Chemical Communication in Horse-Chestnut Leafminer

Cameraria ohridella Deschka & Dimić

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Abstract

SVATOŠ A., KALINOVÁ B., HOSKOVEC M., KINDL J., HRDÝ I. (1999): **Chemical communication in horse-chestnut leafminer** *Cameraria ohridella* Deschka & Dimić. Pl. Protect. Sci., **35**: 10–13.

Females of the horse-chestnut leafminer *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae) produce a highly attractive sex pheromone in the early photophase. The pheromone, luring conspecific males into Delta traps baited with females, is produced in the female abdominal tip (ca 10–50 pg per calling female) and the active components can be extracted with hexane. Both dissected tips and their hexane extracts show high attractiveness for *C. ohridella* males in a wind tunnel behavioural bioassay. According to electrophysiologic recordings the male antennae are notably sensitive to both dissected female abdomens and extracts from them. The strong attractiveness of the pheromone may be useful in control of this pest.

Key words: *Cameraria ohridella*; Lepidoptera; Gracillariidae; chemical communication; EAG; wind tunnel; field trapping

The horse-chestnut leafminer *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae) is presently the most dangerous pest of horse-chestnut, *Aesculus hippocastanum* L., in Southern and Central Europe. This pest gradually radiated from the original place of occurrence in Macedonia (DESCHKA & DIMIĆ 1986) to Austria (PUCHBERGER 1990), Hungary (SZABOKY 1997), Germany (KRAUS 1996), Slovakia and the Czech Republic (SKUHRAVÝ 1998). *C. ohridella* can have up to four generations a year and the infested trees are usually completely defoliated at the end of the season. When trees are defo-liated for several consecutive years they can eventually die, which greatly affects the environment in urban areas.

Current literature reports on occurrence, morphology and possible control methods. So far, chemical communication in *C. ohridella* has not been studied.

MATERIALS AND METHODS

Insects

Insects were collected either as adults from naturally occurring populations in Prague (park Stromovka and Royal Garden of Prague Castle) in the morning hours from 22 July to 28 September, 1998 or infested chestnut leaflets were taken to the laboratory, immersed in water and kept in fine fabric-netted cages (0.4 × 0.4 × 0.4 m) at room temperature. Emerged adults were periodically removed to limit mating. Males and females were kept separately in glass containers with perforated polyethylene stoppers and at a 14:10 h (light/dark) cycle at 24 °C (onset of photophase was set to 09:00 h). Females were observed during photophase to determine their calling period. Males were stored at 5 °C until used in behavioural and electrophysiological (Electro-Antenno-Graphy, EAG) experiments.

Sample Preparation and Extraction

Calling virgin females (1 to 2 days old) were cooled to –20 °C for 5 min. The abdomen of a female was pressed with a pair of forceps under a binocular microscope and the extruded ovipositor was excised between the 7th and 8th abdominal segments, extracted with hexane (ca 10 µl per ovipositor) and the solution was stored at –20 °C.

EAG Recordings

A male to be used in EAG recordings, measured on the set-up described in HOSKOVEC *et al.* (1996), was placed in the tip of a disposable pipette (200 µl, Eppendorf) with a cut end. The exposed head and antennae were fixed in place by melted wax. Electrical activity from the fixed antenna was recorded by using glass Ag/AgCl microelectrodes filled with insect haemolymph saline. Dissected female glands or extracts were applied to a filter paper disc (10 mm) inserted into a Pasteur pipette. Stimuli onto the prepared antennae were delivered by air pulses blown through the pipette.

Wind tunnel bioassay

C. ohridella males (3–4 days old) were flown in a wind-tunnel (HOSKOVEC *et al.* 1996). Air velocity was maintained at 0.4 m/s. The experiments were performed from 2 to 3 h after the beginning of photophase. Males were released from the middle part of the tunnel into an odour plume which was created by pinning a filter paper disc (7 mm, Whatman No. 2) loaded with test stimulus onto a holder placed centrally near the upwind end.

Field tests

The field tests were performed in the upper part of the Royal Garden of Prague Castle from 22 July to 28 September, 1998. Delta traps (25 × 10 cm), baited either with three virgin females or with three males housed in small metallic cages (2 × 1.5 cm) and fitted with sticky inserts (Tanglefoot glue) were suspended in the lower part of the horse-chestnut canopy ca 2.5–3 m above ground level. The traps were checked daily and trapped males were identified and counted. Cages without lure were used as control.

RESULTS

Traps with virgin females caught a substantial number of conspecific males when compared to the control traps (Table 1). By contrast, traps baited with males were not attractive. The insects caught in control traps were either different microlepidoptera species or mixtures of *C. ohridella* males and females.

Table 1. Catches of *C. ohridella* males in Delta traps, Royal garden of the Prague castle (1998)

Lure:	3 Females	3 Males	Control
Date	Moths caught: *C. ohridella* males (other species)		
30. 7.[1]	113 (27)	nd	5 (1)
10. 9.–12. 9.[2]	56 (14)	1(0)	1 (0)
21. 9.–28. 9.[2]	18 (3)	0 (0)	0 (0)

nd – not determined; [1]third generation; [2]fourth generation

When virgin females were observed at the beginning of photophase, more then 80% protrude their ovipositor in the air and up to 50% showed a typical calling position as was described in MOZURAITIS *et al.* (1997). Females were calling from the first post-emergence day for ca 3 h, with a maximum frequency at 1.5 h (Fig. 1), but ceased calling after the 4th day of their life.

