Studies on semiochemicals produced by Gonocerus acuteangulatus for their use as control methods in hazelnut orchards

Studies on Semiochemicals Produced by *Gonocerus acuteangulatus* for Their Use as Control Methods in Hazelnut Orchards

S. T. Moraglio, B. L. Ingegno and L. Tavella
DIVAPRA Entomologia e Zoologia applicate all'Ambiente "C. Vidano"
Università degli Studi di Torino
Via L. da Vinci 44, 10095 Grugliasco (TO)
Italy

Keywords: Hemiptera Coreidae, volatile organic compounds, behavioural response, olfactometry, electroantennography

Abstract

***Gonocerus acuteangulatus* (Goeze) (Hemiptera: Coreidae) is one of the most harmful hazelnut pests in Europe and Turkey, responsible for affecting seriously crop quality with its feeding activity. Chemical control is very difficult to achieve due to its high mobility and polyphagy; however, it is currently the only available method to reduce effectively damage by bugs. Therefore, a two-year study on behavioural responses of *G. acuteangulatus* was carried out with the aim to develop alternative control tactics to protect hazelnut crop. In 2010-2011, individuals of *G. acuteangulatus* were collected in the field, mass-reared in laboratory, and used in behavioural and physiological bioassays. In field surveys, very abundant populations were found on wild bushes, belonging to various plant families, showing a tendency to aggregate on some plant species, different from hazelnut, during fruit ripening. In olfactometer bioassays, females were attractive for both males and females, just after winter diapause and before mating. Moreover, in physiological bioassays the tested compounds were perceived by antennae of both males and females, showing the suitability of the developed electroantennography procedure. In the light of these promising preliminary results, the research is worthy of going on to improve our knowledge on the attractiveness of the volatile compounds produced by both *G. acuteangulatus* and host plants, and on behavioural responses of bugs in field conditions so to implement effective environment-friendly control strategies.**

INTRODUCTION

Bug species, such as *Gonocerus acuteangulatus* (Goeze) (Hemiptera: Coreidae) and *Palomena prasina* (L.) (Hemiptera: Pentatomidae), are main hazelnut pests in Europe and Turkey. They can cause remarkable economic losses affecting nut yield and quality with their feeding activity on kernels (Tavella et al., 2001a, 2001b, 2003; Tuncer et al., 2005; Tuncer, 2009). In particular, both adults and nymphs of *G. acuteangulatus* are responsible for serious damage in Italy: nymphs can indeed complete their development feeding only on hazel (Tavella et al., 2001b). However, this species is a very good flier and has a wide host range. In fact, it can be found not only on hazel but on several other plant species, such as *Arbutus* spp., *Pistacia vera* L. (Anacardiaceae), *Berberis* spp. (Berberidaceae), *Buxus sempervirens* L. (Buxaceae), *Juniperus* spp. (Cupressaceae), *Diospyros kaki* L. (Ebanaceae), *Quercus* spp. (Fagaceae), *Frangula* spp., *Rhamnus* spp. (Rhamnaceae), *Eriobotrya japonica* (Thunb.), *Pyrus malus* L., *Prunus* spp., *Rosa* spp.,

Rubus spp. (Rosaceae), *Taxus* spp. (Taxaceae) (Genduso and Mineo, 1974; Shaefer and Mitchell, 1983; Moulet, 1995).

In hazelnut orchards, *G. acuteangulatus* is sampled throughout the growing season, from late May to late August (*i.e.*, harvest time) (Tavella et al., 1997). Chemical control is very difficult to achieve due to its high mobility and polyphagy that make the infestations unforeseeable; however, it is currently the only available method to reduce effectively damage by bugs. The abundance of beneficial organisms that need to be protected in the hazelnut agro-ecosystem (Viggiani, 1994; AliNiazee, 1998), together with the reduction of insecticides registered on hazel in Europe, make fundamental to develop alternative and environmentally friendly control tactics.

