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Repellency and feeding deterrence activity of *Ageratum Conyzoides* against the stored grain pests *Tribolium Castaneum* and *Sitophilus Oryzae.* Active plant parts and composition*

Miguel Enrique Alonso Amelot**, Marisabel Avendaño, Lianne Aubert, and Jorge Luis Avila

Grupo de Química Ecológica, Departamento de Química, Facultad de Ciencias, Universidad de Los Andes, Mérida 5101, Venezuela.

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Abstract

The anti-insect materials, expressed as contact repellents, feeding inhibitors and modificators of the eficiency of conversion of ingested material (ECI), contained in the forb *Ageratum conyzoides* L. (Magnolionidae, Asteraceae) against the grain pests rice weevil *Sitophilus oryzae* and red flour beetle *Tribolium castaneum*¸ were studied by exhaustive extraction in hexane, dichloromethane, ethyl acetate, methanol and water of separate plant parts and the essential oil of fresh leaves. The primary activity in all orders was found in the low polarity extracts, and chiefly concentrated in the material stemming from the leaves. Bioassay-guided chromatographic isolation of the feeding deterrents yielded the known chromene precocene II as the sole active component. Antifeedant activity of this compound was quantitized as DE_{50} = 1.36 µg/mg in *S. oryzae* and 1.11 µg/mg for *T. castaneum*. The ecological status of *A. conyzoides* as a plant with semiochemical defenses is discussed.

Key words: *Ageratum conyzoides*; antifeedants; chemical ecology; contact repellents; precocene II; *Sitophilus oryzae*; *Tribolium castaneum*.

Repelencia e inhibición alimentaria causada por *Ageratum Conyzoides* sobre los insectos-plaga de granos almacenados *Tribolium Castaneum* and *Sitophilus Oryzae.* Partes activas de la planta y su composición

Resumen

Los materiales anti-insecto, expresados como repelentes al contacto, inhibidores alimentarios y modificadores de la eficiencia de conversión del material ingerido (ECI), contenidos en la herbácea *Ageratum conyzoides* L. (Magnolionidae, Asteraceae) contra las plagas de granos almacenados picudo negro del arroz *Sitophilus oryzae* y gorgojo pardo de las harinas *Tribolium castaneum* se estudiaron por extracción exhaustiva en hexano, diclorometano, acetato de etilo,

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** Autor para la correspondencia. E-mail: alonso@ciens.ula.ve

metanol y agua de partes separadas de la planta, y del aceite esencial de las hojas. La actividad principal en todos los órdenes se encontró en los extractos de baja polaridad y en aquellos provenientes exclusivamente de las hojas. El aislamiento cromatográfico guiado por bioensayo de los fagodepresores, condujo al precoceno II, cromeno conocido, como único material activo. Su actividad antialimentaria se cuantificó como DE_{50} = 1,36 µg/mg en *S. oryzae* y 1,11 µg/mg para *T. castaneum*. Se discute el estatus ecológico de *A. conyzoides* como planta defendida por materiales semioquímicos.

Palabras clave: *Ageratum conyzoides*; fagodepresores; precoceno II; química ecológica; repelentes de contacto; *Sitophilus oryzae*; *Tribolium castaneum*.

1. Introduction

Ageratum (Magnolionidae, Asteraceae) is a genus of weed forbs composed of several species, with two main representatives in Venezuela, *A. houstonianum* and to a much greater extent *A. conyzoides*. Although these plants originated in the neotropics, some of them such as *A. conyzoides* have spread all across the tropical band of the world in natural and managed ecosystems and may be found as far as China. *A. conyzoides* is present in Venezuela in mountain ranges near the northern coast, central Anzoategui state and humid enclaves of the Andes.

