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 the first lacewing pheromone from field-collected males of the goldeneyed lacewing, *Chrysopa oculata* Say; (1*R*,2*S*,5*R*,8*R*)-iridodial is released from thousands of microscopic dermal glands on the abdominal sternum of males, along with comparable amounts of nonanal, nonanol and nonanoic acid. 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 baited traps. Despite their healthy appearance, normal fertility and usual amounts of C_9 compounds, laboratory-reared *C. oculata* males do not produce iridodial. However, we observed that goldeneyed lacewing males caught alive in iridodial-baited traps sometimes try to eat the lure, and in Asia *Chrysopa* spp. males reportedly eat the native plant, *Actinidia polygama* (Siebold & Zucc.) Maxim. (Actinidiaceae) to obtain the iridoid, neomatatabiol. These observations prompted us to investigate why laboratory-reared *Chrysopa* green lacewings do not produce iridodial. Lacewing adult males fed various monoterpenes reduced carbonyls to alcohols and saturated double bonds, but did not convert these compounds to iridodial. Males fed the bicyclic iridoid aphid pheromone component, (4aS,7S,7aR)-nepetalactone, converted ~75% to dihydronepetalactone, but did not produce iridodial; however, wild *C. oculata* males collected in May often contained traces of dihydronepetalactone. On the other hand, adult males fed the second common aphid pheromone component, (1R,4aS,7S,7aR)-nepetalactol converted this compound to iridodial. In California the peak late-season attraction of green lacewings to nepetalactol (the lactone is unattractive) occurs at least a month earlier than the peak in aphid oviparae (the pheromone producing morph of aphids), consistent with the hypothesis that *Chrysopa* males feed on oviparae to obtain nepetalactol as a precursor to iridodial. Adult males from laboratory-reared *C. oculata* larvae fed nepetalactol failed to produce iridodial, and wild *C. oculata* males collected early in the spring produce less iridodial than 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. Whether or not sequestration of iridodial precursors from oviparae and/or iridoid-containing plants is truly the explanation for lack of pheromone in laboratory-reared *Chrysopa* awaits further research .

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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 the first lacewing pheromone from field-collected males of the goldeneyed lacewing, *Chrysopa oculata* Say; (1*R*,2*S*,5*R*,8*R*)-iridodial is released from thousands of microscopic dermal glands on the abdominal sternum of males, along with comparable amounts of nonanal, nonanol and nonanoic acid. 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 baited traps. Despite their healthy appearance, normal fertility and usual amounts of C_9 compounds, laboratory-reared *C. oculata* males do not produce iridodial. However, we observed that goldeneyed lacewing males caught alive in iridodial-baited traps sometimes try to eat the lure, and in Asia *Chrysopa* spp. males reportedly eat the native plant, *Actinidia polygama* (Siebold & Zucc.) Maxim. (Actinidiaceae) to obtain the iridoid, neomatatabiol. These observations prompted us to investigate why laboratory-reared *Chrysopa* green lacewings do not produce iridodial. Lacewing adult males fed various monoterpenes reduced carbonyls to alcohols and saturated double bonds, but did not convert these compounds to iridodial. Males fed the bicyclic iridoid aphid pheromone component, (4aS,7S,7aR)-nepetalactone, converted ~75% to dihydronepetalactone, but did not produce iridodial; however, wild *C. oculata* males collected in May often contained traces of dihydronepetalactone. On the other hand, adult males fed the second common aphid pheromone component, (1R,4aS,7S,7aR)-nepetalactol, converted this compound to iridodial. In California the peak late-season attraction of green lacewings to nepetalactol (the lactone is unattractive) occurs at least a month earlier than the peak in aphid oviparae

(the pheromone producing morph of aphids), consistent with the hypothesis that *Chrysopa* males feed on oviparae to obtain nepetalactol as a precursor to iridodial. Adult males from laboratory-reared *C. oculata* larvae fed nepetalactol failed to produce iridodial, and wild *C. oculata* males collected early in the spring produce less iridodial than 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. Whether or not sequestration of iridodial precursors from oviparae and/or iridoid-containing plants is truly the explanation for lack of pheromone in laboratory-reared *Chrysopa* awaits further research.

INTRODUCTION

With ~ 6000 living species, Neuroptera is one of the smaller orders of insects (Winterton *et al.* 2010), but most larval neuropterans are predacious, often in agricultural systems, lending added importance to this group (Tauber *et al.* 2009). Of foremost agricultural importance are the green lacewings (Chrysopidae), particularly *Chrysoperla* and *Chrysopa* species, whose larvae are voracious predators of aphids and other soft-bodied insects and mites (McEwen *et al.* 2007). The meticulous illustrations of male-specific dermal glands in *Chrysopa* (Principi 1949) by the *grande dame* of neuropterists, Maria Matilde Principi (Pantaleoni 2015), inspired our identification of the first pheromone for green lacewings (Zhang *et al.* 2004).

