EFFECTS OF ORGANIC PLANT EXTRACTS ON BEHAVIOR OF *Sitophilus zeamais* MOTS. (COLEOPTERA: CURCULIONIDAE) ADULTS

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ABSTRACT

The aim of the present study was to evaluate the action of organic plant extracts from different parts of some species on host selection behavior of *Sitophilus zeamais* (Coleoptera: Curculionidade) adults. With the exception of extracts prepared from *Aristolochia paulistana*, all other tested plant extracts showed effect on behavior of *S. zeamais* adults, depending on concentration and solvent used in their preparation. The most pronounced repellent and/or deterrent effects were obtained with nonpolar extracts (in hexane and dichloromethane) of *Annona montana*, *Annona mucosa*, and *Casearia sylvestris*. On the other hand, ethanolic extracts from branches of *C. sylvestris*, leaves of *A. montana*, and leaves and seeds of *A. mucosa* were attractive and/or stimulant of feeding and/or oviposition of *S. zeamais* adults, depending on the concentration used. Thus, these extracts are promising sources of attractive compounds to be used in food baits in monitoring traps or even as repellents of stored grain pests.

Keywords: Maize weevil, allelochemicals, *Annona montana*, *Annona mucosa*, *Casearia sylvestris*

EFEITO DE EXTRATOS ORGÂNICOS DE PLANTAS NO COMPORTAMENTO DE ADULTOS DE *Sitophilus zeamais* MOTS. (COLEOPTERA: CURCULIONIDAE)

RESUMO

Objetivou-se, no presente estudo, avaliar a ação de extratos orgânicos de estruturas vegetais de diferentes espécies no comportamento de adultos de *Sitophilus zeamais* (Coleoptera: Curculionidade). Com exceção dos extratos oriundos de *Aristolochia paulistana*, todos os demais extratos testados causaram efeito no comportamento de adultos de *S. zeamais*, dependendo da concentração e do solvente utilizado na sua preparação. Os efeitos repelente e/ou deterrente mais pronunciados foram obtidos com os extratos mais apolares (em hexano e diclorometano) de *Annona montana*, *Annona mucosa* e *Casearia sylvestris*. Por outro lado, extratos etanólicos de ramos de *C. sylvestris*, de folhas de *A. montana* e de folhas e sementes de *A. mucosa* foram atraentes e/ou estimulantes de alimentação e/ou oviposição de adultos de *S. zeamais*, dependendo da concentração utilizada. Dessa forma, tais extratos são promissoras fontes de compostos atrativos passíveis de serem utilizados em iscas alimentares em armadilhas de monitoramento ou mesmo repelentes de pragas de grãos armazenados.

Palavras-chave: Gorgulho-do-milho, aleloquímicos, *Annona montana*, *Annona mucosa*, *Casearia sylvestris*

INTRODUCTION

The maize weevil, *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae), is a primary pest species of stored grains (LAZZARI & LAZZARI, 2009). *S. zeamais* has a large number of alternative hosts, high biotic potential and capacity to survive at great depths in the grain mass (FARONI, 1992). In addition, the occurrence of cross-infestation, that is, the capacity of the insect to attack grains in the field and in warehouses, increases the damage magnitude of *S. zeamais* (LORINI et al., 2015). In Brazilian conditions, Picanço et al. (2004) reported losses of up to 30% in corn summer crops in Coimbra (MG) during pre-harvest.

The use of synthetic insecticides is the most widely method for the control of *S. zeamais*, mostly because of lack of alternative efficient control methods (BOYER et al., 2012). However, the use of chemical compounds has been compromised due to restricted availability of insecticides registered for the control of this species and the consequent difficulty to switch active ingredients (UPADHYAY & AHMAD, 2011) and occurrence of resistant populations (RIBEIRO et al., 2003). However, some studies have presented the viability of using bioactive compounds obtained from plants for pest control of stored grains (RIBEIRO et al., 2016).

Plants with insecticidal action have been used as an alternative method through products in the form of powders, oils and extracts for control of major pests of stored products in many countries of Latin America, Africa and Asia (GONÇALVES et al., 2015), showing toxicity via contact, ingestion and fumigation (ESTRELA et al., 2006). Plant substances cause mortality, repellency, oviposition inhibition, increased duration of larval development, and reduction in fertility and fecundity of adults (MARTINEZ

& VAN EMDEN, 2001). In addition to the direct application through homemade preparations, elucidation of the chemical structure of plant secondary metabolites (allelochemicals) allow the synthesis of new products more acceptable by the society. Identification, sequencing and cloning of genes responsible for gene expression of allelochemicals may allow the transfer of resistance to agricultural crops, eliminating the use of chemicals (COSTA & MAIRESSE, 2009).

