymatic activities of *S. exigua* matched their postulated mode of action. Thus, F18 affected the activity of the detoxication enzymes during the treatment and post-treatment periods. In contrast, scutecyprol A did not have any significant effect on any of the enzymatic processes. Information regarding the detoxication enzymes involved in the inactivation of terpenoids is scarce. These data showed increased levels of glutathione S-transferase activity after consumption of F18, suggesting that these metabolic enzymes might be involved in the detoxication of limonoids. Ortego *et al*. (1999) found that both glutathione S-transferases and P450 monooxygenases were induced in *L. decemlineata* in response to the consumption of F18. In addition, the toxicity of neem seed kernel extracts to *L. decemlineata* larvae increased after adding the P450 monooxygenase inhibitor piperonyl butoxide (Zehnder and Warthen, 1988). Thus, the metabolic response to terpenoids with toxic mode of action appears to represent a general phenomenon among phytophagous insects, if well the specific detoxication enzymes that are induced depend on the species.

Some plants produce allelochemicals that inhibit the action of detoxication enzymes (Yu, 1984). A significant reduction in esterase activity as a result of F18 consumption was obtained, but normal activity was reached when the larvae were shifted to untreated leaf disks during the post-treatment period. Similar results were obtained when *L. decemlineata* larvae were fed on potato leaves treated with F18 (Ortego *et al*., 1999). Likewise, Smirle *et al*. (1996) reported that ingestion of neem oil significantly reduced esterase activities in larvae and adults of *Choristoneura rosaceana* (Harris). Other secondary metabolites that have been reported to inhibit esterase activity are the hydroxamic acid DIMBOA (Yan *et al*., 1995; Ortego *et al*., 1998), the phenolic compound gossypol and the coumarin umbelliferone (Brattsten, 1987). The finding that ingestion of F18 reduced esterase activities in insects suggest that this mixture of limonoids may be useful control agent and thereby play a relevant role in pest management, particularly when insecticide resistance has developed as a result of elevated esterase activity.

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## References

AHMAD S., BRATTSTEN L.B., MULLIN C.A., YU S.J., 1986. Enzymes involved in the metabolism of plant allelochemicals. In: Molecular aspects of insect-plant

associations (Brattsten L.B., Ahmad S., eds). Plenum Press, London. pp. 73-152.

ASCHER S.K.R., 1993. Non conventional insecticidal effects of pesticides available from the neem tree, *Azadirachta indica*. Arch Insect Biochem Physiol 22, 433-449.

BENTLEY M.D., LEONARD D.E., STODDARD W.F., ZALKOW L.H., 1984. Pyrrolizidine alkaloids as larval feeding deterrents for spruce budworm, *Choristoneura fumiferana* (Lepidoptera: Tortricidae). Ann Entomol Soc Am 77, 393-397.

BERENBAUM M.R., ZANGERL A.R., 1994. Costs of inducible defense: protein limitation, growth and detoxification in parsnip webworms. Ecology 75, 2311-2317.

BLANEY W.M., SIMMONDS M.S.J., LEY S.V., JONES P.S., 1988. Insect antifeedants: a behavioural and electrophysiological investigation of natural and synthetically derived clerodane diterpenoids. Entomol Exp Appl 46, 267-274.

BRADFORD M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72, 248-254.

BRATTSTEN L.B., 1979. Biochemical defense mechanisms in herbivores against plant allelochemicals. In: Herbivores (Rosenthal G.A., Janzen D.H., eds). Academic Press, NY, USA. pp. 200-262.

BRATTSTEN L.B., 1987. Inducibility of metabolic insecticide defenses in boll weevils and tobacco budworm caterpillars. Pest Biochem Physiol 27, 13-23.

BRUNO M., FAZIO C., ARNOLD N.A., 1996. Neo-clerodane diterpenoids from *Scutellaria cypria*. Phytochemistry 42, 555-557.

CABALLERO C., 2004. Efectos de terpenoides naturales y hemisintéticos sobre *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) y *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Doctoral Thesis. Universidad Complutense, Madrid. [In Spanish].

COLE M.D., ANDERSON J.C., BLANEY W.M., FELLOWS L.E., LEY S.V., SHEPPARD R.N., SIMMONDS M.S.J., 1990. *Neo*-clerodane insect antifeedant from *Scutellaria galericulata*. Phytochemistry 29, 1793-1796.

FARRAR R.R., BARBOUR J.D., KENNEDY G.G., 1989. Quantifying food consumption and growth in insects. Ann Entomol Soc Am 82, 593-598.

FEYEREISEN R., 2005. Insect cytochrome P450. In: Comprehensive molecular insect science (Gilbert L.I., Latro K., Gill S., eds). Elsevier, Oxford, UK. pp. 1-77.

GOMORI G., 1953. Human esterases. J Lab Clin Med 42, 445-453.

HABIG W.H., PABST M.J., JAKOBY W.B., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 249, 7130-7139.

