A bioassay for insect deterrent compounds found in plant and animal tissues.

Lee A. Dyer*1,2 Craig D. Dodson2 and Grant Gentry2

*Author to whom correspondence should be addressed.

1Department of Ecology and Evolutionary Biology

Tulane University

New Orleans, LA 70118

2Western Colorado Center For Tropical Research

Department of Physical and Environmental Sciences

Mesa State College

Grand Junction, CO 81501
Abstract

Despite widespread interest among ecologists in plant chemical defenses against insects and the current focus on bioprospecting, there is a dearth of broad field bioassays that can be used to guide efforts to locate insect deterrent natural products found in tropical plants and insects. Here we develop and test the efficacy and sensitivity of a general field bioassay for detecting biologically active compounds in plants and insects. We offered methanolic extracts of 20 plant, and 6 caterpillar species in sucrose solutions to the ponerine ant Paraponera clavata, in order to observe their feeding preferences. The bioassay resulted in 9 plant species and 4 caterpillar species with ant-deterrent extracts, and 11 plant and 3 caterpillar species with neutral or attractant extracts. All plants with ant-deterrent extracts that were chemically investigated in our laboratories or for which chemical literature was available contained secondary metabolites of known deterrence. Both naturally occurring and artificial differences in chemical concentrations can be detected by the bioassay. It provides a means of screening plants and insects for compounds that are insect antifeedants or insect behavior modifiers.
Key words
Bioassay, insect antifeedants, plant secondary metabolites, ants, tropics

Introduction
Tropical plants and animals are acknowledged sources of useful compounds, yet less than one percent of tropical plant species have been chemically investigated (Balick et al. 1995) and an even smaller percentage of tropical insect species have been examined for chemical defenses (Trigo 2000). Widespread interest in the chemistry that mediates many ecological interactions produces a demand for a simple, standard bioassay that can be used to guide efforts to locate and survey natural products found in tropical plants and insects (Trigo 2000). One common bioassay method for assessing deterrence of specific toxins is to offer sugar solutions laced with animal or plant extracts to predaceous ants (e.g., Eisner & Meinwald 1965, Carrel and Eisner 1974, Montllor et al. 1991, Hare & Eisner 1993, Schaffner et al. 1994, Morton & Vencl 1998, Gomez et al. 1999, Vencl et al. 1999). This method has been expanded to discover novel compounds that are deterrent to leaf cutting ants (Wiemer & Ales 1981, Hubert & Wiemer
1985, Chen et al. 1992, Green et al. 1999), but it has not been utilized for more general searches of compounds in plants and animals that are deterrent to omnivorous arthropods.

The primary goal of this study was to develop and test a general field bioassay for detecting compounds in plant and insect tissues that are deterrent to insects in general, or at least some species of insects, using the omnivorous ant, Paraponera clavata (Formicidae: Ponerinae). We examined the relative deterrence of extracts from 20 species of plants and 6 species of Lepidoptera offered to this ant species in sugar solutions. Several species of plants that were identified as deterrent were further examined in the laboratory for the presence of known or novel secondary compounds. Three of these isolated secondary compounds were then directly tested for their repellent qualities in sugar solutions offered to the ants.

**Methods**

*Study site and system*

The research was conducted in secondary forest at the La Selva Biological Station, Costa Rica, from 1998-2001 in both the wet and dry seasons. The station is located at 10°25’N, 84°05’W on the Caribbean
slope of Costa Rica and is described in detail elsewhere (Sanford et al. 1994). The bioassay ant, P. clavata, is generally omnivorous, but at La Selva and at Barro Colorado Island, Panama, it feeds primarily on nectar (Dyer 2002), but it is also considered a scavenger, a nectivore, a generalist predator, and perhaps even an herbivore (Janzen and Carroll 1983, Young and Hermann 1980, Dyer 1995). Nests are usually found at the bases of trees and the ants utilize trails on the tree trunk above the nest. The ants release pheromones for mass recruitment, graded recruitment, and general foraging in the canopy and understory surrounding their nests (Breed and Bennet 1985, Breed et al. 1987).

