Pharmacophagy in green lacewings (Neuroptera: Chrysopidae: *Chrysopa* spp.)?

Jeffrey R Aldrich, Kamal Chauhan, Qing-He Zhang

Green lacewings (Neuroptera: Chrysopidae) are voracious predators of aphids and other small, soft-bodied insects and mites. Earlier, we identified (1*R*,2*S*,5*R*,8*R*)-iridodial from wild males of the goldeneyed lacewing, *Chrysopa oculata* Say, which is released from thousands of microscopic dermal glands on the abdominal sterna. Iridodial-baited traps attract *C. oculata* and other *Chrysopa* spp. males into traps, while females come to the vicinity of, but do not usually enter traps. Despite their healthy appearance and normal fertility, laboratory-reared *C. oculata* males do not produce iridodial. Surprisingly, goldeneyed lacewing males caught alive in iridodial-baited traps attempt to eat the lure and, in Asia, males of other *Chrysopa* species reportedly eat the native plant, *Actinidia polygama* (Siebold & Zucc.) Maxim. (Actinidiaceae) to obtain the monoterpenoid, neomatatabiol. These observations suggest that *Chrysopa* males must sequester exogenous natural iridoids in order to produce iridodial; we investigated this phenomenon in laboratory feeding studies. Lacewing adult males fed various monoterpenes reduced carbonyls to alcohols and saturated double bonds, but did not convert these compounds to iridodial. Only males fed the common aphid sex pheromone component, (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, produced (1*R*,2*S*,5*R*,8*R*)-iridodial. Furthermore, although *C. oculata* males fed the second common aphid sex pheromone component, (4a*S*,7*S*,7a*R*)-nepetalactone, did not produce iridodial, they did convert ~75% of this compound to the corresponding dihydronepetalactone, and wild *C. oculata* males collected in early spring contained traces of this dihydronepetalactone. These findings are consistent with the hypothesis that *Chrysopa* males feed on oviparae (the late-season pheromone producing stage of aphids) to obtain nepetalactol as a precursor to iridodial. In the spring, however, wild *C. oculata* males produce less iridodial than do males collected later in the season. Therefore, we further hypothesize that Asian *Chrysopa* eat *A. polygama* to obtain iridoid precursors in order to make their pheromone, and that other iridoid-producing plants elsewhere in the world must be similarly usurped by male *Chrysopa* species to sequester pheromone precursors.

Pharmacophagy in green lacewings (Neuroptera: Chrysopidae: *Chrysopa* spp.)?

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ABSTRACT

Green lacewings (Neuroptera: Chrysopidae) are voracious predators of aphids and other small, soft-bodied insects and mites. Earlier, we identified (1*R*,2*S*,5*R*,8*R*)-iridodial from wild males of the goldeneyed lacewing, *Chrysopa oculata* Say, which is released from thousands of microscopic dermal glands on the abdominal sterna. Iridodial-baited traps attract *C. oculata* and other *Chrysopa* spp. males into traps, while females come to the vicinity of, but do not usually enter traps. Despite their healthy appearance and normal fertility, laboratory-reared *C. oculata* males do not produce iridodial. Surprisingly, goldeneyed lacewing males caught alive in iridodial-baited traps attempt to eat the lure and, in Asia, males of other *Chrysopa* species reportedly eat the native plant, *Actinidia polygama* (Siebold & Zucc.) Maxim. (Actinidiaceae) to obtain the monoterpenoid, neomatatabiol. These observations suggest that *Chrysopa* males must sequester exogenous natural iridoids in order to produce iridodial; we investigated this phenomenon in laboratory feeding studies. Lacewing adult males fed various monoterpenes reduced carbonyls to alcohols and saturated double bonds, but did not convert these compounds to iridodial. Only males fed the common aphid sex pheromone component, (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, produced (1*R*,2*S*,5*R*,8*R*)-iridodial. Furthermore, although *C. oculata* males fed the second common aphid sex pheromone component, (4a*S*,7*S*,7a*R*)-nepetalactone, did not produce iridodial, they did convert ~75% of this compound to the corresponding dihydronepetalactone, and wild *C. oculata* males collected in early spring contained traces of this dihydronepetalactone. These findings are consistent with the hypothesis that *Chrysopa* males feed on oviparae (the late-season pheromone producing stage of aphids) to obtain nepetalactol as a precursor to iridodial. In the spring, however, wild *C. oculata* males produce less iridodial than do males collected later in the season. Therefore, we further hypothesize that Asian *Chrysopa* eat *A. polygama* to obtain

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64

65 ## INTRODUCTION

66 With ~ 6000 living species, Neuroptera is one of the smaller orders of insects (Winterton *et al.*
67 2010), but most larval neuropterans are predacious, often in agricultural systems, lending added
68 importance to this group (Tauber *et al.* 2009). Green lacewings (Chrysopidae) are the most
69 agriculturally important of the neuropterans because their larvae are generalist predators that
70 actively hunt for aphids, mites, whiteflies, caterpillars, and other small, soft-bodied prey that are
71 common pests on horticultural plants, and in field and tree crops (McEwen *et al.* 2007). While
72 most chrysopids are also predacious as adults, species in the genus *Chrysoperla* feed on nectar
73 and pollen, a characteristic that led to development of artificial diets and mechanized mass
74 rearing of some species (McEwen et al. 2007; Nordlund *et al.* 2001). All stages of *Chrysoperla*
75 are commercially available for augmentative biological pest control in field and greenhouse
76 crops (Pappas *et al.* 2011). In addition, based on volatiles associated with their pollen and nectar
77 consumption, lures for *Chrysoperla* species have been developed to attract wild adults to pest
78 infestations, and to overwintering and egg-laying sites (Koczor *et al.* 2014; Koczor *et al.* 2010;
79 Tóth *et al.* 2009; Wade *et al.* 2008).

