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Screening of Uruguayan plants for deterrent activity against insects

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Abstract

We evaluated the anti-insectan activity of extracts from different vegetative parts of ten plant species native to Uruguay. The selected plants belong to five families: Bignoniaceae: *Clytostoma callistegioides*, *Dolichandra cynanchoides*, *Macfadyena unguis-cati;* Sapindaceae: *Dodonaea viscosa*, *Allophylus edulis*, *Serjania meridionalis*; Lamiaceae: *Salvia procurrens*, *Salvia guaranitica*; Solanaceae: *Lycium cestroides*; and Phytolaccaceae: *Phytolacca dioica*. The extracts were evaluated in independent bioassays against four insect pests and one beneficial insect. Aphid settling inhibition was evaluated with a grass specialist, *Rhopalosiphum padi*, and a feeding generalist, *Myzus persicae* (both Hemiptera: Aphididae). Antifeedant activity was tested with adults of the specialist *Epilachna paenulata* (Coleoptera: Coccinellidae) and larvae of the generalist *Spodoptera littoralis* (Lepidoptera: Noctuidae). Finally, contact toxicity was assessed with honey bees, *Apis mellifera* (Hymenoptera: Apidae). Strong settling inhibition (SI) activity (expressed as % SI, where 100% means complete inhibition by the extract) was found only for the twig extracts of *A. edulis* (Sapindaceae) against *M. persicae* (% SI = 77 ± 4). Antifeedant activity (expressed as % of feeding reduction (FR), where 100% means no consumption on extract-treated diet) against *E. paenulata* was significant for the leaf extracts of *L. cestroides* (Solanaceae) (% FR = 100 ± 0) as well as of all Bignoniaceae and Sapindaceae species. No extracts were active against *S. littoralis* larvae, and most of them were innocuous to honey bees, with the exception of *L. cestroides* and *S. meridionalis* leaf extracts.

Keywords

botanicals; deterrent; Lamiaceae; Bignoniaceae; Sapindaceae; Solanaceae

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Introduction

The increasing world population has generated the need to raise yields of primary production, resulting in turn in an increased use of conventional pesticides to control pest damages in crops. The use of classical pesticides yields effective results in the short term, but it has different drawbacks, such as the development of resistance and the adverse environmental effects on the biotic and abiotic environment. The latest trend in agricultural production calls therefore for the implementation of alternatives to the use of conventional pesticides. Among other approaches to substitute conventional pesticides in Integrated Pest Management programs (pheromones, monitoring, organic production), the developing of new pesticides from natural resources such as undamaged native plants ("botanical pesticides") has been attempted in the past (Isman, 2005).

Although many plant species have been tested in their capacity as anti-insect agents (Grainge & Ahmed, 1988), most efforts have concentrated in species from families that include either the most traditionally used botanical pesticides [Meliaceae, Piperaceae, Asteraceae and Fabaceae (Isman, 2005)], or species with high contents of essential oils including Apiaceae, Lamiaceae, Myrtaceae, Lauraceae, Myristicaceae (Oliveira & Spitzer, 1999).

We have recently implemented a program intended to search for new natural products with anti-insectan activity in plants that are native to the region that includes Uruguay, southern Brazil and Argentina. As additional criteria for selecting the plants to study, we have chosen plant species from families that have not been extensively studied in regards to their anti-insectan capacity, that were readily available within the study area, and showed no obvious damage by herbivores. Here we present our results with plant extracts from ten species of the families Bignoniaceae, Sapindaceae, Lamiaceae, Solanaceae and Phytolaccaceae.

The extracts were evaluated against four pest species chosen to represent different feeding modes (chewing and sucking) and diet breadth (specialist and generalist). The four species are themselves important agricultural pests, either in conventional or organic production (Scatoni & Bentancourt, 1999). Furthermore, to assess the potential negative effect of the use of these plant extracts on the biotic environment, we performed contact toxicity bioassays against a beneficial insect (*Apis mellifera*).

