Entomologia Experimentalis et Applicata **105:** 141–152, 2002.

Role of visual stimuli in host and mate location of the Colorado potato beetle

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Accepted: October 10, 2002

Key words: host and mate location, colour, shape, pattern, orientation, video motion tracking, visual, *Leptinotarsa decemlineata*, Coleoptera, Chrysomeilidae

Abstract

Visual responses of adult male and female Colorado potato beetles CPB, *Leptinotarsa decemlineata* (Say) (Coleoptera, Chrysomelidae) to 10 × 20 cm coloured paper-boards, beetle-sized coloured beads or dead CPB, and to combinations of these in laboratory experiments in an arena of 52 cm diameter were investigated. In addition, visual discriminatory abilities of males exposed to female-specific substances were also measured. The paths of the beetles were recorded on videotape by a computer-aided motion detection system. Parameters of tracks were calculated and the tape-recorded paths were further processed for behavioural analyses and for obtaining mean angular directions by circular statistics. Both sexes showed a strong preference for yellow-black striped, yellow and light-green boards and, to a lesser degree, were also attracted to yellow-green, dark-green, red and white boards, to beetle-sized beads coloured either black or yellow-black striped, and to some combinations of these. Males exposed to female substances immediately prior to entering the arena performed significantly more successful approaches to objects than did males of the control group. These results corroborate field observations on males' mate-searching behaviour and increase our understanding of natural host- and mate-finding processes.

Introduction

Stimuli affecting spatial manoeuvering and sexual recognition are still poorly known in the Colorado potato beetle. Stimulating chemicals of female origin are known to influence male behaviour (Levinson et al., 1979; Jermy & Butt, 1991; Dubis et al., 1987; Otto, 1996), and a close range airborne female sex pheromone is indicated (Edwards & Seabrook, 1997), but details of the chemistry and mode of action are not yet known. Recently, a male-produced volatile aggregation pheromone (Dickens et al., 2002; Oliver et al., 2002) has been identified and synthesized. Within a distance of ca. 60 cm of the plant stand, adults perceive blends of plant odours specific to the potato (Thiery & Visser, 1986; Dickens, 1999, 2000). Adults are also attracted to damaged potato foliage (Bolter et al., 1997) and to foliage parts where a female or larvae were feeding some time earlier (Landolt et al., 1999; Á. Szentesi, unpubl.).

Visual cues also play a key role in sexual and host-finding behaviour, with apparent attraction to conspecifics on potato foliage at a distance of 10 to 20 cm (Á. Szentesi, unpubl.). CPBs respond to certain silhouettes (Jermy et al., 1988), yellow traps (Zehnder & Speese, 1987), and yellow vs. gray paper (van der Ent & Visser, 1991), although earlier work (Hellwig & Ludwig, 1951) concluded that vision was unimportant. It is known, however, that CPB can at least differentiate between 360 and 510 nm wavelengths, based on ERG-measurements (Mischke, 1981), and experiments by Lönnendonker (1991a, b, 1993; Lönnendonker & Scharstein, 1991) using black and yellow striped disks, indicated that both colour and contrast could elicit orientation responses. In spite of this information, little is known about how visual stimuli contribute to reproductive behaviour, and specifically, whether approaching and/or recognizing another individual by sight is possible. It is very likely that chemical cues are not solely responsible for sex encounters. Potato foliage not only provides a platform for depositing or dispersing chemicals (D.C. Weber & J.J. Duan), but also reflects in a wavelength band generally attractive to insects (Zehnder & Speese, 1987).

In the present paper, we investigate the role of vision in locating conspecifics. Specifically, we ask (1) whether CPB are attracted to boards reflecting in different spectral bands of the visible spectrum, including those corresponding to plant surfaces; (2) whether adults react to beetle-sized stationary coloured objects; (3) whether responses of adults can be enhanced by a joint presentation of such objects and boards, and (4) whether prior experience of males with female substances enhances visual responsiveness to objects. We analyse and quantify the responses in different ways. These questions and especially the influence of substances of female origin on the orientation response of males to visual objects have not been investigated previously for the CPB.

Materials and methods

Insects. Sexually mature adults of both sexes and mixed age were used. Due to shortage of laboratory-reared insects, 57% of the adults tested were field-collected from an overwintered population in June, whose members were actively moving on potato plants. The laboratory population was maintained on greenhouse-grown potato around the year at 24–26 °C, and under long daylight (L20:D4). Beetles were fed potato foliage for a minimum of six days to reach sexual maturation before use. Adults were separated by sex and kept apart in cages. The beetles were always deprived from contact with the opposite sex for more than two days, but were never starved. Such sexually mature individuals readily engage in sexually motivated searching behaviour (Szentesi, 1985).

Arena. We used a 52 cm diameter circular arena made of white paper board (Figure 1). Its radius corresponded to the distance from which behavioural responses among beetles were observed in the field. The height of the arena was 50 cm to avoid external visual disturbance. Beetles entered the arena through a centrally located release hole of 2 cm diameter on its platform. The lining of the arena platform (an A2 size white writing-paper sheet) on which the beetles walked was exchanged after each run. The arena was illuminated from above by two fluorescent light sets (Figure 1) providing ca. 2700 lux on the arena platform.

