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DIRECT DEFENSE OR ECOLOGICAL COSTS: RESPONSES OF HERBIVOROUS BEETLES TO VOLATILES RELEASED BY WILD LIMA BEAN (*Phaseolus lunatus*)

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Abstract—In response to feeding damage, Lima bean releases herbivore-induced plant volatiles (HIPV), which are generally assumed to attract carnivorous arthropods as an indirect defense. While many studies have focused on such tritrophic interactions, few have investigated effects of HIPV on herbivores. I used natural herbivores of wild Lima bean and studied their responses to jasmonic acid-induced plants in an olfactometer and in feeding trials. Both *Cerotoma ruficornis* and *Gynandrobrotica guerreroensis* (Chrysomelidae) significantly preferred control plants to induced ones in the olfactometer, and they avoided feeding on induced plants. In contrast, Curculionidae significantly preferred HIPV of the induced plant to those of the control in one plant pair and did not choose in the case of a second pair. In feeding trials, no choice occurred in the first plant pair, while control leaves were preferred in the second. Release of HIPV deterred Chrysomelid herbivores and, thus, acted as a direct defense. This may be an important addition to indirect defensive effects. Whether or not HIPV released by induced plants attracted herbivorous Curculionidae, thus incurring ecological costs, varied among plants. Such differences could be related to various HIPV blends released by individual plants.

Key Words—Indirect defense, induced plant volatiles, plant-herbivore interactions, tritrophic interactions, Mexico.

INTRODUCTION

In response to feeding damage, many plants release herbivore-induced plant volatiles (HIPV), which generally are assumed to attract predators or parasitoids

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and, thus, have the potential to act as an indirect defense. However, HIPV are likely to affect the herbivores' behavior too. HIPV released by herbivore-infested plants may deter further herbivores and thereby act as a direct defense (Dicke and van Loon, 2000; De Moraes et al., 2001; Kessler and Baldwin, 2001), but HIPV may also attract herbivores and thereby incur ecological costs (Bolter et al., 1997; Dicke and van Loon, 2000; Kalberer et al., 2001; Horiuchi et al., 2003). Thus, the ecological role of HIPV may differ among plant and herbivore species.

In this study, herbivore behavior toward induced wild Lima bean (*Phaseolus lunatus* L., Fabaceae) plants was investigated in olfactometer and feeding experiments. Lima bean releases several HIPV, a response that is dependent on the octadecanoid pathway. A transient increase in endogenous jasmonic acid (JA) is involved in the synthesis of HIPV (Koch et al., 1999), and volatile blends similar to those of herbivore-damaged plants are released in response to exogenous JA application (Boland et al., 1995; Dicke et al., 1999). Most studies on effects of HIPV released by Lima bean have focused on responses of carnivorous arthropods. Here, I investigated responses of naturally occurring beetles to volatiles released by JA-treated plants in order to better understand how Lima bean interacts with its herbivores.

METHODS AND MATERIAL

Animals and Plant Material. Adult beetles were collected in the field *c.* 10 km w of Puerto Escondido (coastal area of Oaxaca, Mexico) in the first two weeks of December 2003. Beetles appearing on Lima bean were collected to represent natural ratios of sexes and ages. The beetles were kept in 250 ml plastic cups for 1–3 d with water only (water supplied on cotton, but no food) and then subjected to feeding trials or olfactometer experiments. The beetles used were *Cerotoma ruficornis* (Olivier) and *Gynandrobrotica guerreroensis* Jacoby (Chrysomelidae: Galerucinae: Luperini: Subtribe Diabroticina), and a species of Curculionidae. During November and December 2002 and 2003, these species were the most abundant herbivores on Lima bean at the study site. They were present all day long, exhibiting two peaks of feeding and moving activity in the first hours after dawn (8:00 AM–10:00 AM) and dusk (8:00 PM–11:00 PM), respectively (pers. obser.). Chrysomelids were determined by Astrid Eben (Instituto de Ecología, Veracruz, Mexico).

