Ciência e Tecnologia

Para o Desenvolvimento Ambiental, Cultural e Socioeconômico

Xosé Somoza Medina
(organizador)

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PRÓLOGO

Este libro presenta una colección de artículos de investigación que bajo distintos ámbitos de conocimiento realizan avances de interés en la ciencia y la tecnología. La sociedad del siglo XXI se distingue de la de épocas pretéritas por su capacidad analítica. A diferencia de lo que ocurría en otras épocas, en nuestro mundo contemporáneo tenemos demasiada información y avanzar en el conocimiento significa realizar una investigación original sobre otros antecedentes previos y analizar una gran cantidad de datos para poder extraer conclusiones que signifiquen un desarrollo, un avance entre la situación anterior y la posterior, aunque sea a pequeña escala en un contexto local y en un ámbito científico muy concreto. La suma de miles de esos pequeños avances y la interconexión mundial sostienen a la ciencia y la tecnología del siglo XXI.

Este es el objetivo de este libro, realizar avances en la ciencia y la tecnología para el desarrollo ambiental, cultural y socioeconómico, desde un posicionamiento académico, comprometido con el rigor científico y el desarrollo del ser humano.

Para ello se han compendiado veinticuatro artículos de investigación en dos apartados, ciencia y tecnología. En el primer conjunto nos encontramos con artículos que desde las ciencias ambientales o las ciencias sociales realizan propuestas de mejora de aspectos concretos sobre hidrología, regeneración de suelo agrícola, cuidado ambiental, recursos humanos, ciudades igualitarias o paisajes culturales.

En el segundo bloque, se agrupan trabajos de ingeniería química, ingeniería industrial o ingeniería forestal que relatan avances en distintas tecnologías, relacionadas con el biogás de los vertederos de residuos, los usos de nuevos materiales sintéticos, la química de determinados productos y su toxicidad, o las características bioestructurales de la madera de roble.

Xosé Somoza Medina
Universidad de León, Espanha

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TOXIC EFFECTS OF CONSTITUENTS OF THE FERN *STICHERUS QUADRIPARTITUS* AGAINST *SPODOPTERA FRUGIPERDA* AND *PLODIA INTERPUNCTELLA*

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ABSTRACT: Secondary metabolites produced by plants can interact with insects which can affect at all levels of biological organization, but their action generally disturbs cellular tissues, and physiological processes, e.g., by hormonal regulation, neuronal signalization growth regulators, or reproduction in exposed individuals. A previous chemical investigation of a Patagonian collection of *Sticherus quadripartitus* (Gleicheniaceae) yielded sesqui and diterpenoids that were now evaluated lethal effects of the methanolic extract and the five major diterpenoids for their effects against *Spodoptera frugiperda* Smith, (Lepidoptera: Noctuidae) and *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). In both species were larval and pupal mortality rates, larval nutritional effects as well as adult malformations were registered. Four of the five diterpenoids tested produced 65 to 90% mortality of larvae of *P. interpunctella* at 150 µg per gram of natural diet, with larval LD_{50} ranging from 69.3 to 123.0 µg/g. Our results suggest that the combination of lethal and sublethal effects of sesqui-and diterpenoids may have important implications for the population dynamics of the *Plodia interpunctella.*

KEYWORDS: Diterpenoids. *Sticherus quadripartitus. Lethal and sublethal effects*. Lepidoptera.

EFECTOS TÓXICOS DE LOS CONSTITUYENTES DEL HELECHO *STICHERUS QUADRIPARTITUS* CONTRA *SPODOPTERA FRUGIPERDA* Y *PLODIA INTERPUNCTELLA*

RESUMO: Os metabólitos secundários produzidos por plantas podem interagir com insetos que podem afetar todos os níveis de organização biológica. No entanto, a sua ação geralmente perturba só os tecidos celulares e os processos fisiológicos, como por exemplo, regulação hormonal, reguladores

[1] Corresponding Author.