Recording apparatus. The paths of individual beetles were recorded with a video tracking and motion analysing system (Ethovision ver. 1.70, Noldus et al., 2001). It consisted of a colour video camera (SONY DXC-151P, lens: Canon 6 mm) hooked to a digitiser board (CFG Plus, Visionetics Int.) in a computer and through it to a video recorder (Panasonic AG-7350) and to a video monitor (SONY Trinitron PVM-1440QM). The image included the entire arena. The software continuously followed and stored the path coordinates of an insect until it reached the target or the arena wall or after 10 min.

Objects and their arrangements. Coloured paper boards, painted beetle-sized china-beads, and a dead CPB adult were presented. The boards were 10 × 20 cm size and when in the upright testing position they created a 20° × 40° image as viewed from the release hole. The board types were either painted with glossy enamel (Neolux) or obtained as manufactured coloured paper. Among the former were three types: Yellow (Y) (109U), Black (B), and vertically striped Yellow and Black (YB). (The combined alphanumeric code following a colour in parenthesis is an approximate colour value by Pantone® Color Formula Guide 747XR.) The number and relative width of the stripes corresponded to the pattern of a female beetle's elytra (eight black and ten yellow stripes each 9 mm wide). The rest of the boards [Dark-Blue (DB) (274C), Red (R) (1655U), Light-Green (LG) (355C) and Dark-Green (DG) (3305C), and Yellow-Green (YG) (3955U)] were manufactured colour paper and were almost matte. The china-beads were painted with the same enamels (yellow, black, and yellow-black striped).

The reflectance spectra (from 350 to 800 nm) of boards and other test and reference objects were measured with a Perkin-Elmer MPF 44B spectrofluorometer in 'ratio-mode'. Averages of five spectra from five samples were calculated and smoothed (Figure 2). Saturation and brightness were measured against a gray (443U) and a ZnS plate, respectively (Table 1). Light

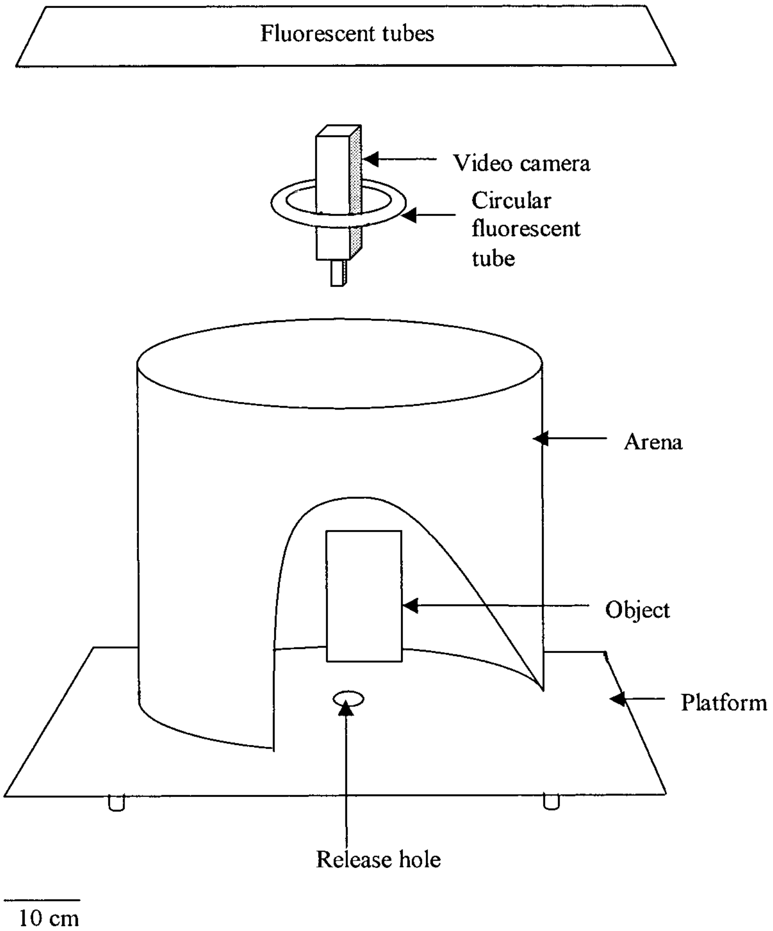


Figure 1. Schematic drawing of the arena used to test orientation responses of walking Colorado potato beetles. Scale: 10 cm.

intensity was measured at the release hole with a light meter positioned perpendicular to the coloured board leaning against the white arena wall. No correction was made for the emission spectrum of the fluorescent light source used. It is noteworthy that several colours' reflectance spectra in this study substantially overlapped with or had similar peak reflectance to those of potato and beetles' elytra.

Tests. With one exception, tests were of no-choice types. The objects were positioned 25 cm from the release hole, so that they touched the arena wall (boards) or were placed 2 cm in front of it (beads) and were rotated for each run in one of four positions (0, 90, 180, or 270 degrees). When boards and beads were tested in combination (e.g., a yellow-green board plus a black bead as an object), the bead was placed 1 cm in front of the board on a 5 × 10 cm piece of board of the same colour as the standing board. In the choice test we wanted to know, whether beetles were able to differentiate between simultaneously presented objects. An empty arena was also tested. The number of tracks (replicates) recorded for testing a particular object was 15 to 82. In 68% of the experiments, adults of mixed origins were used. In the remainder, either field-collected or laboratory-reared beetles were tested. Each insect was used only for one track.