The fact that the aerial parts of *A. conyzoides* have been used in traditional medicine (1-3) has provided impetus for its chemical examination from which several chromenes, one of them unique, sesamin, and caryophylene (4-6), two novel flavones (7, 8) in addition to twelve other known flavones (7, 9, 10), two pyrrolizidine alkaloids (11), aurantiamide acetate (12) and benzofuran (13) have been isolated and characterized from its aerial parts. The phytochemistry of *Ageratum* has been reviewed recently (14). Also, from aqueous to hexane extracts of *A. conyzoides* several physiological properties have been observed, such as muscle myorelaxant (15), cardiac (16), antibacterial (17), antiserotonergic on isolated uterus, bronchodilating (18), antiinflamatory, and analgesic (19, 20) as well as other effects (21, 22). However, to no particular compounds have this bounty of curative virtues been attributed yet.

On the other hand, a strong ecological impact of *A. conyzoides* is also on record, chiefly examined from its problematic weed status in various places (23). In this matter, its phytotoxic potential has been demonstrated (24-27). Inhibition of germination of cultivated seeds is due in part to various volatiles contained in leaves which impart a characteristic odour to the plant but leaching by rain on leaves also has been invoked (27).

Some information on anti-insect activity of *A. conyzoides* also exists on a limited numer of insect species (1, 28) but remains to be explored systematically. *Musca domestica* appeared to be affected by various extracts but the activity disappeared during chromatographic separations. Also, a larvicidal effect on *Culex quinquefasciatus*, the vector of Dancroftian filariasis in India was observed at high concentration of hexane-soluble leaf extracts whereas at lower concentration some developmental defects were induced (29). Fragmentary reports on the insecticidal properties of extracts from this plant on *Drosophila melanogaster, Dysdercus cingulatus, Rhodnius prolixus* (30), *Locusta migratoria* (31), *Sitophilus oryzae* and *Tribolium castaneum* (32, 33) have been consigned but the great majority of these accounts are personal communications to book editors or technical reports of limited circulation and inadequate data.

Very recently, however, (34) the anti-insect effect of the essential oil extract of *Ageratum conyzoides* from Cameroon was studied. It was found to be insecticidal to the maize weevil *Sytophilus zeamays* at low dosage (LD_{50} = 0.09% with respect to grain weight or 0.9 g per kg in 24 h). Unfortunately, the composition of the oil was not determined there but the prevalence of precocene I, precocene II, beta-caryophyllene, gamma-bisabolene, 3,3-dimethyl-5-tert-butylindone and fenchyl acetate in the essential oil extracted from plants growing in three differing habitats in China (27) allows the presumption that these compounds might be present also in the African samples and at least one of them is the purported active material. Indian researchers independently identified 2-(2'-methylethyl)-5,6-dimethoxybenzofuran (5), and (Z)-6-methyl--12-heptadecenoic acid (35) from the essential oil of *Ageratum conyzoides* collected in their geographical region. The latter was characterized as an insecticide for the gregarious desert locust *Schistocerca gregaria*. Besides mortality, the compound also causes disturbances at the nymphal-adult molt, resulting in short fore-wings deformity due to incomplete expansion. These findings imply that *A. conyzoides* synthesizes an array of semiochemical or xenobiotic materials that require bioassay-guided chemical and biological characterization.

Our own interest in plant extracts for the control of insect pests (36-39) led us to such task, and results are now forthcoming. Thus, this work was aimed at: 1) the quantitative characterization of antiinsect effects of *A. conyzoides* essential oil and crude extracts; 2) the isolation and structural determination of the responsible compounds under insect antifeedant bioassays guidance and 3) the assessment of the anti-insect value of the isolated material, against red flour beetle *Tribolium castaneum* Herbst (Coleoptera, Tenebrionidae) and rice weevil *Sitophilus oryzae* L. (Coleoptera, Curculionide), both pests of cereals very common, economically important and difficult to control in the field and under storage.

2. Materials and Methods

a) Biological materials

A. conyzoides whole plants in flowering stage were collected on highly watered soil from Loma San Antonio, 9° 30'N, 71° 10' W, a hilly terrain 5 km south of the city of Mérida, western Venezuela at 1950 m asl on September (peak of flowering season) 1994 and 1998. Voucher specimens are kept at the laboratory herbarium under the key LQE-41. The plants were separated in twigs, leaves, roots, and flowers, spread in wire trays and dried by forced ventilation at room temperature for one week.

