ECOLOGY, BEHAVIOR AND BIONOMICS

Males Are Attracted by Female Traps: A New Perspective for Management of *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae) Using Sexual Pheromone

MAURÍCIO U. VENTURA[1], ELMO P. MELLO[1], ALFREDO R.M. OLIVEIRA[2], FÁBIO SIMONELLI[2], FRANCISCO A. MARQUES[2] AND PAULO H.G. ZARBIN[2]

[1]*Depto. de Agronomia, CCA, Universidade Estadual de Londrina Caixa postal 6001, 86051-970, Londrina, PR, e-mail: mventura@uel.br*
[2]*Depto. de Química, Universidade Federal do Paraná Caixa postal 19081, 81531-990, Curitiba, PR*

Neotropical Entomology 30(3): 361-364 (2001)

Machos são Atraídos por Armadilhas com Fêmeas: Uma Nova Perspectiva para Manejo de *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae) Usando Feromônio Sexual

RESUMO - As respostas de *Diabrotica speciosa* (Germar) para armadilhas com adultos foram estudadas como sugestão da produção de feromônio. Inicialmente, um ensaio para estabelecer a altura adequada para colocação de armadilhas para capturar *D. speciosa* foi conduzido em campo de feijão. Armadilhas colocadas a 0,25 m de altura capturaram cerca de 3,5 vezes mais adultos do que a 0,5; 0,75 e 1,0 m. As respostas para fêmeas e machos virgens de duas idades (mais novos = 24 a 72h e mais velhos = 72 a 120h após emergência de adultos), fêmeas e machos copulados foram avaliadas em campo de feijão consorciado com café, *Coffea arabica* L. Armadilhas com fêmeas virgens capturaram cerca de 22 (mais jovens) e 13 vezes (mais velhas) mais adultos machos do que as testemunhas (armadilhas sem insetos). Capturas foram cerca de 70% mais efetivas usando fêmeas mais jovens. Nenhum dos outros tratamentos aumentou as capturas nas armadilhas. Os resultados indicam que há um feromônio sexual produzido por fêmeas em *D. speciosa.*

PALAVRAS-CHAVE: Insecta, semioquímico, altura de colocação de armadilhas, Luperini, Fucata.

ABSTRACT - The field responses of *Diabrotica speciosa* (Germar) to traps baited with adults were studied as an suggestion of the pheromone production. Firstly, an assay to settle a suitable height placement to capture *D. speciosa* was carried out in common beans, *Phaseolus vulgaris* L., field. Traps placed at 0.25-m height captured ca. 3.5 times more beetles than 0.5, 0.75 and 1.0-m traps. The responses to virgin females and males of two ages (younger = 24 to 72h and older = 72 to 120h after adult emergence), mated females and males were assessed in common bean interplanted among coffee, *Coffea arabica* L., field. Virgin females baited traps captured ca. 22 times (younger) and 13 times (older females) more male beetles than control traps. Captures were ca. 70% more effective using younger females. None of the other treatments improved trap attraction. These results strongly indicated that there is a female-produced sexual pheromone in *D. speciosa*.

KEY WORDS: Insecta, semiochemical, trap height placement, Luperini, Fucata.

In the American continent, *Diabrotica* Chevrolat genus, of neotropical origin, includes several important pests. Two taxonomic groups proposed for *Diabrotica: Diabrotica speciosa* (Germar) in the *fucata* group together with *D. undecimpunctata howardi* Barber, *D. balteata* LeConte and *D. tibialis* Jacoby, among others (Krysan 1986). The *fucata* group species are multivoltine and, as larvae, polyphagous. The *virgifera* group species are univoltine and larval host range is restricted to some grasses (Branson & Krysan 1981). The leaf beetle *D. speciosa* is a polyphagous species associated with a wide range of botanical families and is one of the most important pests of Latin America agriculture. It damages vegetables, fruits, legumes, cucurbits and grasses.

Management strategies for *D. speciosa* are restricted to chemical control. Adult beetles are controlled by foliar spraying. However, insecticides lose their effects quickly and adults reinfest crops. Hence growers use them several times throughout the growing season. Rootworms are controlled by great amounts of soil-applied insecticides that are expensive and an environment hazard.