Field-collected male goldeneyed lacewings, *Chrysopa oculata* Say, release

(1*R*,2*S*,5*R*,8*R*)-iridodial with comparable amounts of nonanal, nonanol and nonanoic acid (Zhang et al. 2004); iridodial-baited traps attracted *C. oculata* males into traps and females to the vicinity of baited traps (Chauhan et al. 2007). Subsequently, we found that the same iridodial stereoisomer similarly attracted adults of *C. nigricornis* Burmeister in the western U.S. (Zhang *et al.* 2006a), and *C. septempunctata* Wesmael in China (Zhang *et al.* 2006b). However, our efforts to pursue pheromone research of exotic chrysopids was thwarted by the discovery by one of us (JRA) that, despite their healthy appearance, normal fertility and usual amounts of C_9 compounds, laboratory-reared *C. oculata* males produced no iridodial (unpublished data). Furthermore, an observation by another of us (Q-HZ) that *C. nigricornis* males caught alive in traps baited with iridodial tried to eat the lure (unpublished observation), combined with previous reports of *Chrysopa septempunctata* eating the iridoid-containing plant known as silver leaf, *Actinidia polygama* (Siebold & Zucc.) Maxim (Actinidiaceae; native to Asia) (Hyeon *et al*. 1968) (Supplemental Figure 1, compounds **5** and prompted us to pursue the feeding studies reported herein in an effort to explain this phenomenon.

MATERIALS AND METHODS

Chemical standards

(*Z*,*E*)-nepetalactone [= (4a*S*,7*S*,7a*R*)-nepetalactone] was prepared from catnip oil, dihydronepetalactone was from hydrogenation of the lactone, (*Z*,*E*)-nepetalactol [= (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol] was from reduction of the lactone, and 1*R*,2*S*,5*R*,8*R*-iridodial was derived from the (*Z*,*E*)-nepetalactone as previously described (Chauhan et al., 200 The standard of 8-hydroxygeraniol was a gift from Dr. Wilhelm Boland

(Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Jena, Germany). Geranyl and farnesyl pyrophosphates were from Sigma-Aldrich (Saint Louis, MO) as were the following volatile standards (≥ 95%) geraniol, citronellol, citronellal, linalool, citral, 6-methyl-5-hepten-2-one, 8-hydroxycitronellol, and 8-hydroxycitronellal.

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 (BARC), 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) 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 with a loose-fitting sponge stopper (4 ml, 15 x 45 mm; Fisher Scientific, Pittsburgh, PA) 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 in 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 ºC, 72% relative humidity, and 16:8 h (L:D) photoperiod.

In addition to chemical feeding trials, some *C. oculata* males were reared as above with access to foliage of *Nepeta cataria* (Catnip) (Mountain Valley Seed Inc., Salt Lake City, UT; lot #G2217); some were antennectomized 1-5 days after emergence; and some larvae were reared as above, plus fed pea aphid clones. Lacewings are unusual among insects in that adults have chewing mouthparts whereas larvae have

139 piercing/sucking mouthparts (Tauber et al. 2009); therefore, some larvae were reared
140 with methylene blue dye in a preliminary experiment to verify that larvae ingested
141 materials from the honey water bottles, as did adults. Adult males from these treatments
142 were subsequently chemically sampled and analyzed as described below.
143

144 ## Scanning election microscopy

145 Live wild *C. oculata* males were anesthetized with CO_2, mounted on copper specimen
146 holders (16 × 29 × 1.5 mm thick) with cryoadhesive, and immersed in liquid N_2. The
147 frozen specimens were transferred to an Oxford CT1500 HF cryo-preparation system,
148 and examined using a low temperature scanning electron microscope (LTSEM; Hitachi
149 S-4100) operated at 2.0 kV (Erbe *et al*. 2003). Micrographs were recorded on Polaroid
150 Type 55 P/N film.
151

152 ## Chemical feeding, extraction of dermal glands, and chemical analysis

153 Each of the chemical standards listed above were individually fed to adult laboratory-
154 reared *C. oculata* males at 1 μg/μl in the 5% aqueous honey solution for ca. 4 days prior
155 to analysis. Abdominal cuticle extracts (segments 3–8) for chemical analyses of *C.*
156 *oculata* male-produced volatiles were prepared the same day as analysis as previously
157 described (Zhang et al. 2004). Wild males collected by sweep net, Beltsville MD, USA,
158 14 May – 1 June, 2009, were dissected in like manner the same day as collected.
159 Gas chromatography (GC) and coinjections were performed in splitless mode
160 using an HP 6890 GC equipped with a DB-5 column (0.25 μm film thickness, 30 m x
161 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 with an Electron impact ionization (EI) mass spectra were obtained at 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

In *C. oculata* adult males the dermal glands (Güsten 1996) are elliptical (~12 x 7.5 μm) with a central slit (Fig. 1), and occur on the 3rd–8th abdominal sternites (~800, 2100, 2500, 2500, 2300 and 1500, respectively); corresponding dermal glands are absent in females (Zhang et al. 2004). Similarly appearing male-specific dermal glands occur on both abdominal tergites and sternites in *C. septempunctata* (Principi 1949), whose males were abundantly captured in iridodial-baited traps in China (Fig. 2) (Zhang et al. 2006b).