Insects: T. castaneum used in these experiments were laboratory cohorts descendants of colonies established from infested cornmeal collected in 1988 and resistant to malathion. *S. oryzae* adults were derived from insects amassed over a three year period (1987-1990) and various dried food sources. Insect taxonomy was determined using existing keys. Insect handling procedures followed modern literature recommendations (43). Unsexed cohorts of 50 *T. castaneum* freshly emerged adults were placed in 500 mL glass jars containing ca 200 g of white wheat flour, with 5% wheat germ, capped with cheesecloth and allowed to feed, copulate and lay eggs undisturbed for 60 days in a growth chamber with environmental control at 28 ± 1°C, 60 – 70% relative moisture and 12 / 12 h light / dark regime. After this time, most of the larvae had emerged into new adults that were used for reproduction by crossing with adults from a different lineage and for bioassays before they were 18 days old. *S. oryzae* were produced similarly but on whole corn, wheat or barley kernels without additional nutrients.

Bioassays: contact repellency: A "sandwitch plate" was used as test arena. This is constructed from two silk paper circles 8 cm

diameter that were dosed on 50% of the surface (one half, 25.2 cm^2) with acetone solutions of extracts, purified materials or essential oil with the aid of a microsyringe (6.3 mg of solute in 100 µL of acetone), so that a concentration of 250 µg/cm^2 was obtained. The remaining half was treated only with acetone. The solvent was allowed to evaporate under ventilation at room temperature. The dried paper disks were placed so that treated and untreated surfaces were facing each other, separated by an aluminum plate 2 mm thick with a centered circular opening 7 cm wide, thus defining a 7 cm circular chamber for insects to perform without possible escape from the paper surfaces laying above and underneath. On both sides of the array, glass plates secured with rubber bands were used to procure an air tight translucent enclosure, after ten unsexed 10-18 days old insects were placed in the working chamber. Five replicates of these "sandwitch plates" for each experiment were set up, plates thus prepared were placed in the dark at 22°C and the position of the insects in treated and control areas of the discs was recorded with minimal perturbation under dim red light, every hour for 6 hours and then 24 h later.

Feeding deterrency, one and two-way assays: the procedure of Alonso-Amelot et al. (37) using flour disks was employed. In two way assays where the choice capacity of insects confronted with treatments and controls simultaneously may be measured, ten unsexed 10-12 days old insects were exposed to a weighed treated disk dosed with a fixed concentration (50–250 µg/cm²) and a control disk with the corresponding solvent, both previously ventilated at 30°C and a forced air current to allow solvents to evaporate completely. After a 60 h feeding period when insects remained undisturbed in the dark (28±1°C) the remainder of disks and insects were weighed and discarded. The Preference Index PI was calculated using equation [1].

$$PI = C(T) \times 100 / [C(T) + C(C)] \quad [1]$$

where C(T) and C(C) are the amount in milligrams of treated and control flour disks consumed, respectively. PI is therefore dimensionless.

Knowing the dosage and the amount of treated disk consumed it was possible to determine the amount of plant material ingested by individudal insects.

In the one way assay the test was performed like the two way assay above except that insects were exposed to only the treated flour disk. Without an edible choice insects face starvation if the treatment is fully rejected, and therefore may be forced to accept the treatment. No preference index was calculated of course. Rather, from the change in insect weight and disk consumption it was possible to estimate the capacity of the ingested plant material along with the feed to disturb insect metabolism, by way of the ECI or eficiency of conversion of ingested food, according to Waldbauer (40) and our own modification in the application to *T. castaneum* adults (37). ECI is calculated by equation [2].

$$ECI = DW / C(T) \quad [2]$$

where W is the body weight gain (mg) during the feeding period and C(T) is the amount (mg) of feed disk accepted. ECI is also dimensionless but reflects the proportion of body weight gain relative to the ingested material, and thus the efficiency of conversion of food into biomass.

b) Extraction and isolation procedures

Three separate extraction procedures were practised. First, whole air dried plants were utilized in a first effort to detect biological activity and a general chemical characterization. Then each plant part was extracted separately to determine the localization of active materials in the plant anatomy. Finally, for the extraction of the essen-

tial oil only fresh leaves and stems were used.