80 Many other lacewings whose adults are predacious are naturally important in agricultural
81 systems, most notably *Chrysopa* species, but efforts to develop artificial diets or lures for these
82 species have been largely unsuccessful (McEwen et al. 2007). Pheromones are potentially useful
83 for attracting generalist predators for augmentative and conservation biological control (Aldrich

84 1999), and there is ample morphological evidence that in many lacewing species males possess
85 exocrine glands likely to produce aggregation pheromones (Aldrich and Zhang 2016; Güsten
86 1996). Based upon the meticulous illustrations of male-specific dermal glands in *Chrysopa*
87 (Principi 1949; Principi 1954), we identified the first attractant pheromone for lacewings (Zhang
88 *et al.* 2004). Field-collected males of the goldeneyed lacewing, *Chrysopa oculata* Say, release
89 (1*R*,2*S*,5*R*,8*R*)-iridodial with comparable amounts of nonanal, nonanol and nonanoic acid (Zhang
90 et al. 2004). Moreover, iridodial-baited traps attracted *C. oculata* males into traps and females to
91 the vicinity of baited traps (Chauhan et al. 2007). Adult *C. oculata* females lack the dermal
92 glands associated with iridodial production, and do not produce iridodial (Zhang et al. 2004).
93 Subsequently, we found that the same iridodial stereoisomer as identified from wild *C. oculata*
94 males also attracted adults of *C. nigricornis* Burmeister in the western U.S. (Zhang *et al.* 2006a),
95 and *C. septempunctata* Wesmael in China (Zhang *et al.* 2006b).
96 The discovery that iridodial powerfully attracted at least three different *Chrysopa* spp.,
97 and that the stereochemically correct isomer of iridodial can be prepared using catnip essential
98 oil as starting material (Chauhan *et al.* 2004), encouraged us to pursue pheromone identifications
99 for other lacewings whose males reportedly possess dermal glands similar to those of *Chrysopa*
100 males; *i.e.* species in the genera *Plesiochrysa*, *Ceratochrysa*, *Nineta*, a[d] *Pseudomallada* (=
101 *Anisochrysa*) (Aldrich and Zhang 2016). But, our plan to pursue pheromone research on some of
102 these chrysopids by rearing them in quarantine was thwarted by the discovery of one us (JRA)
103 that, despite their healthy appearance, normal fertility and usual amounts of C_9 compounds,
104 laboratory-reared *C. oculata* males produced no iridodial. Furthermore, an observation by
105 another of us (Q-HZ) that *C. nigricornis* males caught alive in traps baited with iridodial
106 attempted to eat the lure (unpublished observation), combined with previous reports of *Chrysopa*

septempunctata eating the plant known as silver leaf, *Actinidia polygama* (Siebold & Zucc.) Maxim (Actinidiaceae; native to Asia) to obtain the monoterpene iridoids (neomatatabiols) (Supplemental Figure 1, compounds **5** and **6**) (Hyeon *et al.* 1968), suggested that *Chrysopa* males must obtain certain unknown precursors from their diet in order to produce their pheromone.

The objectives of the present study were to 1) devise techniques to feed suspected pheromone precursors to *C. oculata* males and, 2) discover what precursor compound(s) elicit production of iridodial by *C. oculata* males.

MATERIALS AND METHODS

Lacewing collection and rearing

Adults of *C. oculata* for the laboratory colony were collected in May of 2008 by sweep net from wild herbaceous vegetation bordering deciduous trees at the Beltsville Agricultural Research Center, Prince George's County, Maryland, USA. Quart wide-mouth Mason® canning jars (Mason Highland Brands, LLC, Hyrum, UT) were used to maintain the adult insects. The jars were positioned horizontally, and nylon organdy cloth (G Street Fabrics, Rockville, MD) was held in place by the screw-top rim used to seal the jars. Jars were provisioned with live parthenogenic pea aphids [Homoptera: Aphidae: *Acyrthosiphon pisum* (Harris)] (supplied by Dr. John Reese, Kansas State University), eggs of the Angoumois grain moth (Gelechiidae: *Sitotroga cerealella* (Oliver); Kunafin "the Insectary", Quemado, TX), and a 10% honey solution. A 5 x 12 cm piece of cardboard was used as a feeding platform. Honey solution was provided in a shell vial (4 ml, 15 x 45 mm; Fisher Scientific, Pittsburgh, PA) with a loose-fitting foam stopper secured at one end of the cardboard with a rubber band. An adhesive strip of a Post-it® paper (50

x 40 mm; 3M, St. Paul, MN) was gently applied to the *Sitotroga* eggs, and the paper was glued (UHUstic®, UHU GmbH & Co., Bühl, Germany) to the other end of the cardboard with the band of moth eggs exposed. The cardboard feeding platform thus prepared was inserted into the bottom of the horizontal jar, and live pea aphid clones (up to several hundred) were added to the cage. Ten to twenty adults could be kept per jar, adding fresh aphids and moth eggs every other day or so, and adding fresh honey solution as needed. In jars used as mating cages (5-10 pairs/jar), a piece of light blue colored paper (providing a color contrast to the green eggs that are laid singly on stalks) was inserted inside the length of the jar as an oviposition substrate. Servicing of these jars was accomplished by working in a cage (30 x 30 x 60 cm; BioQuip Products, Rancho Dominguez, CA, USA) open at one end, and illuminated at the top of the other end by a fluorescent light. Adults from mating jars were moved to new jars weekly, the food platform was removed from the jar with freshly laid lacewing eggs, and the eggs that had been laid were allowed to hatch. Using a camel-hair brush, two first-instar larvae were transferred to each plastic cup (3/4 oz., snap-on lids; Solo Cup Company, Urbana, IL) with a layer of *Sitotroga* eggs in the bottom. Cups provisioned with only *Sitotroga* eggs were usually sufficient for both larvae to complete all 3 instars and pupate; more than two larvae per cup usually resulted in cannibalism. Lacewing pupae were transferred to the bottom compartment of mosquito breeders (BioQuip Products) and, upon emergence, the adults were removed from the top compartment. The colony was maintained in an environmental chamber set at 25 ^{o}C, 72% relative humidity, and 16:8 h (L:D) photoperiod. Some *C. oculata* males were reared as just described, plus with access to foliage of *Nepeta cataria* (Catnip) (Mountain Valley Seed Inc., Salt Lake City, UT; lot #G2217); some had their antennae removed (antennectomized) 1-5 days after emergence; and some larvae were reared as above, and fed pea aphid clones.

