# Influence of flower odour compounds on oviposition of the horse chestnut leaf miner *Cameraria ohridella* (Deschka & Dimic)

**A. Bettina Johne, Susanne Sprauer, Bernhard Weißbecker & Stefan Schütz**

**Institute of Forest Zoology and Forest Conservation, Georg-August-University Goettingen**

**Abstract:** Der Einfluss von Blütenduftstoffen auf die Oviposition der Rosskastanienminiermotte *Cameraria ohridella*.

Die Larven von *Cameraria ohridella* (Lepidoptera, Gracillariidae) entwickeln sich als Minierer in den Blättern der gemeinen Rosskastanie, *Aesculus hippocastanum*. In Mitteleuropa können drei Faltergenerationen ausgebildet werden, wobei die erste während der Blütezeit der Wirtspflanze *A. hippocastanum* fliegt. Der Schmetterlingsstrauch *Buddleja davidii* blüht von Sommer bis Herbst während der Flugzeit der zweiten und dritten Generation. Die großen Blütenstände locken zahlreiche Insekten an, die den Nektar als Nahrung nutzen. Mit Hilfe von spurenanalytischen und elektrophysiologischen Methoden (GC-MS, EAG) sowie Verhaltensversuchen wurde der Einfluss von Blütendüften auf die Oviposition der Rosskastanienminiermotte untersucht.
Der Blütenduft von *A. hippocastanum* und *B. davidii* unterschied sich in der Komposition emittierter Komponenten. *C. ohridella* war in der Lage, Blütendüfte zu detektieren. Die untersuchten Blütenduftstoffkomponenten beeinflussten die Oviposition der Miniermotten. Benzaldehyd, Linalool und (*E*)-β-Caryophyllen wurden von Rosskastanienblüten emittiert und steigerten die Oviposition auf den Blättern. Benzylalkohol wurde nicht von der Rosskastanie abgegeben und reduzierte im Gegensatz zu den anderen Duftstoffkomponenten die Eiablage. Der Blütenduft der Rosskastanie kann Faltern der ersten Generation bei der Wirtspflanzensuche helfen. In Zweifachwahltests mit getopften Rosskastaniensämlingen und blühendem Sommerflieder wurden die Blätter der Wirtspflanze bei der Eiablage bevorzugt. Aber auch auf den Blättern des Sommerflieders konnten abgelegte Eier gezählt werden. Bei alleinigem Angebot (Einfachwahltest) stieg die Anzahl der abgelegten Eier auf dem blühenden Sommerflieder. Falter der dritten Generation, die im Herbst auf eine starke Blattverbräunung der Wirtspflanze *A. hippocastanum* durch Minierfraß treffen und dort keine grünen Blattbereiche mehr finden, suchen auf anderen Wirtspflanzen einen geeigneten Eiablageplatz. Dabei können Blütenduftstoffkomponenten die Oviposition der Motten beeinflussen.

**Key words:** *Aesculus hippocastanum*, *Cameraria ohridella*, *Buddleja davidii*, GC-MS, EAG, volatiles, flower scent, bioassay, oviposition

A. B. Johne, B. Weissbecker, S. Schütz, Institut für Forstzoologie und Waldschutz, Georg-August-Universität Göttingen, Büsgenweg 3, D-37077 Göttingen, E-mail: bjohne@gwdg.de

The larval stages of *Cameraria ohridella* develop mining in leaves of the horse chestnut tree *Aesculus hippocastanum*. The insect establishes three generations in Central Europe. During the appearance of the first generation the horse chestnut trees bloom. Further on, parallel to the flying time of all generations other plant species bloom. The olfactory detection of flower odour compounds and the influence on oviposition of *C. ohridella* were examined using trace analytical and electrophysiological methods as well as bioassays.

## Methods and Materials

Odour samples were obtained using the CLSA-method (closed-loop-stripping-analysis) (BOLAND et al., 1984). Blossoms of *A. hippocastanum* were enveloped in a plastic bag directly on trees. Blossoms of *B. davidii* were put into 250 ml glass flasks. Within these enclosures the air circulated through a charcoal filter with a flow of 1 l/min for a sampling time of 4 hours (*A. hippocastanum*) or 45 min (*B. davidii*). The sampling method and time were fitted to each species. Volatiles were eluted with a mixture of 75 µl methylene chloride and methanol (2:1). Four odour samples per species were analysed with a gas

chromatograph (model 6890N, Agilent, Palo Alto, USA) coupled with a mass spectrometer (model 5973N, Agilent). The GC employed the following temperature program: start: 50°C, hold for 1.5 min, ramp 6°C/min to 200°C, hold for 5 min. It was equipped with a split/splitless-injector operated at 250°C in the pulsed-splitless-mode and a HP-5MS column (length 30m, ID 0.25 mm, film thickness 0.25 µm, Agilent). Helium was used as carrier gas at a constant flow of 1 ml/min. For peak identification the NIST mass spectral library (National Institute of Standards and Technology) was used. Subsequently, the spectra and retention time of compounds were compared with those of authentic standards.