1500 g of ground dried whole plants were extracted in a 6 L Ehrlenmeyer flask and moderate heat in a ultrasound bath with three 2 L portions of solvents for 2 h per batch. Solvents employed in succession were: hexane, methanol, ethyl acetate, dicloromethane and water. After solvent evaporation *in vaccuo* 2.81, 0.73, 0.62, 1.38, and 9.38% of crude extract relative to dry plant weight, respectively. Separations by column flash chromatography and thick layer chromatography were performed on silica gel as stationary phase and organic solvent gradients, and the activity of extracts and purified materials were followed by the antiisect bioassays to be described below. The active hexane extract from 1500 g of dried leaf material was flash chromatographed using TLC grade silica gel through a Buchner funnel and a hexane-ethyl acetate gradient, and separated into four fractions A-D: (A): 13.14 g; (B): 1.61 g; (C): 29.58 g; (D): 0.145 g. Fraction (C) containing all the antifeedant activity was again partitioned through the same stationary phase and solvents, yielding three active fractions: (A'): 0.33 g, 8-14% feeding inhibition; (B'): 1.42 g, 14-15% inhibition; (C'): 1.57 g, 99% inhibition. Further chromatography of C' gave 5 fractions of which the fourth (d", 0.600 g) caused >99% feeding inhibition in one way assays on *S. oryzae*. Thick layer chromatography of d" gave a homogeneous band (160 mg) that was fully inhibitory to feeding in both insect species at 6.25 µg/mg of flour disk. From there Precocene was isolated as an amorphous solid (41, 42). ^{1}HRMN (300 MHz, d, ppm) ($CDCl_3$): 1.40 (s, 6H, 2 x methyl), 3.81 (s. 3H, OCH_3), 3.83 (s. 3H, OCH_3), 5.47 and 6.23 (d, 2 x 1H, J = 2.5 Hz, AB-vinyl system), 6.41 and 6.52 (s. 1H, aromatic protons), ppm. The extraction procedure in hexane was repeated for each plant part and after prepurification of the crude extracts using a short path silica gel flash column chromatography and elution with hexane-dichloromethane, the eluted materials were bioassayed as indicated above.

Essential oil: 0.89 mL (0.08%) of slightly greenish essential oil from about 1050 g of *A. conyzoides* fresh leaves and stems was obtained by hydrodistillation after 24 h using a Clevenger separation trap. The oil was used for biotesting without further purification.

Chemicals and apparatus: all solvents were purchased from J.T. Baker in bulk and fractionally distilled prior to use. Chromatographic supports were obtained from Merck Darmstadt, infrared spectra were determined using a Perkin Elmer System 2000 FTIR spectrometer and nuclear magnetic resonance experiments were performed in a Brucker 300 MHz spectrometer located at Laboratorio Nacional de Resonancia Magnética Nuclear, node IVIC, Caracas.

Statistical calculations were performed using the Statistix Analytical Software V. 4.0, St. Paul, Minesota. For the comparison of the means wherever necessary, the Tukey's (α = 0.05) and Kruskal-Wallis nonparametric tests were used. Curve fitting was calculated by use of Microcal Origin V 4.0, Microcal Software Inc, Northampton, Massachussetts.

