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ANTIFEEDANT AND TOXIC EFFECTS OF SESQUITERPENES FROM *Senecio palmensis* TO COLORADO POTATO BEETLE

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Abstract—A bioassay-guided fractionation of the aerial parts of *Senecio palmensis* resulted in the isolation of two sesquiterpenes, 2,10-bisaboladien-1-one and 11β-acetoxy-5-angeloyloxy-silphinen-3-one. The bisabolene and the silphinene represented 0.012% and 0.024% of the plant dry weight, respectively. Both compounds showed antifeedant activity against *Leptinotarsa decemlineata* larvae and adults in short-term choice and no-choice bioassays. Both compounds were also tested against different species of phytopathogenic fungi. The beetles were more sensitive to these compounds in choice than in no-choice assays, with a gradient of increasing sensitivity from second instars to adults. Bisabolene was 45 times less active as an antifeedant than juglone, which was tested as a positive control. The silphinen was more active than the bisabolene, with a range of activity similar to juglone. Furthermore, exposure of fourth instars to these compounds over a 24-hr period resulted in reduced feeding and growth rates. To distinguish between antifeedant and toxic effects, growth efficiencies were calculated as the slope of the regression of relative growth rate on relative consumption rate. The comparison of these

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results with those of antifeedant simulation and contact toxicity bioassays indicates that feeding inhibition is the primary mode of action of the bisabolene, while the silphinene shows both antifeedant and toxic effects.

Key Words—*Senecio palmensis*, Asteraceae, *Leptinotarsa decemlineata*, Coleoptera, Chrysomelidae, antifeedant, toxic, sesquiterpenes.

INTRODUCTION

The chemical control of the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say.), with synthetic insecticides has induced a rapid development of resistance of bettle populations to most of these chemicals, including some natural insecticides such as avermectins and the δ-endotoxin of *Bacillus thuringiensis* (Brattsten, 1991; Whalon et al., 1993).

Insect antifeedants of plant origin may provide an attractive alternative to control this insect since their use may reduce the development of cross-resistance. Furthermore, substances that exert both behavioral (antifeedants) and physiological (toxins) effects would be more useful in reducing the development of resistance (Jermy, 1990).

Plant sesquiterpenes and other terpenoids are major determinants of insect-plant interactions (references in Mullin et al., 1991). Inhibitory cyclic sesquiterpenes and diterpenes for insect herbivores have been identified from at least 28 genera of the terpenoid-rich Asteraceae (Mullin et al., 1991). Additionally, species belonging to the Asteraceae family are an important source of CPB antifeedants (Jermy et al., 1981; Hough-Goldstein, 1990). This family also offers numerous examples of insecticidal and/or antifeedant plant metabolites with additional fungicidal activity (Picman, 1986; Miles et al., 1990). As a result of our ongoing search for natural agrochemicals among Canarian endemic plant species in the Asteraceae, we found that an ethanolic extract of *Senecio palmensis* (Chr. Sm.) had a strong antifeedant activity against *Leptinotarsa decemlineata* larvae.

Here we report the isolation of two sesquiterpenes from a biologically active fraction of *S. palmensis*, along with their antifeedant and antifungal activity against *L. decemlineata* larvae and adults and six species of phytopathogenic fungi. Juglone, a natural compound with insecticidal, antifeedant (Hassanali and Lwande, 1989; Bernays and Cornelius, 1992), and antifungal activity (Marston et al., 1993) was included in the experiments as a positive control. We also report on the effects of those compounds on feeding and growth of fourth-instar *L. decemlineata* larvae and the study of their mode(s) of action through antifeedant simulation.

METHODS AND MATERIALS

Plant Material

Flowering plants were collected in Arico, Tenerife (July 1992). A voucher specimen has been deposited in the Centro de Investigación y Tecnología Agraria (CITA), Tenerife.

