

Detection of oviposition-detering larval tracks in *Chrysopa oculata* and *Chrysopa perla* (Neuroptera: Chrysopidae)

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Abstract. We investigated the ability of females of the aphidophagous chrysopids *Chrysopa oculata* and *Chrysopa perla* to distinguish clean substrates from substrates with tracks of chrysopid first instars after ablation of various sensory organs potentially involved in the detection of oviposition-detering semiochemicals (ODSCs). Also studied were effects of storage time on the degree of oviposition deterrence of substrates contaminated by larvae and by extracts of ODSC in intact females. *C. oculata* and *C. perla* laid significantly fewer eggs on substrates with conspecific larval tracks than on simultaneously provided clean substrates. Females of both chrysopids could perceive ODSCs solely through sense organs on the head. The oviposition of each species was significantly lower on contaminated than on clean substrates when any kind of sense organ on the head was completely removed, i.e. antennae, maxillary palpi, or labial palpi. *C. oculata* could still effectively differentiate substrates after ablation of both maxillary and labial palpi, indicating possible detection of volatiles via the antennae during flight. Only if all three pairs of sensory appendages were removed did females lay similar numbers of eggs on both substrates. In contrast, *C. perla* laid similar numbers of eggs on clean substrates and substrates with either conspecific or *C. oculata* larval tracks when maxillary and labial palpi were removed. Substrates with tracks of first instars of *C. perla* deterred *C. oculata* from oviposition after one year and conspecific females after 1.5 years from contamination. Both species laid significantly fewer eggs on substrates with tracks of *C. oculata* first instars than on clean substrates even after three years. Tracks of *C. oculata* third instars did not deter conspecific females more than tracks of first instars. ODSCs from tracks were easily extracted with water. Thus, precipitation is likely to reduce deterrent effects of contaminated plants. Chloroform extract from *C. oculata* first instars strongly deterred conspecific females from oviposition. Even after 725 days of storage, we found no statistically significant decline in the effect. The extract could be used to redirect egg laying from constructional parts of rearing cages to exchangeable oviposition substrates in mass rearing of chrysopids used for biological control. The hexane extract of third instars was inactive.

INTRODUCTION

Chrysopids are common predators of phytophagous mites and various insects (McEwen et al., 2001). Some species are highly polyphagous and considered effective biological control agents of lepidopteran pests in open fields (Ridgway & Jones, 1969). Many chrysopids specialise on aphids in the larval stage, or in both larval and adult stages. Eggs and larvae of several mass-reared species have been effectively deployed for aphid control in greenhouses (Benuzzi et al., 1991; Ravensberg, 1992). Although the use of these beneficial species for plant protection has resulted in massive reductions of pesticide use in some cases (Hokkanen, 1997), unexplained failures have also been frequent (Senior & McEwen, 2001). In addition to other factors, deterrent effects of chrysopid larvae on conspecific and heterospecific females and larvae have likely contributed to the undesirable dispersal of predators from release sites and declines in expected efficiency.

The first evidence of chemically mediated inhibition of oviposition emerged from experiments with the golden-eye lacewing, *Chrysopa oculata* Say (Růžička, 1994). When substrates with tracks of conspecific larvae were provided, females strongly avoided ovipositing on

them, preferring to lay eggs on substrates without tracks. Oviposition-detering semiochemicals (ODSCs) from larval tracks have since been shown to influence egg distribution in other chrysopid species (Růžička, 1996), in coccinellids (Růžička, 1997b), and in aphidophagous gall midges (Růžička & Havelka, 1998). In contrast to tracks of conspecific larvae, the presence of eggs did not deter chrysopid and coccinellid females from ovipositing (Růžička, 1994; Fréchette et al., 2006). Both chrysopid and coccinellid larvae use the tip of the abdomen as a highly effective pseudopod, especially while traversing the abaxial surfaces of leaves. ODSCs are present in the remains of adhesive secretions used to fix the abdominal discs of larvae to the plant surface (Růžička, 1994; Laubertie et al., 2006).