Analyses of *C. oculata* revealed that nonanal and nonanol were abundant in extracts of the abdominal sternites of males regardless of whether they were collected in the wild or reared in the laboratory; however, iridodial was absent in extracts of laboratory-reared *C. oculata* males (Fig. 3A and B; Table 1). Conspecific females did not produce detectable amounts of the C_9 compounds or iridodial (data not shown). Access of *C. oculata* males to catnip foliage did not stimulate production of iridodial, nor did feeding geranyl or farnesyl pyrophosphates. Removing the antennae of *C. oculata*

185 males had no effect on production of iridodial, and rearing *C. oculata* males in isolation
186 from conspecific males did not result in production of iridodial (data not shown).
187 Providing pea aphid clones to larvae during rearing yielded at most only traces of
188 iridodial in the ensuing adult males, although the methylene blue uptake by larvae
189 verified uptake from the honey water solution. In wild males collected by sweep netting
190 foliage in early spring (*i.e.* not from iridodial-baited traps) the mean iridodial percentage
191 relative to the abundances of nonanal and nonanol was 14.30 % (±SEM = 3.72) (Table
192 1). Analysis of one male caught in one iridodial-baited trap (14 May 200[3], Beltsville,
193 MD) to which the captured males had access to the lure, showed that this male produced
194 more iridodial than the normal mean abundance for nonanol in wild-caught males (64.42
195 ± 4.73; Table 1).
196 Feeding naturally common monoterpene alcohols and aldehydes to *C. oculata*
197 males did not stimulate production of iridodial (Table 2, experiment numbers 1-8).
198 However, this series of feeding trials did reveal that males evidently possess reductase
199 and saturase enzymes capable reducing aldehydes to alcohols, and of saturating double
200 bonds in these molecules. These reactions appeared to be unidirectional; for example,
201 geranial was completely converted to geraniol (Table 2, experiment number [4]) whereas
202 geraniol was slightly isomerized to nerol but aldehydes were not produced (Table 2,
203 experiment number 6). Furthermore, the abundances of C_9 compounds were not affected;
204 nonanal, nonanol and nonanoic acid occurred in ratios within their ranges for wild-caught
205 males for all experiments shown in Table 2. Feeding 8-hydroxygeraniol did not stimulate
206 production of iridodial, nor did feeding geranyl or farnesyl pyrophosphates (data not
207 shown).

208 Feeding male goldeneyed lacewings the common aphid pheromone components,
209 (4a*S*,7*S*,7a*R*)-nepetalactone and (1R,4aS,7S,7aR)-nepetalactol, produced more positive
210 results. While feeding nepetalactone did not result in production of iridodial, about 75%
211 of this lactone was converted to the dihydronepetalactone (Table 2, experiment number
212 9). Interestingly, dihydronepetalactone was detected at low, but unequivocal levels in
213 some samples from wild *C. oculata* males (Supplemental Figures 2 and 3). *Chrysopa*
214 *oculata* males fed (1*R*,4a*S*,7*S*,7a*R*)-nepetalactone converted this compound to
215 (1*R*,2*S*,5*R*,8*R*)-iridodial (82.7%; Table 2, experiment number 10; Fig. 3C), with two later
216 eluting 168 MW compounds accounting for 17.3% of the other newly appearing
217 components, as well as *(Z)*-4-tridecene from the defensive prothoracic glands (Fig. 3C,
218 compound **c**) (Aldrich *et al*. 2009). Two additional feeding experiments were conducted
219 as for experiment 10 (Table 2) except the GC-MS analysis used a 30m HP-5 column; one
220 of these experiments (N = 4 males) resulted in 100% conversion to (1*R*,2*S*,5*R*,8*R*)-
221 iridodial, while the second (N = 9 males) showed 54.90% conversion to (1*R*,2*S*,5*R*,8*R*)-
222 iridodial with two later eluting 168 MW components (14.70% and 30.40%, respectively).
223 The mass spectra of the 168 MW compounds from experiment 10 (Table 2; Fig. 3C) did
224 not match the spectra of the later eluting 168 MW compounds seen in the latter
225 experiment using 9 males analyzed using the HP-5 column.
226
227

228 **DISCUSSION**

229 Coincidence of male-specific dermal glands with extraction of (1*R*,2*S*,5*R*,8*R*)-iridodial
230 from the 3rd–8th abdominal sternites strongly implicates these glands as the pheromone

