Revta brasil. Bot., São Paulo, V.21, n.1, p.101-104, abr. 1998

Effects of ester fractions from leaf epicuticular waxes of *Bauhinia rufa* (Steud.) Bong. and *Stryphnodendron adstringens* (Mart.) Coville from cerrado on the aphid *Rhopalosiphum maidis* (Fitch.)

MARIA ELINA BICHUETTE[1], ELENICE MOURO VARANDA[1] and JOSÉ RICARDO BAROSELA[1]

(recebido em 07/02/96; aceito em 08/07/97)

ABSTRACT - (Effects of ester fractions from leaf epicuticular waxes of *Bauhinia rufa* (Steud.) Bong. and *Stryphnodendron adstringens* (Mart.) Coville from the cerrado on the aphid *Rhopalosiphum maidis* (Fitch.)). Ester fractions were isolated from the leaf epicuticular waxes of *Bauhinia rufa* (Steud.) Bong. and *Stryphnodendron adstringens* (Mart.) Coville collected in the Cerrado de Emas (Pirassununga, SP, Brazil). The bioassays of toxicity and reproductive index were carried out with esters from these species and the aphid *Rhopalosiphum maidis* (Fitch.). It was observed that the ester fractions from *Bauhinia rufa* and *Stryphnodendron adstringens* had a negative effect on the survival of *Rhopalosiphum maidis*. The ester fraction from *Stryphnodendron adstringens* also had a negative effect on the reproductive index of the aphid. These parameters decreased with the increase of ester concentration of artificial diets.

RESUMO - (Efeito de ésteres de ceras foliares epicuticulares de *Bauhinia rufa* (Steud.) Bong. e *Stryphnodendron adstringens* (Mart.) Coville de cerrado sobre afídeos *Rhopalosiphum maidis* (Fitch.)). Os ésteres foram isolados de ceras foliares epicuticulares de *Bauhinia rufa* (Steud.) Bong. e *Stryphnodendron adstringens* (Mart.) Coville, coletadas no Cerrado de Emas (Pirassununga, SP, Brasil). Bioensaios de toxicidade e índice de reprodução foram realizados com os ésteres destas espécies e afídeos *Rhopalosiphum maidis* (Fitch.). Os resultados mostraram que os ésteres de *Bauhinia rufa* e *Stryphnodendron adstringens* têm um efeito negativo na sobrevivência de *Rhopalosiphum maidis* e que os ésteres de *Stryphnodendron adstringens* também diminuem o índice de reprodução dos afídeos. Esses parâmetros decresceram em proporção direta ao aumento da concentração de ésteres em dietas artificiais.

Key words - Esters, *Rhopalosiphum maidis*, *Bauhinia rufa*, *Stryphnodendron adstringens*

Introduction

The thought that the secondary compounds of plants might have evolved to protect them from the attack of herbivores dates from the past century. However, it remained overlooked until Fraenkel (*apud* Rhoades 1979) suggested that those substances controlled the feeding behaviour of herbivores and that their adaptive importance for plants was based on their repellent and/or toxic properties against insects and other herbivores.

Several authors agree that the secondary compounds have ecological importance related to the physical environment (Harborne 1988) and/or to mutualistic and antagonistic relationships among plants and animals (Rosenthal & Janzen 1979).

The action of many secondary compounds against the attack of aphids has been mainly studied in grasses of economic interest such as oat, wheat, barley and broomcorn, due to the fact that those insects represent plagues to plantations. Aconitic acid isolated from wheat, barley, corn and broomcorn acted as a moderator of the resistance degree to the aphids *Rhopalosiphum maidis* and *Melanaphis sacchari* (Rustamani et al. 1992). Gramine, an indole alkaloid, obtained from barley (*Hordeum distichum*) affects the survival and reproduction rate of the aphids *Schizaphis graminum* and *Rhopalosiphum maidis* when they are kept on artificial diets with similar concentrations to those found in leaves of barley (Zúñiga et al. 1988). lt was also demonstrated that hydroxamic acid of cereals decreases the survival and reproduction rate of aphids (Argandoña et al. 1981, Zúñiga et al. 1983, 1985, Corcuera et al. 1985, Niemeyer et al. 1989).