Materials and Methods

Plant material and extracts

The aerial parts (fruits, leaf and twigs) of the plants under investigation (Table 1) were collected in the fall of 2005, at two riverbanks nearby Montevideo city. *D. viscosa* was collected in late spring (November of 2004) in Maldonado (south-eastern Uruguay). Species were identified by us (EAP & MJB), and Voucher specimens (numbers in Table 1) were deposited at the Herbarium of Facultad de Química, Montevideo, Uruguay. All plant material was air-dried before extraction (*S. meridionalis*, *A. edulis* twigs and *D. cynanchoides* were previously grounded). All fixed extracts were performed in Soxhlet with ethanol, then filtered and concentrated under vacuum to give a dried residue. Essential oils were only obtained for the two Lamiaceae (*S. guaranitica* and *S. procurrens*), by steam distillation in a Clevenger apparatus. Extraction yields are given in Table 1.

Insects

Epilachna paenulata Germar (Coleoptera: Coccinellidae): A laboratory colony was maintained on squash (*Cucurbita pepo* L.) under controlled conditions of temperature (20 ± 2 °C) and photophase (14L:10D). The colony was initiated with individuals collected on squash plants

in organic farms nearby Montevideo, and new field-collected individuals have been added every year (Camarano *et al.*, 2006).

Rhopalosiphum padi L. (Hemiptera: Aphididae) were reared on *Hordeum vulgare* L. foliage and maintained at 20 ± 1 °C, > 70% relative humidity, with a photoperiod of (16L:8D) in a growth chamber.

Spodoptera littoralis Boisduval (Lepidoptera: Noctuidae) and *Myzus persicae* Sulzer (Hemiptera: Aphididae) colonies were reared on an artificial diet (Poitout & Bues, 1974) and bell pepper foliage (*Capsicum annuum* L.), respectively. Both colonies were maintained at 25 ± 1 °C, > 70% relative humidity, with a photoperiod of (16L:8D) in a growth chamber.

Apis mellifera L. (Hymenoptera: Apidae) were collected from hives (Canelones, Uruguay) the same day that bioassays were run. They were kept under controlled conditions, and fed candy during the whole experiment (Ruffinengo *et al.*, 2005).

Deterrent activity

Tests against chewing insects (E. paenulata *and* S. littoralis)—Extracts were evaluated in choice-bioassays in Petri dishes (9 cm × 1 cm) lined at the bottom with a layer of agar (2%). Insects were offered four leaf discs (1 cm^2) of the appropriate host plant (*C. pepo* and *C. annuum* for *E. paenulata* and *S. littoralis*, respectively). Two of the discs (T) were coated with 100 μg of the extract (10 μL of a 10% MeOH solution), and the other two (C) were treated with 10 μL of MeOH. For *E. paenulata*, adults, which are faster feeders than larvae (unpublished results), were tested individually (10-15 replicates per extract). In the case of *S. littoralis* (5 replicates per extract), two larvae were placed in each plate (González-Coloma *et al.*, 1996).

To measure deterrent activity, a visual score of area consumed (0, 25, 50, 75 or 100%) was assigned for all discs within the plate, and a percent feeding reduction index (% FR) was determined for each replicate using the formula % FR = [1 - (treatment consumption/ control consumption)] × 100 (González-Coloma *et al.*, 1995; González-Coloma *et al.*, 1996). Since no previous reports of bioassays with *E. paenulata* were available, nicotine and rotenone (100 μg each) were tested under the same conditions for comparison purposes.

Tests against sucking insects (M. persicae *and* R. padi)—The activity against aphid settling was tested in choice experiments as described elsewhere (Gutiérrez *et al.*, 1997). The extracts were tested in plastic boxes (3 × 3 × 1,5 cm) lined at the bottom with 2% agar (20 replicates per extract). Two leaf pieces (*ca.* 1 cm^2) cut from the appropriate host plant were incrusted in the agar and treated either with the extract (100 $\mu g/cm^2$) or the same amount of solvent (MeOH). Ten aphids were placed in the box and the percentage of aphids settled on each surface was recorded after 24 hours of exposure. A settling inhibition index (% SI) was calculated for each extract as % SI = [1 − (%T / %C)] × 100, where %T and % C are the percentage of aphids settled on the treated and control leaf pieces, respectively.