Insect females perceive ODSCs via olfactory or contact chemoreceptors located on various sense organs. The cherry fruit fly, *Rhagoletis cerasi* (L.) (Diptera: Tephritidae), detects the relatively stable oviposition-detering pheromone of conspecifics through tarsal contact receptors (Hurter et al., 1976; Städler et al., 1994). *Pieris brassicae* (L.) and *Pieris rapae* (L.) (Lepidoptera: Pieridae) use tarsal, antennal, and abdominal chemoreceptors to detect non-volatile and volatile components of conspecific ODSCs (Klijstra & Roesingh, 1986; Schoonhoven,

1990). In contrast, *Ceutorhynchus assimilis* (Paykull) (Coleoptera: Curculionidae) uses contact gustatory receptors on the antennae (Ferguson et al., 1999) and *Mono-chamus alternatus* Hope (Coleoptera: Cerambycidae) uses maxillary and labial palpi (Anbutsu & Togashi, 2000, 2001). Parasitoids frequently employ antennae but may also use chemoreceptors on the ovipositor to assess the presence of pheromone markers on the host (van Lenteren, 1972). Aphidophagous coccinellids appear to perceive conspecific and heterospecific larval tracks via maxillary palpi (Růžička, 2003).

Chrysopid females have various sensillae on different body parts, especially on appendages and the tip of the abdomen (Bitsch, 1984). Chemosensillae are particularly numerous on the antennae, and previous studies have concentrated on antennal perception of volatiles. Artificial diets and volatile synomone compounds of prey host plants have been shown to attract some species (Hagen & Tassan, 1966; van Emden & Hagen, 1976; Hagen, 1986). Electroantennogram (EAG) studies have demonstrated a significant response by *Chrysoperla carnea* (Stephens) to 2-phenylethanol emitted from the host plant of the prey, and traps baited with this compound attracted mostly females (Zhu et al., 2005). Other EAG studies with *Chrysopa septempunctata* (Wesmael), *Chrysopa sinica* (Tjeder), and *C. carnea* showed that adults could differentiate between volatiles from different prey host plants (Han & Chen, 2002a, b; Reddy, 2002). Wind tunnel experiments even revealed a preference for certain *Brassica* host plants by *C. carnea* females, but not males (Reddy et al., 2004) and responses to extracts of corn leaves also differed between males and females (Zhu et al., 1999).

Adult chrysopids have shown attraction to components of aphid sex pheromones in an antennogram study (Boo et al., 1998) and *C. carnea* responds strongly to (E)-beta-farnesene, the aphid alarm pheromone (Zhu et al., 1999). Whereas single olfactory sensillae of *C. carnea* react to both 2-phenylethanol and aphid sex pheromone components, those of *C. oculata* respond only to sex pheromone components (Zhu et al., 2005). Both sexes of *C. carnea* respond to a mixture of two major components of the pheromone of *Plutella xylostella* (L.) in an Y-tube olfactometer, but not to the individual compounds (Reddy et al., 2002). These studies indicate species-specific responses to prey-associated semiochemicals.

Chrysopids perceive conspecific semiochemicals also via the antennae. In gas chromatographic-electroantennographic detection (GC-EAD) experiments, males and females of *C. oculata* reacted to four compounds from the abdominal cuticle of males that are absent in females (Chauhan et al., 2004; Zhang et al., 2004). Both males and females were strongly attracted to iridodial, a male-specific compound of *C. oculata*, and aggregated near lures of this compound in nature, suggesting the possibility it could be used to increase oviposition in targeted fields (Chauhan et al., 2007). Iridodial also attracted males of another species, *Chrysopa septempunctata* (Zhang et al., 2006), and the pheromone retained activity

for more than two months. Several other substances also attracted males of different chrysopid species but not females (Hooper et al., 2002). The detection of defensive secretions of *C. carnea* was reported to occur via antennae. Field tests confirmed deterrent effects of the major compound, a tridecene. The avoidance behaviour of predatory ants, observed in tests with the synthetic tridecene, also suggested a defensive function of this substance (Zhu et al., 2000).

Although chrysopids use antennae for perception of some semiochemicals of conspecific origin, the location of sensillae that enable females to detect ODSCs has not been investigated to date. In a comparative study with four chrysopid species, the strongest response of females to tracks of conspecific first instars was in *C. oculata* and the lowest in *C. perla* (Růžička, 1998). Therefore, the present study was undertaken to identify organs for ODSC perception in *C. oculata* and *C. perla*. An associated objective was to compare the persistence of intra- and interspecific effects of ODSCs on females. Because chloroform extracts of larvae and larval tracks had similar oviposition-deterrent effects (Růžička, 1994), the persistence of the extract was studied for its possible use in mass rearing of chrysopids in biological control programs. If stable, the extract could be employed in rearing containers to divert egg laying from structural elements to exchangeable oviposition substrates. The solubility of ODSCs in water was investigated with respect to their persistence on plants.