Despite its neotropical origin and importance to Latin American agriculture, the chemical ecology of *D. speciosa* has been poorly studied. Studies are restricted on the North American species of *Diabrotica,* to which, besides pheromones, natural compounds from host plants (kairomones) have been focused (e.g., Metcalf & Lampman 1989, Deem-Dickson & Metcalf 1995). Pheromones, volatile and non-volatile kairomones have been proposed to monitor and control *Diabrotica* spp (e.g., Deem-Dickson & Metcalf 1995, Herbert *et al*. 1996, Hoffmann *et al*. 1996, Ventura *et al*. 1996, 2000). This study was conducted to suggest the pheromone production by adults of *D. speciosa.* Firstly, we carried out an assay to settle an appropriate trap height placement to capture *D. speciosa* in common bean fields. Using the best trap placement, we compared the attractiveness of virgin males and females of two ages and mated males and females to individuals of both sexes.

Material and Methods

The field experiments were carried out at the Universidade Estadual de Londrina School Farm, in Londrina (latitude 23°19'S, longitude 51°12'W), Paraná State, Brazil. In 1997, traps were placed in common beans, *Phaseolus vulgaris* L., cv. Iapar 57 (sown on February 10, 1997), crop. In 1998, common beans was interplanted (September 15, 1998) among ten-month grown coffee, *Coffea arabica* L. Plastic cups (750 ml) painted with yellow gold Suvinil paint 2450-0103 (Basf S/A São Bernardo do Campo, SP) were placed upside down on wooden stakes. Traps were externally coated with clear insect adhesive Tangle Trap (Tangle Foot Co., Grand Rapids, Mich. MI, USA).

Insect Rearing. Beetles were field collected using sweeping net. Adults were provided with carrot slices and water in rearing cages (40 x 40 x 40 cm). Females laid eggs on a black gauze (Milanez 1995). Twice a week, eggs were removed and placed in corn seedlings in plastic vessels (26 X 16 X 9 cm). Seedlings were replaced once during larval development when vessels were put in adult cages. Adults were collected from cages and sexed before mating every day. Males and females were put in separate vessels. For treatments with mated insects, pairs (age = four days) were confined in the vessels and observed. Only insects that were seen mating were used.

Trap Height Placement. Traps were placed at 0.25, 0.5, 0.75 and 1.00-m above the ground level on March 27, 1997. A 0.25-m minimal height trap placement was used because of strong rain in this season frequently covering the yellow surface of the trap with soil land sparkles. Traps were placed in the field at 3:00 P.M. and removed after 24h.

Pheromone Production Evidence. A plastic cage made with photography film vial (cylinder 3 cm diameter X 5 cm height) containing three adults insects was glued on the bottom of each trap. The cage contained four holes (1 X 3 cm) around. The holes were closed with nylon screen. The screen prevented beetles to fly away and allowed volatile chemicals to emanate. Treatments were: virgin males, 24 to 72h after adult emergence (a.a.e.) or 72 to 120h a.a.e.; virgin females, 24 to 72h a.a.e. or 72 to 120h a.a.e.; mated females and males; and control (no insects). Traps were placed in the field (in the best height placement determined before) at 3:00 P.M. on October 19, 1998 and returned to the laboratory after 48h where the beetles were counted and sexed.

Experimental Design and Statistical Analysis. Experiments were conducted in a four replicate randomized complete block design. Distance among traps was 5-m within a block, and 10-m between blocks. Analysis of variance (ANOVA) was performed on data. Tukey's studentized range test (HSD) was used to compare individual means (SAS Institute 1989). Data were transformed using log (x+1) constant to normalize the data and reduce heterogeneity of variances of the height placement assay. Means and standard errors of means presented are untransformed.

Results and Discussion

Trap Height Placement. Traps placed at 0.25-m height captured ca. 3.5 times more beetles than those placed 0.5-, 0.75- and 1.0-m heigh (Table 1). *Diabrotica* spp. trap height captures, in general, have been recorded as variable, according to crop development. Hoffmann *et al*. (1996) found that in capturing striped cucumber beetle in the small and medium-height squash, traps placed closest to the ground were the most effective. On the other hand, in taller cucurbits they would be positioned at mid- or upper canopy level. Northern corn rootworm, *D. barberi* Smith and Lawrence, exposed outside of cornfields (Ladd *et al*. 1983), was caught more close to the ground level. However *D. virgifera virgifera* LeConte captures were greater in traps placed at ear height than in traps placed at ground level (Weissling & Meinke 1991). Hence *D. speciosa* captures in the lowest trap placement near the level of plant canopy are probably reflecting beetle moving activity.

