Electroantennogram Response of Two Western Corn Rootworm (Coleoptera: Chrysomelidae) Adult Populations to Corn and Soybean Volatiles[1]

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Abstract The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, has adapted to crop rotation in parts of Illinois and Indiana with females now laying eggs in soybean, *Glycine max* L., fields in addition to corn, *Zea mays* L., fields. The electroantennogram (EAG) responses of females from the rotation-adapted population (Illinois) were not significantly different than the EAG responses of females from the 'normal' population (Missouri) for any of nine individual volatile treatments evaluated except to (*E,Z*)-2,6-nonadienal. However, females from the rotation-adapted population had nominally greater EAG responses than females from the 'normal' population for eight of nine treatments. This difference was significant when volatile treatments were combined to analyze the main effect of corn rootworm populations. Differences between populations were consistent across volatile treatments, and the volatile treatments × populations interaction was not significant for the analyses of data from females or males. The EAG responses of males from the rotation-adapted corn rootworm population were not significantly different than the EAG responses of males from the 'normal' population for any of the individual volatile treatments evaluated or in the combined analysis.

Key Words *Diabrotica virgifera virgifera,* EAG, crop rotation, maize, *Zea mays, Glycine max*

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is among the most serious insect pests in the United States (Metcalf 1986), and because of recent changes in its distribution and biology, its impact is growing. This species has now become established in parts of Europe (Sivcev et al. 1994), has developed resistance to methyl parathion and/or carbaryl in parts of Nebraska (Meinke et al. 1998), and has adapted to crop rotation in parts of Illinois and Indiana by laying eggs in soybean, *Glycine max* L. fields in addition to corn, *Zea mays* L., fields in a region where crop rotation is prevalent (Levine and Gray 1996, Levine and Oloumi-Sadeghi 1996). The eggs laid in a soybean field overwinter and hatch in a rotated corn field the following spring. The rotation-adapted population of the western corn rootworm is spreading

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(Onstad et al. 1999). Crop rotation was one of the few management alternatives to insecticides (Levine and Oloumi-Sadeghi 1991) and has been widely practiced on U.S. corn acreage (Bullock 1992), providing strong selection pressure for corn rootworm adaptation. Some time ago, this selection pressure led to the development of rotation-adapted populations of the northern corn rootworm, *Diabrotica barberi* Smith and Lawrence, by selecting for a prolonged egg diapause in which some eggs do not hatch until two winters have passed (Krysan et al. 1986, Levine et al. 1992).

The behavioral mechanism responsible for selection of soybean fields for egg-laying by the rotation-adapted population of the western corn rootworm is unknown. Spencer et al. (1999) suggested that decreased adult western corn rootworm fidelity to corn and/or an increased tendency to leave corn fields are mechanisms consistent with the type of selection imposed by crop rotation. Edwards (1996) proposed that the population of western corn rootworm adults that is laying eggs in soybean fields may be attracted to volatiles from corn residues in soybean fields. While Sammons et al. (1997) concluded from laboratory bioassays that the behavioral variant found in Indiana was attracted to soybean, Spencer et al. (1999) found no evidence for attraction to soybean based on post-flight plant association of Illinois 'problem' beetles in a large wind tunnel. Behavioral studies in manipulated environments such as those in Sammons et al. (1997) and Spencer et al. (1999) can be difficult to interpret when the relative importance of all variables is not completely understood. The objective of the current study was to evaluate the electrophysiological response to corn and soybean volatiles of the rotation-adapted western corn rootworm population and a western corn rootworm population not adapted to rotation using an electroantennogram (EAG) technique, which is a simple method for electrophysiological detection of the responses of insect antennae to volatile semiochemicals (Roelofs 1984).

Materials and Methods

Insect sources. In early August 1999, beetles were field-collected in Illinois and Missouri. Illinois beetles were collected using a sweep net in soybean fields in an area of Illinois (Urbana) in which the rotation-adapted phenotype (laying eggs in soybean) predominates (Levine and Gray 1996). Maintaining healthy western corn rootworm adults on soybean foliage was not possible because soybean is not an optimal host (JLS, unpublished data). These insects were immediately fed young corn and silks and shipped by overnight mail to Missouri for EAG testing. Missouri beetles were field collected in corn because few, if any, are encountered in soybeans there. In Missouri, beetles were prompted to drop into a funnel that was held below corn ears (and into a plastic bag below) by gently kicking the base of the stalk. Adults from both locations were maintained on corn silks, young corn ears, and a water sponge until they were evaluated in the EAG, which was within 1 wk of field capture. Both the Illinois and Missouri populations began adult emergence in early July, and adult numbers present in the field peaked in early August (JLS and BEH, unpubl. data, respectively), so the adults evaluated from the two populations were, on average, of similar age.