MATERIAL AND METHODS

Insects

Experiments were made with two eurytopic chrysopids, the Nearctic *C. oculata* (origin: Nova Scotia, Canada, collected in 1989) and the Palearctic *C. perla* (origin: South Bohemia, Czech Republic, collected in 1992). Both species have been reared continuously in the laboratory since collection at 24 ± 2°C and 18L : 6D photo-phase. Larvae and adults of both species were fed with the pea aphid *A. pisum*. Adults were also supplied with tap water and a complementary diet containing yeast hydrolysate, sucrose, and water. Adult chrysopids were kept in nylon cages 40 × 40 × 40 cm, 300–400 insects per cage. Approximately 50 eggs were collected from each cage three times a week and placed on folded paper strips on the bottom of 0.5-L jars. The inner side of the walls of jars were coated with fluon (polytetrafluoroethylene dispersion in water, Sigma-Aldrich Chemie GmbH) applied to prevent the escape of eclosing larvae. Aphids were added prior to eclosion and on each subsequent day. At the second instar, 25 larvae of similar size were selected for rearing in each jar. Jars were then cleaned daily until pupation and adults were released into cages immediately after emergence.

Experimental design

The oviposition of *C. oculata* and *C. perla* was studied in choice tests in which clean and contaminated substrates were presented simultaneously. Experimental designs of tests were adopted from previous studies (Růžička, 1998; 2001b) and modified for individual experiments as needed. Cylindrical cages (19.5 cm diameter × 10 cm height) were made from firm netting with glass tops and bottoms. Ten chrysopid females, each 10–20 days old, were placed in each cage without males

TABLE 1. Summary of sense organs ablated in various oviposition deterrence experiments with two *Chrysopa* spp.

Ablated organ(s)	Abbreviation for test	Tests with <i>Chrysopa oculata</i>	Tests with <i>Chrysopa perla</i>
None*	BT	+	+
None	0	+	+
One antenna	1a	+	—
Antennae**	2ac	+	—
Antennae	2a	+	+
Labial palpi	2l	+	+
Maxillary palpi	2m	+	+
Antennae, maxillary palpi	2am	+	—
Labial palpi, maxillary palpi	2lm	+	+
Antennae, labial palpi	2al	+	—
Antennae, labial palpi, maxillary palpi	2alm	+	—
One leg of the second pair**	1Lc	+	+

*Clean substrates only; **organs were cut off with a pair of Wecker scissors. Organs were extirpated with a fine pair of forceps in other tests.

but with a surfeit of *A. pisum*. Tap water and the complementary diet were presented in two small plastic dishes, soaked in tampons. Tests were carried out at $24 \pm 2^\circ\text{C}$ and 18L : 6D photophase. Each test lasted 20 h and was replicated 10 times (in some cases, 5). The light source was white fluorescent tubes.

Assessment of sensory organs

The responses of intact females to substrates with tracks of larvae were compared to those of females deprived of different sense organs by surgical removal 2–3 h prior to testing.

Oviposition substrates consisted of four squares of dark blue paper 50×50 mm fixed to the underside of the cage lid with narrow strips of adhesive tape. Two substrates were clean and two had fresh tracks of larvae. Each square of contaminated substrate was exposed to 10 conspecific first instars on the bottom of a glass Petri dish (9 cm diameter) for 4 h prior to the test. The inner side of the wall of the dish was coated with fluon paint to retain the larvae. Substrates of the same kind were located in opposite corners of a hypothetical square, 110×110 mm. The distance between adjacent squares was 10 mm. Egg numbers on both kinds of substrates were compared after 20 h exposure to chrysopid females.

Intact females were used in the blank test (BT), with two pairs of clean squares, and in the control choice test (0), with two clean and two contaminated squares (Table 1). The BT was undertaken to test whether oviposition differed between locations. In other tests, either one or both organs of each type were amputated. In addition, pairs of different organs were simultaneously ablated. All segments of each organ were removed under a binocular microscope. The leg was cut away in the middle of the femur.

Because of differences in perception of ODSCs by *C. oculata* and *C. perla* (see Results), a complementary choice test was performed with *C. perla* females deprived of both kinds of palpi using substrates contaminated by *C. oculata* larvae. To verify the data of the original test with *C. oculata* females without both kinds of palpi and with substrates contaminated by conspecific larvae (test 2lm; see Table 1 for test designations), the same test was repeated.

