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GENERAL GREEN LEAF VOLATILES IN THE OLFACTORY ORIENTATION OF THE COLORADO BEETLE, *LEPTINOTARSA DECEMLINEATA*

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The odour of fully grown potato plants elicits a positive anemotactic response in Colorado beetles, but none of the individual component volatiles alone is attractive. Certain component chemicals or isomers of them disrupt orientation to the host plant, probably by changing the relative proportion of the odour components. These general green leaf volatiles probably occur as important constituants of leaf odours attractive to various phytophagous insects. Such a complex represents "green odour" and confers different modalities on the leaf odours of diverse plant species.

Host selection behaviour of phytophagous insects is a catenary process, which is completed in the acceptance of a plant suitable for oviposition and/or feeding (Schoonhoven, 1968). Because of the spectacular selectivity of oligophagous insects in the final phase, selection behaviour has mainly been described in terms of "acceptance and rejection", resulting in "host plant recognition". Dual discrimination of both nutrients and secondary plant substances is thought to be crucial for the establishment of an insect on its host plant (see Beck, 1965). Acceptance of a plant is brought about at optimal concentrations of a feeding incitant — a "token stimulus" — of feeding stimulants and co-factors, provided that deterrents are absent.

The host plant specificity of the Colorado beetle, *Leptinotarsa decemlineata* Say, has been explained in this way (de Wilde, 1958; Hsiao, 1969). The isolation of a feeding incitant for this insect has been reported several times (Chauvin, 1945; Yamamoto & Fraenkel, 1960; Hsiao & Fraenkel, 1968). However, attempts to purify this "incitant" have failed and its chemical identity remains unsolved, throwing doubts upon its very existence (Ritter, 1967). Since the Colorado beetle feeds on solanaceous plant species lacking deterrents like demissine, tomatine and other related alkaloid-glycosides (Schreiber, 1958), the specificity of this oligophagous insect has been described in terms of rejection rather than acceptance (Jermy, 1961, 1966; Hsiao, 1974).

Since oligophagous insects searching for oviposition and/or feeding sites, are likely to meet suitable plants less frequently than their polyphagous counterparts, they would seem to need means of greater selectivity in approaching plants, as well as after arrival in the final stage of selection. This indicates that in spite of the selectivity in this final phase, host selection behaviour of oligophagous insects may not be explained as merely acceptance and rejection. An initial olfactory orientation which is directed to a relevant part of the vegetation, would increase the effectiveness of host selection behaviour.

The initial olfactory orientation of the Colorado beetle is directed mainly towards solanaceous plant species, thus restricting the final part of the selection process (Visser & Nielsen, 1977). The main components in the essential oil of potato leaves are trans-2-hexen-1-ol, hexanol-1, cis-3-hexen-1-ol, trans-2-hexenal and linalool (Visser *et al.*, 1978). Electroantennogram recordings show that the olfactory system used by the Colorado beetle in olfactory orientation towards its host plant potato, is selective and has a high sensitivity to these generally distributed leaf volatiles (Visser, 1979).

The present study illustrates the effects of these chemicals and some of their isomers, on olfactory orientation of the Colorado beetle, and outlines their rôle in the context of our present knowledge.

MATERIAL AND METHODS

Newly-emerged Colorado beetles were obtained from the laboratory stock culture, and starved for 24 h prior to the experiments. The behavioural responses of individual female beetles to wind-borne volatiles were observed in a low-speed wind tunnel, described in detail by Visser (1976). This instrument (Fig. 1) is housed in a controlled environment room at 25° and 50% R.H. A centrifugal ventilator