The first leaf constituents that herbivores contact is the cuticle. The cuticle layer of leaves of higher plants is composed of cutin, pectic substances and epicuticular waxes and it shows variable thickness. The epicuticular wax frequently appears as crystalline deposits and according to Eglinton & Hamilton (1967) its quantity varies a great deal depending on the species and may come up to 4% of the weight of fresh leaves or over 15% of the weight of dry leaves. Its composition is a complex

1. Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo. Av. Bandeirantes 3900, 14040-901 Ribeirão Preto, SP, Brasil. mebichue@usp.br, emvarand@usp.br, barosela@usp.br.

arrangement of long chain compounds such as hydrocarbons, esters, ketones, free alcohols, and diand triterpenic acids (Eglinton & Hamilton 1967). The primordial function of this wax is to keep a hydric balance in plants. There are, however, other functions which have been assigned to it such as protection of epidermic cells against mechanical damage and ultraviolet light, the inhibition of fungi proliferation and attack by insects (Juniper & Jeffree1983). The presence of secondary compounds associated with epicuticular waxes is relatively common such as those found in several cerrado species. These are supposedly important for the defence against fungi attack which is very frequent in that vegetation (Salatino et al. 1985). Varanda et al, (1 992) isolated the triterpenoid ursolic acid from *Jacaranda decurrens*, a cerrado species, and by testing on *Schizaphis graminum* they noted a decrease in its survival, population growth and reproduction rate.

This work aims at providing evidence for a better understanding about the function of compounds present in leaf waxes of cerrado species. Ester fractions were isolated from the leaf waxes of *Bauhinia rufa* and *Stryphnodendron adstringens* from cerrado. The results of bioassays carried out with esters and the aphid *Rhopalosiphum maidis* are presented.

Material and methods

Leaves of *Bauhinia rufa* (Steud.) Bong. and *Stryphnodendron adstringens* (Mart.) Coville were collected at Cerrado de Emas (Pirassununga, SP, Brazil, 22º02'S, 47º30'W).

Specimens of *Rhopalosiphum maidis* (Fitch.) were kept in laboratory on barley seedlings (*Hordeum distichum* L. var. IAC-75751), which were grown in vases under continuous light conditions. Other specimens were kept in entomological boxes according to Cabette's procedure (1989) in stoves at 25 ± 1ºC and constant light intensity.

After the leaves bad dried in shade and room temperature the waxes were extracted by three times thirty-second immersions in cold chloroform (Croteau & Fergenson 1971). The extracts obtained, after filtering, were submitted to evaporation to dryness, under reduced pressure. The crude wax which was obtained was incorporated in silica for column and submitted to column chromatography. The column was packed by the dry technique and eluted with the following solvents: C_6H_{14}, C_6H_{14} $CHCl_3$ (1:3), CH Cl_3, CH Cl_3 : EtOAc (1:1), EtOAe, EtOAc : MEOH (1:1) and MeOH.

The eluates were submitted to thin layer chroniatography on G-60 silica gei plates (Merck), impregnated with 0.02% sodium fluorescein and developed with C_6H_{14}: CH Cl_3 (7:3) in supersatured chamber. The chromatograms were visualized under long wave ultraviolet light (Salatino & Bonzani 1988). The esters were purified by preparative thin layer chromatography and identified in comparison to authentic samples by TLC and analysed by IR spectrometry (Spectrophotometer Perkin-Elmer, FTIR 1600) in KBr discs.

The esters were dissolved in DMSO, which was characterized as being a non-toxic solvent to aphids up to the concentration of 100 mM (Varanda et al. 1992), and then incorporated to artificial diets. Diets with ester concentration of 0 (control), 2, 3 and 4% were administered to four groups of ten aphids per treatment. These diets were placed between two layers of Parafilm M at one end of acrylic cylinders (6.00 cm x 3.50 cm in diameter) while the other end was covered with a thin netting cloth after the aphids had been placed in (Mittler & Dadd 1964, Auclair 1965).