Naturally produced chemicals that influence insect behaviour (semiochemicals) are powerful tools in integrated pest management programmes (Nordlund, 1981). Pheromones are already known for some Coreidae species, such as sex pheromones of *Leptoglossus clypealis* Heidemann (Wang e Millar, 2000), aggregation pheromone of *L. occidentalis* Heidemann (Blatt and Borden, 1996), alarm pheromones of *L. occidentalis*, *L. zonatus* (Dallas) and *Thasus neocalifornicus* Brailovsky et Barrera (Leal et al., 1994; Blatt et al., 1998; Prudic et al., 2008). Moreover, semiochemicals produced by host and non host plants have been studied. Volatile compounds produced by *Vigna* spp. are repellent for *Clavigralla tomentosicollis* Stål (Koona et al., 2003), while terpenoids of *Melaleuca quinquenervia* (Cav.) S.T. Blake (*e.g.*, trans-nerolidol) are attractive for *Leptoglossus phyllopus* (L.) (Aldrich et al., 1993). Therefore, a two-year study on *G. acuteangulatus* behavioural responses was carried out with the aim to develop alternative control tactics to protect hazelnut crop from bug attacks.

MATERIALS AND METHODS

Insect collection

From April to October in 2010 and 2011, individuals of *G. acuteangulatus* necessary for the experiments were collected in several Piedmont (NW Italy) areas, both cultivated or natural ones, characterized by presence of wild shrub-like vegetation. Collected insects were then transferred to the laboratory, where they were mass-reared in climatic chambers (T 24±1°C, RH 65±5%) in net cages (93 × 47.5 × 47.5 cm) (MegaView, Taichung, Taiwan) containing box trees, and fed with hazelnuts.

Behavioural bioassays

Responses of *G. acuteangulatus* males and females to individuals of the same or opposite sex were evaluated in a dual choice Y-tube olfactometer, using humidified, charcoal filtered air at the rate of 2 L min^{-1}. Olfactometer was a Y-shaped glass tube (∅ 3.7 cm) with a 25 cm long main tube and two 25 cm long arms (tilt angle 70°). Odour sources consisted of a group of three adults of the same sex inserted with wet cotton into a small round glass bottle (volume 500 mL) connected to one olfactometer arm, and only wet cotton (control) inserted in a second bottle connected to the other olfactometer arm.

One male or female, never tested before, was introduced at the base of the main tube of the olfactometer, and the secondary arms in which it walked upwind within 10 min was recorded. Total number of insects that made a choice within 10 min was analyzed using χ^2 test with SPSS 17.0 software (SPSS, Chicago, IL, USA). The null hypothesis was that adults have a 50:50 distribution between the two odour sources. Insects that did not make a choice within 10 min were recorded as no response and were excluded from statistical analysis. The Y-tube was cleaned with neutral soap and alcohol

after 10 tests. Odour sources were inverted after five tests to avoid any positional bias. Males and females were tested in two different but identical Y-tubes.

G. acuteangulatus adult attractiveness to conspecific ones was evaluated also in multichoice bioassays conducted in an experimental anti-insect net cage (5 × 3 × 3 m) under natural atmospheric conditions. As reported in Figure 1, 16 box bushes were placed into the cage, each one with some hazelnuts at the base. Bushes labelled A, B, C, D were enclosed in net cages (40 × 40 × 50 cm), in which the following four treatments were placed by turns for three replicates: 20 females (T1), 20 males (T2), 10 males and 10 females (T3), no insects (T4). For each replicate, 60 *G. acuteangulatus* males were introduced, and their distribution on bushes was recorded three times per day for five days. Percentages of males observed on the three bushes near each encaged box (A, B, C, D) were arcsine square root transformed and then analyzed using one way ANOVA with SPSS 17.0 software (SPSS, Chicago, IL, USA). Means were then separated using Tukey's test. Males on cage walls were excluded from statistical analysis.

Physiological bioassays

To measure physiological responses of *G. acuteangulatus* adults to odour sources, an electroantennography (EAG) recording procedure was set up. Responses (mV) of three right and three left antennae of six males and six females were recorded with a standard EAG apparatus (Syntech® ltd, Hilversum, The Netherlands). Insects were anaesthetized in freezer for 5 min, then one antenna was cut at the level of the scape. Immediately, the base and the tip of the antenna were inserted in two glass micropipettes filled with saline solution (KCl 0.1 M) connected with two silver electrodes. Odour sources consisted in 2 μL of a volatile compound adsorbed on a piece of filter paper (5 × 20 mm) inserted into a Pasteur pipette and put into the constant air flow tube directed to the antenna. For each odour, three stimuli of 0.5 s were repeated with intervals of 10 s, using a stimulus controller (CS-55, Syntech, Hilversum, The Netherlands). Tridecane, a compound produced by *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (Lockwood and Story, 1985; Colazza et al., 2004), was used as odour source, while (E)-2-hexenal, produced by nymphs and adults of other Coreidae species (Aldrich and Yonke, 1975), and an empty pipette were used as positive and negative control, respectively. Mean responses to the three repetitions of each stimulus were normalized to the response obtained with (E)-2-hexenal.

