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Responses to air flow and airborne plant odour in the Colorado beetle

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Abstract

Colorado beetles moved upwind in a wind tunnel. Odour from leaves of potato, nightshade, tomato and bittersweet enhanced the response. The response disappeared after four antennal segments had been amputated. The odour from meadow grass and dandelion repelled the beetles. Beetles 2–3 weeks emerged but not newly emerged beetles were slightly attracted by celerey. In the same wind tunnel, the scent of female beetles attracted males.

Introduction

In 1950, Chin Chun Teh[1] published his thesis on host–plant relations in the Colorado beetle (*Leptinotarsa decemlineata* Say) (Chin, 1950). This paper describes some further progress.

The Colorado beetle is highly selective of host plants, but the sensory basis of this selection has largely been ignored. Chin (1950) found that the larva selects its host plant by a chain process involving several physical and chemical properties of the plant (see also De Wilde, 1958). But infestations depend primarily on selection by the adult female before oviposition. These females may reach the plant by walking or flying. Usually distance will be large and plant odours, which may be of importance in orientation will be carried towards the insect in an air stream. We therefore studied the beetle's behaviour towards air flow and airborne plant odours. A difficulty is that insects may vary in response to plant odour. Jermy (1958) studied the responses of adult *Leptinotarsa* in a Y-tube olfactometer and found attraction to odour of potato leaves only after beetles had eaten potato foliage. Newly emerged, unfed beetles did not show any preference for host plant odour.

Haskell et al. (1962) investigated the responses of larval desert locust (*Schistocerca gregaria*) towards the odour of grasses. They found clear responses in an air stream, whereas previous authors had found no response to plant odour. The use of an air stream seemed decisive. The response of some other phytophagous insects to plant odour may also depend on air flow, so that the experimental method must be adapted to this type of mechanism. Method could have influenced the outcome of Jermy's experiments.

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Materials and methods

The apparatus was a simplified version of the wind tunnel designed by Haskell et al. (1962). The insect chamber, which is 123 cm long, is flat floored and provided with a plate glass inner lining to avoid optical difference. The walls of the chamber are covered with silicon wax to prevent beetles from climbing up. Wind is drawn through to a centrifugal fan. The stream passes first through the test box in which is placed a fixed amount of test material, such as potato leaves, or control material such as cotton wool or filter paper. Odour concentration was not checked. Relative humidity in the wind tunnel was about 65% during the experiments. Preliminary tests showed that phototactic responses did not interfere.

The sexed adult active beetles were newly emerged and unfed or 2–3 weeks emerged and starved for 5 h.

The beetles were placed in the middle of the chamber, and after 5 min their distribution was recorded. Beetles within 5 cm of the intake grid (upwind) were noted as positive choice, beetles within 5 cm from the grid at the outflow (downwind) as negative choice, the rest as indifferent. Every treatment had ten replicates. The temperature during the experiments varied between 27.5° and 27.9°C.

The results were analysed statistically by Wilcoxon's test which compared the excess (positive choice minus negative choice) of the treatment with the excess in the control. All statements about effects, unless otherwise stated, had a reliability of 99%, $p = \frac{1}{2}$.

Results

(a) Experiment with 40 newly emerged female beetles, not fed previously (Table 1). With control materials the beetles showed a significant anemotactic reaction. This response played a role in subsequent experiments. Potato leaves and bittersweet enhanced the response, but celery had no influence on the beetles' tendency to move upwind. The experiments with potato leaves were finished 5 h before the second control and treatments with bittersweet and celery began.

(b) Experiment with 20 female and 20 male beetles starved for 0 and 5 h, 2–3 weeks after emergence (Table 2). It was found that starvation enhances the anemotactic

Table 1. Effect on 40 female unfed Colorado beetles newly emerged of successive treatments with control material and odours of potato, bittersweet and celery

Test material	*Mean choice (number of beetles)*			*Mean excess*	*Effect*
	neg.	*indif.*	*pos.*		
Control 1	0.8	23.5	15.7	+14.9	
Potato (*Solanum tuberosum*)	1.0	15.5	23.5	+22.5	7.6
Control 2	2.2	26.7	11.1	+ 8.9	
Bittersweet (*S. dulcamara*)	1.3	19.0	19.7	+18.4	9.5
Celery (*Apium graveolens*)	2.7	26.3	11.0	+ 8.3	−0.6 (N.S.)

