Effect of Different Condensed Tannins on *Trichoplusia ni* Performance

Azucena González-Coloma
Instituto de Productos Naturales y Agrobiología, CSIC, Ave. Astrofísico F. Sánchez 2, 38206 La Laguna, Tenerife, Spain

Charles S. Wisdom and Philip W. Rundel
Laboratory of Biomedical and Environmental Sciences, University of California, Los Angeles, California, U.S.A. 90024


_Schinopsis quebracho-colorado, Acacia greggii, Euphorbia micromera, Phoradendron californicum, Trichoplusia ni_, Condensed Tannins

To examine the assumption that condensed tannins exhibit uniform activity against herbivores, tannins extracted from four plant species with different in vitro protein binding abilities were fed to larvae of _Trichoplusia ni_ in artificial diets. Larvae were measured for survivorship, growth and food consumption at three tannin concentrations (0.001%, 0.01% and 0.05% w. v. w.). Larval performance was negatively affected by the tannin in a dose-response fashion, giving the different plant-derived tannins different activity thresholds. We did not find any significant correlation between the biological effects of these tannins and their protein binding abilities. This study indicates that different species of tannins do not act uniformly against a tannin sensitive herbivore, and that these differences can not be attributed to their different in vitro protein binding abilities.

**Introduction**

Tannins are common components of many terrestrial plants, and are frequently purported to play a role in defending plants against herbivores [1, 2]. Composed of two subclasses (condensed and hydrolyzable), tannins are a heterogeneous class of phenolic polymers capable of complexing in vitro with proteins and precipitating them from aqueous solutions [3, 4]. These compounds have a purported ecological function of generalized dose-dependent activity, acting uniformly against both vertebrate and invertebrate herbivores. One proposed mechanism of this tannin function is the complexing of dietary and digestive proteins which reduces the digestibility and nutrient content of herbivore food intake [1, 2].

Martin and Martin [3] and Schultz and Baldwin [5] demonstrated however, than tannin quantity and protein binding efficiency (PBE) were uncorrelated and concluded that tannin activity is related to PBE. Furthermore, tannin PBE exhibits a seasonal variation in plants [6, 7]. In contrast, tannin protein complexes are suggested to be unlikely in the in vivo conditions of the insect gut [8–10] although there is some evidence of tannin-protein interaction in the presence of surfactants [11, 12].

In examining the in vitro binding of proteins by tannins, we proposed that the ability of tannins to bind proteins is dependent on both quantity and protein PBE [13]. Consequently, the measurement of tannin quantity in plant material would be insufficient to determine tannin effectiveness if the mode of action of tannins involves protein complexation.

The above hypothesis predicts that tannins with different PBEs will differentially affect the herbivore's biological responses (growth, survivorship, etc.) at equal concentrations. Here we selected a tannin sensitive insect, the cabbage looper _Trichoplusia ni_ (Hübner) (Lepidoptera: Noctuidae), and tested for differences in survivorship, growth and food consumption when presented equal concentrations of different plant species’ tannins with differing in vitro PBEs.

**Materials and Methods**

Fifteen neonate _T. ni_ larvae from a laboratory colony (U.C. Riverside, California, U.S.A.) were used per treatment. The larvae were individually placed in lidded 10 ml cups with approximately 4 g...
of artificial diet (Bio Serv Inc., diet No. F9282) and reared at 21 °C with a 14:10 photoperiod. The diets were supplemented with condensed tannins or carrier solvent alone (acetone) from four different plant genera: *Acacia greggii*, *Euphorbia micromera*, *Phoradendron californicum*, and quebracho (*Schinopsis* sp.). Plant collection and condensed tannin isolation procedures are described in Wisdom *et al.* [13].

Tannin concentrations in the diets were 0.001%, 0.01% and 0.05% wet weight for each species of tannins. Larvae were scored for daily survivorship and were weighed along with the diets at the end of the experiment (14 days).

For data analysis, live larval weights and amounts of diet consumed were normalized with a logarithmic transformation prior to an ANOVA analysis. Differences in mean values were tested with Tukey's studentized range (HSD) test (*p* = 0.05). Mortality data was analyzed as Contingency tables for a Chi-square analysis (*p* = 0.05). The relationship between larval growth and diet consumed was tested with linear regression models.

The PBEs of the condensed tannins were determined using bovine serum albumin (BSA) and are the slopes of the tritation curves (double logarithmic plots) for constant protein concentration against increasing tannin concentrations. The PBEs of the tannins tested are an indicator of their ability to bind BSA protein and were previously determined by Wisdom *et al.* [13] for the same tannin samples.

