# SEX PHEROMONE OF ORIENTAL BEETLE, Exomala orientalis: IDENTIFICATION AND FIELD EVALUATION

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Abstract-A gas chromatograph coupled with a behavioral bioassay was used to identify two sex pheromone components, 7-(Z)- and 7-(E)-tetradecen-2one of the Oriental beetle (OB), Exomala orientalis. Field experiments showed that the blend of the two isomers (Z:E, 7:1) was not significantly more attractive than the Z component alone. The best performance of traps baited with the synthetic sex pheromone was achieved when they were set with the pheromone device at 30 cm above the ground. Catches in traps baited with 1 and 10 mg were not significantly different, but they were higher (2.9-fold) than captures in traps loaded with 0.1 mg of the pheromone. Further investigations by GC-EAD revealed the presence of a possible minor component, but the small amount of material prevented its identification. 2-(E)-Nonenol, with the same retention time as the natural product, did not affect the attractancy of the synthetic sex pheromone. GC-EAD screening of previously identified sex pheromones of scarab beetles showed that male antennae of the Oriental beetle responded to japonilure, but it showed neither synergism nor inhibition to the OB sex pheromone.

Key Words-Exomala orientalis, Blitopertha orientalis, Phyllopertha orientalis, Coleoptera, Scarabaeidae, Oriental beetle, 7-tetradecen-2-one, 6-tetra-

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decen-2-one, 5-tetra decen-2-one, 2-(E)-nonenol, japonilure, GC-EAD, GC-BB

#### INTRODUCTION

The Oriental beetle (OB), *Exomala orientalis* (Waterhouse) (Coleoptera: Scarabaeidae), is probably a native of the Philippine Islands that was carried to Japan and was introduced from Japan to the United States. Sometime before 1908, it was introduced to the Hawaiian Island of Oahu, where it became a serious pest of sugarcane. On the mainland of the United States, adults were first collected in 1920 in a New Haven, Connecticut, nursery, having presumably been imported directly from Japan in infested balled nursery stock (Tashiro, 1987). In Japan, no scientific evidence has been documented in the old literature regarding the origin of the OB (Japanese name: *semadarakogane*). However, the beetle has been recorded for a long time, although its scientific name has changed over the years: *Phyllopertha orientalis* Waterhouse (Murayama, 1950), *Anomala orientalis* (Waterhouse) (Anonymous, 1980), and currently *Blitopertha orientalis* (Waterhouse) (Anonymous, 1987).

A revision of the subfamily Anomalinae (Scarabaeidae) has been proposed for the genus occurring in the United States and Canada, with *Blitopertha* considered a synonym of *Anomala*, tribe Anomalini (Potts, 1974) and, therefore, the OB is named *Anomala orientalis* in the United States (Stoetzel, 1989).

Given the economic importance of the OB in Japan, and the need for alternative methods of control, we initiated a project aimed at identifying the sex pheromone of the beetle and evaluating its potential in practical applications. A similar project was also launched in the laboratory of Dr. Wendell L. Roelofs (Cornell University at Geneva, New York) with the American population of OB. Although these beetles were supposed to be a different species, the facts that "Anomala orientalis" males responded in a wind tunnel to a sex pheromone identified from "Blitopertha orientalis" (Leal, 1993) and that the active chemical isolated from the former had the same capillary GLC retention times and MS as the latter (Leal et al., 1993a; to be reported in detail by Roelofs' group), led us to compare the two species taxonomically.

Specimens were sent to the United States, and the taxonomists consulted referred to a recent revision of the genus *Blitopertha* (Baraud, 1991), in which *B. orientalis* is newly classified as *Exomala orientalis*. As Baraud's paper refers only to the species occurring in Japan, Korea, and Hawaii, it appears that the external characters of aedeagus that led to the new genus classification have not been examined in the American population of OB.

We describe here the identification and field evaluation of the sex pheromone of the Japanese population of the OB (semadarakogane, formerly Blitopertha orientalis), Exomala orientalis.

