SCIENTIFIC NOTE

Adult Diapause Morph of the Brown Stink Bug, *Euschistus servus* (Say) (Heteroptera: Pentatomidae)

MIGUEL BORGES[1,2], AIJUN ZHANG[3], MARY J. CAMP[4] AND JEFFREY R. ALDRICH[5]

[1]USDA-ARS/EMBRAPA/LABEX, Bldg 007.
Insect Chemical Ecology Laboratory - Agricultural Research Center-West.
Beltsville, Maryland 20705.
[2]Empresa Brasileira de Pesquisa Agropecuária, Embrapa, SAIN, Parque Rural Final,
Av. W/3 Norte, Caixa postal 040315, 70770-901, Brasília, DF. Brazil.
[3,5]Insect Chemical Ecology Laboratory, USDA-ARS, Bldg 007.
Agricultural Research Center-West. Beltsville, Maryland 20705.
[4]Biometrical Consulting Service, USDA-ARS, Bldg 005, Beltsville Agricultural
Research Center-West Biometrical Consulting Service.

Neotropical Entomology 30(1): 179-182 (2001)

Diapausa em Adultos do Percevejo Marrom Neártico, *Euschistus servus* (Say) (Heteroptera: Pentatomidae)

RESUMO - O percevejo marrom neártico, *Euschistus servus* (Say), é abundante em toda a Região Leste da América Norte e é encontrado geralmente alimentando-se em culturas de soja, mullein, feijão, tomate, ervilha, algodão, trigo, milho, tabaco e pêssego. A mudança da coloração de verde para marrom demonstrou ser um indicativo de diapausa reprodutiva em *E. servus*. Os insetos de coloração marrom viveram por mais tempo que os indivíduos de coloração verde, as fêmeas não colocaram nenhum ovo, e os machos não produziram feromônio sexual. A alta taxa de mortalidade registrada para a colônia de indivíduos verdes de adultos de *E. servus* foi interpretada como custo fisiológico associado à reprodução. O componente principal do feromônio dessa espécie foi confirmado como 2*E*, 4*Z*-decadienoato de metila, de acordo com trabalho realizado anteriormente. A primeira geração dessa espécie se desenvolve em plantas silvestres e a segunda geração migra frequentemente para culturas onde podem exceder os níveis de dano econômico. Porém, técnicas semioquímicas podem ser empregadas para suprimir populações da segunda geração de *E. servus*. Armadilhas ou culturas armadilhas iscadas com feromônio podem capturar ou concentrar as fêmeas da espécie para serem destruídas, ou mesmo a técnica do confundimento, pode ser utilizada para diminuir a possibilidade de acasalamentos.

PALAVRAS-CHAVE: Insecta, coloração, feromônio, 2*E*, 4*Z*-decadienoato de metila.

ABSTRACT - The brown stink bug, *Euschistus servus* (Say), is abundant throughout most of eastern North America and is commonly found feeding on soybean, mullein, beans, tomatoes, peas, cotton, wheat, corn, tobacco and peach. Color change in *E. servus* from green to reddish-brown was shown to be an indicator of reproductive diapause. Reddish-brown insects lived longer than green individuals, females laid no eggs, and males did not produce pheromone. The high mortality registered for the green colony of *E. servus* adults was associated with the physiological cost associated with reproduction. The main pheromone component of this species is methyl 2*E*,4*Z*-decadienoate, in agreement with previous work. The first generation of this species develops on noncrop hosts and the second generation often migrates to crops where they may then exceed economic damage thresholds. Traps or trap crops baited with pheromone to catch or concentrate females for destruction, or even a pheromone-based disruption of orientation behavior to decrease the mating success, are possible semiochemical techniques to suppress populations of second generation of *E. servus*.

KEY WORDS: Insecta, coloration, pheromone, methyl 2*E*,4*Z*-decadienoate.

The brown stink bug, *Euschistus servus* (Say), is abundant throughout most of the eastern North America and is commonly found feeding on soybean, mullein, beans, tomatoes, peas, cotton, wheat, corn, tobacco and peach (Munyaneza & McPherson 1994). This bivoltine species overwinters as an adult in the fall (Rolston & Kendrick 1961, McPherson 1982), the normal situation for other pentatomid species in temperate climates. The following spring the bugs mate and deposit eggs; the first generation normally develops on wild (noncrop) hosts, while the second generation typically develops on cultivated crops (Ehler 2000).