**Bioassay procedures**

From January 1998 to July 2001 in rainy season months (June-August, October-December), we offered extracts in sugar water to P. clavata colonies at La Selva. We prepared extracts of 20 plant species in 9 families, 6 species of Lepidoptera in 4 families, a sample of tobacco (Nicotiana tabacum Solanaceae) as a toxicity reference, and a mixture of three synthetic compounds. The plants used for the bioassay were identified with help from the La Selva botanist (O. Vargas), and vouchers were prepared and deposited at Mesa State College,
Colorado. The caterpillars are also well-known by the authors, and adult vouchers were deposited at Tulane University, Louisiana. For all plants, leaves (no other plant parts were used) were completely dried at approximately 25°C in an air-conditioned laboratory; the tobacco was obtained from Dos Pinos brand cigarettes. A 15 g (dry weight) sample of leaf material of each species of plant was extracted overnight in 250 ml methanol. While not all compounds are soluble in methanol, it is a good general solvent that will extract a wide range of both polar and non-polar compounds and is also palatable to Hymenoptera when small volumes are presented in a sucrose solution (using a t-test to compare consumption of sugar water with 1% methanol versus control, \( T = 1.4, \) \( \text{DF} = 141, \) \( P = 0.18 \)). The bioassay we describe is also amenable to the use of other solvents (Dyer 1995). For the caterpillar extracts, newly molted, fifth instar caterpillars were starved for 24 hours (to ensure the absence of plant material in their guts), freeze-dried, ground, and extracted overnight in methanol (60 mg/ml). These extracts were presented to ants in sugar feeders, which were 2.5 ml microcentrifuge tubes containing sugar water and extract or sugar water and solvent. Extracts were diluted in a 20% sucrose solution (0.1 ml extract in 2.25 ml solution), while control tubes contained only the sugar water solution.
and 0.1 ml methanol (the extracting solvent). All water used in the procedures described in this paper was nanopure deionized water and the sucrose was table sugar purchased in Puerto Viejo, Costa Rica.

A bioassay trial consisted of simultaneously offering sugar solutions containing extracts and controls to all colonies of P. clavata. Fifteen colonies were used along a 1 km transect, and each colony was at least 25 m from another. For each species tested, at least 5 trials were conducted at each colony (usually within 1-2 days), using the same extract but new sugar solutions. The vials were attached via paper clips to the tree trunk containing the main foraging trail of each colony and were approximately 1.5 m and 2 m from the nest entrance. Controls and experimentals were randomly placed in the upper versus lower position. The tubes were weighed (at 0.01 g sensitivity), given to the ants for one hour and then weighed again. All trials were conducted between 7AM and 3PM. In previous trials (Dyer and Floyd 1993, Dyer 1995, Dyer unpublished data), the amount of sugar water consumed by ants was unaffected by position (1.5 m versus 2 m, T = 0.02, DF = 2418, P = 0.98) or time of day (morning versus afternoon, T = 1.0, DF = 1867, P = 0.3), nor was there evaporation from vials placed on trees.
without foraging ants for one hour (N = 400 vials, mean mass lost = 0.0 mg).

The mass of liquid taken from each tube was determined (mass before minus mass after) and an adjusted consumption difference (ACD) calculated (consumed control - consumed extract) / (consumed control + consumed extract) for each species as per Dyer (1995). This variable ranges from -1 to 1, with values from 0 to 1 indicating a more unpalatable extract (because the ants are consuming more control than extract) and values from 0 to -1 indicating a more palatable extract (because the ants are consuming more extract than control). For each species or extract type, an average ACD value was determined for each colony. One tailed t-tests were used to determine if ACD values were significantly greater than zero. The sample size for these tests was always 15 (based on the number of ant colonies, not on the number of extracts made) for each species or extract type. A Bonferoni correction was applied to correct inflated type II error introduced by multiple tests (20 plant and 6 caterpillar species); thus ? was 0.0025 for plants and 0.007 for caterpillars. Species were categorized into different palatability categories based on their mean ACD values; species with values significantly greater than 0 were deterrent, all others were
neutral or attractant. Because the bioassay was developed to detect deterrent compounds, no statistical differentiations were made between neutral or attractant extracts.

