BIOLOGICAL ACTIVITIES OF *Trewia nudiflora* EXTRACTS AGAINST CERTAIN ECONOMICALLY IMPORTANT INSECT PESTS


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Abstract—An ethanol extract of *Trewia nudiflora* (Euphorbiaceae) seed was tested as an agent for controlling several economically important insects. Results suggest that this plant extract acts as an antifeedant for the spotted cucumber beetle (*Diabrotica undecimpunctata howardi* Barber) and the European corn borer (*Ostrinia nubilalis* (Hübner)) but not for the other insects tested. Also indicated were morphogenic effects on the codling moth (*Laspeyresia pomonella* (L.)), disruption of the normal life cycle of the redbanded leafroller (*Argyrotaenia velutinana* (Walker)), and reduction in the progeny of the plum curculio (*Conotrachelus nenuphar* (Herbst)). In addition, the extract was toxic to the striped cucumber beetle (*Acalymma vittatum* (F.)) and gave 100% control of the chicken body louse (*Menacanthus stramineus* (Nitzsch)) from 5 to 28 days. Fractionation of the extract was monitored by a bioassay using *O. nubilalis*. This fractionation yielded six pure compounds, the most abundant of which was trewiasine. Its LD_{50} was 7.4 ppm when incorporated into the diet of *O. nubilalis*. Dose–mortality relationships for the other compounds with *O. nubilalis* are presented.

Key Words—Pest control agent, plant extract, *Trewia nudiflora*, trewiasine, control agent, antifeedant, morphogenic agent.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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INTRODUCTION

Ethanolic extracts of *Trewia nudiflora* (Euphorbiaceae) (false white teak) seed have shown significant activity in vitro against human carcinoma of the nasopharynx (KB) and in vivo against P388 lymphocytic leukemia (PS) (Powell et al., 1981). These extracts also inhibited initiation and growth of crown-gall tumors on potato disks (Galsky et al., 1980). In what appears to be the first insecticidal use of *T. nudiflora*, we observed previously that this extract produced high mortality when incorporated into the larval diet of the European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae) (Freedman et al., 1979). We have now tested *T. nudiflora* extracts against a number of insects to determine their potential as a pest control agent. We have included several Lepidoptera and Coleoptera which are pests of fruit and vegetables, as well as the chicken body louse, *Menacanthus stramineus* (Nitzsch) (Mallophaga: Menoponidae). In addition, we report further testing with *O. nubilalis*.

Fractionation of the extract was guided by a dual monitoring arrangement; both antitumor (KB cell culture and PS leukemia) and *O. nubilalis* systems were employed. As detailed by Powell et al. (1981), this fractionation (Figure 1) culminated in the isolation of at least six new maytansinoid compounds; the structures of three of these are shown in Figure 2. The structures of the other three are not yet certain, although they are being investigated intensively. Results of these bioassays are presented in this paper.

METHODS AND MATERIALS

*Trewia nudiflora* Extract.

Extraction and fractionation of *T. nudiflora* seed has been described by Powell et al. (1981). The procedure used for the fractionation of the ethanol extract of this seed is summarized in Figure 1. Trewiasine is by far the most abundant of the maytansinoids in *T. nudiflora* and was purified in quantities sufficient for testing against *O. nubilalis* at several levels. Five less abundant congeners of trewiasine (demethyltrewiasine, dehydrotrewiasine, treflorine, trenudine, and *N*-methyltrenudone) have been purified in quantities sufficient only for more limited bioassays.