153 Lacewings are unusual among insects in that adults have chewing mouthparts whereas
154 larvae have piercing/sucking mouthparts (Tauber et al. 2009); therefore, some larvae were reared
155 with methylene blue dye added to the honey solution to verify that larvae ingested materials from
156 the honey water bottles, as did adults. Adult males from these treatments were subsequently
157 chemically sampled and analyzed as described below.
158

159 ## Scanning election microscopy

160 Live wild *C. oculata* males were anesthetized with CO_2, mounted on copper specimen holders
161 (16 × 29 × 1.5 mm thick) with cryoadhesive, and immersed in liquid N_2. The frozen specimens
162 were transferred to an Oxford CT1500 HF cryo-preparation system, and examined using a low
163 temperature scanning electron microscope (LTSEM; Hitachi S-4100) operated at 2.0 kV (Erbe *et*
164 *al.* 2003). Micrographs were recorded on Polaroid Type 55 P/N film.
165

166 ## Chemical standards

167 (*Z,E*)-Nepetalactone [= (4a*S*,7*S*,7a*R*)-nepetalactone] was prepared from catnip oil;
168 dihydronepetalactone was from hydrogenation of the lactone; (*Z,E*)-nepetalactol [=
169 (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol] was from $NaBH_4$ reduction of the lactone; and 1*R*,2*S*,5*R*,8*R*-
170 iridodial was derived from the (*Z,E*)-nepetalactone as previously described (Chauhan et al.
171 2004). Geranyl and farnesyl pyrophosphates were from Sigma-Aldrich (Saint Louis, MO), as
172 were the following volatile standards (≥ 95%): geraniol, citronellol, citronellal, linalool, citral, 6-
173 methyl-5-hepten-2-one, 8-hydroxycitronellol, and 8-hydroxycitronellal. *(Z)*-3-Octen-1-ol was
174 from Bedoukian Research, Inc. (Danbury, CT).
175

Chemical feeding, extraction of dermal glands, and chemical analysis

Chemical standards were individually fed to adult laboratory-reared *C. oculata* males at 1 µg/µl in the 5% aqueous honey solution for ca. 4 days prior to analysis. For extraction, *C. oculata* adult males were anesthetized with CO_2, eviscerated under tap water, the abdominal cuticle (segments 1-8) was removed with microscissors, cleaned of fat under water with micro-forceps, then removed from the water, and dried briefly with tissue paper. Cuticle from a single male was extracted in 10-15 µl of CH_2Cl_2 (≥ 99.9%; Sigma-Aldrich) in a Waters Alliance Total Recovery Vial® (deactivated, 12x32 mm; Taunton, MA/USA) or the minimum amount of solvent necessary to submerge the cuticles for pooled samples of several males (ca. 50-150 µl) (Zhang et al. 2004). Wild males collected by sweep net, Beltsville MD, May – June, 2008 and 2009, were dissected in like manner the same day as collected.

Gas chromatography (GC) and coinjections were performed in splitless mode using an HP 6890 GC equipped with a DB-5 column (0.25 µm film thickness, 30 m x 0.32 mm ID; J & W Scientific, Folsom, CA). Helium was used as the carrier gas, programming from 50◦C/2 min, to 250◦C at 10◦C/min, then held for 10 min. GC-mass spectrometry (GC-MS) analyses were performed in splitless mode with an electron impact ionization (EI) of 70 eV with an Agilent Technologies 5973 mass selective detector interfaced with 6890N GC system equipped with either an HP-5MS (30 m × 0.25 mm i.d. × 0.25 µm film) column programmed from 50◦C/2 min, rising to 230◦C at 15◦C/min, then held for 15 min, or using a DB-WaxETR column (0.25 µm film thickness, 30 m × 0.25 mm ID; J &W Scientific, Folsom, CA) programmed at 50◦C/2 min, rising to 230◦C at 15◦C/min, then held for 15 min.

RESULTS

199 *Chrysopa* adults are ca. 1.5-2 cm in length, and males are readily attracted to and captured in
200 sticky traps (Fig. 1) (Zhang et al. 2006b). In adult *C. oculata* males the dermal glands (Güsten
201 1996) are elliptical (~12 x 7.5 µm) with a central slit (Fig. 2), and occur on the 3rd–8th
202 abdominal sternites (~800, 2100, 2500, 2500, 2300 and 1500, respectively); corresponding
203 dermal glands are absent in females (Zhang et al. 2004).
204 Analyses of *C. oculata* revealed that nonanal and nonanol were abundant in extracts of
205 the abdominal sternites of males regardless of whether they were collected in the wild or reared
206 in the laboratory; however, iridodial was absent in extracts of laboratory-reared *C. oculata* males
207 (Fig. 3A and B; Table 1). Rearing *C. oculata* males in isolation from conspecific males did not
208 result in production of iridodial (Table 1), and removing the antennae of *C. oculata* males had no
209 affect on production of iridodial (Supplemental Figure 2). Access of *C. oculata* males to *Nepeta*
210 *cataria* (catnip) foliage in the laboratory did result in a detectable level of iridodial
211 (Supplemental Figure 3); however, this level was far below that seen for wild *C. oculata* males
212 (Table 1). In wild males collected by sweep netting foliage in early spring (*i.e.* not from
213 iridodial-baited traps) the mean iridodial percentage relative to the abundances of nonanal and
214 nonanol was 14.30 % (±SEM = 3.72) (Table 1). Analysis of one male caught in an iridodial-
215 baited trap (14 May 2008, Beltsville, MD) to which the captured males had access to the lure,
216 showed that this male produced much more iridodial (40.71 %) than the normal mean abundance
217 of iridodial in wild *C. oculata* males (Table 1). *(Z)*-3-Octen-1-ol was used as an internal standard
218 to quantitate pheromone production per wild *C. oculata* males collected in May 2008; extracts of
219 single males contained 20.42 ± 6.88 ng iridodial/male (mean ± SEM; N = 8) (Supplemental Data,
220 Iridodial Quantitation).
221 Feeding naturally common monoterpene alcohols and aldehydes to *C. oculata* males did

not stimulate production of iridodial (Table 2, experiment numbers 1-8). However, this series of feeding trials did reveal that males evidently possess reductase and saturase enzymes capable of reducing aldehydes to alcohols, and of saturating double bonds in these molecules. These reactions were essentially unidirectional; for example, geranial was completely converted to geraniol (Table 2, experiment number 2), whereas geraniol was only slightly isomerized to nerol but aldehydes were not produced (Table 2, experiment number 6). Furthermore, the abundances of C_9 compounds were not affected; nonanal, nonanol and nonanoic acid occurred in ratios within their ranges for wild-caught males for all experiments shown in Table 2.