General

IR spectra were recorded in CH_2Cl_2 solutions using a Perkin-Elmer spectrometer. ^1H and ^{13}C NMR spectra in $CDCl_3$ were measured in a Bruker AMX 400 MHz spectrometer (chemical shifts reported are relative to residual $CDCl_3$, 7.26 ppm for ^1H and 77.0 ppm for ^{13}C). Mass spectra were obtained at 70 eV on a VG micromass ZAB-2F instrument. Optical rotations were recorded with a Perkin Elmer 241 polarimeter. HPLC chromatography was performed on a Beckman System Gold apparatus. The GC-MS analysis was done on a Perkin-Elmer Sigma 3B with a Supelco capillary silica column SPB-1 (30 m × 0.25 mm and 0.25 μm mesh) and the conditions were as follows: temperature gradient 110–250°C and 150–280°C with 5°C/min increments for compounds **1** and **2**, respectively.

Isolation of Compounds

Dried aerial parts of *S. palmensis* (4.7 kg) were exhaustively extracted with EtOH in a Soxhlet apparatus. The extract (808.75 g) was chromatographed on a silica gel vacuum liquid chromatography column (VLC column 9 × 5 cm, packed with silica gel 5–40 μm mesh) with an *n*-hexane–ethyl acetate–methyl alcohol gradient to give one biologically active fraction A (6.35 g, *n*-hexane–EtOAc, 9:1). This fraction was further chromatographed on silica gel (VLC column 7 × 5 cm, packed with silica gel 5–40 μm mesh) and eluted with an *n*-hexane–EtOAc gradient to provide two biologically active fractions, A1 (1.41 g, *n*-hexane–EtOAc, 6:4) and A2 (2.10 g, *n*-hexane–EtOAc 2:8). Both fractions were subsequently purified by preparative normal-phase HPLC using a 250 × 20 mm silica column (Gasukuro Kogyo Inertsil ODS-2, 5 μm particle size) and an isocratic system of Cl_2CH_2–CH_3CN, 93:7, at a flow rate of 12 ml/min. Peaks were detected at 254 nm. Two pure bioactive compounds (**1**, 475 mg; and **2**, 850 mg) were obtained from fractions A1 and A2, respectively, as a result of the HPLC purification.

(6S)-2,10-bisaboladien-1-one **(1).** Oil, $[\alpha]_D = -34°$ (EtOH; c 0.34); EI-MS (70 eV, *m/z*, rel. int.): 220 $[M]^+$, 205 (7), 163 (13), 137 (100), 135 (55), 110 (44), 109 (35), 95 (27), 82 (31), 67 (15), 55 (13), 53 (15). ^1H NMR

($CDCl_3$, 400 MHz): δ, 5.86 (1H, q, J = 1.2 Hz, H-2), 5.06 (1H, brt, J = 5.8 Hz, H-10), 2.37 (1H, m, H-7), 2.31 (2H, m, H-4), 2.14 (1H, ddd, J = 12.0, 4.2 Hz, H-6), 2.01 (1H, dt, J = 7.2 Hz, H-9), 1.91 (1H, m, H-5α), s-1.89 (3H, s, H-14), 1.76 (1H, m, H-5β), 1.63 (3H, d, J = 1.0 Hz, brs, H-13), 1.55 (3H, brs, H-12), 1,24 (2H, dt, J = 7.0 Hz, H-8), 0.79 (3H, d, J = 6.8 Hz, H-15). ^{13}C NMR ($CDCl_3$, 100 mHz): 200.89 (C-1), 126.96 (C-2), 161.05 (C-3), 30.77 (C-4), 22.32 (C-5), 49.73 (C-6), 30.18 (C-7), 34.57 (C-8), 26.12 (C-9), 124.4 (C-10), 131.2 (C-11), 17.55 (C-12), 25.9 (C-13), 24.01 (C-14), 15.56 (C-15).

11β-Acetoxy-5-angeloyloxy-silphinen-3-one **(2).** Oil, $[\alpha]_D$ = −66.6° (EtOH; c 0.15); IR ($CDCl_3$) 1735 (ester), 1706, 1603, 1458, 1377, 1238, 1131, 1086 cm^{-1}. EI-MS (70 ev, *m/z*, rel. int.): 374 (M^+) (10), 291 (24), 275 (51), 231 (18), 215 (90), 203 (10), 189 (14), 187 (16), 173 (13), 161 (20), 145 (10), 83 (100), 55 (57).

Insect Bioassays

The *L. decemlineata* colony was reared on potato foliage (cv. Desirée) and maintained at 24 ± 1°C, >70% relative humidity with a photoperiod of 16:8 hr (L:D) in a growth chamber.