Activity of known compounds

**Piper** is one of the most common genera of understory plants at La Selva (Dyer pers. obs.), and a large number of **Piper** species worldwide have been investigated for natural products (Parmar et al. 1997). As of 1997, 592 individual structures from every major group of secondary metabolites had been isolated from this genus. At least four species at La Selva contain amides of a type characteristic of the genus, and thus referred to as **Piper** amides. A total of 16 **Piper** amides have been demonstrated to be insecticidal or to have insect anti-feedant qualities (Parmar et al. 1997). We tested the bioassay by examining three **Piper** amides found in the ant plant, *P. cenocladium*: piplartine, 4'-desmethylpiplartine, and cenocladamide (Dodson et al. 2000). Plants with high levels of these amides experience low levels of herbivory (Dyer and Letourneau 1999), and they negatively affect development of various generalist herbivores (Dyer unpublished data). Piplartine, demonstrated potent cytotoxic activity in vitro (Duh et al.
1990), and two simple derivatives of piplartine, 12-desmethoxypiplartine
and its 3,4-epoxy derivative piplaroxide, are known to be repellent to
the leafcutting ant, *Atta cephalotes* (Capron and Wiemer 1996).

The procedures for testing the bioassay by using amides were
the same as for plant material (above) with synthetic amides used in
place of plant extract. Amides were synthesized at Mesa State College
(Richards et al. 2001). A mix of piplartine (0.44% dry weight of the
sugar solution), 4′-desmethylpiplartine (0.36%), and cenocladamide
(0.26%) was tested. The amide concentrations used in these solutions
were adjusted to mimic those that are normal for *P. cenocladum* plants
at La Selva (Dodson et al. 2000, Dyer et al. 2001). In addition to the
tests of synthetic amides, plants with natural differences in amide
concentration were extracted and tested. Dodson et al. (2000) and Dyer
et al. (2001) found that concentrations of amides in *P. cenocladum*
were significantly different between fragments without mutualistic ants
(highest: x = 1.74%), fragments with ants (intermediate: x = 1.40%), and
shrubs with ants (lowest: x = 0.41%). We tested the sensitivity of the
bioassay to naturally occurring amide concentrations in *P. cenocladum*
by assaying the plant material from these different plant/insect
combinations.
Sensitivity of the assay

We altered the sensitivity of the assay by adjusting the concentration of the sucrose solution. We used a caterpillar species, Hypothyris euclea (Nymphalidae: Ithomiinae), that we had previously found to be deterrent and put it through the standard bioassay using three different concentrations of sucrose: 10%, 20%, and 30%. The low concentration was tested to create a more sensitive assay, and the high concentration was used to create a less sensitive assay (to identify only very deterrent extracts). Untransformed ACD values for the three groups were compared with Analysis of Variance (ANOVA) followed by Tukey’s multiple comparisons. The ACD values met normality assumptions of ANOVA (Anderson Darling $A^2 = 0.6$, $P = 0.1$).

