IDENTIFICATION OF (Z)-4-TRIDECENE FROM DEFENSIVE SECRETION OF GREEN LACEWING, *Chrysoperla carnea*

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Abstract—We report the identification of a defensive secretion from the green lacewing, *Chrysoperla carnea*. By using combined gas chromatography–electroantennographic detection (GC-EAD), we found one major compound in the solvent extract of this secretion that elicited a significant EAD response from the antenna. Based upon its characteristic fragments from gas chromatography–mass spectrometry (GC-MS) analysis, the compound was identified as a tridecene. Dimethyldisulfide derivatization suggested that a double bond was located between positions 4 and 5 in the carbon chain. Thus, the compound was tentatively identified as a 4-tridecene. Coinjection of the extract with a mixture of the Z or E form of the synthetic 4-tridecene revealed that the unknown was (Z)-4-tridecene. EAG dose–responses showed a direct correlation to dose. Single sensillum recordings from sensilla trichodea situated on the antennae suggested the presence of receptor neurons specifically responding to this compound. An arrestment behavior was observed when tested in the Y-tube olfactometer. Preliminary field trapping results indicate that the compound is an antagonist to attraction. The avoidance behavior of predatory ants, observed when tested with the synthetic compound of this secretion further suggested a defensive function.

Key Words—Defensive secretion, green lacewing, *Chrysoperla carnea*, (Z)-4-tridecene, electroantennography, single sensillum recording, alarm pheromone, Chrysopidae, Neuroptera.

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2421

INTRODUCTION

The Chrysopidae, the so-called green lacewings have been extensively studied because many species are predators of agricultural insect pests (New, 1975; Tauber et al., 2000). Many are resistant to a wide range of insecticides commonly used in crop pest control, which has led to interest in their use in integrated control systems (Ridgway and Kinzer, 1974). Larvae or adults of several chrysopids are predators that feed on soft-bodied insects or their eggs. Lacewings are subject to predation by other invertebrates, such as ants, or parasitized by some hymenopteran species (Evans, 1978; Blum, 1981; Ruberson et al., 1995). Many arthropods have evolved to optimize the effectiveness of their defensive system as means of deterring predators. We observed that adults of the green lacewing, *Chrysoperla carnea*, discharge a secretion with a distinctive odor from a pair of glands situated in the anterior part of the prothorax. Sometimes, the entire head is covered with this offensive-smelling, pale yellowish fluid. The constituents of this secretion could function as defensive compounds against insectivorous invertebrates or perhaps vertebrates.

Although there has been a steady and significant increase in our knowledge of the chemical communication in arthropods over the past 30 years, much is yet to be learned, particularly in the defensive chemistry of invertebrates. In the present paper, we report on recordings of coupled gas chromatography–electroantennographic responses from antennae of adult *C. carnea* to their own exuduates. We also identified the main constituent that may function as an alarm pheromone for defensive purposes. In addition, we made single sensillum recordings from sensilla trichodea of antennal neurons responsive to the identified chemical and one selected plant volatile, (Z)-3-hexenyl acetate. Behavioral assays with the synthetic compound conducted in a Y-tube olfactometer indicated that lacewing adults exhibit arrestment of upwind movement to this compound, and field trapping tests demonstrated antagonistic effects on attraction of adults in the field.

METHODS AND MATERIALS

Insects and Defensive Secretion Extraction. Larvae of *Chrysoperla carnea* were purchased from Rincon-Vitova (Ventura, California) and reared on a regime of 20°C and 16L : 8D period. Females were mated and produced eggs. Larvae were fed with pea aphids, *Acyrthosiphon pisum*, and frozen *Ephestia kuekniella* eggs until pupation. Emerged adults were sexed and kept in separate cages with a supply of aphid honeydew coated leaves and water until they were used in experiments.

Two methods were used to collect defensive secretions. Three 4-day-old adults were either dissected into three parts, head with thorax, abdomen, and

wings, or adult lacewings were forced to secrete defensive fluids by clasping their body and wings with a pair of soft foreceps. The fluids were then collected with a 10-μl micropipet. Both body parts and collected fluids were extracted with distilled hexane containing an internal standard, (Z)-8-tridecenyl acetate, for qualitative and quantitative analyses. The extracts were stored at $-20°C$.

