Antifeedant and Insecticidal Activity of Endemic Canarian Lauraceae

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Although botanical insecticides have been used for hundreds of years to protect crops against insects, they have been almost completely replaced over the past 50 years by cheaper and more effective synthetic compounds. However, an increased awareness of the problems associated with these chemicals such as cross resistance, public health risks and damage to the environment, has prompted a number of groups to promote the investigation of plants for the presence of natural products with insect-control properties. In addition, many botanical pesticides have the advantage of providing novel modes of action that can reduce the risk of cross resistance (BERENBAUM, 1989), as well as offer new leads for the design of target-specific molecules. This resurgence of interest in plant-derived insecticides has focused primarily on tropical floras, but non-tropical native plants have also developed defense systems in response to herbivore...
pressure (Bergerbaum, 1989) and have the advantage of being locally available. This consideration is particularly important when dealing with island ecosystems.

The islands of Macaronesia (the Canary Islands, Madeira and the Azores) are a well-preserved example of the flora that was present in the Tethyan region during the first half of the Tertiary period (Bramwell, 1976). The Canarian Laurel forest, a climactic ecosystem, is a unique plant community whose dominant Lauraceae evergreen tree species are: *Apollonia barbushana*, *Laurus azorica*, *Persea indica* and *Ocotea foetens*. The potential chemical richness of this forest however, remains largely unexplored. *Persea indica* has been recently reported as a potential source of natural pest-control agents (González-Coloma et al., 1992) due to its content in ryanoid diterpenes, which are insecticides acting on a non-neuronal target (Ishigai et al., 1977; Pessah, 1989; González-Coloma et al., 1990).

This finding led us to a more detailed exploration of the potential of the Canarian Lauraceae species as a source of natural antifeedants and insecticides. In the present study the four above mentioned plant species were tested for their antifeedant activity against *Spodoptera litura* larvae, a polyphagous crop pest, and *Reticulitermes speratus*, a termite pest; for growth inhibition against *S. litura* larvae; for contact toxicity against adults of *Drosophila melanogaster* and for larvicidal activity against *Aedes aegypti*.

**MATERIALS AND METHODS**

*Plant material and extracts.* Oven-dried (50°C, 48 h) terminal branches (avg. 20 cm) of the plant samples were ground in a Wiley mill to pass a 2 mm sieve and extracted with boiling ethanol in a Soxhlet. The list of plant species, extraction yield and collection sites is given in Table 1.

**Bioassays.** Five different bioassays were performed with each plant extract.

1) *Spodoptera litura* leaf-disk choice bioassay: This bioassay was used to determine the feeding deterrence of the plant extracts to *S. litura* larvae (3rd instar). The insects came from a laboratory colony and the bioassay was performed as described by Escoubas et al. (1993). According to the literature, a feeding ratio (FR = % of treated disks consumed) of control disks consumed) was calculated for each treatment and an arbitrary level (FR < 0.25) was used as the criterion to determine very effective deterrents (Mikolajczack and Reed, 1987).

2) *Spodoptera litura* diet incorporation bioassay: To test for chronic growth inhibition, we incorporated the plant extracts into a commercial artificial diet (Insecta LF, Nihon Nosan Kogyo Co.) (0.1% w/w) using cellulose as an inert carrier (10% dry wt.). Twenty neonate larvae were fed on the treated and control diets at 27°C in the darkness for seven days, and then their weights were recorded. Three controls were run for each experiment: solvent plus cellulose, cellulose alone and no cellulose. Differences in live larval weights between treatment and control were analyzed by the non-parametric Mann-Whitney U-test.

3) Termite choice and no-choice filter paper disk bioassay: These bioassays were used to determine deterrence and toxicity of the plant extracts to *Reticulitermes speratus* workers. Termites were collected from infested pine logs near Kagoshima city, Kyushu (Japan) and only workers older than the third instar were used. Feeding substrates were 2 cm diameter Whatman No. 1 filter paper disks. Each assay consisted of a 55 mm diameter plastic Petri dish coated with agar (15%) covered with sand (Nacalai Tesque Sea Sand C), one (no-choice) or two (choice) paper disks treated with 25 μl of plant extract (1%) or solvent (control) on top of an aluminum foil disk, and 30 *R. speratus* workers in three replicates.