Short-Term Choice and No-Choice Feeding Assays: These experiments were conducted with a newly emerged second (L2) and fourth instar (L4) *L. decemlineata* larvae and adults. Potato leaf disks (1 cm^2) were treated on the upper surface with 10 μl of the test substance. For choice assays, four treated and four control disks were arranged alternatively on five 2.5% agar-coated Petri dishes (9 cm diameter); for no-choice assays, eight treated or control disks were used per dish. Ten second instars, three fourth instars, or three adults were placed in each dish following three hours of starvation and allowed to feed in a growth chamber (environmental conditions as described above). Feeding was terminated after consumption of 50–75% of the control disks, and the uneaten leaf disk surfaces were measured according to Escoubas et al. (1993) with a computer-interfaced scanner (Escoubas, personal communication).

Calculations of amounts of treated and control disks eaten were made by subtracting the surface area of the remaining portion from the initial surface for the appropriate test. Percent feeding reduction (%FR) was determined for each arena by the equation %FR = [1 − (treatment consumption/control consumption)] × 100 (Bentley et al., 1984). For the crude extract and its fractions, an arbitrary level of %FR > 75 was considered indicative of a strong feeding deterrency.

The two isolated compounds and commercial juglone (Sigma) were tested in a dose–response experiment to calculate their relative potencies (EC_{50} values, the effective dose for 50% feeding reduction), which were determined from log

probit analysis (Finney, 1971). Two dose series were used: 10, 25, 50, 75, and 100 $\mu g/cm^2$ for compound **1** and 0.1, 0.25, 0.50, 0.75, 1.0, 10 and 25 $\mu g/cm^2$ for compound **2** and juglone.

Long-Term Feeding Assays. These experiments were performed under the same environmental conditions as above. One L4 larva (less than 24 hr old) and eight potato leaf disks (1 cm^2) were placed on 2.5% agar-coated Petri dishes (9 cm diam.). The upper surfaces of the disks were treated with either 10 μl of the test solution or the solvent carrier alone (acetone control), and 15 plates per treatment were used in all feeding experiments.

To distinguish between the antifeedant and toxic effects of these compounds, antifeedant simulation assays with different levels of starvation and dose–response assays were run simultaneously (Blau et al., 1978). At the end of the feeding experiments larval feeding indexes and growth efficiencies were calculated.

Feeding Indexes and Growth Efficiency. The feeding indexes were calculated on a dry weight basis. Initial larval dry weights were estimated from the regression of fresh on dry weight of additional newly molted L4 larvae. Initial dry weight of food was calculated similarly using additional leaf disks. At the end of the feeding experiments, the remaining food and larvae were lyophilized and weighed.

The relative consumption rate (RCR) and the relative growth rate (RGR) were calculated according to Farrar et al., (1989). Growth efficiency (GE) was calculated as the slope of the regression of RGR on RCR, assuming a common intercept determined by the RGR of the starved control larvae (Blau et al., 1978). The relative chemical consumption (RC) was estimated as RCR $\times$ dose (in milligrams) (Liu et al., 1990).

Antifeedant Simulation. Fifteen individually weighed *L. decemlineata* L4 larvae were fed for 24 hr on zero, two, four, six, and eight preweighed potato leaf disks (1 cm^2). At the end of the experiment, the weights of larvae and remaining leaf disks were recorded. With these data, a calibration curve was constructed by calculating the regression of RGR on RCR ranging from total starvation to abundance.

Dose–Response Experiments. Fifteen individually weighed L4 CPB larvae were fed during 24 hr on eight preweighed leaf disks treated with 0, 10, 25, 50, 75, and 100 $\mu g/cm^2$ of each terpene. At the end of the experiment, uneaten food and live larval weights were recorded. To study the posttreatment growth, the same larvae were allowed to feed ad libitum on untreated potato foliage for another 24 hr and then reweighed.

Contact Toxicity. The contact toxicity of the compounds was investigated by topical application using 15 preweighed L4 larvae. A drop (2 μl) of each compound (20 μg) dissolved in acetone was applied to the back of each larva. Treated larvae were fed on eight preweighed potato leaf disks (1 cm^2) for 24

hr, and then reweighed, along with the remaining food, to calculate their relative consumption and growth rates as above.