For electrophysiological investigations insect antenna was fixed in an antenna holder (FÄRBERT et al. 1997). A minimum of three antennae originated from different moths were tested per compound and resulting signals were amplified by a factor of 100. Dose-response series of insect antennae were measured by manual puffing of air from glass syringes which contained pieces of filter paper drenched with $10^{-7}$ up to $10^{-2}$ standard dilutions of stimulus compounds in paraffin oil. The following substances were measured: 2-phenyl ethanol (Merck-Schuchardt), linalool (Acros), benzylalcohol (Sigma-Aldrich), 1.8-cineole (Merck-Schuchardt), benzaldehyde (Acros), (*Z*)- and (*E*)-linalooloxide (furanoid) (Sigma-Aldrich) and (*E*)-*β*-caryophyllene (Sigma-Aldrich).

For bioassays a lighted (1 klx) cage (180×75×75 cm) was used. In "dual choice tests" individual olfactory active compounds were tested regarding effect on oviposition. On leaflets of two green twigs of *A. hippocastanum* filter papers covering 30-50 % of the leaf surfaces were fixed with a needle. The filter papers of one twig were soaked with a paraffin oil solution of one olfactory active odorant compound ($1 \cdot 10^{-3}$) whereas the filter papers of the second twig were treated with pure paraffin oil in order to serve as a control. In this way, the volatile pattern of host tree leaves was overlaid with an additional flower odorant. In the beginning, 250 moths were released in the middle of the cage. Fresh odour solution was added every 6 hours (benzaldehyde) or 12 hours (control-bioassay, other compounds). Fresh eggs on leaves were counted after 24 hours (benzaldehyde) or 48 hours (other bioassays). In another "dual-choice-test", a potted seedling of *A. hippocastanum* with green leaves and a potted flowering *B. davidii* were offered to moths of *C. ohridella*. In an "no-choice-test" only a potted flowering *B. davidii* was offered. The adults in the last both choice tests were released in five groups of 100 animals, one group very second day. After ten days the fresh eggs on the leaves were counted and the leaf surface was measured. The statistical analysis of bioassays was carried out with Chi-square Test ($\alpha = 0.05$) using the Statistica 6.1. software. One oviposited egg was assessed as one decision.

**Results**

More than 20 odorant compounds could be identified in the flower scent of *A. hippocastanum* and *B. davidii*. Both species differed in the composition of released compounds. The present study focus on some selected compounds (Fig. 1). Adults of *C. ohridella* were able to detect flower compounds of both plants (Fig. 2). In dual-choice-tests olfactory detectable compounds of flower scent influenced the oviposition of the insect on *A. hippocastanum* (Fig. 3). In a further dual-choice-test *C. ohridella* reduced oviposition on leaves of blooming *B. davidii* compared to green leaves of *A. hippocastanum*. If the host plant *A. hippocastanum* is not available, oviposition rate on blooming *B. davidii* increased (no-choice-test, Fig. 4).

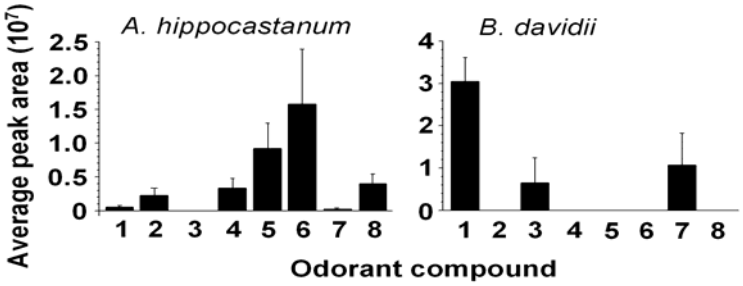


Fig. 1: Gas chromatographic quantification of *A. hippocastanum* and *B. davidii* flower compounds: **1**=benzaldehyde, **2**=1.8-cineole, **3**=benzyl alcohol, **4**=(*E*)-linalooloxide, **5**=(*Z*)-linalooloxide, **6**=linalool, **7**=2-phenyl ethanol, **8**=(*E*)-β-caryophyllene; for each bar n=4. Error bars indicate the standard error.