The dishes were incubated at 27°C for 14 days in darkness. Termite mortality was then recorded and the remaining surface of the paper disks was measured as described by Escoubas et al. (1993). The feeding ratio indexes were calculated as described above for the *S. litura* choice bioassay. The differences in consumption between treated and
control paper disks were evaluated by the Chi-square Goodness of Fit test.

4) Mosquito larvae toxicity bioassay: Each assay consisted of ten second-instar Aedes aegypti larvae, from a laboratory colony, placed in a tissue-culture well containing 10 ml of distilled water, 100 µl of plant extract (1.0%) in acetone and 5 µl of dimethyl sulfoxide (DMSO). Control batches received only acetone and DMSO. Two replicates per treatment were used and the larval mortality was recorded after 24 h. The mortality values were corrected according to Abbott's formula (Abbott, 1925).

5) Drosophila toxicity bioassay: Each assay consisted of 10 Drosophila melanogaster adults, from a laboratory colony, placed in a 3 cm diameter glass Petri dish coated with 100 µl of plant extract (1.0%) or solvent alone for control, and a cotton plug with 50 µl of a 10% sucrose solution. Two replicates per treatment were used and the mortality was recorded after 24 h. The mortality values were corrected as described above.

RESULTS AND DISCUSSION

The plant extracts tested exhibited different degrees of antifeedant and toxic activities depending on the target species. *P. indica* strongly deterred feeding by S. littoralis larvae in the leaf-disk bioassay, followed by *O. foetens*, which we did not consider a strong feeding deterrent, with an FR value over 0.25 (Table 2). In the diet-incorporation bioassay, *A. barbriana* showed statistically significant growth inhibition activity against this insect (32% growth reduction in treated larvae) followed by *P. indica* (26.5% growth reduction) (Fig. 1).

When tested for anti-termite activity, *O. foetens* and *L. azorica* strongly deterred feeding in the choice test (Table 3). In the no-choice experiment, *P. indica* significantly reduced the feeding activity.

Table 2. Feeding ratios (FR) for 1% crude ethanolic extracts tested in choice leaf-disk tests against *S. littoralis* larvae

<table>
<thead>
<tr>
<th>Plant species</th>
<th>FR*</th>
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<tbody>
<tr>
<td>A. barbriana</td>
<td>0.91 (0.10)</td>
<td></td>
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<tr>
<td>L. azorica</td>
<td>0.74 (0.15)</td>
<td></td>
</tr>
<tr>
<td>O. foetens</td>
<td>0.43 (0.14)</td>
<td></td>
</tr>
<tr>
<td>P. indica</td>
<td>0.00 (0.05)</td>
<td></td>
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</tbody>
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* Average FR (standard deviation) of three replicates, N=15.

Table 3. Feeding ratios (FR) and surface eaten for 1% extracts tested in choice and no-choice paper disk tests against *R. speratus*

<table>
<thead>
<tr>
<th>Plant species</th>
<th>FR choice</th>
<th>% Eaten no-choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. barbriana</td>
<td>0.40 (0.26)*</td>
<td>54.20 (9.32)*</td>
</tr>
<tr>
<td>L. azorica</td>
<td>0.04 (0.02)</td>
<td>53.31 (16.45)</td>
</tr>
<tr>
<td>O. foetens</td>
<td>0.05 (0.02)</td>
<td>42.45 (0.60)</td>
</tr>
<tr>
<td>P. indica</td>
<td>0.61 (0.45)</td>
<td>19.69 (4.16)*</td>
</tr>
<tr>
<td>Control</td>
<td>0.48 (0.12)</td>
<td>50.61 (4.29)</td>
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* Average FR and (STD) of three replicates (N=90).

Table 4. Mosquito larvae and fly mortality caused by the different plant extracts assayed

<table>
<thead>
<tr>
<th>Plant species</th>
<th>A. aegypti mortality (%)</th>
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<tbody>
<tr>
<td>A. barbriana</td>
<td>0</td>
<td>5.0 (3.53)</td>
</tr>
<tr>
<td>L. azorica</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>O. foetens</td>
<td>0</td>
<td>5.0 (3.53)</td>
</tr>
<tr>
<td>P. indica</td>
<td>5.0 (3.53)</td>
<td>55.5 (3.9)</td>
</tr>
</tbody>
</table>

* Mortality values (STD) corrected according to Abbott.
activity of the termites when compared to the control (Table 3).

None of the plant extracts exhibited any significant mosquito larvicidal activity, and only *P. indica* killed some of the *Drosophila* flies in the contact toxicity test (55.5% mortality, Table 4).