Insect Rearing and Testing

*Codling Moth*, Laspeyresia pomonella (*L.*) (Lepidoptera: Olethreutidae). Larvae were reared and tested at 26.6 ± 2°C, 60 ± 5% relative humidity, continuous light, on a modified Shorey's pinto bean diet (Shorey and Hale, 1965). *T. nudiflora* extract was combined with this diet to provide
concentrations of 0.1, 0.05, 0.01, and 0.005% by weight. The two higher concentrations were prepared by adding 40 mg and 20 mg of extract, respectively, to 2 ml of 95% ethanol, and emulsifying with a Brinkmann Polytron (PCR-2) for 5 sec. Each of the resulting suspensions was added to 40 g of hot diet and stirred thoroughly. To make the two lower concentrations, 10 mg of the extract was suspended in 10 ml of 95% ethanol, and 4 ml and 2 ml, respectively, of this suspension were added to 40 g of hot diet. The resulting hot diet mixtures were drawn up into a 5-mm-ID glass tube to harden. The diet was then expelled from the tubes, sectioned into 30- to 40-mm lengths, and evacuated at 17 mm of mercury for 15 min at 40°C to eliminate solvent. Two neonate *L. pomonella* larvae were placed onto each diet piece in a jelly cup with a plastic-coated cardboard cap. Each treatment was replicated 10
times. Larvae were transferred to untreated diet after 2 days. Mortality
determinations were made after 7, 9, and 11 days. Ten replicates of two larvae
on untreated diet were used for controls.

Striped Cucumber Beetle, Acalymma vittatum (Fabricus) (Coleoptera:
Chrysomelidae). Beetles were reared and tested at 29.5 ± 2°C, 60 ± 5%
relative humidity, 15:9 light:dark cycle, on cantaloupe leaves. A 1% solution
was prepared by adding 50 mg of extract to 1 ml acetone, emulsifying with a
Polytron homogenizer, and diluting to 5 ml with distilled water containing
0.01% Tween 20. Disks from cantaloupe leaves ca. 20 mm in diameter were cut
with a cork borer, dipped for 10 sec in the appropriate solution, air dried, and
placed in a cotton-stoppered shell vial containing a single beetle. Ten adult
beetles which had recently emerged, preferably female, were tested per
treatment. Feeding deterrence was evaluated by comparing the amount of
treated leaf consumed in comparison with untreated leaf. Mortality was
determined after 1, 4, and 5 days. Controls replicated 10 times, with one beetle
per test, were fed on disks dipped into formulation blank only.

Spotted Cucumber Beetle, Diabrotica undecimpunctata howardi Barber
(Coleoptera: Chrysomelidae). Beetles were reared as described for A.
vittatum. Testing was also done with treated cantaloupe leaf disks. To a Petri
dish containing two treated and two untreated disks, five beetles were
introduced. This was replicated twice. Both feeding deterrence and mortality
were evaluated.

Redbanded Leafroller, Argyrotaenia velutinana (Walker) (Lepidoptera:
Tortricidae). Larvae were reared and tested at 26.6 ± 2°C, 60 ± 5%
relative humidity, continuous light, on an artificial alfalfa leaf meal diet (Redfern,
1964). The diet was swabbed with the test material, and 8 neonate larvae, one
per cup, were tested for each treatment. Mortality for the treatments was
determined after controls had emerged as adults.
**Plum Curculio**, Conotrachelus nenuphar (Herbst) (*Coleoptera: Curculionidae*). Beetles were reared and tested at 26.6 ± 2°C, 60 ± 5% relative humidity, and continuous light. Adults, 3 days after emergence, were tested on apples which had one treated and one untreated side. After adding five adult beetles to each, apples were checked periodically until death of adults and/or emergence of progeny. This procedure was replicated four times.

**Chicken Body Louse**, *M. stramineus*. These lice were tested by the procedure of Hoffman and Hogan (1972) except that two rather than four chickens were treated. Also, the extract was tested at only one concentration (1% in 100% ethanol).

**European Corn Borer**, *O. nubilalis*. Larvae were reared and tested as previously described (Freedman et al., 1979) except that mortality was determined after 5 days and 11 days only.

**Method for Determining LD<sub>50</sub> of Trewiasine for O. nubilalis**. Estimations of the LD<sub>50</sub> of trewiasine for *O. nubilalis* were made using the standard probit analysis of Daum (1970). Trewiasine in 6 doses was mixed with the larval diet. To each 4 g of treated diet, five 7-day-old larvae were added. This was replicated four times. In addition, each dose was replicated twice. Mortality was determined after 5 and 11 days. Abbott's formula was used to adjust for control mortality.

