Effect of Ursolic Acid from Epicuticular Waxes of Jacaranda decurrens on Schizaphis graminum

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EFFECT OF URSOLIC ACID FROM EPICUTICULAR WAXES OF *JACARANDA DECURRENS* ON *SCHIZAPHIS GRAMINUM*

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ABSTRACT.—Ursolic acid from *Jacaranda decurrens* showed toxicity and feeding deterrency towards the greenbug *Schizaphis graminum*. Biological activity was determined by analyzing ursolic acid effects on the survival, reproductive index, and population growth rate of the greenbug. Survival and reproductive index decreased in direct proportion to ursolic acid content in the diet. The population growth rate decreased markedly when the aphids were fed on barley leaves sprayed with ursolic acid dissolved in DMSO, in comparison to leaves sprayed only with DMSO. The feeding behavior of the greenbug was also affected by ursolic acid. Ingestion time on diet with 0.1 mM was reduced about 30% in relation to the ingestion time on control diet.

Several classes of compounds show toxicity and/or feeding deterrency towards herbivores and insects. For example, alkaloids (1), flavonoids and related compounds (2), and diterpenes (3) affect the aphid *Schizaphis graminum* (Rondani) (Homoptera: Aphididae). The effects of several classes of terpenoids on insects are well known (4). However, very little has been said about the action of pentacyclic triterpenoids (amyrin-type terpenoids) on herbivores. The relatively common occurrence of such triterpenoids in epicuticular waxes is not frequently cited in the literature (5). Yet these compounds, among other components, predominate in the waxes of leaves of woody species of the Brazilian Cerrados (a characteristic vegetation in Central Brazil similar to savannas), such as *Didymopanax vinosum*, *Aspidosperma tomentosum*, and *Tocoyena brasiliensis* (6). The biological role of these compounds in plants is unknown.

In this paper we report the isolation of ursolic acid from *Jacaranda decurrens* Chams (Bignoniaceae), a frequent species in the Cerrados, and describe the effect of the compound on *S. graminum*, a polyphagous aphid species.

RESULTS AND DISCUSSION

TERPENOIDS.—Leaves of *J. decurrens* yielded about 0.23% (wet wt) of total triterpenoids and 0.11% of ursolic acid (3 mmol/kg wet wt of leaf material), embedded in the epicuticular wax. Therefore, *J. decurrens*, as well as other species from the Brazilian Cerrado, has foliar epicuticular waxes with high contents of terpenoids (6).

EFFECT OF TERPENOIDS ON APHIDS.—Because ursolic acid is H_2O-insoluble, it was dissolved in DMSO and then added to insect diet. In order to test the effects of DMSO, aphids were fed on diets containing 0–100 mM DMSO. No significant differences in the survival of *S. graminum* were detected at any of the

concentrations tested. Thus, DMSO proved to be an adequate solvent for bioassays of H_2O-insoluble compounds.

Survival of greenbug fed with artificial diets containing ursolic acid decreased with an LD_{50} of 0.19 mM at 24 h (Figure 1). It was observed that survival rates of aphids at 0.5 mM were higher than at 0.1–0.3 mM (Figure 2). These results suggest that ursolic acid had a feeding deterrent effect at high concentrations (0.4 mM and above) and a toxic effect at low concentrations. At low concentrations, the compound was ingested, causing deleterious effects on aphids. At high concentration, the compound was not ingested because of its feeding deterrent effect. In addition, the ursolic acid decreased the reproductive index of *S. graminum* (Figure 3).

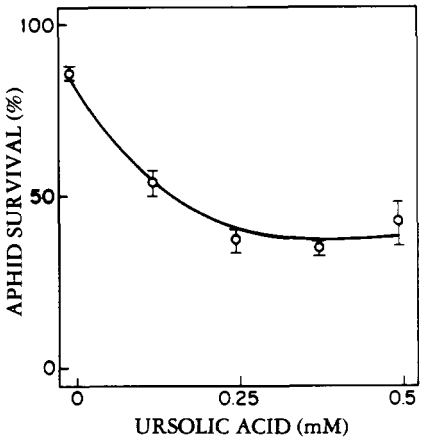

FIGURE 1. Effect of ursolic acid upon survival of *Schizaphis graminum* reared on artificial diets. Survival is expressed as per cent of initial individuals measured after feeding the aphids for 24 h. Each point is the average of three samples of ten aphids each.

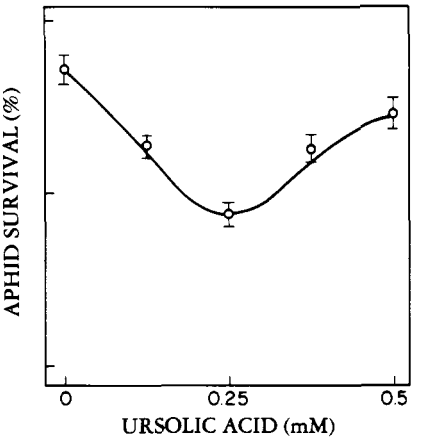

FIGURE 2. Toxicity and feeding deterrency of ursolic acid. Survival was measured after feeding the aphids for 6 h with diets plus ursolic acid and transferring to control diets for 24 h. Each value is the mean of three samples of ten aphids each.

