Pergamon

PII: S0031-9422(97)00076-9

Phytochemistry, Vol. 45, No. 5, pp. 919–923, 1997

0031-9422/97 $17.00 + 0.00

DIURNAL EMISSION OF VOLATILE COMPOUNDS BY JAPANESE BEETLE-DAMAGED GRAPE LEAVES

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(*Received in revised form* 26 *November* 1996)

Key Word Index—*Vitis labrúsca*; Vitaceae; grape; *Popillia japonica*; diurnal; kairomone; plant–insect interactions; semiochemical; terpene; terpenoid.

Abstract—The volatile compounds liberated by Japanese beetle (*Popillia japonica* Newman) feeding on grape leaves (*Vitis labrúsca* L.) were studied. Ten consecutive collections of 3 hr duration were performed on live vines starting at 09:00 and continuing until 15:00 the following day. Release of most compounds followed a diurnal pattern, with the period of peak emission from 12:00 to 15:00 and the period of least emission from 00:00 to 03:00. Nineteen compounds were identified from the beetle-damaged vines, most of which were aliphatic aldehydes, alcohols and esters as well as terpene hydrocarbons. During periods of peak emission, volatile production from beetle-damaged vines was about 50-times higher than that of undamaged vines. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Volatile compounds liberated by arthropods feeding on angiosperm leaves have been associated with the attraction of predators and parasitoids. For instance, two spotted spider mite (*Tetranychus urticae*) feeding on lima bean (*Phaseolus lunatus*) leaves induces volatiles which are attractive to the predatory mite *Phytoseiulus persimilis* [1]. In another case, beet armyworm (*Spodoptera exigua*) feeding on corn (*Zea mays*) leaves attracts the hymenopterous parasitoid *Cotesia marginiventris* [2]. It has been proposed, therefore, that this response has evolved as a means whereby plants actively interact with the third trophic level by recruiting natural enemies of herbivorous pests [3].

At the second trophic level, however, odours released by insect feeding on leaves can act as aggregation kairomones for certain insects. *Maladera matrida* feeding on *Duranta repens* leaves, for example, attracts other members of this species [4]. Striped cucumber beetles (*Acalymma vittatum*) aggregate on cucurbit seedings and mature plants [5]. While in the latter case volatile attractants have been identified from cucurbit flowers [6], in neither case have the kairomones released by damaged leaves been identified.

Recently, we found that detached crab apple (*Malus* × *domestica*) leaves with overnight feeding damage by Japanese beetles (*Popillia japonica*) or fall webworms (*Hyphantria cunea*) were significantly more attractive to Japanese beetles than were undamaged leaves, artificially damaged leaves or leaves freshly damaged by Japanese beetles [7]. Leaves that received overnight feeding damage produced a complex mixture of volatile aliphatic compounds, aromatic compounds and terpenoids. In comparison, artificially damaged leaves or freshly damaged leaves produced a less complex blend of compounds, mainly consisting of green-leaf volatiles.

Later, we investigated Japanese beetle aggregation on grape vines (*vitis Labrúsca*) [8]. In field trials, many more beetles were recruited to vines with overnight feeding damage than to undamaged vines, undamaged vines with nonfeeding beetles or freshly damaged vines. Subsequently, we examined the volatile compounds emitted from grape vines *in situ*. While we presented identifications of some compounds [8], here we present data for yields of volatile compounds and show a periodicity for their induction.