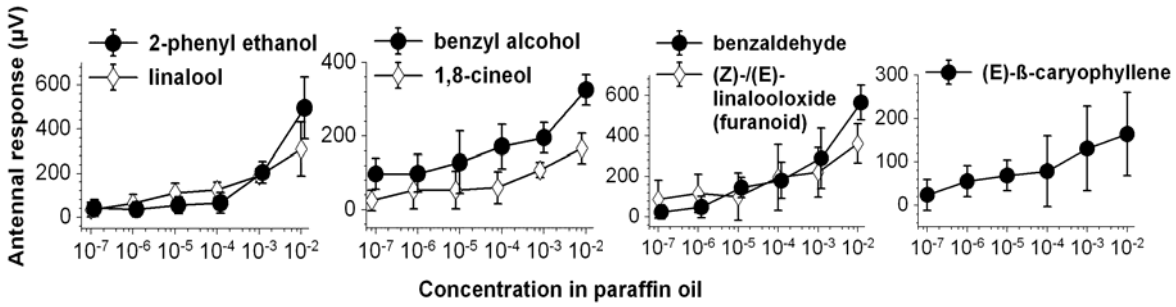


Fig. 2: Dose-response relation of compounds released by blossoms of *A. hippocastanum* and *B. davidii* stimulating three *C. ohridella* antennae originated from different moths. Puffs of 5 ml air loaded with reference standards (pulses 0.5s). Error bars indicate the standard deviation.

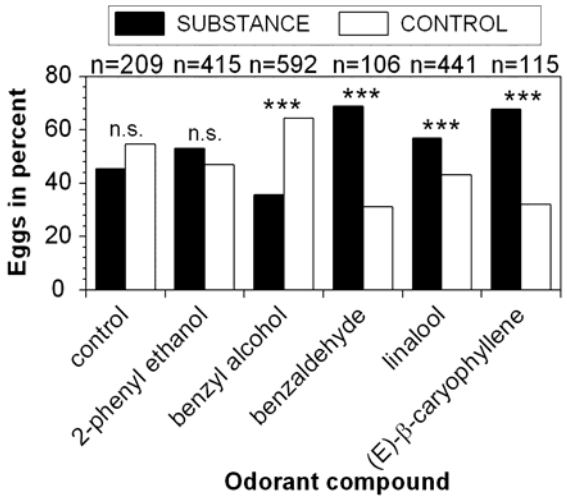


Fig. 3: Oviposition of *C. ohridella* on leaves of *A. hippocastanum* treated with floral volatiles solved in paraffin oil ($1 \cdot 10^{-3}$, SUBSTANCE) and pure paraffin oil (CONTROL) in dual-choice-tests;
chi²-test: n.s. not significant, *** $p \leq 0.001$.

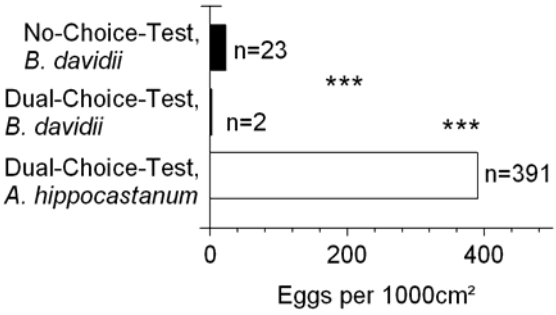


Fig. 4: Oviposition (eggs per 1000 cm²) of *C. ohridella* in no-choice and dual-choice-tests;
chi²-test: *** $p \leq 0.001$.

**Discussion**

*A. hippocastanum* and *B. davidii* released flower odour compounds typical for plants pollinated by insects. The composition of released flower compounds differed between the plant species (ANDERSON et al. 2002). *C. ohridella* was able to detect compounds of flower scent. Floral volatiles modified the oviposition of moths. Benzaldehyde, linalool and (*E*)-β-caryophyllene were released from blossoms of the host tree and increased oviposition. Linalool was one of the dominating compounds identified in the flower scent of *A. hippocastanum*. In contrast, benzyl alcohol which was not emitted from the flowers of horse chestnuts reduced oviposition rate. The flower scent may help the first generation in finding and selection of host tree.

*C. ohridella* has a proboscis with numerous sensillae which may have a tactile or gustatory function (ZUNKE 2003). So far, there is no evidence of nectar consumption by *C. ohridella*. The longevity of adults differs in each generation and amounts 4-5 days under laboratory conditions (BLAESER & SENGONCA 2004). This is a short reproduction phase. Lepidoptera species absorbing nectar have a life time of e.g. 13 days (*Pieris rapae*) or 21 days (*Gonepteryx rhamni*) (WIKLUND et al. 2001). However, the detection of flower scent might help moths in orientation towards food resources or can be seen as a rudimental ability in an evolutionary context.