Wild Lima bean belonging to the Mesoamerican gene pool grows abundantly in the study area (Heil, 2004). Young seedlings were collected from the same site and grown in 250 ml plastic cups filled with natural soil for at least 3 wk prior to the experiment. At the time of the experiment, plants were 40–60 cm tall, had 10 to 15 leaves, and were easily identifiable based on the typical trifoliate and extrafloral nectaries-bearing leaves (Heil, 2004). Plants were sorted by pairs according to shoot length, leaf number, and average leaf size; then one plant per pair was selected randomly and induced between 08:00 and 09:00 AM, i.e., sprayed with 5 ml of a 1 mmol aqueous solution of JA (leaves were sprayed once until completely soaked). Control plants received equal amounts of water. Plants were allowed to dry, and then were put into PET bags (‘Bratenschlauch’, Toppits, Minden, Germany, a PET foil that does not emit detectable amounts of volatiles even after exposure to temperatures of up to 150°C), and placed so that they received a natural photoperiod without being exposed to direct sunlight.

Olfactometer Experiments. Tests on the beetles’ behavior toward HIPV were conducted in a Y-olfactometer. Inflowing air was cleaned by charcoal filters (1.5 mg of charcoal, CLSA-Filters, Le Ruissaeu de Montbrun, France) and then passed plants placed in PET foil bags for 12 hr prior to the experiment. Air was kept flowing (ca 3 l min^{-1}) by means of a ventilator at the end of the olfactometer. All experiments were conducted in the dark between 06:00 PM and 11:00 PM at a temperature of 28–30°C (air humidity >90%). Directional movement of the beetles was achieved by one lamp (40 W) placed 50 cm in front of the olfactometer. Each beetle was tested individually, and only beetles entering one of the arms within 5 min were counted as having made a choice. The arms of the olfactometer were exchanged after every fifth beetle. Each experiment consisted of one choice situation (control vs. induced plant, [C:I]) and three controls (empty arm vs. empty arm [0:0], empty arm vs. control plant [0:C], and control plant vs. control plant [C:C]). The experimental setup was conducted twice on two consecutive days, each with different plant pairs and different sets of beetles for the Chrysomelids, and with the same two plants pairs yet different sets of beetles for the Curculionids.

Feeding Experiments. Single beetles were placed in 250 ml plastic cups, each containing one leaflet of an induced plant and one of a control plant. Only lateral leaflets were used for this experiment, and only beetles that fed within 24 hr were evaluated. Leaflets were scanned to calculate missing leaf area using standard image-processing software, and the two leaflets exposed to the same beetle were used as a pair for data evaluation.

Gas Chromatography-Mass Spectrometry. Treated and control plants were generally tested for successful induction by using a portable GC (ZNose® Model 4100 Vapor Analysis Systems, Newbury Park, CA 91320). However, Curculionid beetles responded differently to odors of two induced plants, and volatiles released by these plants were collected after the olfactometer experiments over the next 24 hr on charcoal filters in a closed-flow stripping, resolved in dichloromethane to which *n*-bromodecane (200 ng μl^{-1}) had been added as internal standard (IS), and then subjected to GC-Trace-MS (2000 series, Thermo Quest, program Xcalibur 1.2, Finnigan Corp.; see Koch et al., 1999 for details and Hopke et al., 1994 for original identification of compounds).

RESULTS AND DISCUSSION

Whereas only traces of *cis*-ß-ocimene, C_{11} homoterpene, and $C_{10}H_{14}$ (compounds 1, 3, and 5 in Figure 1) and MeSA were released by control plants, JA treatment of wild Lima bean significantly induced release of several HIPV (data not shown, but see Figure 2 in Heil, 2004 for typical chromatograms of induced and control plants). Both Chrysomelids significantly preferred HIPV of controls to those of induced plants ($P < 0.01$ for both species on two days each, Figure 1). The beetles never showed a significant choice when offered two empty arms or two arms containing control plants, and they significantly preferred control plants vs. an empty arm (Figure 1). Therefore, these beetles' behavior represents an ecologically relevant avoidance of HIPV released by induced plants, at least when non-induced plants are also available. In the feeding experiments, these species preferred control leaflets ($P < 0.05$ in two independent feeding experiments conducted for each species, see Table 1).