Extracts and synthetic compounds. Corn silk is olfactorily attractive to western corn rootworm adults (Prystupa et al. 1988). While green silk elicited no significant EAG responses, senescing portions (the brown portion that protrudes from the tip of the ear) of corn silks were previously demonstrated to elicit strong EAG responses from western corn rootworm adults (Abou-Fakhr et al. 1996). An extract of brown silk served as one of the treatments in the current study and was prepared as previously described (Hibbard et al. 1997a). Soybean blossoms and leaves were field collected at peak flowering and were each extracted using similar techniques. Five gram-equivalents (fresh weight before extraction) of each extract was evaluated with adult males and females from the two rootworm populations along with a solvent control.

Because plant material (and its extracts) may be somewhat subject to environmental conditions, synthetic compounds known to be attractive and/or EAG-active to western corn rootworm adults were tested in addition to corn and soybean extracts. The following synthetic compounds were tested: 4-methoxycinnamaldehyde (Lancaster Synthesis, Windham, NH), tridecan-2-one, (*E,Z*)-2,6-nonadienal, (*E*)-2-hexenal (Aldrich, Milwaukee, WI), and 2-phenyl-1-ethanol (Eastman Kodak, Rochester, NY). The compound 4-methoxycinnamaldehyde was previously demonstrated to be highly attractive to western corn rootworm adults (Metcalf and Lampman 1989, Metcalf and Metcalf 1992). The compound (*E*)-2-hexenal was previously isolated from soybean as one of the attractants for the Mexican bean beetle, *Epilachna varivestis* Mulsant (Liu et al. 1989). The compounds tridecan-2-one, (*E,Z*)-2,6-nonadienal, and 2-phenyl-1-ethanol were all among the most EAG-active compounds from brown corn silk (Hibbard et al. 1997a) and/or buffalo gourd, *Cucurbita foetidissima* H.B.K., root powder, the biologically-active component of a commercial bait used for adult rootworm control. Buffalo gourd root powder is attractive to corn rootworm adults (Cossé and Baker 1999), but the primary biologically-active components in the buffalo gourd are non-volatile cucurbitacins which are feeding stimulants for rootworm adults (Metcalf and Metcalf 1992). The overall number of treatments used for EAG evaluation was limited to nine treatments so that all could be tested twice with a single antenna.

Electroantennogram tests. Responsiveness of the antennae of western corn rootworm beetles from the rotation-adapted population from Illinois and a ‘normal’ population from Missouri to volatile extracts of senescing corn silk, soybean blossoms, and soybean leaves, as well as several synthetic compounds were determined using the EAG apparatus described by Bjostad (1988). EAG methods were similar to those in Abou-Fakhr et al. (1996), Hibbard et al. (1997a, b), Hibbard and Webster (1993), and Cossé and Baker (1999). Briefly, the head was removed from an adult rootworm and the base was held in contact with the reference electrode while the tip of one antenna was placed in contact with the recording electrode. Five μl aliquots (50 μg for synthetic materials and 5 gram-equivalents for extracts) of each treatment were transferred to a small piece of filter paper (~0.7 cm × 3.8 cm), and the solvent was allowed to completely evaporate (5 min was allowed for extracts and 2 min for synthetic material). The filter paper was then placed in a Pasteur pipette, and a 5-ml polyethylene syringe was used to puff 3 ml of air through the pipette into an air stream (~100 ml/min) that flowed over the antenna. The entire treatment series was tested twice with each insect (same antenna). Because the responsiveness of an antenna gradually decreased over time, the order of testing was reversed the second time. The average of the two responses for a given insect was considered one replication. The complete series of treatments was evaluated with 15 males and 15 females from each population. The control consisted of evaporated solvent. As mentioned, synthetic material was delivered to the filter paper in 5 μl solvent. For the extracts, 50 g wet weight of plant material was originally extracted in 100 ml of dichloromethane, so the 5 gram-equivalents tested in the experiment were extracted in 10 ml of dichloromethane (note the 50 g was fresh, wet weight and contained mostly unextractable material). The solvent control consisted of an evaporated concentrate of 10 ml dichloromethane that was concentrated in the same manner as the extracts. No EAG response was usually detected for the solvent control (limit of detection was approximately 0.05 mV due to baseline noise). The quantities of materials chosen for evaluation (50 μg) may seem large, but it should be noted that traps baited for diabroticite beetles are typically loaded with 100 mg of "neat" material on dental wicks (Hammack 1996, Hammack et al. 1999), and trap catches go up with increasing dosages to 100 mg and higher (Metcalf and Metcalf 1992). EAG responses with corn rootworm adults also increase with increasing doses from 0.01 μg to 10 μg and 100 μg was used for rootworm EAG evaluations when comparing responses of different compounds (Cossé and Baker 1999).