Analysis of plants for secondary metabolites

A chemical literature search was performed on all plant species listed in Table 1 in order to determine which species had been phytochemically investigated, which had not, and what secondary metabolites had been found in those species or at least in that genus.
Four of the six *Solanum/Lycianthes* species, *Cecropia obtusifolia* and *Lonchocarpus oliganthus* were all screened for the presence/absence of alkaloids in the following manner. The leaves were dried at approximately 25°C in an air-conditioned laboratory. Five gram samples of the dried leaves were ground to a fine powder in a coffee grinder and wetted with saturated aqueous sodium bicarbonate to ensure that the alkaloids would be in the organic soluble free base form. This material was extracted twice with 95% aqueous ethanol, overnight, at room temperature, with stirring. The solvent of the combined extracts was removed *in vacuo* and the residue was redissolved in 0.01 M sulfuric acid with heating on a steam bath. The acidic layer was extracted with three portions of chloroform and then made basic to pH 10 with sodium hydroxide. The free bases were extracted from the basic aqueous layer with three portions of chloroform and the solvent from the combined chloroform layers was removed *in vacuo*. The aqueous layer was then made acidic to pH 2 with concentrated sulfuric acid and stirred over powdered zinc for 2 hours to reduce to free bases any N-oxides that may have been present. The alkalinity was readjusted to pH 10 again, the now reduced N-oxides were extracted into chloroform, and the solvent was removed. The
alkaloid and reduced N-oxide residues were dissolved in minimal chloroform and spotted, entirely, on a silica gel TLC plate that was developed in 1:1 chloroform/methanol. Developed plates were visualized using short wave UV absorbance, long wave UV fluorescence, and an iodoplatinic acid alkaloid-specific spray reagent. A positive spray test can be obtained on a minimum of 60 micrograms of most alkaloids, thus a positive result is expected for any alkaloid present at a concentration greater than 12 ppm. Only S. adherens was alkaloid positive. A 20 g sample of this plant was treated, as above, and a combination of GC/MS and NMR revealed the presence of the known compound solapalmatine, and its congener with one less methylene group in the alkyl chain (Kupchan et al. 1969).

Piper cenocladum, P. imperiale and P. melanocladum were fully investigated in our laboratory. Piper amides and prenylated benzoic acid derivatives were isolated and identified using methods described in Dodson et al. (2000).

Results

The bioassay resulted in 9 plant and 4 animal species with ant-deterrent extracts, and 11 plant and 3 animal species with neutral or
attractive extracts (Tables 1 and 2). The mixture of synthetic *Piper* amides previously found in *P. cenocladium* was also deterrent (Table 1). The results for 8 of the 9 deterrent plant species can be explained by the known chemistry of those species, either from work in our laboratories, or from the phytochemical literature for that species or a closely related species in that genus (Table 1). The deterrence of *Neea psychotrioides* remains unexplained due to a complete lack of chemical data for this species as well as the entire genus.

Reporting the neutral/attractant results is more difficult since a lack of data in the chemical literature does not indicate that the plant is devoid of secondary metabolites – it may also be due to the fact that it simply has not been investigated. Our chemical investigations of some of these plants did not yield any deterrent compounds. We screened three of the 11 neutral plants for alkaloids, with negative results, and the compounds we isolated from *P. melanocladium* are known compounds and were shown to not be deterrent to Attine ants (Ampofo et al. 1987). Secondary metabolites reported in the literature to be present in the other neutral/attractant plants exhibit a variety of bioactivities but none are known to be insecticidal (Table 1).
For *Hypothyris euclea* extracts, the solutions with 10% and 20% sucrose were deterrent but those with 30% sucrose were not (Table 2). All three solutions had significantly different ACD values ($F_{[2,159]} = 29.0$, $P < 0.0001$). All Tukey’s pairwise comparisons were significant, ($P < 0.05$), with the most sensitive assay (10%) yielding the highest ACD value, and the least sensitive (30%) yielding the lowest value (Table 2). The 30% sucrose solutions were such a valuable resource for the ants that even a very deterrent extract could not keep them from feeding, while the 10% sucrose solution was avoided almost completely when the same extract was used, thus the 20% sucrose solution appears best for general use.

