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Predator–prey interactions: olfactory adaptations of generalist and specialist predators

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Abstract

1. Olfactory responses of the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), a generalist predator, *Podisus maculiventris* (Say) (Hemiptera, Heteroptera: Pentatomidae) (*Pm*), and a specialist predator, *Perillus bioculatus* (F.) (Hemiptera, Heteroptera: Pentatomidae) (*Pb*) were investigated. Volatiles tested included 20 compounds emitted by undamaged potato plants (*Solanum tuberosum*), plants that had been artificially damaged, or plants damaged by feeding by CPB larvae.
2. Coupled gas chromatography/electroantennogram detector (GC/EAD) recordings revealed five compounds for which reliable responses were recorded from CPB antennae: (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, (±)-linalool, nonanal, methyl salicylate, and indole. Both *Pm* and *Pb* responded selectively to the same compounds as the CPB with exceptions: (1) (*Z*)-3-hexenyl butyrate elicited reliable responses for both *Pm* and *Pb,* and (2) (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol were inactive for *Pm* and *Pb* under these conditions. Dose–response curves showed that CPB was at least 100 times more sensitive to (*E*)-2-hexen-1-ol than were the predators. Both predators were more sensitive to each of the other compounds than were CPB. Both CPB and *Pm* were attracted to a five component blend comprising (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, (±)-linalool, nonanal and methyl salicylate. However, attraction of CPB to the blend occurred only with lower doses of (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol.
3. These results show that the herbivore (CPB) has olfactory receptors which are more sensitive to constitutive host plant volatiles, e.g. green leaf volatiles, while both generalist (*Pm*) and specialist (*Pb*) predators are more sensitive to systemic volatiles produced in response to prey feeding.

Keywords Colorado potato beetle, constitutive compounds, host plant, induced compounds, olfaction, *Perillus bioculatus*, *Podisus maculiventris*, predator, prey, tritrophic.

Introduction

Upon feeding by an herbivore, plants release volatiles into the atmosphere which are attractive to predators and parasitoids (Turlings *et al*., 1990). For example, lima bean plants, *Phaseolus lunatus*, infested with the spider mite, *Tetranychus urticae*, emit a blend of volatiles which attract the predatory mite, *Phytoseiulus persimiliss* (Dicke *et al*., 1990a,b). A mixture of four components of this volatile blend [3,7-dimethyl-1,6-octadien-3-ol, (*E*)-β-ocimene, (3E)-4,8-dimethyl-1,3,7-nonatriene, and methyl salicylate] is attractive to *P. persimiliss* females. Volatile compounds emitted not from the wound site but systemically from the entire cotton plant are attractive to both the generalist parasitoid, *Cotesia marginiventris*, and the specialist parasitoid, *Microplitis croceipes* (Röse *et al*., 1998). Among the systemic volatiles were (*Z*)-3-hexenyl acetate (*E*)-β-ocimene, linalool, (*E*)-4,8-dimethyl-1,3,7-nonatriene, (*E*)-β-farnesene, (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, indole, and (*Z*)-3-hexenyl butyrate. While both the generalist and specialist parasitoids were attracted to volatiles emitted by insect damaged plants, only the generalist parasitoid was attracted to artificially

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damaged plants. Oral secretions associated with insect feeding elicit *de novo* synthesis and release of acyclic terpenes including linalool, (*E*)-β-farnesene, (*E*)-β-ocimene and other volatiles within the isoprenoid biosynthetic pathway, and indole, a product of the tryptophan pathway (Paré & Tumlinson, 1997a, b).

The Colorado potato beetle (CPB) *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) is a major pest of potatoes and related solanaceous plants, e.g. tomatoes. The ability of CPB to develop resistance to various insecticides and other control measures has lead to a continuing search for alternative methods for population regulation. Biological control measures such as predaceous insects (Hough-Goldstein *et al.*, 1993) and attractants (Levinson *et al.*, 1979; Jermy & Butt, 1991; Otto, 1996) have been investigated as biorational environmentally preferable tactics. However, while predaceous insects have been shown to reduce CPB populations substantially under certain conditions, a synthetic attractant for CPB does not yet exist.

Adult CPB are attracted by volatiles emitted by mature potato plants (McIndoo, 1926; Schanz, 1953; De Wilde *et al.*, 1969; Visser, 1976). Potato plants fed upon by either CPB larvae or larvae of the moth *Spodoptera exigua* were attractive to CPB adults (Bolter *et al.*, 1997). CPB larvae feeding on potato plants (var. Surprise) elicited emission of a number of compounds including the green leaf volatile derivatives (*Z*)-3-hexenol and (*Z*)-3-hexenyl butyrate, and the sesquiterpenes, β-caryophyllene and β-selenine (Bolter *et al.*, 1997). Coupled gas chromatography/electroantennographic detector recordings showed that CPB antennae respond to at least 18 compounds released by damaged plants (var. Granola) (Schütz *et al.*, 1997). Benzene methanol and 2-benzene-ethanol were emitted by beetle damaged plants but were not released by mechanically damaged plants. Several volatiles emitted by CPB damaged potato plants 24 h following removal of the insects included linalool and indole (Bolter *et al.*, 1997).

Both the spined soldier bug *Podisus maculiventris* (*Pm*) (Heteroptera: Pentatomidae), a generalist predator (McPherson, 1982; O'Neil, 1988), and the two-spotted bug *Perillus bioculatus* (*Pb*) (Heteroptera: Pentatomidae), a specialist predator (Saint-Cyr & Cloutier, 1996), have been tested as potential biocontrol agents of CPB. An aggregation pheromone secreted by the dorsal abdominal gland of *Pm* was identified [principally (*E*)-2-hexenal, α-terpineol, and benzyl alcohol with lesser amounts of (±)-linalool, terpinen-4-ol, *trans*- and *cis*-piperitol; Aldrich *et al.* (1984)] that attracted both nymphal and adult stages (Sant'Ana *et al.*, 1997). The pheromone has been used to enhance its effectiveness of *Pm* for biocontrol of CPB

Table 1 Source and purity of chemicals used in electrophysiological experiments, behavioural studies, and their presence in volatiles emitted from *Solanum tuberosum* var. Surprise (Bolter *et al.*, 1997) and *S. tuberosum* var. Granola (Schütz *et al.*, 1997).