Antifungal Activity Assays

The antifungal activity of the terpenes isolated was tested against six species of plant pathogens (*Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *Botrytis cinerea*, *Phytophthora syringae*, and *Ascochyta lentis*) and estimated as mycelial growth inhibition (Murabayashi et al., 1991). The test substances were dissolved in acetone and incorporated in a PDA culture medium (5% final concentration of solvent). For each experiment, 2-cm-diameter disks were cut from the corresponding culture medium and inoculated with the appropriate fungal species. Eight replicates of each culture were incubated at 27°C in darkness and the colony diameters measured after 48 hr. Control experiments consisted of inoculated untreated culture-medium incubated under the same conditions. EC_{50} values were determined from log probit analysis (Finney, 1971). Two dose series were used: 0.01, 0.05, 0.1, 0.25, and 0.5 mg/ml for compounds **1** and **2**; and 0.005, 0.01, 0.025 mg/ml plus the same as above for juglone.

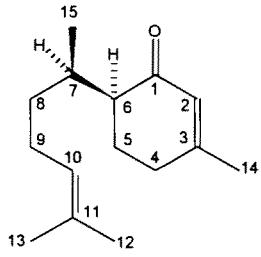
Statistical Analysis

All dry weight measures were log-transformed prior to ANOVA analysis to test for treatment effects on the feeding indexes, chemical consumption, and contact toxicity. Differences between treatment means were checked with Duncan's multiple range test. The toxicity of these compounds was determined by regressing RGR on RCR and comparing each treatment slope with the control slope (antifeedant simulation experiment) (Blau et al., 1978; Berenbaum and Feeny, 1981; Miller and Feeny, 1983). The slopes of the regression lines were compared with a *t* test for parallelism (Tallarida and Murray, 1981). The basic assumption of this analysis is that the RGR of starved larvae does not differ among treatments within an experiment. Treatments in which the slope of the regression is significantly lower than that of the calibration curve can be categorized as toxins. The slope of the regression of RGR on RCR is one estimate of growth efficiency (GE) on any particular treatment.

RESULTS

Bioassay-Guided Isolation of Compounds. The crude alcoholic extract of the aerial parts of *S. palmensis* was a strong antifeedant against *L. decemlineata* fourth instars in choice tests (97 ± 3% feeding reduction at 100 $\mu g/cm^2$). A bioassay-guided fractionation of the extract resulted in the isolation of two active sesquiterpenes (**1** and **2**, Figure 1) by VLC column, normal-phase column, and

Compound 1



Compound 2

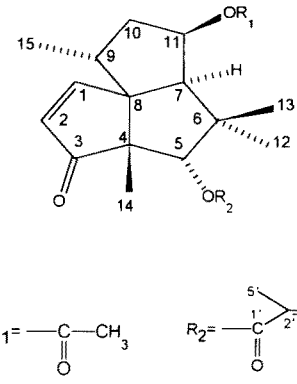


FIG. 1. Structures of compounds isolated from *S. palmensis*.

normal-phase HPLC chromatography. Their structures were established by IR, mass (EI-MS) GC-MS, and ^{1}H and ^{13}C NMR spectroscopic experiments, including two-dimensional methods (HMCQ and HMBC) (Table 1), and by comparison with previously reported data.

Compound **1** was identified as (6*S*)-2,10-bisaboladien-1-one, previously isolated from *Stevia purpurea* and *Cymbopogon citratus* (Asteraceae) (Bohlmann et al., 1976; Abegaz and Yohannes, 1983). Compound **2,** two times more abundant in the plant than **1,** was identified as 11β-acetoxy-5-angeloyloxy-silphinen-3-one (see Table 1), previously isolated from *Cineraria geifolia* var. glabra (Asteraceae) (Jakupovic and Abraham, 1985).

