The Repellence of *Aristolochia* aff. *orbicularis* Roots against the Corn Borer *Sitophilus zeamais*

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The repellence of Aristolochia aff. *orbicularis* root, a native of Xochipala, Guerrero, Mexico, to the corn borer *Sitophilus zeamais* (Coleoptera) was investigated. The essential oil was isolated from the aromatic root and its repellent effect was assessed. About 40 components of the oil were identified using gas chromatography-mass spectrometry and other spectroscopic methods. The repellence of the roots, the oil and the chromatography fractions were also evaluated. Some fractions had a higher repellence than the total oil.

Introduction

The intensification of agriculture is one of the predominant changes produced in this century. The use of high yield varieties, fertilization, irrigation and pesticides have contributed to the tremendous increase of food production in the last 50 years. However, these practices damage the ecosystem by altering the biotic interactions and this can produce unexpected local, regional and global consequences in the environment. The use of ecological methods for crop protection might increase the sustainability of the agricultural production, reducing the undesirable environmental unbalance.

Products of secondary plant metabolism may be responsible for the chemical communication between plants and insects. Volatile plant constituents have attractive or repellent properties to insects or microorganisms, show repellence or allelophatic activities. Allelochemicals have been considered as potential natural insecticides and can be used for insect management in integrated control (Jilani and Su, 1983; Qureshi *et al.*, 1986). Since most insecticides are toxic not only to pest insects, but also to the natural enemies of pest species, the study of plant constituents that might contribute to avoid the use of insecticides for pests control is important.

The genus *Aristolochia* includes about 500 species, native to tropical, subtropical and Mediterranean regions. Among the species of *Aristolochia*, many aromatic plants are found. The terpenic lactones aristolone and aristolactone are volatile compounds of *Aristolochia* species (Kasuhito *et al.*, 1992; De Pascual, 1983). Aristolochic acid, another constituent of members of this genus, is a nitrophenanthrene derivative that has long been used in traditional medicine (Hegnauer, 1966).

Infusions or suspensions of some *Aristolochia* species have been used as natural insecticides for example, *A. bracteata* for flies (*Musca domestica*) insecticide and *A. brasiliensis* for cockroaches (*Periplaneta americana* and *Blatella germanica*) (Arenas, 1992). The interactions of other *Aristolochia* species with insects was assessed by Lajide *et al.* (1993) who studied the antifeedant activity of *A. albida* to the tobacco cutworm *Spodoptera litura* (Lepidoptera) using a leaf disc choice bioassay. These researchers found antifeedant activity in the extract of *A. albida* at 0.1% concentrations: methyl aristolochiate and 6-hydroxyaristolochiate prepared by diazomethane treatment of the acids, had lower activity than the extract, and azadirachtin, a known insecticide from *Azadirachta indica* (Indian neem tree) revealed comparative levels of activity (Zanno *et al.* 1975; Tomlin, 1994). Hayashi *et al.* (1987) found that the volatile components

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of *Aristolochia debilis* were attractants to *Byasa alcinous* (Lepidoptera), *Lueddorfia japonica* (Lepidoptera) and *Hebomoia glaucippe* (Lepidoptera).

Aristolochia aff. *orbicularis* (Durch) grows in Xochipala, the central part of Guerrero, Mexico. Local inhabitants use the roots of *A.* aff. *Orbicularis,* which have an intense aroma, as an analgesic for stomach ailments. Roots of this species growing in different localities are thought to have different curative activity. Roots are the most aromatic part of the plant. In Xochipala, they are also considered insecticidal.

Sitophilus zeamais is one of the main stored maize infestants. The effect of *A. orbicularis'* roots and essential oil as repellent of the corn borer, *Sitophilus zeamais* was studied in this work.

Materials and Methods.

Aristolochia aff. *orbicularis* (Durch) was identified by Dr. F. González Garavito, from the Institute of National Sciences, Bogotá, Colombia. Specimens of the plant are found in the National Autónomous University Botanical Gardens (herbarium voucher AG1636) and at the School of Sciences, (herbarium voucher AG1881). The roots of the material for this study were collected from two zones in Xochipala, Guerrero, Mexico: "Encinal", on the highlands, and "Lagunitas", at a lower altitude. The air-dried roots were grounded in a mill and the powder was used in all experiments.