Effects of prior exposure of males to female substances. We hypothesized that experience with females' substances would make males change their behaviour. The abilities of males to differentiate between two simultaneously presented combined objects (YG-board+B-bead vs. LG-board+YB-bead) in a choice test were tested immediately after exposing them to a surface contaminated with female elytra substances (Jermy & Butt, 1991). Elytra from a live female were gently rubbed against a piece of filter paper. This pa-

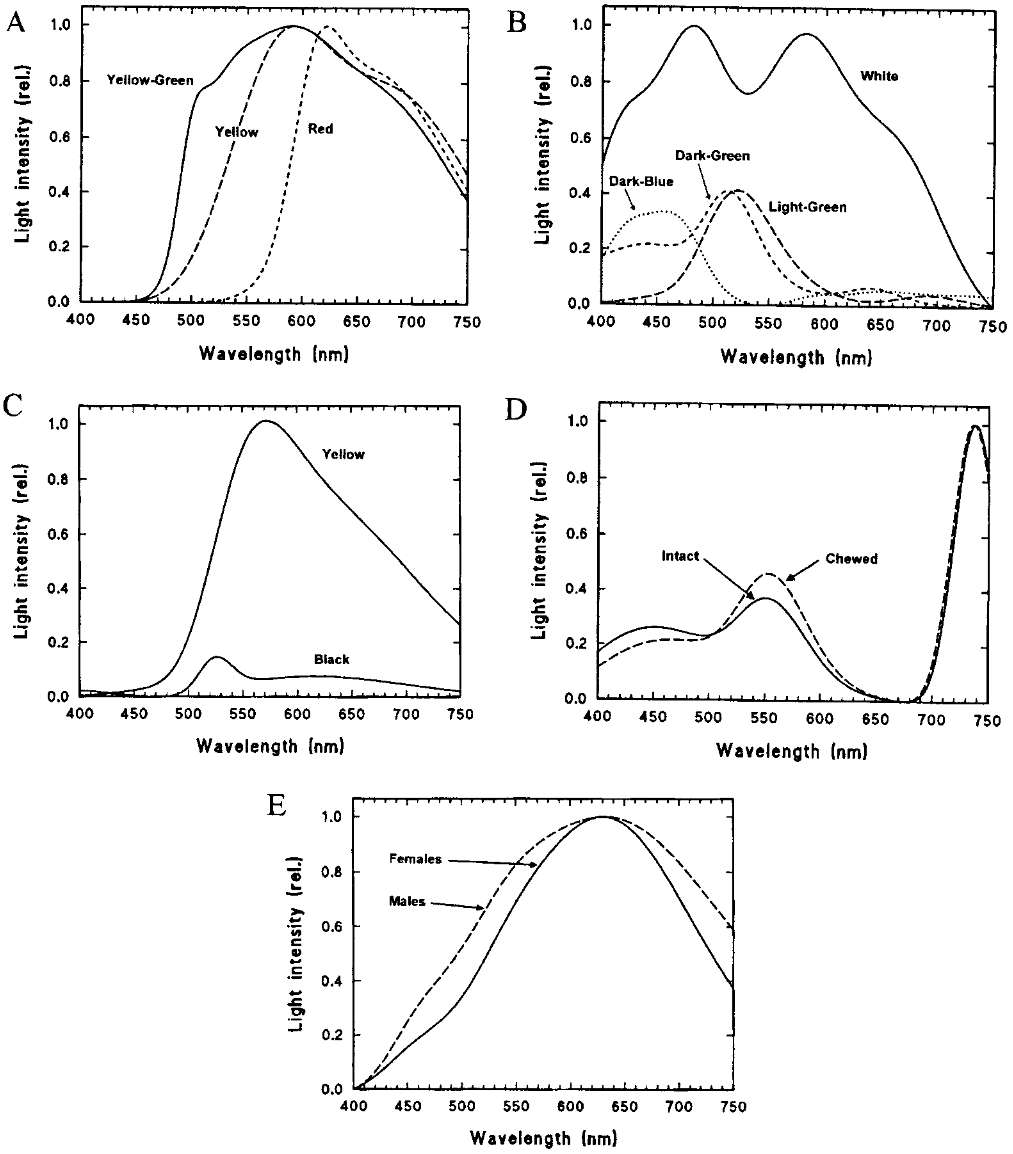


Figure 2. Reflectance spectra of boards (A and B), beads (C), live potato foliage (D), and yellow stripes on live adult CPBs' elytra (E). Each curve is a mean of five measurements of five samples.

per was placed on the release platform. This procedure was repeated two to three times with different females during the runs. There were 42 runs performed before and 49 after the application of the substances and the responses were evaluated by Chi^2 test. Only those runs were taken into consideration in which the male investigated the treated paper before entering the arena, i.e., initiated local search accompanied by intensive antennation and palpation.

Track analysis. Subsequent evaluation of the recorded paths by various program modules of Ethovision (Noldus et al., 2001) allowed inspection of individual tracks, to make some minor corrections and to obtain track attributes: duration (in s), length (in cm), as well as the speed of animals (total length of track/total run-time in cm/sec).