Toxicity against honey bees

The toxic activity against *A. mellifera* was tested by a contact bioassay with extracts applied on Petri dishes (9 cm × 1 cm) as described elsewhere (Ruffinengo *et al.*, 2005). Dishes were swept with the extract solutions (in ethanol) to yield 100 $\mu g/cm^2$ of applied material (same concentration as in the deterrent bioassays). After the ethanol evaporated, five worker bees were placed in each dish (N= 6 replicates/extract), and the number of bees alive and knockdown (dead and non-responsive) were recorded after 24 hours. Negative and positive controls were

performed with ethanol and cypermethrin (40 ng/cm^2), respectively. The bees were fed with a candy made up of flour sugar and honey throughout the experiment.

Results

Table 2 shows the results for all the plant extracts evaluated and the five insect species used in the bioassays. For the settling inhibition bioassays, more extracts showed activity against the generalist *M. persicae* than against the specialist *R. padi*. However, inasmuch as previous work with this bioassay indicates that good anti-settling activity is achieved only with % SI greater that 70% (García *et al.*, 2007), most extracts can be considered as moderately active. Only the twig extracts from *A. edulis* showed a settling inhibition effect greater than 70% (% SI = 78 ± 2, against *M. persicae*), although it was inactive against *R. padi* (% SI = 25 ± 7). In the case of chewing species, several extracts showed significant feeding reduction against *E. paenulata*: all Bignoniaceae, all Sapindaceae with the exception of *D. viscosa* fruits, and *L. cestroides*. The feeding reduction observed for these extracts against *E. paenulata* is similar to or even greater than the reduction produced by two commonly used botanicals (nicotine, % FR = 82 ± 10; rotenone % FR = 87 ± 7). None of these extracts showed activity against *S. littoralis* larvae.

Regarding the contact toxicity against honey bees, no extract was as toxic as cypermethrin, which was tested at a dosage three orders of magnitude below that of the extracts. In fact, most extracts were as inactive as the negative control, with the exception of *L. cestroides* (Solanaceae) and one of the Sapindaceae, *S. meridionalis*, which showed moderate activity.

Discussion and conclusions

To our knowledge, with the exception of *D. viscosa* as cited by Ignacimuthu (2007), the plant species included in this study have not been previously tested in their anti-insectan capacity. Moreover, the five families that include these plants have not been extensively studied in this regard either. Some Sapindaceae species have shown antifeedant activity against coleopterans (Alonso-Amelot *et al.*, 1994; Jayasinghe *et al.*, 2003), as well as larvicidal activity against mosquitoes (Garcia Da Silva *et al.*, 2004), and lepidopterans (Boiça Jr *et al.*, 2005). In the case of Bignoniaceae, previous studies report anti-insect activity for both general extracts and specific compounds of some species. Indeed, this family is known for producing high amounts of iridoid glycosides (von Poser *et al.*, 2000) such as catalpol, ipolamiide, and specioside. These metabolites, as well as the derivatives catalposide and aucubin, have been the focus of studies concerning anti-insectan activity against lepidopterans, ants and grasshoppers (El-Naggar & Doskotch, 1980; Bernays & De Luca, 1981; Chan *et al.*, 1987; Puttick & Bowers, 1988; de la Fuente *et al.*, 1994). The species included in this study, however, belong to the Bignonieae, a tribe in which iridoids are relatively uncommon (von Poser *et al.*, 2000), suggesting that other, non-iridoidal compounds may be involved, as already reported for the family (Grace *et al.*, 1987; Varanda *et al.*, 1992). Members of the Lamiaceae are well known to present essential oils, and there have been many studies on the anti-insectan activity of these volatile secondary metabolites, particularly as repellents (Isman, 2005; Regnault-Roger, 2005; Isman, 2006). In the case of the Phytolaccaceae, to our knowledge there have been no reports on anti-insectan activity, even though extracts from members of the family have been tested (Villani & Gould, 1985; Vickerman & de Boer, 2002). For the Solanaceae, members of the family are well known for their alkaloid content, and indeed nicotine from tobacco has been regularly used in organic production despite its toxicity to mammals. Other alkaloids related to nicotine, such as anabasine have been also extensively studied (Philogène *et al.*, 2005; Regnault-Roger, 2005).

