Naturwissenschaften (2000) 87:216–219

SHORT COMMUNICATION

Caroline Müller · Monika Hilker

The effect of a green leaf volatile on host plant finding by larvae of a herbivorous insect

Received: 19 October 1999 / Accepted in revised form: 28 February 2000

Abstract The role of a general green leaf volatile (glv) in host finding by larvae of the oligophagous chrysomelid *Cassida denticollis* was investigated using a new bioassay which takes into account the need for neonate larvae of this species to climb fresh host plants from the ground. A "stem arena" was designed in which plant stems of the host, tansy (*Tanacetum vulgare*), and stem dummies (tooth picks), both wrapped in perforated filter paper, were offered to neonate larvae. The wrapping allowed olfactory responses to be tested by preventing access to contact stimuli of stems and dummies. Larvae significantly preferred to climb the wrapped tansy stems over dummies after a period of 15 min. The test glv, (*Z*)-3-hexen-1-ol, was not attractive when applied to dummies. However, when the glv was applied to the bottom of the arena, the ability of larvae to discriminate between host stems and untreated dummies was significantly enhanced. More larvae climbed wrapped host stems than dummies even within 5 min. While numerous other herbivorous insects are known to be directly attracted by glv, this study shows that a singly offered glv on its own is unattractive to an herbivore but enhances the herbivore's ability to differentiate between host and nonhost plants.

Introduction

The importance of so-called general green leaf volatiles (glv) has been demonstrated in several interactions between plants, herbivores, and antagonists of herbivorous insects. General green leaf volatiles are well-known to be released especially after leaf damage, subsequent hydrolysis of glycosides, and oxidative degradation of leaf lipids to six carbon aldehydes, alcohols, and esters of the latter (Karban and Myers 1989; Mattiacci et al. 1995; Merkx and Baerheim Svendsen 1990; Visser and Avé 1978). They have been shown to mediate information between and within trophic levels. For example, herbivorous insects may be attracted by glv of their host plants (Mitchell and McCashin 1944; Schütz et al. 1997; Visser and Avé 1978), and glv can modify responses of insects towards their aggregation or sex pheromones (Dickens et al. 1990, 1992, 1993; Landolt and Phillips 1997; Light et al. 1993; Zhang et al. 1999). They are also secreted by abdominal glands in some heteropteran species and can act as pheromones or defensive devices (Aldrich et al. 1984; Hamilton et al. 1985), and several parasitoid species of herbivorous insects use glv for host finding (Howse et al. 1998).

This study examined whether a glv may influence the host finding behavior of larvae of the leaf beetle species *Cassida denticollis* Suffr. (Coleoptera, Chrysomelidae). *C. denticollis* feeds on various Asteraceae such as *Tanacetum vulgare* L. (tansy), *Achillea millefolium* L., and *Artemisia campestris* L. Females lay their eggs in spring on dry plant material that remains from annual plants of the previous year. Therefore neonate larvae must find fresh host plants by climbing the stems of green plants to reach the leaves for feeding. In our study (*Z*)-3-hexen-1-ol was tested as a representative of the glv's known to be released by numerous plant species after leaf damage (Merkx and Baerheim Svendsen 1990; Visser et al. 1979). The tested host plant tansy also releases (*Z*)-3-hexen-1-ol (gas chromatography–mass spectroscopy analysis by Müller, unpublished data). The amounts of glv are known to vary between plant species (Schoonhoven et al. 1998). Even within a species, amounts of glv released vary between individual plants depending on age, temperature, and extent of damage. We studied the following questions: (a) Do larvae of *C. denticollis* recognize host (tansy) stems by their volatiles? (b) Does a glv [here: (*Z*)-3-hexen-1-ol] affect larval ability to discriminate between host and

C. Müller · M. Hilker (✉)
Institut für Biologie, Freie Universität Berlin,
Haderslebenerstrasse 9, 14163 Berlin, Germany
e-mail: hilker@zedat.fu-berlin.de
Fax: +49-30-83853897

nonhost stems? (c) Is (*Z*)-3-hexen-1-ol itself attractive to larvae of *C. denticollis*?

Materials and methods

Insects

In May 1997 adults of *C. denticollis* were collected at ruderal sites in Berlin, Germany, and placed in plastic containers (20×20×6 cm, Gerda, Schwelm, Germany) with a gauze lid (120 μm mesh). The bottom of each container was covered with filter paper and leaves of *T. vulgare* were offered as food plant. Egg batches (containing three to six eggs), which were laid on the filter paper, were transferred into glass vials (5×2.5 cm). Larvae were tested 5–7 h after hatching. Rearing conditions were 20 °C, 75% relative humidity and a cycle of L16:D8.