Analysis of behavioural events. Behavioural recordings were made by the Observer program (ver. 3.0, Noldus et al., 2000) during video replays. For each

Table 1. Saturation(%), brightness (%), and mean reflected light intensity (±SD, lux) of coloured boards

Hue of boards	Saturation	Brightness	Reflected light intensity[a]
ZnS-plate (as standard)	–	100.0	–
Gray (as standard)	100.0	45.6	–
White	90.0	115.4	2477 ± 26
Yellow-green	89.1	90.2	2507 ± 30
Yellow	81.6	74.5	2437 ± 26
Red	57.8	88.7	2302 ± 29
Dark-green	52.8	41.2	2250 ± 26
Dark-blue	46.3	33.6	2237 ± 33
Light-green	40.3	41.6	2287 ± 25
Black	6.9	4.3	2250 ± 26
Empty arena	–	–	2570 ± 22

[a] An average of four readings corresponding to the four testing positions within the arena separated by 90°.

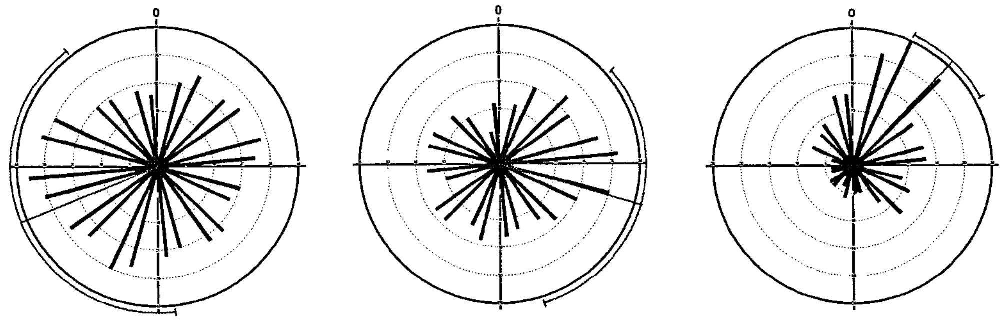
track, the frequency and duration of three behavioural events (stop, move, fly), and a 'response score' were measured and established. These were as follows: 1 = the beetle did not move during the entire test period; 2 = the path was opposite to or distant from the object; 3 = the insect went in the direction of the object, but passed it by some centimetres; 4 = the same as #3, but it passed the object closely or approached it from front, however, did not touch it; 5 = it approached and touched the object or climbed on it.

Statistical analyses. Runs of > 500 s were excluded from the analyses because most of these insects moved little or not at all. Raw data, when needed, were transformed to improve homogeneity. However, mostly non-parametric tests were applied [homogeneity testing (Levene-test; Milliken & Johnson, 1984), followed by Kruskal-Wallis one-way ANOVA]. Since the sex and origin (whether field collected or laboratory-reared) of CPB adults were also known, track attributes were analysed by sex and origin by the Wald-Wolfowitz Runs test (a nonparametric alternative to the t-test, Statistica, 1994).

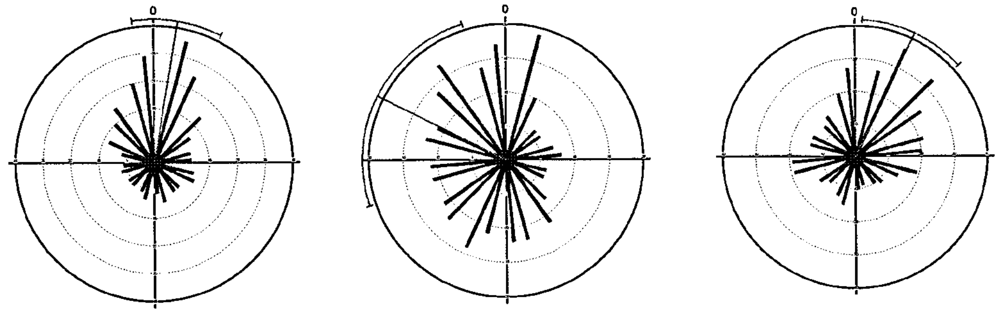
Circular statistics. In order to evaluate orientation angle of beetles in relation to objects in the arena, all tracks of no-choice tests were replayed. An acetate film mask was fitted on to the video monitor screen divided into 24 radially arranged fields of 15 degrees each. The mask could be turned around according to the position of the test object in the arena and the 0° was always assigned to the object. Thus, the tracks were evaluated by common rules regardless to the rotational position of objects. In case of the empty arena (no object present), the 0° was always in the same position, i.e., North. The duration a beetle was within any of the 24 fifteen-degree fields was recorded and processed to obtain mean angular direction (MAD), which comprises the mean vector (angle) of direction and its length. These data were processed in Oriana software (ver.1.0, 1994) to produce circular histograms (Zar, 1996). The histograms show MADs as if objects were always in the arbitrarily chosen 0° (North) position of the arena. In a 45-degree interval, both to the left and to the right of the 0° position, MADs were considered as referring to a positive response, from 45 to 135° (or between 225 and 315°) as no preference-no avoidance, and only below these (between 135 and 225°) was a MAD qualified as avoidance. Circular statistics (Rayleigh's test of uniformity and Watson's F-tests, respectively; Zar, 1996) were applied to MAD values. The Rayleigh test evaluated the null hypothesis that the vectors of a given track were uniformly distributed amongst all 24 fields. Watson's F-test executed pairwise comparisons of MADs assuming the null hypothesis that circular means obtained with two different objects did not differ.