Electrophysiological and Chemical Analysis. A Hewlett Packard 5890 Series II gas chromatography was equipped with a DB-5 column (30 m $\times$ 0.25 mm ID; J&W Scientific, Folsom, California). An effluent split at 1:1 ratio allowed simultaneous flame ionization (FID) and electroantennographic detection (EAD) responses of extracts. Helium was used as the carrier gas with a flow rate of approximately 30 ml/min for both FID and EAD. Extracts were injected in the splitless mode. The injector temperature was 250°C, and the split valve was opened 1 min after injection. The column temperature started at 80°C for 1 min following the injection and then linearly increased to 250°C at a rate of 20°C/min. The outlet for the EAD was continuously supplied with a purified airstream flowing over the antennal preparation at a speed of 0.5 m/sec. A dissected lacewing head with its antennae was used for EAD recordings. A capillary recording Ag–AgCl electrode filled with the electrode gel (Spectra 360, Parker Laboratories, New Jersey) was placed in contact with the cut tip of the antenna. The other electrode, filled with the same gel, was connected with the head and served as the ground. The EAD setup with a high-impedance DC amplifier with automatic baseline drift compensation was purchased from Syntech (Hilversum, The Netherlands). A GC-EAD program (version 2.3) developed by Syntech was used to record and analyze the amplified EAD and FID signals on a Pentium II MICRON computer.

GC-MS analyses of the GC-EAD active defensive compound were performed by using a Hewlett Parkard 5890 Series II gas chromatograph linked to a Hewlett Parkard 5972 mass selective detector (MSD), which was equipped with a second DB-5 column used for the GC-EAD analysis described above. The GC operating conditions were the same as those described for the GC-EAD analysis. Spectra were recorded from 30 to 550 amu after electronic impact ionization at 70 eV. The chemical identification of the defensive compound was performed by comparing the retention time and mass spectrum with that of the synthetic standard.

Electroantennogram Responses and Single Sensillum Recording. EAG recordings were made by connecting an electrogel-filled (Spectro 360, Parker Laboratory, New Jersey) glass electrode to the cut distal end of a dissected lacewing antenna; the other electrode filled with the same gel was placed in connection with the base of the antenna. Antennal responses to different doses of the identified synthetic defensive compound from both sexes were recorded. Serial dilutions of the tested compound were made in redistilled HPLC-grade hexane at dosages of 1, 10, 100, and 1000 μg. The tested synthetic compounds were applied to filter-paper strips (0.5 $\times$ 2.5 cm, Whatman No. 1) in 10 μl of solvent.

The filter-paper strips were inserted into Pasteur pipet (15 cm long). A control puff of hexane was applied after each puff of a tested stimulus. The sequence of exposure to the stimulus compound on each antenna proceeded from the lowest to the highest concentration.

Single sensillum recordings were performed by using the penetration technique (Hubel, 1957) with tungsten electrodes. Tungsten wire electrodes (0.2 mm diam.) were sharpened electrolytically to a tip diameter <0.3 μm. A C. carnea adult was restrained with wire loops on a dental wax stub, and the antennae were fixed in the desired position. The tip of the recording electrode was inserted at the sensillum base by using a micromanipulator; the other electrode was inserted into the lacewing abdomen with a second micromanipulator. The recording electrode was connected to a high impedance amplifier (Syntech) that was also connected to a loudspeaker to provide an indication of the quality of the contact. The signal was recorded on a Vetter model 420F four-channel FM recorder. The recorded responses were transferred from tapes to a Micron Millennia Pentium computer and analyzed by using a Syntech analog-to-digital interface with Autospike for Windows 95 software (Syntech).

Alkylthiolation for Determining Double Bond Position. Dimethyldisulfide (DMDS) derivatization of the extract was conducted as described in Buser et al. (1983). First 100 μl of DMDS (Sigma) and 10 μl of iodine solution (120 mg I_2 in 4 ml diethyl ether) were added to the head and thorax extracts of 10 lacewing equivalents. The reaction was performed at approximately 40°C for 12 hr. After adding 100 μl $Na_2S_2O_3$ solution to reduce the excessive amount of iodine to iodide, 1 ml hexane was added to extract the product. The hexane layer was then transferred and concentrated to 200 μl, and 2 μl were injected for GC-MS analysis.