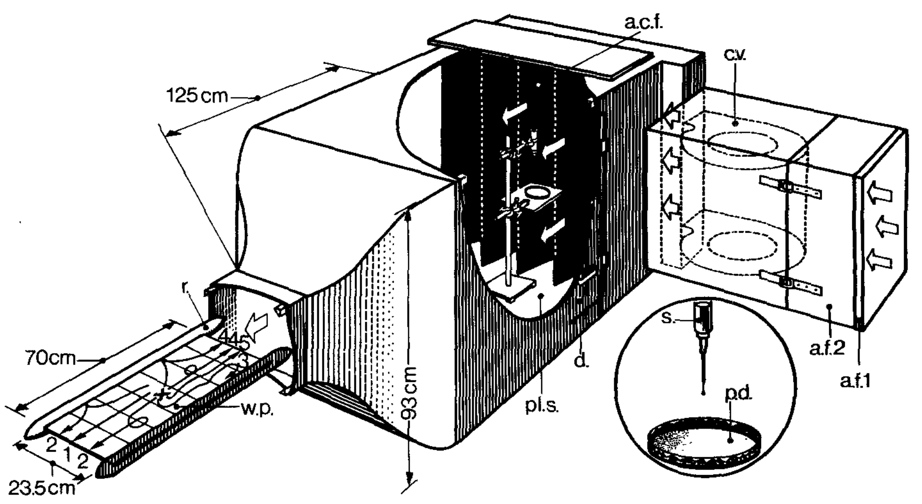


Fig. 1. Diagram of wind tunnel. a.c.f.: activated charcoal filter; a.f.1 — a.f.2.: air filters; c.v.: centrifugal ventilator; d.: door; p.d.: petri dish; pl.s.: plant section; r.: streamlined rails; s.: syringe; w.p.: walking plate. Outlines of insect test section not shown. A support holding syringe filled with a test chemical solution, stands in plant section. Inset illustrates the way test chemical is released into the wind. Arrows indicate direction of air flow. Beetles were released at the cross drawn on walking plate. 1—5: categories of response. See text for further explanation.

sucks air through two air filters, the air is purified by an activated charcoal filter and flows into the plant section. This dark section held the odour source: four pots of fully grown potato plants (cultivar Eigenheimer), and a support holding a syringe from which test chemicals could be injected into the air stream. The syringe was filled with pure paraffin oil (Merck Uvasol) in control treatments, or with a test chemical solution in paraffin oil in experimental situations. After the plunger had been removed, the solution dripped out of the syringe at a rate of 1.1 ml per hour into a petri dish, thus releasing volatiles continuously into the wind. In order to maintain the vapour concentration within certain limits in the course of an experiment, the petri dish was replaced every hour. The concentrations of the test chemicals in paraffin oil refer to the concentrations of the original solutions (volume/volume). The vapours were distributed over the total cross-sectional area of the wind tunnel by turbulence, which originated from the folded construction of the activated charcoal filter. This resulted in a uniform vapour concentration in the insect test section, downwind of the odour source (Visser, 1976). A glass walking plate was fixed with streamlined rails at half height in the test section, where the velocity of the main stream was set at 80 cm/sec.

In each experiment one beetle was placed in the centre of the walking plate. After it started walking, it was observed for 5 min., or until it reached one of the edges. So as to discriminate the behavioural responses, the observed tracks were divided into five categories, reported in detail by Visser & Nielsen (1977): 1 — straight to the downwind edge; 2 — indirectly to the downwind edge; 3 — reaching neither of the edges; 4 — indirectly to the upwind edge; 5 — straight to the upwind edge. In addition, the time needed to reach the upwind edge was noted. The same beetle was subjected to control and experimental situations. The resultant scores were analysed statistically by the Sign Test (Siegel, 1956) on the basis of the categories of responses called criterion A, and the periods required to reach the upwind edge called criterion B.

The test chemicals were obtained from commercial sources: the hexenols (98%—99%) from Roth, trans-2-hexenal (99%) from Koch-Light Lab., linalool (99%) from Fluka and the hexanols (96%—98%) from Merck.

RESULTS AND DISCUSSION

The individual responses of female Colorado beetles to wind, to wind-borne potato plant odour, and to wind supplied with the vapours of test chemicals, are shown in Table I. The potato plants strongly attract the beetles. The host plant odour elicits positive anemotactic responses in these insects; odour-conditioned positive anemotaxis is illustrated in the shift of the response categories from indifferent (3) to moving upwind (4 and 5); its significance is expressed in criterion A. Potato plant odour also increases the beetle's speed of locomotion or direct chemo-orthokinetic response; its significance is reflected in criterion B. As a consequence, the beetles reach the upwind edge faster in wind with potato plant odour than in an air flow devoid of vapours. The behavioural responses of

TABLE I

Individual responses of female Colorado beetles to wind, to wind plus potato plant odour and to wind plus test chemicals[1]