One dissected female abdomen, presumably containing the pheromone gland, was highly attractive for *C. ohridella* males in wind tunnel experiments (Fig. 2). Large numbers (90%) of tested males took off and flew in the odour plume, 20% reached the odour source and made copulatory attempts. When a similar preparation (from three females) was inserted into a glass Pasteur pipette and blown over male antennae, significantly higher EAG potentials (0.28 mV) were measured than for the control preparation (0.02 mV).

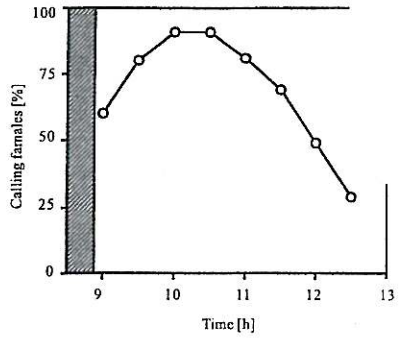

Fig. 1. Calling behaviour of one-day-old *C. ohridella* females (n = 10) emerged from infested leaflets. The onset of photophase was set to 9 am (stippled insert)

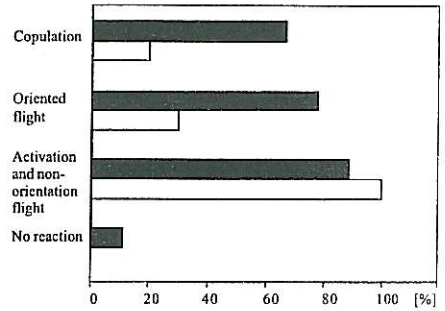

Fig. 2. Percentage of *C. ohridella* males responding to excised female abdomen (open bars, n = 10) and hexane extracts (3 female equiv., black bars, n = 18) in wind tunnel bioassay. No males react to control stimulation (hexane, n = 10)

Hexane extracts of three female abdominal tips loaded on a paper disc was very attractive for *C. ohridella* males in the wind tunnel bioassay, and the attractiveness was comparable to that elicited with the abdominal preparation (Fig. 2). The extract (from one female tip) elicited an EAG response comparable to excised female abdomens.

A preliminary examination of the hexane extract using gas chromatography coupled either to EAG or mass spectrometer showed that females produce minute amounts (estimated to be ca 10–50 pg per calling female) of pheromone. Their structure is being elucidated in our laboratory.

DISCUSSION

To our knowledge this is the first report on the existence of a sex pheromone in the genus *Cameraria*. There are several reports on sex attractants in Gracillariidae (ARN *et al.* 1998), and sex pheromones were identified for three *Phyllonorycter* species. Females of the tentiform leafminer, *P. mespilella* Hübner, produce E10-12:Ac accompanied by trace amounts of E4E10-12:Ac (GRIES *et al.* 1993). For the tentiform leafminer moth, *P. ulmifoliella* Hübner, Z10-14:Ac was identified as the sex pheromone (MOZURAITIS *et al.* 1997). Similarly for the apple leafminer, *P. ringoniella* Matsumura, where the pheromone is a mixture of E4Z10-14:Ac and Z10-14:Ac in 6/4 ratio (BOO & JUNG 1996). Structural similarities of sex pheromones in related species lead us to the assumption that *C. ohridella* can, in part, employ similar chemicals.

When a series of 12:Ac and 14:Ac monoenes and their mixtures were tested in field, none of those chemicals attracted *C. ohridella* males, nevertheless, several different related insect species were trapped (LIŠKA *et al.* – unpublished). Interestingly, some of the tested compounds showed notable EAG potentials (Z10-14:Ac >> E6-12:Ac).

From the presented data it is evident that *C. ohridella* females produce a highly attractive sex pheromone during 1–2 h after onset of photophase. The amounts of the active compound(s) are minute, ca 10–50 pg per calling female. We believe that the sex pheromone can be useful for monitoring and as a potential tool in Integrated Pest Management of *C. ohridella* on horse-chestnut.

Acknowledgements

We are highly indebted to Mrs. J. TITZENTALEROVÁ and to Mr. O. BLAŽEK for skilful technical assistance and to staff of the Prague Royal Garden for help in trapping tests. Financial support from our Institute is acknowledged with pleasure.

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Received for publication February 15, 1999
Accepted for publication March 1, 1999

Souhrn

SVATOŠ A., KALINOVÁ B., HOSKOVEC M., KINDL J., HRDÝ I. (1999): **Chemická komunikace klínénky jírovcové** *Cameraria ohridella* Deschka & Dimić. Pl. Protect. Sci., **35**: 10–13.

Samice klínénky jírovcové *Cameraria ohridella* Deschka & Dimić (Lepidoptera: Gracillariidae) vylučují v časných ranních hodinách velmi atraktivní sexuální feromon. Feromon lákající do delta lapáků, navnaděných samičkami, samce klínénky, je vytvářen na zadečku samice (ca 10–50 pg na volající samici) a aktivní složku z něj lze vyextrahovat hexanem. Jak vypreparované zadečky, tak i jejich hexanové extrakty vykazovaly vysokou atraktivnost pro samce v trubicovém olfaktometru (tzv. větrném tunelu). Samčí tykadlo je značně citlivé (podle elektroantenografického měření) jak k vypreparovaným zadečkům, tak i k odpovídajícím extraktům. Existence feromonu a jeho vysoká aktivita se zdá být příslibem pro případné využití v boji proti klínénce jírovcové.

Klíčová slova: *Cameraria ohridella*; Lepidoptera; Gracillariidae; chemická komunikace; EAG; větrný tunel; polní testy

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