Chemical	Purity (%)	Source[1]	Kovàts' indices[2]	Presence in intact or damaged plant[3]
Green leaf volatiles				
(*Z*)-3-hexenol	98	A	858	IP I, II; DP I, II
(*E*)-2-hexenol	97	A	887	IP II, DP II
(*Z*)-3-hexenyl acetate	98	B	1009	DP I
(*Z*)-3-hexenyl butyrate	99	C	NA	DP I
Aliphatic aldehydes				
nonanal	95	A	1104	IP I, II; DP I, II
decanal	95	A	1207	IP I, DP I, II
Monoterpenes				
1,8-cineole	99	A	1030	DP I
(*R*)-(+)-limonene	97	A	1033[2a]	IP I (±); DP I
(*S*)-(–)-limonene	96	A	1033[2a]	IP I (±); DP I
(±)-linalool	97	A	1100	IP I, DP I, II
myrcene	94	A	992	IP I, DP I, II
1R-(+)-α-pinene	97	A	937[2a]	IP I (±); DP I
1S-(–)-α-pinene	97	A	937[2a]	IP I (±); DP I
Sesquiterpenes				
β-caryophyllene	99	D	1455	IP I, DP I, II
α-humulene	99	D	1489	DP I
(+)–longifolene	98	A	NA	DP I (±)
β-selinene	86	E	1436	IP I; DP I
Other compounds				
2-furaldehyde	99	A	NA	DP I
indole	98	A	1292	DP I
methyl salicylate	99	A	1234	DP I

[1] A – Aldrich Chemical Co., St. Louis, MO, U.S.A.; B – Sigma Chemical Co., St Louis, MO; C – Bedoukian Research Inc., Danbury, CT; D – ICN Biomedicals Inc., Aurora, OH; E – Dr D. M. Light, USDA, ARS, WRRC, Albany, CA.

[2] Kovàts' retention indices (Kovàts, 1958) on DB-5 column (Acree & Arn, 1997); NA = not available;

[2a] Kovàts' index given is for racemic compound.

[3] IP=intact plant, DP=damaged plant; I – Bolter *et al.*, 1997; II – Schütz *et al.*, 1997.

(Aldrich *et al*., 1997). The major glandular secretion identified in *Pb* males as 6,10,13-trimethyltetradecyl isovalerate (Aldrich *et al*., 1986) was inactive in field tests (J. R. Aldrich, personal communication). Little is known about chemical cues used by either *Pm* (the generalist predator) or *Pb* (the specialist predator) to locate potential prey.

The study presented here determines chemical signals emitted by potato plants which are reliably detected by the CPB, its generalist (*Pm*) and specialist (*Pb*) predators. Dose–response curves show differing sensitivities for these volatiles among the predators and insect herbivore. Laboratory bioassays show attraction of the CPB and *Pm* to blends of these constitutive and systemic volatiles.

Methods

Insects

Adult Colorado potato beetle (CPB) *Leptinotarsa decemlineata,* were obtained from a laboratory colony which was annually infused with feral insects or were collected directly from the field. CPB were maintained on potato, *Solanum tuberosum* var. Kennebeck, for both larval and adult stages. Spined soldier bugs *Podisus maculiventris* (*Pm*), were obtained initially either as late instar nymphs or adults from Dr J. R. Aldrich, USDA, ARS, Insect Chemical Ecology Laboratory, Beltsville, MD. *Perillus bioculatus* (*Pb*) were obtained from either Dr T. A. Coudron, USDA, ARS, Biological Control of Insects Laboratory, Columbia, MO, as second-instar nymphs or field collected on potatoes grown on the Beltsville Agricultural Research Center. Both *Pm* and *Pb* were maintained on immature or adult forms of CPB. In general, all insects used in electrophysiological and behavioural studies were at least six days post emergence.

Odorous stimuli

Chemicals used in this study were chosen based on their presence in emissions of potato plants, *S. tuberosum* var. Surprise (Bolter *et al*., 1997). Some of the same chemicals are also emitted by *S. tuberosum* var. Granola (Schütz *et al*., 1997). The source and purity of the chemicals used are listed in Table 1.

Experimental protocol

For the first experiment, an 'isosynthetic' potato plant (i.e. a blend of chemicals found in emissions of potato plants but present in approximately equal amounts in a solvent solution) was prepared based on volatiles found in *S. tuberosum* var. Surprise (Bolter *et al*., 1997). In this approach, equal amounts of 20 chemicals previously identified from emissions of *S. tuberosum* var. Surprise were mixed with hexane to form a solution in which each compound was ≈ 10 µg/1 µL of the total solution. Serial dilutions of this stock solution were prepared and used in coupled gas chromatography (GC)/electroantennographic detector (EAD) studies with CPB, *Pm*, and *Pb*. One microlitre samples of serial dilutions of the 'isosynthetic' potato plant were injected into a Hewlett Packard^{TM} Model 5890 GC equipped with an HP-5 capillary column (Crosslinked 5% PH ME Siloxane; film thickness 0.25 µm; length 30 m; i.d. 0.25 mm). The column was split at the end using Gerstel^{TM} GraphPack-3D/2 flow splitter with a split ratio of ≈ 1 (FID):4 (EAD). After an initial temperature of 50°C which was held for 2 mins following injection, the GC oven was programmed to increase at 15°C/min to 235°C which was held for 2 mins. An effluent conditioning assembly to carry the GC effluent over the insect antennal preparation, hardware, and software for data collection and analyses using a mini-computer were obtained from SYNTECH (Hilversum, the Netherlands). Both EAD and electroantennogram (EAG) responses were recorded using a modification of an earlier technique (Schneider, 1957). In brief, an excised antenna was mounted between two glass capillary electrodes filled with 0.1 M NaCl. Ag–AgCl wires in the glass capillaries connected the preparation to the SYNTECHsystem.