	Category of response					Compared with wind Criterion		Compared with potato Criterion	
	1	2	3	4	5	A	B	A	B
wind	0	5	21	4	0				
potato	0	0	1	23	6	**	**		
cis-3-hexen-1-ol	0	4	19	7	0	ns	ns	**	**
wind	0	1	13	2	0				
potato	0	0	0	13	3	**	**		
trans-3-hexen-1-ol	0	3	12	1	0	ns	ns	**	**
cis-2-hexen-1-ol	1	4	7	4	0	ns	ns	**	**
wind	0	5	9	2	0				
potato	0	0	1	7	8	**	**		
trans-2-hexenal	0	3	10	3	0	ns	ns	**	**
wind	0	2	12	2	0				
potato	0	0	1	10	5	**	**		
trans-2-hexen-1-ol	0	5	10	1	0	ns	ns	**	**
linalool	0	5	9	2	0	ns	ns	**	**
wind	0	2	10	3	0				
potato	0	0	0	10	5	**	**		
hexanol-1	0	6	6	3	0	ns	ns	**	**
hexanol-2	0	7	5	3	0	ns	ns	**	**
hexanol-3	1	5	6	3	0	ns	ns	**	**

[1] Concentration of test chemical is 1 μl/ml of paraffin oil, 10^{-3}.
ns: not significant at $P > 0.01$
**: significant at $P < 0.001$

Colorado beetles to the test chemicals are neutral, these individual volatiles being neither attractive nor repellent.

In spite of these "negative" results, further experiments were conducted in order to assess possible effects of these volatiles on the orientation response of Colorado beetles to potato plants.

Female beetles were tested successively with wind alone, with wind-borne potato plant odour, and with wind bearing both potato plant odour and the vapour of a test chemical (Fig. 2). In a control experiment Colorado beetles were tested once in wind and twice with wind-borne host plant odour, the interval between the first and second test with potato odour being 6 hr (Fig. 2a). It is observed that in the course of this experiment, the behavioural responses of Colorado beetles to the attractive potato plant odour do not change. Potato plants combined with trans-3-hexen-1-ol, cis-2-hexen-1-ol, trans-2-hexen-1-ol or trans-2-hexenal (Fig. 2c, d, f, h, j) are not attractive, that is the beetles no longer react with an odour-conditioned positive anemotaxis (criterion A). Thus, a disruption of the olfactory orientation is attributed to the chemicals applied. The geometrical isomer trans-3-hexen-1-ol is the most effective chemical in this respect (at both 10^{-3} and 10^{-4} in paraffin oil). It is striking that direct orthokinetic responses of the beetles are not affected by these chemicals (criterion B). The beetles are more active than in a pure air flow, consequently several beetles reach the upwind edge of the walking plate. At the