Chemicals. (Z)-4-Tridecene was prepared by a Wittig reaction as described below. Potassium t-butoxide (13.4 g, 120 mmol) was dissolved in 100 ml tetrahydrofuran (THF), and n-butyltriphenylphosphonium bromide (16 g, 40 mmol) was added to the stirred solution. After reflux for 30 min, the t-butanol formed was distilled off (10 ml). The reaction was cooled to room temperature, and a solution of octanal (5.68 g, 40 mmol) in THF (6 ml) was added. The resulting mixture was stirred for 2 hr at 25°C before it was poured into cold saturated NH_4Cl (100 ml). The organic phase was separated, and the aqueous phase extracted with pentane (2 × 50 ml). The combined organic phases were then dried with $MgSO_4$. After filtration, the product was subjected to fractional distillation. The yield was 4.4 g in an isomeric purity of 94% of Z isomer by GC-MS. ^{1}H NMR (CDCl$_3$) δ: 5.31–5.40 (m, 2H), 1.98–2.04 (m, 4H), 1.27–1.41 (m, 14H), 0.90 (t, 3H), 0.88 (t, 3H). ^{13}C NMR (CDCl$_3$ δ: 130.1, 129.6, 31.9, 29.8, 29.5, 29.3, 29.3, 27.2, 27.2, 22.9, 22.7, 14.1, 13.8. MS m/z (relative intensity): 182 (M, 18), 125 (3), 111 (10), 97 (20), 83 (33), 69 (39), 56 (39), 55 (80), 43 (43), 41 (100).

Behavioral Response Y-Tube Olfactometer, Defensive Evaluation, and Field Trapping. The behavioral response of adult lacewings to the identified defensive secretions and/or alarm pheromone was first conducted under laboratory conditions (23°C and daylight intensity) in the Y-tube olfactometer. In the olfactometer, freshly field-collected lacewings were offered a choice between the synthetic (Z)-4-tridecene (500 μg) and the control (hexane). The numbers of *C. carnea* adults entering either chamber were recorded. The duration of each choice test was limited to no more than 5 min. Control runs were also performed in the absence of any test stimuli to ensure that there was no bias for a particular chamber of the olfactometer.

The defensive value of this compound was evaluated by placing one honey bar inside each of two Petri dishes with *Crematogester* and *Tapinoma* ant species ($N = 20$). The honey bar in one of the dishes was encircled by a 3-mm-wide ring of the synthetic (Z)-4-tridecene (5 mg). The circle, outlined in pencil, was about 10 mm from the edge of the honey bar. The other Petri dish served as the control, with only the solvent application. The ants' forging behavior was observed, and the number of times ants avoided or retracted from the treated source after an attempted approach was recorded.

A field trapping test to investigate possible antagonistic effects of the compound on lacewing attraction was conducted in an alfalfa field in Ames, Iowa (May–July, 1999). Synthetic compounds were prepared in hexane or methylene chloride, and medical peerless cotton rolls (5 cm long, 100% cotton) were used for dispensers. The loading dosage for baits containing 2-phenylethanol (a known attractant for *C. carnea*) (Zhu et al., 1999) or (Z)-4-tridecene was 10 mg. Traps similar to Rebell-type traps, made of four Pherocon AM sticky traps back to back, were used for trapping in the field test (Trécé Inc.). Traps were hung from the bent end of metal posts, 10–15 cm above the canopy of alfalfa plants. Within a replicate ($N = 3$), traps were set at least 10 m apart. Traps were checked every other day for six days, and the trap position within a series was randomzied to minimize the effects of habitat heterogeneity.

Scanning Electron Microscopy. Antennae of adult *C. carnea* were first fixed in 70% ethanol, then dehydrated in absolute ethanol, critical point-dried, and coated with gold–palladium alloy (60 : 40) before examination under a Jeol 5800LV scanning electron microscope operated at 10–15 kV.