**Results**

Cabbage looper mortality varied significantly among the different tannins used. At the 0.001% level, there was no mortality from any of the four tannin diets (Fig. 1). At the 0.01% concentration, only the *Euphorbia* treated larvae differed significantly from the control (Fig. 1). At the 0.05% concentration however, larval mortality from all four experimental diets differed significantly from the control except for *Phoradendron* (Fig. 1). Larvae on the quebracho diet (PBE = 0.47) exhibited the sharpest response to the increase from 0.01% to 0.05% concentration, indicating a strong threshold in quebracho tannin activity (Fig. 1). In contrast, the mortality level of the larvae fed *Phoradendron*, with a similar PBE value (0.40), was not distinguishable from the controls at any of the concentrations tested (Fig. 1). The larval mortalities for the *Acacia* and *Euphorbia* diets, with PBEs of 0.31 and 0.28 respectively, were intermediate (Fig. 1).

Larval growth was differentially affected by tannin type and was also dependent on the concentrations used. At the 0.05% level, larvae fed quebracho tannins grew the least, followed by those fed *Euphorbia* and *Acacia* tannins. At the 0.01% concentration, the *Euphorbia* tannins produced the

![Fig. 1. Mortality of *T. ni* larvae fed diets containing condensed tannins from *Euphorbia* (*E*), *Acacia* (*A*), *Phoradendron* (*P*) and quebracho (*Q*) species. * Significantly different from the control (Chi-square goodness of fit test, *p* = 0.05).](image)
highest larval growth reduction, followed by those from *Acacia* and quebracho. Finally, at the 0.001% concentration, all the tannins assayed did not produce significant larval growth reductions compared with the control (Table I).

The food consumption on all the treated diets varied significantly from the control and depended on tannin concentration (Table I). We also observed some variation in consumption among the different tannins at the 0.01% and 0.001% concentrations, the *Phoradendron*-based diet showing the highest consumption in both cases (Table I).

We found significant correlations between consumption and larval growth for all the tannins tested at 0.001%, and only for quebracho and *Acacia* tannin at 0.01% (Table II). At the highest dose (0.05%), only the *Phoradendron* diet fed larvae showed a significant, but weak, relationship between growth and amount of food eaten (Table II).

**Discussion**

The effects that the different tannins had on *T. ni* could be attributed to feeding deterreny, digestibility reduction and/or toxicity. We observed deterreny at the 0.001% dose (no mortality, reduced consumption, strong relationship between growth and consumption) although we cannot separate the behavioral versus the post-ingestive component of this effect. The toxic and antinutritive effects became more evident as the tannin concentration increased (higher larval mortality and

### Table I. Mean values and standard errors (SE) of larval growth (% of control, Grwt.) and food consumption (% of control, Cons.) of neonate *T. ni* larvae fed diets containing condensed tannins from *Euphorbia* (E), *Acacia* (A), *Phoradendron* (P) and quebracho (Q) species with different protein binding efficiencies (PBE), plus control (C).

<table>
<thead>
<tr>
<th>Plant sp.</th>
<th>PBE</th>
<th>0.05% Grwt.</th>
<th>0.05% Cons.</th>
<th>0.01% Grwt.</th>
<th>0.01% Cons.</th>
<th>0.001% Grwt.</th>
<th>0.001% Cons.</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>0.28</td>
<td>3.63a</td>
<td>35.72a</td>
<td>13.63a</td>
<td>36.73a</td>
<td>64.54a</td>
<td>38.57a</td>
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<tr>
<td></td>
<td></td>
<td>(0.74)</td>
<td>(0.40)</td>
<td>(39.92)</td>
<td>(11.62)</td>
<td>(24.02)</td>
<td>(3.31)</td>
</tr>
<tr>
<td>A</td>
<td>0.31</td>
<td>8.63a</td>
<td>28.83a</td>
<td>33.55b</td>
<td>38.96ab</td>
<td>90.91a</td>
<td>51.13ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.18)</td>
<td>(0.87)</td>
<td>(12.35)</td>
<td>(2.06)</td>
<td>(32.71)</td>
<td>(5.92)</td>
</tr>
<tr>
<td>P</td>
<td>0.40</td>
<td>44.54b</td>
<td>37.81a</td>
<td>105.15c</td>
<td>56.99c</td>
<td>178.54a</td>
<td>67.87b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14.92)</td>
<td>(1.97)</td>
<td>(18.01)</td>
<td>(9.74)</td>
<td>(48.25)</td>
<td>(10.33)</td>
</tr>
<tr>
<td>Q</td>
<td>0.47</td>
<td>1.81</td>
<td>33.22</td>
<td>36.23b</td>
<td>39.82b</td>
<td>77.21a</td>
<td>40.40a</td>
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<td></td>
<td></td>
<td>(10.4)</td>
<td>(2.04)</td>
<td>(19.4)</td>
<td>(2.51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.0</td>
<td>100c</td>
<td>100b</td>
<td>100c</td>
<td>100d</td>
<td>100a</td>
<td>100c</td>
</tr>
<tr>
<td>Sig. level</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
<td>ns</td>
<td>p &lt; 0.001</td>
<td>ns</td>
</tr>
</tbody>
</table>