Individuals from a laboratory-reared *E. servus* colony were observed to sometimes change color in the entire ventral surface, from green to reddish-brown. Therefore, behavioral and physiological studies were conducted to determine whether coloration in *E. servus* could be used as an indicator of diapause. We compared the reproductive activity, mortality, weight, and pheromone production among the green and reddish-brown *E. servus*.

E. servus were field collected from May through June, 1998 and 1999, feeding on new growth of Empress-trees, *Paulownia tomentosa* (Thunb.) Steud. (Scrophulariaceae), and reared in an incubator (Percival Scientific Inc., Model 1-30BLL, Boone, Iowa), at temperatures of 26 and 23°C, humidities of 75 and 61% for 14:10 day and night cycle, respectively. The insects were kept on a diet of sunflower seeds, soybeans and green beans. A second field collection was made during the fall (October/1999) on soybean plots at the maturation R8 stage of development or harvest maturity (Fehr *et al.* 1971).

In January 1999, only a few egg-masses were being produced from the approximately 75 adult females in the colony, and most of the individuals in the colony had reddish-brown sterna, with only a few insects having green sternal coloration. The colony was separated into two groups according to these color differences. Fecundity, weight, pheromone production, and mortality were recorded daily for both colonies. The two colonies were monitored for color changes of their adults and, if changes occurred, the adults were transferred to the matching colony.

Pheromone was collected from groups of 15 virgin individuals ranging from 15 to 20 day-old males *E. servus*. Both green and brown colored bugs were introduced into separate aeration apparatuses, which consisted of four-neck glass bottles (1000 ml) connected to two Super Q traps (15 cm X 0.6 cm OD, 200 mg each, Alltech Associates, Inc. Deerfield, Illinois). The air was filtered with charcoal traps (Activated Carbon, 6-14 mesh, Fisher Scientific; 2 cm X 1.5cm OD) before being drawn through the apparatus by vacuum at ~1 liter/min. The bugs were aerated continuously for several days, and were provided fresh green beans as needed. Super Q traps were changed every 24h. The airborne volatiles were extracted by percolating each Super Q trap with four portions of GC-grade methylene chloride (0.5 ml/each), and the resultant methylene chloride solutions were stored at - 4°C in a freezer until further analysis.

Capillary GC analysis of the pheromone was performed on a Hewlett Packard 6890 gas chromatograph equipped with a DB-5 capillary column (60 m X 0.25-mm ID, 0.25-um film thickness; J & W Scientific, Inc. Folsom, CA.) in the splitless mode. The oven temperature was programmed at 50°C for 2 min, then increased at 15°C/min to 300°C and held for 10 min. Injector and detector temperatures were set at 310°C. Nitrogen was the carrier gas, with the flow rate of 2 ml/min. GC-mass spectrometry (GC-MS) was carried out with a Hewlett Packard 6890 gas chromatograph coupled to a HP 5973 Mass Selective Detector using the same capillary column and conditions as above, but with helium as the carrier gas.

Statistical analysis reported in the results were performed using PROC's Mixed Lifetest (SAS 1997) and StatXact (Cytel – Mehta & Patel 1996).

The abdominal sterna of *E. servus* adults collected by one of us (J.R.A.) in the spring were yellowish-green and bright-green coloration for males and females, respectively (hereinafter referred to as "green"). A total of 23 adult *E. servus* were collected in soybean plots during the summer and fall of 1999. All bugs collected during the summer were green (n = 16), whereas five were brown in coloration for those collected at the beginning of fall (n = 7).

Seventy egg masses were obtained from the green colony, while none were produced by females from the brown colony of *E. servus*. The fecundity of green females was highest during the second week (n = 45 females) of adulthood when nearly one egg mass/female was being laid, compared with 0.2 egg mass/female (n = 53 females), and 0.7 egg mass/female (n = 31 females) for the first and third week, respectively.