Pheromone Production Evidence. Significant differences were found only in captures of males (Fig. 1). None of the treatments improved trap attraction to females. Virgin females-baited traps attracted significantly more male beetles

Table 1. Mean number ± SEM of adults of *D. speciosa* caught by yellow stick traps placed at 0.25, 0.5, 0.75 and 1.00 m above the ground after 24h (March 24, 1997).

Height (m)	Beetles
0.25	8.6 ± 2.90a
0.50	2.4 ± 0.50b
0.75	2.1 ± 0.40b
1.00	2.4 ± 0.75b

Means with different letter are significantly different from Tukey's studentized range test ($P<0.05$); n = 4.

than male-baited traps and control. Virgin female-baited traps captured ca. 22 and 13 times more male beetles than the control (24 to 72h a.a.e. and 72 to 120h a.a.e., respectively). Virgin males and mated insects (females and males) did not improve the males caught. Therefore, these results suggest that virgin females produce a sexual pheromone. The female-produced sex pheromone system seems to be wide spread in *Diabrotica* genus. In general, virgin females exhibit a potent attraction to males (Guss *et al*. 1982). Sex pheromones also have been isolated and identified from virgin females. In the *virgifera* group, the compound racemic 8-methyl-2-decanol propanoate was isolated from *D. virgifera virgifera* virgin females. Besides *D. virgifera virgifera*; *D. v. zeae* Krisan and Smith, *D. longicornis barberi* Smith and Lawrence, *D. longicornis longicornis* (Say) and *D. porracea* Harold were attracted to 8-methyl-2-decanol propanoate (Guss *et al*. 1982). Another chemical identified was the 6,12-dimethylpentadecan-2-one, from *D. balteata* LeConte virgin females (Chuman *et al*. 1987). And in the *fucata* group, 10-methyl-2-tridecanone was isolated and identified from *D. undecimpunctata howardi* virgin females. This compound also attracted males of *D. undecimpunctata undecimpunctata* Mannerhiem (Guss *et al*. 1983). Synthetic procedures for 8-methyl-2-decanol propanoate (Sonnet *et al*. 1985) and 10-methyl-2-tridecanone (Senda & Mori 1983) have provided more opportunities for use of these compounds in integrated pest management programs.

D. speciosa younger virgin females (24 to 72h a.a.e.) captured 70% more male beetles than older (72 to 120h a.a.e.) ones (Fig. 1). This may indicate greater pheromone production in the onset of the calling behavior. Pheromone extraction methods in *D. speciosa* must consider these differences. Johnson *et al*. (1985) caught 16,000 male beetles on 10 pheromone-baited sticky traps in 24 days, compared to 70 beetles caught on unbaited traps (ca. 133 male beetles per 48h). In our study, means were 398.5 and 233.0 males per trap (24 to 72h a.a.e. and 72 to 120h a.a.e., respectively) baited with three females during 48h. Hence it seems that virgin female-produced sex pheromone is a strong attractant to *D. speciosa* males as well.

Isolation of a pheromone produced by *D. speciosa* females may confirm the female system of sexual attraction. It creates new challenges for management of *D. speciosa.* This species is a serious rootworm pest mainly in potato and corn. Growers are unable to settle control levels. Traps to *D. speciosa* had been used with visual and gustatory cues (Nishida *et al*. 1986, Roel & Zatarin 1989, Nishida & Fukami 1990, Ventura *et al*. 1996), hence pheromone isolation, identification and synthesis may establish a suitable tool for use in lure traps to improve male beetle captures. In the same

Figure legend:
YVF = Younger virgin females: 24 to 72h after adult emergence (a.a.e.)
OVF = Older virgin females: 72 to 120h a.a.e.
YVM = Younger virgin males: 24 to 72h a.a.e.
OVF = Older virgin females: 72 to 120h a.a.e.
MF = Mated females
MM = Mated males