RESULTS AND DISCUSSION

Insect collection

In the two-year field surveys, very abundant populations of *G. acuteangulatus* were found on wild bushes belonging to different plant families, always during fruit ripening, confirming high poliphaghy of this bug species (Genduso and Mineo, 1974; Shaefer and Mitchell, 1983; Moulet, 1995). Moreover, adults and nymphs showed an evident tendency to aggregate on some plants, such as *B. sempervirens*, *Frangula alnus* Miller, *Rhamnus cathartica* (L.), *Prunus* spp., *Rosa* spp. When ripening fruits of different plant species were present at the same time and in the same site, *G. acuteangulatus* showed a preference for some plant species; in particular, high numbers of adults and nymphs were found on wild bushes [*e.g.*, *Cornus sanguinea* L. (Cornaceae)] surrounding hazel groves, rather than on hazel bushes. Further research should verify if and how the presence of more favourite bushes can influence the nut damage rate, and consequently could address to a correct management of the agro-ecosystem.

Behavioural bioassays

In olfactometer bioassays, females were attractive for both males and females (Fig. 2), just after diapause and before mating. In Coreidae, males are generally responsible for the production of attractive pheromones, as observed for *L. clypealis*, whose males produce sex pheromones (Wang and Millar, 2000), and for *L. occidentalis*, whose males produce aggregation pheromones just after winter diapause (Blatt and Borden, 1996). However, in some species females have appropriate glands to produce aggregation pheromones (Pavis, 1987). In our bioassays, *G. acuteangulatus* females seemed to be able to produce an aggregation pheromone, but these preliminary results need to be further confirmed.

In multichoice bioassays in the experimental net cage, *G. acuteangulatus* males were often found on the net walls, in particular on the North-facing wall, probably due to the overheated and dry microclimatic conditions occurring in the cage. Anyway, presence of 20 females (T1) was significantly more attractive for males than presence of 20 males (T2) or presence of 10 males and 10 females (T3).

Physiological bioassays

Antennae of *G. acuteangulatus* showed to perceive and respond to the stimuli provided in EAG as the typical evident responses to the positive control proved (Fig. 3). Mean antennal responses of six males and six females to the three stimuli are reported in Figure 4. No differences were found between responses observed from right and left antennae, and between responses observed from males or females, to any stimulus. The procedure set up in our bioassays showed to be suitable for *G. acuteangulatus*; therefore, further investigations on volatile compounds produced by the insect or by host plants could be supported by physiological bioassays to assess antennal perception.

CONCLUSIONS

In the light of these promising preliminary results, the research is worthy of going on to improve our knowledge on the attractiveness of the volatile compounds produced both by *G. acuteangulatus* adults and by host plants. However, attractive pheromones of Heteroptera are very difficult to identify because they are often masked by defence compounds, produced in greater quantities (McBrien and Millar, 1999; Millar, 2005). Furthermore, to implement effective environment-friendly control strategies, a special attention should be paid to the correct management of the hazel agro-ecosystem, and to the presence of more favourite bushes that could influence bug behaviour.

Literature Cited

Aldrich, J.R., and Yonke, T.R. 1975. Natural products of abdominal and metathoracic scent glands of coreoid bugs. Ann. Entomol. Soc. Am. 68: 955-960.

Aldrich, J.R., Waite, G.K., Moore, C., Payne, J.A., Lusby, W.R., and Kochansky, J.P. 1993. Male-specific volatiles from Nearctic and Australasian true bugs (Heteroptera: Coreidae and Alydidae). J. Chem. Ecol. 19: 2767-2781.

AliNiazee, M.T. 1998. Ecology and management of hazelnut pests. Ann. Rev. Entomol. 43: 395-419.

Blatt, S.E., and Borden, J.H. 1996. Evidence for a male-produced aggregation pheromone in the western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae). Can. Entomol. 128: 777-778.