Nymphs of *Rhopalosiphum maidis* were used to carry out the toxicity tests with diets containing esters as well as control diets for a period of 48 hours, after which the survival percentage was recorded (Argandoña et al. 1981).

In order to verify the possible repellent effect, the aphid nymphs were fed on diets containing different ester concentrations for 6 hours and after that period they were transferred to a control diet for a period of 24 hours after which the nuniber of surviving insects was observed (Argandoña et al. 1981).

Adult aphids kept on diets with increasing concentration of esters for 50 hours were used to calculate the reproduction rate, which was defined as the relation between the number of nymphs produced and the average number of surviving adult aphids (Zuñiga & Corcuera 1986).

All bioassays were made in triplicate. The results obtained from these three bioassays were statistically analyzed through Kruskall-Wallis' test (Hollander & Wolfe 1973) for more than two comparisons.

Results and Discussion

The IR spectrum of the fraction purified from *Bauhinia rufa* showed strong bands indicating ester function (1 735 and 1 1 75 cm^{-1}) and long carbon chain (2915, 1415 and 720 cm^{-1}).

The isolated fraction of *Stryphnodendron adstringens* was also shown to be esters with long carbon chain (bands at 1733 and 1172 cm^{-1} and 2917, 1471 and 719 cm^{-1}).

During the bioassays the survival percentage was observed to have a small decrease from 81 to 65% as the ester concentration of *Bauhinia rufa* increased in the diet (figure 1). The esters of *Stryphnodendron adstringens* also caused a decrease from 66 to 30% in the survival rates of *Rhopalosiphum maidis* (figure 2). Thus the esters fractions of *Bauhinia rufa* and *Stryphnodendron adstringens* are toxic to *Rhopalosiphum maidis* since the results obtained were statistically significant.

In the repellence tests it was verified that the esters of *Bauhinia rufa* and *Stryphnodendron adstringens* did not show significant differences among the different concentrations which were offered to *Rhopalosiphum maidis*.

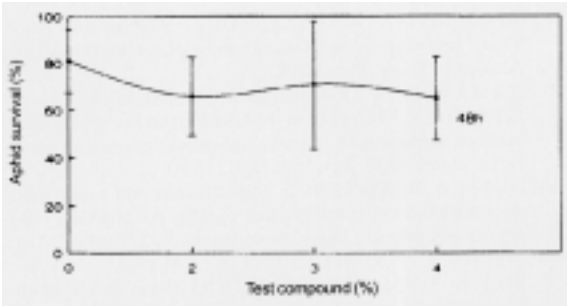

Figure 1. Effect of esters of *Bauhinia rufa* (Steud.) Bong. on survival of nymphs of *Rhopalosiphum maidis* (Fitch.) maintained on artificial diets for 48 h. Vertical bars represent S.D.

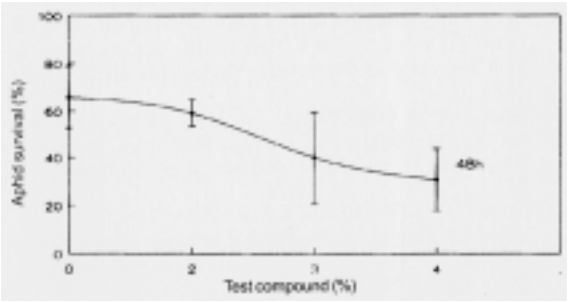

Figure 2. Effect of esters of *Stryphnodendron adstringens* (Mart.) Coville on survival of nymphs of *Rhopalosiphum maidis* (Fitch.) maintained on artificial diets for 48 h. Vertical bars represent S.D.