3. Results

Contact repellency: Insect preference for treated paper surfaces vs controls in the two way sandwitch plates assays differed greatly depending on insect species and plant extract, ranging from no appreciable activity in the polar end to maximum activity in the lipid extract. *Tribolium castaneum* and *Sitophilus oryzae* showed equal aversion for the treated field in the dichloromethane extract along all the 24 h observation period (Figure 1b), so no habituation behavior was observed. However, *S. oryzae* was more resilient in the ethyl acetate and methanol extracts of *A. conyzoides* than *T. castaneum*, which after 24 h escaped fully from the treatment (Figures 1c and 1d). In the end, it was the hexane soluble material

that caused the greatest repulse to both insect species early on in the observation period (Figure 1a). Preference indexes PI for these extracts measured in the two way repellency tests clearly showed the increased tendency towards repulse from the polar to the non polar plant materials (Figure 2). However, one way contact assays using the sandwitch plates led to marginal mortality only. Similar results were obtained with the essential oil at elevated concentration (250 $\mu g/cm^2$).

Feeding assays of crude extracts. Two way choice tests: flour disks were dosed with 250 $\mu g/cm^2$ of *A. conyzoides* crude extracts prepared as indicated above and presented simultaneously to insects along control disks of the same size. All extracts were markedly rejected in favor of the control disks, affecting specially *S. oryzae*, as Figures 3A and 3B show. In this insect, the preference index (PI) did no surpass the 0.04 mark meaning that only 4% of the treated disk relative to the control was acceptable to the insects. Moreover, the ethyl acetate extract elicited complete rejection in the *S. oryzae* case. The treatments were, however, rejected to a lesser extent by *T. castaneum* (Figure 3a), showing a different pattern. Not only there was less aversion towards the ethyl acetate extract of *A. conyzoides* but it was the non polar end (hexane and dichloromethane) that caused the greatest effect, as did the essential oil whose composition, albeit undetermined, was inferred to contain active components of similar nature. However, no mortality was recorded.

One way flour disk feeding deterrency assay: In these tests, insects are presented only with treated disks and face starvation if the feed is refused, so conditions for rejection are more strenuous and life threatening for the test animal, and therefore provide more information as regards to the behavior of the insect pest in its natural environment, a grain storage in the case of our model insect species. With *S. oryzae*, the pattern of treated feed acceptance changed starkly relative to the two way assay (Figure 4). Not only the water extract appeared not to affect the feeding activity of this insect, but as the plant materials became less polar the feeding inhibition increased dramatically. 250 $\mu g/cm^2$ or 1.25% of hexane and dichloromethane crude extracts on the disks caused 98% feeding inhibition in the rice weevil (Table 2). Although no significant mortality was recorded during the feeding period, such low levels of food intake were likely to force the demise of the insect population shortly thereafter. The assay with this insect species cannot provide other feeding variables because *S. oryzae* feeding pattern may be described as largely exploratory. That is, the insects disperse the material from the disk (or grain) and ingest only part of the separated feed, leaving crumbs and fragments uneaten.

Assays with *T. castaneum*, nonetheless, were of more significance to feeding parameters, as this insect only separates from the feeding disk whatever it actually ingests, allowing for the calculation of ECI and from there the assessment of physiological stress induced by the feeding treatment. Phagoinhibitory experiments committed to this end gave the results of Table 3. It became evident not only that the polar material did not cause any inhibition but rather it was phagostimulatory, with as much as 141% increment in the feeding activity. This not only indicated a greater tolerance of plant extract solutes by the insect and the probable presence of glycosidic feeding elicitors or cues, but also that there was a net body weight gain of 6% during the 60 hours of the experiment. As opposed to this, low polarity solutes from *A. conyzoides* forced 97-99% feeding inhibition which led to a net loss of about 7% of insect body weight, where the viability of the insect is seriously compromised. Notably, the ethyl acetate extract induced a moderate feeding inhibition, that was compensated with a 36% increment in the conversion of the ingested food into insect biomass (ECI), whose end result was a net gain

of 5% in body weight, comparable to that of the control. Compounds there contained may facilitate the digestion and/or absorption of nutrients in the disk or stimulate the metabolism of enteric bacterial symbionts in the insect gut. The essential oil at the same concentration caused moderate insect weight loss, and some degree of physiological stress as evidenced by the 50% decrease in ECI.