This study evaluated the action of organic extracts of plants with insecticidal activity at different stages of host selection behavior of adults of *S. zeamais* in choice tests using corn seeds as a substrate.

MATERIAL AND METHODS

Establishment and maintenance rearing of *Sitophilus zeamais*

The rearing of *S. zeamais* was established from specimens obtained from a population kept in the Laboratory of Food Irradiation and Radioentomology of Centro de Energia Nuclear na Agricultura (CENA/USP), in Piracicaba, São Paulo, Brazil.

Insects used in bioassays were reared in an air-conditioned room at 25 ± 2^{o}C, relative humidity 60 ± 10%, photophase of 14 h, and average luminosity 172 lux in 3-L glass bottles with the top sealed with fine cloth (*voile*). Wheat grains were used as substrate for the maintenance rearing of *S. zeamais*, which were previously exposed to -10^{o}C, for at least 48 h, in a domestic freezer to eliminate insect contaminants (SILVA-AGUAYO et al., 2006). After fumigation, the grains were kept in a temperature-controlled room, under the conditions mentioned previously, for 30 d to reach hygroscopic balance before use (RIBEIRO et al., 2013).

Obtaining species and preparing plant extracts

Data collection of plant species used in extract preparation are shown in Table 1. After collection, an exsiccate was obtained from each species, which were forwarded to specific identification to the following institutions: Department of Systematics and Evolution of Higher Plants/University of Vienna and Bioscience Institute of University of São Paulo (IB/USP), for identification of species of Annonaceae; Department of Biological Sciences of ESALQ/USP, for identification of species of Aristolochiaceae and E.S.A. Museum of the Department of Biological Sciences of ESALQ/USP, for identification of species of Salicaceae.

Table 1. Species, botanical families and plant parts used in the study on *Sitophilus zeamais*, with their respective locations and collection dates

Species	Botanical family	Plant part collected	Collection location	Collection data
Annona montana Macfadyen	Annonaceae	Leaves, branches and seeds	Campus ESALQ/USP, Piracicaba, SP (Lat.: 22⁰42' 29''S, Long.: 47⁰37'36''O)	01/22/2010
Annona mucosa Jacquin	Annonaceae	Leaves, branches and seeds	Campus ESALQ/USP, Piracicaba, SP (Lat.: 22⁰42' 26''S, Long.: 47⁰37'39''O)	01/22/2010
Aristolochia paulistana Hoehne	Aristolochiaceae	Branches	Dist. Coronel Finzito, Erval Seco, RS (Lat.: 27⁰31' 54''S, Long.: 53⁰33'13''O)	01/10/2010
Casearia sylvestris Swartz	Salicaceae	Leaves and branches	Campus ESALQ/USP, Piracicaba, SP (Lat.: 22⁰42' 50''S, Long.: 47⁰37'36''O)	01/16/2010

For the preparation of extracts, the plant structures collected were dehydrated in an oven at 40ºC for 48-72 h. Later, the materials were ground in a knife mill and plant powders were obtained, which were stored separately by species and plant structure in a hermetically sealed glass until use.

Organic extracts were obtained by maceration, using as solvents (at ratio 1:5, w/v), in crescent order of polarity, hexane (polarity: 0.06), dichloromethane (polarity: 3.4) and ethanol (polarity: 5.2). For each solvent, extraction was made until exhaustion, using then the solvent with polarity immediately above. At each change of solvent, the macerate was filtered through filter paper and solvent of the remaining sample was eliminated in rotary evaporator at 40ºC and pressure -600 mmHg. After complete evaporation of the solvent in the airflow chamber, the extraction yield for each structure of plant species in the distinct solvents used was determined.

Bioassays

Bioassays were conducted in an air-conditioned room at 25 ± 2ºC, relative humidity 60 ± 10%, photophase of 14 h and average luminosity 172 lux, under a complete randomized design. As a substrate to carry out the tests, whole corn seeds of hybrid AG 1051 (toothed yellow; semi-hard) were used, which were previously selected manually.

To evaluate the effect of extracts on attractiveness of *S. zeamais* adults, 10 arenas (repetitions) were used, each consisting of five plastic Petri dishes (6 cm in diameter and 2 cm high) mounted on a plastic base (30 x 30 cm). In each arena, one of the dishes is fixed at the base center and connected to the others by means of plastic tubes of equal length.