Curculionid beetles significantly preferred the induced plant of one pair and did not choose in the case of the second pair (Figure 1). This pattern was repeated using the same plants yet different beetles one day later. HIPV released by these induced plants differed strongly from each other (Figure 1): Plant 1 was slightly induced and released only few HIPV at amounts lower than the IS. Plant 2, which was strongly induced, released *trans*-ß-ocimene, *cis*-jasmone, ß-caryophyllene, and TMTT at rates as high as or higher than the IS, and several other HIPV at lower rates (Figure 1). HIPV released in response to intense weevil feeding are usually dominated by trans-ß-ocimene, *cis*-jasmone, and TMTT (unpublished data obtained with ZNose), and all these compounds were released in high amounts by plant 2. In feeding experiments (same plant individuals), the Curculionids preferred the control plant to the induced one of pair 2 (heavily induced plant; $P = 0.057$) but showed no detectable preference in the case of pair 1 (slightly

TABLE 1. RESULTS OF FOOD CHOICE TESTS ON JA-INDUCED AND CONTROL LEAFLETS OF LIMA BEAN

	Cerotoma		*Gynandrobrotica*		Curculionidae	
	C:I	**P**	**C:I**	**P**	**C:I**	**P**
Trial 1	7:0	0.008	7:0	0.008	9:7	n.s.[1]
Trial 2	6:1	0.014	12:2	0.011	12:6	0.057[2]

C:I = number of control leaflets damaged: number of induced leaflets damaged, P = result of Wilcoxon test for paired samples on leaf area removed. Different trials were conducted with different plant pairs on different days.
[1]Plant pair 1, [2]Plant pair 2 (see Figure 1).

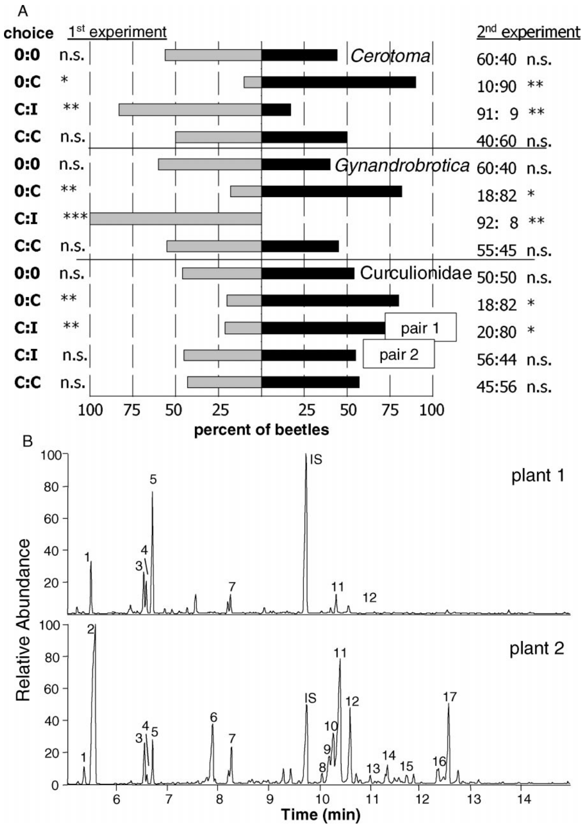

FIG. 1. Responses of beetles in different choice situations in a Y-tube olfactometer (A) and HIPV released by two different induced plants (B). (A) Behavior of three beetle species in different choice situations. Bars indicate percent of beetles that chose during the first experiment, trials (order from top to bottom indicates the temporal order of the experiment) were 0:0 (empty vs. empty), 0:C (empty vs. control), C:I (control vs. induced) and C:C (control vs. control). Results of tests against binomial distribution are indicated for both experiments (n.s. not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$). Data are graphically presented for the first experiment, while ratios are given for the second experiment. Sample sizes are: *Cerotoma*, 12 on day 1 and 15 on day 2; *Gynandrobrotica*, 10 on day 1 and 11 on day 2; Chrysomelidae, 16 on day 1 and 20 on day 2. (B) HIPV released by the induced plants of pairs 1 and 2 used in the experiment on weevils. Peaks are: 1: *cis*-ß-ocimene, 2: *trans*-ß-ocimene, 3: C_{11} homoterpene [4,8-dimethylnona-1,3,7-trien], 4: *p*-mentha-1,5,8-triene, 5: $C_{10}H_{14}$ [all-trans-2,6-dimethyloctatetraen], 6: *cis*-hexenylbutanoat, 7: $C_{10}H_{16}O$ [2,6-dimethyl-3,5-7-octatrien-2-ol], IS: *n*-bromodecane, 8: α-copaene, 9: *cis*-hexenylhexanoate(??), 10: hexenylbenzoate (??), 11: *cis*-jasmone, 12: ß-caryophyllene, 13: α-humulene, 14: germacrene, 15: α-farnesene, 16: nerolidol, 17: TMTT [(3E, 7E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene].