General bioassay design

To examine the role of (*Z*)-3-hexen-1-ol in the host finding behavior of *C. denticollis*, a "stem arena" was designed in which stems (2.5 cm) of the host plant tansy or wooden stem dummies (tooth picks) with various volatile treatments were offered to larvae in an open petri dish (diameter 5.5 cm; Fig. 1). The bottom of the petri dish was filled with soil covered by filter paper to fix the stems and dummies. To study responses of larvae to volatiles only, both plant stems and stem dummies were wrapped with perforated filter paper tubes (diameter 0.5 cm) to prevent direct contact. Prior to each bioassay all solvents used were allowed to evaporate for 2 min. Stems were cut from the middle part of about 30-cm-high, growing plants and bioassayed immediately after cutting. When starting the bioassay, 20 larvae were placed into the center of the stem arena. The numbers of larvae that had climbed the wrapped stems or stem dummies were recorded 5, 10, and 15 min after larvae had been placed into the arena. Larvae were not removed from a stem after climbing it. Instead, they were allowed to climb up and down more than one stem. However, they did not move to another stem more than once within a period of 5 min. Each bioassay was replicated 20 times and conducted at 20 °C.

Experiments

In the first experiment we examined whether neonate larvae are able to find their host plant stems by olfaction. Three tansy stems and three untreated wooden stem dummies were offered to the larvae in an alternating sequence. To keep conditions of this bioassay comparable to those used in the bioassay of the second experiment, 30 μl hexane were pipetted onto the center of the bottom of the stem arena (Fig. 1).

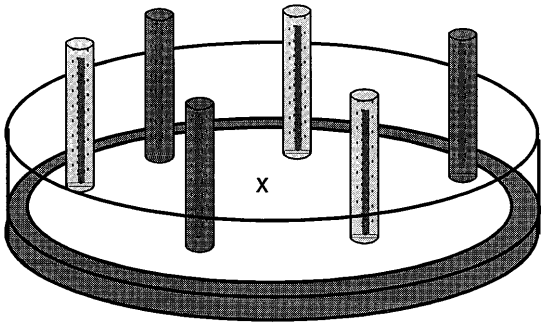

Fig. 1 Stem arena used for bioassays with larvae of *Cassida denticollis*. *Dark gray* test stems; *light gray* control stems (each 2.5 cm long); arena diameter 5.5 cm, 1 cm high. Test and control stems were placed 1.5 cm away from the center (×) of the dish and were wrapped with tubes (diameter 0.5 cm) of perforated filter paper to prevent larvae from making direct contact. The bottom of the arena was filled with soil and covered by a filter paper

In the second experiment we studied whether the presence of the glv (*Z*)-3-hexen-1-ol affects the ability of neonate larvae to find the host plant tansy. Larvae were offered the same arena with the same stems and dummies as in the bioassay of the first experiment, but 30 μl of a solution of (*Z*)-3-hexen-1-ol in hexane was applied onto the center of the arena. Two concentrations (0.3 and 1 μl/ml) were tested.

In the third experiment we examined whether (*Z*)-3-hexen-1-ol itself is attractive to the larvae. The responses of neonate (1-day) and 5-day-old larvae were tested by offering three test stem dummies treated with (*Z*)-3-hexen-1-ol and three control stem dummies treated with hexane only in an alternating sequence. From a solution of (*Z*)-3-hexen-1-ol in hexane 15 μl was pipetted over the whole length of each test stem dummy at the concentrations of 0.3 and 1 μl/ml.

Statistics

Data were evaluated statistically by the Wilcoxon signed-rank test for paired differences when comparing the numbers of larvae climbing test and control stems. The total numbers of larvae climbing stems in experiment 1 at 5, 10, and 15 min were compared to the total numbers of larvae climbing stems at the same time intervals in experiment 2 by the 2×2 table χ^2 test. The same test was used to compare the total number of (neonate) larvae climbing stems in experiment 1 at 15 min and the total number of neonates (1 day old) climbing in experiment 3 at both the low and high glv concentration. The threshold value for significance was $P=0.05$.

Results

When offered tansy stems and dummies, neonate larvae of *C. denticollis* significantly preferred to climb their host plant stems after 15 min (Fig. 2A). Within 5–10 min some larvae climbed up both plant stems and stem dummies, but no preference was detectable within these short periods.