de crescimento de sinalização neuronal ou reprodução em indivíduos expostos. Uma investigação química realizada a uma coleção patagônica de Sticherus quadripartitus (Gleicheniaceae) produziu sesqui e diterpenóides, onde foram agora avaliados efeitos letais do extrato metanólico e os cinco principais diterpenóides pelos seus efeitos contra Spodoptera frugiperda Smith (Lepidoptera: Noctuidae) e Plodia interpunctella Hübner (Lepidoptera: Pyralidae). Em ambas as espécies foram registadas taxas de mortalidade larval e pupal, efeitos nutricionais larvais, bem como malformações adultas. Quatro dos cinco diterpenóides testados produziram 65 a 90% de mortalidade de larvas de P. interpunctella a 150 µg por grama de dieta natural, com DL50 larval variando de 69,3 a 123,0 µg/g. Os nossos resultados sugerem que a combinação de efeitos letais e subletais de sesqui- e diterpenóides pode ter implicações importantes para a dinâmica populacional de Plodia interpunctella.
PALAVRAS-CHAVE: Diterpenóides. *Sticherus quadripartitus. Efeitos letais e subletais.* Lepidoptera.

1 INTRODUCTION

Synthetic insecticides have been widely developed and are extensively used because of their effectiveness; easy application and storage. Their frequent use has brought environmental disturbances, pest resistance, lethal effects on non-target organisms, and toxicity to users (Gill and Garg 2014; Rimoldi *et al.,* 2018). On the other hand, the use of environmentally friendly botanical insecticides has gained importance in recent years (Raghavendra *et al*., 2016).

Sticherus quadripartitus (Poir.) Ching (=*Gleichenia quadripartite* Moore) is a bitter-tasting fern of the family Gleicheniaceae endemic to southern Argentina and Chile (Rodríguez *et al*., 2018). Some species of Gleicheniaceae have been chemically investigated and many glycosylated labdanes and clerodanes have been described from them. These compounds display antifeedant effects on phytophagous insects (Klein Gebbinck *et al.,* 2002; Topçu and Gören 2007). A methanolic extract of *S. quadripartitus* furnished 15 diterpenoid glycosides that were structurally investigated by exhaustive use of spectroscopic methods and X-ray diffraction analysis (Socolsky *et al.,* 2007).

Many secondary metabolites from plants as terpenoids (Corzo *et al*., 2012; Sosa *et al*, 2019), steroids (Salgado Garciglia *et al*., 2008) flavonoids (Su *et al.,* 2017) acetogenins among (Ruiz Hidalgo *et al*., 2015), have shown to produce high mortality rates and sublethal effects at low concentrations on a number of insects (Ajaha *et al*., 2019).

Spodoptera frugiperda Smith (Lepidoptera: Noctuidae) is a polyphagous insect who causes, in larval stages, severe damages in maize, soybean, beans, and cotton crops in South America (Silva *et al.,* 2017). *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) is a moth well adapted to storage. The larvae are able to penetrate and infest a wide

range of packaged foods and can economically affect the production of dry fruits, nuts, almonds, pistachios, and cereal grains (Mohandass *et al.*, 2007; Borzoui *et al.,* 2016).

Lethal effects of the methanolic extract and the five major diterpenoids of *Sticherus quadripartitus* (Gleicheniaceae) were evaluated on *S. frugiperda* and *P. interpunctella* when incorporated to the insect diets at different concentrations (150 - 50 µg/ g of diet). Larval and pupal mortality rates, larval nutritional effects as well as adult malformations were registered.

2 MATERIALS AND METHODS

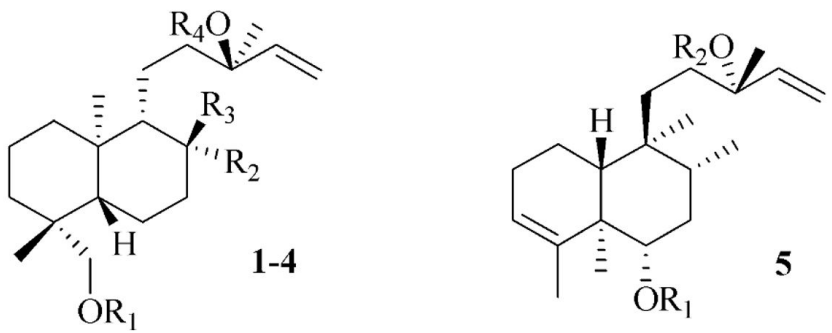
2.1 PLANT MATERIAL

Frond of *S. quadripartitus* were collected in Rio Negro, Argentina and a voucher specimen was deposited at Fundación Miguel Lillo, Tucumán, Argentina (LIL607333).