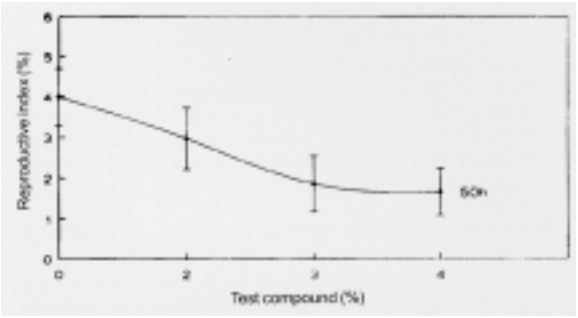

Figure 3. Effect of ester of *Stryphnodendron adstringens* (Mart.) Coville on the reproductive index of adults of *Rhopalosiphum maidis* (Fitch.) maintained on artificial diets for 50 h. Vertical bars represent S.D.

The esters of *Stryphnodendron adstringens* had a negative effect upon the reproduction rate of *Rhopalosiphum maidis*, causing a decrease in the number of nymphs produced as the concentration increased in the diet (figure 3).

Therefore, according to the results, it was verified that isolated esters of *Bauhinia rufa* and *Stryphnodendron adstringens* influenced negatively the survival, and the esters from *S. adstringens* decreased the reproduction rate of *Rhopalosiphum maidis*. This species and others of the same genus have been utilized for tests with various substances. Thus the indole alkaloid gramine decreased the survival of *Rhopalosiphum maidis* (Corcuera 1984, Zuñiga & Corcuera 1986), for the number of survivors decreased as the concentration of the compound increased in the diet. Survival decrease of *Rhopalosiphum padi* was registered when these aphids were fed on artificial diets to which DIBOA with increasing concentrations up to 7 mM had been added (Barria et al. 1992). Aconitic acid isolated from wheat, barley, corn and broomcorn decreased the resistance degree of the aphids *Rhopalosiphum maidis* and *Melanaphis sacchari* (Rustamani et al. 1992). Ursolic acid isolated from *Jacaranda decurrens* and incorporated to artifical diets decreased the survival, reproduction rate and population growth of *Schizaphis graminum* (Varanda et al. 1992). Survival and reproduction rate of *Rhopalosiphum maidis* were observed to drop when they were fed on barley seeds (Robinson 1992).

It can be suggested that due to their toxic effect on the reproduction rate of *Rhopalosiphum maidis* the esters from the studied species may represent a first barrier against attack by phytophagous insects on leaves. Since the observed effects cannot be generalized to other herbivores, these results indicate that further investigations with such compounds and herbivores found in cerrado may lead to a better understanding about the roles of the compounds present in leaf waxes on the protection against attacks by phytophagous insects.

Acknowledgements - The authors thank Antonio Salatino and Maria Luiza Salatino for their help in the esters isolation, CNPq for providing a scientific initiation scholarship to M.E. Bichuette (Proc. 501486-7), and Nei Aparecido Padovan for recording the IR spectra.

References

ARGANDOÑA, V. H., NIEMEYER, H.M. & CORCUERA, L.J. 1981. Effects of content and distribution of hidroxamic acids in wheat on infestation by aphid *Schizaphis graminum*;. Phytochemistry 20:673-676.

AUCLAIR, J.L. 1965. Feeding nutrition of the pea aphid *Acyrthosiphon pisum* (*Homoptera, Aphididae*), on chemically defined diets of various pH and nutrient levels. Ann. Entomol. Soc. Am. 58:855-875.

BARRIA, B.N., CORCUERA, L.J. & NIEMEYER, H.N. 1992. Occurrence of DIBOA in wild *Hordeum* and its relation to aphid resistance. *Phytochemistry* 31:89-91.

CABETTE, H.S.R. 1989. Capacidade reprodutiva e sobrevivência do pulgão do sorgo granífero (*Sorghum bicolor*), *Schizaphis graminum* (Rondani 1852) (Homoptera, Aphididae) no campo e no laboratório. Dissertação de mestrado, Universidade de São Paulo, Ribeirão Preto.

CORCUERA, L.J. 1984. Effects of indole alkaloids from Gramineae on aphids. Phytochemistry 23:539-541.