Tabel 1. Effect van geur van aardappel, bitterzoet en selderie op 40 juist uitgekomen, niet eerder gevoede wijfjes

Table. 2. Difference in response to wind of 20 female and 20 male Colorado beetles 2–3 weeks after emergence before and after starving for 5 h

Sex	*Starving*	*Mean choice (number of beetles)*			*Mean excess*	*Effect*
		neg.	*indif.*	*pos.*		
♂♂	0 h	0.0	15.6	4.4	+4.4	
	5 h	0.6	12.2	7.2	+6.6	2.2
♀♀	0 h	1.1	15.3	3.6	+2.5	
	5 h	2.1	8.6	9.3	+7.2	4.2

Tabel 2. Effect van 5 uur hongeren op 20 ♀♀ en 20 ♂♂, 2–3 weken oud

response with control materials. There was no significant difference between the sexes.
(c) The terminal segments of the antennae were successively cut off from the 20 males. The anemotactic reaction disappeared after the fourth segment had been removed.
(d) Forty female beetles were starved for 5 h 2–3 weeks after emergence and tested successively with solanaceous (bittersweet, black nightshade) and other plants (grass, dandelion, alder, celery) (Table 3). Potato, tomato, bittersweet, black nightshade and celery attracted beetles. Beetles did not respond to alder but were repelled by grass and dandelion.
(e) When the antennal segments of 20 of the females were amputated one by one, the attraction of potato leaves disappeared with the fourth antennal segment.

Table 3. Effects of the scent of solanaceous and other plants on 40 female Colorado beetles starved for 5 h 2–3 weeks after emerging

Stimulus	*Mean choice (number of beetles)*			*Mean excess*	*Effect*
	neg.	*indif.*	*pos.*		
Control 1	1.6	27.6	10.8	+ 8.8	
Potato (*S. tuberosum*)	2.0	18.5	19.5	+17.5	8.7
Control 2	3.7	28.3	7.8	+ 4.1	
Nightshade (*S. nigrum*)	4.7	21.7	13.6	+ 8.9	4.8
Control 3	2.5	31.3	6.2	+ 3.7	
Tomato (*S. esculentum*)	3.7	25.1	11.2	+ 7.5	4.2
Control 4	0.4	29.4	10.2	+ 9.8	
Bittersweet (*S. dulcamara*)	0.4	24.5	15.2	+14.7	4.9
Control 5	1.9	28.6	10.5	+ 8.6	
Alder (*Alnus incana*)	4.9	22.9	12.2	+ 7.3	−1.2 (N.S.)
Control 6	4.2	22.4	13.4	+ 9.2	
Dandelion (*Taraxacum officinale*)	7.2	22.0	10.8	+ 3.6	−5.6
Control 7	2.5	23.9	13.6	+11.1	
Grass (*Poa annua*)	1.9	30.9	7.2	+ 5.3	−5.8
Control 8	0.4	29.4	10.2	+ 9.8	
Celery (*Apium graveolens*)	1.7	23.9	14.4	+12.7	2.9

Tabel 3. Effect van de geur van diverse planten, solanaceae en niet-solanaceae op 40 ♀♀, 2–3 weken oud, na hongeren gedurende 5 uur

(f) As the presence of female beetles greatly influenced the olfactory response of the males, we attempted to measure this factor. The female beetles were found to produce an odour which attracted the males, so we placed 40 females in the test box; 20 males placed in the chamber were attracted (mean excess 8.3 males with female odour compared with 2.7 males for control material). The difference disappeared after two terminal segments had been amputated from the male antennae.

Discussion

Adult Colorado beetles respond to the odour of solanaceous plants in an air stream by an increased tendency to move upwind. Even without plant odours, both males and females show anemotactic responses. The response is enhanced by starvation. The receptors are on the four terminal segments of the antennae.
Odour of potato and bittersweet attracted newly emerged beetles. However, an alien plant like celery did not, whereas older beetles were attracted.
Beetles emerged 2–3 weeks were attracted towards four different *Solanum* spp. but not to several other plants except celery.
The response of older beetles to celery raises the question whether the odour of solanaceous plants is chemically related to that of celery, which is a mixture of terpenes.
Schanz (1953) found that the 5 terminal segments of the antennae of *Leptinotarsa* are the seat of olfactoceptors. Yet we found that removal of four segments prevented response to wind or plant odour. Perhaps the fifth segment provides only a small contribution, not detected by our method.
Female odour enhanced the anemotactic response of males. The receptors were on the two terminal segments of the male's antennae.
The information the Colorado beetle receives from air flow seems to be integrated with that from plant odour and female sex pheromone. The combined response to olfactory stimuli and air flow is of special interest for the study of host plant selection by the Colorado beetle and probably by many other phytophagous insects.
These responses were only tested with the walking insect, but may also apply during flight.

Samenvatting

Reactie van Coloradokevers op luchtstroming en plantegeur

In een windtunnel werden proeven gedaan over de reactie van Coloradokevers op de geur van diverse planten. In controleproeven werd een positief anemotactische reactie bij de kevers waargenomen, die werd versterkt door de geur van bladeren van aardappel, nachtschade, tomaat en bitterzoet. In beide gevallen verdween de anemotactische reactie na amputatie van 4 leden van de antennae. Gras en paardebloem beïnvloedden het anemotactisch effect in negatieve zin. Selderij bleek enigszins aantrekkelijk te zijn voor kevers van 2–3 weken oud. Met behulp van hetzelfde apparaat werd aangetoond dat de wijfjes een voor de mannetjes aantrekkelijke geur produceren.

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