Overall, the plant extracts studied here show different activity according to the insect used, with no clear trend related to feeding style or diet breadth. In the case of sucking insects, even

though there seems to be greater activity against the generalist *M. persicae*, only the twig extracts from *A. edulis* (Sapindaceae) showed a strong settling inhibition effect. Such relatively low activity against aphids may indicate that these plant species are not aphid-resistant or that aphid settling inhibition activity may be found in more lipophilic compounds such as those located on the leaf surface, which may have not been extracted in our study. Alternatively, since host-plant selection by aphids is a complex behaviour involving several chemical and physical cues (Powell *et al.*, 2006), the stimuli needed for a plant to be rejected may become relevant at a later step in the process, therefore not detected in our bioassay. Concerning the activity against chewing insects, the extracts of several species showed a significant deterrent activity against *E. paenulata*, similar to the standard deterrents nicotine and rotenone. In the case of the two Lamiaceae species, the essential oils of *S. procurrens* and *S. guaranitica* showed no deterrent activity, and the later even stimulated consumption (Table 2). However, the ethanolic extract of *S. procurrens* was strongly deterrent, indicating antifeedant activity in non-volatile, polar components. Since *E. paenulata* is an important pest in the local organic production of cucurbits, these results may have immediate practical implications for the local control of this pest. Conversely, our feeding reduction tests clearly failed to find activity against a generalist lepidopteran larva (*S. littoralis*). Insect deterrents act at the level of gustatory receptors, causing the insect to reject a food source after direct contact (Mansson & Schlyter, 2004). Here the differential deterrent effect against two chewing insects may be explained by the different diet breadth of these two herbivores. As a generalist, *S. littoralis* is able to ingest a variety of plant foods, and it is therefore expected to exhibit greater tolerance or even resistance toward plant chemical defenses. This trait, however, would not be expected for specialists such as *E. paenulata*, which has only been selected to withstand secondary metabolites from its food plants (Cucurbitaceae) (Bernays, 1999). On the other hand, being a specialist, *E. paenulata* may be prompted to select its food based on the presence of secondary metabolites predictably found in Cucurbitaceae, and it is therefore possible that the plant compounds applied to the *C. pepo* leaf discs hindered these positive cues.

In general, the plant extracts tested showed no toxicity to honey bees, with the exception of the leaf extracts of *S. meridionalis* and *L. cestroides*, which showed some toxicity (less than cypermethrin). Incidentally, *S. meridionalis* and *L. cestroides* showed also good activity against aphids and *E. paenulata*, which may suggest that the anti-insectan effects of plant extracts are non-discrimatory. However, our results also show that extracts that are active against some pests (for instance *C. callistegioides*) can be inactive against a beneficial insect, and are hence more promissory for the development of botanical pesticides.

As a final remark, it is worth noticing that two out of three inactive extracts against chewing insects (*P. dioica* and *D. viscosa* fruits) showed anti-settling activity against both aphid species, reinforcing the notion that a variety of target insects is important when evaluating the activity of plant extracts (Cole, 1994; Pascual-Villalobos & Robledo, 1998).

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Table 1

Plant species and parts evaluated in their anti-insectan activity, and extraction yields as percent of plant dry weight.*

Family	Species (Voucher numbers)	Organ extracted	Yield	
			EE (%)	EO (%)
Lamiaceae	*Salvia guaranitica* St.Hilaire ex Benth. (4320 MVFQ)	leaves	no	1.0
	Salvia procurrens Benth. (4319 MVFQ)	leaves	7.8	NA
Bignoniaceae	*Clytostoma callistegioides* Bureau ex Griseb. (4311 MVFQ)	leaves	5.1	-
	Dolichandra cynanchoides Cham. (4318 MVFQ)	leaves	11.6	-
	Macfadyena unguis-cati (L.) A.H.Gentry (4312 MVFQ)	leaves	8.8	-
Phytolacaceae	*Phytolacca dioica* L. (4313 MVFQ)	fruits	12.9	-
Sapindaceae	*Allophylus edulis* (A. St.-Hil.) Radlk. ex Warm. (4316 MVFQ)	leaves	10.3	-
		twigs	9.4	-
	Dodonaea viscosa (L.) Jacq. (4314 MVFQ)	leaves	19.6	-
		twigs	3.4	-
		fruits	20.5	-
	Serjania meridionales Camb. (4315 MVFQ)	leaves	16.5	-
Solanaceae	*Lycium cestroides* Schldl. (4317 MVFQ)	leaves	11.7	-

* EE = ethanolic extract, EO = essential oil, NA = yield not available, - = not obtained

Table 2

Bioassay results for the different anti-insectan activities evaluated in the plant extracts (settling inhibition, feeding reduction and contact toxicity). Results are shown as mean ± standard error.