RESULTS AND DISCUSSION

GC-EAD analyses of the secretion exuded by both sexes of *C. carnea* demonstrated the presence of one minor and one major peak, with only the latter eliciting a significant EAG response from antennae (Figure 1). GC-MS analyses of extracts showed that the main compound contained characteristic ions of
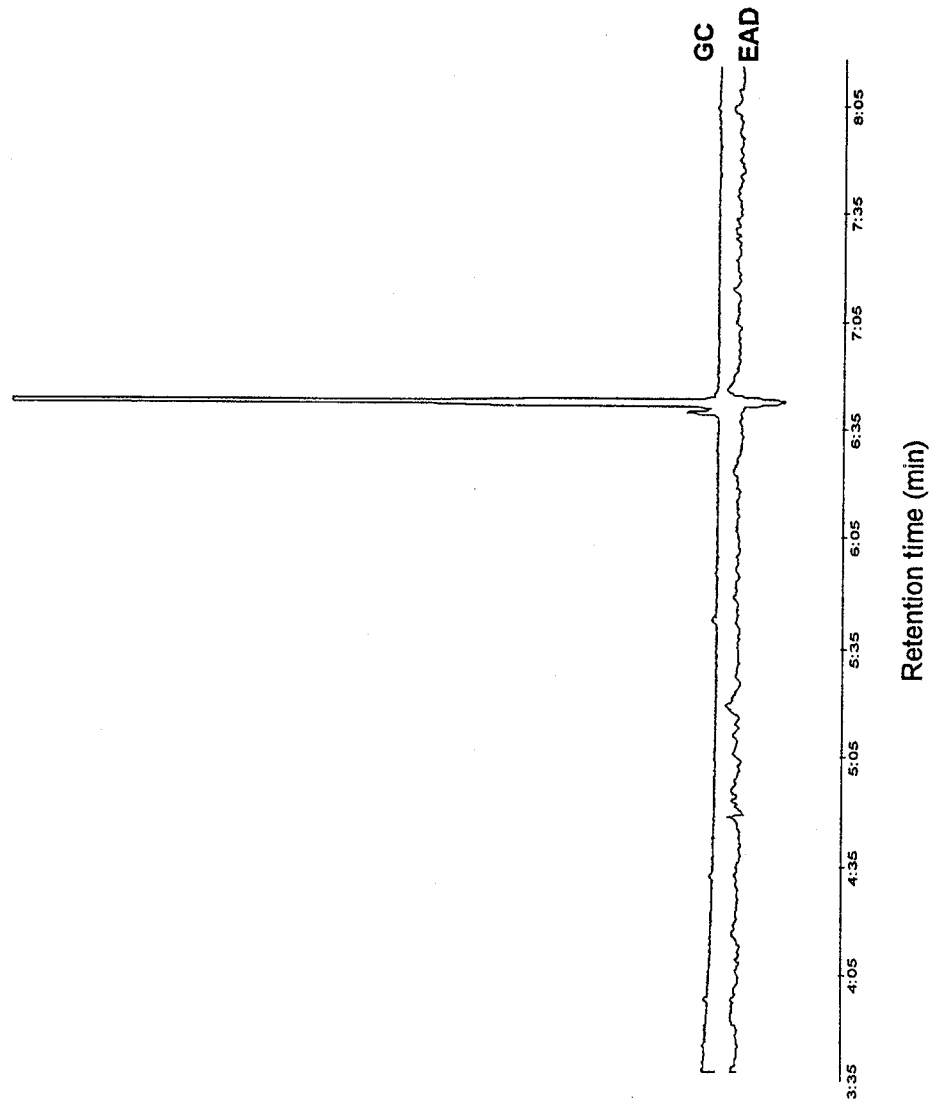
FIG. 1. Combined gas chromatography–electroantennographic detection (GC-EAD) from the antenna of an adult green lacewing, *Chrysoperla carnea*, stimulated with extracts of the defensive secretion.
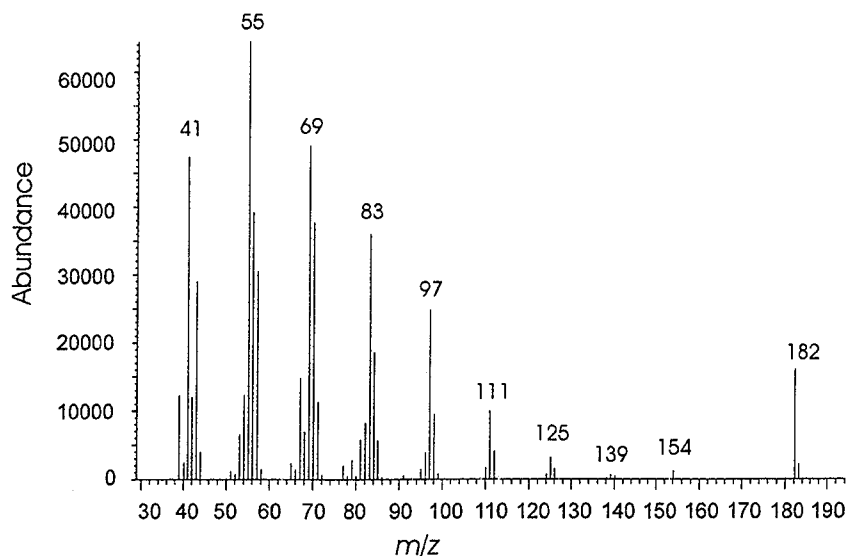
FIG. 2. Mass spectrum of the EAD active compound from extracts of the defensive secretion of adult green lacewings, *Chrysoperla carnea.*