The presence of the glv, (*Z*)-3-hexen-1-ol, in the arena enhanced the larvae's ability to differentiate between host plant stems and dummies. At either low concentration (Fig. 2B) or high concentration (Fig. 2C) larvae distinguished significantly between host stems and stem dummies even after 5 min. In comparison, no such differentiation between tansy stems and dummies was observed after 5 and 10 min in the absence of additional glv (Fig. 2A).

When offered a choice of test dummies treated with (*Z*)-3-hexen-1-ol and dummies with solvent alone, the glv itself was not attractive to the larvae. At both glv concentrations neither neonate larvae nor 5-day-old larvae preferred to climb the treated stem dummies (Fig. 3).

To provide information on the climbing activity of larvae, percentages of larvae climbing test and controls from all tested larvae are given in Figs. 2 and 3. From the 400 larvae tested in each experiment, the proportions ranged from 21.5% at the observation time of 5 min (Fig. 2A) to 40.5% at the observation time of

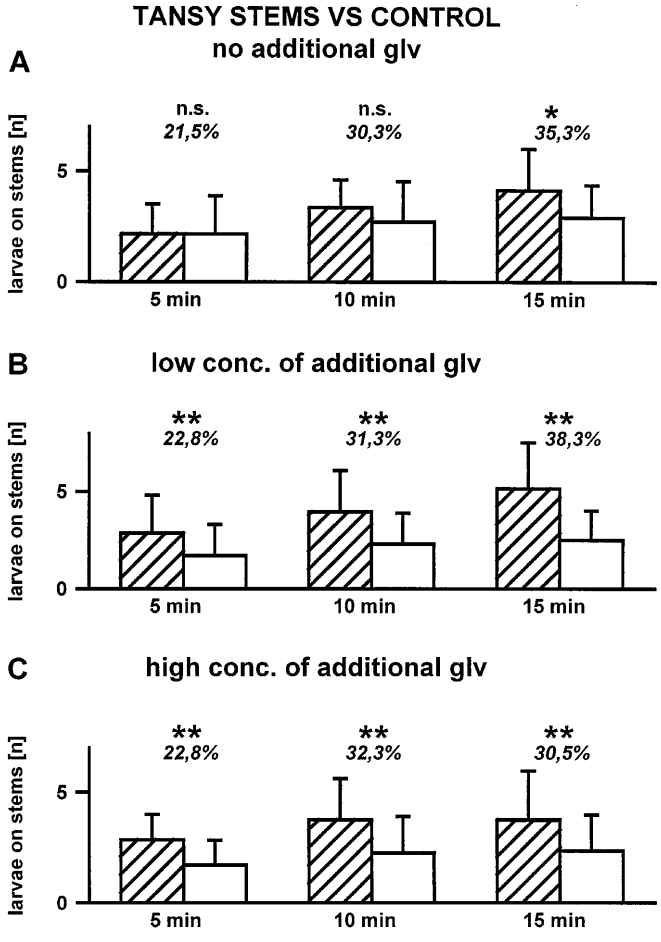

Fig. 2 Response of neonate larvae of *Cassida denticollis* to odor of host stems (*Tanacetum vulgare*, tansy) in the absence (A) and presence (B, C) of an additional green leaf volatile (*glv*). Filter paper at the bottom of the test arena was treated with 30 µl hexane (A, experiment 1), 30 µl of a solution of (*Z*)-3-hexen-1-ol in hexane at low concentration of 0.3 µl/ml (B, experiment 2), 30 µl of a solution of (*Z*)-3-hexen-1-ol in hexane at high concentration: 1 µl/ml (C, experiment 2). Mean number ± SD of neonate larvae on filter paper-wrapped tansy stems (*hatched bars*) and on wrapped stem dummies (*white bars*) at 5, 10, and 15 min. $n = 20$ replicates per bioassay. $^{**}P \leq 0.01$, $^{*}P \leq 0.05$; *n.s.* not significant; Wilcoxon signed-rank test for paired differences, two-tailed. Percentages represent the proportion of the number of larvae having climbed up a stem (both test and control stems). 100% = 400 larvae = sum of larvae tested in 20 replicates