induced plant; see Table 1). These beetles feed on a slightly induced plant as they do on an uninduced one, and appear to use the HIPV of the slightly induced plant to locate it, yet they avoid heavily induced plants. Similar behavior has recently been reported for spider mites, which are also attracted to volatiles released by slightly induced Lima bean leaves, yet repelled by heavily induced leaves (Horiuchi et al., 2003). Releasing HIPV by slightly induced Lima beans, thus, can incur ecological costs by attracting, rather than deterring, herbivores.

Odors of control plants were more attractive than those of induced plants in all cases in which beetles preferred controls. Although beetles tend to be attracted to HIPV (pers. comm. by T. Turlings; see also Bolter et al., 1997; Kalberer et al., 2001), HIPV released by JA-induced wild Lima bean plants deterred both herbivorous Chrysomelid species and, thus, acted as a direct defense. In contrast, the Curculionids showed mixed responses. Why did conspecific plants respond differently to the same treatment? Are other herbivores also attracted by slightly induced plants yet deterred when HIPV release exceeds a given level? Further studies are required to determine if HIPV released by induced Lima beans under natural conditions act as a direct defense by deterring herbivores or if, instead, they attract herbivores, thus incurring ecological costs.

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REFERENCES

BOLAND, W., HOPKE, J., DONATH, J., NÜSKE, J., AND BUBLITZ, F. 1995. Jasmonic acid and coronatin induce odor production in plants. *Angew. Chemie Int. Ed.* 34:1600–1602.

BOLTER, C. J., DICKE, M., VAN LOON, J. J. A., VISSER, J. H., AND POSTHUMUS, M. A. 1997. Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *J. Chem. Ecol.* 23:1003–1023.

DE MORAES, C. M., MESCHER, M. C., AND TUMLINSON, J. H. 2001. Caterpillar-induced nocturnal plant volatiles repel nonspecific females. *Nature* 410:577–580.

DICKE, M. AND VAN LOON, J. J. A. 2000. Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomol. Exp. Appl.* 97:237–249.

DICKE, M., GOLS, R., LUDEKING, D., AND POSTHUMUS, M. A. 1999. Jasmonic acid and herbivory differentially induce carnivore-attracting plant volatiles in Lima Bean plants. *J. Chem. Ecol.* 25:1907–1922.

HEIL, M. 2004. Induction of two indirect defences benefits Lima Bean (*Phaseolus lunatus,* Fabaceae) in nature. *J. Ecol.* 92: in press.

HOPKE, J., DONATH, J., BLECHERT, S., AND BOLAND, W. 1994. Herbivore-induced volatiles—The emission of acyclic homoterpenes from leaves of *Phaseolus lunatus* and *Zea mays* can be triggered by a beta-Glucosidase and jasmonic acid. *FEBS Letters* 352:146–150.

HORIUCHI, J.-I., ARIMURA, G. I., OZAWA, R., SHIMODA, T., TAKABAYASHI, J., AND NISHIOKA, T. 2003. A comparison of the responses of *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus* *persimilis* (Acari: Phytoseiidae) to volatiles emitted from Lima Bean leaves with different levels of damage made by *T. urticae* or *Spodoptera exigua* (Lepidoptera: Noctuidae). *Appl. Entomol. Zool.* 38:109–116.

KALBERER, N. M., TURLINGS, T. C. J., AND RAHIER, M. 2001. Attraction of a leaf beetle (*Oreina cacaliae*) to damaged host plants. *J. Chem. Ecol.* 27:647–661.

KESSLER, A. AND BALDWIN, I. T. 2001. Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291:2141–2144.

KOCH, T., KRUMM, T., JUNG, V., ENGELBERTH, J., AND BOLAND, W. 1999. Differential induction of plant volatile biosynthesis in the Lima Bean by early and late intermediates of the octadecanoid-signaling pathway. *Plant Physiol.* 121:153–162.