231 source (Zhang et al. 2004). Surprisingly, only males are caught in traps baited with this
232 iridodial (Zhang et al. 2004; Zhang et al. 2006a; Zhang et al. 2006b); however, females
233 are drawn to the vicinity of, but seldom enter, iridodial-baited traps (Chauhan *et al*.
234 2007), presumably because the close-range substrate-borne vibrational signals to which
235 females are ultimately attracted are disrupted by trapping males (Henry 1982). The C_9
236 compounds are unattractive to *C. oculata*, quantitatively much less variable than
237 iridodial, and inhibitory to iridodial attraction, suggesting these compounds play a role
238 independent from that of iridodial (Zhang et al. 2004).
239 Previous laboratory rearing studies with *Chrysopa oculata* showed that males
240 produced fertile matings when fed only sugar and water, whereas females needed to feed
241 on pea aphid clones in order to mate and produce fertile eggs (Tauber and Tauber 1973).
242 Our results support these finding, but also make it clear that *C. oculata* males are unable
243 to make pheromone on this feeding regimen. Iridodial production in *C. oculata* males
244 was not stimulated by 1) antennectomy of sexually mature *C. oculata* males, which in
245 some group-reared insects stimulates pheromone production (e.g. Dickens *et al*. 2002); 2)
246 providing access to catnip plants, *Nepeta cataria*, containing the nepetalactone aphid
247 pheromone component (Pickett *et al*. 2013); or 3) rearing *C. oculata* males in isolation,
248 which in some insects is required for maximal pheromone production (Ho *et al*. 2005;
249 Khrimian *et al*. 2014).
250 Feeding monoterpene alcohols and aldehydes to *C. oculata* males did not
251 stimulate production of iridodial either, but this series of feeding trials revealed that
252 males are capable of reducing aldehydes to alcohols and of saturating double bonds.
253 Feeding 8-hydroxygeraniol, which is a precursor to biosynthesis of iridodials in some

254 insects (Hilgraf *et al*. 2012), did not stimulate production of iridodial, nor did feeding 8-
255 hydroxycitronellol. On the other hand, males fed the common aphid pheromone
256 component, (4a*S*,7*S*,7a*R*)-nepetalactone, converted ~75% to dihydronepetalactone, and
257 males fed the other common aphid pheromone component, (1R,4aS,7S,7aR)-nepetalact
258 converted this bicyclic iridoid to (1*R*,2*S*,5*R*,8*R*)-iridodial. Interestingly, analyses of wild
259 *C*. *oculata* males collected in May often revealed the presence of dihydronepetalactone.
260 One interpretation of these data is that *C*. *oculata* males must eat aphid oviparae
261 to obtain nepetalactol in order to make their pheromone. Indeed, in northern California
262 the peak late-season attraction of green lacewings to nepetalactol (nepetalactone is
263 unattractive) occurs at least a month earlier than the peak in aphid oviparae (Symmes
264 2012), consistent with the hypothesis that *Chrysopa* males feed on oviparae to obtain
265 nepetalactol as a precursor for iridodial. These dynamics indicate there is sufficient time
266 for *Chrysopa* males to feed on oviparae, produce iridodial, mate, and have conspecific
267 females' offspring reach the prepupal overwintering stage (Uddin *et al*. 2005). However,
268 adult males from laboratory-reared *C*. *oculata* larvae fed nepetalactol still failed to
269 produce wild-type levels of iridodial even though wild *C*. *oculata* males collected early in
270 the spring produce less iridodial than do males collected later in the season (Zhang et al.
271 2004). Although some aphids produce oviparae under stressed conditions in summer
272 (Hardie 1985), it seems unlikely that these oviparae are a reliable or abundant enough
273 source to sustain *Chrysopa* male pheromone production. Therefore, we further
274 hypothesize the *raison d'être* that Asian *Chrysopa* eat fruit and foliage of silver leaf (*A*.
275 *polygama*) is to obtain iridoid precursors necessary to make their pheromone; other
276 iridoid-producing plants (e.g. Hilgraf et al. 2012; Prota *et al*. 2014) elsewhere in the

277 world must be similarly usurped by male *Chrysopa* species to sequester iridoid
278 pheromone precursors.