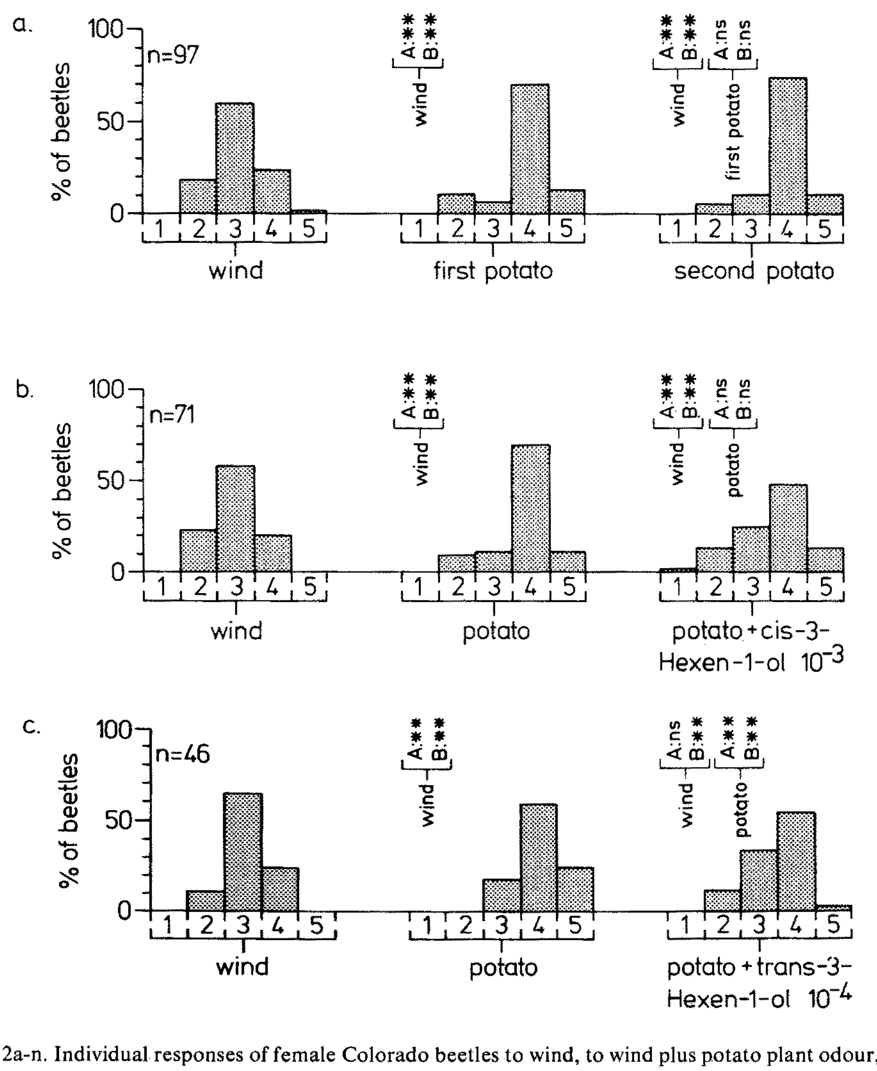
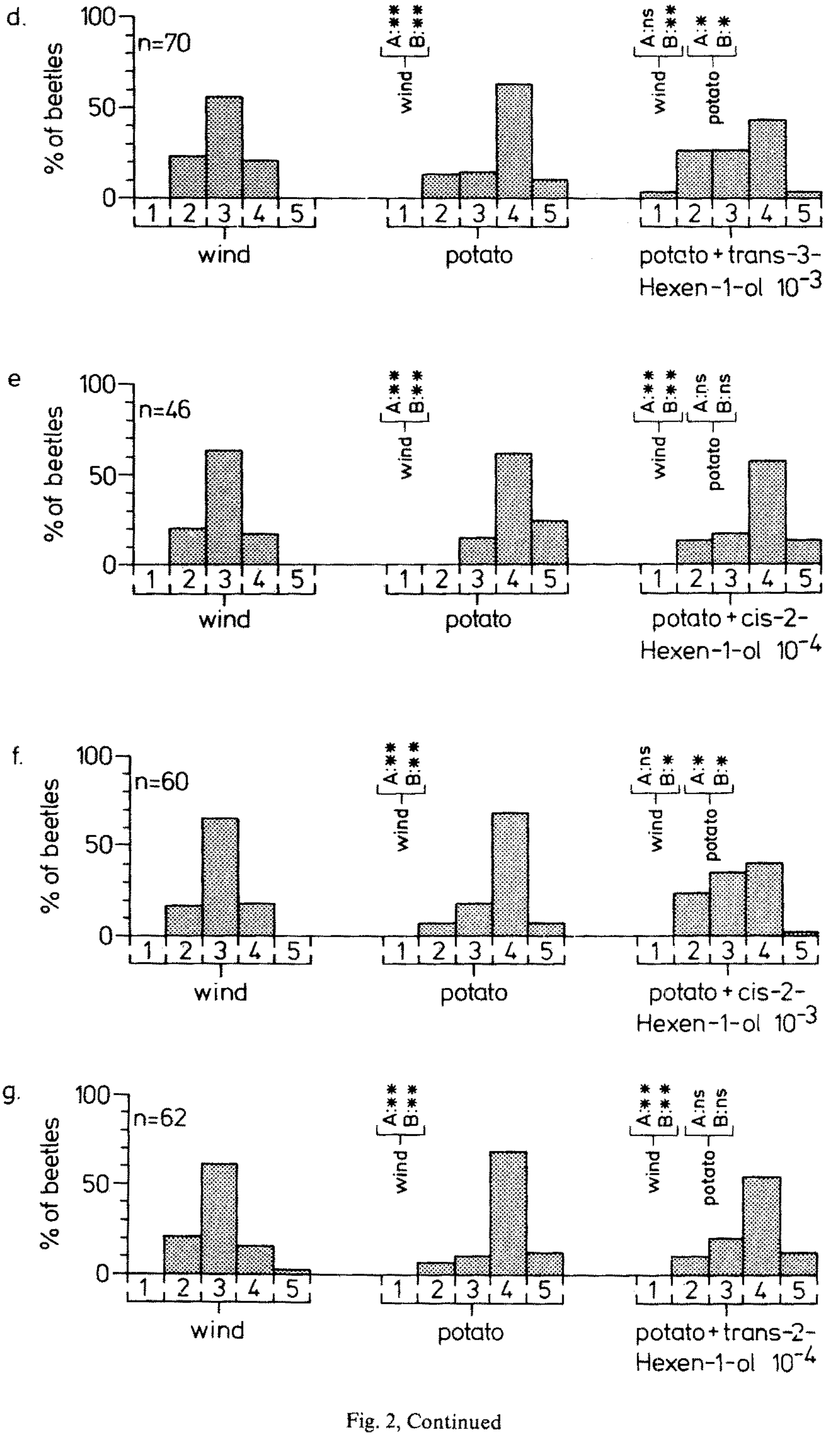