Plant extract[1]	Insect species								
	M. persicae[2]			*R. padi*[2]			*S. littoralis*[3]	*E. paenulata*[3]	*A mellifera*[4]
	% T	% C	%SI	% T	% C	%SI	% FR	% FR	Knock-down bees
S. guaranitica (EO, L)	nt			nt			22 ± 23	-50 ± 22‡	nt
S. procurrens (EO, L)	nt			nt			17 ± 10	15 ± 19	nt
S. procurrens (EE, L)	28 ± 4	72 ± 4*	51 ± 10	40 ± 5	60 ± 5	-35 ± 54	11 ± 15	80 ± 20*	0,20 ± 0,20[a] ◆
C. callistegioides (EE, L)	36 ± 5	64 ± 5*	-0,6 ± 39	40 ± 4	60 ± 4*	25 ± 10	21 ± 19	100 ± 0*	0,33 ± 0,21[a]
D. cynanchoides (EE, L)	41 ± 4	59 ± 4*	11 ± 20	44 ± 3	56 ± 3	11 ± 11	19 ± 18	60 ± 19*	0,33 ± 0,33[a]
M. unguis cati (EE, L)	41 ± 3	59 ± 3*	22 ± 9	43 ± 4	57 ± 4	13 ± 15	7 ± 7	75 ± 14*	0,0 ± 0,0[a]
P. dioica (EE, F)	35 ± 13	65 ± 13*	33 ± 10	36 ± 3	64 ± 3*	32 ± 12	35 ± 18	10 ± 28	0,33 ± 0,21[a]
A edulis (EE, L)	48 ± 4	52 ± 4	-14 ± 19	42 ± 3	58 ± 3	16 ± 11	5 ± 5	90 ± 6*	0,50 ± 0,34 [a,b]
A edulis (EE, T)	17 ± 2	83 ± 2*	77 ± 4	49 ± 5	51 ± 5	-27 ± 25	18 ± 19	60 ± 20*	0,17 ± 0,17[a]
D. viscosa (EE, F)	42 ± 3	58 ± 3*	19 ± 9	38 ± 3	62 ± 3*	29 ± 10	3 ± 3	6 ± 22	0,17 ± 0,17[a]
D. viscosa (EE, L)	41 ± 5	59 ± 5	4 ± 21	40 ± 4	60 ± 4*	15 ± 14	43 ± 16	78 ± 14*	0,50 ± 0,34[a,b]
D. viscosa (EE, T)	38 ± 3	62 ± 3*	30 ± 9	46 ± 4	54 ± 4	-4 ± 14	16 ± 8	79 ± 14*	0,0 ± 0,0[a]
S. meridionalis (EE, L)	26 ± 4	74 ± 4*	54 ± 10	45 ± 4	55 ± 4	-3 ± 17	17± 11	100 ± 0*	2,33 ± 0,61[c]
L cestroides (EE, L)	27 ± 3	73 ± 3	59 ± 6	27 ± 4	73 ± 4*	52 ± 11	30 ± 20	100 ± 0*	2,00 ± 0,68[b,c]

[1] See Table 1 for details.

[2] Settling inhibition in percentage (SI) = [1-%T/%C] × 100; aphids settled on treated leaves (%T) or control (%C) leaves (n=20 replicates).

[3] Feeding reduction in percentage (FR) = [1-(T/C)] × 100; consumption of treated (T) and control (C) discs; n=10-15 for *E. paenulata* and n=5 for *S.littoralis*.

[4] Knock-down refers to the absolute number of bees dead or non-responsive after 24 hours (out of five bees per replicate, n=6 replicates). nt, not tested.

‡ denotes a significant phagostimulant effect ($p < 0.05$, Wilcoxon Rank Test).

* denotes significant differences between C and T (deterrent, $p < 0.05$, Wilcoxon Rank Test).

◆ different letters indicate significant differences among treatments (extracts and controls) in post-ANOVA pair wise comparisons ($p < 0.05$, Tukey's error rate). Negative control = ($0,17 \pm 0,17^{a}$); positive control ($4,83 \pm 0,17^{d}$).