279 Thus, *Chrysopa* spp. lacewings, whose adults are predacious distinguishing them
280 from closely aligned green lacewings in the genus *Chrysoperla* whose adults are not
281 predacious (Tauber et al. 2009), appear to exhibit pharmacophagy: that is, they "search
282 for certain secondary plant substances directly, take them up, and utilize them for specific
283 purpose other than primary metabolism" (Boppré 1984). A prime example of
284 pharmacophagy are male *Bactrocera* fruit flies (Tephritidae) that feed on plants to obtain
285 their pheromone precursor, methyl eugenol (Tan and Nishida 2012). Indeed, males of
286 certain lacewings [*i.e. Ankylopteryx exquisite* (Nakahara) (Pai *et al*. 2004), and *Mallada*
287 *basalis* (Walker) (Oswald 2015; Suda and Cunningham 1970)] are also powerfully
288 attracted to methyl eugenol for unknown reasons (Tan and Nishida 2012). In addition,
289 certain chrysomelid beetle larvae discharge iridoid allomones that may be synthesized *de*
290 *novo*, which is considered ancestral, or produced via the more evolutionarily advanced
291 mechanism, sequestration from plants (Kunert *et al*. 2008). Increasingly, pharmacophagy
292 is being recognized as a widespread phenomenon in insects, and Wyatt (2014) has
293 extended the concept of pharmacophagy to include molecules produced by bacteria that
294 are used as pheromones, such as locust phase-change pheromones produced by gut
295 bacteria. If male *Chrysopa* spp. lacewings actually do seek out aphid oviparae to obtain
296 nepetalactol as a precursor to iridodial, and in this regard it should be noted that only
297 *Chrysopa* males are attracted to nepetalactol (Koczor *et al*. 2015), then the concept of
298 pharmacophagy must be further extended to include this type of predator/prey interaction.
299 Whether or not sequestration of iridodial precursors from oviparae and/or iridoid-

containing plants is truly the explanation for lack of pheromone in laboratory-reared *Chrysopa* awaits further research.

CONCLUSIONS

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

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427

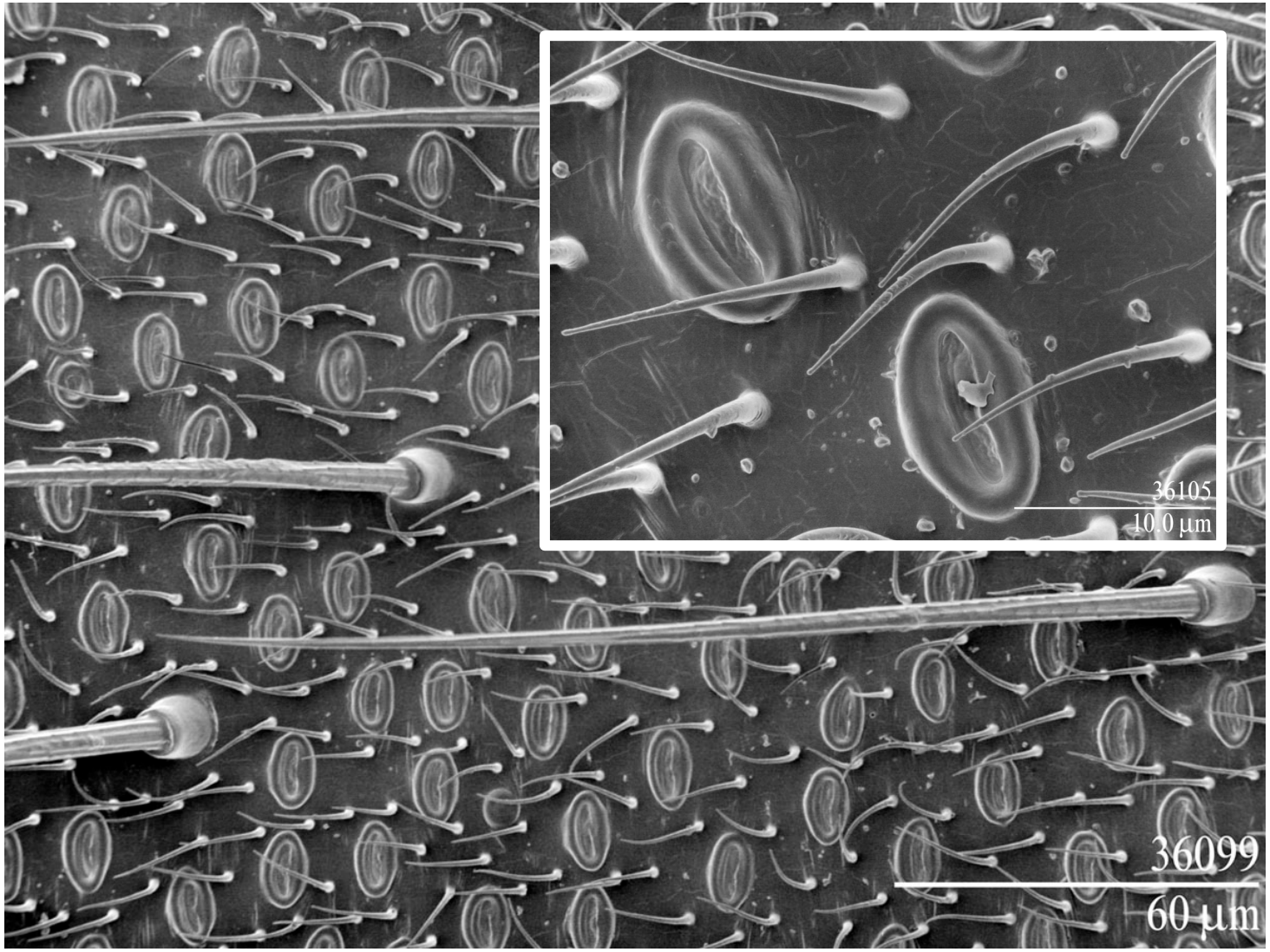


Figure 1 Scanning electron micrographs of the male-specific dermal glands of Chrysopa oculata. Low temperature scan (Erbe et al., 2003) with insert showing close-up of two dermal glands.