In the second experiment, individual chemicals found to be active in coupled GC/EAD studies in the initial experiment were selected for more detailed examination. Dose–response curves were constructed from EAG responses to serial stimulus loads (0.005–5 µg on filter paper) of (*E*)-2-hexen-1-ol, (*Z*)-3-hexenyl butyrate, methyl salicylate, nonanal, (±)-linalool, and indole. Serial dilutions of experimental odourants were made in nanograde hexane. Stimuli were delivered as volatiles emanating from 5-µL aliquots of serial dilutions placed on Whatman no. 1 filter paper (7 × 18 mm) in glass odour cartridges (80 mm long × 5 mm i.d.). Odour cartridges were orientated towards the antenna from a distance of less than 1 cm. Molecules evaporating from the filter paper were carried over the preparation by dry, hydrocarbon-free air (gas purifier obtained from Alltech Associates, Deerfield, Illinois) at a flow rate of 0.3 m/s. Stimulus duration was 1 s; interstimulus time intervals of 2–3 min allowed for recovery of the antennal receptors. Stimuli were presented in order from the lowest to the highest stimulus load. Three replicates were obtained for each sex of each species. (*Z*)-3-hexenyl acetate (5 µg stimulus load) was used as a standard to normalize all responses, so that responses within an individual and among individuals could be compared (Payne, 1975). Millivolt responses were converted into a percentage of the mean of two nearest responses to the standard (Dickens, 1978). Stimulations with a solvent control (5 µL of the hexane on filter paper evaporated to dryness) were made at the beginning and end of each dilution series. Mean responses to the control were subtracted from each intervening EAG. Maximal depolarization of the EAG during the stimulation period was used as a measure of antennal stimulation. Threshold was considered to be the lowest dose at which the lower limit of the standard error of the mean response is greater than the upper limit of the standard error for the lowest dilution tested.

For the third experiment, a blend of the odourants found to be active in coupled GC/EAD and dose–response experiments was tested for behavioural activity in an open Y-track olfactometer modified after Visser & Piron (1998). In brief, the 'Y' was modified to a T with extensions at 45° angles to the top of the 'T'. All parts of the 'T' were constructed of copper tubing (6 mm o.d.). The vertical segment of the 'T' was 12 cm in length; both the horizontal part of the 'T' and 45° extensions were 6 cm long. Odorous stimuli emanating from filter paper discs (2.5 cm diameter; Whatman^{TM} No. 1 filter paper) in Ehrlenmeyer flasks were delivered to either side of the device through glass tubes (1.7 cm i.d.) placed over the ends of the 45° extensions of the horizontal part of the 'T'. Hydrocarbon-free air that was humidified by passing through distilled water carried the odour molecules to either arm of the bioassay apparatus. Airflow was regulated to 1 L/min by flowmeters. Teflon tubing connected all parts of the apparatus. A light source was placed at the top of the 'T', so when an insect was released at the bottom, it was stimulated to walk up the 'T' toward the light source and also by its tendency to be negatively geotropic. Experiments were conducted in a darkened room at 22°C in which the only source of light was that associated with the bioassay device. Behavioural experiments were performed in like manner for CPB and *Pm.* A five-component blend comprised of (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, nonanal (±)-linalool, and methyl salicylate prepared in paraffin oil (25 μL of a ≈ 0.5 μg/μL of each compound) was tested on one side of the olfactometer vs. the solvent (paraffin oil) control. A second five-component blend tested on CPB was identical to the initial blend except quantities of (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol were ten times less than in the initial blend (0.05 μg/μL). For all bioassays, 20 males and 20 females of each species were tested.

Statistical analyses

EAGs were evaluated by analysis of variance for two factors (Ostle, 1969), namely species and dose. Where differences were noted, comparisons were made using Duncan's new multiple range test (Duncan, 1955). Results from laboratory bioassays were assessed by the hypothesis on proportions based on the standard normal approximation (Brase & Brase, 1983).

Results

No sexual differences were noted in electrophysiological or behavioural responses of the Colorado potato beetle (CPB)