RESULTS

Emission of most volatiles from the beetle-damaged grape vines exhibited diurnal periodicity, with maximum and minimum levels of emission from 9:00

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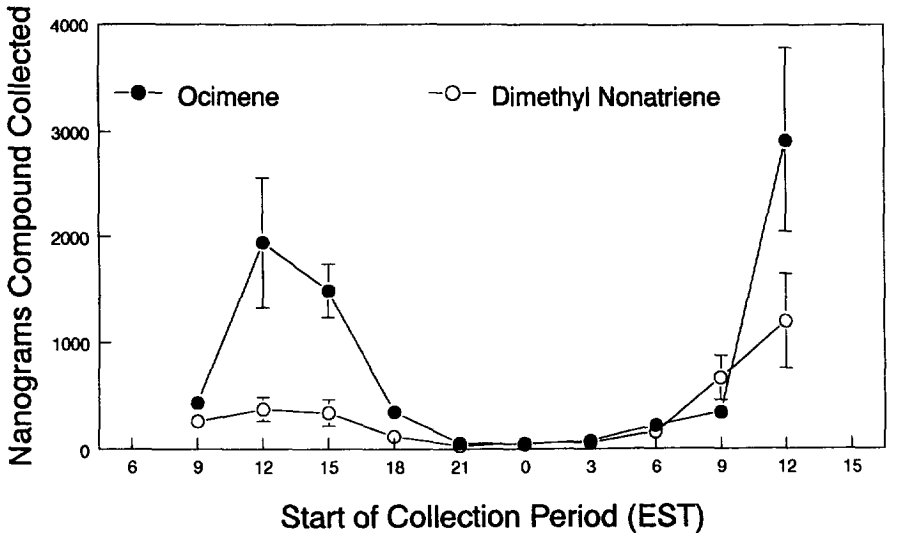

Fig. 1. Emission of (*E*)-β-ocimene and (*E*)-4,8-dimethyl 1,3,7-nonatriene by Japanese beetle damaged grape vines. Data represent the mean of five determinations ± s.e.m.

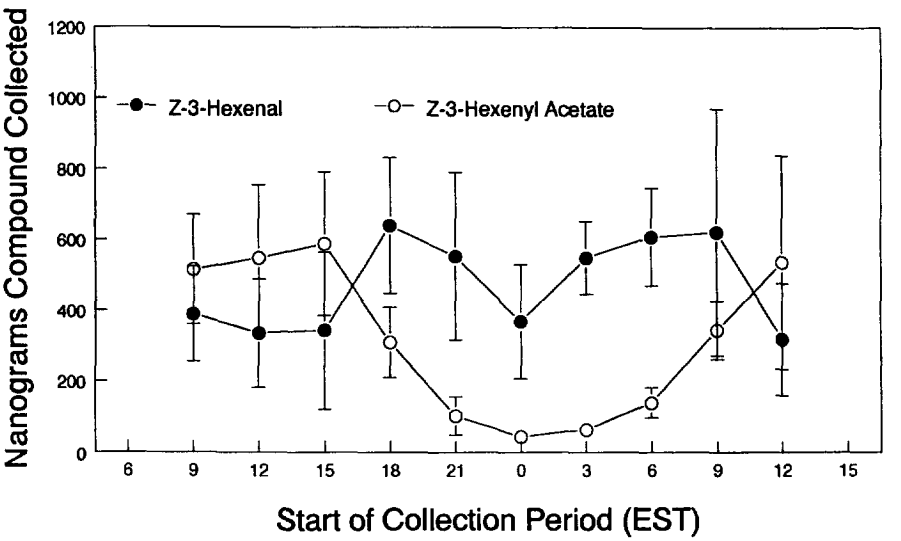

Fig. 2. Emission of (*Z*)-3-hexenal and (*Z*)-3-hexenyl acetate by Japanese beetle damaged grape vines. Data represent the mean of five determinations ± s.e.m.

to 15:00 hr and 21:00 to 03:00 hr, respectively. The periodicity of emission for the terpene hydrocarbons (*E*)-β-ocimene and (*E*)-4,8-dimethyl 1,3,7-nonatriene is illustrated in Fig. 1.

Similar periodicity occurred for emission of aliphatic alcohols and esters. For instance, the emission of (*Z*)-3-hexenyl acetate followed a pattern similar to that noted for terpenoids (Fig. 2). Interestingly, emission of the precursor to the aliphatic alcohols and esters, (*Z*)-3-hexenal [9], did not exhibit periodicity.