TABLE 1. ^{1}H, ^{13}C, HMQC, AND HMBC NMR DATA OF **2** (400 MHz, $CDCl_3$)[a]

Proton		Correlated carbon	
		HMQC	HMBC
1	7.56 d (7.6)	167.8 d	C-8, C-4, C-2
2	6.12 d (7.6)	131.9 d	C-8, C-4, C-3
		211.1 s (C-3)	
		63.9 s (C-4)	
5	5.43 s	86.3 d	C-13,C-1′(167.5),
		42.5 s (C-6)	C-3, C-8
7	2.23 d (4.0)	61.6 d	C-5, C-13
		57.1 s (C-8)	
9	2.61 ddq (12.5, 6, 7)	35.1 d	C-15, C-8, C-4
10α	1.60 dt (13.8, 2.6)	41.7 t	
10β	2.12 dd (12.8, 6)		C-7
11α	5.25 t (3.4)	71.6 d	
12	0.90 s	25.5 q	C-6, C-7
13	0.95 s	23.7 q	C-11
14	1.24 s	19.9 q	C-8, C-4, C-3
15	0.93 d (7.2)	15.9 q	C-10, C-7
3′	6.11 q (7.2)	138.7 d	C-1′ (167.5)
4′	2.04 dd (7.2)	15.8 q	C-2′, C-3′
5′	1.94 t (1.4)	20.7 q	C-2′, C-3′, C-1′
C=O		170.1 s	
|			
CH_3	2.16 s	21.7 q	

[a]Carbon multiplicities were established by DEPT pulse sequence. The coupling constants in parenthesis are in Hz.

Antifeedant Effects of Sesquiterpenes. Both chemicals strongly deterred feeding of CPB larvae (98% feeding reduction at 100 $\mu g/cm^2$ in choice assays). Their relative potencies (EC_{50}) are presented in Table 2 along with that of juglone. The degree of antifeedant activity of the chemicals tested varied with the insect's developmental stage, in agreement with previous reports (Bentley et al., 1988; Liu et al., 1989; Hough-Goldstein, 1990). In general, a gradient of increasing sensitivity from second instars to adults was shown. Larvae and adults generally appeared more selective in choice than in no-choice experiments, in contrast with the effect of limonin (Alford et al., 1987), and similar to the effect of polygodial and warbuganal (Caprioli et al., 1987).

Compound **1** showed the lowest antifeedant activity (Table 2) with a relative potency range similar to limonin, a known CPB antifeedant with EC_{50}

TABLE 2. RELATIVE ANTIFEEDANT POTENCIES OF COMPOUNDS **1** AND **2** AND JUGLONE (JU) AGAINST *L. decemlineta*

Test[a]	L2	L4	Adults
1 A	20.34 (17.03, 24.29)[b]	4.00 (2.73, 5.86)[b]	14.64 (3.55, 60.40)[b]
B	35.21 (21.0, 59.05)[b]	29.86 (26.55, 33.6)[b]	11.66 (1.65, 82.04)[b]
2 A	12.55 (4.87, 32.34)[b]	1.69 (1.36, 2.11)[b]	0.27 (0.16, 0.46)[b]
B	30.90 (16.62, 57.45)[b]	14.43 (3.01, 69.14)[b]	2.67 (1.38, 5.18)[b]
JU A	—	0.83 (0.25, 2.80)[b]	0.32 (0.06, 1.81)[b]
B	—	20.41 (16.93, 24.59)[b]	3.14 (0.04, 9.42)[b]

[a]Test A, choice; test B, no-choice.
[b]Lower and upper 95% confidence intervals of EC_{50}.

values of 34.92 and 9.34 $\mu g/cm^2$ against L4 larvae in choice and no-choice tests, respectively (Alford et al., 1987).

Compound **1** decreased *L. decemlineata* consumption and growth rate. The responses were not dose-dependent, but significant reductions of RGR at 100 $\mu g/cm^2$ and RCR at 75 and 100 $\mu g/cm^2$ were observed (Table 3). The effects of compound **1** on posttreatment growth rate and consumption were similar (Table 3).

The growth efficiencies of CPB larvae treated with different concentrations of **1** were similar to those of the starved control (Table 3). Each treatment dose gave significant linear relationships between RCR and RGR (0 $\mu g/cm^2$: $r = 0.88$; $P < 0.00001$; 10 $\mu g/cm^2$: $r = 0.87$; $P < 0.00001$; 25 $\mu g/cm^2$; $r = 0.71$, $P < 0.00001$; 50 $\mu g/cm^2$: $r = 0.78$; $P < 0.00001$; 75 $\mu g/cm^2$: $r = 0.57$, $P = 0.00075$; 100 $\mu g/cm^2$: $r = 0.88$, $P < 0.00001$) and parallel to the calibration curve (Figure 2, Table 3).