an alkene, with a molecular ion at m/z 182 (Figure 2). The fragmentation patterns and mass spectrum suggested a tridecene. Thus, an alkylthiolation was carried out to determine the double-bond position. After dimethyldisulfide (DMDS) derivatization of the extracts, the characteristic mass fragments with ions at m/z 103 and 173 and the molecular ion at m/z 276 indicated that the double bond of this tridecene was between carbons 4 and 5 (Figure 3). Geometric isomerism of the 4-tridecene was determined by coinjection of synthetic 4-tridecene containing both the Z and E isomers together with the lacewing extracts. The E isomer eluted earlier than the Z isomer, and the Z isomer coeluted with the EAG active compound, which, therefore, was assigned as the (Z)-4-tridecene. Quantitative analyses of extracts showed about 5.5 μg of (Z)-4-tridecene from the secretion of a single female and 3.9 μg from one male.

EAG responses of both sexes of *C. carnea* to (Z)-4-tridecene increased with increasing doses, and the highest response was observed at a dose of 1000 μg (Figure 4). By using the tungsten recording technique, we successfully recorded the response to (Z)-4-tridecene from single sensilla trichodea situated on the antennae (Figure 5), but not to one of the most dominant components from alfalfa, (Z)-3-hexenyl acetate (Figure 6). To our knowledge, this is the first single sensillum recording conducted on a lacewing species. The response to (Z)-4-tridecene consisted of a large variety of action potential amplitudes, which
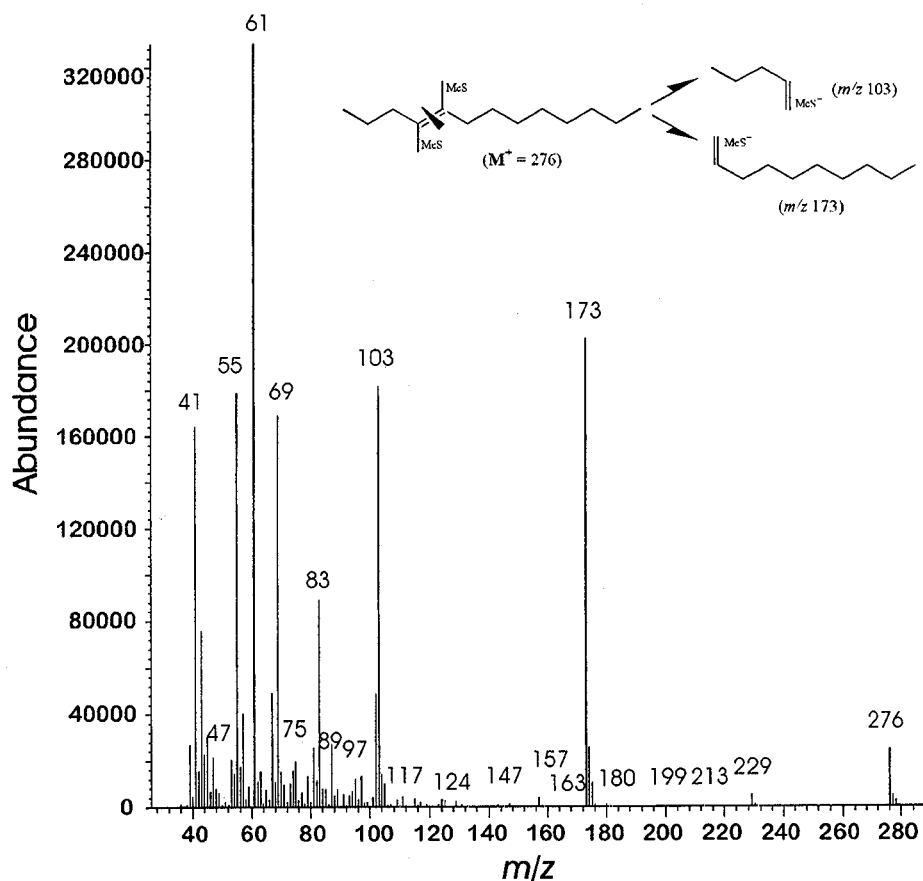
FIG. 3. Mass spectrum of DMDS-derivates of the EAD active compound from extracts of the defensive secretion of adult *Chrysoperla carnea* showing diagnostic mass spectral fragments of 4-tridecene.

suggests the presence of at least two receptor neurons in the trichoid sensillum.