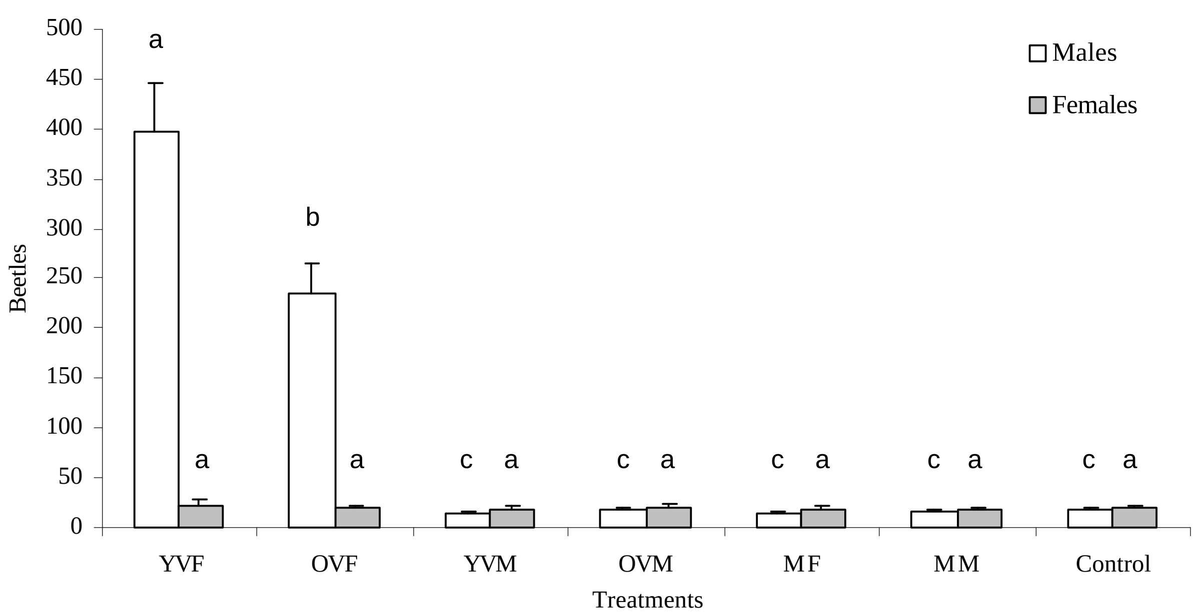

Figure 1. Mean number ± SEM of males and females of *D. speciosa* caught by yellow stick traps in response to virgin females and males of different ages and mated females and males after 48h (October 19, 1998). Bars with the same color with different letter are significantly different from Tukey's studentized range test ($P<0.05$); n = 4.

way, using 8-methyl-2-decyl-propanoate in lure traps, Meinke *et al*. (1989) captured significantly more *D. virgifera virgifera* beetles than control traps throughout the season. For other *fucata* group species, *D. undecimpunctata howardi,* the female produced pheromone 10-methyl-2-tridecanone was more suitable attractant to monitor beetle population changes in peanut fields than the TIC mixture (1,2,4-trimethoxybenzene, indole and trans-cinnamaldehyde), a simplified blend of *Curcubita maxima* Duchesne blossoms volatile (Herbert *et al.* 1996).

Considering results obtained in our studies, laboratory analyses are being carried out to isolate and to establish the chemical structure of the pheromone.

Acknowledgments

We thank David C. Tramontina (Laboratory of Entomology, Universidade Estadual de Londrina, Londrina, PR, Brazil) for logistical help, José Maria Milanez (Empresa de Pesquisa Agropecuária e Extensão Rural, Chapecó, SC, Brazil) for insect rearing assistance and CNPq for a scholarship granted to Elmo P. Mello.

Literature Cited

Branson, T.F. & J.L. Krysan. 1981. Feeding and oviposition behavior and life cycle strategies of *Diabrotica*: an evolutionary view with implications for pest management. Environ. Entomol. 10: 826-831.

