

SPECIFICITY IN THE OLFACTORY ORIENTATION OF THE COLORADO BEETLE, *LEPTINOTARSA DECEMLINEATA*

BY

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Wind induces positive anemotactic responses of unfed newly-emerged female Colorado beetles. These responses are enhanced by the volatiles of several solanaceous species i.e. *Solanum tuberosum*, *S. nigrum*, *S. luteum*, *S. dulcamara*, *S. lycopersicum*, *Nicotiana tabacum*, *Capsicum annum* and *Petunia hybrida*, and a non-solanaceous species, *Tropaolum majus*. *Brassica oleracea*, *B. pekinensis*, *Raphanus sativus* and *Phaseolus vulgaris* are neutral in this respect. Unfed post-diapause Colorado beetles also react to the vapours of undamaged potted potato plants. It is concluded that the olfactory orientation will mainly lead the Colorado beetle towards solanaceous species. When contact results, further steps in host plant selection will occur.

In the host selection of phytophagous insects, the existence of an initial olfactory orientation phase has not been overlooked but, owing the difficulties in experimentation, has been poorly studied. The Colorado beetle, *Leptinotarsa decemlineata* Say, is one of the exceptions in this respect. McIndoo (1926), Chin (1950), Schanz (1953), Grison (1957), Jermy (1958), de Wilde *et al.* (1969), Bongers (1970) and de Wilde (1974) reported attraction of larvae and adult Colorado beetles by potato plant volatiles. However, the rôle of this attraction in the host selection of this oligophagous insect was estimated very unequally. Most of this divergence is related to the various methods of stimulus application. Visser (1976) described the design of a low-speed wind tunnel, and making use of this equipment, he showed attraction of adult Colorado beetles towards potted potato plants being caused by odour-conditioned anemotaxis. The wind, dispersing the plant volatiles through the atmosphere, guides the insects towards the source of the stimulus. Under field conditions, the scale of turbulence and the thickness of the boundary layer over the underlying substrate will impair anemotactic responses of insects.

The present study gives more extended information of the olfactory orientation of adult female Colorado beetles under laboratory conditions. Our observations concentrate on the specificity in this initial phase of host selection.

MATERIAL AND METHODS

Female Colorado beetles were obtained from the laboratory stock culture. After emergence they were separated from the males. Prior to the experiments they

were isolated in vials and starved for several hours at an 18 hour photoperiod, 25° and 60% relative humidity. In the wind tunnel described by Visser (1976), the responses of these individuals to wind-borne volatiles of several plant species were examined. This low-speed wind tunnel comprises two main parts, namely a low velocity compartment and the insect test section. In experiments on olfactory orientation, undamaged potted plants were placed in the low velocity compartment at a distance of 125 cm from the test section. Small plants were raised, so that the foliage was positioned at half height in this section. In the insect test section, a rough glass walking plate was fixed with streamlined rails, and was illuminated from above by six fluorescent lamps (Philips TL MF 40W/33RS). The distribution of light intensities over the walking plate was measured by a Hartmann & Braun EBLX3 photometer, fitted with a circular detector of 66 mm diameter, and is shown in Fig. 1. Six red lines, spaced equally at 10 cm intervals, were drawn on the underside of the walking plate (see Fig. 2).

In each experiment one female beetle was placed carefully in the centre of the walking plate and was allowed to walk around. After the beetle started locomotion, it was observed for 5 minutes, or until it reached one of the edges of

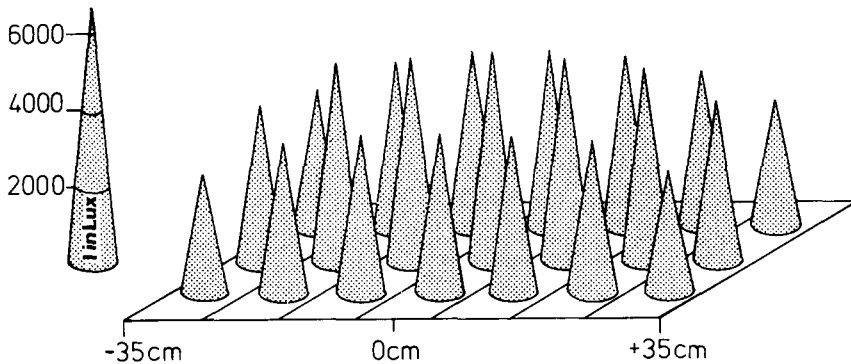


Fig. 1. The distribution of light intensities over the walking plate (represented in skew perspective). Ordinate: intensity. Abscissa: +35 cm = upwind edge, 0 cm = centre and -35 cm = downwind edge of the walking plate.