The relationship between MAD and response score is that, while the former gives the average of all directions with a 15° field resolution within the arena during the entire test period, the latter gives a single value for the final position reached by the insect in relation to the object.

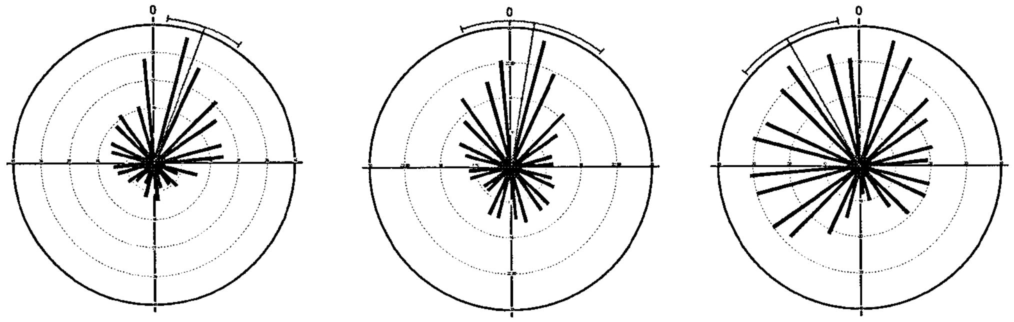
Boards



Black, P = 0.14 Dark-Blue, P = 0.03 Dark-Green, P < 0.01



Light-Green, P < 0.01 Red, P = 0.01 White, P < 0.01



Yellow, P < 0.01 Yellow-Black-striped, P < 0.01 Yellow-Green, P < 0.01

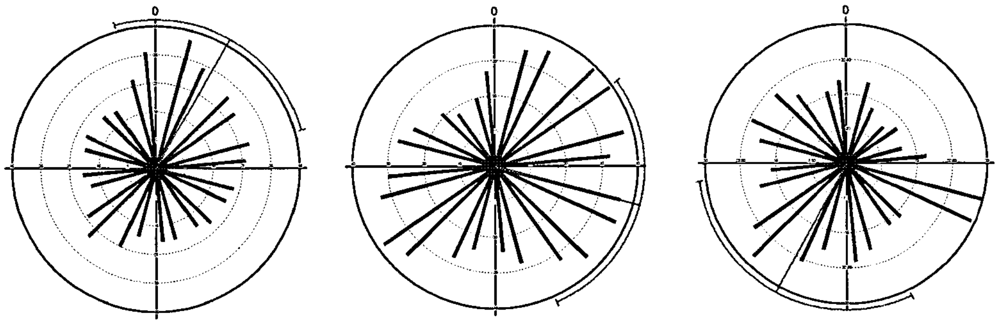
Figure 3. Circular histograms of various stimulus situations. They present the frequency and distribution of vectors of duration of stays, mean vector directions, and their 95% confidence limits in angles. Objects' positions are always projected to 0° (top of the circle, North), although they were placed at each run in one of the four main directions. The thin line running beyond the outermost circle is the mean angular direction (MAD) with 95% confidence limits. P-values below the subfigures show whether a MAD is significantly different from the uniform angle distribution assumed under the null-hypothesis (Rayleigh-test, P = 0.05).

Results

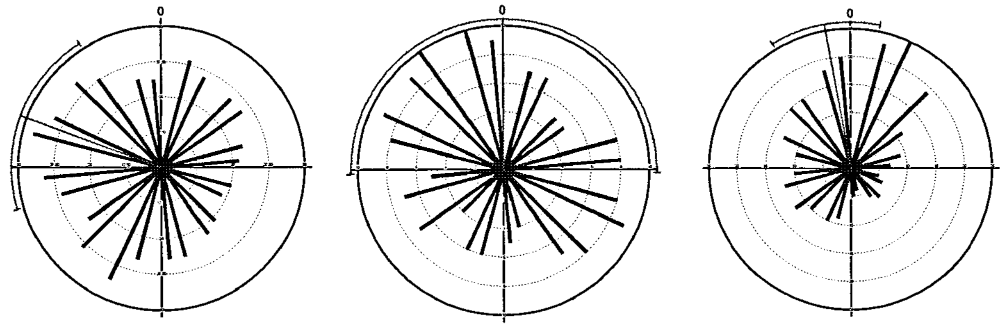
The runs in the empty arena resulted, for unknown reasons, in a slight mean angular direction towards NE. We compensated for this slight spatial asymmetry by placing the test objects after each run sequentially in one of four directions (0, 90, 180 and 270 degrees), however, without reference to the bias. Therefore, all tests can be compared with each other by Watson's F-test, as they contain objects of similar class.

The results below refer to tests where both sexes were used, and the results are not differentiated by sex except as noted. When the mean MAD value was close to 0°, the insect was attracted to the object, it walked by shorter and straighter path (less tortuosity), and spent less time in the arena.