Chuman, T., T.L. Guss, R.E. Doolittle, J.R. Mclaughlin, J.L. Krysan, J.M. Schalk & J.H. Tumlinson. 1987. Identification of a female-produced sex pheromone from banded cucumber beetle, *Diabrotica balteata* LeConte (Coleoptera: Chrysomelidae). J. Chem. Ecol. 13: 1601-1616.

Deem-Dickson, L. & R.L. Metcalf. 1995. Attractants for adult corn rootworm monitoring and control, pp. 93-100. In Proceedings, Illinois Agricultural Pesticide Conference, 3-6 Jan 1995, U. Illinois, Urbana.

Guss, P.L., J.H. Tumlinson, P.E. Sonnet & A.T. Proveaux. 1982. Identification of a female-produced sex pheromone of the western corn rootworm. J. Chem. Ecol. 8: 545-556.

Guss, P.L., J.L. Tumlinson, P.E. Sonnet & J.R. Mclaughlin. 1983. Identification of a female-produced sex pheromone from the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber. J. Chem. Ecol. 9: 1363-1375.

Herbert, D.A. Jr., B.N. Ang & R.L. Hodges. 1996. Attractants for adult southern corn rootworm (Coleoptera: Chrysomelidae) monitoring in peanut and relationship of trap catch to pod damage. J. Econ. Entomol. 89: 515-525.

Hoffmann, M.P., J.J. Kirkwyland, R.F. Smith & R.F. Long. 1996. Field tests with kairomone-baited traps for cucumber beetles and corn rootworms in cucurbits. Environ. Entomol. 25: 1173-1181.

Krysan, J.L. 1986. Introduction: Biology, distribution and identification of pest *Diabrotica,* p.1-23. In J.L. Krysan & T.A. Miller (eds.). Methods for study of pest *Diabrotica.* New York: Springer-Verlag, 260p.

Ladd, T.L. Jr., B.R. Stiner & H.R. Krueger. 1983. Influence of color and height of eugenol-baited sticky traps on attractiveness to northern corn rootworm beetles (Coleoptera: Chrysomelidae). J. Econ. Entomol. 77: 652-654.

Meinke, L.J., Z.B. Mayo & T. Weissling. 1989. Pheromone delivery system: western corn rootworm (Coleoptera: Chrysomelidae) pheromone encapsulation in a starch borate matrix. J. Econ. Entomol. 82: 1830-35.

Milanez, J.M. 1995. Técnicas de criação e bioecologia de *Diabrotica speciosa* (Germar, 1824) (Coleoptera: Chrysomelidae). Tese de Doutorado, Escola Superior de Agricultura "Luiz de Queiroz"/Universidade de São Paulo, Piracicaba, 102p.

Nishida, R. & H. Fukami. 1990. Sequestration of distasteful compounds by some pharmacophagous insects. J. Chem. Ecol. 16: 151-164.

Nishida, R., H. Fukami, Y. Tanaka, B.P. Magalhães, M. Yokoyama & A. Blumenschein. 1986. Isolation of feeding stimulants of brazilian leaf beetles (*Diabrotica speciosa* and *Cerotoma arcuata*) from the root of *Ceratosanthes hilariana.* Agric. Biol. Chem. 50: 2831-2836.

SAS Institute. 1989. SAS/stat. User's guide, Version 6, 4th. ed. SAS Institute, Cary, NC.

Senda, S. & K. Mori. 1983. Synthesis of (R)-(-)-10-Methyl-2-tridecanone, the pheromone of the southern corn rootworm. Agric. Biol. Chem.47: 795-798.

Sonnet, P.E., R.L. Carney & C. Henrick. 1985. Synthesis of stereoisomers of 8-methyl-2-decanol and esters attractive to several *Diabrotica* sp. J. Chem. Ecol. 11: 1371-1387.

Ventura, M.U., M. Ito & R. Montalván. 1996. An attractive trap to capture *Diabrotica speciosa* (Ger.) and *Cerotoma arcuata tingomariana* Bechyné. An. Soc. Entomol. Brasil 25: 529-535.

Ventura, M.U., M.C. Martins & A. Pasini. 2000. Responses of *Diabrotica speciosa* and *Cerotoma arcuata tingomariana* (Coleoptera: Chrysomelidae) to volatile attractants. Florida Entomol. 83:403-410.

Received 14/04/00. Accepted 10/06/01.