2.2 EXTRACTION AND PURIFICATION OF DITERPENOIDS

The air-dried fronds of *S. quadripartitus* were ground and extracted successively with Et_2O and MeOH. A part of the MeOH extract was evaluated on its effects when added to the *S. frugiperd*a larval diet. Diterpenoid glycosides were obtained from the MeOH extract by chromatographic procedures (Socolsky *et al*., 2007) and the major pure compounds shown in Figure 1 were also evaluated on their effects on *S. frugiperda* and *P. interpunctella.*

Figure 1: Clerodane and labdane-type diterpenoid glycosides tested for their toxicity against *S. frugiperda* and *P. interpunctella.*



1-R1=H,R2=OH,R3=CH3, R4=β-glucopyranosyl.
2-R1=H,R2=OH, R3=CH3, R4=α-rhamnopyranosyl-[1→2]-β-quinovopyranosyl.
3- R1=α-rhamnopyranosyl, R2=OH, R3=CH3, R4=α – rhamnopyranosyl.
4-R1=α-rhamnopyranosyl, R2=OH, R3=CH3, R4= α-rhamnopyranosyl-[1→2]-β-quinovopyranosyl.
5- R1=β-quinovopyranosyl, R2=β-glucopyranosyl.

2.3 INSECTS

Larvae of *S. frugiperda* were obtained from the laboratory of Estación Experimental Obispo Colombres Tucumán, Argentina. Colonies were reared under controlled conditions of temperature (26±1°C), relative humidity (60-70%), and photoperiod (L12:12D), during ten generations. Larvae fed on an artificial diet (Corzo *et al*., 2012; Ruiz Hidalgo *et al.,* 2016).

Larvae of *P. interpunctella* were originally obtained from warehouses of small and medium farmers from Antinaco-Los Colorados valley, La Rioja province, Argentina (29°10'00"S, 67°30'00"W). There were reared in our laboratory during six generations fed with chopped nuts and maintained at controlled conditions of temperature (25 ± 1°C), relative humidity (60-65%), and photoperiod (16:8 h, L:D) Corzo *et al.,* 2020; Borzoui *et al.,* 2016.

2.4 *SPODOPTERA FRUGIPERDA* MORTALITY TEST

A portion of the artificial diet was impregnated with ethanol and, after solvent removal, was employed as a control diet. Another portion was treated with an ethanolic solution of the MeOH extract, in order to leave 1.000 µg and 10.000 µg per g of diet after solvent removal. A third portion of the diet was impregnated with ethanolic solutions of pure compounds in order to leave 50 and 100 µg per g diet of each compound after solvent removal.

Portions of control and treated diets (5 g) were placed separately in test tubes (20 replicates for control and 20 for each treatment). Second stage larvae of homogeneous size were placed individually in each tube at the beginning of the experiment and kept in the tube until the emergency of adults. Test tubes were covered with a small piece of moistened cotton to prevent diet desiccation and then kept under rearing conditions (Corzo *et al*., 2012; Ruiz Hidalgo *et al*., 2016). Larval and pupal mortality, as well as adult malformations were recorded. Larvae were considered dead if they did not move when touched with a little brush for 30 seconds. Pupae were considered dead when desiccated or when the adults do not emerge after 14 days of the pupal period. An adult was considered malformed if abnormal legs and/or abdomen, and/or wings are clearly observed (Zarate *et al*., 2010).