The number of bug deaths for the four color and sex groups were analyzed first as a survival analysis. The tests for homogeneous strata [Log-Rank, Wilcoxon and –2log (LR)] were all statistically significant at $P \leq 0.0001$, showing that the survival curves were not all the same for the groups. Because the number of dead were observed only at two discrete points, week 2 and week 3, rather than on a continuous or more approximately continuous scale, and the data were heavily censored as many bugs were still alive at week 3, it was decided that a survival analysis was not the best analysis of the data. A second analysis looked at the number of alive or dead at the end of week 3. The hypothesis that proportion of dead was equal for green and reddish-brown bugs was tested for each sex. The Fisher exact test for two independent binomial populations was done. The proportions dead (n = 22 and n = 2 for the green and brown males respectively) were very different for males (FI = 33.13 P = 0.0000). The proportions for females (n = 13 and n = 1 for the green and brown females respectively) were different (FI = 4.1719 P = 0.0522) but not as overwhelmingly so as for males.

The weigh of the bugs was analyzed as a two-factor general linear model with color and sex as the factors. The analysis showed that only color was statistically significant (F = 19.43 P = 0.0001) in accounting for the difference in insect weights (n = 10, for each color group). The weight of the males and females within a color are nearly identical, i.e., 0.185 and 0.184 g for green male and female respectively, whereas the brown insects weighted 0.158 and 0.160 g for brown males and females respectively. The means were

compared using the Fisher protected t-test.

When pheromone production was contrasted between the two groups, only green males produced the characteristic pheromone component, methyl 2*E*,4*Z*-decadienoate (Aldrich *et al*. 1991) (Fig. 1). Females, regardless of coloration, never produced any pheromone, which is consistent with earlier research on *Euschistus* spp. pheromone production (Aldrich *et al*. 1991). Dissection of a female *E. servus* by one of us (J.R.A.) revealed that reddish-brown insects have undeveloped oocytes and more extensive fat body compared with green females.

The high mortality registered for the green colony of *E. servus* adults may represent a physiological cost associated with reproduction. For example, sexually active male and female southern green stink bugs, *Nezara viridula* (L.), had shortened life spans by six and two times compared with unmated cospecifics (Mitchell & Mau 1969), and Harris *et al*. (1984) reported that diapausing *N. viridula* lived longer than non-diapausing individuals. For the Neotropical brown stink bug, *E. heros* (F.), longevity varied in a like manner according to sex and sexual activity (Costa *et al*. 1998). Hiding behavior (Harris *et al*. 1984) may be associated with lower food consumption and could explain the lighter weight for the reddish-brown insects in this study.

Only green *E. servus* males produced pheromone; none was detected from reddish-brown males. Since pheromone production in male stink bugs is an important component of mate-finding (Mitchell & Mau 1971, Harris & Todd 1980, Borges *et al.* 1987, Borges & Aldrich 1994, Borges 1995, Borges *et al*. 1998), the absence of pheromone production in reddish-brown *E. servus* males supports the observed color association with diapause. Thus, reddish-brown coloration may be used as a visible indicator of diapause in adult *E. servus*.

Color change associate with diapausing have been reported for other pentatomids species such as *Nezara viridula* (Harris *et al*. 1984) and for the Neotropical brown stink bug, *E. heros* (Mourão & Panizzi 2000a and b). However, although this variation in color was known for *E. servus*, it was never related with diapause for this species (J.E. McPherson - personal communication).

Egg masses were collected only from the colony of green individuals and none were recorded from females in the reddish-brown colony, indicating that color is associated with reproductive status.

Seventy one percent of the *E. servus* adults collected in the field during the fall of 1999 were brown in color; this result corroborates that of Rolston & Kendrick (1961) who reported that the species enters hibernation in the fall. Therefore, the discovery of the morphs raises several questions whose answers might be highly relevant to stink bug management: what triggers diapause to begin in the fall, and to end in the spring? At what time of year does pheromone production begin and end? The new information on diapause supports the argument that mid-summer is the best time to use pheromone intervention, which would also be the time