Among the results described above, the growth-inhibition and the antifeedant plus growth-inhibition activities of *A. barbuse* and *P. indica* respectively against *S. litura* along with the antifeedant activities of *O. foetens* and *L. azorica* against *R. speratus* were strong enough for these plants to be considered as potential sources of natural pesticides. Although the fly mortality caused by *P. indica* was somewhat interesting, we did not consider it to be strong enough to warrant future bioassay-guided chemical investigation.

This is the first time that a biological activity has been described for *A. barbuse*, and the chemicals responsible for such action remain unknown. On the contrary, *P. indica* has been previously described as having growth inhibition effects against a specialist (*Macaronesia fortunata*) and a generalist (*Heliothis armigera*) Lepidoptera (GONZÁLEZ-COLOMA et al., 1992), and these activities have been attributed to the presence of the insecticidal ryanoids ryanodol and cinnecyanol (WATERHOUSE et al., 1987; GONZÁLEZ-COLOMA et al., 1990) in the plant extract but this plant has never been described as being a potent antifeedant.

In a no-choice situation, *S. litura* was able to overcome the repellency caused by *P. indica*, feeding on the treated diet and subsequently showing reduced fitness. This observation could be attributed to the presence of two classes of compounds, or to the dual activity of the same compounds. This would represent a primary defense mechanism preventing the insect from feeding, and a secondary mechanism functioning as a stomach poison following ingestion. This type of defense would be acting on both the sensory and physiological levels to reduce insect herbivory. The growth inhibition observed could also be explained by detoxification of the plant defense compounds by the insect, diverting energy from growth.

A similar scenario has been previously described for *S. littoralis* whose larvae, after having emerged from egg clusters on *P. americana* leaves, died within 48 h, while those transferred to the plant in their third instar were able to feed and develop (SHEEH and GROSS, 1981) probably because of their ability to detoxify the plant toxins. The genus *Persea* seems to be a good source of a variety of biologically active compounds, for example the toxicity of *P. americana* against several other insect species has been attributed to its high content in estragole (STEIN and KLINGEB, 1990) and the bark of *P. major* contained cytotoxic and anti-tumor δ-lactones (MA et al., 1990).

The timber from the genus *Oostea* has been previously described as being resistant to termites. Among the resistant species are *O. ovifolia*, *O. rodaiae* and *O. acetangula* (JACOBSON, 1975), all of them native to Central and South America, but there is no reference to the active compounds involved.

*L. azorica* also showed effective anti-termite activity. This is the first time that such activity has been described for this plant species or genus. Within this genus, comprised of *L. azorica* and *L. nobilis*, the bay laurel has been previously described as an anti-parasite (JACOBSON, 1975) and its essential oil as being effective as a fumigant against the coleoptera *Rhizophytha dominica* (SHAAYA et al. 1991); but the composition of the essential oils of both species is rather different (HOKWERDA et al., 1982). Again, further investigation is needed in order to isolate the active components of *L. azorica*.

Interestingly, when forced to feed, the termites were susceptible to the toxins present in *P. indica*, but in the choice test this plant did not show significant feeding deterreny against this insect, as opposed to *S. litura*. This result indicates that different taste receptors may be present in these insect species, and that any study targeting natural antifeedants should be conducted with a number of different test species.

In conclusion, this study reveals the existence of significant anti-insect activities in the Canarian Lauraceae. Among them we should emphasize the strong antifeedant activity of *P. indica* against *S. litura* and the promising anti-termite activities of *O. foetens* and *L. azorica*. We believe that this information will be very useful in the future search for natural products with pesticidal activities.

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REFERENCES


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**Diapause Response in *Samia cynthia***

**Subspecies and Their Hybrids (Lepidoptera: Saturniidae)**

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*Samia cynthia* DRURY is a giant silkmoth with several domesticated subspecies. *Samia cynthia ricini* DONOVAN has been commercially reared for many centuries in India. *Samia cynthia pyreri* BUTLER is widely distributed in Japan. It has been generally known that *S. c. ricini* is a non-diapausing saturniid silkmoth species, whereas the *S. c. pyreri* undergoes diapause in its pupal stage. It has been reported that the F1 hybrids between *S. c. ricini* and *S. c. pyreri* do not undergo diapause in any stage of development under natural photoperiodic conditions (Kawaguchi, 1946; Yamaguchi, 1949; Kawai, 1957). However, diapause characteristics, such as the length of critical photoperiods, have not been clarified.

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