2.5 DETERMINATION OF GROWING ALTERATIONS AND DIET CONSUMPTION OF *SPODOPTERA FRUGIPERDA* LARVAE

Portions of accurately weighed control and treated diets were placed separately in test tubes (20 replicates for control and 20 for each treatment). Second stage larvae

were accurately weighed and placed individually in each tube. Test tubes were covered with a small piece of moistened cotton to prevent diet desiccation and then kept under rearing conditions for ten days. Every addition of diet with the corresponding weight was recorded as well as the larval weight at the end of the experiment (10 days).

In order to determine the larval weight gained per day, the following equation was used:

GR = (A-B)/t

GR= Average daily weight gain during the experiment

A= final larval weight, ***B***= initial larval weight, t= 10 days

The amount of diet consumed by larvae was also calculated for the same period like

CR = D/t

CR= Average daily diet consumption during, ***D***= total weight of food consumed,

t= 10 days

Then the larvae continued to feed on treated or control diets to pupate. Bioassays were checked at 24-h intervals until mortality occurred or adult emergency (Corzo *et al*., 2012; Hidalgo *et al*., 2016; Ramirez *et al*., 2010).

2.6 BIOASSAYS ON *PLODIA INTERPUNCTELLA*

The diet of *P. interpunctella* was chopped nuts previously sterilized at -18 °C for one week, (Souza Aguiar *et al.,* 2003). A portion of the nuts was impregnated with ethanol (diet control); another three portions (50 g each) were impregnated with ethanol solutions of each pure compound in order to leave 50, 100 and 150 µg each per g diet. After impregnation of both diets (control and treated) solvent was completely removed *in vacuo*. Nutritional indexes were not determined for this species.

2.7 FEEDING TOXICITY ASSAY ON *P. INTERPUNCTELLA*

Known portions (50 g) of control and treated diets were placed in 500 mL Erlenmeyer flask and 20 second instar larvae were placed on the diet. Flasks were located in controlled conditions, until the emergence of 1st generation adults. Pupae were considered dead when observed desiccation in abdominal segments. The larval and pupal periods, the median lethal dose (LD_{50}), mortality and deformed adults were recorded. An adult was considered deformed if it was unable to shed from the pupal exuviae, or if it did not have normal wings (Zarate *et al*., 2010).

2.8 STATISTICAL ANALYSIS

Duration of the larval, pupal, and adult emergence stages was reported as Mean ± SEM. Differences in mean values were evaluated by analysis of variance (ANOVA). The Tukey test was used for all pairwise multiple comparisons of groups. The P> 0.05 was considered not significant. Probit analysis was carried out using the program MINITAB® release 14 in order to LD_{50} for, and the median lethal time (LT_{50}) only *S. frugiperda,* STATISTIX 7.1 (2000).

3 RESULTS

3.1 TOXICITY OF MEOH EXTRACT OF *S. QUADRIPARTITUS* ON *S. FRUGIPERDA*

The MeOH extract of *S. quadripartitus* added at 10.000 µg/g of diet produced an increase in the length of the larval period of 50 % in relation to control, while the treatment at 1000 µg/g caused no significant changes in the larval period. The larval mortality rises to 20 and 40% when the diet is treated with 1.000 and 10.000 µg/g respectively, while a 10 and 30% pupal mortality is observed at same concentrations of the methanol extract of *S. quadripartitus*,. The treatments produce 21.4 and 25% malformations in the adult wings at 1.000 and 10.000 µg/g (Table 1).

Table 1. Duration life cycle of *S. frugiperda* larvae fed with MeOH extract of *S. quadripartitus* added to diet. *Mean ± SD followed by different letters within a column followed by the same letter are not significantly different (*P > 0.05*, Tukey multiple range test).Lp: larval period.