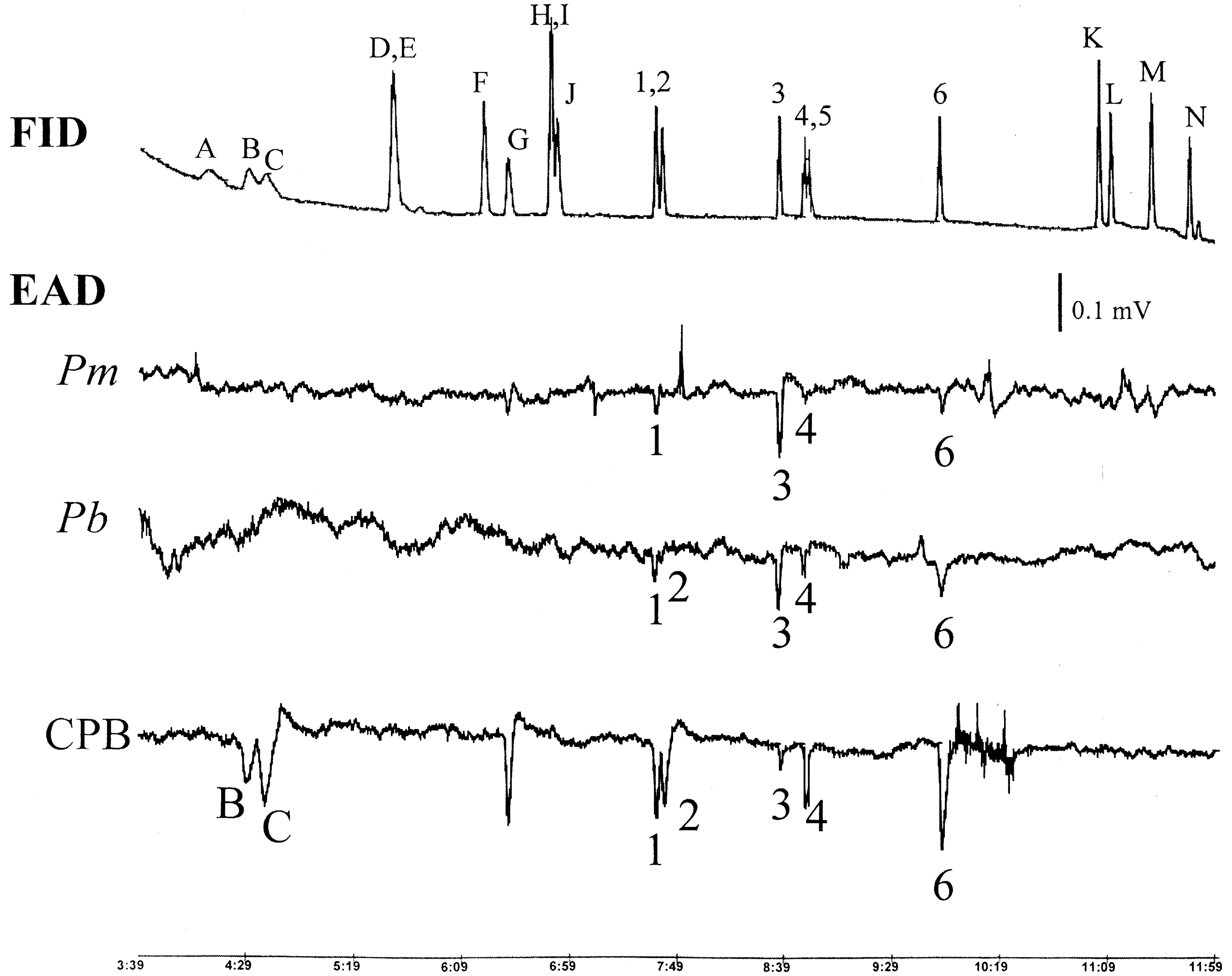

Figure 1 Coupled GC (FID)/EAD responses of the Colorado potato beetle (CPB) *Leptinotarsa decemlineata*, the generalist predator, *Podisus maculiventris (Pm.)*, and the specialist predator, *Perillus bioculatus (Pb)*, to equal amounts (5 ng) of 20 volatiles emitted by potato plants. Note response of CPB to two green leaf volatile alcohols (*Z*)-3-hexen-1-ol (B) (*E*) = 2=hexen-1-ol (C). 1 = (±)-linalool, 2 = nonanal, 3 = (Z)-3-hexenyl butyrate, 4 = methyl salicylate, 5 = decanal, 6 = indole. A = 2-furaldehyde, B = (*Z*)-3-hexen-1-ol, C = (*E*) = 2=hexen-1-ol, D,E = (+)-α-pinene and (–)-α-pinene, F = myrcene, *G* = (Z)-3-hexenyl acetate, H,I = (R)-(+)-limonene and (S)-(–)-limonene, J = 1,8-cineole, K = (+)-longifolene, L = β-caryophyllene, M = α-humulene, and N = β-selenine. Scale at bottom of figure is retention time in minutes and seconds. Vertical bar is 0.1 mV.

Leptinotarsa decemlineata, and the spined soldier bug *Podisus maculiventris* (*Pm*). Sexual differences did occur in EAGs for *Perillus bioculatus* (*Pb*) in response to the 5 μg stimulus load of (±)-linalool and the 0.5 μg and 5 μg stimulus loads of indole ($P < 0.05$). Therefore, EAGs and behavioural responses of male and female CPB, *Pm* and *Pb* (except for the above mentioned stimuli) were combined for further analyses and graphic representation.

Selectivity

Coupled GC (FID)/EAD studies. Typical GC/EAD responses of CPB, *Pm*, and *Pb* to 5 ng quantities of 20 volatiles emitted by potato plants are shown in Fig. 1. Only in CPB were reliable EAD responses recorded to the green leaf volatile alcohols, (*E*)-2-hexen-1-ol (B) and (*Z*)-3-hexen-1-ol (C), at this dose. EAD responses were also noted less reliably for (*Z*)-3-hexenyl acetate. Reliable EAD responses could also be noted for the CPB, and two predators, *Pm*, and *Pb,* for 5 ng quantities of (±)-linalool (1), nonanal (2), methyl salicylate (4), and indole (6) ‘(Figs 1 and 2). EADs of CPB and *Pb* to these five potato volatiles were similar. Both *Pm* and *Pb* gave more reliable responses to (*Z*)-3-hexenyl butyrate than did CPB. While responses were apparent to most of these compounds for *Pm* at the 5 ng level, they were smaller than those for CPB and *Pb*.

Sensitivity

Dose–response curves to serial stimulus loads of six potato volatiles active in coupled GC/EAD studies revealed differences in sensitivity of receptors in the CPB and the two predators. CPB were at least 100 times more sensitive to the green leaf volatile alcohol (*E*)-2-hexen-1-ol, than either *Pm* or *Pb* (Fig. 3A). EAG responses elicited by each stimulus load of this compound exceeded responses of both *Pm* and *Pb* ($P < 0.01$). Dose–response curves of *Pm* and *Pb* to (*E*)-2-hexen-1-ol were not significantly different at any stimulus load.

Pm were more responsive to the 0.5 μg stimulus load of (*Z*)-3-hexenyl butyrate than either *Pb* or CPB ($P < 0.05$) (Fig. 3B). While EAGs to the remaining stimulus loads of this compound did not differ for *Pm*, *Pb* or CPB, EAGs of the predators exceeded those of CPB at every stimulus load.