Feeding male goldeneyed lacewings the common aphid pheromone components, (4a*S*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, produced positive results. While feeding nepetalactone did not result in production of iridodial, about 75% of this lactone was converted to the dihydronepetalactone (Table 2, experiment number 9). Furthermore, dihydronepetalactone was detected at low, but unequivocal levels in some samples from wild *C. oculata* males (Supplemental Figures 4 and 5). *Chrysopa oculata* males fed (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol converted this compound to (1*R*,2*S*,5*R*,8*R*)-iridodial (82.7%; Table 2, experiment number 10; Fig. 3C), with two later eluting 168 MW compounds accounting for 17.3% of the other newly appearing components, as well as *(Z)*-4-tridecene from the defensive prothoracic glands (Fig. 3C, compound **c**) (Aldrich *et al.* 2009). Two additional feeding experiments were conducted as for experiment 10 (Table 2); one of these experiments using the same GC-MS conditions (N = 9 males) showed 54.90% conversion to (1*R*,2*S*,5*R*,8*R*)-iridodial with the two later eluting 168 MW components totaling 40.10%, and the second experiment (N = 4 males) analyzed using a 30m HP-5 column resulted in 100% conversion to (1*R*,2*S*,5*R*,8*R*)-iridodial (Supplemental Data, Table 2, #10a & b).

Finally, feeding experiments conducted with *C. oculata* larvae failed to stimulate more than trace levels of iridodial in the resulting male adults. Providing pea aphid clones to larvae during rearing yielded at most only traces of iridodial in the ensuing adult males (Supplemental Data, Larvae Fed Aphids). While *Chrysopa oculata* larvae provided with honey water solution containing methylene blue turned decidedly blue, verifying this method as an appropriate means to feed suspected pheromone precursors to larvae, feeding geranyl or farnesyl pyrophosphates did not stimulate detectable production of iridodial in the ensuing adult males (Supplemental Data, Larvae Fed Terpene Phosphates). Feeding *C. oculata* larvae with (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, which in laboratory-reared adult males resulted in wild-type levels of (1*R*,2*S*,5*R*,8*R*)-iridodial, produced trace levels of iridodial far lower than wild-type levels of the pheromone (Supplemental Data, Larvae Fed Nepetalactol).

DISCUSSION

Coincidence of male-specific dermal glands with extraction of (1*R*,2*S*,5*R*,8*R*)-iridodial from the 3rd–8th abdominal sternites strongly implicates these glands as the pheromone source (Zhang et al. 2004). Surprisingly, only males are caught in traps baited with this iridodial (Zhang et al. 2004; Zhang et al. 2006a; Zhang et al. 2006b); however, females are drawn to the vicinity of, but seldom enter, iridodial-baited traps (Chauhan *et al.* 2007). Presumably, females stop short of entering traps because the close-range substrate-borne vibrational signals to which females are ultimately attracted (Henry 1982) are disrupted by trapping males. The C_9 compounds are unattractive to *C. oculata*, quantitatively much less variable than iridodial, and inhibitory to iridodial attraction, suggesting these compounds play a role independent from that of iridodial (Zhang et al. 2004).

Previous laboratory rearing studies with *Chrysopa oculata* showed that males produced fertile matings when fed only sugar and water, whereas females needed to feed on pea aphid clones in order to mate and produce fertile eggs (Tauber and Tauber 1973). Our results support these finding, but also make it clear that *C. oculata* males are unable to make pheromone on this feeding regimen. Iridodial production in *C. oculata* males was not stimulated by 1) antennectomy of sexually mature *C. oculata* males, which in some group-reared insects stimulates pheromone production (e.g. Dickens *et al.* 2002); 2) providing access to catnip plants, *Nepeta cataria*, containing the nepetalactone aphid pheromone component (Pickett *et al.* 2013) or; 3) rearing *C. oculata* males in isolation, which in some insects is required for maximal pheromone production (Ho *et al.* 2005; Khrimian *et al.* 2014).

Cyclopentanoid natural products based on an iridoid structure are widespread in plants and insects (Hilgraf *et al.* 2012; Lorenz *et al.* 1993), and incorporation of [^{14}C]mevalonolactone by the stick insect, *Anisomorpha buprestoides* (Stoll) (Phasmatodea: Pseudophasmatidae), and the catnip plant (*N. cataria*) demonstrated that biosynthesis of their respective iridoids, anisomorphal and nepetalactone, proceed via parallel terpene pathways from acyclic precursors, particularly geraniol (Meinwald *et al.* 1966). Larvae of leaf beetles (Coleoptera: Chrysomelidae) from four different genera showed that biosynthesis of the iridoid defensive compound, chrysomelidial, proceeds from geraniol via an ω-oxidation sequence to 8-hydroxygeraniol, with the eventual cyclization of 8-oxocitral to form the characteristic iridoid cyclopentanoid ring structure (Hilgraf et al. 2012; Lorenz et al. 1993; Veith *et al.* 1994). Certain rove beetles (Coleoptera: Staphylinidae: *Philonthus* spp.) also produce defensive secretions containing iridoids (*e.g.* plagiodial), but unlike enzymes from iridoid-producing leaf beetle larvae, the *Philonthus* enzyme is able to oxidize and cyclize saturated substrates such as citronellol (Weibel

291 *et al.* 2001). In plants , including a catnip species (*N. racemosa*) (Hallahan *et al.* 1995), the
292 cyclization reactions to iridoids proceed via 10-hydroxygeraniol and 10-oxogeranial rather than
293 8-hydroxygeraniol/al (Geu-Flores *et al.* 2012). Furthermore, Hilgraf *et al.* (2012) stressed that
294 there are still many open questions concerning the biosynthesis of iridoids, particularly
295 "saturated" iridoids such as iridodial.