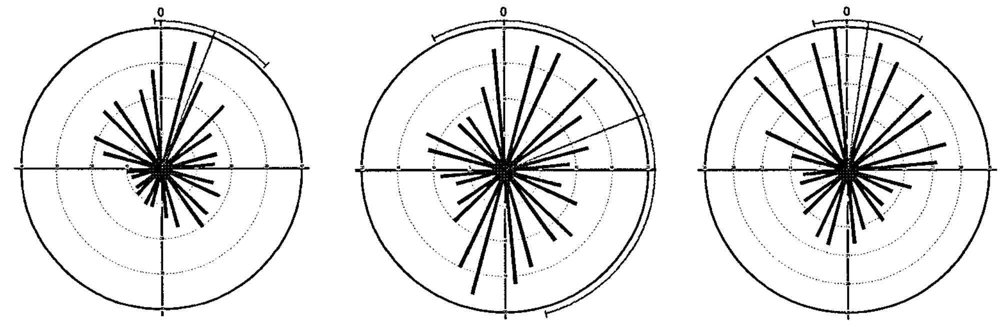
Beads, Dead Beetle and combinations of Boards and Beads



Black bead, P = 0.01 Dead beetle, P = 0.01 Yellow bead, P = 0.03



Yellow-Black-striped bead, P < 0.01 Dark-Blue board + Yellow bead, P = 0.27 Light-Green board + Yellow-Black bead, P < 0.01



Yellow-Green board + Black bead, P < 0.01 Yellow-Green board + Dead Beetle, P = 0.31 Yellow-Green board + Yellow-Black bead, P < 0.01

Figure 3. Continued.

Responses to coloured boards. Five boards (YG, YB, LG, Y, and W) were consistent in evoking a positive response both in terms of mean angular direction (all within 45° of 0°) and high mean response scores (Table 2; Figure 3a). Other boards evoked either a weaker positive response (DG), or neither a positive nor negative response, in terms of MAD, and the lowest response score was earned by the black board. Results for tortuosity were not consistent within these groups. Kruskal-Wallis ANOVAs were significant for duration of runs (in s, $H = 31.04$, d.f. $= 9$, $P = 0.0003$) and for path lengths (in cm, $H = 22.28$, d.f. $= 9$, $P = 0.008$), respectively. However, beetles walked with the same speed along the paths regardless of the colour of the board (Kruskal-Wallis ANOVA: $H = 11.61$, d.f. $= 9$, $P = 0.2365$). There were significant differences among the means of time spent while standing ($H = 74.73$, d.f. $= 9$, $P \leq 0.0001$) or moving

Table 2. Track characteristics of CPB adults responding to coloured boards (means). N for runs = 15-42, for data = 171-2820

Hues and objects	Mean angular direction of path (°, mean±SE)[a]	Tortuosity of path[b]	Response score[c]
Boards			
Light-Green (LG)	9.3 ± 7.0a	0.27a	4.5a
Yellow-Black striped (YB)	9.3 ± 11.ab	0.45b	4.5a
Yellow (Y)	20.5 ± 5.8ab	0.43b	4.2a
White (W)	25.5 ± 8.8b	0.51b	4.4a
Dark-Green (DG)	43.3 ± 7.0c	0.40ab	3.7b
Dark-Blue (DB)	106.3 ± 21.8d	0.43b	3.1b
Black (B)	247.8 ± 28.8c	0.48b	2.8c
Red (R)	296.8 ± 17.5f	0.25a	3.9b
Yellow-Green (YG)	330.8 ± 8.0g	0.42b	4.3a
Beads			
Black	29.5 ± 17.5a	0.58a	2.8a
Dead CPB	105.0 ± 19.5a	0.53a	1.5a
Yellow	208.5 ± 21.0a	0.49a	3.1a
Yellow-Black striped	291.0 ± 14.0a	0.60a	3.1a
Board + Bead			
Dark Blue + Yellow	0 ± 35.3a	0.30a	2.9b
Yellow-Green + YB-sriped	8.3 ± 8.0a	0.46a	4.4a
Yellow-Green + Black	21.5 ± 9.3a	0.35a	3.9a
Yellow-Green + Dead CPB	68.0 ± 37.3b	0.37a	4.0a
Light-Green + YB-striped	349.8 ± 8.3a	0.36a	3.9a

[a] Ten minutes long runs are not included. Values followed by the same letter within object type are not significantly different (Watson's F-test, $P > 0.05$).

[b] Tortuosity = ratio of the ideal to real path length. Values followed by the same letter within object type are not significantly different (LSD with $P > 0.01$ for boards). Parametric tests were applied (Levene-test is satisfied at $P > 0.05\%$ for boards (d.f. = 8, F = 1.587, P = 0.130), for beads (d.f. = 3, F = 0.259, P = 0.855), and for board + bead (d.f. = 4, F = 1.748, P = 0.144); ANOVA for boards: d.f. = 8,223, F = 2.930, P = 0.004, for beads: d.f. = 3,64, F = 0.470, P = 0.704, for board + bead: d.f. = 4,112, F = 1.035, P = 0.392.

[c] Kruskal-Wallis ANOVAs. For boards: H = 49.368, d.f. = 8; $P < 0.0001$, for beads: H = 2.996, d.f. = 3, P = 0.392, and for board + bead: H = 14.343, d.f. = 4, P = 0.006. Values followed by the same letter within object type are not significantly different.