Green leaves of *A. hippocastanum* were more attractive for oviposition than a blooming plant of a non host species. Green leaf volatiles and their typical composition in host plant odour are important for herbivore orientation behaviour (VISSER & AVÉ 1978). Old leaves of *A. hippocastanum* or leaves infested by the leaf miner emit additional volatiles that are typical for decaying processes. Heavy browned leaves are not attractive for moths (JOHNE 2003). In autumn for example, the total leaf surface of *A. hippocastanum* can be browned and leaves fall off the tree (TOMICZEK & KREHAN 1998). Consequently, moths of last generations can meet conditions in which the leaves of the host plant are not available for oviposition. The insect has to explore new resources. For several times it was observed that *C. ohridella* oviposited on non host species close to heavily browned trees of *A. hippocastanum* (SKUHRAVÝ 1999). If the host plant is not available, moths may be attracted by the green leaf odour of non host species. Further on, the scent of blossoms may affect the oviposition of moths. The present study showed that flower scent compounds can modify the oviposition rate of *C. ohridella* adults.

**Acknowledgements**

A. B. JOHNE is grateful for the financial support by the ev. Studienwerk "eV Villigst". Moreover, we want to thank the members of our institute for their aid. Thanks to STEFAN SCHWAB, Friedrich Alexander University Nürnberg/Erlangen-Nürnberg, Institute for Zoology, for exchange of literature. Finally, we are grateful VOLKER MENG (Forest botanical garden of Georg August University) and MARTIN LEVIN (Revierförsterei Hainberg) for appropriation of trees.

**References**

ANDERSSON, S., NILSSON, L.A., GROTH, I. & BERGSTRÖM, G. (2002): Floral scents in butterfly-pollinated plants: possible convergence in chemical composition. – Botanical Journal of the Linnean Society 140: 129-153.

BLAESER, P. & SENGONCA, C. (2004): Laboratory and semi-field studies on development, longevity and fecundity of the Horse-Chestnut Leafminer *Cameraria ohridella* DESCHKA & DIMIC (Lep., Gracillariidae) under climatic conditions of the city of Bonn. – Mitt. Dtsch. Ges. allg. angew. Ent. 14: 355-360.

BOLAND, W., NEY, P., JAENICKE, L. & GRASSMANN, G. (1984): A „Closed-Loop-Stripping" technique as a versatile tool for metabolic studies of volatiles. – In: SCHREIER, P.: Analysis of volatiles. Walter de Gruyter, Berlin.

FÄRBERT, P., KOCH, U.T., FÄRBERT, A., STATEN, R.T. & CARDÉ, R.T. (1997): Pheromone concentration measured with electroantennogram in cotton fields treated for mating disruption of *Pectinophora gossypiella* (Lep.: Gelechiidae). – Environ. Entomol. 26: 1105-1116.

JOHNE, A.B., FÜLDNER, K., WEIßBECKER, B. & SCHÜTZ, S. (2003): Kopplung der phänologischen Entwicklung der Rosskastanie *A. hippocstanum* mit Lebenszyklus und Verhalten der Kastanienminiermotte *C. ohridella*. – Nachrichtenbl. Deut. Pflanzenschutzd. 55: 213-220, 299.

SKUHRAVÝ, V. (1999): Zusammenfassende Betrachtung der Kenntnisse über die Rosskastanienminiermotte, *Cameraria ohridella* DESCHKA & DIMIC (Lep., Gracillariidae). – Anz. Schädlingskunde. 72: 95-99.

TOMICZEK, C. & KREHAN, H. (1998): The horsechestnut leafmining moth (*Cameraria ohridella*): a new pest in Central Europa. – Journal of Arboriculture 24: 144-148.

VISSER, J.H. & AVÉ, D.A. (1978): General green leaf volatiles in the olfactory orientation of the colorado beetle *Leptinotarsa decemlineata*. – Ent. exp. & appl. 24: 538-549.

WIKLUND, C., KARLSSON, B. & LEIMAR, O. (2001): Sexual conflict and cooperation in butterfly reproduction: a comparative study of polyandry and female fitness. – Proc. R. Soc. Lond. B. 268: 1661-1667.

ZUNKE, U. & DOOBE, G. (2003): Neue Erkenntnisse zur Rosskastanien-Miniermotte durch das Hamburger *Cameraria*-Projekt. – In: Jahrbuch der Baumpflege 2003 (7): 176-194.