Rearing of insects

Males and females of *Sitophilus zeamais* (200 specimens), were gathered from infested maize cobs found in a crop field in Xoxocotla, Morelos, México. Insects were kept in 5 liter containers and fed with "cacahuazintle", a soft variety of maize. After 60 days, the second generation of insects was isolated. The healthiest insects were selected to carry out the bioassays.

Bioassays

Repellence bioassays were carried out with *Sitophilus zeamais* adults using a glass olfactometer (Bestmann *et al.*, 1991) consisting of a Y tube 23 cm length, with a ground glass joint attached to a 7 cm test tube which contained the insects at the beginning of the experiment. The ground glass joint connection allows the researcher to exchange the insects easily. The tube was placed horizontally. One of the Y branches contained the test sample covered with filter paper and the other branch contained only a piece of filter paper. Insects were placed in the base of the olfactometer and allowed to enter the opened branches of the olfactometer during 30 min. In another series of experiments carried out with root powder, both branches of the olfactometer were connected to Kitasato flasks full with maize. Twenty insects were used for each root sample, oil or oil fraction. At least 5 bioassays were carried out for each root sample, oil concentration or oil fraction.

Oil fractions were tested using oil solutions in *n*-hexane (1 mg/ml); 0.8 ml of this solution were placed on filter paper in one of the olfactometer branches and pure hexane in the other. The bioassay was carried out after evaporation of the hexane.

Repellence indexes expressing the ratio between repelled and attracted insects were calculated. Five double solvent and blank control experiments were carried out with *S. zeamais* in an empty olfactometer showing that in 30 min, 91.2+ 0.25 of the insects distribute statistically in both branches of the olfactometer. The remaining of the insects (8.8%) moved very slowly, or did not react, staying in the olfactometer base.

Volatile extraction

Samples of 80–100 g of dried, ground roots of *Aristolochia* aff. *orbicularis* were subjected to simultaneous steam distillation-extraction in a modified Shultz *et al.* (1977) apparatus for 30 min. Dichloromethane was used as the extraction solvent.

Chromatography and spectroscopic analysis

Column chromatography was carried out with 0.8 g essential oil on a silica gel 60, (70–230 mesh) column using hexane as eluting solvent. Fractions of 50 ml were collected. Silica gel 60F-254 on aluminum thin layer plates were used to monitor the separation. Visualization was carried out with UV 254 and or 336 nm light. Gas chromatography was carried out with a Varian 3300 instrument using a DB-5 phenylmethyl silicon capillary DB-5 column. The temperature of the column was programmed from 50 to 300 °C at 5 °C/min.

Gas chromatography-mass-spectrometry analyses were carried out in a Finnigan MAT 90 with an ICIS data system. SE30, SE52 and SE54 capillary columns all 25 m in a gas chromatograph (Varian 300) were used. Helium at 22 cm^3/sec was used as a carrier gas.

Gas-chromatography-IR spectroscopy was carried out in a Bruker IFS48 apparatus with a Carlo Erba GC 600 Vega 15 m fused silica DB-5 glass capillary column, film thickness 1 μm.

NMR spectra were carried out with a JEOL JNM-LGXx400 FT-NMR or with JEOL PS 100 instruments.

Results and Discussion

Preliminary bioassays to determine the optimal dose of *Aristolochia* aff. *orbicularis* roots collected in the Encinal zone, showed that 60 mg of the root produced the highest repellence index (8.5) (see Table I). Since controlled experiments showed that more than 90% of the insects go to both branches of the olfactometer, we considered that insects staying in the olfactometer base as repelled by the root. In fact, a careful observation of the experiment showed that some insects entered the "root branch" and then returned to the base of the olfactometer apparently without being affected by the root. Repellence indexes were calculated considering that 90% of the insects in the base of the olfactometer were repelled insects.

The repellence index (Table I) increases as the dose of root increases from 0.015 g to 0.060 g remains almost constant if the dose is 0.120 g but diminishes at higher doses. The presence of attractive compounds along with the repellent compounds in the roots might explain the results: since a mixture of volatile compounds is present in the root, at higher doses some attractive compounds might manifest their activity. Experiments carried out with the same amounts of the essential oil isolated from dry and fresh roots, which showed a higher repellence index for the dry roots oil (8.7) than for fresh roots (5.6), seem to support this proposal.