1 Values in column followed by the same letter are not statistically different, Tukey's studentized range (HSD) test, p = 0.05.
2 Not enough degrees of freedom.
3 Significance level of one-way ANOVA analysis.

### Table II. Parameters of the linear regressions of *T. ni* larval weight on consumption of artificial diets supplemented with plant tannins for 14 days (I, intercept; s, slope; r, coefficient). Plant species are: E, *Euphorbia*; Q, quebracho; P, *Phoradendron* and A, *Acacia*.

<table>
<thead>
<tr>
<th>Plant sp.</th>
<th>0.05%</th>
<th>0.01%</th>
<th>0.001%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>s</td>
<td>r²</td>
</tr>
<tr>
<td>E</td>
<td>-5.38</td>
<td>0.25</td>
<td>0.67</td>
</tr>
<tr>
<td>A</td>
<td>-12.68</td>
<td>0.74</td>
<td>0.41</td>
</tr>
<tr>
<td>P</td>
<td>-195.48</td>
<td>6.34</td>
<td>0.70</td>
</tr>
<tr>
<td>Q²</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a Probability level of estimation (*, p < 0.05; **, p < 0.01).
b Not enough degrees of freedom for the model at the 0.05% level.
lack of correlation between growth and food consumption).

If a tannin-protein complexation mechanism was involved in the biological effects observed in this experiment, we should have found a negative relationship between them and the PBEs of the tannins tested. On the contrary however, comparing the ranking of both larval growth and food consumption data with their correspondent PBE value, we observed that Euphorbia tannin, with the lowest PBE (0.28) produced the highest growth and consumption inhibition at the 0.01% level, followed by quebracho and Acacia (PBEs of 0.47 and 0.31 respectively). At the 0.05% dose, quebracho, Euphorbia and Acacia ranked similarly, while the Phoradendron-treated diets, with a PBE similar to that of quebracho (0.40), ranked the lowest in terms of larval growth and consumption reduction for all the concentrations tested.

Zucker [14] predicted that chemical changes in tannin structure would lead to selective abilities to complex specific proteins. Other authors [15–17] have reported differences in tannin’s abilities to complex proteins or inhibit enzymes related to their different plant sources. Recently, Clausen et al. [18] found that the structural differences in condensed tannins from bitterbrush (Purshia tridentata) and blackbrush (Coleogyne ramosissima) lead directly to differences in the physical and/or chemical properties of the resultant tannin-protein complexes.

We found that the different tannins assayed had different PBEs with bovine serum albumin (BSA) [13] although we could not correlate such values with their biological effects on T. ni. Alternative modes of activity for tannins have been advanced [19], focusing instead on the toxic-like effects of tannins on herbivorous insects, binding to the gut lining and causing damage and mortality by bacterial invasion of the hemolymph [20–22] or having detrimental effects on their performance without affecting their digestibility [23–25]. From the above mentioned theories two hypothesis could explain our results: A) the PBEs changed with the type of protein available as a substrate in the insect’s gut (plant proteins, gut proteins, etc.). B) a toxic-like activity of these tannins, unrelated to their PBEs. Both cases would explain the lack of correlation between the tannin’s biological effects observed and their PBEs.

In conclusion, we have shown that there is not a generalized protein-binding related effect of different plant species’ condensed tannins on T. ni. The biological activity of the tannins varied with the plant species and it was dose-dependant. The differences in biological activity observed among the different plant species’ tannins could be related to differential feeding deterrency at sublethal doses (0.001%) and to differences in the substrate-specificity of these tannins to the proteins available in the insect’s gut or to a toxic-like action at higher doses (0.01 and 0.05%). Studies using purified tannin molecules instead of mixtures will lead to a better understanding of their defensive properties.

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