## METHODS AND MATERIALS

Chromatographic, Mass, and Infrared Spectral (MS, IR), Analyses. GC analyses were performed on a Hewlett-Packard 5890 equipped either with a DB-wax column (30 m  $\times$  0.25 mm; 0.25  $\mu$ m) or an HP-1 column (12 m  $\times$ 0.2 mm; 0.33  $\mu$ m), operated in a splitless mode at 50°C for 1 min, programmed at 4°C/min to 180°C, held at this temperature for 1 min, programmed again at 10°C/min to 230°C and held at this temperature for 20 min [i.e., 50(1)-180(1)/ 4-230(20)/10]. Mass spectra were recorded on a Hewlett-Packard 5891 mass selective detector using the same type of capillary columns under the same conditions as described for the GC. GC-FTIR was done on a Hewlett-Packard 5965B equipped with a DB-wax capillary column operated at 70(1)-150(1)/5-240(10)/10. The light pipe was operated at 250°C and the transfer line at 270°C.

Gas Chromatography-Electroantennographic Detector (GC-EAD). The response of Exomala orientalis antennae were recorded with a GC-EAD system (Leal et al., 1994a). The previously described acrylic EAD station (Leal et al., 1992a) was modified in order to have an adjustable opening space between the two holes (for holding the pedicel and last flagellum) to be regulated according to the size of the antenna.

Gas Chromatography-Behavior Bioassay (GC-BB). Coupled chromatographic resolution and behavioral observations were done as previously described (Leal et al., 1992b). Males (10) were placed in a plastic box ( $17 \times 12 \times 6$ cm) fixed to the outlet of the GC (Figure 1) and their response to GC eluents was observed.

Insects. Eggs laid by field-collected female beetles were transferred to wet sand (10% water) in ice cream cups (60 ml), which were kept at 25°C. After hatching, grubs were individually kept in ice cream cups loaded with a 1:1 mixture of sand and humus obtained from leaves of *Quercus acutissima* (a kind of oak; *kunugi* in Japanese). This was humidified (ca. 10% water) and the grubs supplied with slices of sweet potato. After the third stage, grubs reached the yellow stage (stop feeding) and were chilled to 10°C for over two months. The temperature was then raised again to 25°C. Adults were kept in culture dishes (90 mm OD  $\times$  60 mm high) at 25°C, 70% relative humidity, and 14L:10D photoperiod, and provided with saturated sucrose solution on cotton.

Aeration. The airborne volatiles of either male or female beetles were collected according to a previously reported method (Leal et al., 1992a).

Isolation of Pheromone. Crude extract of female volatiles was separated on a silica gel column (Wako C-200) by successive elution with hexane-ether mixtures: 100:0, 95:5, 90:10, 80:20, 50:50, and 0:100. Pheromonal activity was monitored by a simplified bioassay (Leal et al., 1992c). Males were placed inside culture dishes (90 mm OD  $\times$  60 mm high), the bottom of which was



FIG. 1. Behavioral response of E. orientalis males in a GC-BB. (A) Males randomly walking inside the arena and (B) gathered on the outlet of the GC system in response to female-released semiochemicals separated on the GC capillary column.

covered with wet filter paper. Samples were transferred to a filter paper (1  $\times$  1 cm), set inside the dish, and the insect response was recorded.

Synthesis. 7-Tetradecen-2-one was synthesized as reported (Leal, 1993). Pure Z isomer was obtained by separation on silver nitrate on a silica gel (10%, 200 mesh) column.

5- and 6-Tetradecen-2-one were prepared by Wittig reaction of dicarboethoxy aldehydes with appropriate ylides. These dicarboethoxy products were transformed into the corresponding acids by hydrolysis, and then they were treated with methyl lithium to yield the desired ketones.

5-Tetradecen-2-one was synthesized starting from the reaction of ethyl malonate with sodium hydride in DMSO, followed by coupling with 2-bromoacetaldehyde diethylacetal to produce 3,3-dicarboethoxypropionaldehyde diethylacetal. After deprotection of the aldehyde, it was reacted with the ylide formed by the action of butyl lithium in THF on nonyltriphenylphosphonium bromide. Alkaline hydrolysis of the product, 1,1-dicarboxy-3-dodecene, followed by heating (135°C), gave 4-tridecenoic acid. Reaction of the acid with methyl lithium in THF yielded 5-tetradecen-2-one (85% Z; 15% E).

Synthesis of 6-tetradecen-2-one (90% Z; 10% E) was achieved by the same route, starting from 3-bromopropionaldehyde ethylene acetal and using octyl-triphenylphosphonium bromide in the Wittig reaction.