Figure 2 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).

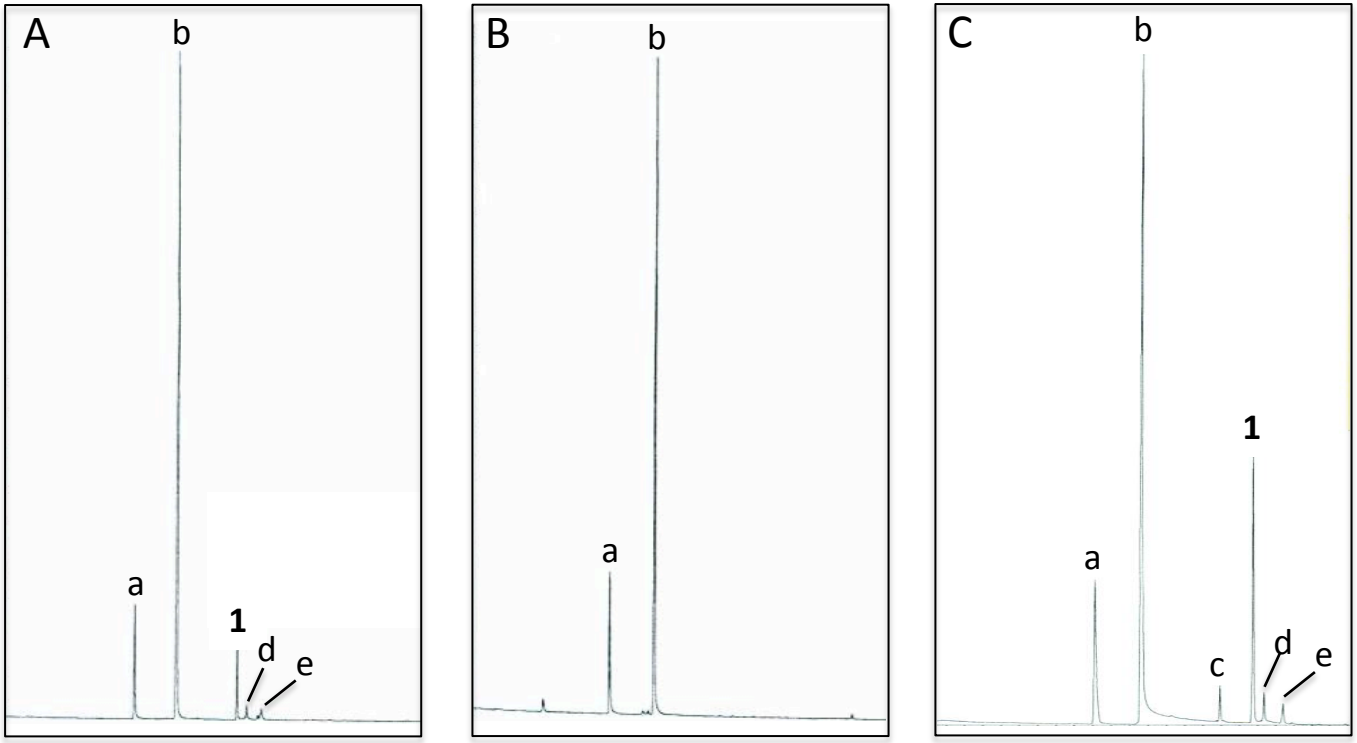


Figure 3 Total ion chromatograms of abdominal cuticular extracts of male *Chrysopa oculata*; A) field-collected, B) laboratory-reared and, C) laboratory-reared fed (1*R*,4*S*,4a*R*,7*S*,7a*R*)-dihydronepetalactol (see Table 2). (Column = 30m DB-WAXetr: a = nonanal ; b = nonanol ; c = *(Z)*-4-tridecene; 1 = (1*R*,2*S*,5*R*, 8*R*)-iridodial; d & e = 168 MW isomers.)

Table 1 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. Abdominal cuticle (segments 3–8) for chemical analyses were prepared as described previously (Zhang et al., 2004).