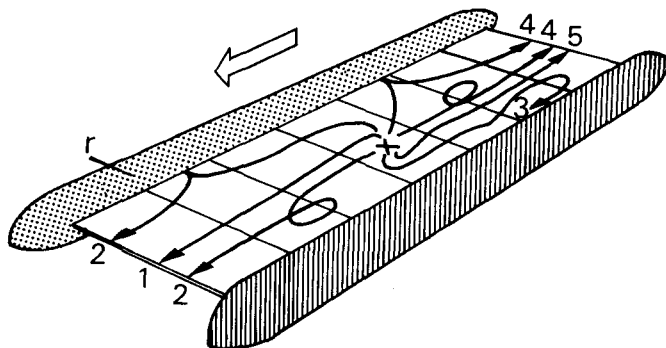


Fig. 2. Walking plate fixed with streamlined rails (*r*). Beetles were released at the cross. 1—5: category of response. Arrow indicates the direction of the air flow.

the walking plate. The tracks of the beetles were divided into five categories (Fig. 2):

1. straight to the downwind edge; crossing each of the three lines once and not touching the rails.
2. indirectly to the downwind edge; crossing a line more than once or touching the rails.
3. reaching neither of the edges.
4. indirectly to the upwind edge; crossing a line more than once or touching the rails.
5. straight to the upwind edge; crossing each of the three lines once and not touching the rails.

The same beetle was subjected to control and experimental situations. The results were analysed statistically by the Sign Test (Siegel, 1956); using criterion A, the categories of responses, and criterion B, the length of the periods required to reach the upwind edge.

Undamaged greenhouse-grown potted plants were used i.e. *Solanum tuberosum* L. (cultivar Eigenheimer), *Solanum nigrum* L., *Solanum luteum* Mill., *Solanum dulcamara* L., *Solanum lycopersicum* L., *Nicotiana tabacum* L., *Petunia hybrida* (Hook.) Vilm., *Capsicum annuum* L., *Phaseolus vulgaris* L., *Raphanus sativus* L., *Brassica oleracea* L. var. *gemmifera* DC., *Brassica pekinensis* Rupr. and *Tropaeolum majus* L.. The number of plants used per experiment was arranged to give approximately the same leaf area.

The experiments were conducted in a controlled environment room at 25° and 60% R.H.. In the insect test section the wind speed was set at 80 cm/s.

RESULTS AND DISCUSSION

Two groups of 42 female Colorado beetles each, one group starved for 24 hours and the other starved for 72 hours, were tested in still air and in wind without plant odour (Table I). Wind induced positive anemotactic responses of these unfed newly-emerged beetles (criterion A), and the individuals starved for 72 hours were

TABLE I

Individual responses of unfed newly-emerged female Colorado beetles, in still air and in wind without plant odour

		Category of response					Criterion	
		5	4	3	2	1	A	B
starved for 24 hours	no wind	0	5	26	11	0		
	wind	1	14	21	6	0	*	ns
starved for 72 hours	no wind	1	7	29	5	0		
	wind	4	19	16	3	0	*	*

ns: not significant at $P > 0.01$

*: significant at $P < 0.01$

faster in reaching the upwind edge (criterion B). Schanz (1953), de Wilde *et al.* (1969), de Wilde (1974) and Visser (1976) reported positive anemotaxis of Colorado beetles in an air flow devoid of plant vapours. However, it is plausible that this type of anemotaxis is in fact a transformed anemomenotaxis (Linsenmair, 1969 and 1973; Visser, 1976).

As the same individuals were used in different situations, it was important to make sure that the responses of individual beetles were not influenced by earlier experience in the test section of the wind tunnel. For this purpose two groups of female Colorado beetles were observed. Group A was tested successively three times in pure wind and once with wind-borne volatiles of four fully grown potato plants standing in the low velocity compartment of the wind tunnel. Group B was subjected alternately to a pure air flow and to potato plant odour (Table II). Wind-borne potato plant odour strongly attracted the unfed newly-emerged Colorado beetles. These responses and those to pure wind were not altered by previous tests. Visser (1976), making use of different criteria, showed attraction of fed male and female Colorado beetles by potato plant volatiles in this wind tunnel. In the present investigation, the light intensity over the walking plate was raised ten times (Fig. 1), without affecting the responses.