In one of the Petri dishes (control), it was placed 10 g of corn seeds treated with the same solvent used for resuspension of extracts (control), while in other cases, it was placed corn samples treated with the respective extracts chosen at random within each bioassay. In the central recipient, 50 adults of *S. zeamais* were released, not sexed and aged between 10 and 20 d. After 24 h, the number of insects in each container was assessed.

Data analysis

The treatments were compared to each other by means of the repellency/deterrence index (R.I./D.I.) adapted from Lin et al. (1990), calculated by the formula R.I./D.I. = 2 G/(G + P), where G = % of insects in grain treated with the extract under test and P = % of insects in grain in the control group. Based on R.I./D.I. and standard deviation obtained, it was determined the classification interval (Class.I.) for the averages of the treatments by the formula: Class.I. = $1 \pm t_{(n-1;\alpha: 0.05)} x (SD/\sqrt{n})$; where t = value of "t" charted; SD = standard deviation; n = number of repetitions. The extracts were considered neutral when the R.I./D.I. value remained within the Class.I. evaluated; repellent and/or deterrent when the R.I./D.I. value was below the lowest value obtained for Class.I.; and attractive and/or phagodeterrent when the R.I./D.I. values were higher than the highest Class.I. calculated.

RESULTS AND DISCUSSION

The time of evaluation adopted did not allow to distinguish between the possible effects of the extracts tested in the different stages of the host selection process of *S. zeamais*. As this step is an initial screening of a large number of extracts, the objective was only to detect bioactive effects, without the aim to characterize them in this first moment. The characterization of possible effects of plant extracts on host selection of *S. zeamais* should be the subject of future investigations.

Containers with corn seeds treated with hexane extracts at 300 ppm of branches of *A. montana* and seeds and branches of *A. mucosa* showed fewer insects attracted compared to boxes containing only corn (control) (Table 2), differing significantly according to the index adopted (R.I./D.I.). This shows the existence of repellent and/or deterrent effect of food and/or oviposition layer of these extracts for adult of *S. zeamais*. However, at 1,500 ppm, all hexane extracts were classified as repellents and/or deterrents of food and/or oviposition for adult *S. zeamais*, except for the hexane extract of branches of *A. paulistana* (Table 2). At this concentration, the extract of branches of *C. sylvestris* was the treatment that caused greater repellent and/or deterrent effect. These results show a bioactive effect of nonpolar extracts (hexanes) that depend on the concentration.

At a concentration of 300 ppm, only the dichloromethane extract prepared from seeds of *A. mucosa* caused significant effect on adults of *S. zeamais*, reducing the number of insects attracted compared to boxes containing only corn (control) (Table 3). With the increase of the concentration to 1,500 ppm, however, except for dichloromethane extracts of branches of *A. paulistana* and leaves and the branches of *A. mucosa*, all others reduced the attractiveness of *S. zeamais adults* for the treated samples (Table 3).

On the other hand, ethanolic extracts of leaves of *A. montana* and leaves and seeds of *A. mucosa* were classified as attractive and/or stimulants for feeding and/or oviposition when tested at a concentration of 300 ppm (Table 4). However, at the concentration of 1,500 ppm of seed extract from *A. mucosa*, it was repellent and/or deterrent for the weevil under study, while branch extracts of *C. sylvestris* caused attractive and/or stimulating effect of food and/or oviposition (Table 4). Therefore, not only does this finding highlights the behavior change of the insect due to the extract concentration used, but it also shows the diversity of substances in extracts obtained from the same plant structure and species, which are likely to cause different behavioral effects to *S. zeamais*, depending on the solvent used that leads to significant changes in the chemical profile of the derivatives obtained and, consequently, changes in their bioactivity. Similarly, these results confirm the chemical separation provided by organic solvents through the extraction method used, which was already found in chromatographic profiles obtained by thin-layer chromatography (TLC) and the spectroscopic analyses carried out previously (RIBEIRO et al., 2013, 2014).

The action of plant compounds can present itself at different stages of host selection behavior (VENDRAMIM & CASTIGLIONI, 2000) by means of differential performance on the physiological mechanisms of insects. Thus, the repellent activity is revealed in plant extracts that have compounds with unpleasant and irritant odors to insects (PETERSON & COATS, 2001), which act on olfactory receptors (SUKUMAR et al., 1991). However, food deterrence is a disorder associated to sensory mechanisms that cause reduced food consumption (ISMAN, 2006). For this author, the feeding behavior of insects depends on the integration of the central nervous system with the chemoreceptors located on the tarsi, buccal parts and oral cavity. The authors also reports that certain substances, such as azadirachtin, present in neem extracts, can act on the chemoreceptors, stimulating "specific deterrent cells" or blocking the phagostimulants, such as the "sugar receptor cells", inhibiting feeding. Nevertheless, some substances may affect the substrate characteristics, making it unsuitable for oviposition or even influencing the levels of oviposition stimulants in grains or susceptible varieties (GOMEZ et al., 1983), thus, causing oviposition deterrence.