At this point it was necessary to proceed either with the essential oil or the extracts to isolate the active components. To make a decision in this respect, a dose-response study of the oil in the 2.5% - 0.31% concentration range relative to the feed disk was undertaken with the results of Table 4. The weakness of the response was interpreted as a high dilution of the possible active components in this material, as opposed to expectations in view of early experience recorded elsewhere.

It was thus clear that the fraction of anti-insect activity, expressed as contact repellency and feeding deterrency, was concentrated in the hexane-soluble fraction. It remained to be established which part of the plant, if any specific one, contained the maximum activity. Hexane extracts of root, reproductive organs, twig and leaf excisions were obtained separately, dosed on flour disks at 250 µg/cm^2 and subject to the one way feeding test against *T. castaneum*. As Figure 5 shows, only leaves elicited strong feeding inhibition whereas there was no difference in response between the rest of the plant parts and the controls. *S. oryzae* was similarly inhibited in its feeding activity of flour disks (Figure 5B).

The hexane extract of 1500 g of plant leaves gave 29.58 g of solutes (1.97%) Successive chromatography in column and flat layer arrays of 3.32 g of this material yielded a series of increasingly active fractions until a single compound (160 mg, 0.095%) was isolated. The very simple spectral data (see materials and methods section) was compatible only with the structure of the known chromene precocene II (41, 42).

The phagoinhibitory effect of this compound was examined at various concentrations in the 10 to 500 µg per cm^2 of disk and was found to vary marginally with beetle species. Thus, *S. oryzae* showed a sharp drop on feeding activity as soon as precocene II was added to the diet disk. The ED_{50} could not be calculated by standard logit/probit analysis as the dose-response curve did not follow a linear regression but an exponential decrease sequence (Figure 6a) which was best described by equation [3].

$$y = e^{a+bx} \quad [3]$$

where y = feeding amount (mg/insect)

x = dosage (µg/mg of feed)

parameters:

a = -0.07739

b = -0.38678

X^2 = 0.02977

$$x = (\ln y - a) / b \quad [4]$$

Application of the solved equation for **x** when **y** = 0.546 mg/insect [4] or 50% of the ingested control disk gave DE_{50} = 1.36 µg/mg in *S. oryzae* or 0.136% for precocene II. Similarly, *T. castaneum* showed an exponential decrease feeding behavior (Figure 6b) governed by equation 1 with the following parameter values:

a = -0.37505

b = -0.56976

χ^2 = 0.00146

Hence, the effective dosage was calculated as DE_{50} = 1.11 µg/mg for *T. castaneum*.

4. Discussion

Wild *Ageratum conyzoides* shows little evidence of herbivory in its natural habitat. The markings left in the leaves appear to cor-

relate well with the pattern of generalist insects that devour only small bits of the leaf surface in a small cirle away from the leaf margin or inwards from the edge, but in all cases leaving most of the surface intact. Such behavior is compatible with the existence of feeding deterrents or toxins that limit the tolerance to the ingestion of particular plant parts. Previous chemical studies and our own field observations strongly suggested that *A. conyzoides* in our geographical area potentially contained feeding deterrent chemicals in sufficient quantity to elicit the anotated feeding behavior of generalists or capable of fending off other invertebrate herbivores altogether. Field observations extend this rejection to large vertebrates such as bovines. Although *A. conyzoides* may contain more than one specific antiherbivore compound, which in that case must be concentrated in the low polarity group of its secondary metabolites, our experiments revealed that only one of the components, precocene II, was responsible for all the phagoinhibitory activity of this plant, as witnessed by *S. oryzae* and *T. castaneum*. The sharp separation pattern as revealed by disparate feeding inhibition in the two insect species, of contiguous chromatographic fractions, and the wide spectrum of solvents employed for extraction, left little doubt that other compounds of importance might have passed without notice. In spite of some pigment, darkish and polyphenolic materials that came along with the initial active fractions that might have obstructed perhaps the way to other potentially antiinsect components, to no other individual compound in our samples could be attributed any antiinsect activity in these two insect species. This result does not rule out the existence of other feeding inhibitors active against other folivore invertebrates, since there is no evidence that *A. conyzoides* ever evolved under the pressure of our two model insect species.