Data	Control	Treated (1000 µg/g)	Treated (10000 µg/g)
Lp[d]	16.7 ± 1.1a	19.0±1.4a	25.5 ± 3.1b
Larval mortality (%)	5	20	35
Pupal mortality (%)	10	30	35
Adult malformation (%)	0	50	55

3.2 TOXICITY OF DITERPENOIDS OF *S. QUADRIPARTITUS* ON *S. FRUGIPERDA*

Pure diterpenoids incorporated into the diet at 100 µg/g do not significantly affect the larval period but larval mortalities (10 to 45%) and pupal mortalities (35 to 65 %) are detected in the treatment at the same concentration. At 100 µg/g all pure compounds produced mortality on the larval stage for *S. frugiperda*. In treated pupae by compounds **2**, **3** and **4** the major sublethal effects observed were desiccation of pupal abdominal segments that produced subsequent mortality. Adults who

survived treatment presented malformations in the wings and abdomen. At the lower concentration (50 µg/g), pupal mortality and adult malformation was reduced except for compounds **3** and **5** (Table 2).

Table 2. Toxicity diterpenoids *S. quadripartitus* on *S. frugiperda.* *Pupae mortality includes deformed pupae and normal pupae that had not emerged after 14 days; combines total mortality during larval and pupal stages.

Compounds	Larval Duration (Days)	Larval mortality 100 µg/g (%)	Pupal mortality 100 µg/g	Larval mortality 50 µg/g (%)*	Pupal mortality 50 µg/g (%)
Control	22.9±3.0	15	5	5	0
1	20.3±2.9	25	35	15	5
2	22.8±2.4	25	50	10	10
3	22.5±2.0	10	65	25	0
4	19.3±2.0	25	65	20	0
5	22.5±2.5	45	50	25	0

3.3 NUTRITIONAL INDICES

As shown in Table 3, the only significant decrease (59.6%) in the growth rate of *S. frugiperda* larvae is produced by incorporation of the MeOH extract of *S. quadripartitus* at 10,000 µg/g. Both treatments (1,000 and 10,000 µg/g) produced a significant increment in the consumption rate in relation to control of 15,6 and 13 %, respectively.

A significant decrease (66.7%) in the efficiency of conversion of the consumed diet was observed when 10,000 µg/g of extract were incorporated into the larval diet.

Table 3: Values of Nutritional indices of S. *frugiperda* fed diet added MeOH extract on *S. quadripartitus* *Mean ± SD followed by different letters within a column followed by the same letter are not significantly different (*P > 0.05*, Tukey multiple range test)*

Compounds	GR* mg/d	CR*	EDC*
Control	5.20 ± 1.92a	41.06±2.98a	0.12 ± 0.04a
MeOH extract 1,000 µg/g	4.76 ± 1.82a	47.58 ± 2.35b	0.09 ± 0.03b
MeOH extract 10,000 µg/g	2.10 ± 0.69b	46.39 ± 1.54b	0.04 ± 0.01b

3.4 TREATMENT OF PURE COMPOUNDS ADDED TO THE DIET ON *PLODIA INTERPUNCTELLA*

The compounds showed a clear dose-dependent toxicity when probed against *P. interpunctella.* The highest percentage mortality larval was produced by glycosides diterpenoids **3** and **4** at 150 µg/g. Malformed adults are generated at 100 µg/g by 2, 3 and 4 compounds. At the same concentration the compounds 2, 3, 4 and 5 produce larval mortality superior at 35%. Larval and pupal duration have no significant differences in all concentrations (Table 3).

Table 4. Alterations in life cycle in days, mortality percentage and malformations on *S. frugiperda* fed with diet added pure compounds of *S. quadripartitus* *Mean ± SD followed by different letters within a column followed by the same letter are not significantly different (*P > 0.05*, Tukey multiple range test). Duration larval (LD), Pupal duration (PD) in days, Larval mortality(LM), Pupal mortality (PM), Malformated adults (MFA).