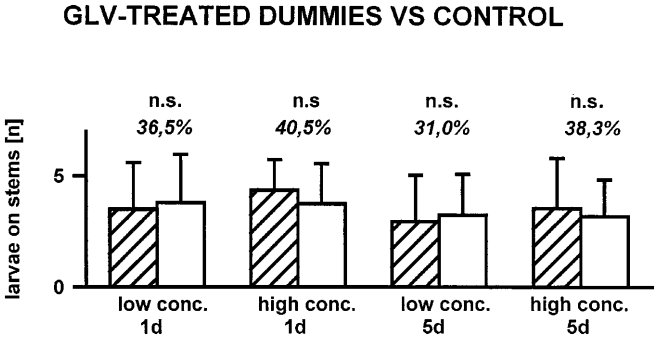

Fig. 3 Response of larvae of *Cassida denticollis* to a general green leaf volatile (*glv*; experiment 3). Mean number ± SD of neonate and 5-day-old larvae on filter paper-wrapped stem dummies treated with 15 µl of a solution of (*Z*)-3-hexen-1-ol at 0.3 µl/ml or at 1 µl/ml (*hatched bars*) and on wrapped stem dummies with 15 µl hexane (*white bars*), respectively, at 15 min. $n = 20$ replicates per bioassay. *n.s.* Not significant; Wilcoxon signed-rank test for paired differences, two-tailed. Percentages represent the proportion of the number of larvae having climbed up a stem (both test and control stems). 100% = 400 larvae = sum of larvae tested in 20 replicates

15 min (Fig. 3). The total numbers of climbing larvae in experiment 1 did not significantly differ from the numbers in experiment 2 with low or high concentration, at the comparable times. Also, no significant differences were detected between the total number of neonate (1-d-old) larvae climbing test and control stems in experiment 1 at the 15 min observation time (35.3%; Fig. 2A) and the number of 1-day-old larvae climbing stems in experiment 3 at the tested low glv concentration (36.5%, Fig. 3) and the high glv concentration (40.5%, Fig. 3), respectively. Thus, application of (*Z*)-3-hexen-1-ol did not enhance climbing activity of the larvae in any of the experiments.

Discussion

Our results show that larvae of *C. denticollis* are able to distinguish by olfaction between host stems and nonhosts within 15 min (Fig. 2A). Since plant stems were wrapped with filter paper, larvae could not have recognized their hosts by contact stimuli. Volatiles from tansy stems might have attracted larvae of *C. denticollis*, or larvae encountered tansy stems by chance and were stimulated to climb when they perceived volatiles from the tansy stem.

The glv (*Z*)-3-hexen-1-ol on its own was neither attractive to the larvae nor did it stimulate climbing (Fig. 3). However, larval ability to differentiate between host and nonhost stems was enhanced when this glv was present in addition to tansy stem volatiles. In the presence of (*Z*)-3-hexen-1-ol, the time needed for larvae to distinguish significantly between host stems and dummies was reduced from 15 min to 5 min (compare Fig. 2A to Fig. 2B, C). In the field, conspecifics of *C. denticollis* and other herbivores may cause the release of glv including (*Z*)-3-hexenol from tansy and other plants within the habitat. Thus the presence of glv might indicate that food resources are decreasing. A possible response is to accelerate location of the food plant in order to start feeding as soon as possible. Neonate larvae of *C. denticollis* need to find their host plants (various Asteraceae) among numerous other plants and dried plant material from the past year at ruderal sites, the typical habitats of this species. If glv were themselves attractive, neonate larvae would often climb "wrong" plants. Larvae of *C. denticollis* would therefore waste time and energy by climbing up nonhost structures. However, enhancement of discriminative ability in host location by exposure to a glv might

be advantageous, since larvae would spend less time and energy climbing "wrong" stems. As explained by Bernays (1996, 1999), herbivorous insects benefit from maximized speed and accuracy in host location.

All other studies on attraction of herbivorous insects to glv have depended on measuring direct anemotactic responses to the glv (Visser and Avé 1978) or by higher numbers trapped by baits after addition of glv (Dickens et al. 1993; Metcalf and Metcalf 1992). Studies of several insect species have revealed that glv and nonhost odors may mask the attractive odor of the host (Hartlieb and Anderson 1999; Thiery and Visser 1986). In our study, additional (*Z*)-3-hexen-1-ol obviously did not mask the host plant odor towards larvae of *C. denticollis*. Studies of the physiological mechanisms of olfactory perception of glv in herbivorous insects have shown that both highly specific and so-called generalist chemosensory cells respond to glv. Responses by various glv-sensitive cells may interact with one another and with other responses of chemosenory cells perceiving host-specific volatiles. These interactions may be either synergistic or suppressive (Hansson and Christensen 1999; Mitchell 1994). Most studies of perception of host plant odor and glv have been conducted with adult insects. Only a few investigations have considered orientation of herbivorous larvae in response to volatiles (Hartlieb and Anderson 1999). The physiological mechanisms of olfaction in *C. denticollis* have not yet been studied for either adults or larvae. Future studies are needed to understand how information from glv and specific host plant odor is physiologically integrated to result in observed responses.