Fig. 2a-n. Individual responses of female Colorado beetles to wind, to wind plus potato plant odour, and to wind plus a combination of potato plant odour and a test chemical. Concentrations refer to test chemical solutions in paraffin oil (v/v). n. number of individuals tested. 1—5: categories of response. Response criteria A. and B. (see text) are compared with responses to wind and to potato. ns: not significant at $P > 0.01$; * : significant at $P < 0.01$; ** : significant at $P < 0.001$..



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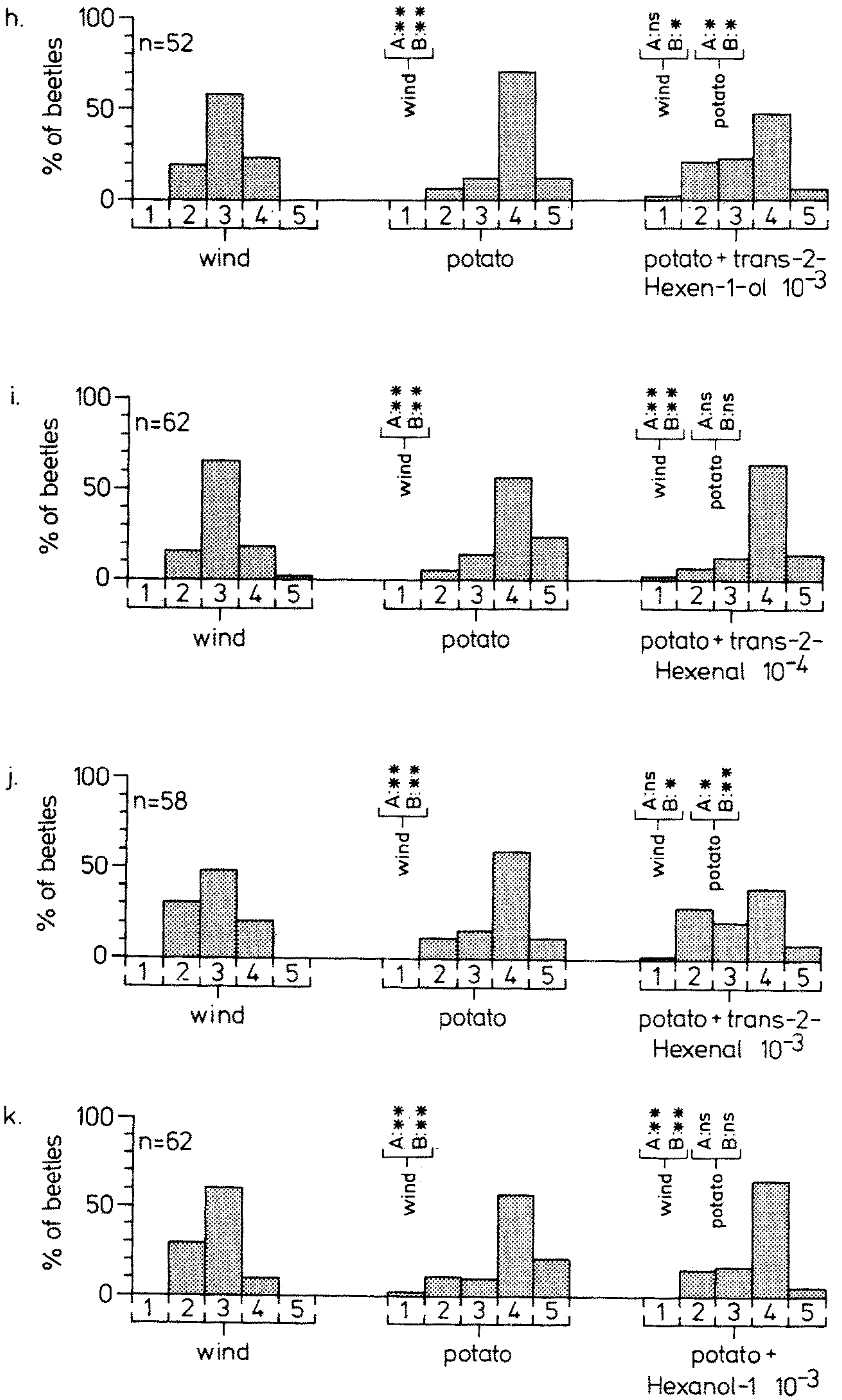