Surprisingly, no precocene I, a monomethoxy analog of precocene II with powerful feeding inhibitory activity earlier found in *Ageratum* plants from other parts of the world, could be detected in our samples. Mortality was not recorded either, be it during body contact tests with paper layers impregnated with plant components, or by ingestion of these materials, as opposed to earlier observations with *Drosophila melanogaster, Dysdercus cingulatus, Rhodnius prolixus* (30), *Locusta migratoria* (31) *Sitophilus oryzae* and *Tribolium castaneum* (32, 33). This apparent contradiction of results may be solved by consideration of: 1) the likely inhomogeneity of plant secondary metabolism in response to different unanotated phenotypes from various regions of the world, 2) particular sources of unrecorded stress of plant material studied, 3) variations in the growth stage of the plant samples brought in for analysis, and 4) sensitivity of insect cohorts employed in each one of these previous studies. In addition, without LD_{50} or DE_{50} data from these other reports, it is virtually impossible to draw constructive comparisons with the data we present here. As for the interference in insect hormonal development (protheletic action) expected for precocenes such as *Ageratum*'s agerochromene or precocene II (42), our experiments only used adult insects which are therefore phenologically beyond the action of the molting or JH hormone mimics.

The strong rejection of surfaces treated with *A. conyzoides* extracts in both *T. castaneum* and *S. oryzae* revealed by our two way flatbed paper assays suggested the intervention of a sensory mechanism in the insects to elicit the observed aversion, in agreement with Azambuja et al. (9). However, neither the forced contact in the one way assay, nor the spraying of acetone preparations of these extracts on confined insect groupings (undescribed results) did lead to any significant mortality or detectable contact toxicity effects, with the implication that insects reacted not by acquired antagonism through internal toxicity of possible xenobiotic chemicals in the plant, but by simple activation of the sensory re-

sponse. A similar conclusion may be reached from the results of the two way feeding assay. All extracts of *A. conyzoides* were rejected in favor of the untreated diet, in spite that only the hexane- and dichloromethane- soluble plant materials were of any risk to the animals as indicated by the forced one way test.

Ageratum leaves showed the greater antiherbivory protection, implying that it is the leaves among the the plant tissues at greater risk of herbivory. The sampled plants were at an advanced phenological stage in which abundant inflorescences were present. At this stage, a greater proportion of aerial biomass is placed in structural tissues -twigs and stems- and reproductive structures, than in younger plants, in which not only flowers are obviously absent but leaves are clearly the dominant structure.

The survival of our test insects, the grain pests *S. oryzae* and *T. castaneum*, becomes compromised when exposed to relatively low dosages of precocene II. However, at around the DE_{50} value, *T. castaneum* is still capable to maintain or marginally increase its body weight (table 3) and only when the concentration of this xenobiotic is doubled (2 x DE_{50}) is there a descent in body mass. It is this decrease that may be related to loss of viability and reproductive capacity of the insect, which will result in the eventual elimination of the insect colony in the treated grain deposit. This key quantity, 2 x DE_{50} (0.236%) means a dosage of 2.36 g/Kg of precocene II. Therefore, in spite of *A. conyzoides* chemical attributes that may serve the plant well in the wild, and although this is only 2.6 times the dosage (LD_{50}) claimed by Bouda et al. (34) to kill the related *Sytophilus zeamais* in Africa (as essential oil), the practical applications of its primary xenobiotic for grain protection against *S. oryzae* and *T. castaneum* adults as a stand alone material are as such probably not granted. Further investigations on the effects of these extracts on larval forms of these serious pests and chemical derivatization of precocenes may increase their potential as feed protectants.

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