Of special interest is the discovery that both sexes of *C. carnea* can detect (Z)-4-tridecene, based on the significant EAG responses elicited from the antennae during stimulation. Furthermore, both sexes responded behaviorally to (Z)-4-tridecene, with 87% of both deterred from entering the arm of the Y-tube olfactometer with (Z)-4-tridecene (Figure 7). This suggests that the compound may also function as an alarm signal for nearby conspecific individuals, in addition to being a possible defensive compound against predators. In addition, 93% of tested ants avoided the sugar bar surrounded with the synthetic (Z)-4-tridecene.
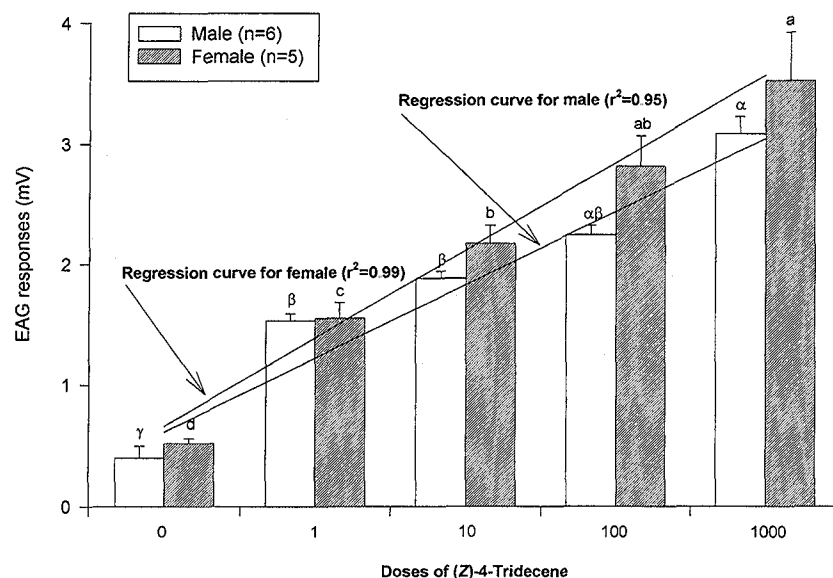
FIG. 4. EAG dose–response of male and female green lacewings, *Chrysoperla carnea*, to the identified defensive component, (Z)-4-tridecene. Means (± standard error) with different letters are significantly different (ANOVA followed by FPLSD test, $P < 0.05$).

In contrast, more than 95% of tested ants fed on the sugar bar without the application (Figure 8). Our preliminary field trapping results demonstrated antagonistic effects, with only two lacewings caught in traps baited with the compound together with 2-phenylethanol, a newly identified attractant (Zhu et al., 1999). In contrast, an average of more than 15 lacewings were captured in traps containing only 2-phenylethanol.