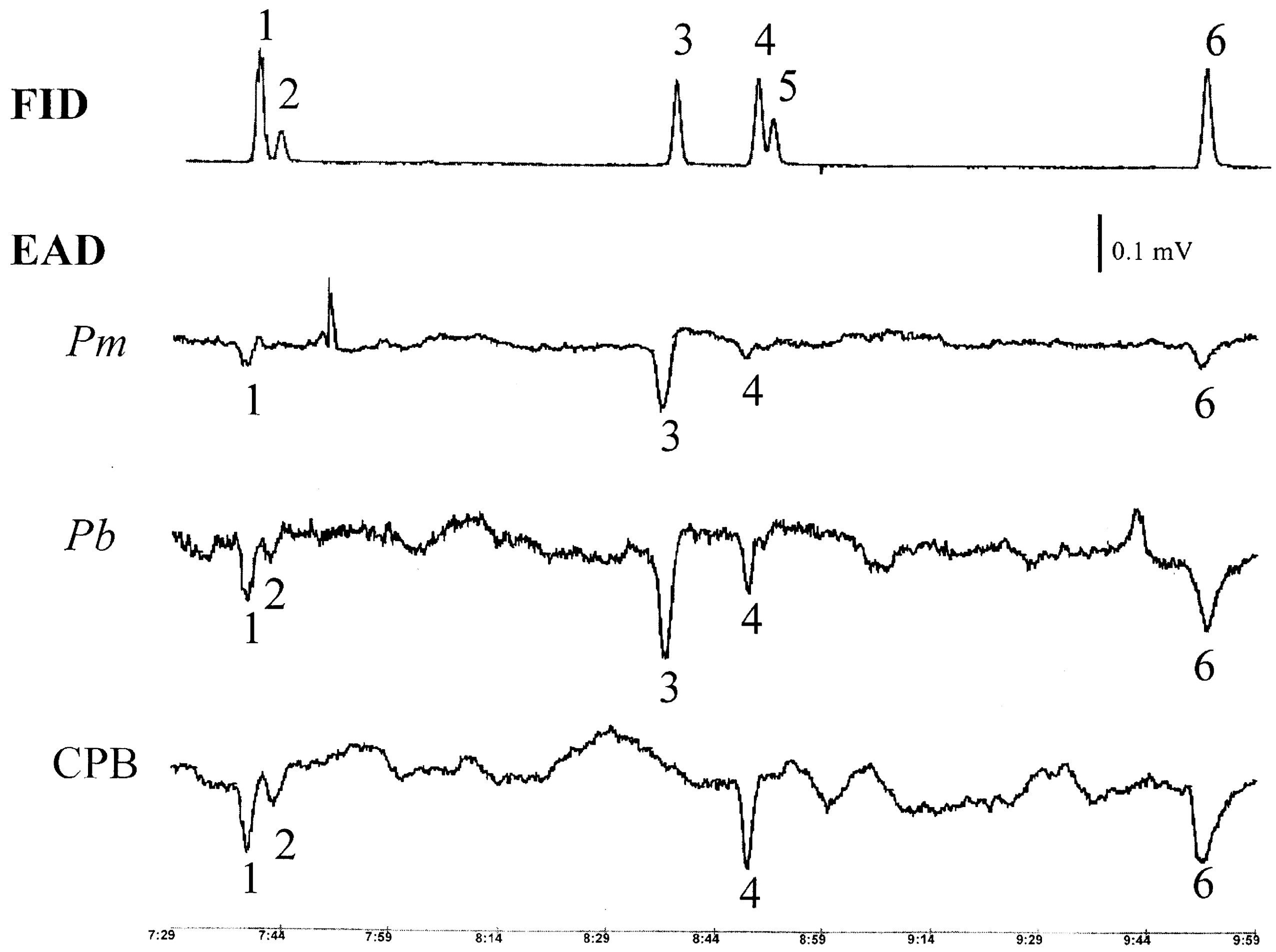

Figure 2 Close-up of coupled GC-FID/EAD responses of the Colorado potato beetle (CPB) *Leptinotarsa decemlineata*, the generalist predator, *Podisus maculiventris* (*Pm*), and the specialist predator, *Perillus bioculatus* (*Pb*), to equal amounts (5 ng) of six volatiles emitted by potato plants. 1 = (±)-linalool, 2 = nonanal, 3 = (*Z*)-3-hexenyl butyrate, 4 = methyl salicylate, 5 = decanal, 6 = indole. Scale at bottom is retention time. Vertical bar is 0.1 mV.