Since the growth efficiencies of larvae treated with **1** were similar to those of the starved control, we conclude that feeding inhibition at the behavioral level is the primary mode of action of **1**. Additionally, this compound did not show contact toxicity (Table 4).

The antifeedant effect of the 24-hr assay with compound **1** (50% feeding inhibition at 100 $\mu g/cm^2$) was lower than that of short-term (<6 hr) no-choice

TABLE 3. FEEDING INDEXES, GROWTH EFFICIENCY, POSTTREATMENT GROWTH AND CONSUMPTION MEAN VALUES AND STANDARD ERRORS (SE) OF FOURTH-INSTAR CPB LARVAE (N = 15) FEEDING ON COMPOUND **1**.

Dose (μg/cm^2)	RCR[a]	RGR[b]	post-RGR[c]	RC1[d]	GE[e]
0	0.88 (0.13)	0.36 (0.05)	0.49 (0.03)	0.00 (0.01)	0.482
10	1.04 (0.11)	0.22 (0.08)	0.42 (0.06)	0.080a (0.009)	0.443
25	1.00 (0.07)	0.26 (0.04)	0.43 (0.02)	0.185b (0.01)	0.344
50	0.77 (0.07)	0.22 (0.02)	0.42 (0.02)	0.309c (0.03)	0.399
75	0.62 (0.09)	0.15[f] (0.08)	0.40 (0.03)	0.374d (0.06)	0.297
100	0.44[f] (0.08)	0.02[f] (0.04)	0.35[f] (0.03)	0.356d (0.06)	0.438
Starved control					0.371
F[g]	5.387	4.910	1.376	19.287	
P	0.0002	0.0005	0.24	<0.0000 1	

[a]RCR = $I/(BI) \times T$, I = food consumed (mg), T = feeding period (days), BI = initial insect weight (mg).
[b]RGR = $\Delta B/(BI) \times T$, ΔB = change in insect body weight (mg).
[c]Growth efficiency (GE), calculated as the slope of the regression of RGR on RCR.
[d]RGR of insects fed for 24 hr on untreated food after the treatment.
[e]Relative consumption (RC) of compound **1**: RCR × dosage (mg). Values followed by a different letter are significantly different (Duncan's multiple range test, $P = 0.05$).
[f]Denotes a significant difference from the control, Duncan's multiple range test, $P = 0.05$.
[g]ANOVA parameters (F statistic and probability level, P).

tests (95% feeding reduction at 100 μg/cm^2). An explanation of the decreased effect of antifeedant **1** with longer exposure time (over 6 hr) could be a behavioral desensitization. A similar effect has been described for behavioral desensitization of *Spodoptera frugiperda* to aristolochic acid in no-choice situations (Raffa and Frazier, 1988).

Compound **2** showed higher activity levels than compound **1** (Table 2). Compound **2** strongly deterred adult beetles from feeding and had relative potencies within the range of juglone. When compared with limonin, compound **2** was 23 times more active against L4 larvae in choice tests, but less active in no-choice tests, with an effective dose of **2** that was 1.5 times higher than

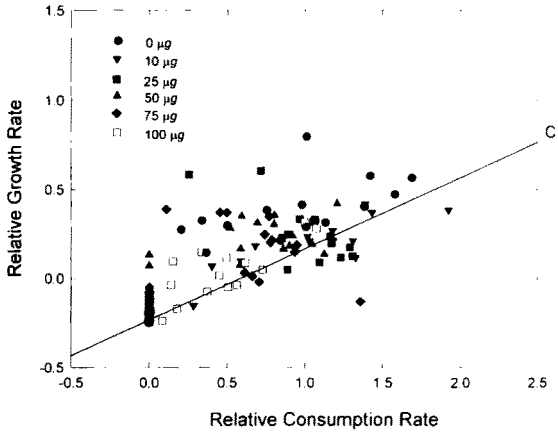


FIG. 2. Plot of the relative growth rate (RGR) on the relative consumption rate (RCR) for *L. decemlineata* L4 larvae fed for 24 hr on leaf disks treated with compound **1**. The line represents the calibration curve ($y = 0.371x - 0.220$, $r = 0.84$, $P < 0.0001$).

limonin (Alford et al., 1987). The activity of juglone was also stronger than limonin (42 and 2.2 times stronger against L4 larvae in choice and no-choice tests, respectively) (Alford et al., 1987). Therefore, commercial juglone can be considered an effective CPB antifeedant suitable for use as a positive control.