Alkenes, such as 4-tridecene, have been reported as constituents of the Dufour's gland secretion of several ant species (Lanne et al., 1988). These chemicals combined with other straight chain or branched hydrocarbons or terpenes function as alarm, territory, or trail signals or act as defense signals in several subfamilies of Formicidae (Attygalle and Morgan, 1984). This study is the first report of (Z)-4-tridecene identified from the putative defensive secretion of a lacewing species. Adults of *C. carnea* are active mostly at night. During the day, when they are resting on vegetation, they can become prey to invertebrate predators, such as ants, wasps, and beetles (Blum and Wallace, 1973; Jones et al., 1977; Evans, 1978; Clancy, 1946). Because of the possible alarm and defensive nature of (Z)-4-tridecene in ant species, *C. carnea* might be able to use this compound for defensive purposes. It might also be effective for defense against vertebrate predators, for example, birds and bats.
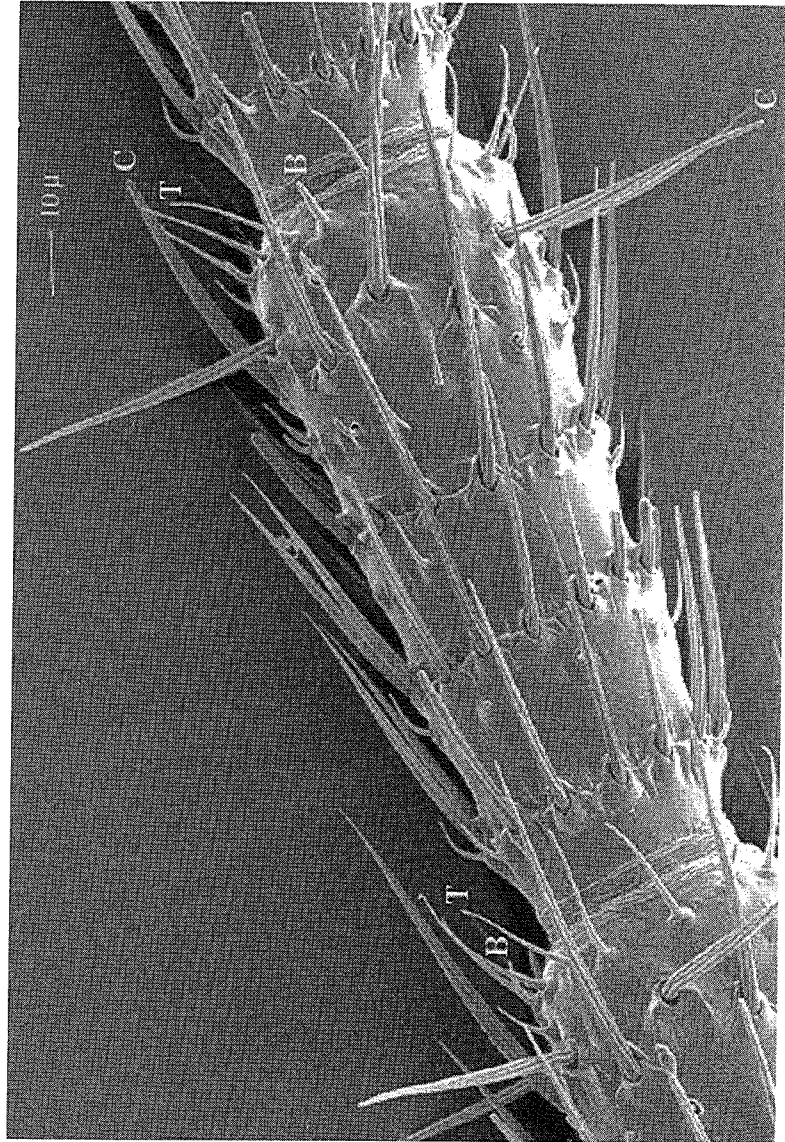
FIG. 5. One of the flagella segments of female *Chrysoperla carnea*, showing different types of sensilla. T, sensillum trichodeum; C, sensillum chaeticum; B, sensillum basiconicum. Scale bar = 10 μm.
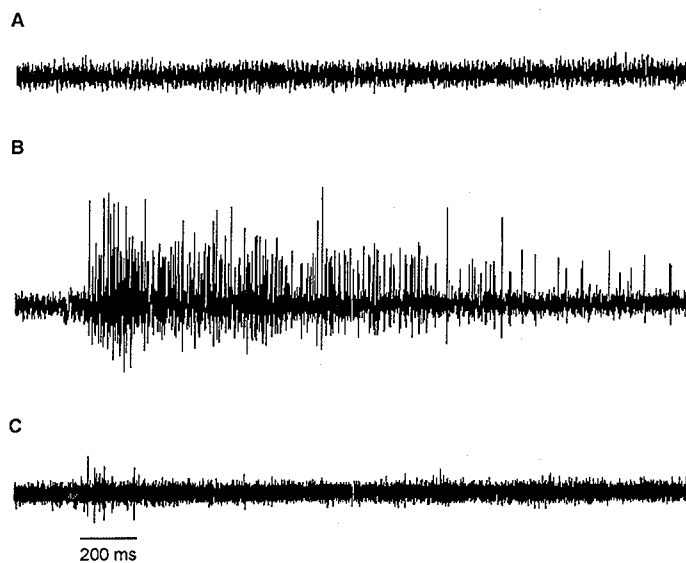
A