2-(E)-Nonenol was obtained by the reduction of 2-(E)-nonenal with LiAlH<sub>4</sub> in dry ether (Leal et al., 1992b). 2-Tetradecanone was commercially available (Tokyo Kasei Kogyo Co., Tokyo).

Field Experiments. Evaluation of baits was conducted at the fields of NISES (Tsukuba) and Chiba Prefectural Agricultural Experiment Station (Chiba) in the summer of 1993. Funnel traps (Japan Tobacco Inc., Tokyo), 7 or 10 m apart, were baited with synthetic lures incorporated into plastic pellets (4–5 mm in diameter) made of a polyethylene-vinyl acetate. These pellets were placed inside pellet holders (Fuji Flavor Co., Tokyo) and set 2 cm above the trap lip. Unless otherwise mentioned, traps were positioned with the pheromone dispenser at 30 cm above the ground. The candidate lures were replicated in randomized blocks and capture data were transformed to log (x + 1) before differences among means were tested for significance by ANOVA with JMP software (Version 2) (SAS Institute, 1989). In this paper, treatments followed by the same letters are not significantly different at the 5% level in the Tukey-Kramer honestly significant difference test. In the figures, means of captures are untransformed and error bars show one SE.

## **RESULTS AND DISCUSSION**

Identification of Sex Pheromone. Pheromonal activity was observed in the crude extract of airborne volatiles collected from the headspace of 20 female beetles, and this activity was recovered in a hexane-ether (90:10) fraction after separation on a silica gel column. In order to identify the active peak(s) (out of at least 38 candidates), this fraction was analyzed by GC-BB. While inactive compounds were eluted, male beetles walked randomly inside the arena (Figure 1A), and they gathered in the outlet of the GC system (Figure 1B) in response to peak(s) appearing at ca. 31.5 and 29.1 min on an HP-1 and DB-wax columns,

respectively. The same activity was elicited only with the crude extract and 90:10 fraction.

GC-MS analyses of the 90:10 fraction demonstrated the occurrence of at least four possible peaks in the active region by GC-BB (Figure 2A). The peaks at  $R_r$  32.51, 32.41, 29.25, 27.67, and 25.86 were ruled out because they were found also in the (inactive) crude extract collected from male beetles. The peaks at  $R_r$  31.73 and 31.91 min gave similar mass spectra (Figure 2B and C). The occurrence of a base peak at m/z 43 was initially considered due to an acetate structure (with the m/z 61 peak missed), but the fact that the compounds did not undergo alkaline hydrolysis ruled out this possibility. Hydrogenation with Adam's catalyst gave rise to 2-tetradecanone, which was also found in the volatiles of male and female beetles (peak at  $R_r$  32.41 min, Figure 2A). Therefore, the major peak was considered to be tetradec-?-en-2-one. The peak at m/z58 was small because the position of the double bond may not favor McLafferty rearrangement. The occurrence of peaks at m/z 145 and 159 in the MS of the dimethyl disulfide derivative suggested that the major peak was either 6- or 7-tetradecen-2-one.

Although not available at the time of the structure elucidation, the vapor phase IR of the major peak (Figure 3A and B) gave a characteristic profile of a long-chain ketone: C—H stretching (2935-6 cm<sup>-1</sup>) predominant over the carbonyl band (C=O st, 1731-2 cm<sup>-1</sup>). A library search suggested a possible structure to be 2-undecanone (Figure 3C), which basically differed from the natural product in that the former did not show the band at 3013-4 cm<sup>-1</sup> of a double bond in the *cis* configuration (Leal, 1991; Leal et al., 1992d).

Synthetic 7-(Z)-tetradecen-2-one was identical to the major peak in terms of  $R_t$ , MS, and pheromonal activity in the GC-BB, whereas 6-tetradecen-2-one differed not only in the MS, but also in retention times. Based on its  $R_t$  on the two capillary columns as well as on its MS, the minor peak at  $R_t$  31.91 min was characterized as 7-(E)-tetradecen-2-one. Therefore, the sex pheromone of *Exomala orientalis* was identified as a mixture of 7-(Z)- and 7-(E)-tetradecen-2-one in a natural ratio of 7:1.