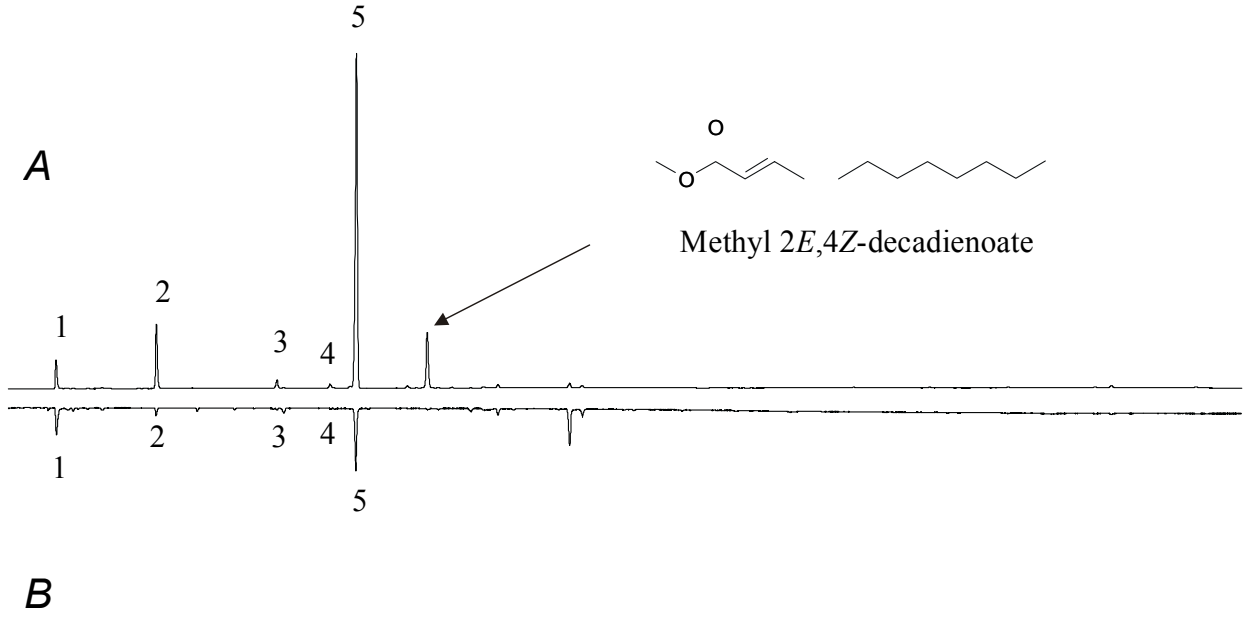

Figure 1. Gas chromatograms of the airborne pheromone extract of males *E. servus* males. (**A**) yellowish-green (non-diapausing), and (**B**) reddish-brown (diapausing). Compounds numbers **1** = 4-oxo-(*E*)-2-hexenal, **2** = (*E*)-2-octenal, **3** = dodecane, **4** = (*E*)-2-decenal, and **5** = tridecane are previously known metathoracic scent gland components (Aldrich 1995).

that the bugs switch crop hosts, while using pheromones in spring or fall might be ineffective.

Furthermore, combining the knowledge of diapausing in *E. servus* with Rolston & Kendrick's (1961) finding that the first generation of this species develops on noncrop hosts may lead to new semiochemical strategies to manage populations of this pest. For instance, traps baited with pheromone to catch females, or even a pheromone-based disruption of orientation behavior to decrease mating success (McBrien *et al*. 1996), are possible semiochemical techniques to suppress populations of second generation of *E. servus* before they migrate to cultivated crops.

Acknowledgements

Karen Collins and Carolyn Smith helped with field collecting and laboratory rearing of the bugs, for which we are grateful. We also thank the anonymous reviewers whose comments enhanced the contents of this manuscript.

Literature Cited

Aldrich, J.R. 1995. Chemical communication in the true bugs and parasitoid exploitation. In: "Chemical Ecology of Insects II", p. 318-363, ed. By R. T. Carde and W. J. Bell. Chapman & Hall, New York.

Aldrich, J.R., M.P. Hoffmann, J.P. Kochansky, W.R. Lusby, J.E. Eger & J.A. Payne. 1991. Identification and attractiveness of a major pheromone component for Nearctic *Euschistus* spp. Stink bugs (Heteroptera: Pentatomidae). Environ. Entomol. 20: 477-483.