Statistical analysis. Males and females were analyzed separately because major differences between the EAG responses of males and females to treatments within the study were previously known (Hibbard et al. 1997a), and evaluating these differences was not among the goals of the experiment. A completely-randomized design was used with treatments arranged in a 9 × 2 factorial by using the statistical package SAS (SAS Institute Inc. 1989). The factorial included the nine volatile treatments and the two egg-laying types (from rotation-adapted and 'normal' western corn rootworm populations). Because there were only two population types, no multiple range test was needed for this factor. Dunnett's (1964) test was used to compare EAG responses to each volatile with the control response. Comparisons among volatile treatments were not part of the goals of the experiment and were not analyzed because differences in volatility of the synthetics and dose (when comparing extracts to synthetics) would make comparisons meaningless. Beyond the standard ANOVA for the factorial, we preplanned to compare responses of the rotation-adapted population to the 'normal' population within each volatile treatment (males and females again analyzed separately), and this was done with a *t*-test within the SAS program.

Results

Females from the rotation-adapted western corn rootworm population had greater EAG responses than females from the 'normal' population for eight of nine treatments (Fig. 1), and this difference was significant ($F = 9.98$; df = 1,252; $P = 0.0018$) in the combined analysis. In addition to the significant population main effect, the main effect of volatile treatment was also significant ($F = 39.5$; df = 8,252; $P < 0.0001$) in the analysis of female EAG responses. The EAG average response of females from the two populations of western corn rootworm adults was significantly greater than the solvent control for all three extracts, 2-phenyl-1-ethanol, and 4-methoxycinnamaldehyde (Fig. 1). Differences between populations were consistent across treatments, and the treatments × populations interaction was not significant ($F = 0.58$; df = 8, 252; $P = 0.79$). In preplanned comparisons, the EAG responses of females from the rotation-adapted population were not significantly different than the EAG responses of females from the 'normal' population for any of the individual volatile treatments evaluated except for (*E,Z*)-2,6-nonadienal, for which the rotation-adapted females had a significantly greater response than the 'normal' females ($P = 0.0091$).

The EAG responses of males from the rotation-adapted population were not significantly different from the EAG responses of males from the 'normal' population in the combined analysis ($F = 0.00$; df = 1,252; $P = 0.9534$). Although the main effect of population was not significant for males, the main effect of volatile treatment was significant ($F = 59.62$; df = 8,252; $P < 0.0001$). The EAG responses of males from the rotation-adapted population of western corn rootworm adults were significantly