**RESULTS**

**Biological Activity of *T. nudiflora***

*L. pomonella* (*Codling Moth*). Both the crude ethanol extract and chloroform solubles at the two higher doses showed 100% mortality (Table 1); however, the two lower doses of the chloroform solubles appear to be more effective, reflecting a higher concentration of the active materials in the chloroform solubles. These bioassays showed that a high level of control of *L. pomonella* larvae was possible at low doses even with relatively crude fractions of *T. nudiflora*. We also observed that the extract produced morphogenic changes in the larvae. This manifested itself in prolonged larval development which usually resulted in an abnormally high mortality of pupae or adults.

*A. vittatum* (*Striped Cucumber Beetle*). Neither the ethanol extract (I, Figure 1) nor the active fraction obtained after HPLC (III) were effective as antifeedants against *A. vittatum*. Consumption of cucumber leaves treated with the extract by these beetles did result in 90% mortality, but only at relatively high dose levels and only after 5 days (Table 2). *A. vittatum* thus showed greater tolerance to the extract than did *L. pomonella*.

*D. undecimpunctata* (*Spotted Cucumber Beetle*). A 0.5% solution of the extract was highly effective as an antifeedant against these beetles. A 79%
TABLE 1. TOXICITY OF Trewia nudiflora TO Laspeyresia pomonella LARVAE\(^a\)

<table>
<thead>
<tr>
<th>Concentration weight % in diet</th>
<th>7 days</th>
<th>9 days</th>
<th>11 days</th>
<th>7 days</th>
<th>9 days</th>
<th>11 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.005</td>
<td>28</td>
<td>35</td>
<td>82</td>
<td>61</td>
<td>88</td>
<td>100</td>
</tr>
<tr>
<td>0.01</td>
<td>67</td>
<td>65(^c)</td>
<td>94</td>
<td>89</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.05</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

\(^a\)Each dose was mixed with artificial diet to which two neonate larvae were added. This was replicated 10 times.

\(^b\)Data adjusted for control mortality by Abbott's formula. Mortality in controls after 7 days was 10%; after 9 days and 11 days, 15%.

\(^c\)The apparent decrease in \% mortality from 7 to 9 days is due to the fact that mortality among control larvae enters into computation of the values listed.

Differences in consumption were observed between treatment and control. Of the fruit and vegetable insects examined in this study, *D. undecimpunctata* was the only insect against which *T nudiflora* extract acted as an antifeedant. A 0.5% solution of the extract also resulted in 50% mortality of this insect after 1 day.

*A. velutinana* (*Redbanded Leafroller*). A 0.5% solution of the extract when applied to the diet of these larvae prevented all larvae on treated diets from becoming adults. Control diets in the same test permitted 75% of the larvae to emerge as adults.

*C. nenuphar* (*Plum curculio*). A 0.5% solution of the extract was ineffective as a feeding and oviposition deterrent on treated apples exposed to

TABLE 2. EFFECT OF Trewia nudiflora EXTRACT ON Acalymma vittatum ADULTS\(^a\)

<table>
<thead>
<tr>
<th>Dose, % solution (w/v)</th>
<th>1 day</th>
<th>4 days</th>
<th>5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.125</td>
<td>0</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>0.25</td>
<td>0</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>0.5</td>
<td>10</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>1.0</td>
<td>10</td>
<td>60</td>
<td>90</td>
</tr>
</tbody>
</table>

\(^a\)Each dose was tested with ten beetles. Extract was applied to beetles' diet.