Compounds emitted by the beetle-damaged vines at the period of peak emission on the first and second day are summarized in Table 1, along with levels emitted by undamaged vines during the same period. Nineteen compounds were identified from the damaged plants. Of the terpenoids produced by the beetle-damaged vines, all were acyclic with the exception of limonene. Production of volatiles by damaged vines averaged about 36- and 64-times that of undamaged vines on the first and second day of volatile collection, respectively.

DISCUSSION

In field trials studying Japanese beetle aggregation [8], we compared the relative attractiveness of undamaged grape vines, vines with 15 female and 15 male beetles which were not allowed to feed, vines with 15 female and 15 male beetle confined to a single leaf (fresh feeding damage), and vines on which 200 beetles had been placed overnight and then, after removal of the beetles, had 15 female and 15 male beetles confined to a single leaf. Vines with nonfeeding beetles were included to test for attraction to sex or aggregation pheromones, whereas freshly damaged vines were

Table 1. Volatile compounds emitted by grape plants sampled from 12:00 to 15:00 hr

Compound	Nanograms compound collected* Undamaged	First day of damage	Second day of damage	P†
	Aliphatic Aldehydes, Alcohols and Esters			
(*Z*)-3-Hexenal	25.4±1.3	335±171‡	318±176‡	0.887
Hexanal	11.0±0.4	57.8±23.7‡	73.8±33.0‡	0.465
(*E*)-2-Hexenal	5.8±4.3	309±170‡	1010±306‡	0.014
(*Z*)-3-Hexenol	§	323±175‡	619±232‡	0.390
Hexanol	16.3±1.7	69.6±31.7‡	143±87.7‡	0.290
(*E*)-2-Hexenol		52.3±7.2‡	279±133‡	0.071
(*Z*)-3-Hexenyl acetate	17.4±8.4	548±231‡	536±337‡	0.961
Hexyl acetate		34.3±20.2‡	43.7±25.5‡	0.352
(*E*)-2-Hexenyl acetate		12.6±9.9‡	12.2±10.5‡	0.912
	Terpenoids			
Limonene	29.2±8.2	18.6±5.4	127±104‡	0.356
(*E*)-β-Ocimene	10.8±5.8	1940±685‡	2910±969‡	0.416
Linalool	13.2±2.6	59.7±25.6‡	85.0±33.3‡	0.124
(*E*)-4,8-Dimethyl 1,3,7-nonatriene	6.8±4.2	374±124‡	1200±499‡	0.113
(*E,E*)-α-Farnesene	2.5±2.5	121±33.6‡	408±155‡	0.208
Nerolidol		22.9±5.8‡	72.7±67.2‡	0.734
	Aromatics			
Benzyl alcohol	§	211±44.3‡	353±80.9‡	0.954
Methyl salicylate	8.5±5.2	104±37.5	94.4±49.5	0.750
Indole	3.0±3.0	39.4±16.3	134±83.3‡	0.293
(*Z*)-3-Hexenyl benzoate	8.7±6.1	68.3±28.3‡	72.7±30.9‡	0.507

* Data represent the mean of five determinations ± standard error of the mean.

† Probability that level of compound differs from the first to the second day of feeding damage by a two-tailed paired *T*-test.

‡ Indicates that level of compound differs from that of undamaged control by one-tailed unpaired *T*-test at $P = 0.05$.

§ Blank indicates that compound was not detected or less than 2 ng of compound was collected.

included as a control for the possibility that production of pheromones was higher by feeding beetles.

Many more beetles were attracted to vines with overnight feeding damage ($\bar{x} = 25.2$) than to undamaged vines, undamaged vines with nonfeeding beetles or freshly damaged vines ($\bar{x} = 0.2$, 1.0 and 5.1, respectively). This indicated that Japanese beetles are more likely attracted to feeding-induced volatiles than to an aggregation or sex pheromone.

Previous studies on herbivore-induced volatile release demonstrated qualitative differences in the types of volatiles emitted during early and late stages of damage. Turlings *et al.* [2], for example, found that early stages of caterpillar feeding on corn leaves were characterized by release of aliphatic aldehydes and relatively low levels of terpenoids. Later, reduced and esterified forms of aliphatic compounds predominated; along with greater amounts of terpenoids.