		Compound (%)			
Source / Date	**N**[a]	**Nonanal**	**Nonanol**	**Iridodial**[b]	**% ∑**[c]
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**	
Lab / 27 June 2008[d]	8	21.28	76.26	0	97.54
Lab / 13 Aug 2008[d]	5	21.37	69.34	0	90.71
Lab / 24 Nov 2008[d]	6	11.20	86.12	0	97.32
Lab / 24 Nov 2008[d]	7	18.60	75.74	0	94.34
Lab / 5 Jan 2009[e]	5	16.58	79.42	0	96.00
	Mean:	**17.81**	**77.38**	**0**	**95.18**
	±SEM:	**1.88**	**1.73**		

[a] In samples where N>1, multiple males were pooled and analyzed as a single sample by GC-MS on a 30 m DB-WaxETR column.

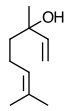
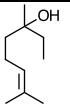
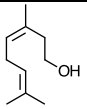
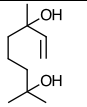
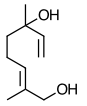
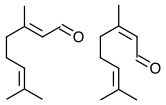
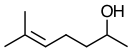
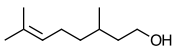
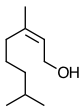
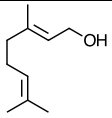
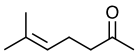
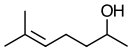
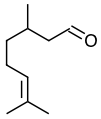
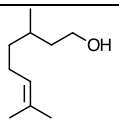
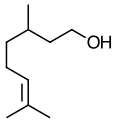
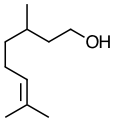
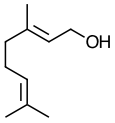
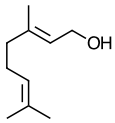
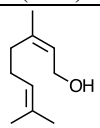
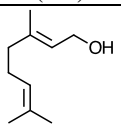
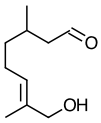
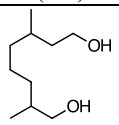
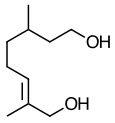
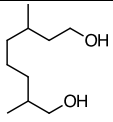
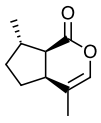
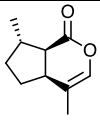
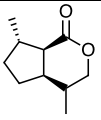
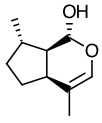
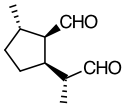
[b] (1*R*,2*S*,5*R*,8*R*)-Iridodial (Chauhan et al., 2004).

[e] Percentage of total volatiles; nonanoic acid (poorly resolved chromatographically) accounted for the majority of non-included volatiles.

[d] Reared singly as adults.

[e] Reared in a group as adults.

Table 2 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.

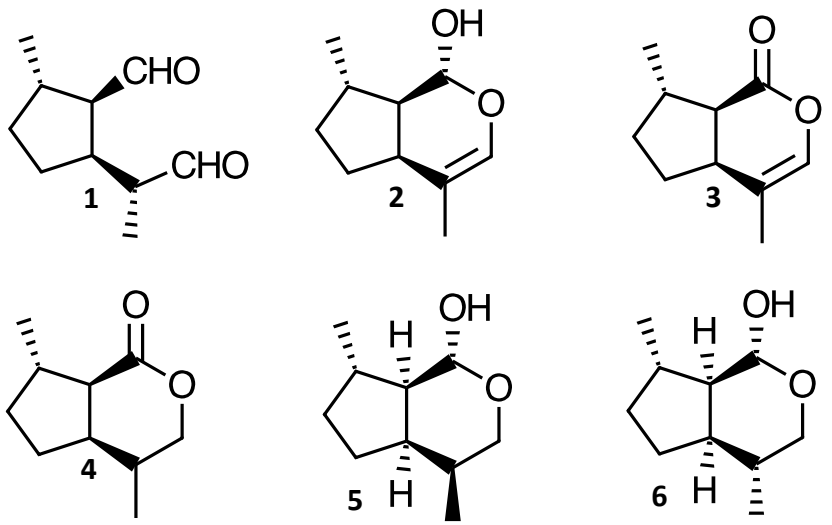
No.	N[a]	Compound fed[b]	Compound(s) produced from treatment (%)[c] a	b	c	d
1	8		 (16)	 (3.3)	 (9.7)	 (71)
2	12		 (9.9)	 (8.3)	 (42.3)	 (39.5)
3	9		 (100)			
4	10		 (100)			
5	7		 (95.3)	 (4.7)		
6	5		 (4.3)	 (95.7)		
7	15		 (100)			
8	15		 (100)			
9	12		 (23.3)	 (76.7)		
10	10		 (82.7)			

[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 $\geq$ 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*,7*S*,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.