Some experiments were carried out to evaluate whether or not the effect of the roots on the insects is manifested even in the presence of maize. To this end, both branches of the olfactometer, one of them carrying 0.060 g of root powder, were connected to Kitasato flasks filled with maize. Five bioassays were carried out, using 20 insects for each bioassay. Results were as follows: 2+0.2 insects went to the branch with the root sample (attracted insects), 15+ 4 insects went to the empty branch (repelled insects) and 2+ 0.4 insects stayed) in the olfactometer base (90% were considered repelled insects). The calculated index was 8.7. These results showed that the effect of the roots remains even in the presence of maize, which attracts the insect.

Volatile oil

Roots of *A. orbicularis* were collected from two zones in Xochipala. The amount of oil in the Lagunitas dry root was 1.45%, and the Encinal's dry root had 1.05% of essential oil. The oil isolated from fresh materials constituted around 0.2% of both roots. The root with a larger amount of essential oil, which was collected in a region of lower altitude, produced a higher repellence to the insects. These data seem to indicate that the volatile oil is responsible of repellence of the root.

Gas chromatography-mass spectrometry was used to identify some of the components of the oil. Percentage, Kovats index, and the identification method of the most abundant compounds are shown in Table II. The oil contained mainly mo-

Table I. Effect of different *A. orbicularis* root doses on *S. zeamais.**

Dose (g)	Number of Insects/test	Insects in the root branch	Insects in the blank branch	Insects in the olfactometer base	Repellence index
0.015	20	4.2+1.5	13.2+2.2	2.6+1.2	3.7
0.030	20	4.4+2	10.6+3.2	5+1.6	3.43
0.060	20	2+1.2	8+1.7	10+2.1	8.5
0.120	20	2.2+1	13.8+2.2	4+2.1	7.9
0.180	20	5+2	3.4+2.3	11.6+2.2	2.76
0.240	20	4.4+3	9.6+2.3	6+1	3.4

* Each data represents the result of at least 5 experiments.

Table II. Composition of the of *Aristolochia* aff. *orbicularis* volatile oil.*

Peak Number	Compound	Percentage**	Retention index	Identification method
1	camphene	traces	944	Ms
2	β-pinene	traces	979	Ms
3	6-methyl-5-hepten-2-ol	traces	990	Ms
4	limonene	traces	1024	Ms,ri
5	cineol	0.62	1025	Ms,ri
6	α-terpinolene	traces	1085	Ms,ri
7	2,4-dimethyl-2,4-heptadienal	traces	1130	Ms
8	canphene hydrate	traces	1140	Ms
9	borneol	7.32	1159	Ms,ri,IR
10	terpinen-4-ol	0.24	1172	Ms,ri
11	α-terpineol	0.35	1188	Ms,ri
12	$C_{10}H_{18}O$ alcohol	5.56	1200	Mc,IR
13	β-cyclocitral	traces	1220	Ms
14	bornyl formiate	5.24	1222	Ms
15	bornyl acetate	2.60	1282	Ms,ri,IR
16	jasmone isomer	traces	1292	Ms
17	jasmone	0.10	1317	Ms
18	cis-carvil acetate	3.66	1328	Ms,ri,IR
19	δ-elemene	0.20	1334	Ms,ri
20	terpinyl acetate	2.35	1347	Ms,ri,IR
21	α-cubebene	traces	1350	Ms,ri
22	ylangene	0.66	1366	Ms,ri
23	trans-carvyl acetate	0.71	1372	Ms,ri
24	α-copaene	traces	1372	Ms,ri
25	β-bourbonene	traces	1386	Ms,ri
26	β-elemene	1.06	1388	Ms,ri,IR
27	caryophyllene isomer	traces	1402	Ms
28	caryophyllene	5.24	1413	Ms,ri,IR
29	β-guriunene m.w.180	traces	1426	Ms,ri
30	dihydro-α-ionone	traces	1435	Ms
31	δ-guaiene	2.32	1440	Ms,IR
32	α-Ionone	traces	1440	Mc
33	dihydro-β-ionone	traces	1445	Ms
34	humulene	0.40	1450	Ms
35	γ-muurolene	traces	1475	Ms
36	germacrene	9.37	1478	Ms,ri,IR
37	β-ionone	5.97	1486	Ms,ri,IR
38	α-elemene	0.91	1488	Ms
39	cadina-4–9-diene	0.66	1505	Ms
40	γ-cadinene	2.40	1511	Ms,ri,IR
41	δ-cadinene	1.00	1517	Ms,ri

* (**ms** stands for mass spectra., **mc** stands for coupled chromatography-mass spectrometry, **ri** stands for retention index, **IR** stands for infrared spectra.