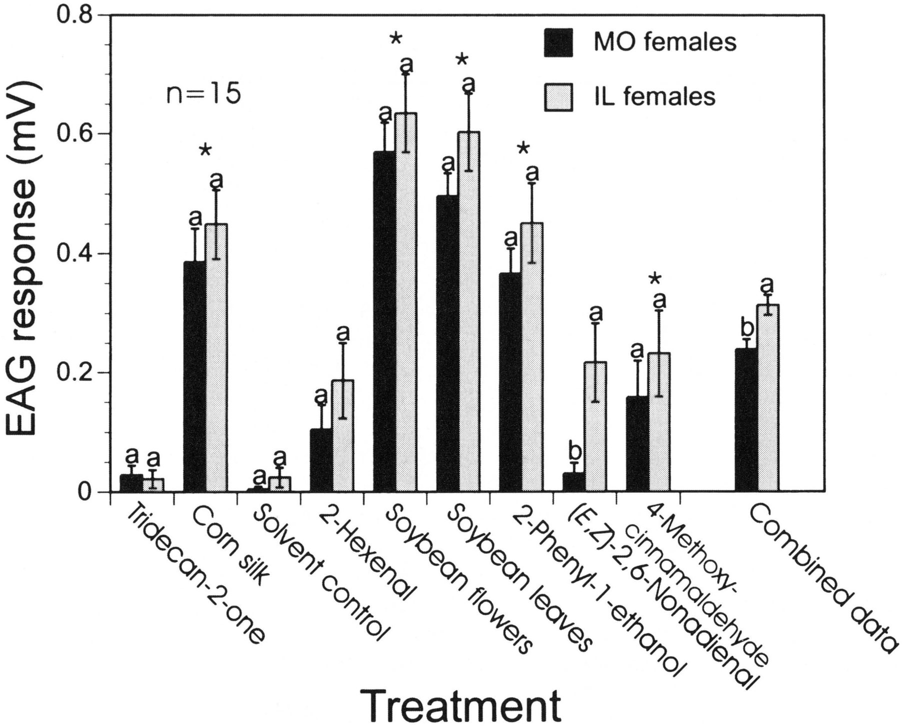

Fig. 1. Electroantennogram response of female beetles from the Illinois rotation-adapted population and the Missouri 'normal' population to volatiles in corn and soybean extracts as well as synthetic compounds. Different lower-case letters indicate significant differences between the two populations within the volatile treatment. An * indicates a significant difference between the control and the volatile treatment in question. Error bars represent standard error.

greater than the solvent control for all treatments except (*E*)-2-hexenal (Fig. 2). Because some of the responses (~1/3) of males to tridecan-2-one were off the scale of the detector (maximum response was 1.84 mV under the conditions of the experiment), the average response for tridecan-2-one is actually higher than the 1.6 mV (Fig. 2). Differences between populations were consistent across volatile treatments, and the volatile treatments × populations interaction was not significant ($F = 0.15$; df = 8,252; $P = 0.9966$). In preplanned comparisons, the EAG responses of males from the rotation-adapted population were not significantly different than the EAG responses of males from the 'normal' population for any of the individual volatile treatments evaluated.

Discussion

Data from Sammons et al. (1997) indicated that soybean volatiles were attractive to western corn rootworm adults from the 'problem' area, but not so for adults from

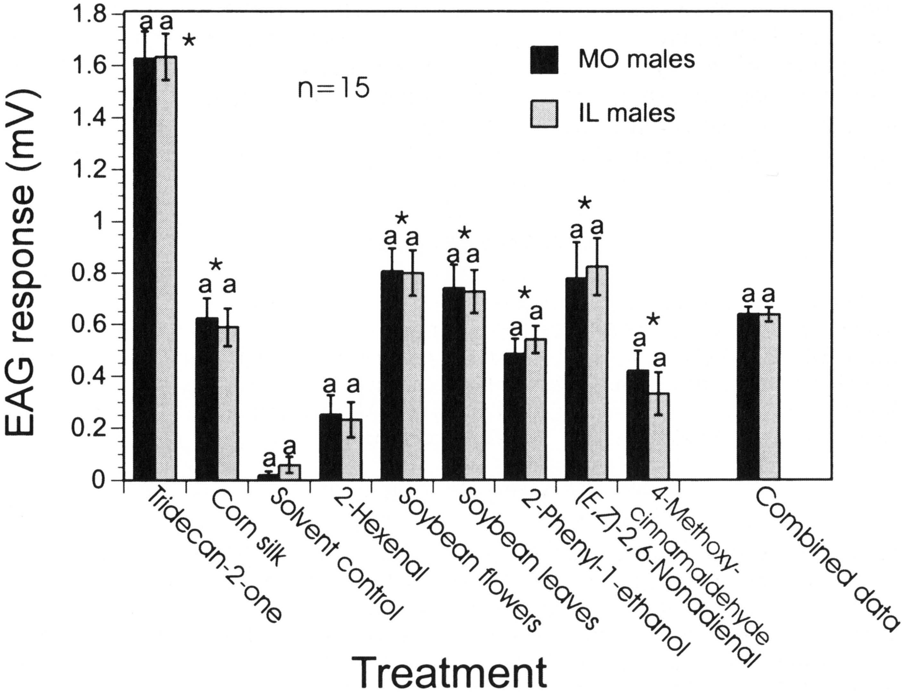

Fig. 2. Electroantennogram response of male beetles from the Illinois rotation-adapted population and the Missouri 'normal' population to volatiles in corn and soybean extracts as well as synthetic compounds. Different lower-case letters indicate significant differences between the two populations within the volatile treatment. An * indicates a significant difference between the control and the volatile treatment in question. Error bars represent standard error.