LÓPEZ-OLGUÍN J.F., 1998. Actividad de productos de *Trichilia havanensis* (Jacq.) y *Scutellaria alpina* subesp. *javalambrensis* (Pau), sobre *Leptinotarsa decemlineata* (Say) y *Spodoptera exigua* (Hübner). Doctoral Thesis. Universidad Politécnica, Madrid. [In Spanish].

MASTERS B.S.S., KAMIN H., GIBSON Q.H., WILLIAMS C.H. Jr., 1965. Studies on the mechanism of microsomal triphosphopyridine nucleotide-cytochrome c reductase. J Biol Chem 240, 921-931.

MIZUTANI J., 1999. Selected allelochemicals. Crit Rev Plant Sci 18, 653-671.

MORDUE (LUNTZ) A.J., BLACKWELL A., 1993. Azadirachtin: an update. J Insect Physiol 39, 903-924.

ORTEGO F., RODRÍGUEZ B., CASTAÑERA P., 1995. Effects of *neo*-clerodane diterpenes from *Teucrium* on feeding behavior of Colorado potato beetle larvae. J Chem Ecol 21, 1375-1386.

ORTEGO F., RUIZ M., CASTAÑERA P., 1998. Effect of DIMBOA on growth and digestive physiology of *Sesamia nonagrioides* (Lepidoptera: Noctuidae) larvae. J Insect Physiol 44, 95-101.

ORTEGO F., LÓPEZ-OLGUÍN J.F., RUIZ M., CASTAÑERA P., 1999. Effects of toxic and deterrent terpenoids on digestive protease and detoxication enzyme activities of Colorado potato beetle larvae. Pest Biochem Physiol 63, 76-84.

PICKETT J.A., 1991. Lower terpenoids as natural insect control agents. In: Ecological chemistry and biochemistry of plant terpenoids (Harborne J.B., Tomas-Barberan F.A., eds). Proc. Phytochemistry Soc. Europe, 31. Clarendon Press, Oxford, UK. pp. 297-313.

POITOUT S., BUES R., 1970. Élevage de plusieurs espèces de Lépidoptères Noctuidae sur milieu artificiel riche et sur milieu artificiel simplifié. Ann Zool Ecol Anim 2, 79-91. [In French].

RANSON H., CLAUDIANOS C., ORTELLI F., ABGRALL C., HEMINGWAY J., SHARAKHOVA M.V., UNGER M.F., COLLINS F.H., FEYEREISEN R., 2002. Evolution of supergene families associated with insecticide resistance. Science 298, 179-181.

RODRÍGUEZ B., DE LA TORRE M.C., RODRÍGUEZ B., BRUNO M., PIOZZI F., SAVONA G., SIMMONDS M.S.J., BLANEY W.M., PERALES A., 1993. *Neo*-clerodane insect antifeedants from *Scutellaria galericulata.* Phytochemistry 33, 309-315.

ROSSELLI S., MAGGIO A., PIOZZI F., SIMMONDS M.S.J., BRUNO M., 2004. Extremely potent antifeedant neo-clerodane derivatives of scutecyprol A. J Agric Food Chem 52, 7867-7871.

SIMMONDS M.S.J., BLANEY W.M., LEY S.V., SAVONA G., BRUNO M., RODRÍGUEZ B. 1989. The antifeedant activity of clerodane diterpenoids from *Teucrium*. Phytochemistry 28, 1069-1071.

SMIRLE M.J., LOWERY D.T., ZUROWSKI C.L., 1996. Influenze of neem oil on detoxication enzyme activity in the obliquebanded leafroller, *Choristoneura rosaceana*. Pest Biochem Physiol 56, 220-230.

SNYDER M.J., GLENDINNING J.I., 1996. Causal connection between detoxification enzyme activity and consumption of a toxic plant compound. J Comp Physiol 179A, 255-261.

WEIMIN L., SCHULER A., BERENBAUM M.R., 2003. Diversification of furanocoumarin-metabolizing cytochrome P450 monooxygenases in two papilionids: specificity and

substrate encounter rate. Proc Natl Acad Sci USA 100, 14593-14598.

WHEELER G.S., SLANSKY F. Jr., YU S.J., 2001. Food consumption, utilization and detoxification enzyme activity of larvae of three polyphagous noctuid moth species when fed the botanical insecticide rotenone. Ent Exp Appl 98, 225-239.

YAN F., XU C., LI S., LIN C., LI J., 1995. Effects of DIMBOA on several enzymatic systems in Asian corn borer, *Ostrinia furnicalis* (Guenée). J Chem Ecol 21, 2047-2056.

YU S.J., 1984. Interactions of allelochemicals with detoxication enzymes of insecticide-susceptible and resistant fall armyworms. Pestic Biochem Physiol 22, 60-68.

ZEHNDER G., WARTHEN J.D., 1988. Feeding inhibition and mortality effects of neem-seed extract on the Colorado potato beetle (Coleoptera: Chrysomelidae). J Econ Entomol 81, 1040-1044.