(H = 57.92, d.f. = 9, $P \leq 0.0001$), but not while flying (H = 0.0, d.f. = 9, P = 1.0).

Responses to coloured beads and to dead CPB. Beads or dead beetles presented without boards evoked a generally weaker response in terms of response score. MADs were variable, with only the black bead evoking a positive numerical MAD, but with no significant difference amongst treatments (Table 2; Figure 3b). Tortuosity was similar and relatively high across all treatments, suggesting undirected movement. There were also no significant differences detected by the Kruskal-Wallis test in the duration of path lengths (in s, H = 4.37, d.f. = 3, P = 0.22), the distance walked (in cm, H = 1.38, d.f. = 3, P = 0.71), or in the speed of beetles (in cm/sec, H = 3.10, d.f. = 3, P = 0.38).

Responses to combinations of coloured boards + beads/dead CPB. Boards plus bead combinations evoked positive responses in terms of mean angular direction, except for the combination including the dead CPB: all were within 45° of 0°. MAD values indicated strong preference for LG-board + YB-bead, YG-board + B-bead, and YG-board + YB-bead combinations. Response scores were also generally high relative to beads presented alone, and only the dark blue board plus yellow bead was significantly less than others in terms of response score.

Lumping together all runs and analysing the relationships between two path parameters we found (1) that the faster the beetles move the straighter the path (Spearman $R = +0.4068$, $N = 734$, $t = 12.05$, $P \leq 0.001$), and (2) that straighter the paths the shorter the duration of moving (Spearman $R = -0.107$, $N = 731$, $t = 2.91$, $P = 0.004$).

Effect of prior experience of males with female substances. Males before entering the arena investigated (antennated and palpated) the female-treated filter paper very thoroughly. Of the 49 runs where female smear was used, 43 runs received a response score of 5, whereas from 42 runs performed among identical circumstances without female smear, only 23 received the same scores ($\text{Chi}^2 = 12.36$, d.f. = 1, $P = 0.0004$). Both groups were tested in a choice situation (YG-board+B-bead vs. LG-board+YB-bead). No track parameters differed between the two groups.

Differences in responses by sexes and by origin. Data from both sexes were pooled under the assumption that males (M) and females (F) equally performed orientation to objects. However, differences in responses to visual stimuli were also detected by the Wald-Wolfowitz Runs test. Females took several centimetres shorter paths to boards ($Z = -2.506$ $P = 0.012$) and produced shorter stops in general, and field-collected females in particular ($M = 15.2$ s, $F = 5.9$ s, $Z = -3.638$, $P = 0.0003$). As for the origin of adults, among field-collected beetles there were more differences between sexes: mean time spent moving was larger ($M = 11.1$ s, $F = 13.6$ s, $Z = -2.720$, $P = 0.0065$) and path lengths shorter ($M = 61.0$ cm, $F = 51.7$ cm, $Z = -3.026$, $P = 0.025$) for females, and they also took straighter paths (tortuosity: $M = 0.43$, $F = 0.39$, $Z = -2.086$, $P = 0.037$).

Discussion

The behavioural analyses support the conclusion that Colorado potato beetles make use of reflectance patterns in their searching behaviour. At spots of the most attractive reflectance, the probability of occurrence of host or a potential mate can be higher than elsewhere. In this process, the beetles exercise preference, and the spatial arrangement of various objects substantially influences the visual response. It is likely that edge effect or visual contrast also contributes. Experience of female-specific substances greatly enhances males' tendency to respond positively.

Boards which evoked a positive response corresponded in reflectance properties to the vegetation. An exception was the Yellow-Black board, which was considered as an oversized beetle dummy. This was based on Lönnendonker (1993) who found that a yellow-black, vertically striped object of 10° (ca. 2 cm) size was attractive to walking CPB adults.

The contrast component of visual stimuli must play an important role in the response to striped patterns since it was frequently observed that beetles went to borders of adjacent black and yellow stripes or to edges of the boards that were standing in front of a white background. It might explain the attractive effect of white board too (contours contrasting to the background). This supports Lönnendonker's (1993) results where contrast information appeared to be important, although van der Ent and Visser (1991) considered colour more important than contrast.

When considering the relative importance of hues for the orientation response of the CPB, one can accept Mischke's (1981) findings that the CPB has real colour discrimination at only 360 nm (UV) and 510 nm (green), or those of Lönnendonker's (1993) that the basic colour is a mixture of yellow, orange, and green. Then there is little probability that the beetles are able to differentiate among hues such as red and dark-blue used in our experiment. They may affect movement more or less in the same way as black, i.e., may provide information for menotactic orientation. On the contrary, all other hues (yellow, yellow-green, and light-green) tested could elicit telotactic responses. This is in agreement with findings of preference for a yellow-related hue (Stüben, 1972; Zehnder & Speese, 1987; van der Ent & Visser, 1991; Pelletier, 1995) and is not different from the general picture gained for many herbivorous insects that they react to 'insect yellow' (Prokopy & Owens, 1983). Thus, it may be concluded that responses to yellow, yellow-green, and light-green boards are equivalent with responses to the plant foliage on which the CPB most frequently moves. This is also supported by the reflectance spectra obtained for these boards and potato leaves, since the peak reflectance values of the yellow and yellow-green boards are close to or overlap with that of the potato foliage (550 nm) (Zehnder & Speese, 1987; Figure 2). The reflectance spectra of the yellow stripes of the beetles' elytra (Figure 2) also match with the reflectance bands of the yellow and yellow-green boards and beads used in the experiments. Among the boards, there were two groups with an additional possibly important difference between them, i.e., the glossy or matte character of surface. As many, and specifically the curved and shiny, surfaces polarize light to some degree (Land, 1993; Horváth & Zeil, 1996), the difference could serve as one of the sources of variability. Although the detection and utilization of polarized light have not been experimentally demonstrated in the CPB yet, its perception was suggested in a study of the sun-compass orientation of the species (Jermy, 1961).