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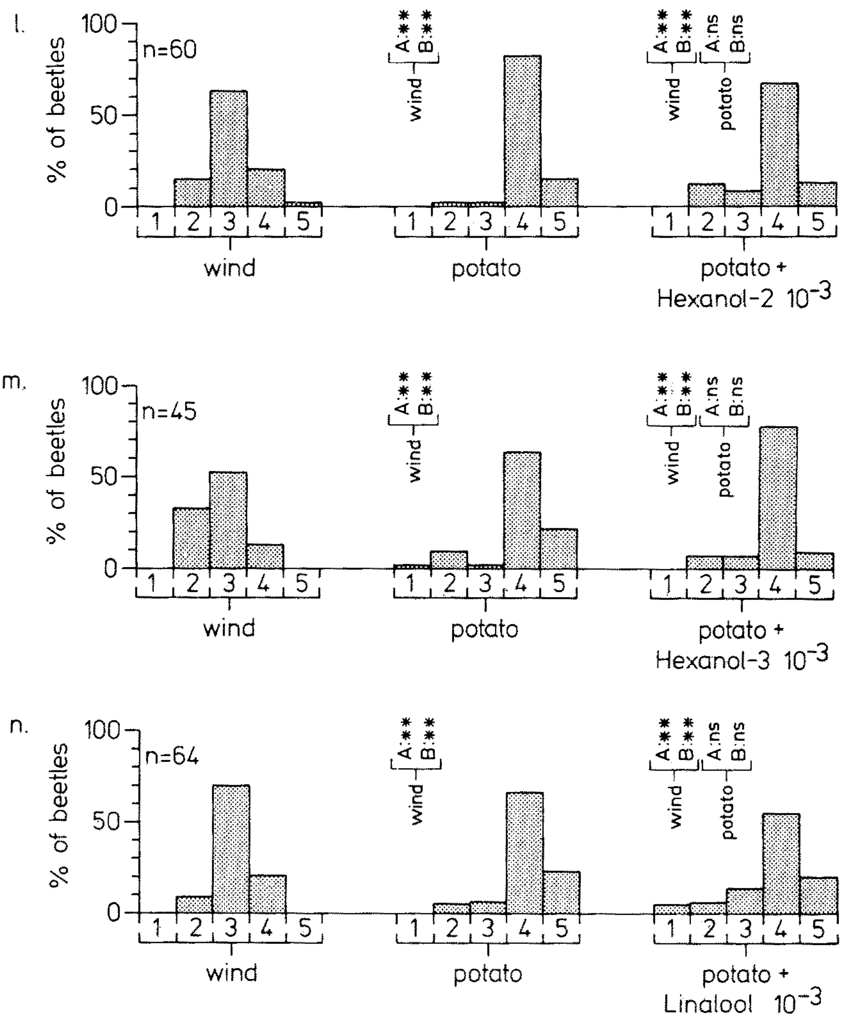

Fig. 2, Continued

concentration tested (10^{-3}), cis-3-hexen-1-ol, hexanol-1, hexanol-2, hexanol-3 and linalool (Fig. 2b, k, l, m, n) do not affect either of the responses of Colorado beetles to potato plant odour.

Duality of long-range olfactory orientation

In distant attraction, for the main part, odour-conditioned anemotaxis guides insects towards the odour source. The primary effect of attractive volatiles on insects, a chemo-orthokinesis, is also a requisite for this behaviour (see review by Kennedy, 1977). However, when chemo-orthokinesis is separated from the anemotactic responses its effectiveness in long-range olfactory orientation is doubtful. Several volatiles "turn off" the odour-conditioned anemotaxis in Colorado beetles, but do not affect the orthokinetic responses, underlining the duality in long-range olfactory orientation. Thus, potato plant odour combined with some chemicals, is no longer effective for distant attraction.