Persistence of larval tracks

The persistence of intra- and interspecific oviposition-deterrence effects of tracks of *C. oculata* and *C. perla* larvae was studied in standard choice tests. Substrates were strips of printer paper 4×20 cm, folded every 1 cm to a total length of 13 cm. Contaminated substrates were exposed in pairs to either 40 *C. oculata* or 40 *C. perla* first instars in glass Petri dishes, 18.5 cm in diameter, coated with fluon paint on the inner side of the

wall. Ten clean and ten contaminated substrates were prepared simultaneously for each period of storage and then stored separately at room temperature. The substrates were used only once after either 1, 5, 10, 30, 180, 360, 540, 720, and 1100 days of storage. In each test, one clean and one contaminated substrate were fixed parallel to the cage lid, 25 mm apart. Two clean substrates were provided for oviposition in blank tests.

Effect of larval age and solubility of ODSCs

The oviposition-deterrence effects of tracks of first and third instar *C. oculata* were compared. In addition, the solubility and persistence of ODSCs in water, chloroform and hexane were studied in choice tests.

The oviposition substrates comprised two clean and two contaminated round filter papers (5.5 cm diameter). The arrangement of substrates on cage lids was the same as in experiments with squares of dark blue paper. Contaminated substrates were soaked with one of the extracts; clean substrates were treated with an equal volume of corresponding solvent. In tests with fresh chloroform extract of larvae, two strips of folded filter paper were provided for oviposition as in the experiments on the persistence of larval tracks.

To compare the deterrent effects of tracks of first and third instars of *C. oculata*, eight filter paper substrates were exposed either to 50 first instars or to 40 third instars on the bottom of a Petri dish, 18.5 cm in diameter.

To obtain a water extract of ODSCs from tracks, 100–150 first instars were placed in a 22-ml glass vial for 24 h, and larval tracks on the glass were then extracted with distilled water for 10 min. The procedure was repeated on three consecutive days using a new vial on each occasion. Fresh extract was used for contamination of substrates in five replicates. In another five replicates, substrates were soaked with an extract stored at -32°C for 30 days. A total volume of 0.5 ml of water extract was applied to each substrate. Tests were initiated several hours after contamination, once substrates had dried. The volume of water extract applied to each cm^2 of substrate corresponded to the tracks left by 0.7 larvae.

To obtain a chloroform extract, groups of 100–340 larvae were extracted for 2 min each. With the exception of the first experiment with fresh chloroform extract, the extract was stored first at -30°C and later at room temperature. The first test was performed with chloroform extract kept for 120 days at -30°C and then for 10 days at room temperature. The second test was performed with the extract kept for 120 days at -30°C and then for 630 days at room temperature. The volume of the extract applied to each cm^2 of substrate corresponded to 0.3 extracted larvae.

To obtain a hexane extract of larvae, 100 third instars were extracted in 3 ml of solvent for 2 min. The total volume of 2.5 ml of clear hexane extract was applied on 20 substrates. A small fraction of heavier, brownish fluid was not used. The volume of hexane extract applied to each cm² of substrate corresponded to 0.15 extracted larvae.

Data analysis

The differences between numbers of eggs laid on treated vs. untreated substrates within choice tests were evaluated using the non-parametric Wilcoxon signed paired-sample test. Differences between tests in the percentage of eggs laid per contaminated substrate were analysed by Student's t-test following arcsine transformation.

RESULTS

Sensory organs

Intact females of *C. oculata* and *C. perla* laid similar numbers of eggs on pairs of clean paper substrates in blank tests ($P = 0.557$ and $P = 0.652$). The total numbers of eggs laid by both species in blank tests were also similar ($P = 0.167$).

In choice tests with intact females (0), both species laid significantly lower numbers of eggs on substrates with fresh tracks of conspecific larvae than on clean substrates ($P = 0.002$). Although the total numbers of eggs laid by both species on paper substrates were similar ($P = 0.229$), *C. oculata* laid a lower proportion of eggs on contaminated substrates than did *C. perla* ($P < 0.0001$).