The relative size of the objects (20° × 40° and 4° for boards and beads, respectively) seems important, because beads alone received very few visits. The stripe widths of YB-board or the entire 10 mm diameter beads gave an image of 4° size viewed from the release hole. This value was above the resolving power of 2° established for the CPB (van der Ent & Visser, 1991). However, the stripes of the YB-bead were surely below the 2° threshold at ca. one-tenth of the stripe-width on the YB-board. The beads' overall size must be close to the 2° limit (van der Ent & Visser, 1991) of the beetles' resolving power from the distance of the release hole. Thus, the relatively high response score of some board + bead combinations (Table 2) can be explained by the effects of boards alone, although not in all cases. For instance, whereas DB-board alone was neutral in its effect (Table 2 and Figure 3a), in combination with a yellow bead (that was a negative stimulus by its own) the two produced very straight paths, a mean MAD of 0°, but with high variability (confidence limits ± 90°) and a low response score. The combination attracted the attention of beetles, i.e., it provided a contrasting fixation point, and although the beetles generally selected the objects' direction, nevertheless they passed it from a considerable distance, because the board was not a preferred colour. This makes it probable that a single trait of an object cannot adequately explain the response observed and that complex mechanisms may play roles in the orientation. We also think that, when the values of MAD and response score indicate a strong preference, but the tortuosity is high or vice versa (e.g., YB-board or DB-board+Y-bead, Table 2), it might refer to yet unknown factors influencing the outcome.

Differences observed between the sexes in path-length and tortuosity indicated that females, especially field-collected females, took shorter paths to attractive boards, with less hesitation (shorter stops) and straighter paths (lower tortuosity) than comparable male beetles. This could be associated with a higher affinity of females for the host plant, which is also observed in their reduced movement (compared to males) between plants in the field, and lesser net dispersal within a field (Szentesi, 1985; Boiteau, 1988; Weber & Ferro, 1994). The responsiveness could be enhanced for field-collected females because of prior experience with plant location in the field. Males, by contrast, showed significantly more success in reaching attractive board-and-bead combinations if exposed beforehand to female substances. This gives some insight into the complex and sequential interaction of olfactory and/or taste stimuli with subsequent response to visual patterns.

This study has also demonstrated that paths can be characterized by various parameters that individually or in combination adequately refer to stimulus situations. Average speed and tortuosity, or periods spent moving and tortuosity interacted in a complex way. Preferred objects (like a yellow-black-striped board) elicited shorter and straighter paths and faster movements, in between which there are stops when scanning and readjusting the direction may take place. Similarly, the relationship between stops and moves can take any of the two possibilities: if one of them increases the other decreases, or both change into the same direction. The latter case may refer also to a perceptual change that we assumed, e.g., to the stop-move patterns of beetles in an empty arena where circular movement dominates due to lack of any fixation (Lönnendonker & Scharstein, 1991). An even finer resolution of individual paths would have been required to prove that directional changes from second to second are due to the stopping-scanning-redirecting sequence performed by the beetles. It could reveal the preferred and dominant directionality of path even if it did not end with a hit. This has been possible on a locomotion-compensator giving much higher resolutions of CPB beetles' orientational responses under various modalities (e.g., Visser & Thiery, 1985). We provided mean angular direction (MAD) by a 15° resolution only. Nevertheless, it revealed that, in some cases, even if beetles arrived at beads or dead CPB, mean path directions were directed away from the objects (Figures 3a,b). Beads and dead CPB did not evoke a strong positive response without an attractive background. Alternately, as also indicated by earlier observations (Hsiao, 1988), had we kept 'moving' the beetle-sized objects within the arena, they might have provided a stronger stimulus situation similar to the increase in arousal achieved by exposing male beetles to female substances before entering the arena.

Acknowledgements

We thank Béla Böddi (Department of Plant Anatomy, L. Eötvös University, Budapest) for preparing the reflectance spectra, the Swiss National Fund grant No. 7UNPJ041519, and the Hungarian National Grant OTKA T25794 for financial support. Elizabeth A. Bernays, Joseph C. Dickens, John R. McLaughlin, Dénes Schmera, Mark W. Brown, and two anonymous reviewers made helpful and valuable comments and improvements on earlier versions of the manuscript. Mrs. Katalin Újj provided important and reliable technical assistance. Miss V. Takács and Miss M. Messmer helped in the evaluation of an earlier set of data.

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