Acknowledgements This study was financially supported by a grant from the Universities of Berlin. Critical comments on earlier drafts of this manuscript were kindly provided by Dr. J. Ruther and Dr. J. Steidle (Freie Universität Berlin), Dr. U. Schaffner (CABI Bioscience, Switzerland), and anonymous reviewers. Frank Müller (Freie Universität Berlin) helped to draw the figures.

References

Aldrich JR, Kochansky JP, Abrams CB (1984) Attractant for a beneficial insect and its parasitoid: pheromone of the predatory spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae). Environ Entomol 13:1031–1036

Bernays EA (1996) Selective attention and host-plant specialization. Entomol Exp Appl: 125–131

Bernays EA (1999) Plasticity and the problem of choice in food selection. Ann Entomol Soc Am 92:944–951

Dickens JC, Jang EB, Light DM, Alford AR (1990) Enhancement of insect pheromone responses by green leaf volatiles. Naturwissenschaften 77:29–31

Dickens JC, Billings RF, Payne TL (1992) Green leaf volatiles interrupt aggregation pheromone response in bark beetles infesting southern pines. Experientia 48:523–524

Dickens JC, Smith JW, Light DM (1993) Green leaf volatiles enhance sex attractant pheromone of the tobacco budworm, *Heliothis virescens* (Lep., Noctuidae). Chemoecology 4:175–177

Hamilton JGC, Gough AJE, Staddon BW, Games DE (1985) Multichemical defense of plant bug *Hotea gambiae* (Westwood) (Heteroptera: Scutelleridae), (*E*)-2-hexenol from abdominal gland in adults. J Chem Ecol 11:1399–1409

Hansson BS, Christensen TA (1999) Functional characteristics of the antennal lobe. In: Hansson BS (ed) Insect Olfaction. Springer, Berlin Heidelberg New York, pp 126–164

Hartlieb E, Anderson P (1999) Olfactory-released behaviours. In: Hansson BS (ed) Insect Olfaction. Springer, Berlin Heidelberg New York, pp 315–350

Howse P, Stevens I, Jones O (1998) Insect Pheromones and Their Use in Pest Management. Chapman Hall, London

Karban R, Myers JH (1989) Induced plant responses to herbivory. Annu Rev Ecol Syst 20:331–348

Landolt PJ, Phillips TW (1997) Host plant influences on sex pheromone behavior of phytophagous insects. Annu Rev Entomol 42:371–391

Light DM, Flath RA, Buttery RG, Zalom FG, Rice RE (1993) Host-plant green leaf volatiles synergize the synthetic sex pheromones of the corn earworm and codling moth (Lepidoptera). Chemoecology 4:145–152

Mattiacci L, Dicke M, Posthumus MA (1995) β-Glucosidase: an elicitor of herbivore induced plant odor that attracts host-searching parasitic wasps. Proc Natl Acad Sci USA 92:2036–2040

Merkx YM, Baerheim Svendsen A (1990) Glycosidically bound volatile aliphatic and aromatic alcohols: a common feature in the vegetable kingdom? J Ess Oil Res 2:207–208

Metcalf RL, Metcalf ER (1992) Plant kairomones in insect ecology and control. Chapman Hall, New York

Mitchell BK (1994) The chemosensory basis of host-plant recognition in Chrysomelidae. In: Jolivet P H, Cox ML, Petitpierre E (eds) Novel Aspects of the Biology of Chrysomelidae. Kluwer, Dordrecht, pp 141–152

Mitchell BK, McCashin BG (1994) Tasting green leaf volatiles by larvae and adults of Colorado potato beetle, *Leptinotarsa decemlineata*. J Chem Ecol 20:753–769

Schoonhoven LM, Jermy T, van Loon JJA (1998) Insect-Plant Biology. From Physiology to Evolution. Chapman Hall, London

Schütz S, Weissbecker B, Klein A, Hummel HE (1997) Host plant selection of the Colorado potato beetle as influenced by damage induced volatiles of the potato plant. Naturwissenschaften 84:212–217

Thiery D, Visser JH (1986) Masking of host plant odour in the olfactory orientation of the Colorado potato beetle. Entomol Exp Appl 41:165–172

Visser JH, Avé DA (1978) General green leaf volatiles in the olfactory orientation of the Colorado potato beetle, *Leptinotarsa decemlineata*. Entomol Exp Appl 24:538–549

Visser JH, Van Straten S, Maarse H (1979) Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the Colorado potato beetle. J Chem Ecol 5:13–25

Zhang QH, Schlyter F, Anderson P (1999) Green leaf volatiles interrupt pheromone response of spruce bark beetle, *Ips typographus*. J Chem Ecol 25:2847–2861