Borges, M. 1995. Attractant compounds of the southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae). An. Soc. Entomol. Brasil. 24: 215-225.

Borges, M. & J.R. Aldrich. 1994. Attractant pheromone for Nearctic stink bug, *Euschistus obscurus* (Heteroptera: Pentatomidae): insight into a Neotropical relative. J. Chem. Ecol. 20: 1095-1102.

Borges, M., K. Mori, M.L.M., Costa & E.R. Sujii. 1998. Behavioural evidence of methyl-2,6,10-trimethyltridecanoate as a sex pheromone of *Euschistus heros* (Het., Pentatomidae). J. Appl. Entomol. 122: 335-338.

Borges, M., P.C., Jepson & P.E. Howse. 1987. Long-range mate location and close-range courtship behaviour of the green stink bug, *Nezara viridula* and its mediation by sex pheromones. Entomol. Exp. Appl. 44,: 205-212.

Costa, M.L., M. Borges & E.V. Vilela. 1998. Biologia reprodutiva de *Euschistus heros* (F.) (Heteroptera: pentatomidae). An. Soc. Entomol. Brasil. 27: 559-568.

Ehler, L.E. 2000. Farmscape ecology of stink bugs in Northern California. Thomas Say Publications: Memoirs. Entomol. Soc. Amer. Lanhan, MD. 59p.

Fehr, W.R., Cavines, C.E., Burmood, D.T. & J.S. Pennington. 1971. Stage of development descriptions for soybeans, *Glycine max* (L.) Merrill. Crop Sci. 11: 929-931.

Harris, V.E. & J.W. Todd. 1980. Male-mediated aggregation of male, female and 5th instar southern green stink bugs and concomitant attraction of a tachinid parasite, *Tricopoda pennipes*. Entomol. Exp. Appl. 27: 117-126.

Harris, V.E., J.W. Todd & B.G. Mullinix. 1984. Color change as an indicator of adult diapause in the southern green stink bug, *Nezara viridula*. J. Agric. Entomol. 1: 82-91.

Mehta, C. & N. Patel. 1996. StatXact 3 for Windows, Cambridge, MA: Cytel Software Corporation, 795p.

McBrien, H. L., G. J. R., Judd & J. H. Borden. 1996. Potential for pheromone-based mating disruption of mullein bug, *Campylomma verbasci* (Meyer) (Heteroptera: Miridae). Can. Entomol. 128: 1057-1064.

McPherson, J.E. 1982. The Pentatomoidea (Hemiptera) of northeastern North America with emphasis on the fauna of Illinois. So. Illinois Univ. Press, Carbondale and Edwardsville. 240p.

Mitchell, W.C. & R.F.L. Mau. 1969. Sexual activity and longevity of the green stink bug, *Nezara viridula*. Ann. Entomol. Soc. Am. 62: 1246-1247.

Mitchell, W.C. & R.F.L. Mau. 1971. Response of the female southern green stink bug and its parasite, *Trichopoda pennipes*, to male stink bug pheromones. J. Econ. Entomol. 64: 856-859.

Mourão, A.P.M. & A.R. Panizzi. 2000a. Diapausa e diferentes fomas sazonais em *Euschistus heros* (Fabr.) (Hemiptera: Pentatomidae) no Norte do Paraná. An Soc. Entomol. Brasil 29: 205-218.

Mourão, A.P.M. & A.R. Panizzi. 2000b. Estagios ninfais fotossensiveis a indução da diapausa em *Euschistus heros* (Fabr.) (Hemiptera: Pentatomidae). An. Soc. Entomol. Brasil 29: 219-225.

Munyaneza, J. & J.E. McPherson. 1994. Comparative study of life histories, laboratory rearing, and immature stages of *Euschistus servus* and *Euschistus variolarius* (Hemiptera: Pentatomidae). Great Lakes Entomol. 26: 263-274.

Rolston, L.H. & R.L. Kendrick. 1961. Biology of the brown stink bug, *Euschistus servus* Say. J. Kansas Entomol. Soc. 34: 151-157.

SAS Institute. 1997. SAS/STAT® Software: Changes and Enhancements through release 6.12, Cary, NC: SAS Institute Inc., 1167p.

Received 6/XII/99. Accepted 07/01/2001.