Compound **2** showed a strong and significant reduction in feeding and growth of *L. decemlineata* larvae with increasing concentrations, with 100%

TABLE 4. FEEDING INDEXES MEAN VALUES AND STANDARD ERRORS OF FOURTH-INSTAR CPB LARVAE ($N = 15$) TOPICALLY TREATED WITH 20 µg OF COMPOUNDS **1** AND **2**

Treatment	RCR[a]	RGR[b]
1	0.75 (0.03)	0.12 (0.01)
2	0.65 (0.07)	0.09 (0.02)
Control	0.72 (0.04)	0.13 (0.02)
F[c]	1.028	1.397
P	0.366	0.266

[a] As in Table 3.
[b] As in Table 3.
[c] See footnote *g* in Table 3.

feeding inhibition at the maximum dose tested (Table 5). The posttreatment larval relative growth rate decreased without following a dose response pattern and was significantly lower than the control starting from a dose threshold of 25 $\mu g/cm^2$ (Table 5). The chemical consumption of **2** peaked at 50 $\mu g/cm^2$, decreasing to zero at 100 $\mu g/cm^2$ (Table 5).

The growth efficiencies of the larvae treated with **2** were significantly lower than the starved control, also starting from a dose threshold of 25 $\mu g/cm^2$. We could not calculate the growth efficiency for the highest dose of **2** (100 $\mu g/cm^2$) since the corresponding RCR was zero (Table 5). For compound **2**, the linear relationships between RCR and RGR were not always significant (0 $\mu g/cm^2$: $r = 0.51$, $P = 0.003$; 10 $\mu g/cm^2$: $r = 0.91$, $P < 0.00001$; 25 $\mu g/cm^2$: $r = 0.61$, $P < 0.001$; 50 $\mu g/cm^2$: $r = 0.40$, $P = 0.001$; 75 $\mu g/cm^2$: $r = 0.22$, $P = 0.14$), and their slopes were lower than the slope of the calibration curve at a dose of 25 $\mu g/cm^2$ (Figure 3, Table 5), indicating that compound **2** has a toxic action.

TABLE 5. FEEDING INDEXES, GROWTH EFFICIENCY, POSTTREATMENT GROWTH AND CONSUMPTION MEAN VALUES AND STANDARD ERRORS OF FOURTH-INSTAR CPB LARVAE ($N = 15$) TREATED WITH COMPOUND **2**[a]

Dose ($\mu g/cm^2$)	RCR[a]	RGR[a]	post-RGR[a]	RC2[a]	GE[a]
0	0.62 (0.13)	0.32 (0.03)	0.52 (0.03)	0.00	0.320
10	0.64 (0.10)	0.20[a] (0.02)	0.46 (0.07)	0.05a (0.008)	0.350
25	0.33[a] (0.05)	−0.05[a] (0.01)	0.34[a] (0.02)	0.04a (0.007)	0.174[b]
50	0.18[a] (0.03)	−0.09[a] (0.01)	0.34[a] (0.03)	0.08b (0.014)	0.125[b]
75	0.05[a] (0.03)	−0.06[a] (0.01)	0.34[a] (0.02)	0.04a (0.02)	0.165[b]
100	0.00[a]	−0.01[a] (0.01)	0.34[a] (0.03)	0.00	
Starved control					0.358
F[a]	14.519	76.633	2.832	7.357	
P	<0.00001	<0.00001	0.020	<0.00001	

[a] See footnotes *a* to *g* in Table 3.
[b] Significantly different from the starved control (t-test, $p < 0.05$).
[c] Slope could not be calculated.