\(^b\)No dead beetles in controls during the test.
### TABLE 3. Mortality of *Ostrinia nubilalis* Larvae Treated with Compounds from *Trewia nudiflora*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg)</th>
<th>5 days</th>
<th>11 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trewiasine</td>
<td>0.15</td>
<td>13</td>
<td>87S</td>
</tr>
<tr>
<td></td>
<td>0.075</td>
<td>18</td>
<td>69S</td>
</tr>
<tr>
<td></td>
<td>0.038</td>
<td>24G</td>
<td>51S</td>
</tr>
<tr>
<td>Demethyltrewiasine</td>
<td>0.20</td>
<td>6</td>
<td>69S</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>24G</td>
<td>54S</td>
</tr>
<tr>
<td></td>
<td>0.0125</td>
<td>22G</td>
<td>34S</td>
</tr>
<tr>
<td>Treflorine</td>
<td>0.20</td>
<td>18</td>
<td>100S</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>11</td>
<td>100S</td>
</tr>
<tr>
<td></td>
<td>0.0125</td>
<td>7</td>
<td>40S</td>
</tr>
<tr>
<td>Dehydrotrewiasine</td>
<td>0.20</td>
<td>1</td>
<td>60S</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>17</td>
<td>47S</td>
</tr>
<tr>
<td></td>
<td>0.0125</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>N-methyltrenudone</td>
<td>0.20</td>
<td>30G</td>
<td>100S</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>17</td>
<td>94S</td>
</tr>
<tr>
<td></td>
<td>0.0125</td>
<td>22G</td>
<td>94S</td>
</tr>
<tr>
<td>Trenudine</td>
<td>0.20</td>
<td>9S</td>
<td>100S</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>53S</td>
<td>100S</td>
</tr>
<tr>
<td></td>
<td>0.0125</td>
<td>10</td>
<td>100S</td>
</tr>
</tbody>
</table>

*a* Each dose was mixed with 4 g diet to which five 7-day-old larvae (third instar) were added. This was replicated four times.  
*b* Data adjusted for control mortality by Abbott's formula. Mortality in controls after 5 and 11 days was 5 and 11%, respectively.  
*c* S indicates that the mean mortality for that treatment is significantly different from control at *P* = 0.05, as determined by a range test criterion (Freedman et al., 1979).  
*d* G indicates that the mean mortality for that treatment is suggestive of being different from control at *P* = 0.05, as determined by least significant difference (Freedman et al., 1979).

*C. nenuphar* adults. It did reduce the progeny from those insects which ate treated apples, with only 32% of larvae emerging.

*M. stramineus* (*Chicken Body Louse*). A 1% solution of *T. nudiflora* extract when sprayed on chickens provided 90% control of the chicken body louse within a 5-day period and 100% control for 28 days.

**Bioassay of Compounds from *T. nudiflora* with *O. nubilalis***. Table 3 shows the results of testing all six maytansinoid compounds, each at three dose levels. These data and those in Table 4 indicate that several of the compounds may be more active than trewiasine, the most abundant. Further investigation will be needed to clarify this point. *O. nubilalis* larvae exposed to diet containing *T. nudiflora* extract, derived fractions, or pure maytansinoids crawled away from the diet as though they were avoiding it. When live or dead
TABLE 4. Mortality of Ostrinia nubilalis Larvae Treated with Trewiasine

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>5 days</th>
<th>11 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.009</td>
<td>17G</td>
<td>28S d</td>
</tr>
<tr>
<td>0.019</td>
<td>17G</td>
<td>41S</td>
</tr>
<tr>
<td>0.038</td>
<td>18S</td>
<td>50S</td>
</tr>
<tr>
<td>0.075</td>
<td>18S</td>
<td>61S</td>
</tr>
<tr>
<td>0.15</td>
<td>21S</td>
<td>94S</td>
</tr>
<tr>
<td>0.3</td>
<td>25S</td>
<td>92S</td>
</tr>
</tbody>
</table>

a Each dose was mixed with 4 g diet to which five 7-day-old larvae were added. This was replicated four times. Each dose was replicated twice.
b Data adjusted for control mortality by Abbott’s formula. Mortality in controls after 5 and 11 days was 1 and 3%, respectively.
c G indicates that the mean mortality for that treatment is suggestive of being different from control at $P = 0.05$, as determined by least significant difference.
d S indicates that the mean mortality for that treatment is significantly different from control at $P = 0.05$, as determined by a range test criterion.

d Larvae were detected after a number of days, they were invariably much smaller in size than controls, as if they had eaten little or none of the diet.