The oviposition of *C. oculata* females remained significantly lower on substrates with tracks than on clean paper when one antenna ($P = 0.002$) or both antennae were ablated (test 2a, $P = 0.002$; test 2ac, $P = 0.006$). Similarly, the oviposition on contaminated substrates remained lower after removal of one leg of the second pair ($P = 0.002$), labial palpi ($P = 0.002$), maxillary palpi ($P = 0.002$), antennae and labial palpi ($P = 0.004$), or antennae and maxillary palpi ($P = 0.002$). The oviposition of females without labial and maxillary palpi on substrates with conspecific tracks was lower in both consecutive tests ($P = 0.002$). Females laid 29.2% of eggs on contaminated substrates in the first test and 29.9% in the second test performed 54 months later. Oviposition on clean and contaminated substrates was similar only when antennae, labial palpi, and maxillary palpi were all ablated ($P = 0.846$; Fig. 1A).

If palpi of only one kind were removed, the proportion of eggs laid by *C. oculata* females on contaminated substrates was similar to that of intact females (maxillary palpi, $P = 0.499$; labial palpi, $P = 0.531$). Whereas ablation of one antenna did not affect the proportion of eggs laid on contaminated substrates ($P = 0.107$), ablation of both antennae significantly reduced it relative to intact females (test 2ac, $P = 0.009$; test 2a, $P = 0.0001$). Females without labial and maxillary palpi laid a proportion of eggs on contaminated substrates similar to that of females without antennae (test 2ac, $P = 0.756$; test 2a, $P = 0.202$).

C. perla laid lower numbers of eggs on substrates with fresh tracks of conspecific larvae than on clean substrates after the ablation of both antennae ($P = 0.002$), one leg of

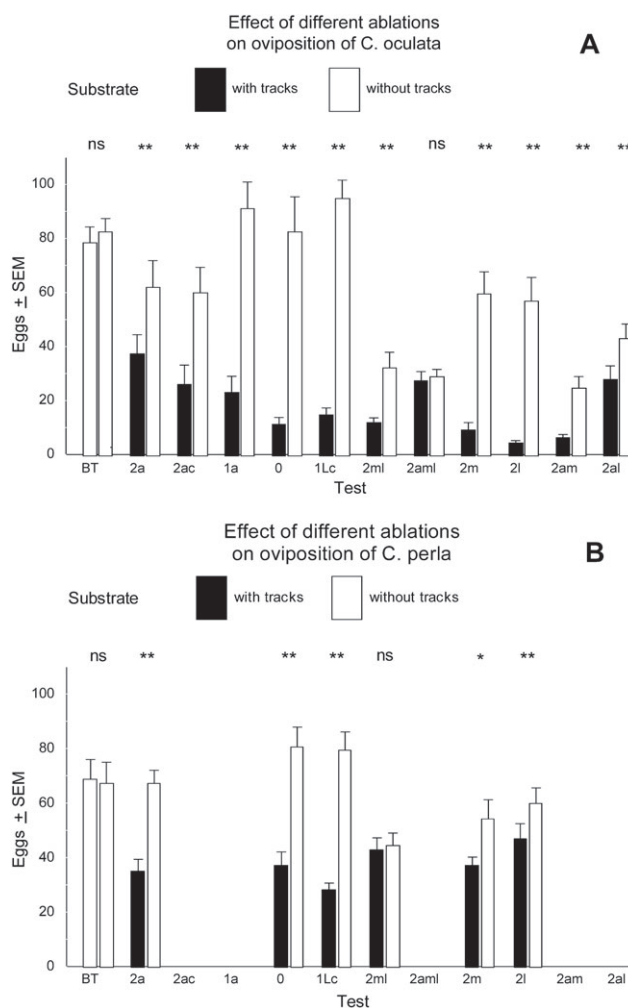


Fig. 1. Effects of different ablations on the choice of oviposition substrates by *Chrysopa oculata* (A) and *Chrysopa perla* (B). Ten females were tested in each of 10 replicates in each test. Tracks of first instars were used. Wilcoxon paired-sample test, ** $P < 0.01$; * $P < 0.05$; ns = not significantly different ($P > 0.05$). Details on ablations in Table 1.

the second pair ($P = 0.002$), both labial palpi ($P = 0.004$), or both maxillary palpi ($P = 0.0195$). In contrast to *C. oculata*, *C. perla* laid similar numbers of eggs on contaminated and clean substrates when both labial palpi and maxillary palpi were removed ($P = 0.557$; Fig. 1B). *C. perla* lacking both pairs of palpi also laid similar numbers of eggs on clean substrates and substrates with tracks of *C. oculata* first instars ($P = 0.695$).