Compounds	LD (d) 150µg/g	PD(d) 150 µg/g	(%) LM 150 µg/g	(%) PM 150 µg/g	MFA 150 µg/g	LD (d) 100 µg/g	PD (d) 100 µg/g	(%) LM 100 µg/g	(%) PM 100 µg/g	MFA 100 µg/g	LD (d) 50 µg/g	PD (d) 50 µg/g	(%) LM 50 µg/g	(%) PM 50 µg/g	MFA 50 µg/g
Control	12.4± 1.8a	11.4 ± 1.8a	10	0	0	12.8 ± 2.1a	11.4 ± 1.1a	5	5	0	13.4 ± 2.7a	9.5 ± 1.5a	10	5	0
1	10.7±1.5a	11.3 ± 2.3a	65	5	0	11.7 ± 2.2a	11.2 ± 1.9a	35	15	0	13.9 ± 2.1a	9.3 ± 1.6a	20	10	0
2	11.6±2.1a	11.6 ± 1.1a	75	0	0	12.7 ± 2.8a	10.6 ± 1.4a	40	5	9.1	-	-	-	-	-
3	12.0±1.4a	9.5 ± 0.7a	90	0	0	12.2 ± 2.6a	9.5 ± 1.7a	80	0	25	14.1 ± 3.2a	9.6 ± 1.1a	30	10	8.3
4	13.5±2.2a	10.0 ± 1.4a	90	0	0	13.4 ± 2.3a	9.8 ± 1.2a	65	0	28.6	13.6 ± 2.1a	9.3 ± 1.1a	40	5	0
5	10.8±1.8a	9.8 ± 1.3a	75	0	0	12.1 ± 1.5a	10.1 ± 1.4a	55	5	0	14.5 ± 3.0a	10.6 ± 1.8a	25	20	18.2

The values based on LD_{50}, the effects toxicity of compounds evaluated ranging from 69.3 to 123.0 µg/g obtained of *S. quadripartitus* against *P. interpunctella*, with clear overlapping between confidence intervals. The compounds **3** and **4** showed the most aggressiveness (Table 5).

Table 5. Median Lethal Concentration (LD_{50}) at 24h after *P. interpunctella* were exposed with diterpenoids incorporated in the larval diet of (n= 20). [a]CI_{95}: confidence intervals 95%, d: day.

Compounds	LD_{50} (CI_{95})[a][µg/g]
Control	-
1	*123.0 (95.2-184.0)
2	70.8 (38.8;90.0)
3	69.3 (20.6-92.5)
4	96.5 (62.7-126.6)
5	119.0 (103.5-136.8)

[a]CI_{95}: confidence intervals 95%, d: day

4 DISCUSSION

From *S. quadripartitus* were isolated and identified diterpenes, clerodanes and labdane-type. Coll and Tandron (2005) as well as Gonzalez- Coloma *et al.* (2005) demonstrated that clerodans obtained from *Teucrium nudicaule* H., exerted a strong effect on *Spodoptera littoralis,* rated by larval consumption and growth on a dry weight basis. These later authors also demonstrated that clerodans reduced biomass gains without decreasing food consumption. Results similar to those obtained in the present work (Table 1). The mortality observed in this study, was similar to that obtained by Kumari *et al*., 2003; Abbaszadeh *et al.,* 2014 and who worked with clerodane diterpenoids isolated of *Clerodendron infortunatum* on larvae of the polyphagous lepidopteran *Helicoverpa armigera*. Also, Hernandez and Gamboa-Angulo (2019) had similar results for the species *Plutella xylostella*.

In reference to the species *Plodia interpunctella*, another polyphagous insect the toxicity was caused mainly by compounds **3** and **4** in larvae and pupae (table 4). Kumari *et al*., 2003 evaluated neo-clerodane diterpenoids isolated from Clerodendron species (Verbenaceae) on *S. litura* and showed that the main effect occurred in the larval period of this species. They observed significant increases in the larval period and subsequent larval mortality. Compared with our work the larval mortality was significant for the above compounds at 100 and 150 µg/g. The use of secondary metabolites is an alternative method for the control of insects on integrated pest management programs and a good strategy

for cultures and stored products. Over 400 natural and semi-synthetic clerodanes have been examined in laboratory assays, yielding several compounds with potent antifeedant activity against various insect species**.** This is the first report on biological activity of diterpenoid glycosides from *S. quadripartitus* against *S. frugiperda* and *P. interpunctella,* key pests in walnuts in Province of La Rioja- Argentina.

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SOBRE O ORGANIZADOR

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