TABLE II

Effect of previous tests on the individual responses of unfed newly-emerged female Colorado beetles, starved for 24 hours

	Successive tests	Category of response					Criterion ²	
		5	4	3	2	1	A	B
group A	wind	1	7	8	2	0		
18 beetles	wind	1	4	10	3	0	ns	ns
	wind	0	4	13	1	0	ns	ns
	wind + potato	9	9	0	0	0	**	**
	group B	wind	0	6	8	3	0	
17 beetles	wind + potato	11	6	0	0	0	**	**
	wind	0	8	4	5	0	ns	ns
	wind + potato	12	5	0	0	0	**	**

²) the levels of significance were compared with the first test pure wind

ns: not significant at $P > 0.01$

** : significant at $P < 0.001$

To investigate the specificity of the olfactory orientation, female Colorado beetles were subjected to the vapours of several plant species. On the same day, six different experimental situations were presented in random order. Results from three series are given in Table III. All the solanaceous species tested i.e. *S.tuberosum*, *S.nigrum*, *S.luteum*, *S.dulcamara*, *S.lycopersicum*, *N.tabacum*, *C.annuum* and *P.hybrida*, elicited substantially more and faster upwind movements of unfed newly-emerged Colorado beetles than a pure air flow. One non-solanaceous plant species, *Tropaolum majus*, also attracted these beetles. *Brassica oleracea*,

B. pekinensis, *Raphanus sativus* and *Phaseolus vulgaris* were neutral in this respect. To a certain extent, unfed newly-emerged female Colorado beetles possess olfactory discriminative capacities, enabling them to select plants at a distance.

TABLE III

Individual responses of unfed newly-emerged female Colorado beetles, starved for 24 hours, to several plant species

	Number of plants	Category of response					Compared with wind criterion		Compared with potato criterion	
		5	4	3	2	1	A	B	A	B
wind		0	6	10	4	0				
<i>Solanum tuberosum</i>	4	5	15	0	0	0	**	**		
<i>Raphanus sativus</i>	12	2	7	10	1	0	ns	ns	*	*
<i>Brassica oleracea</i>	6	0	10	7	3	0	ns	ns	**	**
<i>Brassica pekinensis</i>	12	0	11	6	3	0	ns	ns	**	**
<i>Tropaeolum majus</i>	4	4	11	4	1	0	**	**	ns	**
wind		0	4	13	3	0				
<i>Solanum tuberosum</i>	4	7	12	1	0	0	**	**		
<i>Solanum nigrum</i>	6	3	12	5	0	0	*	*	ns	ns
<i>Solanum luteum</i>	6	6	12	2	0	0	**	**	ns	ns
<i>Solanum dulcamara</i>	4	3	14	3	0	0	**	*	ns	**
<i>Solanum lycopersicum</i>	5	4	14	2	0	0	**	**	ns	ns
wind		0	7	12	1	0				
<i>Solanum tuberosum</i>	4	6	14	0	0	0	**	**		
<i>Nicotiana tabacum</i>	4	9	11	0	0	0	**	**	ns	ns
<i>Capsicum annuum</i>	6	3	15	1	1	0	*	**	ns	ns
<i>Petunia hybrida</i>	5	9	9	2	0	0	**	**	ns	ns
<i>Phaseolus vulgaris</i>	12	2	8	8	2	0	ns	ns	*	*

ns: not significant at $P > 0.01$

*: significant at $P < 0.01$

** : significant at $P < 0.001$

In order to examine whether previous feeding will affect the specificity of olfactory orientation, newly-emerged female Colorado beetles were fed on *S. tuberosum* or *S. dulcamara* for 48 hours. Subsequently, they were starved for 24 hours and subjected to wind-borne odours of several plant species (Table IV). The number of plants used in these series were the same as mentioned in Table III. *Brassica oleracea* and *Solanum nigrum* evoked no significant attraction of the fed beetles. *S. tuberosum*, *S. luteum* and *S. dulcamara* still elicited more upwind movements of beetles fed on potato or bittersweet than wind without plant odour. However, the responses of potato-fed beetles towards *S. dulcamara* were slower than towards *S. tuberosum*. Beetles fed on bittersweet reacted equally towards potato and bittersweet. De Wilde *et al.* (1969) reported olfactory orientation of potato-fed female Colorado beetles towards leaves of *S. nigrum* and *Apium*

graveolens L.. Newly emerged female beetles were not attracted by the leaves of *A. graveolens*. It is concluded that the specificity of olfactory orientation is not distinctly affected by previous feeding of female Colorado beetles shortly after emergence.