** Weight % of the named compound in the whole essential oil.

noterpenes and sesquiterpenes. The major oxygenated monoterpenes that could be identified were borneol (11), bornyl formiate (16), and bornyl acetate (22). In addition we also found carvyl acetate, terpenyl acetate and trans carvyl acetate.

A larger amount of sesquiterpenes than of monoterpenes was found: germacrene (50) (9.4%) as the main component of the oil, followed by caryophyllene (38) (5.2%) and cadinene (57) (2.40%). Besides β-ionone (51), other ketones were found: dihydro-α-ionone (42), α-ionone (44) and dihydro-β-ionone (46). Other phenylpropanoids found as components of other *Aristolochia* species oil components, for instance, eugenol, isoeugenol, apiol, safrol, were not found in the oil of *A.* aff. *orbicularis.*

The repellence effect of 0.1 mg/ml and 0.01 mg/ml solutions of the essential oil in hexane towards *S. zeamais* showed repellence indexes of 5.26 and 5.55 respectively.

Using column chromatography of the essential oil, nine oil fractions were prepared, that had different effect on the insect. The fraction which produced the greatest repellence was fraction 2 (R_f = 0.16–0.31) (repellence index = 25.6). The major components of this fraction are presented in Table III. The fraction consists mainly of β-ionone (51) (43.9%), α-ionone and their hydrogenated analogs: dihydro-β-ionone (46) and dihydro-α-ionone, *cis*-and *trans*-carvyl acetates and terpinyl and bornyl acetates.

Samples of pure β-ionone (51) and bornyl acetate (22) were tested as repellents using bioassays as before. Their activity was much weaker than the activity of the complete fraction. A mixture of (51) and (22) was also tested and its activity was also weaker than those of the complete fraction. These results showed that the most abundant components alone are not responsible of the repellence effects.

The volatile components of other Aristolochiaceae have been previously studied: Sagrero Nieves *et al.* (1993) identified the volatile constituents of *A. asclepiadifolia*. Linalool and its oxides, borneol and its oxides, and isomers of guaiol were the major components, accompanied by bisabolol, ledol, terpineol, limonene and its oxides; vainillin was a minor component.

Leitao *et al.* (1991), on the other hand, studied several Brazilian Aristolochiaceae and found caryophyllene and germacrene as abundant constituents in *A. birostris* and *A gigantea*, humulene in *A. birostris* and *A. papilaris*; geraniol, nerolidol, linalool, nerol, and farnesene were also found in *A. gigantea*. In the last plant they also reported trans α-begamotene. They found calarene and cadinenes in *A. papilaris* and α- copaene in *A. rodriguesia*. Limonene and phelandrene were in *A. ringens* and bisabolene in *A. triangularis*.

Two new pinane monoterpenes: the alcohol and the acetate derived from pinene and other known compounds together with calarene, maaliol and 1(10 aristolen)-2-one were isolated by De Pascual *et al.* (1983) from *A. longa*, and Priestap (1990) found 20 components, predominantly sesquiterpenes, in *A. triangularis*, an Argentinian plant.

The possible uses of *Aristolochia* aff. *orbicularis* as a natural control medium of other stored grain pests is to be studied.

Table III. Composition of the most attractive A. orbicularis oil fraction*.

Peak Number	Compound	Percentage**	Index	Identification method
8	camphene hydrate	0.35	1140	Ms
9	borneol	3.12	1159	Ms,ri,IR
13	β-cyclocitral	0.50	1220	Ms
14	bornyl formiate	2.37	1222	Ms
15	bornyl acetate	3.73	1282	Ms,ri,IR
18	*cis*-carvil acetate	8.05	1328	Ms,ri,IR
20	terpinyl acetate	1.55	1347	Ms,ri,IR
23	trans-carvyl acetate	1.74	1372	Ms,ri
30	dihydro-α-ionone	0.11	1435	Ms
32	α-ionone	0.55	1440	Mc
33	dihydro-β-ionone	4.00	1445	M
37	β-ionone	47.92	1486	Ms,ri,IR

* Abreviations as in Table II.
** Weight % of each compound in the most attractive oil fraction.

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