Colorado Potato Beetle (Leptinotarsa decemelineata) Orientation: unpublished Servosphere Studies from 1983

Hans J. Visser, Denis Thiéry

Colorado Potato Beetle (*Leptinotarsa decemelineata*) Orientation: unpublished Servosphere Studies from 1983

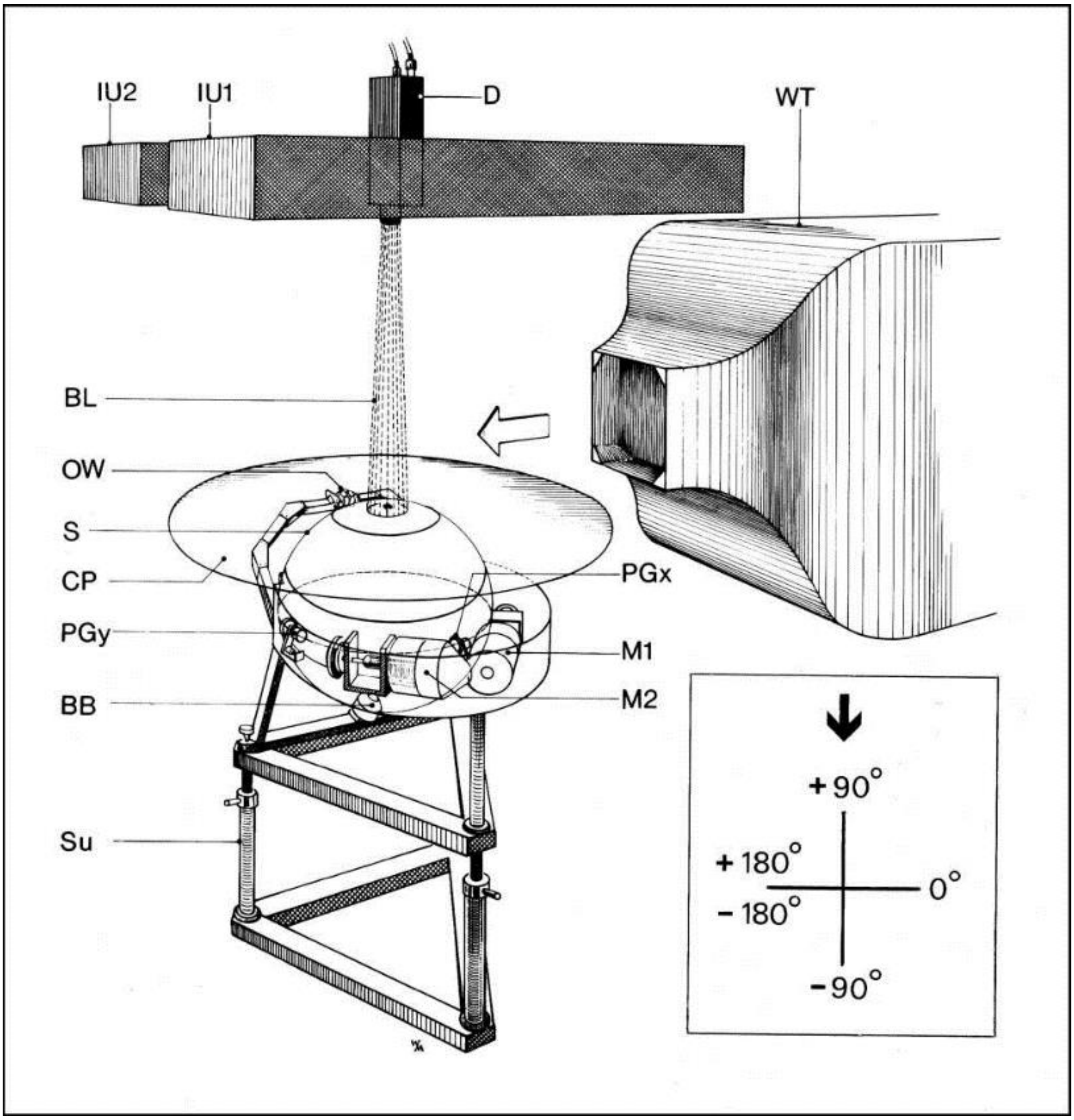
Female Colorado potato beetle freely walking on the servosphere apparatus