The possibility of chemically related compounds being used as minor components was also exploited. Although the saturated ketone, 2-tetradecanone, was found to be released by both male and female beetles, its attractancy was tested in the field in Tsukuba (June 17-20). There was no significant difference in captures of the OB in traps baited with the synthetic sex pheromone alone, 7-(Z)-tetradecen-2-one, or in combination with 2-tetradecanone (Figure 4A).

Because the occurrence of 6-tetradecen-2-one as a minor component could not be ruled out on the basis of the MS of the dimethyl disulfide derivative, this compound was tested in the field (Tsukuba, June 25–28). A combination of 7-(Z)-tetradecen-2-one and 6-tetradecen-2-one was not a significantly better lure than 7-(Z)-tetradecen-2-one alone (Figure 4B).



FIG. 2. Reconstructed total ion monitor profile of the active hexane-ether (90:10) fraction separated on a DB-wax capillary column (A). EI-MS of the two female-specific active peaks at  $R_i$  31.73 min (B) and 31.91 min (C).



FIG. 3. Vapor-phase infrared spectrum of the major peak (A, B) compared to the best library fitting (C).

Although we do not have any direct evidence of the biosynthetic pathways, some sex pheromones of scarab beetles seem to be derived from fatty acids (Leal et al., 1994a). Based on this biochemistry reasoning, 5-tetradecen-2-one was considered to be a possible minor component. Since it possessed a retention time that is very close to 7-tetradecen-2-one, the candidate chemical was tested in the field in Tsukuba (June 25-29). Interestingly, trap catch of OB to its sex pheromone was reduced by the presence of 5-tetradecen-2-one. It has been found (Ono, unpublished data) that captures of the soybean beetle, *Anomala rufocuprea*, were greatly decreased by the use of its sex pheromone, methyl 5-(Z)-



FIG. 4. Effect of possible minor components (A) 2-tetradecan-one, (B) 6-tetradecen-2one, and (C) 5-tetradecen-2-one on the catches of the OB.

tetradecenoate, in combination with japonilure, the sex pheromone of the Japanese beetle. Therefore, we considered that 5-tetradecen-2-one may be involved in the pheromonal communication of related species.

Evaluation of Synthetic Sex Pheromone. Preliminary field tests of the synthetic sex pheromone (5 mg) loaded in a rubber septum (July 30-August 12) revealed that a Z:E (7:1) mixture was attractive to males of the OB (Leal, 1993). It remained unclear, however, whether both geometric isomers were essential for attraction. Experiments conducted in Chiba (July 15-30, 1993) demonstrated that captures in traps baited with the Z:E mixture were not significantly different from the catches with the Z isomer only (Figure 5).

The effect of the dosage on the catches of the OB was tested in field experiments in Chiba (June 24-27) by comparing the captures with 0.1, 1, and

10 mg of a Z: E (7:1) mixture of the sex pheromone. Traps baited with 1 mg of the pheromone captured significantly more beetles (2.9-fold) than those with 0.1 mg of the lure. However, there was no significant difference in catches in traps with 1 and 10 mg of the synthetic sex pheromone (Figure 6). We have found that catches of *Anomala octiescostata* with the synthetic sex pheromone were not significantly different in traps baited with 1 and 10 mg, but 100 mg captured significantly more beetles than 1 mg (Leal et al., 1994b). So far, we



FIG. 5. Captures of *E. orientalis* male and female beetles with the two naturally occurring geometric isomers and the *Z* component only.



FIG. 6. Effect of the dosage on the catches of the E. orientalis.

have not found any case of a decrease in catches of scarab beetles due to the dosage of the pheromone in the range from 0.1 to 100 mg.

Catches of *Exomala orientalis* were compared in experiments conducted in Chiba (July 2-9), setting traps with the pheromone dispenser at 30, 100, and 160 cm above the ground. The best performance was achieved with traps at 30 cm (Figure 7), in which 86% of the male beetles were captured. This has also been shown to be the best height for captures of *Anomala schonfeldti* with synthetic sex pheromone (Hasegawa et al., 1993). Interestingly, 11% of the OB captured in these experiments were female beetles. Furthermore, nearly the same ratio was found in another experiments (Figure 5). In our experiments, these female catches are significantly higher in traps baited with the synthetic sex pheromone than in control traps. Therefore, it seems that male-released semi-ochemicals may also be involved in the communication of scarab beetles (see Leal et al., 1994a for further discussion).