TABLE IV

Responses of female Colorado beetles, previously fed on potato or bittersweet, to several plant species

	Category of response					Compared with wind criterion		Compared with potato criterion	
	5	4	3	2	1	A	B	A	B
fed on potato:									
wind	1	10	7	2	0				
<i>Solanum tuberosum</i>	7	13	0	0	0	**	**		
<i>Solanum nigrum</i>	1	14	5	0	0	ns	ns	*	**
<i>Solanum luteum</i>	3	14	3	0	0	**	*	ns	ns
<i>Solanum dulcamara</i>	3	16	1	0	0	*	ns	ns	**
<i>Brassica oleracea</i>	1	12	7	0	0	ns	ns	**	**
fed on bittersweet:									
wind	1	11	5	3	0				
<i>Solanum tuberosum</i>	9	11	0	0	0	**	**		
<i>Solanum nigrum</i>	4	9	6	1	0	ns	ns	*	**
<i>Solanum luteum</i>	7	13	0	0	0	*	*	ns	ns
<i>Solanum dulcamara</i>	9	11	0	0	0	**	**	ns	ns
<i>Brassica oleracea</i>	2	11	4	3	0	ns	ns	**	**

ns: not significant at $P > 0.01$

*: significant at $P < 0.01$

** : significant at $P < 0.001$

TABLE V

Responses of unfed female Colorado beetles, brought out of diapause 24 hours earlier

	Category of response					Compared with no-wind criterion		Compared with wind criterion	
	5	4	3	2	1	A	B	A	B
no wind	1	3	12	13	1				
wind	1	16	11	2	0	**	**		
potato	3	24	1	2	0			**	**

** : significant at $P < 0.001$

Jermy (1958) noted that post-diapause Colorado beetles reacted only to cut pieces of potato leaf, not to intact leaves. He concluded that olfactory stimuli play a minor rôle in host plant selection. However, experiments with post-diapause

beetles (Table V) demonstrated positive anemotactic responses in a pure air flow, which were enhanced by the introduction of four potato plants to the wind tunnel.

In his Y-tube olfactometer, McIndoo (1926) showed that adult Colorado beetles were directed more towards unbruised potato plants than towards the volatiles emanating from soil. In addition, he noted attraction towards water extracts and steam distillates of potato plant leaves, *Solanum carolinense* L., *Hyoscyamus niger* L., *S.lycopersicum* and *Datura stramonium* L.. However, these responses were liable to substantial variation. Chin (1950) performed screen test experiments with larvae and reported arresting effects of potato, *Solanum demissum* Lindl. and *P.hybrida*. No responses were elicited by *Doronicum pardalianches* L.. Schanz (1953) using a Y-tube olfactometer, established olfactory responses of adult Colorado beetles to potato plant leaves. Air humidity was excluded as the causal factor of this attraction. Grison (1957) mentioned that in screen tests, potato leaves were more attractive to adult beetles than leaves of neutral plant species like *Pisum sativum* L. and cabbage. Jermy (1958) noted that newly-emerged Colorado beetles needed first to have been fed on potato in order to prefer potato leaf odour to the vapours of lucerne, *Medicago sativa* L., in a Y-tube olfactometer. De Wilde *et al.* (1969) found that the leaves of potato, *S.lycopersicum*, *S. dulcamara*, *S.nigrum* and *Apium graveolens* enhanced the upwind movements of adult female Colorado beetles in a wind tunnel. Leaves of *Alnus incana* (L.) Vill. did not elicit olfactory responses,

TABLE VI

Plant species being attractive, neutral and repellent to Leptinotarsa decemlineata

Attractive	Neutral	Repellent
Umbelliferae i.e. <i>Apium graveolens</i> ^h	Corylaceae i.e. <i>Alnus incana</i> ^h	Compositae i.e. <i>Taraxacum officinale</i> ^h
Tropaeolaceae, i.e. <i>Tropaeolum majus</i> ³	Papilionaceae i.e. <i>Medicago sativa</i> ^d <i>Pisum sativum</i> ^c <i>Phaseolus vulgaris</i> ³	Gramineae i.e. <i>Poa annua</i> ^h
Solanaceae i.e. <i>Solanum tuberosum</i> ^{a-h,3} <i>dulcamara</i> ^{h,3} <i>luteum</i> ³ <i>nigrum</i> ^{h,3} <i>lycopersicum</i> ^{e-h,3} <i>carolinense</i> ^e <i>demissum</i> ^b	Cruciferae i.e. <i>Brassica</i> sp. ^c <i>oleracea</i> ³ <i>pekinensis</i> ³ <i>Raphanus sativus</i> ³	Compositae i.e. <i>Doronicum pardalianches</i> ^b
<i>Nicotiana tabacum</i> ³		
<i>Capsicum annum</i> ³		
<i>Petunia hybrida</i> ^{b,3}		
<i>Hyoscyamus niger</i> ^e		
<i>Datura stramonium</i> ^e		