Effective concentration

The geometrical isomer trans-3-hexen-1-ol interferes with the host plant odour at a concentration as low as 100 ppm in the syringe positioned upwind, and when released into the air flow this chemical is diluted, the resultant vapour concentration in the wind of the test section is at most 2.3×10^9 molecules per ml of air. As this concentration is near the threshold for an electroantennogram response of the Colorado beetle (2.3×10^8 molecules per ml of air at the same wind speed; Visser, 1979), the observed interference with potato plant odour is not caused by extreme stimulus strengths.

Masking of potato plant odour

The air over cut fully grown potato leaves, which is attractive to Colorado beetles (see e.g. Schanz, 1953; de Wilde *et al.,* 1969), differs from the essential oil (Visser *et al.,* 1978) in that it contains cis-3-hexen-1-ol (100%), cis-3-hexenylacetate (59%), trans-2-hexenal (37%) and trans-2-hexen-1-ol (16%), as identified by GLC-MS studies (Visser & Schaefer, unpubl.). The geometrical isomers trans-3-hexen-1-ol and cis-2-hexen-1-ol are not detected in either the essential oil or the headspace of cut potato leaves. The potato leaf volatiles trans-2-hexen-1-ol and trans-2-hexenal, and the geometrical isomers trans-3-hexen-1-ol and cis-2-hexen-1-ol, interfere with the attractive potato plant odour. As none of these chemicals applied singly elicits repellent responses in Colorado beetles, it is concluded that they mask the host plant odour. This masking is explained as a disturbance of the attractive complex by artificially changing the relative proportions of the components. The particular ratio of components, constituting an essential aspect of the attractive odour, is distorted by adding small quantities of its minor components, trans-2-hexen-1-ol and trans-2-hexenal. The geometrical isomers trans-3-hexen-1-ol and cis-2-hexen-1-ol may interfere with the perception of this complex. This has an obvious resemblance to the distortion of insect pheromone blends, as their ratios are changed or isomers added (Birch, 1974). A complex of volatiles rather than one single compound, is essential for olfactory orientation of the Colorado beetle towards its host plant.

General green leaf volatiles

The components involved in the attraction of Colorado beetles, also constitute a considerable proportion of the leaf vapours of numerous plant species (Visser *et al.,* 1978). They are formed by oxidative degradation of leaf lipids, and their biosyntheses are illustrated in Fig. 3. Owing to plant ageing and injury these volatiles are continuously released into the surrounding air. The observation that fully grown potato plants are more attractive to Colorado beetles than young plants

(Visser, 1976), may be related to the increase in the levels of these volatiles with age (Visser & Schaefer, unpubl.).

These general green leaf volatiles elicited behavioural responses in several phytophagous insects. Silkworm larvae, *Bombyx mori* are attracted by 3-hexen-1-ol and 2-hexenal (Watanabe, 1958). Adults of the vegetable weevil *Listroderes costirostris obliquus,* were attracted by 3-hexen-1-ol, and both 3-hexen-1-ol and hexanol-1 attracted the larvae (Matsumoto & Sugiyama, 1960). Trans-2-hexenal isolated from oak leaves, is an incitant for the "calling" behaviour of female