Iowa or Nebraska in laboratory bioassays. Spencer et al. (1999) found no evidence for attraction to soybeans in larger-scale wind tunnel assays. Data from the current work support the conclusions of Spencer et al. (1999) in that differences between populations were relatively consistent across treatments (no treatment × population interaction). The significantly greater EAG response of females from the rotation-adapted population (not specific to soybeans but in general) compared to the EAG response of 'normal' females could be interpreted to be consistent with the suggestion of Spencer et al. (1999) that the behavioral mechanism leading to increased oviposition in soybean fields in an increased tendency to leave corn fields (higher responding antennae may be more likely to perceive and respond to a broader range of olfactory stimuli). Additional evidence which supports this view is that insects from the rotation-adapted population are not simply found in soybeans and corn. Western corn rootworm adults in the "problem" area are found in a number of other crops, including alfalfa, clover, wheat, and oats (E.L., unpubl. data). Whatever the mechanism for this behavioral shift toward laying eggs in soybean fields, these data indicate that the rotation-adapted western corn rootworm population is not significantly more responsive specifically to soybean volatiles, nor is it less capable of detecting corn volatiles. Females from the rotation-adapted population of western corn rootworm simply responded more strongly to the volatiles tested (significantly so overall and to (*E,Z*)-2,6-nonadienal). Thus, these data provide no support for the hypothesis that specific attraction to volatiles from soybeans is the mechanism responsible for movement into soybean fields and subsequent egg-laying by the rotation-adapted western corn rootworm population.

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References Cited

Abou-Fakhr, E. M., B. E. Hibbard, D. K. Jewett and L. B. Bjostad. 1996. Electroantennogram responses of western corn rootworm (Coleoptera: Chrysomelidae) adults in relation to corn silk morphology and phenology. Environ. Entomol. 25: 430-435.

Bjostad, L. B. 1988. Insect electroantennogram responses to semiochemicals recorded with an inexpensive personal computer. Physiol. Entomol. 13: 139-145.

Bullock, D. G. 1992. Crop rotation. Crit. Rev. Plant Sci. 11: 309-326.

Cossé, A. A. and T. C. Baker. 1999. Electrophysiologically and behaviorally active volatiles of buffalo gourd root powder for corn rootworm beetles. J. Chem. Ecol. 25: 51-66.

Dunnett, C. W. 1964. New tables for multiple comparisons with a control. Biometrics 20: 482.

Edwards, C. R. 1996. The dramatic shift of the western corn rootworm to first-year corn, Pp. 14-15. *In* 1996 Illinois Agricultural Pesticides Conference. Coop. Extension Service, Univ. of Illinois, Urbana-Champaign.

Hammack, L. 1996. Corn volatiles as attractants for northern and western corn rootworm beetles (Coleoptera: Chrysomelidae: *Diabrotica* spp.). J. Chem. Ecol. 22: 1237-1254.

Hammack, L., B. E. Hibbard, C. W. Holyoke, M. Kline and D. M. Leva. 1999. Behavioral response of corn rootworm adults to host plant volatiles perceived by western corn rootworm (Coleoptera: Chrysomelidae). Environ. Entomol. 28: 961-967.

Hibbard, B. E. and F. X. Webster. 1993. Enantiomeric composition of grandisol and grandisal produced by *Pissodes strobi* and *P. nemorensis* and their electroantennogram response to pure enantiomers. J. Chem. Ecol. 19: 2129-2141.

Hibbard, B. E., T. L. Randolph, E. J. Bernklau, E. M. Abou-Fakhr and L. B. Bjostad. 1997a. Electroantennogram-active components of maize silk for western corn rootworm (Coleoptera: Chrysomelidae) adults. Environ. Entomol. 26: 285-295.

Hibbard, B. E., T. L. Randolph, E. J. Bernklau and L. B. Bjostad. 1997b. Electroantennogram-active components of buffalo gourd root powder for western corn rootworm adults (Coleoptera: Chrysomelidae). Environ. Entomol. 26: 1136-1142.