296 In contrast to other iridoid-producing insects and plants whose biosynthetic pathways
297 have been investigated, *Chrysopa* males are evidently incapable of cyclizing geraniol or other
298 acyclic analogs to form the cyclopentanoid ring structure characteristic of iridoid compounds.
299 Thus, feeding acyclic monoterpene alcohols and aldehydes to *C. oculata* males did not stimulate
300 production of iridodial. However, our feeding trials revealed that *C. oculata* males are capable of
301 reducing aldehydes to alcohols and of saturating double bonds. Moreover, males fed the common
302 aphid pheromone component, (4a*S*,7*S*,7a*R*)-nepetalactone, converted ~75% to
303 dihydronepetalactone, and males fed the other common aphid pheromone component,
304 (1R,4aS,7S,7aR)-nepetalactol, converted this bicyclic iridoid to (1*R*,2*S*,5*R*,8*R*)-iridodial.
305 Interestingly, analyses of wild *C. oculata* males collected in May often revealed the presence of
306 dihydronepetalactone.

307 One interpretation of these data is that *C. oculata* males must eat aphid oviparae to obtain
308 nepetalactol in order to make their pheromone. Indeed, in northern California the peak late-
309 season attraction of green lacewings to nepetalactol (nepetalactone is unattractive) occurs at least
310 a month earlier than the peak in aphid oviparae (Symmes 2012), consistent with the hypothesis
311 that *Chrysopa* males feed on oviparae to obtain nepetalactol as a precursor for iridodial. These
312 dynamics indicate there is sufficient time for *Chrysopa* males to feed on oviparae, produce
313 iridodial, mate, and have conspecific females' offspring reach the prepupal overwintering stage

(Uddin *et al.* 2005). However, adult males from laboratory-reared *C. oculata* larvae fed nepetalactol still failed to produce wild-type levels of iridodial even though wild *C. oculata* males collected early in the spring produce less iridodial than do males collected later in the season (Zhang et al. 2004). Although some aphids produce oviparae under stressed conditions in summer (Hardie 1985), it seems unlikely that these oviparae are a reliable or abundant enough source to sustain *Chrysopa* male pheromone production. Therefore, we further hypothesize the *raison d'être* that Asian *Chrysopa* eat fruit and foliage of silver leaf (*A. polygama*) is to obtain iridoid precursors necessary to make their pheromone; we believe that other iridoid-producing plants (e.g. Hilgraf et al. 2012; Prota *et al.* 2014) elsewhere in the world must be similarly usurped by male *Chrysopa* species to sequester iridoid pheromone precursors.

Contrary to *Chrysoperla* green lacewings whose adults are not predacious, *Chrysopa* spp. lacewing adults are predacious (Tauber et al. 2009), and appear to exhibit pharmacophagy; that is, they "search for certain secondary plant substances directly, take them up, and utilize them for a specific purpose other than primary metabolism" (Boppré 1984). A prime example of pharmacophagy are male *Bactrocera* fruit flies (Tephritidae) that feed on plants to obtain their pheromone precursor, methyl eugenol (Tan and Nishida 2012). Indeed, males of certain lacewings [*i.e. Ankylopteryx exquisite* (Nakahara) (Pai *et al.* 2004), and *Mallada basalis* (Walker) (Oswald 2015; Suda and Cunningham 1970)] are also powerfully attracted to methyl eugenol for unknown reasons (Tan and Nishida 2012). In addition, certain chrysomelid beetle larvae discharge iridoid allomones that may be synthesized *de novo*, which is considered ancestral, or produced via the more evolutionarily advanced mechanism, sequestration from plants (Kunert *et al.* 2008). Increasingly, pharmacophagy is being recognized as a widespread phenomenon in insects, and Wyatt (2014) has extended the concept of pharmacophagy to include

337 molecules produced by bacteria that are used as pheromones, such as locust phase-change
338 pheromones produced by gut bacteria. If male *Chrysopa* spp. lacewings actually do seek out
339 aphid oviparae to obtain nepetalactol as a precursor to iridodial, and in this regard it should be
340 noted that only *Chrysopa* males are attracted to nepetalactol (Koczor *et al.* 2015), then the
341 concept of pharmacophagy must be further extended to include this type of predator/prey
342 interaction. Whether or not sequestration of iridodial precursors from oviparae and/or iridoid-
343 containing plants is truly the explanation for lack of pheromone in laboratory-reared *Chrysopa*
344 awaits further research.
345

346 ## CONCLUSIONS

347 Goldeneyed lacewing males, *Chrysopa oculata* (Neuroptera: Chrysopidae), produce
348 (1*R*,2*S*,5*R*,8*R*)-iridodial as an aggregation pheromone from specialized dermal glands on the
349 abdomen; however, seemingly normal laboratory-reared males of *C. oculata* do not produce
350 iridodial. Feeding studies with *C. oculata* showed that males of these predatory insects fed one of
351 the common aphid sex pheromone components, (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, sequester this
352 compound and convert it to the stereochemically correct lacewing pheromone isomer of
353 iridodial. These data, combined with literature accounts of other *Chrysopa* species from the
354 Oriental region that feed on iridoid-producing plants, suggest these (and some other) lacewing
355 species must obtain precursors from aphid oviparae and/or certain plants containing iridoids in
356 order to make pheromone. The phenomenon, known as pharmacophagy, whereby an insect
357 searches for certain secondary plant substances and sequesters the chemicals for a specific
358 purpose other than primary metabolism, is widespread among phytophagous insects but, to our
359 knowledge, is unknown among lacewings or other predacious insects. Our findings, if verified,

360 have significant implications for lacewing-based biological control of aphids and other small
361 arthropod pests.
362

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374

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Figure 1(on next page)

Male *Chrysopa septempunctata* captured in pheromone-baited trap, Shengyang, China (Zhang et al., 2006).

Chrysopa females come to the vicinity of iridodial-baited traps, but are seldom caught (Chauhan et al., 2007).