Table 2. Attractiveness of *Sitophilus zeamais* adult to corn samples (10 g) treated with hexane extracts prepared from different species and/or plant parts at two concentrations*. Temperature: 25±2°C; R.H.: 60±10%; photophase: 14 h; average luminosity: 172 lux

Plant species	Plant part	R.I./D.I.[1] (Mean±SD)	Class.I.[2]	Classification
		300 ppm		
Annona montana	Seeds	0.88±0.33	1±0.24	Neutral
	Leaves	0.97±0.18	1±0.13	Neutral
	Branches	0.88±0.12	1±0.09	Repellent and/or deterrent
Annona mucosa	Seeds	0.63±0.28	1±0.20	Repellent and/or deterrent
	Leaves	0.89±0.24	1±0.17	Neutral
	Branches	0.74±0.26	1±0.19	Repellent and/or deterrent
Aristolochia paulistana	Branches	1.01±0.49	1±0.35	Neutral
Casearia sylvestris	Leaves	0.88±0.51	1±0.36	Neutral
	Branches	0.99±0.42	1±0.30	Neutral
		1,500 ppm		
Annona montana	Seeds	0.39±0.38	1±0.27	Repellent and/or deterrent
	Leaves	0.47±0.42	1±0.30	Repellent and/or deterrent
	Branches	0.68±0.48	1±0.34	Repellent and/or deterrent
Annona mucosa	Seeds	0.21±0.19	1±0.13	Repellent and/or deterrent
	Leaves	0.53±0.50	1±0.36	Repellent and/or deterrent
	Branches	0.44±0.35	1±0.25	Repellent and/or deterrent
Aristolochia paulistana	Branches	1.09±0.35	1±0.25	Neutral
Casearia sylvestris	Leaves	0.48±0.30	1±0.21	Repellent and/or deterrente
	Branches	0.12±0.16	1±0.11	Repellent and/or deterrent

[1] R.I./D.I.: repellence and/or deterrence index;
[2] Class.I.: Classification interval;
* In a solution volume of 30 L t^{-1}.

Table 3. Attractiveness of *Sitophilus zeamais* adult to corn samples (10 g) treated with dichloromethane extracts prepared from different species and/or plant parts at two concentrations*. Temperature: 25±2°C; R.H.: 60±10%; photophase: 14 h; average luminosity: 172 lux

Plant species	Plant part	R.I./D.I.[1] (Mean±SD)	Class.I.[2]	Classification
		300 ppm		
Annona montana	Seeds	0.82±0.48	1±0.34	Neutral
	Leaves	1.14±0.35	1±0.25	Neutral
	Branches	1.21±0.42	1±0.30	Neutral
Annona mucosa	Seeds	0.65±0.32	1±0.23	Repellent and/or deterrent
	Leaves	1.09±0.27	1±0.19	Neutral
	Branches	0.84±0.46	1±0.33	Neutral
Aristolochia paulistana	Branches	1.24±0.52	1±0.37	Neutral
Casearia sylvestris	Leaves	1.09±0.58	1±0.41	Neutral
	Branches	0.85±0.72	1±0.51	Neutral
		1,500 ppm		
Annona montana	Seeds	0.35±0.33	1±0.23	Repellent and/or deterrent
	Leaves	0.89±0.45	1±0.32	Neutral
	Branches	0.31±0.30	1±0.21	Repellent and/or deterrent
Annona mucosa	Seeds	0.29±0.37	1±0.26	Repellent and/or deterrent
	Leaves	0.73±0.38	1±0.28	Neutral
	Branches	0.71±0.44	1±0.32	Neutral
Aristolochia paulistana	Branches	0.93±0.39	1±0.28	Neutral
Casearia sylvestris	Leaves	0.40±0.36	1±0.26	Repellent and/or deterrent
	Branches	0.18±0.20	1±0.14	Repellent and/or deterrent

[1] R.I./D.I.: repellence and/or deterrence index;
[2] Class.I.: Classification interval;
* In a solution volume of 30 L t^{-1}.