Krysan, J. L., D. E. Foster, T. F. Branson, K. R. Ostlie and W. S. Cranshaw. 1986. Two years before the hatch: rootworms adapt to crop rotation. Bull. Entomol. Soc. Am. 32: 250-253.

Levine E. and H. Oloumi-Sadeghi. 1991. Management of diabroticite rootworms in corn. Annu. Rev. Entomol. 36: 229-255.

1996. Western corn rootworm (Coleoptera: Chrysomelidae) larval injury to corn grown for seed production following soybeans grown for seed production. J. Econ. Entomol. 89: 1010-1016.

Levine, E. and M. Gray. 1996. First-year corn rootworm injury: east-central Illinois research progress to date and recommendations for 1996, Pp. 3-13. *In* 1996 Ill. Agric. Pestic. Conf., Coop. Ext. Serv., Univ. of Illinois at Urbana-Champaign.

Levine, E., H. Oloumi-Sadeghi and J. R. Fisher. 1992. Discovery of multiyear diapause in Illinois and South Dakota northern corn rootworm (Coleoptera: Chrysomelidae) eggs and incidence of the prolonged diapause trait in Illinois. J. Econ. Entomol. 85: 262-267.

Liu, S., D. M. Norris and P. Lyne. 1989. Volatiles from the foliage of soybean, *Glycine max,* and lima bean, *Phaseolus lunatus*: their behavioral effects on the insects *Trichoplusia ni* and *Epilachna varivestis.* J. Agric Food Chem. 37: 496-501.

Meinke, L. J., B. D. Siegfried, R. J. Wright and L. D. Chandler. 1998. Adult susceptibility of Nebraska western corn rootworm (Coleoptera: Chrysomelidae) populations to selected insecticides. J. Econ. Entomol. 91: 594-600.

Metcalf, R. L. 1986. Foreword, Pp. vii-xv. *In* J. L. Krysan and T. A. Miller [eds.], Methods for the study of pest Diabrotica. Springer-Verlag, New York.

Metcalf, R. L. and R. L. Lampman. 1989. Estragole analogues as attractants for corn rootworms (Coleoptera: Chrysomelidae). J. Econ. Entomol. 82: 123-129.

Metcalf, R. L. and E. R. Metcalf. 1992. Plant kairomones in insect ecology and control. Contemporary topics in Entomology 1. Chapman and Hall, New York.

Onstad, D. W., M. G. Joselyn, S. A. Isard, E. Levine, J. L. Spencer, L. W. Bledsoe, C. R. Edwards, C. D. Di Fonzo and H. Wilson. 1999. Modeling the spread of western corn rootworm (Coleoptera: Chrysomelidae) populations adapting to soybean-corn rotation. Environ. Entom. 28: 188-194.

Prystupa, B., C. R. Ellis and P. E. A. Teal. 1988. Attraction of adult *Diabrotica* (Coleoptera: Chrysomelidae) to corn silks and analysis of the host-finding response. J. Chem. Ecol. 14: 635-651.

Roelofs, W. L. 1984. Electroantennogram assays: rapid and convenient screening procedures for pheromones, Pp. 131-159. *In* H. E. Hummel and T. A. Miller [eds.], Techniques in Pheromone Research. Springer-Verlag, New York.

Sammons, A. E., C. R. Edwards, L. W. Bledsoe, P. J. Boeve and J. J. Stuart. 1997. Behavioral and feeding assays reveal a western corn rootworm (Coleoptera: Chrysomelidae) variant that is attracted to soybean. Environ. Entomol. 26: 1336-1342.

SAS Institute, Inc. 1989. SAS/STAT User's Guide, 4th edition. SAS Institute Inc., Cary, NC.

Sivcev I., B. Manojlovic, S. Krnjajic, N. Dimic, M. Draganic, F. Baca, Z. Kaitovic, R. Sekulic and T. Keresi. 1994. Distribution and harmful effect of *Diabrotica virgifera* LeConte (Coleoptera: Chrysomelidae), a new maize pest in Yugoslavia. Zastita Bilja. 45: 19-26.

Spencer, J. L., S. A. Isard and E. Levine. 1999. Free flight of western corn rootworm (Coleoptera: Chrysomelidae) to corn and soybean plants in a walk-in wind tunnel. J. Econ. Entomol. 92: 146-155.