Investigation of Occurrence of Minor Component(s). Sex pheromone systems of nine scarab species have been identified so far (Leal et al., 1994a), of which only four have been identified as binary mixtures (the others were single components). In the cupreous chafer, the occurrence of two components has been demonstrated not only by chemical identification (Leal et al., 1993b), but also by the existence of two receptor cells in the pheromone-sensitive sensilla as well as the occurrence of two spike amplitudes in the impulses from the pheromone receptor (Leal and Mochizuki, 1993). We believe that, as a rule, minor component(s) may be involved in the pheromonal communication of scarab



Fig. 7. Performance of the sex pheromone-baited traps on the captures of the OB at different heights.

beetles, although the existence of minor constituent(s) may be masked by a highly attractive major pheromone (Leal et al., 1993c).

As minor components are unlikely to be detected by GC-BB, we investigated the occurrence of minor component(s) in the pheromone system of *Exo*mala orientalis by means of GC-EAD. Male antennae of the OB (settled in an improved EAD station) responded to two peaks in the crude extract of the airborne volatiles from female beetles (Figure 8). The major peak at  $R_{r}$  28.12 min was confirmed to be 7-(Z)-tetradecen-2-one, whereas the minor peak had the same retention time as 2-(E)-nonenol (22.05 min) on the polar capillary column. Due to the small amount of this EAD-active peak, it was not possible to obtain an MS out of pooled extract from the headspace of female beetles. Nevertheless, the fact that some scarab species utilize sex pheromone of other species as a minor component of their own blends (Leal et al., 1993d; 1994a) and that 8% of the total capture in traps baited with 2-(E)-nonenol, the sex pheromone of A. schonfeldti, were males of the OB (Hasegawa, unpublished) led us to test its attractiveness.

Field experiments carried out in Tsukuba (June 17-20) showed that there was no significant difference in catches of the OB with 7-(Z)-tetradecen-2-one alone or in combination with 2-(E)-nonenol (Figure 9A). Further experiments will be carried out in the next seasons in order to characterize the EAD-active peak at  $R_t$  20.02 min and to clarify its role.

The use of traps baited simultaneously with the sex pheromones of two different species, although desirable for economic reasons, has not been possible in some cases due to the antagonism caused by the sex pheromone of one species on the captures of the other. Simultaneous monitoring of *E. orientalis* and *A. schonfeldti* may be possible, but the effect of the combined lure on the catches of the latter are yet to be tested.

An EAD screening of the previously identified sex pheromones of scarab



FIG. 8. Coupled GC-EAD response of male antenna to the airborne volatiles of virgin female beetles showing two EAD-active peaks. Upward and downward arrows indicate the peak of 7-(Z)-tetradecen-2-one and an unidentified chemical, respectively.



FIG. 9. Effect of the pheromones of two other scarab species on the catches of E. orientalis: (A) 2-(E)-nonenol, the sex pheromone of A. schonfeldti, and (B) japonilure, the sex pheromone of *Popillia japonica*.

beetles (Leal et al., 1994a) was carried out to explore possible attractants or antagonists. Only japonilure gave a significant and reproducible EAD response. Both the signal-to-noise ratio and reproducibility of the response generated by 2-(E)-nonenol were very low. Field tests in Tsukuba (June 28-30) demonstrated, however, that japonilure neither increases nor decreases trap catch of the sex pheromone of *E. orientalis*.

In conclusion, 7-(Z)- and 7-(E)-tetradecen-2-one were identified as sex pheromone constituents of the Japanese population of the OB, *E. orientalis*. This pheromone system is a potent lure for studies on the chemical ecology as well as management of the OB.

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#### REFERENCES

- ANONYMOUS. 1980. Major Insect and Other Pests of Economic Plants in Japan. Japan Plant Protection Assoc., Tokyo.
- ANONYMOUS. 1987. Major Insect and Other Pests of Economic Plants in Japan. Society of Applied Entomology and Zoology (ed.). Japan Plant Protection Assoc., Tokyo.