LD$_{50}$ of Trewiasine. Table 4 shows the mortality of O. nubilalis larvae when treated with trewiasine. With almost every dose, considerably higher mortality was achieved after 11 days compared to 5 days, indicating that even pure trewiasine acts relatively slowly. On the basis of the data in Table 4, the LD$_{50}$ of trewiasine was determined to be 0.0297 mg/4 g of diet, which is equivalent to 7.4 ppm. The 95% confidence interval of 0.0185–0.0424 mg corresponds to 4.6–10.6 ppm. The slope was 1.405.

DISCUSSION

Possible Antifeedant Action of T. nudiflora. The mechanism by which T. nudiflora causes mortality in O. nubilalis is not known. Our observations indicate that it may act as an antifeedant and/or repellent. This avoidance behavior contrasts with that noted with other toxic plant extracts where dead larvae were usually found within the diet (Freedman et al., 1979). In terms of their activity towards O. nubilalis larvae, there appear to be no very striking differences among these six maytansinoids despite certain structural variations. We previously found that neriifolin, a plant-derived compound, had an LD$_{50}$ of 0.12 mg (30 ppm) against O. nubilalis (McLaughlin et al., 1980). Trewiasine is thus four times as active against O. nubilalis as is neriifolin,
making it the most potent naturally occurring compound we have so far discovered in our tests with this insect.

*Comparisons of the Effect of T. nudiflora on Different Insects.* The wide variety of activity exhibited by *T. nudiflora* makes it interesting as a potential pest control agent. Its effect on *L. pomonella* appears to be concentration dependent. At the doses shown in Table 1, it acts mainly as a toxicant; at lower doses, as a morphogenic agent. Unlike the antifeedant effect observed with *O. nubilalis*, no antifeedant effect was observed with *L. pomonella* even at the higher doses. This may partially explain why toxic effects appear more rapidly than with *O. nubilalis*, a greater amount of material being consumed immediately.

Several other biological effects, such as suppression of adult emergence with *A. velutinana* and reduction in progeny with *C. nenuphar*, were also noted. With *D. undecimpunctata*, the extract was an antifeedant, as these insects largely avoided eating diet treated with it. Analysis of the data presented in Tables 1, 2, and 3 reveals that *L. pomonella* may be more sensitive to *T. nudiflora* than *O. nubilalis* and is definitely affected by lower doses of the extract compared to *A. vittatum*. For both *A. vittatum* and *O. nubilalis*, 90% or greater mortality was not achieved until the insects had been exposed to treated diet for 5–11 days. This suggests the effects produced by the extract occur relatively slowly.

In summary, the active components of *T. nudiflora* act as antifeedants, reduce or suppress adult emergence or progeny, or produce morphogenic changes in several insect species. Further experimentation is needed to define these modes of action more precisely. This initial study indicates the extract may have potential for controlling a number of economically important insects; however, further testing, including ultimately field tests, will be required to establish this potential.

The exotic structure of the maytansinoids, together with their biological activity, has prompted intensive synthetic efforts from several groups; at least two of these have already culminated in success (Corey et al., 1980, Meyers et al., 1980). *Trewia nudiflora*, the source of our active material, appears to be fairly abundant in its native habitat (India). However, from the standpoint of supply, the most significant development may be the discovery by a group of Japanese workers (Asai et al., 1979) that maytansinoids can be produced by fermentation with a *Nocardia* sp. This development opens up possibilities for a relatively cheap method for producing the complex macrocyclic ring of maytansinoids; these compounds could then be modified by attachment of various ester sidechains.

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REFERENCES