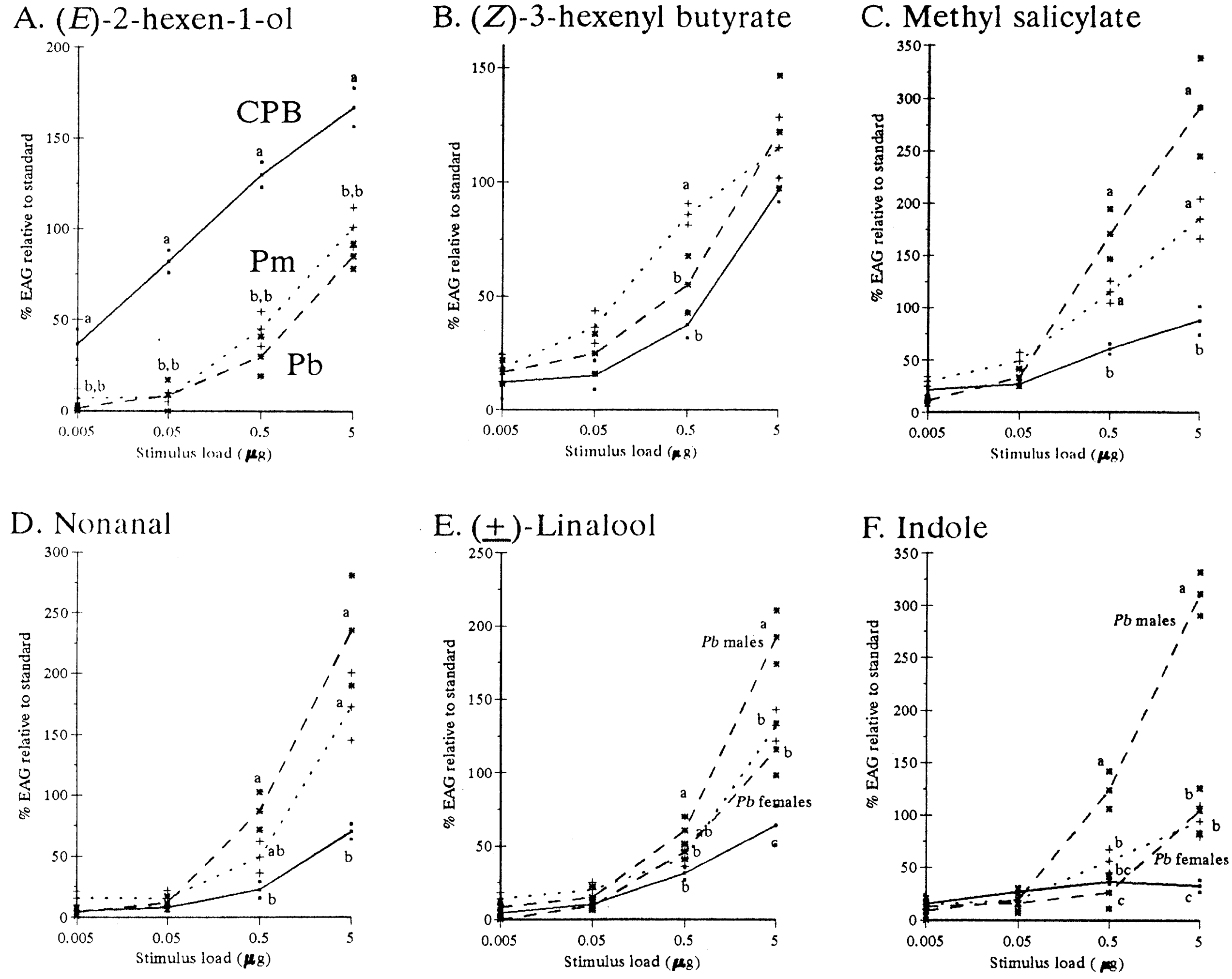

Figure 3 Dose–response curves of Colorado potato beetle Leptinotarsa decemlineata (CPB), the generalist predator, *Podisus maculiventris* (*Pm*), and the specialist predator, *Perillus bioculatus* (*Pb*), to serial stimulus loads of potato volatiles that elicited reliable GC/EAD responses. Responses of species followed by different letters for a dose are significantly different ($P < 0.05$, Duncan's multiple range test).

EAG responses of *Pb* and *Pm* to serial stimulus loads of methyl salicylate and nonanal did not differ (Fig. 3C,D). Responses of both *Pb* and *Pm* exceeded those of CPB for either one or two stimulus loads (0.5 μg and/or 5 μg) for both odourants. *Pm* and *Pb* were at least ten times more sensitive to these compounds than CPB.

Pm and *Pb* were more responsive to both (±)-linalool and indole than CPB (Fig. 3E,F). Although EAG responses of *Pm* and *Pb* females to these compounds did not differ, responses of *Pm* males to these compounds exceeded those of both of these groups ($P < 0.05$).

Behavioural bioassays

Pm was attracted to the five-component blend of (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, nonanal, (±)-linalool and methyl salicylate which contained equal amounts of each compound (Fig. 4). CPB were not attracted to this blend, but did respond to a similar blend that contained ten times less of the green leaf volatiles (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol. Sufficient numbers of *Pb* were not available for testing.

Discussion

Selectivity studies presented here show enhanced sensitivity of CPB to constitutive compounds, e.g. the green leaf volatiles (GLVs) (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol (Fig. 1) emitted by plants that are artificially damaged or damaged by insect feeding (Turlings *et al.*, 1990; Bolter *et al.*, 1997; Schütz *et al.*, 1997). Neither the generalist predator, *Podisus maculiventris* (*Pm*), nor the specialist predator, *Perillus bioculatus* (*Pb*), were sensitive to these odourants in coupled gas chromatography/electroantennogram detector (GC/EAD) preparations, and dose–response curves revealed both predators to be at least 100 times less sensitive to these compounds than CPB. GLVs are common plant volatiles that are released by intact plants at low levels (Schütz *et al.*, 1997) or at higher levels at the wound site in response to artificial damage or damage by insect feeding

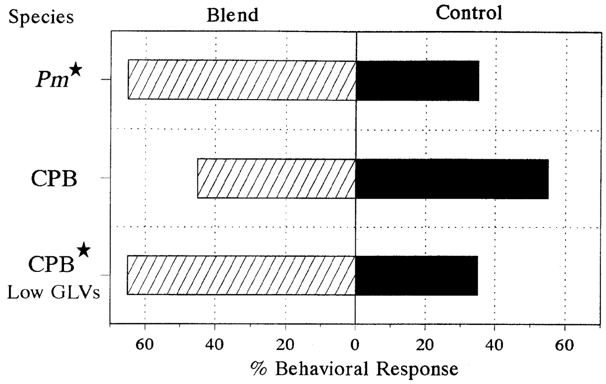

Figure 4 Laboratory behavioural responses (% of total number of insects, $n = 40$, 20 males + 20 females for each treatment) of the Colorado potato beetle (CPB) *Leptinotarsa decemlineata,* and the generalist predator, *Podisus maculiventris* (*Pm*), to a five-component blend of volatiles emitted by potato plants comprised of (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol, nonanal, (±)-linalool, and methyl salicylate prepared in paraffin oil (25 mL of ≈0.5 mg/mL of each compound) vs. a solvent control (25 mL of paraffin oil). Behavioural responses of CPB to the same five-component blend with decreased quantities of (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol (25 mL of a ≈0.5 mg/mL of each compound except (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol which were 0.05 mg/mL) (CPB Low GLVs). Star indicates response to blend differs significantly from control ($P < 0.05$, hypothesis on proportions based on standard normal distribution). See text for details.