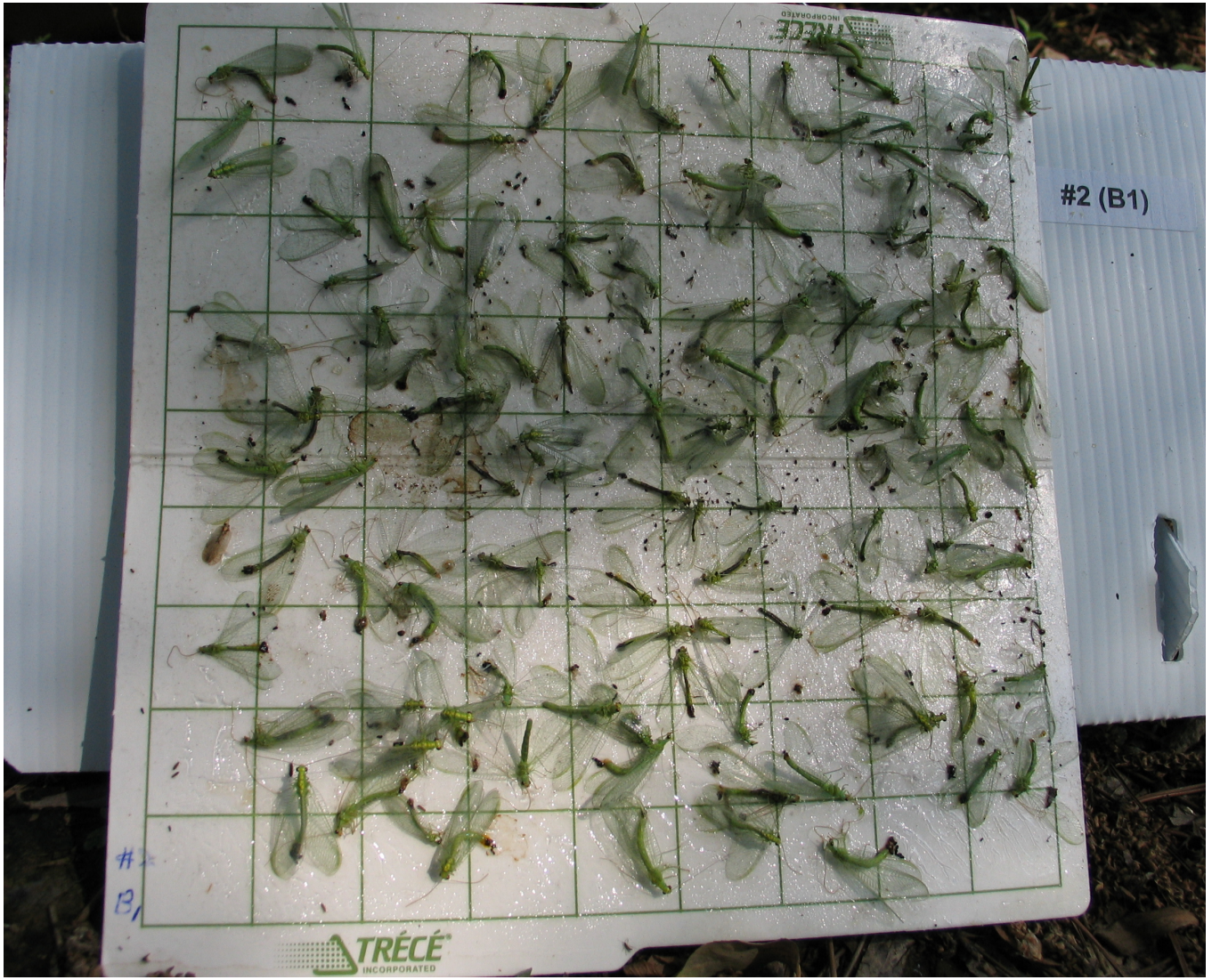
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Figure 2(on next page)

Scanning electron micrographs of the male-specific dermal glands of *Chrysopa oculata*.

Low temperature scan (Erbe et al., 2003) with insert showing close-up of two dermal glands.

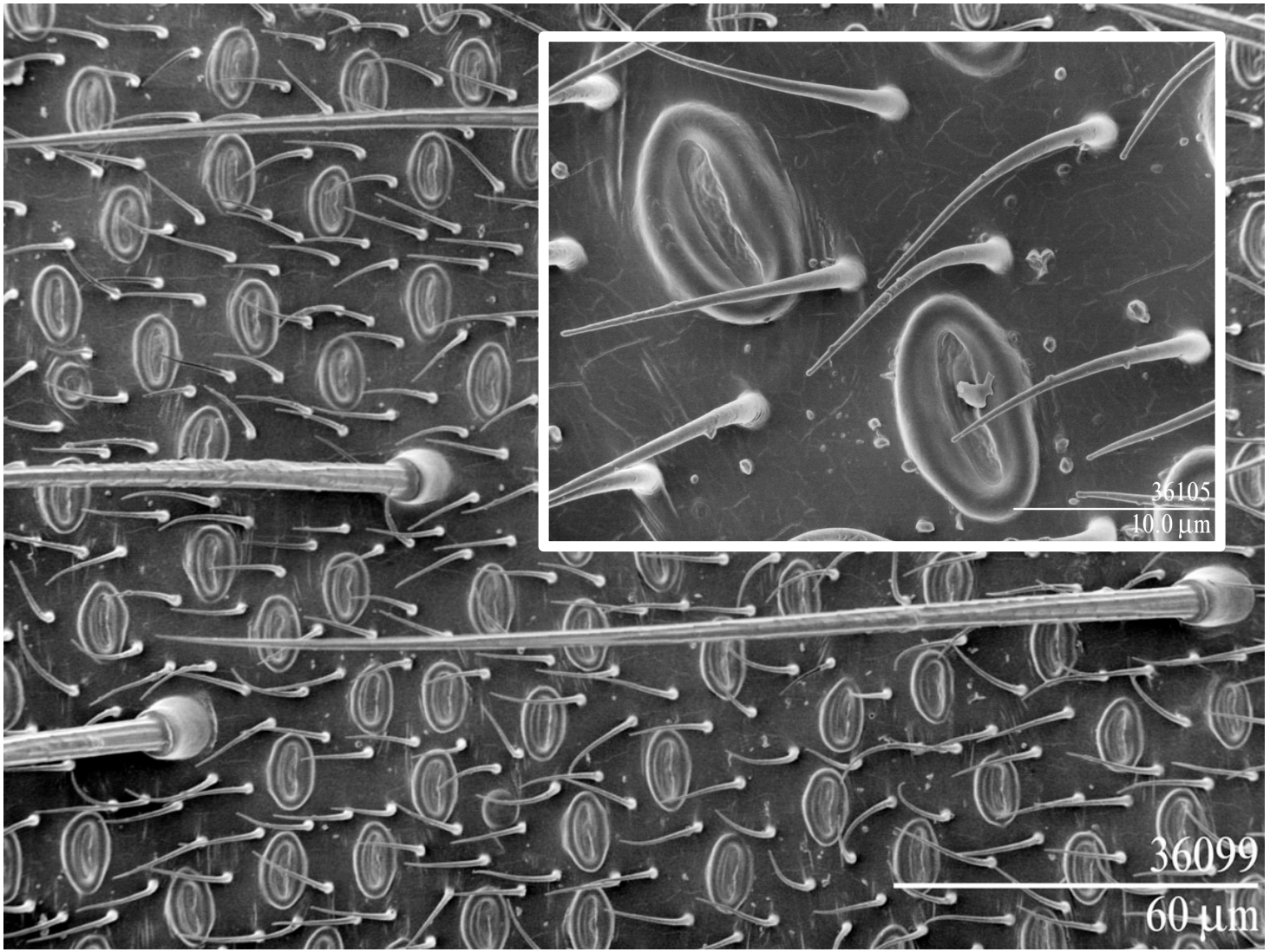

36105
10.0 µm
36099
60 µm

Figure 3(on next page)