*: Bongers, 1970; b: Chin, 1950; c: Grison, 1957; d: Jermy, 1958; e: McIndoo, 1926; f: Schanz, 1953; g: Visser, 1976; h: de Wilde *et al.*, 1969; 3: present investigation

whereas the beetles were repelled by leaves of *Taraxacum officinale* Weber and *Poa annua* L. Bongers (1970) showed olfactory responses of adult beetles to potato leaves in screen tests and in an olfactometer. The information of these studies and of the present investigation is classed in Table VI.

It is obvious that all the solanaceous species tested evoked olfactory responses of *Leptinotarsa decemlineata*. Their odours probably include some identical components attractive to Colorado beetles. However, only *S.tuberosum*, *S.dulcamara*, *S.lycopersicum*, *S.carolinense* and *H.niger* are able to support feeding, growth and reproduction of Colorado beetles (Chin, 1950; Jermy, 1961; Hsiao & Fraenkel, 1968a,b; Bongers, 1970; Hsiao 1974). *S.luteum* and *S.nigrum*, plant species highly attractive for oviposition, are nevertheless not eaten (de Wilde *et al.*, 1960 Hsiao & Fraenkel, 1968b; Bongers, 1970). *S.demissum* is resistant and *P.hybrida* toxic to the larvae (Chin, 1950). *N.tabacum*, *C.annuum* and *D.stramonium* are unacceptable for support of growth and oviposition (Jermy, 1961; Hsiao & Fraenkel, 1968a,b). The non-solanaceous plants *A.graveolens* and *T.majus* also attracted Colorado beetles. *A.graveolens* is a non-host plant (Hsiao & Fraenkel, 1968b) and *T.majus* has never been recorded as being attacked by the Colorado beetle. The neutral and repellent plant species listed in Table VI cannot be regarded as natural host plants (Chin, 1950; Jermy, 1958, 1961; Hsiao & Fraenkel, 1968a,b).

The present investigation revealed that fed and unfed newly-emerged, as well as unfed post-diapause beetles responded to the vapours of several solanaceous species and one non-solanaceous plant. In these experiments, the stimulus was applied in the most natural way by using undamaged potted specimens, as cutting leaves changes not only the quantitative but also the qualitative composition of the plant odour in the air (Weurman, 1969). Possibly the observed repellent actions of *T.officinale* and *P.annua*, and the attraction by *A.graveolens* (de Wilde *et al.*, 1969) resulted from such effects.

Despite two exceptions, it is clear that the initial olfactory orientation will mainly lead the Colorado beetle in the field towards solanaceous species. Further steps in host plant selection will occur by the combined action of feeding incitants, feeding stimulants, feeding co-factors and inhibitors (de Wilde, 1958; Jermy, 1961, 1966; Hsiao, 1969, 1974).

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ZUSAMMENFASSUNG

SPEZIFITÄT BEI DER GERUCHSORIENTIERUNG DES KARTOFFELKÄFERS, LEPTINOTARSA DECEMLINEATA

Wind induziert positive anemotaktische Reaktionen von frisch geschlüpften, noch nicht gefütterten Weibchen des Kartoffelkäfers. Diese Reaktionen werden verstärkt durch flüchtige Verbindungen

verschiedener Solanaceen, nämlich *Solanum tuberosum*, *S. nigrum*, *S. luteum*, *S. dulcamara*, *S. lycopersicum*, *Nicotiana tabacum*, *Capsicum annuum* und *Petunia hybrida* und eine Nichtsolanacee, *Tropaeolum majus*. *Brassica oleracea*, *B. pekinensis*, *Raphanus sativus* und *Phaseolus vulgaris* waren in dieser Hinsicht neutral. Ungefütterte Postdiapause-Kartoffelkäfer reagieren ebenfalls auf flüchtige Verbindungen von ungeschädigten, getopften Kartoffelpflanzen. Es wird geschlossen, daß die geruchliche Orientierung den Kartoffelkäfer vorwiegend zu Solanaceen-Arten leitet. Sobald sich ein Kontakt ergibt, werden weitere Schritte in der Wirtspflanzenwahl erfolgen.

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