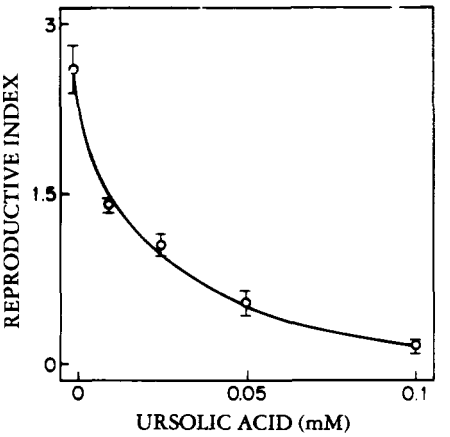

FIGURE 3. Effect of ursolic acid on the reproductive index of *Schizaphis graminum* reared on artificial diet, measured after feeding adults for 72 h.

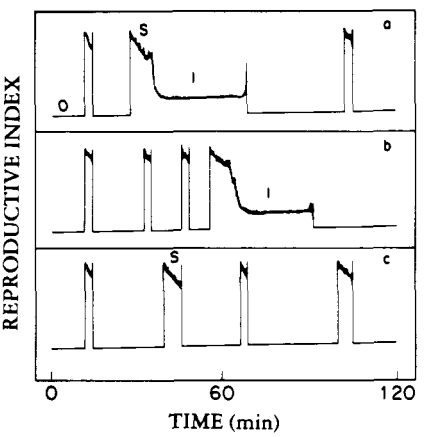

FIGURE 4. Effect of ursolic acid on feeding behavior of *Schizaphis graminum*. a = control, b = 0.1 mM, c = 0.5 mM, O = base line, S = salivation, I = ingestion.

FEEDING BEHAVIOR OF APHIDS.—The feeding behavior of *S. graminum* on diets with DMSO (control) and ursolic acid (0.1 and 0.5 mM) was determined as described in the Experimental section. The aphids were kept for 2 h on the diet, and their feeding behavior was recorded. The aphids on the diets without terpenoid fed for 35 min, while aphids with 0.1 mM ursolic acid fed for only 25 min. Aphids kept on diets with 0.5 mM ursolic acid did not feed, showing only short periods of salivation (Figure 4).

PROTECTION OF BARLEY SEEDLINGS BY URSOLIC ACID.—DMSO solutions of ursolic acid at different concentrations were sprayed onto barley leaves. Control leaves were sprayed with solvent only. Seedlings of each group were infested by two apterous adult *S. graminum* per plant. After 5 days, the control group showed a high population growth rate, while the population growth rate decreased in groups treated with terpenoids (Table 1). These results suggest that an addition of ursolic acid to barley leaves may protect them from infestation by aphids.

The vegetation of the Cerrado may be an important source of compounds for studying ecological relationships between plants and insects. Plant breeding programs of crop plants aiming at the introduction of the genetic ability to include triterpenoids in the epicuticular waxes may be an effective way of controlling infestation by aphids.

TABLE 1. Population Growth Rate of *Schizaphis graminum* on Barley Seedlings Sprayed with Ursolic Acid.[a]

Ursolic acid (mM)	Population growth rate per day
0	0.43 ± 0.01
0.5	0.40 ± 0.01
1.0	0.37 ± 0.01
2.0	0.33 ± 0.01
3.0	0.29 ± 0.01

[a]Ten-day-old barley seedlings were sprayed with DMSO containing different ursolic acid concentrations. Then each group of plants was infested with ten adult aphids. The population growth rate was determined after 5 days. Each value is the mean on five samples of five seedlings each ± 1 SE. Population growth rate = ln (Nf/Ni)/t.

EXPERIMENTAL

COMPOUND.—Ursolic acid was obtained from the epicuticular waxes of *J. decurrens* (voucher EMV-269/SPF, deposited in the Herbarium of Dep. Botânica, Instituto de Biociências/USP) and identified by means of ^{13}C-nmr spectrometry according to procedures described previously (6).

APHIDS ASSAY.—Nymphs of *S. graminum* were reared on barley plants (*Hordeum distichum* L. cv. Aramir) and kept under conditions of continuous light at 25 ± 2°. For toxicity, feeding deterrency, and reproductive assays, the aphids were fed from diet as described by Argandoña *et al.* (7) at pH 8.0. Control diet and diet plus terpenoid at various concentrations were placed between two sheets of Parafilm M (8). The experiments were performed at 25 ± 1° and continuous light. In order to test toxicity and/or deterrency, aphids were fed for 6 h on diets with ursolic acid and then transferred to diets without the terpenoid. After 24 h, the survival rate of aphids was determined.

ELECTRICALLY-MONITORED ASSAYS.—These assays were performed according to McLean and Kinsey (9) Argandoña *et al.* (10). Aphid probing activity was studied as a function of ursolic acid concentration in the diets (0.1 and 0.5 mM).

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