Differences in relative concentrations of deterrent *Piper* amides found in the three *P. cenocladum* shrub/fragment/ant/no-ant combinations were readily detected by the bioassay. The ACD values reflected the total concentration of amides in plants (Table 1) with the highest total amide concentration (1.74%) being the most deterrent and the lowest concentration (0.41%) being the least deterrent. The mixture of synthetic amides had a total concentration roughly equal to that of the plant with an intermediate concentration of amides and its ACD was also roughly the same as that of the plant extract. This is a good
indication that the deterrent compounds in the crude extracts are indeed the isolated and later synthesized amides.

**Discussion**

The bioassay proved to be efficient and practical. The entire process takes about 10 hours per species tested, and only requires access to methanol, vials, any species of predatory ant, and a scale that weighs to 0.01g. This assay was also useful for screening plants for secondary compounds with activity directed at insects. Plants that were identified as distasteful to ants were found to have insect deterrent secondary compounds or were previously known to have deterrent secondary compounds. The relevance of ants as a bioassay for antiherbivore compounds might seem questionable because they are not herbivores. However, antiherbivore compounds often exhibit very broad insecticidal activities (Harborne 1988), which is why sequestration by herbivores is a very successful defensive strategy against ant predators (de la Fuente et al. 1994, Dyer 1995, Dyer and Bowers 1996). The sensitivity of this assay to very small changes in concentration of antiherbivore compounds in *P. cenocladum* supports this hypothesis. Neutral plants that we preliminarily investigated did not
yield any interesting compounds based on our procedures. Some of the neutral plants were known from the literature to contain compounds with a variety of activities against human pathogens or in human cells but none had activities directed against insects. For example, the literature indicates *Pothomorphe peltata* (Gustafson et al. 1992; Desmarchelier et al. 1997; Desmarchelier et al. 2000; Ferreira-da-Cruz et al. 2000) and some species of *Cissampelos* (e.g., *C. pareira*; Morita et al. 1993b; Morita et al. 1993c; Morita et al. 1993a) are rich sources of complex natural products with powerful activity against a host of human pathogens and diseases but no reported activities in insects. This is consistent with our goal of identifying tissues with compounds deterrent only to insects. The genus *Costus* is a rich source of saponins that are known to be deterrent to insects yet it yielded a neutral extract in our assay. The literature indicates that the saponins of several *Costus* species are isolated from the roots only and not the leaves, which were used for our extracts, thus explaining the negative results (da Silva et al. 1999; Lin et al. 1996; Lin et al. 1997; Bohme et al. 1997). The fact that *Cecropia* is known to contain relatively high levels of tannins (Coley 1986) and yet has a neutral extract is a positive aspect of this assay. Tannins are ubiquitous “quantitative” defenses with effects on insects.
that vary from beneficial to mildly deterrent (Ayres et al. 1997), and the fact that the assay does not identify plants with tannins reduces the number of false positive results that it produces.

Various modifications of this assay have been used successfully in other studies to identify chemically defended plants and insects (Dyer and Floyd 1993; Dyer 1995; Gentry and Dyer 2002). One strength of the assay is the adaptability of the procedure. The concentration of the sugar solutions can be modified to make the assay more or less sensitive depending on the research questions. Extracts can be made with solvents other than methanol, different insect omnivores could be used as assay animals, and the assay can be used to find insect attractant compounds as well. The choice of the solvent methanol over the less toxic ethanol is based upon cost, purity and availability of ethanol in Costa Rica. Absolute (i.e. 100%) ethanol is available but is much more expensive than methanol. Denatured ethanol is readily available and inexpensive, however in Costa Rica it is denatured by adding 6% diethyl phthalate. This denatured ethanol was shown to be very deterrent in the bioassay and thus was deemed unsuitable.

It is clearly not an omniscient general assay that will uncover all secondary compounds, but it is a flexible assay that can guide chemical
ecologists towards systems with interesting chemistry. An examination of Tables 1 and 2 reveals that approximately half of the species assayed can be classified as deterrent, and warrant further study.