B

C

200 ms

FIG. 6. Single-sensillum recordings from sensillum trichodeum of a female green lacewing, *Chrysoperla carnea*, stimulated for 0.2 sec (bar) with hexane (A), 100 µg of (Z)-4-tridecene (B), and 100 µg of (Z)-3-hexenyl acetate (C).
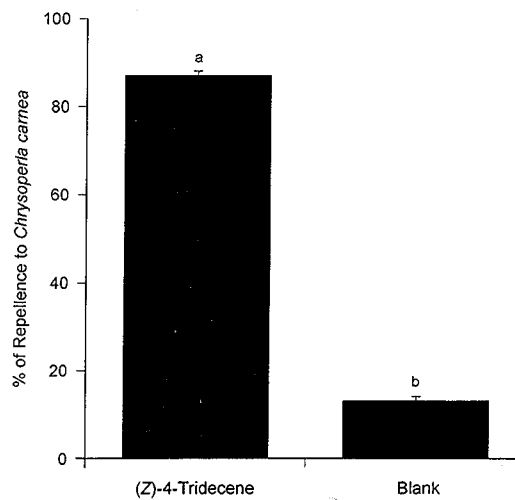
FIG. 7. Behavioral response of *Chrysoperla carnea* to 500 µg of (Z)-4-tridecene in the Y-tube olfactometer. Bars represent the mean ± SE. Different letters at the top of bars indicate means are significantly different at $P < 0.05$ (Student's t test).
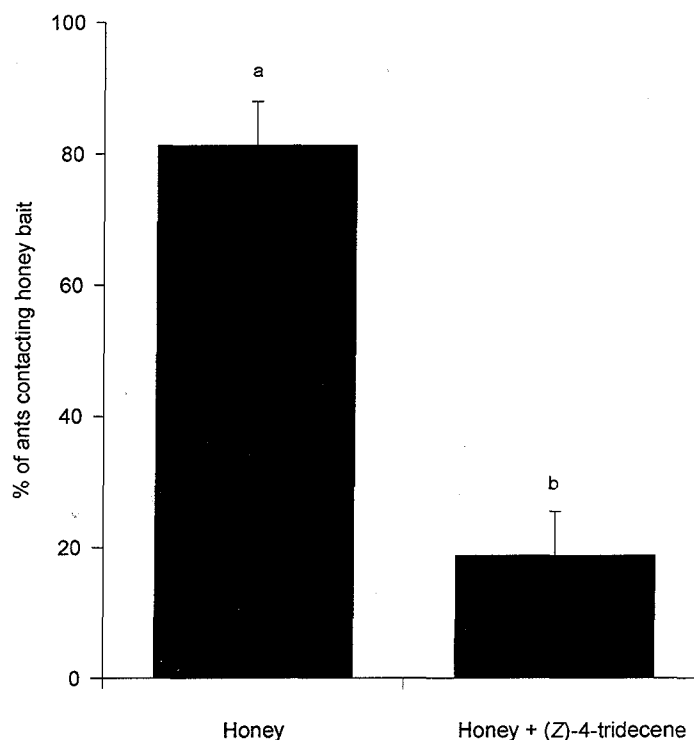
FIG. 8. Percentages of ants touching the sugar sources with or without treatment with (Z)-4-tridecene. Bars represent the mean ± SE. Different letters at the top of bars indicate means are significantly different at $P < 0.05$ (Student's t test).

Blum and Wallace (1973) found skatole and 1-tridecene in the secretions of another lacewing species, *Chrysopa oculata*. It is interesting that the two different lacewing species produce quite specific defensive secretion constituents. One possible explanation could be that these two species have different predatory ant species, which produce different alarm pheromones, since both 1-tridecene and (Z)-4-tridecene have been identified from the Dufour glands of several ant species (Lanne et al., 1988). In contrast to many other species of chrysopids not producing defensive secretions, the currently investigated *C. carnea* and previously reported *C. oculata* do produce secretions and have been cited as being the only two odorous lacewing species (McLachlan, 1874). The advantage of producing defensive secretions could be one of the selective forces for maintaining their populations, and partly explain why they are two of the more common species in Europe and North America.

Sound communication between the two sexes of conspecific lacewing species has been widely studied (Henry, 1979). However, chemical communication in lacewings has not been as well studied as in other invertebrate species, such as moths, ants, and wasps. Two recently published papers reported that adult *C. carnea* and *C. cognata* respond to volatiles released from either their aphid prey or plants (Boo et al., 1998; Zhu et al., 1999). Here we demonstrate that *C. carnea* adults not only produce defensive secretions against predation, but probably also use this compound as an alarm pheromone.

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