- BARAUD, J. 1991. Nouvelle classification proposée pour les espèces du genre Blitopertha Reitter (1903) (Coleoptera: Rutelidae). Lambillionea 91:46-62.
- HASEGAWA, M., LEAL, W.S., and SAWADA, M. 1993. Field evaluation of Anomala schonfeldti Ohaus (Coleoptera: Scarabaeidae) synthetic sex pheromone. J. Chem. Ecol. 7:1453-1459.
- LEAL, W.S. 1991 (R,Z)-5-(-)-(Oct-1-enyl)oxacyclopentan-2-one, the sex pheromone of the scarab beetle Anomala cuprea. Naturwissenschaften 78:521-523.
- LEAL, W.S. 1993. (Z)- and (E)-Tetradec-7-en-2-one, a new type of sex pheromone from the Oriental beetle. Naturwissenschaften 80:86-87.
- LEAL, W.S., and MOCHIZUKI, F. 1993. Sex pheromone reception in the scarab beetle Anomala cuprea. Enantiomeric discrimination by sensilla placodea. Naturwissenschaften 80:278-281.
- LEAL, W.S., MOCHIZUKI, F., WAKAMURA, S., and YASUDA, T. 1992a. Electroantennographic detection of Anomala cuprea sex pheromone. Appl. Entomol. Zool. 27:289-291.
- LEAL, W.S., HASEGAWA, M., and SAWADA, M. 1992b. Identification of Anomala schonfeldti sex pheromone by high-resolution GC-behavior bioassay. Naturwissenschaften 79:518-519.
- LEAL, W.S., HASEGAWA, M., MOCHIZUKI, F., and YASUDA, T. 1992c. Behavioral and electrophysiological evidence of sex pheromone(s) in *Anomala schonfeldti* Ohaus (Coleoptera: Scarabaeidae). Appl. Entomol. Zool. 27:592-594.
- LEAL, W.S., MATSUYAMA, S., SUZUKI, T., and OZAWA, T. 1992d. GC-FTIR potential for structure elucidation. J. Braz. Chem. Soc. 3:25-29.
- LEAL, W.S., ROELOFS, W.L., ZHANG, A., VILLANI, M., SAWADA, M., and HASEGAWA, M. 1993a. Sex pheromone of the Oriental beetle, *Exomala orientalis*. Program, 10th Annual ISCE Meeting, Clearwater Beach, Florida, p. 53.
- LEAL, W.S., SAWADA, M., and HASEGAWA, M. 1993b. The scarab beetle Anomala cuprea utilizes the sex pheromone of Popillia japonica as a minor component. J. Chem. Ecol. 19:1303-1313.
- LEAL, W.S., SAWADA, M., MATSUYAMA, S., KUWAHARA, Y., and HASEGAWA, M. 1993c. Unusual periodicity of sex pheromone production in the large black chafer *Holotrichia parallela*. J. Chem. Ecol. 19:1381-1391.
- LEAL, W.S., SAWADA, M., and HASEGAWA, M. 1993d. The scarab beetle Anomala daimiana utilizes a bland of two other Anomala spp. sex pheromones. Naturwissenschaften 80:181-183.
- LEAL, W.S., KAWAMURA, F., and ONO, M. 1994a. The scarab beetle Anomala albopilosa sakishimana utilizes the same sex pheromone blend as a closely related and geographically isolated species, Anomala cuprea. J. Chem. Ecol. 20:1667-1676.
- LEAL, W.S., HASEGAWA, M., SAWADA, M., ONO, M., and UEDA, Y. 1994b. Identification and field evaluation of Anomala octiescostata (Coleoptera: Scarabaeidae) sex pheromone. J. Chem. Ecol. 20:1643-1655.
- MURAYAMA, J. 1950. Phyllopertha orientalis, p. 1319, in T. Esaki, T. Ishii, T. Kawamura, S. Kinoshita, S. Kuwayama, T. Shiraki, and S. Uchida (eds.). Iconographia Insectorum Japonicorum. Hokuryu-kan Publishing Co., Tokyo.
- POTTS, R.W.L. 1974. Revision of the Scarabaeidae: Anomalinae. 1. The genera occurring in the United States and Canada. *Pan-Pac. Entomol.* 50: 148-154.
- SAS Institute. 1989. Software for Statistical Visualization on the Apple® Macintosh. JMP® Introductory Guide. SAS Institute, Inc., Cary, North Carolina.
- STOETZEL, M.B. 1989. Common Names of Insects & Related Organisms. Entomology Society of America, Lanham, Maryland.
- TASHIRO, H. 1987. Turfgrass Insects of the United States and Canada. Cornell University Press, Ithaca, New York, 474 pp.