Total ion chromatograms of abdominal cuticular extracts of male *Chrysopa oculata*.

A) Field-collected, B) laboratory-reared and, C) laboratory-reared fed (1R,4S,4aR,7S,7aR)-dihydronepetalactol (see Table 2). (Column = 30m DB-WAXetr: **a** = nonanal ; **b** = nonanol ; **c** = (Z)-4-tridecene; **1** = (1R,2S,5R,8R)-iridodial; **d** & **e** = 168 MW isomers.)

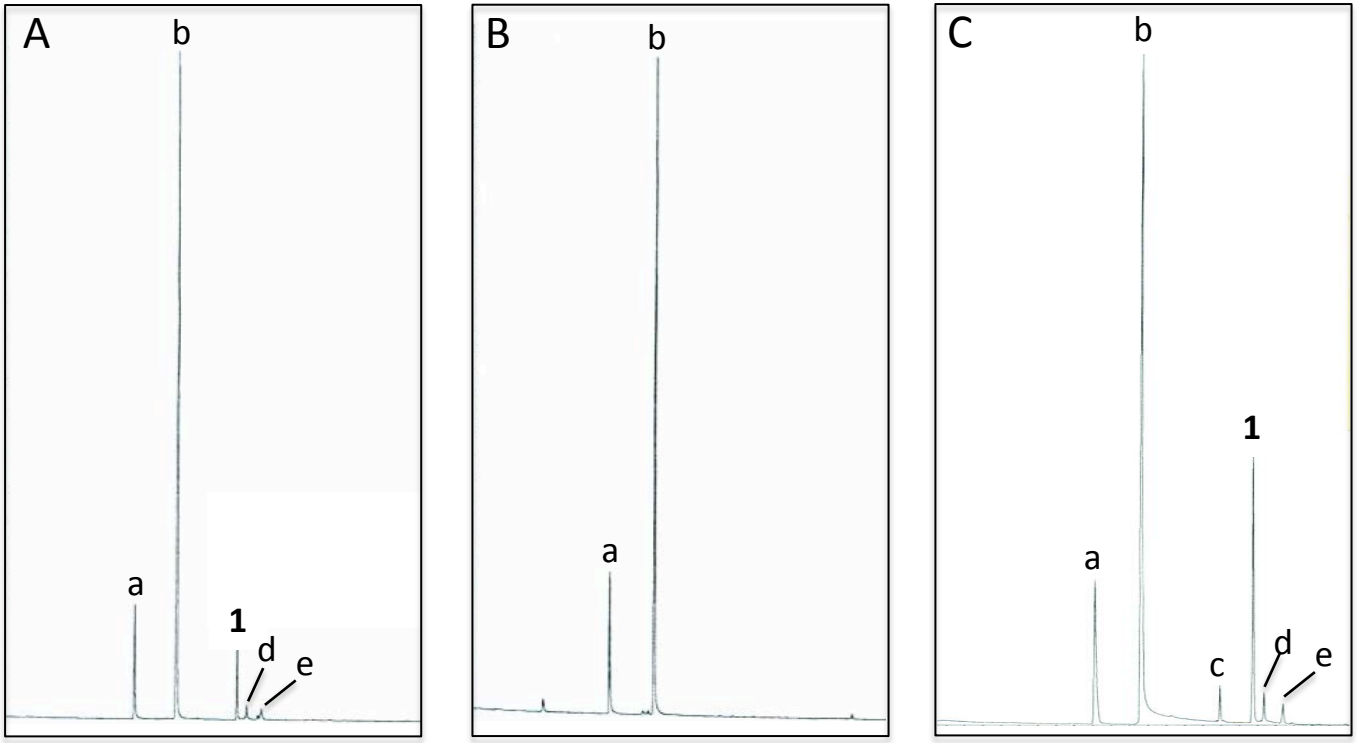

A
b
a
1
d
e
B
b
a
C
b
1
a
c
d
e

Table 1(on next page)

Volatiles from abdominal cuticle of field-collected and laboratory-reared *Chrysopa oculata* males.

Wild *C. oculata* males were collected by sweep net, Beltsville, Maryland, and *C. oculata* laboratory-reared males (see text for details) were sampled for comparisons. One *C. oculata* male was from a field trap baited with a lure including synthetic iridodial. Abdominal cuticle (segments 3–8) for chemical analyses were prepared as described previously (Zhang et al., 2004).

1

Source / Date	N [a]	Compound (%)			%∑[c]
		Nonanal	**Nonanol**	**Iridodial** [b]	
Field / 14 May 2009	4	13.06	80.68	2.35	96.09
Field / 18 May 2009	2	15.81	80.16	2.12	98.09
Field / 22 May 2009	1	10.31	42.01	38.13	90.45
Field / 28 May 2009	1	30.09	50.06	16.11	96.26
Field / 28 May 2009	1	13.56	67.55	16.19	97.30
Field / 28 May 2009	1	8.84	74.88	14.06	97.78
Field / 1 June 2009	1	32.24	54.82	9.94	97.00
Field / 1 June 2009	1	13.69	65.20	15.53	94.42
Mean:		**13.95**	**64.42**	**14.30**	**95.92**
±SEM:		**3.81**	**4.73**	**3.72**	
Field Trap[d] / 13 May 2008	1	16.43	38.93	40.71	96.07
Lab / 27 June 2008[e]	8	21.28	76.26	0	97.54
Lab / 13 Aug 2008[e]	5	21.37	69.34	0	90.71
Lab / 24 Nov 2008[e]	6	11.20	86.12	0	97.32
Lab / 24 Nov 2008[e]	7	18.60	75.74	0	94.34
Lab / 5 Jan 2009[f]	5	16.58	79.42	0	96.00
Mean:		**17.81**	**77.38**	**0**	**95.18**
±SEM:		**1.88**	**1.73**		

2 [a] In samples where N>1, multiple males were pooled and analyzed as a single

3 sample by GC-MS on a 30 m DB-WaxETR column.