CORCUERA, L.J., QUEIROLO, B. C. & ARGANDOÑA, H. V. 1985. Effects of 2-D-glycosyl-4-hydroxy-7-metoxyl 4-benzoxazin-3 on *Schizaphis graminum* (Rondani) (Inscet, Aphididae) feeding on artificial diets. Experientia 41:514-516.

CROTEAU, R. & FERGENSON, I.S. 1971. The chemical composition of cuticular waxes of cranberry. Phytochemistry 10:3239-3245.

EGLINTON, G. & HAMILTON, R.J. 1967. Leaf epicuticular waxes. Science 156:1322-1335.

HARBORNE, J.B. 1988. Introduction to Ecological Biochemistry. Academic Press, Cambridge.

HOLLANDER, M. & WOLFE, D.A. 1973. Nonparametric Statistical Methods. John Wiley & Sons, New York.

JUNIPER, B.E., & JEFFREE, C.E. 1983. Plant Surfaces. Edward Arnold, London.

MITTLER, T.E. & DADD, R.H. 1964. An improved method of feeding aphids on artificial diets. Ann. Entomol. Soc. Am. 57:139-40.

NIEMEYER, H.M., PESEL, E., FRANKE, S. & FRANCKE, W. 1989. Ingestion of the benzoxazinone DIMBOA from wheat plants by aphids. Phytochemistry 28:2307-2310.

ROBINSON, J. 1992. Modes of resistance in barley seedlings to six aphid (Homoptera: Aphididae) species. J. Econ. Entomol. 85:25 10-2515.

RHOADES, D.F. 1979. Evolution of plants chemical defense against herbivores. In Herbivores - Their interaction with secondary plant metabolites. (G.A. Rosenthal & D.H. Janzen, eds.). Academic Press, New York, p.4-56.

ROSENTHAL, G.A. & JANZEN, D.H. 1979. Herbivores - Their interaction with secondary plant metabolites. Academic Press, New York.

RUSTAMANI, M.A., KANEHISA, K., TSUMUKI, H. & SHIRAGA, T. 1992. Further observations on the relationship between aconitic acid contents and aphid densities on some cereal plants. Bull. Res. Inst. Bio. 1:9-20.

SALATINO, A. & BONZANI, J. 1988. Método para a separação de constituintes comuns de ceras vegetais por cromatografia em camada delgada. Bolm. Botânica, Univ. S. Paulo 10: 1-6.

SALATINO, A., SALATINO, M.L.F., AMARAL, M.C.E., ROQUE, F. N. & VILEGAS, W. 1985. Ceras de Ias superficies adaxial e abaxial de hojas de dicotiledoneas del cerrado brasilero. Medio Ambiente 7:15-20.

VARANDA, E.M., ZÚÑIGA, G.E., SALATINO, A., ROQUE, N. F. & CORCUERA, L. J. 1992. Effect of ursolic acid from epicuticular waxes of *Jacaranda decurrens* on *Schizaphis graminum* (Rondani). J. Nat. Prod. 55:800-803.

ZÚÑIGA, G.E., ARGANDOÑA, V.H., NIEMEYER, H.M. & CORCUERA, L.J. 1983. Hidroxamic acids content in wild and cultived Gramineae. Phytochemistry 22:2665-2668.

ZÚÑIGA, G.E. & CORCUERA, L.J. 1986. Effect of gramine in the resistance of barley seedlings to aphid *Rhopalosiphum maidis*. Entomol. Exp. Appl. 40:259-262.

ZÚÑIGA, G.E., CORCUEPA, L.J. & VARANDA, E.M. 1988. Effect of gramine on the feeding behavior of the aphids *Schizaphis graminum* and *Rhopalosiphum padi*. Entomol. Exp. Appl. 47:161-165.

ZÚÑIGA, G.E., SALGADO, M.S. & CORCUEPA, L.J. 1985. Role of indole alkaloid in the resistance of barley seedling to aphids. Phytochemistry 24:945-947.