Blatt, S.E., Borden, J.H., Pierce, H.D., Gries, R., and Gries, G. 1998. Alarm pheromone system of the western conifer seed bug, *Leptoglossus occidentalis*. J. Chem. Ecol. 24: 1013-1031.

Colazza, S., McElfresh, J.S., and Millar, J.G. 2004. Identification of volatile synomones, induced by *Nezara viridula* feeding and oviposition on bean spp., that attract the egg parasitoid *Trissolcus basalis*. J. Chem. Ecol. 30: 945-964.

Genduso, P., and Mineo, G. 1974. Difesa del Nocciuolo dagli artropodi dannosi - X - Ricerche bio-etologiche sul *Gonocerus acuteangulatus* (Goeze) (Rhynchota-Heteroptera-Coreidae). B. I. Entomol. Agrar. Oss. Fitopatol. Palermo 9: 23-75.

Koona, P., Osisanya, E.O., Lajide, L., Jackai, L.E.N., and Tamo, M. 2003. Assessment of chemical resistance of wild and cultivated *Vigna* species to the brown pod-bug *Clavigralla tomentosicollis* Stal (Hem., Coreidae). J. Appl. Entomol. 127: 293-298.

Leal, W.S., Panizzi, A.R., and Niva, C.C. 1994. Alarm pheromone system of leaf-footed bug *Leptoglossus zonatus* (Heteroptera: Coreidae). J. Chem. Ecol. 20: 1209-1216.

Lockwood, J.A., and Story, R.N. 1985. Bifunctional pheromone in the first-instar of the southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae): its characterization and interaction with other stimuli. Ann. Entomol. Soc. Am. 78: 474-479.

McBrien, H.L., and Millar, J.G. 1999. Phytophagous bugs. In: Hardie, J., Minks, A.K., (eds) Pheromones of non-lepidopteran insects associated with agricultural plants. CABI publishing: pp. 277-304.

Millar, J.G. 2005. Pheromones of true bugs. Top. Curr. Chem., 240: 37-84.

Moulet, P. 1995. Hémiptères Coreoidea (Coreidae, Rhopalidae, Alydidae), Pyrrhocoridae, Stenocephalidae Euro-Méditerranéens. Faune de France, 81. Fédération Française des Sociétés de Sciences Naturelles, Paris.

Nordlund, D.A. 1981. Semiochemicals: A Review of the Terminology, In: D.A. Nordlund, R.L. Jones and W.J. Lewis (Eds.), Semiochemicals: Their Role in Pest Management, Wiley, New York, pp.13-28.

Pavis, C. 1987. Les sécrétions exocrines des Hétéroptères (allomones et phéromones). Une mise au point bibliographique. Agronomie 7 (8): 547-561.

Prudic, K.L., Noge, K., and Becerra, J.X. 2008. Adults and nymphs do not smell the same: the different defensive compounds of the giant mesquite bug (*Thasus neocalifornicus*: Coreidae). J. Chem. Ecol. 34: 734-741.

Schaefer, C.W., and Mitchell, P.L. 1983. Food plants of the Coreoidea (Hemiptera: Heteroptera). Ann. Entomol. Soc. Am. 76: 591-615.

Tavella, L., Arzone, A., Sargiotto, C., and Sonnati, C. 1997. Pentatomidae and Coreidae harmful to hazelnut in North Italy (Rhynchota: Heteroptera). Acta Hort. 445: 503-509.

Tavella, L., Sonnati, C., and Arzone, A. 2001a. Rilevamento di coreidi e pentatomidi in corileti piemontesi (Heteroptera). Inftore Fitopatol. 51 (3): 55-59.

Tavella, L., Arzone, A., Miaja, M.L., and Sonnati, C. 2001b. Influence of bug (Heteroptera, Coreidae and Pentatomidae) feeding activity on hazelnut in northwestern Italy. Acta Hort. 556: 461-468.

Tavella, L., Migliardi, M., Sonnati, C., and Miaja, M. L. 2003. Effetti dell'attività trofica delle cimici sulle nocciole in relazione al periodo di attacco. Inftore Fitopatol. 53 (11): 47-51.

Tuncer, C., Saruhan, I, and Akça, I. 2005. The insect pest problem affecting hazelnut kernel quality in Turkey. Acta Hort. 686: 367-375.