In subsequent studies, it was shown for cotton (*Gossypium hirsutum*) [10] and corn [3] that emission of induced volatiles exhibits diurnal periodicity with maximum and minimum levels of emission in the photophase and scotophase, respectively. The difference in the type of volatiles emitted between early and late stages of damage, therefore, is likely due to this periodicity since experiments examining herbivore-induced volatile release usually started feeding damage late in the afternoon [1, 2, 10], a time when the production of induced volatiles should be declining anyway. The relationship between the speed of induction for reduced aliphatics and terpenoids and time of day when feeding starts has not been examined, however.

In the present experiments, beetle-damaged grape vines quickly produced high levels of volatiles in response to feeding damage and few significant differences were noted in the levels of individual compounds from the first to the second day of feeding damage. This result indicates that production of feeding-induced volatile compounds is more dependent on the time of day at which feeding occurs than how long the plant has been damaged.

Japanese beetles start to fly on sunny mornings when the temperature reaches about 21°. Late in the afternoon, flight slackens and the beetles land on host plants where they remain overnight [11]. The

maximum release of volatile semiochemicals by beetle-damaged grape vines, therefore coincides with the period of greatest flight activity. In the absence of volatile attractants Japanese beetle flight is not-directed and they tend to drift with the wind [11, 12].

Japanese beetles are attracted to essential oils with floral- and fruit-like odours [13] and often aggregate on flowers and fruit in the field [11]. The relative amounts of fruit-like esters and floral terpenoids (i.e. linalool and nerolidol) produced by insect-damaged plants are likely to determine attractiveness to the Japanese beetle. Floral- and fruit-like compounds may also affect susceptibility to Japanese beetles by acting as phagostimulants. Major and Tietz [14], for example, found that applying a solution of eugenol to *Gingko biloba* leaves increased Japanese beetle feeding on this normally resistant species. These same compounds are also likely to be attractive to parasitic Hymenoptera that attack insect larvae, because they are dependent on nectar for food [15].

Terpene hydrocarbons, on the other hand, are more likely to be involved in direct defence against arthropods. Plant apparency theory predicts that terpene hydrocarbons should be more effective in defence against generalist herbivores than against specialists [16]. Blends of terpenes characteristic of a plant are often complex, which may render detoxification more difficult for a nonadapted species [17]. In addition, complex volatile blends may also serve as reliable host indicators to specialist herbivores [16].

The production of acyclic terpenes, however, seems to be a ubiquitous response of angiosperm leaves to arthropod feeding damage [18]. Acyclic terpene hydrocarbons may play a specific role in defense against arthropod herbivores by acting as insect juvenile hormone analogues [19, 20]. Recently, we examined the volatiles produced by leaves of maple (*Acer*) species in response to Japanese beetle feeding [21]. Maple species resistant to defoliation by Japanese beetles (*A. rubrum*, *A. saccharinum*) produced significantly higher levels of cyclic terpenes than did susceptible maples (*A. palmatum*, *A. platanoides*). Leaves of both susceptible and resistant maples produced high amounts of acyclic terpenes. Given that the production of acyclic terpenes appears to be a ubiquitous response of angiosperm leaves to arthropod herbivory [18] and that these compounds may affect larval development [19, 20], it would be interesting to know if adult generalists such as the Japanese beetle are affected less by acyclic terpenes in the diet than by cyclic terpenes.

In conclusion, feeding-induced volatile compounds may often alleviate the damage caused by herbivorous pests by attracting predators and parasitoids [1–3]. In the case of insects such as the Japanese beetle, however, they can lead to greater damage by acting as aggregation kairomones. Induced volatile compounds may possibly affect susceptibility to the Japanese beetle directly by acting as feeding deterrents [16, 17] and phagostimulants [14]. Feeding-induced volatiles, emitted in relatively large amounts during the period of beetle flight, may serve as a reliable guide to conspecifics and suitable host plants.