The chemical investigation of tropical flora has largely been driven by the desire to find new drugs to treat human disease. The bioassay described here provides a means of screening plants and insects for compounds with non-medical applications, in an ecologically relevant manner. It is a cheap, quick, and relevant method to screen crude extracts for compounds that may be of use as insecticides or insect behavior regulating agents. Although the pharmaceutical industry has extensive experience in the screening of plant/animal extracts for biological activity in both cell/organism based and receptor binding based bioassays, typically only one compound goes to clinical trials for every 10,000 extracts tested and of these compounds only 1 in 4 ever becomes a prescribed drug (Balick et al. 1995). This low success ratio (0.01% to clinical trials and only 0.0025% to market) is in part explained by the simple fact that these newly discovered compounds are useful as human medicines not by evolutionary design, but completely by chance. Because many plant secondary compounds evolved in response to herbivory by insects (Rhoades 1979, Mauricio and Rausher
1997), the search for chemicals used to deter insect herbivory should be much more successful.

We are particularly hopeful that this assay will help guide research on chemical defense in insects. Several authors (Dyer 1995, Dyer and Gentry 1999, Gentry and Dyer 2002, Blum et al. 1990) have found that chemical defenses are the most important predictors of mortality caused by natural enemies, yet it is difficult to ascertain if an herbivore is actually chemically defended or protected by unrelated behavioral or morphological defenses (Bowers 1993, Dyer 1995, Dyer 1997). Gross generalizations are often made about the toxicity of herbivores in particular taxonomic groups (e.g., all Papilionidae, Sime and Brower 1998) or particular regions (e.g. tropical insects, Gauld and Gaston 1994) without a significant amount of information about these systems. More exploration of the existence and role of chemicals in plant-herbivore-carnivore interactions would help resolve many current theoretical questions concerning insect diversity, density, evolution, and diet breadth. A simple bioassay can make this type of investigation more accessible and more common.
Acknowledgments

We thank many Earthwatch volunteers, H. Garcia, M. Rathbone, A. Schaefer, J. Legac, D. Rosen, K. McClendon, J. Fleet, B. Hutchins, H. Rosenberg and J. D. Searcy for help with data collection. The Organization for Tropical Studies provided facilities and valuable logistical help. This work was supported by grants from Earthwatch Institute, National Geographic, and the National Science Foundation (NSF, DEB-0074806).

References


ecological and evolutionary constraints on foraging Chapman & Hall, New York, pp 331-371


Ferreira-da-Cruz MD, Adami YL, Espinola-Mendes ED, Figueiredo MR, Daniel-Ribeiro CT (2000) The intraperitoneal Plasmodium berghei-Pasteur infection of Swiss mice is not a system that is able to detect the antiplasmodial activity in the Pothomorphe plant extracts that are used as antimalarials in Brazilian endemic areas. Experimental Parasitology 94:243-247


Ripperger H, Porzel A (1992) 2a-hydroxysoladulcidine from *Lycianthes biflora*. Phytochemistry 31:725-726


Schaffner U, Boeve JL, Gfeller H, Schlunegger UP (1994) Sequestration of veratrum alkaloids by specialist *Rhadinoceraea*
nodicornis konow (Hymenoptera, Tenthredinidae) and its ecoethological implications. Journal of Chemical Ecology 20:3233-3250


TABLE 1. Results of bioassay using *Paraponera clavata* to assess the relative palatability of the species and a mixture of synthetic secondary compounds. Everything classified as deterrent had a greater than zero (P < 0.0025).