Persistence of larval tracks

Females of *C. oculata* and *C. perla* laid similar numbers of eggs on each of two clean substrates in blank tests. The repellency of conspecific larval tracks persisted longer in *C. oculata* than in *C. perla* (Table 2). Although the proportion of eggs laid by *C. oculata* on contaminated substrates gradually increased over time, females laid a significantly higher proportion of eggs on clean substrates in all tests, even after 1100 days. In contrast, the preference of *C. perla* females for clean substrates over those

TABLE 2. Duration of oviposition-deterrent effects of larval tracks following various periods of storage. Tracks of first instars were used.

Oviposition	<i>Chrysopa oculata</i>						<i>Chrysopa perla</i>					
Larval tracks	<i>Chrysopa oculata</i>			<i>Chrysopa perla</i>			<i>Chrysopa perla</i>			<i>Chrysopa oculata</i>		
Substrate	clean	clean		clean	clean		clean	clean		clean	clean	
Blank test ^a	41 ± 7	45 ± 7	ns	41 ± 7	45 ± 7	ns	13 ± 1	17 ± 2	ns	13 ± 1	17 ± 2	ns
Substrate	with tracks	clean		with tracks	clean		with tracks	clean		with tracks	clean	
Storage (days)												
0	4 ± 1	61 ± 7	**	9 ± 3	45 ± 12	**	6 ± 1	26 ± 3	**	9 ± 2	21 ± 2	**
1	3 ± 1	61 ± 11	**	13 ± 3	40 ± 3	**	9 ± 3	35 ± 3	**	7 ± 1	29 ± 6	**
5	8 ± 2	58 ± 10	**	22 ± 7	51 ± 5	**	13 ± 1	31 ± 5	**	8 ± 2	28 ± 3	**
10	4 ± 1	54 ± 10	**	12 ± 2	49 ± 6	**	11 ± 2	32 ± 7	*	9 ± 2	29 ± 3	**
30	9 ± 3	63 ± 11	**	18 ± 3	38 ± 9	*	18 ± 5	34 ± 8	**	10 ± 4	28 ± 4	**
180	26 ± 6	90 ± 11	**	61 ± 9	101 ± 9	*	14 ± 2	38 ± 5	**	10 ± 1	26 ± 3	**
360	56 ± 8	116 ± 12	**	59 ± 17	84 ± 20	*	12 ± 2	36 ± 5	**	20 ± 4	36 ± 4	**
540	40 ± 6	89 ± 14	**	58 ± 5	93 ± 20	ns	21 ± 3	41 ± 7	**	27 ± 4	64 ± 5	**
720	63 ± 9	89 ± 6	*	64 ± 8	77 ± 13	ns	25 ± 5	30 ± 3	ns	40 ± 5	53 ± 8	ns
1100	54 ± 11	77 ± 11	*	60 ± 5	66 ± 6	ns	39 ± 7	58 ± 5	ns	36 ± 4	57 ± 6	**

Mean number (per replicate) ± SEM of eggs laid by chrysopid females in choice tests. Ten replicates by each test, 10 females in one replicate. Numbers of eggs compared with Wilcoxon paired-sample tests (two-sided *P* value): ***P* < 0.01; **P* < 0.05; ns = not significantly different (*P* > 0.05). ^a Two clean substrates, storage 0 days.

contaminated by conspecific first instars disappeared after 540 days of storage.

Substrates contaminated with tracks of *C. perla* lost their repellency to *C. oculata* females faster than did those of the reciprocal combination. While *C. oculata* laid fewer eggs on strips with tracks of *C. perla* larvae than on clean substrates after 360 days of storage, *C. perla* laid fewer eggs on strips with tracks of *C. oculata* larvae even after 1100 days (Table 2).

Effect of larval age and solubility of ODSCs

Females of *C. oculata* laid fewer eggs on substrates with tracks of first and third instar larvae (Mean ± SE = 4 ± 1.1 and 10.6 ± 1.5, respectively) than on simultaneously provided clean substrates (78.6 ± 8.1 and 58.7 ± 4.7, respectively), a significant result in both tests (*P* = 0.002). On average, only 5.0% of eggs were laid on substrates contaminated by first instars, and 15.4% on substrates contaminated by third instars.