4 [b] (1*R*,2*S*,5*R*,8*R*)-Iridodial (Chauhan et al., 2004).

5 [e] Percentage of total volatiles; nonanoic acid (poorly resolved

6 chromatographically) accounted for the majority of non-included volatiles.

7 [d] This *C. oculata* male was collected in a trap baited with 5 mg of iridodial plus 1 mg of skatole

8 per 50 µl of octane to the well of gray rubber septa (5-mm sleeve-type, The West Co., Lititz,

9 PA); the trap used was as previously described (Zhang et al. 2004), and it was deployed at the

10 Agricultural Research Center-West, B Beltsville, MD.

11 [e] Reared singly as adults.

12 [f] Reared in a group as adults.

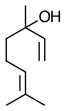
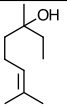
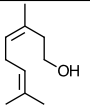
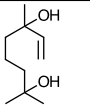
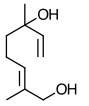
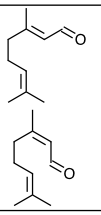
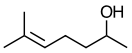
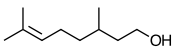
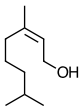
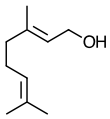
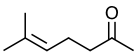
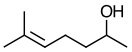
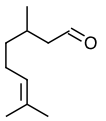
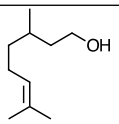
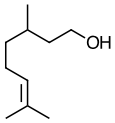
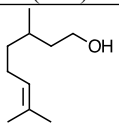
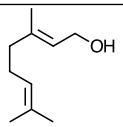
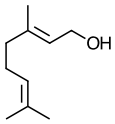
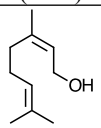
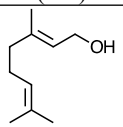
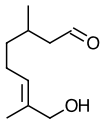
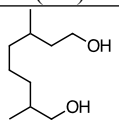
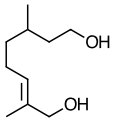
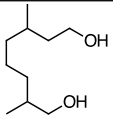
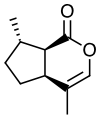
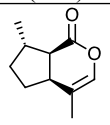
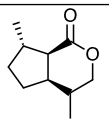
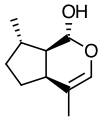
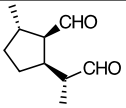
13

Table 2 (on next page)

Compounds produced by laboratory-reared *Chrysopa oculata* males fed various exogenous terpenoids.

Sampling and rearing methods described in text; 1 *µg/µl* test compound in honey water, analyzed by gas chromatography-mass spectrometry using a 30 m DB-WaxETR column.

1

No.	N[a]	Compound fed[b]	Compound(s) produced from treatment (%)[c] a	b	c	d
1	8		 (16)	 (3.3)	 (9.7)	 (71)
2	12		 (9.9)	 (8.3)	 (42.3)	 (39.5)
3	9		 (100)			
4	10		 (100)			
5	7		 (95.3)	 (4.7)		
6	5		 (4.3)	 (95.7)		
7	15		 (100)			
8	15		 (100)			
9	12		 (23.3)	 (76.7)		
10	10		 (82.7)			

2 [a] Number of males pooled for analysis.

[b] Sources of standards listed in text; 1) 3,7-dimethyl-1,6-octadien-3-ol (linalool), 2) (*Z/E*)-3,7-dimethyl-2,6-octadienal (citral: 43% *Z*-isomer, neral + 57% *E*-isomer, geranial), 3) 6-methyl-5-hepten-2-one, 4) 2,6-dimethyl-5-heptenal (citronellal), 5) 2,6-dimethyl-5-heptenol (citronellol), 6) (*E*)-3,7-dimethyl-2,6-octadien-1-ol (geraniol), 7) (*E*)-3,7-dimethyl-8-hydroxy-6-octen-1-al (8-hydroxycitronellal), 8) (*E*)-2,6-dimethyloct-2-ene-1,8-diol (8-hydroxycitronellol), 9) (4a*S*,7*S*,7a*R*)-nepetalactone and, 10) (1*R*,4*S*,4a*R*,7*S*,7a*R*)-dihydronepetalactol. Purities of all standards (except for iridodial) were ≥ 95%; synthetic and natural iridodial analyzed by GC existed with two later eluting 168 MW isomers (Fig. 3; compounds d and e), here accounting for 10.2% and 7.1%, respectively, of the 168 MW compounds.

[c] Abdominal cuticle (segments 3–8) for chemical analyses of *C. oculata* male-produced volatiles were prepared as described previously (Zhang et al., 2004). Compounds produced from fed precursors for which synthetic standards were available were verified by coinjections: 2c & 6a) nerol; 2d, 5b & 6b) geraniol; 4a & 5a) citronellol; 9a) (4a*S*,7*S*,7a*R*)-nepetalactone; 9b) (4a*S,7S,*7a*R*)-dihydronepetalactone and, 10a) (1*R*,2*S*,5*R*,8*R*)-iridodial. Other compounds were tentatively identified by near matches to mass spectra of compounds in the National Institute of Standards and Technology (NIST) mass spectral library: 1a) 3,7-dimethyl-6-octen-3-ol (1,2-dihydrolinalool); 1b) (*Z*)-3,7-dimethyl-2,6-octadien-1-ol; 1c) 2,6-dimethyl-7-octene-2,6-diol; 1d) (*E*)-2,6-dimethyl-2,7-octadiene-1,6-diol; 2a & 3a) 6-methyl-5-hepten-2-ol; 2b) 3,7-dimethyl-6-octen-1-ol.

Compound 7a and 8a yielded a less than a perfect match for 3,7-dimethyl-1,7-octanediol; based upon previously seen glandular reactions, this compound is likely 2,6-dimethyl-1,8-octanediol.