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>ACD</th>
<th>SE</th>
<th>Known Chemistry</th>
<th>Known Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solanaceae</td>
<td><em>Nicotiana tabacum</em></td>
<td>0.4</td>
<td>0.01</td>
<td>Nicotine.</td>
<td>Toxic/deterrent to insects</td>
</tr>
<tr>
<td>Solanaceae</td>
<td><em>Solanum adherens</em></td>
<td>0.4</td>
<td>0.01</td>
<td>Alkaloids detected: Solapalmitine and congeners.</td>
<td>Cytotoxic to insects</td>
</tr>
<tr>
<td>Solanaceae</td>
<td><em>Solanum jamacense</em></td>
<td>0.3</td>
<td>0.006</td>
<td>No known compounds for this species; the genus is a rich source of secondary metabolites.</td>
<td>None</td>
</tr>
<tr>
<td>Piperaceae</td>
<td><em>Piper cenocladum</em></td>
<td>0.3</td>
<td>0.01</td>
<td>3 <em>Piper</em> amides: piplartine, 4'-desmethylpiplartine and cenocladamide at high concentrations (total 1.74% dry weight).</td>
<td>Deterrent to insects</td>
</tr>
<tr>
<td>Nyctaginaceae</td>
<td><em>Neea psychotrioides</em></td>
<td>0.2</td>
<td>0.004</td>
<td>Novel clerodane diterpenes and cucurbictacins.</td>
<td>Cucurbit deterrent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Family</td>
<td>Species</td>
<td>Concentration</td>
<td>Ant Activity</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------</td>
<td>---------------</td>
<td>--------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Leguminosae</td>
<td><em>Lonchocarpus oliganthus</em></td>
<td>0.09</td>
<td></td>
<td>No alkaloids detected and no literature for this species; related Central American species are a rich source of rotenoids and flavanoids.</td>
<td></td>
</tr>
<tr>
<td>Piperaceae</td>
<td><em>Piper auritum</em></td>
<td>0.09</td>
<td>0.003</td>
<td>Oxoaporphine alkaloids; Propenylphenols (Elemicin, Eugenol, Myristicine and Safrole); Monoterpenes including camphor and camphene.</td>
<td></td>
</tr>
<tr>
<td>Piperaceae</td>
<td>Synthetic mixture of 3 amides</td>
<td>0.07</td>
<td>0.003</td>
<td>Piplartine, 4’-desmethylpiplartine and cenocladamide at 1.06% total concentration.</td>
<td></td>
</tr>
<tr>
<td>Piperaceae</td>
<td><em>Piper cenocladum</em> (fragments with ants)</td>
<td>0.06</td>
<td>0.01</td>
<td>Piplartine, 4’-desmethylpiplartine and cenocladamide at medium concentration (1.40% total).</td>
<td></td>
</tr>
<tr>
<td>Solanaceae</td>
<td><em>Lycianthes synanthera</em></td>
<td>0.06</td>
<td>0.01</td>
<td>No alkaloids detected and no literature for this species; the related species, <em>L. biflora</em>, contains the steroidal alkaloids 2’?hydroxy-soladulcidine, soladulcidine, and solasodine as well as the sapogenins, gitogenin and neogitogenin.</td>
<td></td>
</tr>
<tr>
<td>Piperaceae</td>
<td><em>Piper imperiale</em></td>
<td>0.06</td>
<td>0.004</td>
<td>Three <em>Piper</em> amides, two new structures and Piplarox.</td>
<td></td>
</tr>
</tbody>
</table>
Dyer et al. – Bioassay 35