The oviposition of *C. oculata* was lower on substrates soaked with water extracts of the conspecific tracks of first instars than on clean substrates. Females laid fewer eggs on substrates soaked with fresh and with 30-day-old extracts (0.8 ± 0.4 and 0.4 ± 0.2, respectively) than on simultaneously provided clean substrates (9.2 ± 2.6 and 29.4 ± 3.0, respectively), both significant results (*P* = 0.006). On average, females laid 9.0% of eggs on substrates contaminated with fresh water extract and 1.4% on substrates contaminated with extract stored for 30 days at -30°C.

C. oculata laid fewer eggs on substrates treated with fresh chloroform extract of first instars than on substrates treated with the solvent alone (0.4 ± 0.2 vs 28.7 ± 5.3, *P* = 0.002). Females laid an average of 1.0 ± 0.3 eggs on substrates soaked with extract stored for 120 days in the freezer and later held for 10 days at room temperature versus 30.4 ± 5.5 eggs on substrates treated with solvent

alone (*P* = 0.002). When the chloroform extract was held for 120 days in the freezer and then 630 days at room temperature, females laid 2.8 ± 0.8 eggs on contaminated substrates versus 73.3 ± 3.6 on solvent-treated substrates (*P* = 0.002). In these successive tests, *C. oculata* laid on average 2.8%, 3.3%, and 3.8% of total eggs on contaminated substrates. In contrast, the fresh hexane extract of third instars did not deter conspecific females from laying eggs (*P* = 0.1055).

DISCUSSION

Chrysopids are not considered to be strong fliers, but some species frequently cover long distances with the help of winds. Reproductively active females do not necessarily stay in one field even when plenty of food is available. The dispersal tendency of chrysopids has been described as a continuous "downwind nomadism" (Duelli, 1984). Whereas this process may be largely passive, olfactory orientation to volatiles emanating from prey or artificial attractants appears to play an important role in the selection of landing sites (Duelli, 1980).

An ability of chrysopid females to detect volatile ODSC components via their antennae could enable them to respond to the presence of chrysopid larvae during flight and thus affect their selection of landing sites. Substrates exposed for 4 h to a volatile ODSC from fresh tracks of *C. oculata* first instars deterred conspecific females from ovipositing, indicating females can respond to volatile ODSC components. While substrates contaminated with volatiles from tracks of *C. oculata* larvae deterred conspecific females for only a few days, those actually contacted by larvae retained their deterrent effects for weeks. Tracks of first instars deter oviposition even after exposure to 140°C for one hour (Růžicka, 1997a), indicating considerable thermal stability of the compound. After landing, females obviously respond to

more persistent semiochemical residues, probably through palpation like coccinellids (Růžicka, 2003).

Detection of ODSCs through individual pairs of sense organs was more complex in *C. oculata* than in *C. perla*. *C. oculata* females failed to differentiate clean from contaminated substrates solely when deprived of antennae and both kinds of palpi. The ablation of maxillary palpi had no significant effect on the preference for clean substrates over contaminated ones. Likewise, the ability of females deprived of labial palpi to prefer clean substrates was similar to that of intact females. Females retained the ability to detect conspecific ODSCs, apparently via the antennae, even after ablation of both pairs of palpi. The behaviour of females with only antennae did not differ from that of females deprived solely of antennae. These results demonstrate redundant perception of ODSC's via multiple sensory structures.

In contrast to *C. oculata*, *C. perla* females did not respond to ODSCs via antennae when both pairs of palpi were removed. After ablation of only one kind of palpi, females always laid significantly fewer eggs on contaminated substrates, indicating that palpi of each kind bear sensillae capable of detecting conspecific tracks. Tracks of first instar *C. oculata* deterred *C. perla* females from oviposition more than did tracks of conspecific first instars (Růžicka, 1998). One may speculate whether this was due to a quantitative effect (a higher amount of deterrent substance in tracks of *C. oculata* larvae) or a qualitative one (a different compound with stronger activity). *C. perla* lacking both kinds of palpi also laid similar numbers of eggs on substrates with tracks of *C. oculata* larvae as on clean substrates, indicating an inability to use the antennae to detect even the stronger ODSC of heterospecific larvae.

Experiments with females deprived of one leg of the second pair showed the excellent capability of *C. oculata* and *C. perla* to discriminate between clean and contaminated substrates after severe injury and by decreased mobility. Similarly, ablation of one leg of the second pair had no significant effect on the degree of preference for clean substrates by females of aphidophagous coccinellids *C. limbifer* and *C. undecimnotata* (Růžicka, 2003).