Tuncer, C. 2009. Arthropod pest management in organic hazelnut growing. Acta Hort. 845: 571-578.

Viggiani, G. 1994. Current management of hazelnut diseases and pests. Acta Hort. 351: 531-541.

Wang, Q., and Millar, J.G. 2000. Mating behavior and evidence for male-produced sex pheromones in *Leptoglossus clypealis* (Heteroptera: Coreidae). Ann. Entomol. Soc. Am. 93: 972-976.

Figures

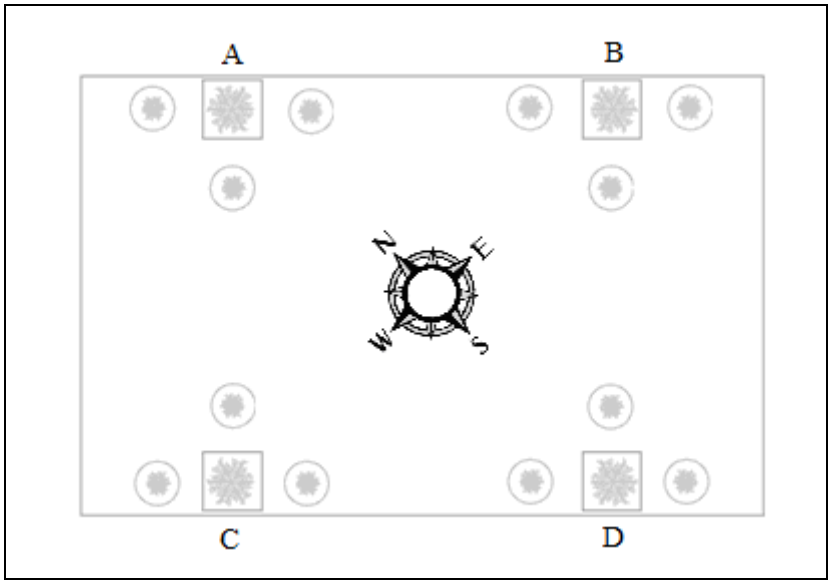

Figure 1. Scheme of multichoice bioassays conducted in the experimental net cage under natural atmospheric conditions to assess the behaviour of 60 males of *Gonocerus acuteangulatus*. Boxes labelled A, B, C, D were enclosed in net cages and contained the four treatments being tested by turns.

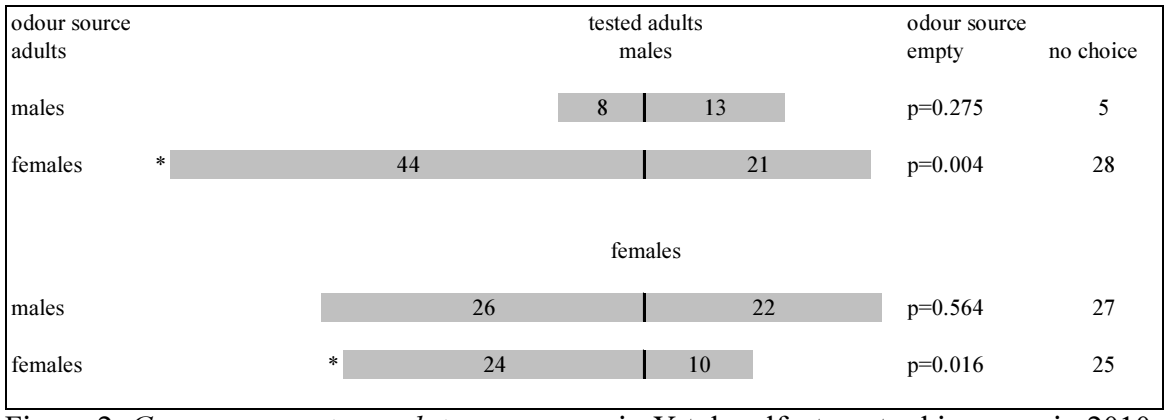

Figure 2. *Gonocerus acuteangulatus* responses in Y-tube olfactometer bioassays in 2010-2011. Odour sources were three males or three females in a glass bottle *vs* an empty bottle. Total number of insects that made a choice within 10 min was analyzed using χ^2 test. Insects that did not make a choice within 10 min were recorded as "no choice" and were excluded from statistical analysis.