(Turlings *et al.*, 1990; Schütz *et al.*, 1997). While GLVs alone would not be a reliable indicator of potential prey for a hungry predator, it is likely, as shown in behavioural bioassays presented here, that their presence would not interfere with prey location.

In general, reliable coupled GC/EAD responses were present for the same compounds for the insect herbivore (CPB), its generalist (*Pm*) and specialist (*Pb*) predators (Fig. 2). With the exception of responses to (*Z*)-3-hexenyl butyrate, responses of *Pb* nearly mirrored those of its prey CPB to (±)-linalool, nonanal, methyl salicylate and indole, while responses of *Pm*, although present, were generally smaller. Both *Pm* and *Pb* were sensitive to (*Z*)-3-hexenyl butyrate at this dose. (*Z*)-3-hexenyl butyrate is released by potato plants (var. Surprise) after feeding by CPB larvae within 2 h but is not present 24 h following removal of the larvae from the plant (Bolter *et al.*, 1997). The presence of (*Z*)-3-hexenyl butyrate and green leaf volatiles mentioned above could signal to the predators the presence of actively feeding prey.

Linalool, nonanal, methyl salicylate and indole are emitted by potato plants following several hours of larval feeding, and continue to be emitted by the plants even 24 h following removal of the larvae (Bolter *et al.*, 1997). Dose–response curves to these systemic compounds show that the predators (*Pm* and *Pb*) are in each instance more sensitive to them than the herbivore (CPB) (Fig. 3). This increased sensitivity of both the generalist and specialist predators for these compounds would enhance their ability to locate their prey or prey habitat since the compounds may continue to be emitted 24 h following prey removal. It should also be noted that sensitivity of the specialist, *Pb*, to these systemic compounds generally slightly exceeded sensitivity of the generalist, *Pm*, and responses of *Pb* males were significantly greater that either *Pb* females or *Pm* for (±)-linalool and indole (Fig. 3). Sensitivity of CPB to these compounds would also be important due to its narrow range of suitable host plants.

Chemical signals used by generalist predators to locate prey on different host plants may be similar. Linalool (a product of the isoprenoid pathway) and indole (a product of the trytophan pathway and a precursor of the plant hormone indole acetic acid), both synthesized *de novo* in response to insect feeding on cotton plants (Paré & Tumlinson, 1997a,b), are released by potato plants following removal of CPB larvae (Bolter *et al.*, 1997). (*Z*)-3-hexenyl butyrate and (*Z*)-3-hexenyl acetate are released systemically in response to insect feeding on cotton (Paré & Tumlinson, 1998) and constitutively by potatoes in response to feeding by larval CPB (Bolter *et al.*, 1997).

The attraction of CPB and a generalist (*Pm*) predator to the blend of constitutive and systemic volatiles emitted by potato plants is not surprising (Fig. 4). It is already known that CPB are attracted by both artificially damaged and insect damaged plants (Bolter *et al.*, 1997). Since CPB is narrowly oligophagous, a blend of volatiles might enhance the specificity of the signal given off by the plant in a turbulent environment, much like blends of chemicals used by other insects in sexual communication. Attraction of the generalist predator to the same blend would ensure the presence of prey feeding on the plant. Preliminary bioassays indicated that the specialist predator, *Pb*, might also be attracted to the blend ($n = 8$; 75% to five-component blend).

The lack of attractiveness to CPB of the five-component blend with the high dose of the GLVs (*E*)-2-hexen-1-ol and (*Z*)-3-hexen-1-ol, could have adaptive value. For example, relatively high concentrations of GLVs would occur at the initiation of feeding by conspecific CPB and other herbivores (Bolter *et al.*, 1997). These high concentrations of GLVs may facilitate spacing of the insects on the plant, thus enhancing resource utilization. Previously, it was shown that CPB were attracted by mature potato plants; this attraction could be masked by addition of other host plant chemicals such as GLVs that changed the proportions of natural odour components (Visser & Avé, 1978). While we made no effort to duplicate natural potato odour, we did observe a change in behaviour of CPB in response to two odourous blends differing only in quantities of green leaf volatiles (Fig. 4).

In conclusion, both the CPB and its predators are sensitive to specific components of volatile blends emitted by potato plants. The herbivore (CPB) has olfactory receptors which are more sensitive to constitutive volatiles, e.g. GLVs, while both generalist (*Pm*) and specialist (*Pb*) predators are more sensitive to systemic volatiles produced in response to prey feeding. Both herbivore and predators are attracted to a blend of volatile compounds emitted by potato plants. Additional behavioural bioassays and field tests are underway to determine other attractive blends for use in modification of the behaviour of CPB, *Pm* and *Pb*, in potato ecosystems. The discovery of a synthetic attractant for CPB and knowledge of chemical cues used by its predators should lead to biorational control measures and novel management strategies for pestiferous CPB populations.

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References

Acree, T. & Arn, H. (1997) *Flavornet*. Cornell University, Ithaca, NY. *http: //www.nysaes.cornell.edu/flavornet/*

Aldrich, J.R., Kochansky, J.P. & Abrams, C.B. (1984) Attractant for a beneficial insect and its parasitoids: pheromone of a predatory spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae). *Environmental Entomology*, **13**, 1031–1036.

Aldrich, J.R., Oliver, J.E., Lusby, W.R. & Kochansky, J.P. (1986) Identification of male-specific exocrine secretions from predatory stink bugs (Hemiptera, Pentatomidae). *Archives of Insect Biochemistry and Physiology*, **3**, 1–12.

Aldrich, J.R., Zanuncio, J.C., Vilela, E.F., Torres, J.B. & Cave, R.D. (1997) Field tests of predaceous pentatomid pheromones and semiochemistry of *Podisus* and *Supputius* species (Hemiptera: Pentatomidae: Asopinae). *Anais de Sociedade Entomologica du Brasil*, **26**, 1–14.

Bolter, C.J., Dicke, M., Van Loon, J.J.A., Visser, J.H. & Posthumus, M.A. (1997) Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. *Journal of Chemical Ecology*, **23**, 1003–1023.