Although females have no receptive sensillae on their legs, tarsi apparently play a certain role in ODSC detection. Females likely contaminate tarsal segments with ODSCs and other semiochemicals during walking on the plant surface. Since both aphidophagous chrysopids and coccinellids frequently clean the tarsi of the first pair of legs with the mouthparts, this cleaning behaviour is likely to transfer semiochemicals from tarsi to gustatory sensory organs and thus enhance detection of conspecific and heterospecific tracks.

Although females of both *C. oculata* and *C. perla* responded to tracks of conspecific larvae, the response of the latter species was considerably lower, corresponding with previous findings (Růžicka, 1996, 1998). In laboratory rearing and experiments, *C. oculata* frequently lays eggs in rows closely spaced, whereas *C. perla* usually lays single eggs with greater distance between them. Pre-

sumably, neonate larvae of *C. oculata* would encounter higher densities of conspecifics than those of *C. perla*. Whether the intensity of response to larval tracks is related to a species-specific propensity for cannibalism remains to be tested. Substrates contaminated by *C. oculata* larvae remained deterrent to females of both species considerably longer than those contaminated by *C. perla* larvae, in accordance with stronger initial deterrence. These results suggest that ODSC may persist for considerable periods on plants in nature under favourable conditions. Oviposition in experiments with conspecific and heterospecific tracks generally increased with time of storage on contaminated substrates as well as on clean substrates, though less on the latter, indicating overall gradual decrease of deterrence of tracks on oviposition inside cages.

The deterrent effects of tracks of first and third instars were surprisingly similar, in accordance with preliminary results on ODSCs in tracks of chrysopid larvae (Růžicka, 1994). It remains unclear, however, why third instars cause less contamination of substrates with ODSCs than first instars. Numbers of eggs laid by the coccinellid *Adalia bipunctata* (L.) also did not differ among Petri dishes previously containing an excess of aphids and either first, second, third, or fourth conspecific instars (Doubila et al., 1998). However, although the fresh tracks of first and second instar *Coccinella septempunctata* had strong oviposition-deterrence effects, only tracks of the second instars deterred conspecific females from ovipositing after 24 h (Růžicka, 1997b). Similarly, *C. oculata* females responded more to substrates with tracks of older instars of the coccinellid *Coleomegilla maculata lengi* Timberlake than to those of younger ones (Chauhan & Weber, 2008).

The water extract of tracks of *C. oculata* first instars deterred conspecific females from oviposition. Fresh extract and extract stored for one month in a freezer deterred females similarly. The solubility of ODSCs in water and effects of weathering factors such as solar radiation could thus potentially diminish the persistence of larval tracks on plants in the field.

Substrates treated with chloroform extract of *C. oculata* larvae had an extremely strong deterrent effect on oviposition by conspecific females. This extract could potentially be exploited in the rearing of chrysopids, especially in their mass production for biological control programmes. If coated with chloroform extract, constructional parts of breeding cages would effectively repel females and promote egg laying on exchangeable substrates provided specifically for oviposition.

In coccinellids, fresh tracks of *C. limbifer* larvae exhibited strong oviposition-deterrence for conspecific females (Růžicka, 2001a, 2003) and also repelled conspecific larvae (Růžicka & Zemek, 2008), but simultaneously prolonged the residence time of conspecific females on sites with tracks (Růžicka & Zemek, 2003). In contrast, fresh tracks of larvae of the coccinellid *C. undecimnotata* had the opposite effect on the residence time of *C. limbifer* females, although they also deterred oviposition

(Růžicka, 2001a, 2003). Whether chrysopid larval tracks also deter conspecific larvae or influence searching behaviour of chrysopid females should be investigated.

In conclusion, females of aphidophagous chrysopids employ multiple sense organs for detection of ODSCs in tracks of conspecific larvae, and these organs are all located on the head. Interestingly, the role of antennae was species-specific. Whereas only palpi were involved in the detection of ODSCs by *C. perla*, *C. oculata* could detect them via the antennae. Choice tests indicate an absence of sensillae for ODSC detection on tarsi or other parts of the body in both species. Further research is needed to confirm the perception of volatile ODSC components by *C. oculata* via antennae during nomadic flight, but some volatility of ODSC components is evident. Chloroform extracts of ODSCs are easy to collect and retain persistence for long periods of storage, rendering them potentially useful for manipulation of oviposition in the mass-rearing of chrysopid species.

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