In this study, the extracts of branches of *C. sylvestris* in hexane and dichloromethane were the treatments with the lowest R.I./D.I. and consequently the most promising effects. Hypothetically, this bioactive effect observed on *S. zeamais* may be related to the occurrence of clerodanic diterpenes, which have been frequently isolated in different structures of *C. sylvestris* (CARVALHO et al., 2009; WANG et al., 2009). According to Rosselli et al. (2004), clerodanic diterpenes isolated from aerial part of

Scutellaria sieberi (Lamiaceae) cause a potent anti-feeding effect to larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), which corroborates the hypothesis raised.

Table 4. Attractiveness of *Sitophilus zeamais* adults to corn samples (10 g) treated with ethanolic extracts prepared from different species and/or plant parts at two concentrations*. Temperature: 25±2°C; R.H.: 60±10%; photophase: 14 h; average luminosity: 172 lux

Plant species	Plant part	R.I./D.I. [1] (Mean±SD)	Class.I.[2]	Classification
300 ppm				
Annona montana	Seeds	0.83±0.28	1±0.23	Neutral
	Leaves	1.37±0.31	1±0.26	Attractive and/or stimulant
	Branches	1.25±0.42	1±0.35	Neutral
Annona mucosa	Seeds	1.33±0.26	1±0.21	Attractive and/or stimulant
	Leaves	1.35±0.21	1±0.17	Attractive and/or stimulant
	Branches	1.15±0.31	1±0.26	Neutral
Aristolochia paulistana	Branches	1.13±0.38	1±0.31	Neutral
Casearia sylvestris	Leaves	1.14±0.44	1±0.36	Neutral
	Branches	1.09±0.39	1±0.32	Neutral
1,500 ppm				
Annona montana	Seeds	0.75±0.45	1±0.37	Neutral
	Leaves	1.17±0.52	1±0.43	Neutral
	Branches	1.17±0.54	1±0.45	Neutral
Annona mucosa	Seeds	0.33±0.34	1±0.28	Repellent and/or deterrent
	Leaves	1.16±0.46	1±0.38	Neutral
	Branches	0.90±0.51	1±0.42	Neutral
Aristolochia paulistana	Branches	1.08±0.57	1±0.47	Neutral
Casearia sylvestris	Leaves	1.28±0.41	1±0.34	Neutral
	Branches	1.31±0.34	1±0.28	Attractive and/or stimulant

[1] R.I./D.I.: repellence and/or deterrence index;
[2] Class.I.: Classification interval;
* In a solution volume of 30 L t^{-1}.

Studies on the effect of substances extracted from Annonaceae on insect behavior are rather scarce. Ukeh et al. (2008) found that the application of seed powder of *Monodora myristica* to corn seeds provides significant oviposition deterrence for *S. zeamais* and suppresses entirely the F_1 progeny at doses greater than 5% (w/w). More promising results were obtained with essential oil of *Mkilua fragrans*, which caused repellent activity to *Anopheles gambiae* Giles (Diptera: Culicidae) more pronounced than DEET (N,N-diethyl-3-toluamide), active ingredient of synthetic commercial repellents (ODALO et al., 2005).

On the other hand, extracts from branches of *A. paulistana* caused no effect on the behavior of *S. zeamais* at any of the concentrations tested for the three solvents used. Unlike the results obtained, Pinto et al. (2009) found that the aristolochic acid extracted from *A. chilensis* causes stimulating effect to feeding in larvae and oviposition to females of *Battus polydamas archidamas* (Boisduval) (Lepidoptera: Papilionidae) and that this substance may even act on determining oviposition sites of females of this Lepidoptera during host selection. Lajide et al. (1993), on the other hand, found phagodeterrent effect of root extract of *A. albida* to *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) larvae, and this activity was attributed to a free carboxylic group situated near a nitro group of aristolochic acid structure. Therefore, it is observed that the compounds present in *Aristolochia* can present distinct effects depending on the species of the target insect.

CONCLUSIONS

Nonpolar extracts (in hexane and dichloromethane) of *A. montana*, *A. mucosa* and *C. sylvestris* cause pronounced repellent and/or deterrent effects to feeding and/or oviposition of *S. zeamais*. In addition, ethanolic extracts from branches of *C. sylvestris*, leaves of *A. montana* and leaves and seeds of *A. mucosa* are attractive and/or stimulant of feeding and/or oviposition of adults of *S. zeamais*, depending on the concentration used. Therefore, the results obtained in this study point to prospects for more detailed studies on the use of these compounds with attractive and/or stimulating effects on feeding baits in monitoring traps, enabling more efficient and early detection of infestations. In addition, repellent compounds can be used to keep insects out of factories and warehouses, away from products to be protected.

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