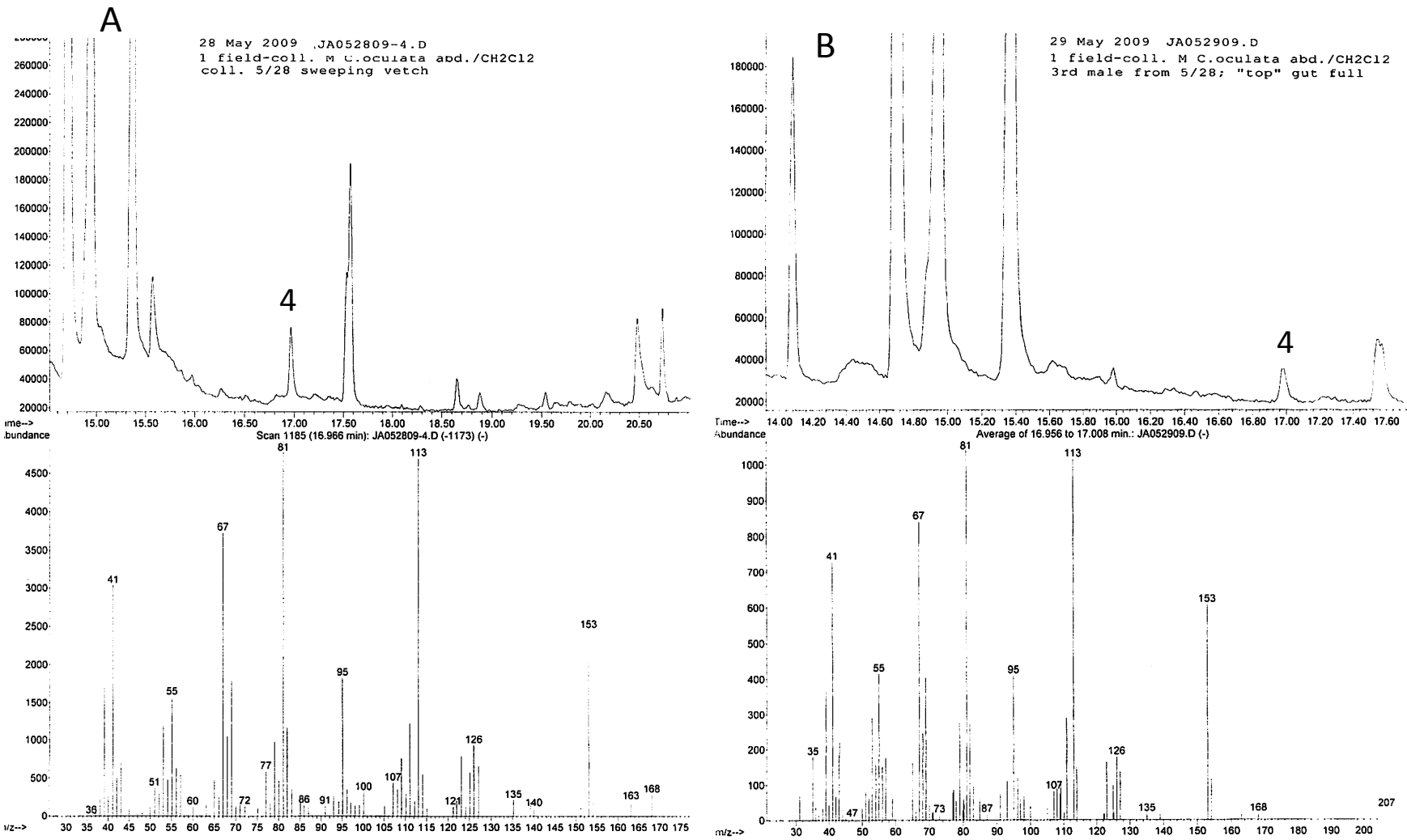
Compound 7a and 8a yielded a less than a perfect match for 3,7-dimethyl-1,7-octanediol; based upon previously seen glandular reactions, this compound is likely 2,6-dimethyl-1,8-octanediol.



Suppl. Figure 1 Structures of *Chrysopa* semiochemicals: **1:** (1*R*,2*S*,5*R*,8*R*)-iridodial, **2**:(1*R*,4*S*,4a*R*,7*S*,7a*R*)-dihydronepetalactol, **3**: (4a*S*,7*S*,7a*R*)-nepetalactone, **4**: dihydronepetalactone, **5**: (1*R*,4*S*,4a*R*,7*S*,7a*R*)-dihydronepetalactol , **6**: (1*R*,4*R*,4a*R*,7S,7a*R*)-dihydronepetalactol

Suppl. Figure 2 GC and MS data of abdominal cuticular extracts from *Chrysopa Oculata* males a) & b) collected 28 May, 2009, sweeping vetch, Beltsville, MD.

(4 = dihydronepetalactone (column = 30m HP-5; conditions described in text)



Suppl. Figure 3 GC-MS data for dihydronepetalactone (4), 2 July 2014.
Analyzed on an HP 6890N GC coupled in series with an HP 5973 mass selective detector using a 30m DB-5 capillary column (250 µm x 0.25 µm film Thickness; Agilent Technologies, Wilmington, DE, USA), 50 °C for 5 min, to 280 °C at 10 °C/min, hold 3 min.