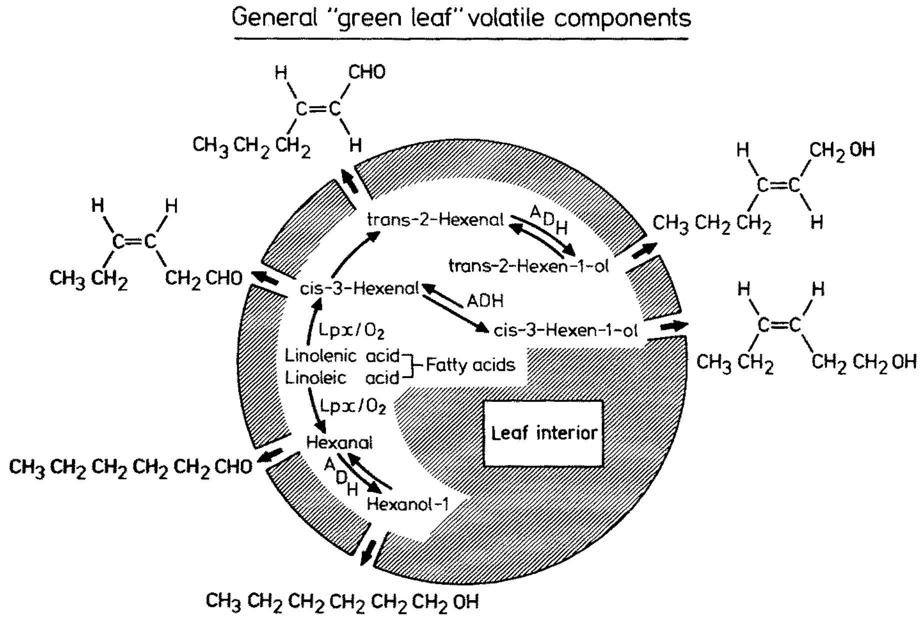

Fig. 3. Biosyntheses of the general green leaf volatiles (Visser *et al.*, 1978). ADH: Alcoholdehydrogenase; Lpx: Lipoxygenase.

polyphemus moths, *Antheraea polyphemus* (Riddiford, 1967). The phagostimulatory nature of *Solanum campylacanthum* for the larvae of *Epilachna fulvosignata* is attributed to a fraction of the essential oil, containing hexanol-1, cis-3-hexen-1-ol and cis-2-hexen-1-ol (Murray *et al.*, 1972), however data supporting the biological activity were not presented, and the identification of cis-2-hexen-1-ol is controversial (Visser *et al.*, 1978). The known behavioural responses to these components, and the more extended information concerning their perception by insects (see Visser, 1979), point to the conclusion that the complex of general green leaf volatiles represents "green odour" for various phytophagous insects.

Modality

The relative proportions of the components in the "green odour" complex vary in and between different plant species, as influenced by the expression of the enzymes involved. The predominant vapour component of several cruciferous species is cis-3-hexenylacetate, while cis-3-hexen-1-ol and hexylacetate are minor constituents and trans-2-hexenal is absent (Wallbank, 1972; Wallbank & Wheatley, 1976). In this way the cruciferous "green odour" contrasts with the potato "green odour", and accordingly different modalities, or qualities, are conferred on the "green odours" of diverse plant species.

As a consequence, the "background odour" of other plant species in the field may prevent an initial olfactory orientation of the Colorado beetle towards susceptible plants by masking the attractive "green odour modality" peculiar to these susceptible plants. This interference should depend both on the density and variety of plants and the opposing natures of the several "green odours" involved. Information concerning the capability of the antennal olfactory system of the Colorado beetle to discriminate different modalities, is presented by Ma & Visser (1978).

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RÉSUMÉ

SUBSTANCES VOLATILES DES FEUILLES VERTES ET ORIENTATION OLFACTIVE CHEZ LE DORYPHORE, LEPTINOTARSA DECEMLINEATA

On a cherché à déterminer l'effet sur l'orientation olfactive de doryphores femelles des composants principaux identifiés dans l'huile des feuilles de pomme de terre, trans-2-hexen-1-ol, hexanol-1, cis-3-hexen-1-ol, trans-2-hexenal et linalool, et les isomères trans-3-hexen-1-ol, cis-2-hexen-1-ol, hexanol-2 et hexanol-3. Si des pieds de pomme de terre ayant atteint leur développement complet provoquent des réactions anémotactiques positives, causées par leur odeur, chez le doryphore, aucune de ces substances chimiques, appliquée isolément, n'est attrayante. Associés à des pieds de pomme de terre, les composés suivants masquent l'odeur attrayante de la plante-hôte: trans-2-hexen-1-ol, trans-2-hexenal, trans-3-hexen-1-ol et cis-2-hexen-1-ol. L'isomère géométrique trans-3-hexen-1-ol est à cet égard la substance la plus efficace. Les doryphores ne réagissent plus par une anémotaxie provoquée par l'odeur, mais ils sont pourtant activés. Le masquage de l'odeur de la plante-hôte s'explique par une perturbation du complexe attrayant par suite du changement des proportions relatives de ses composants. Ces substances volatiles générales des feuilles vertes constituent probablement une partie importante des odeurs de feuille qui attirent différents insectes phytophages et représentent "l'odeur verte".

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