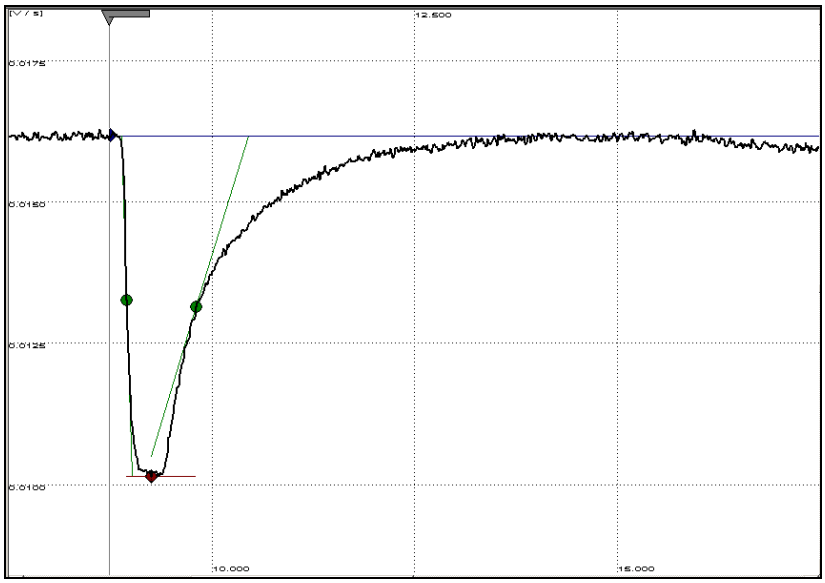

Figure 3. Typical antennal response (mV) of *Gonocerus acuteangulatus* adult to (E)-2-hexenal recorded with the electroantennographic apparatus.

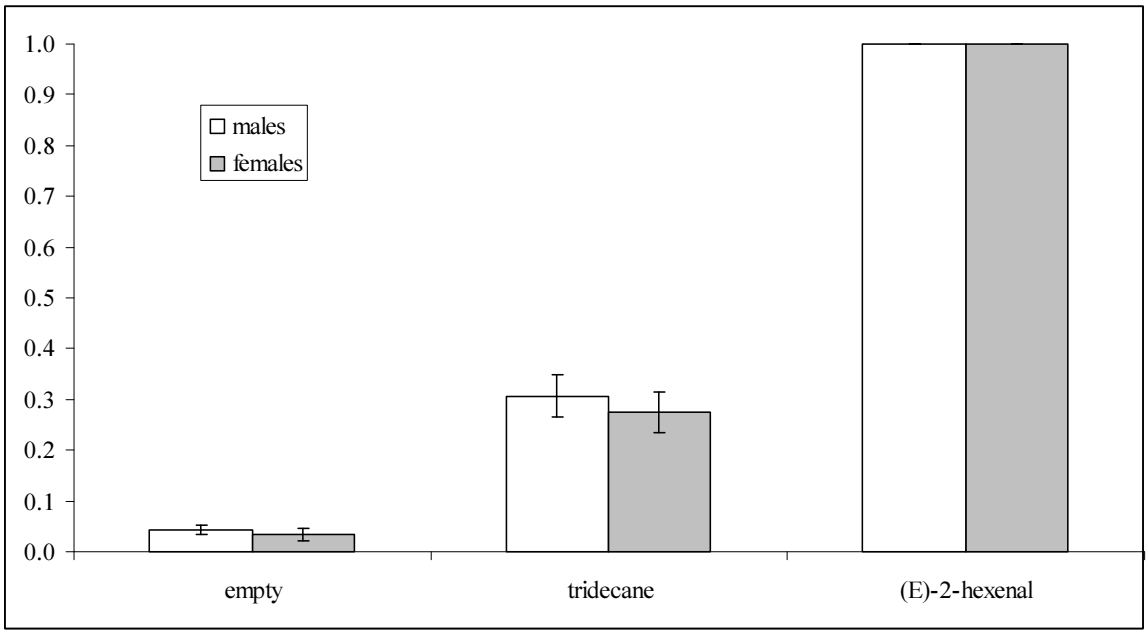

Figure 4. Mean responses with standard error bars to each odour stimulus of *Gonocerus acuteangulatus* males (n=6) and females (n=6) recorded with the electroantennographic apparatus. Values are normalized to the response obtained with (E)-2-hexenal.