<table>
<thead>
<tr>
<th>Plant Family</th>
<th>Species</th>
<th>Alkaloids Detected</th>
<th>Tannins Concentration</th>
<th>Activity</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moraceae</td>
<td><em>Cecropia obtusifolia</em></td>
<td>0.03</td>
<td>0.003</td>
<td>None</td>
<td>No alkaloids detected; high tannin concentrations.</td>
</tr>
<tr>
<td>Urticaceae</td>
<td><em>Myriocarpa longipes</em></td>
<td>0.03</td>
<td>0.002</td>
<td>None</td>
<td>None for this species nor this genus.</td>
</tr>
<tr>
<td>Piperaceae</td>
<td><em>Piper cenocladium</em> shrubs</td>
<td>0.02</td>
<td>0.01</td>
<td>Deterrent</td>
<td>Piplartine, 4’-desmethylpiplartine and cenocladamide at low concentration (0.41% total).</td>
</tr>
<tr>
<td>Solanaceae</td>
<td><em>Solanum rudepanum</em></td>
<td>0.02</td>
<td>0.009</td>
<td>None</td>
<td>No alkaloids detected and no literature for this species.</td>
</tr>
<tr>
<td>Piperaceae</td>
<td><em>Piper melanocladum</em></td>
<td>0.02</td>
<td>0.003</td>
<td>Activity</td>
<td>Two known prenylated benzoic acid derivatives previously found in <em>P. auritum</em>.</td>
</tr>
<tr>
<td>Solanaceae</td>
<td><em>Solanum enchlyosum</em></td>
<td>0.01</td>
<td>0.002</td>
<td>None</td>
<td>None for this species.</td>
</tr>
<tr>
<td>Solanaceae</td>
<td><em>Witheringia asterotricha</em></td>
<td>0.002</td>
<td>0.003</td>
<td>Anti-tum</td>
<td>The genus contains some anti-tumor compounds.</td>
</tr>
<tr>
<td>Solanaceae</td>
<td><em>Solanum rugosum</em></td>
<td>-0.009</td>
<td>0.001</td>
<td>None</td>
<td>No alkaloids detected and no literature for this species.</td>
</tr>
<tr>
<td>Family</td>
<td>Species</td>
<td>IC50</td>
<td>EC50</td>
<td>Activity Description</td>
<td>Notes</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------------------------</td>
<td>------</td>
<td>------</td>
<td>--------------------------------------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Piperaceae</td>
<td>Pothomorphe peltata</td>
<td>-0.03</td>
<td>0.002</td>
<td>Peltatols and other catechol derivatives.</td>
<td>No known activity in insects;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>activity against human pathogens.</td>
</tr>
<tr>
<td>Menispermaceae</td>
<td>Cissampelos tropaeolifolia</td>
<td>-0.04</td>
<td>0.002</td>
<td>No literature for this species.</td>
<td>None</td>
</tr>
<tr>
<td>Zingiberaceae</td>
<td>Costus laevis</td>
<td>-0.04</td>
<td>0.002</td>
<td>No literature for this species. The genus is a rich source of steroidal saponins of the diosgenin type in the roots and flavonol glycosides from the leaves.</td>
<td>No known activity against the leaf glycosids.</td>
</tr>
</tbody>
</table>
TABLE 2. Results of bioassay using *Paraponera clavata* to assess the relative palatability of the species. All caterpillars classified as deterrent had ACD values significantly greater than zero. Caterpillars were assayed using a 20% sucrose solution. *Hypothyris euclea* was assayed using several different concentrations of sucrose.

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>ACD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deterrent caterpillars</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nymphalidae</td>
<td><em>Hypothyris euclea</em> – 10% solution</td>
<td>0.6</td>
<td>0.01</td>
</tr>
<tr>
<td>Pericopidae</td>
<td><em>Agarea</em> sp.</td>
<td>0.3</td>
<td>0.009</td>
</tr>
<tr>
<td>Nymphalidae</td>
<td><em>Hypothyris euclea</em> – 20% solution</td>
<td>0.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Pyralidae</td>
<td>“Red social” undescribed species</td>
<td>0.1</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Neutral/attractant caterpillars</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyralidae</td>
<td><em>Panthographa expansalis</em></td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Pericopidae</td>
<td><em>Dysshemia leucophaeae</em></td>
<td>0.01</td>
<td>0.004</td>
</tr>
<tr>
<td>Riodinidae</td>
<td><em>Emesis lucinda</em></td>
<td>-0.01</td>
<td>0.002</td>
</tr>
<tr>
<td>Nymphalidae</td>
<td><em>Hypothyris euclea</em> – 30% solution</td>
<td>-0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>