Brase, C.H. & Brase, C.P. (1983). *Understanding Statistics*. D. C. Heath and Co., Lexington, MA.

De Wilde, J., Hille Ris Lambers-Suverkropp, K. & Van Tol, A. (1969) Responses to airflow and airborne plant odour in the Colorado beetle. *Netherlands Journal of Plant Pathology*, **75**, 53–57.

Dicke, M., Sabelis, M.W., Takabayashi, J., Bruni, J. & Posthumus, M.A. (1990b) Plant strategies of manipulating predator–prey interactions through allelochemicals: prospects for application in pest control. *Journal of Chemical Ecology*, **16**, 3091–3118.

Dicke, M., Van Beek, T.A., Posthumus, M.A., Ben Dom, N., Van Bokhoven, H. & De Groot, A.E. (1990a) Isolation and identification of volatile kairomone that affects acarine predator–prey interactions. *Journal of Chemical Ecology*, **16**, 381–396.

Dickens, J.C. (1978) Olfactory perception of pheromone and host-plant enantiomers by *Ips typographus* L. (Col. Scolytidae). *Entomologia experimentalis et applicata*, **24**, 136–142.

Duncan, D.B. (1955) Multiple range and multiple F tests. *Biometrics*, **11**, 1–42.

Hough-Goldstein, J.A., Heimpel, G.E., Bechmann, H.E. & Mason, C.E. (1993) Arthropod natural enemies of the Colorado potato beetle. *Crop Protection*, **12**, 324–334.

Jermy, T. & Butt, B.A. (1991) Method for screening female sex pheromone extracts of the Colorado potato beetle. *Entomologia experimentalis et applicata*, **59**, 75–78.

Kovàts, E. (1958) Gas-chromatographische Charakterisierung organisher Verbindungen. *Helvetica Chimica Acta*, **41**, 1915–32.

Levinson, H.Z., Levinson, A.R. & Jen, T.-L. (1979) Sex recognition by a pheromone in the Colorado beetle. *Naturwissenschaften*, **66**, 472–473.

McIndoo, N.E. (1926) An insect olfactometer. *Journal of Economic Entomology*, **19**, 545–571.

McPherson, J.E. (1982) *The Pentatomidae (Hemiptera) of Northeastern North America with Emphasis on the Fauna of Illinois*. University Press, Carbondale, IL.

O'Neil, R.J. (1988) A model of predation by *Podisus maculiventris* (Say) on Mexican bean beetle, *Epilachina varivestis* Mulsant, in soybeans. *Canadian Entomologist*, **120**, 601–8.

Ostle, B. (1969) *Statistics in Research*. Iowa State University Press, Ames, Iowa.

Otto, D. (1996) Further evidence for the presence of a female sex pheromone in the Colorado potato beetle *Leptinotarsa decemlineata* Say. and its biological characterization. *Practice Oriented Results on Use and Production of Neem Ingredients and Pheromones IV* (ed. by H. Kleeberg and V. Micheletti), pp. 135–147. Trifolio-M GmbH.

Paré, P.W. & Tumlinson, J.H. (1997a) *De novo* biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiology*, **114**, 1161–1167.

Paré, P.W. & Tumlinson, J.H. (1997b) Induced synthesis of plant volatiles. *Nature*, **385**, 30–31.

Paré, P.W. & Tumlinson, J.H. (1998) Cotton volatiles synthesized and released distal to the site of insect damage. *Phytochemistry*, **47**, 521–526.

Payne, T.L. (1975) Bark beetle olfaction. III. Antennal olfactory responsiveness of *Dendroctonus frontalis* Zimmerman and *D. brevicomis* Le Conte (Coleoptera: Scolytidae) to aggregation pheromones and host tree terpene hydrocarbons. *Journal of Chemical Ecology*, **1**, 233–242.

Röse, U.S.R., Lewis, W.J. & Tumlinson, J.H. (1998) Specificity of systemically released cotton volatiles as attractants for specialist and generalist parasitic wasps. *Journal of Chemical Ecology*, **24**, 303–319.

Saint-Cyr, J.-F. & Cloutier, C. (1996) Prey preference by the stinkbug *Perillus bioculatus*, a predator of the Colorado potato beetle. *Biological Control*, **7**, 251–258.

Sant'Ana, J., Bruni, R., Abdul-Baki, A.A. & Aldrich, J.R. (1997) Pheromone-induced movement of nymphs of the predator, *Podisus maculiventris* (Heteroptera: Pentatomidae). *Biological Control*, **10**, 123–128.

Schanz, M. (1953) Der Geruchssinn des Kartoffelkäfers (*Leptinotarsa decemlineata* Say). *Zeitschrift für Vergleichende Physiologie*, **35**, 353–379.

Schneider, D. (1957) Elektrophysiologische Untersuchungen von Chemo-und Mechanorezeptoren der Antenne des Seidenspinners *Bombyx mori* L. *Zeitschrift für Vergleichende Physiologie*, **40**, 8–41.

Schütz, S., Weiβbecker, B., Klein, A. & Hummel, H.E. (1997) Host plant selection of the Colorado potato beetle as influenced by damage induced volatiles of the potato plant. *Naturwissenschaften*, **84**, 212–217.

Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science (Wash.)*, **250**, 1251–1253.

Visser, J.H. (1976) The design of a low-speed wind tunnel as an instrument for the study of olfactory orientation in the Colorado potato beetle (*Leptinotarsa decemlineata*). *Entomologia experimentalis et applicata*, **20**, 275–288.

Visser, J.H. & Avé, D.A. (1978) General green leaf volatiles in the olfactory orientation of Colorado beetle, *Leptinotarsa decemlineata*. *Entomologia experimentalis et applicata*, **24**, 538–549.

Visser, J.H. & Piron, P.G.M. (1998) An open Y-track olfactometer for recording aphid behavioural responses to plant odours. *Proceedings of the Section Experimental and Applied Entomology of the Netherlands Entomological Society Amsterdam*, **9**, 41–46.

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