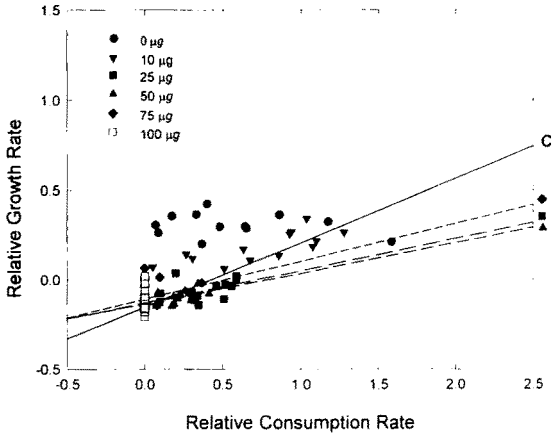


FIG. 3. Plot of the relative growth rate (RGR) on the relative consumption rate (RCR) for *L. decemlineata* L4 larvae fed for 24 hr on leaf disks treated with compound **2.** Represented are the lines of the calibration curve C ($y = 0.376x - 0.156$, $r = 0.82$, $P < 0.00001$) and the treatment lines with slopes significantly different from the control C.

Antifungal Activity: Both terpenes were tested against soil-borne (*Fusarium* sp.) and foliar (*Botrytis*, *Phytophthora*, and *Ascochyta* sp.) phytopathogenic fungi. They showed only mild activity against *B. cinerea* (EC_{50} values of 0.15 and 0.03 for **1** and **2**, respectively) and *A. lentis* (EC_{50} values of 0.19 and 0.33 for **1** and **2**, respectively). Juglone showed the highest overall antifungal activity (EC_{50} values of 0.007 and 0.04 for *B. cinerea* and *A. lentis*, respectively).

DISCUSSION

Two effective CPB sesquiterpene antifeedants have been isolated from *S. palmensis*. Compound **1** belongs to the chemical class of bisabolenes and has an antifeedant action lower than juglone but similar to limonin. Compound **2** is a silphinene sesquiterpene with an activity level similar to juglone and higher than limonin. Both compounds showed mild antifungal activity, suggesting a target-specific interaction.

Bisabolenes are a class of sesquiterpenoids mainly isolated from Asteraceae plants (Kreiser, 1991). In contrast, silphinenes have only been described in the genera *Cineraria* and *Callilepis* (Bohlmann and Zdero, 1982; Jakupovic and Abraham, 1985) and in *Silphium* sp. along with some bisabolenes (Bohlmann and Jakupovic, 1980).

This is the first report of an antifeedant silphinene (compound **2**). In con-

trast, some natural bisabolenes are effective antifeedants. For example bisabolangelone, isolated from *Angelica silvestris*, has strong antifeedant properties against several insect species including *L. decemlineata* (Muckenstrum et al., 1981; Nawrot et al., 1984).

Another bisabolene structurally related to compound **1**, zingiberene, appears to be responsible for the toxicity of the wild tomato plant *Lycopersicon hirsutum* f. *hirsutum* to CPB and represents a potential new source of insect resistance in the cultivated tomato *L. esculentum* (Carter et al., 1989). The search for structurally related natural bisabolenes could be a promising source of new leads for the development of CPB host-plant resistance.

These two compounds were more effective in choice that in no-choice tests, indicating behavioral avoidance, in addition to their deterrent effect. The greater effect that both compounds had against adult CPB indicates that these chemicals may alter the host selection process through behavioral avoidance by the adults, which are more mobile and are the primary finders of hosts (Hough-Goldstein, 1990).

Furthermore, compound **1** and the starvation simulation test both had a similar effect of reduction in the growth and feeding of the larvae, which suggests an antifeedant effect at the behavioral level. The association between larval feeding and growth reduction caused by compound **2** did not parallel that of the starved control, indicating both antifeedant and toxic effects. Additionally, larval contact with compound **2** was associated with detrimental effects. We also observed a decrease in larval posttreatment growth rate that did not correlate with chemical consumption. These observations suggest that the toxic action of **2** is not postingestive, in contrast to limonin, an antifeedant and toxic limonoid with a negative postingestive effect on *L. decemlineata* larvae associated with relative chemical consumption (Mendel et al., 1991). Compound **2**, with antifeedant and toxic activity, could be more promising than compond **1** as a control agent against CPB because both activities together require behavioral and physiological adaptations in order to develop resistance (Jermy, 1990). Further research is needed in order to understand the toxic mode of action of **2**.

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