EXPERIMENTAL

Insects and plants. One-year-old clonal grape root stocks (*Vitis labrusca* L. cv. 'Beta'; Gurney's, Yankton SD) were planted in 30-cm-diameter pots and maintained in a greenhouse. Japanese beetles were captured in the field using traps baited with food-type lures containing a 1:1.1:2.2 ratio of geraniol, phenethyl propionate and guenol (Trécé Inc., Garwood, NJ). Beetles were removed from the traps within 2 hr of capture and held without food in wire mesh screen cages until placed on plants.

Volatile collections. The emission of volatile compounds from the grape vines was examined in late July through early August using an apparatus similar to that developed by Heath and Manukian [22, 23]. It consisted of a 15-cm-diameter by 40-cm-tall glass sleeve with polyvinyl couplers at the top and bottom to accommodate a purified air inlet line and eight concentrically-arranged vacuum lines for volatile collection traps, respectively. The terminal 0.5 m of a vine was tied to a wooden dowel and placed in the apparatus. About 20 min before the start of volatile collections designed to examine the emission of volatiles from beetle damaged vines, 15 male and 15 female beetles were placed in the glass sleeve, the bottom of which was covered in nylon mesh to prevent escape of the beetles. During collections, air flow was passed at 5.0 l min^{-1} through multiple layers of compressed activated charcoal-infused fabric (Lewcott Corp., Milbury, MA) located in the top coupler.

Sequential 3-hr collections were accomplished using solenoid valves controlled by 12 V DC solid state relays operated under the control of software developed by the first author. The software was written in Visual Basic for MS-DOS (Microsoft, Pullman, WA), which allowed operation on a computer with an Intel 8086 or higher microprocessor and which also simplified programming the user interface.

Volatile collections were performed in a white-washed greenhouse with no supplemental lighting and the collection apparatus was screened with a shade cloth with an 80% transmittance to reduce heat buildup in the glass sleeve. Temp. during the volatile collections was not continuously monitored but ranged from 32 to 38° during the day and 20 to 24° at night. In order to follow the course of volatile release and determine the periods of peak emission, volatile collections were started at 9:00 hr EST and continued until 15:00 the following day. During collections, air was pulled through traps consisting of individual glass tubes containing 50 mg Super Q adsorbent (Alltech Associates, Deerfield IL) at a rate of 1.0 l min^{-1}. After the first collection series from beetle-damaged vines showed that the period of maximum volatile emission occurred from 12:00 to 15:00 hr, collections were per-

formed on undamaged vines during this same time period alternately with those of damaged vines. These experiments were replicated 5× for both beetle-damaged and undamaged plants.

Compounds were eluted from the traps using 250 μl of an 80:20 (v/v) mixt. of hexane and CH_2Cl_2. One μg cumene was added as an internal standard and compounds were analyzed by injection on a gas chromatograph equipped with a 60 m × 0.32 mm DB-5 column with a 1.0 μm film thickness. Gas chromatograph operating conditions were as follows: oven temp. 60° for 1 min, then programmed at 2° min to 220°; injector 220°; flame ionization detector 240°; He carrier linear flow velocity 21 cm sec^{-1}.

Mass spectroscopy was performed on a Hewlett-Packard mass ion detector interfaced to a gas chromatograph equipped with a 25 × 0.25 mm DB-5 column operated under the following conditions: oven temp. 40° for 1 min, then programed at 2°–220°; injector 220°. Compounds were identified as previously described [7].

Acknowledgements—We thank David Held, Brian Kunkel and Randy Collins for technical assistance and Gwyn Ison for manuscript preparation. We also thank Owen Loughrin and Terry McIlvain of the University of Kentucky Center for Robotics and Manufacturing Systems for technical advice. This work was supported by USDA SRIPM grant 94-34103-0185. This is manuscript no. 96-08-121 of the University of Kentucky Agricultural Experiment Station and is published with the approval of the director.

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