J.H. Visser and D. Thiéry

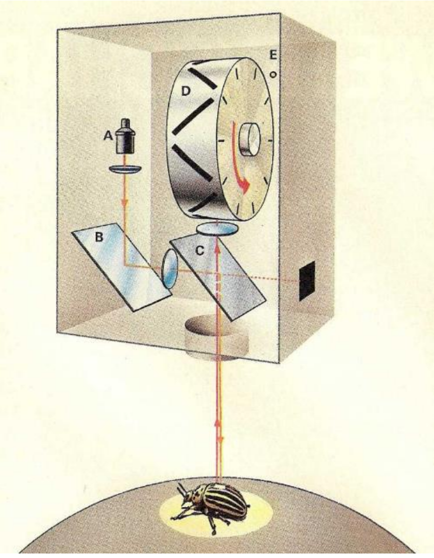
Department of Entomology, Wageningen Agricultural University, The Netherlands

In the seventies the olfactory orientation of the Colorado potato beetle in response to plant odours has been studied on a walking plate, which measured 70 cm long and 23.5 cm wide, in front of a wind tunnel (Visser, 1976; Visser & Nielsen, 1977; Visser & Avé, 1978). As individual beetles moved around they frequently touched the rails on the sides and soon reached the upwind or downwind edge of the walking plate. In this setup the beetles' olfactory orientation could only be studied for a limited amount of time. Furthermore, walking tracks were affected by the limited size of the walking plate. For these reasons the first author introduced the locomotion compensator, servosphere, for further studies on Colorado potato beetle orientation. This instrument allows the beetle to walk continously without reaching any object, and enables detailed analyses of movements and walking tracks.

The servosphere operates as follows (see illustrations below): a tiny piece of reflective material is fixed on the back of the beetle. The beetle is placed on top of the sphere and is observed by a position detector. The detector projects a beam of visible light onto the beetle, which is reflected and transformed into signals that operate two motors which rotate the sphere in the opposite direction. In this way the beetle stays on top of the sphere while walking. The rotations of the sphere are recorded by two pulse generators, and every second the computer stores and calculates the beetles' position, walking speed and orientation angle. In addition to beetles (Visser & Thiéry, 1985; Thiéry & Visser, 1986), this setup has been used for tiny predatory mites and aphids (Visser & Taanman, 1987), and for very fast moving cockroaches (Piek et al., 1984, 1989).

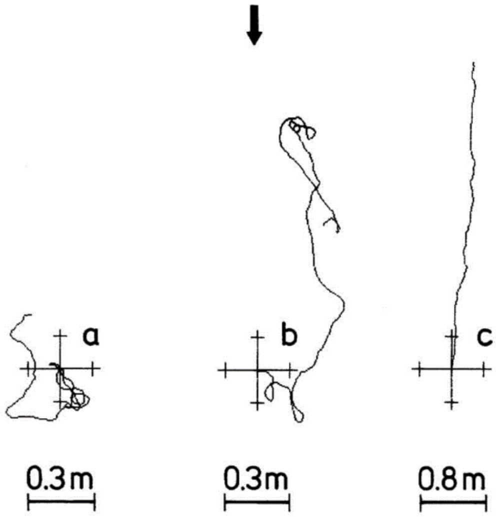


Servosphere legend: *servosphere positioned at the outlet of wind tunnel WT and high-frequency illumination units IU1 and IU2. Arrows indicate wind direction. Inset depicts co-ordinates and corresponding angles. Further abbreviations: ball bearing BB, beam of light BL, circular plate CP, position detector D, motors for compensation M1 and M2, omnidirectional wheels OW, pulse generators for registration PGx and PGy, 50 cm sphere S and support Su. The sphere was coated with an easily washable black Nextel suède coating 3101 B21 (3M Company), in this way walking insects had normal grip on the surface. The circular white plate CP was installed in order to improve the symmetry of the beetles' visual surroundings. Drawing by W.C.T. Middelplaats.*



Position detector of servosphere: *light source A, mirror B, half-silvered mirror C, rotating drum D, reference light E. Piece of self-adhesive reflective material (high gain reflective sheeting type 7610, 3M Company) is fixed on the beetles' elytra. The drum rotates at 60 rps and on its periphery 5 pairs of diagonal slits are present. Inside the drum photocell PC1 is directed downward and, thus, pulses when the beetles' reflection is projected through a slit. Reference pulses originate from photocells PC2 & PC3 inside the drum when stimulated by the reference light. Any x,y-position change of the beetle from the centre is recorded by the time intervals between PC1 and PC2 pulses (x), and between PC1 and PC3 pulses (y). Flip-flops and sample and hold circuits are connected to power amplifiers which operate 2 motors rotating the sphere in the opposite direction. The position detector is fast since 300 x,y positions per second are used for the compensation of walking.*

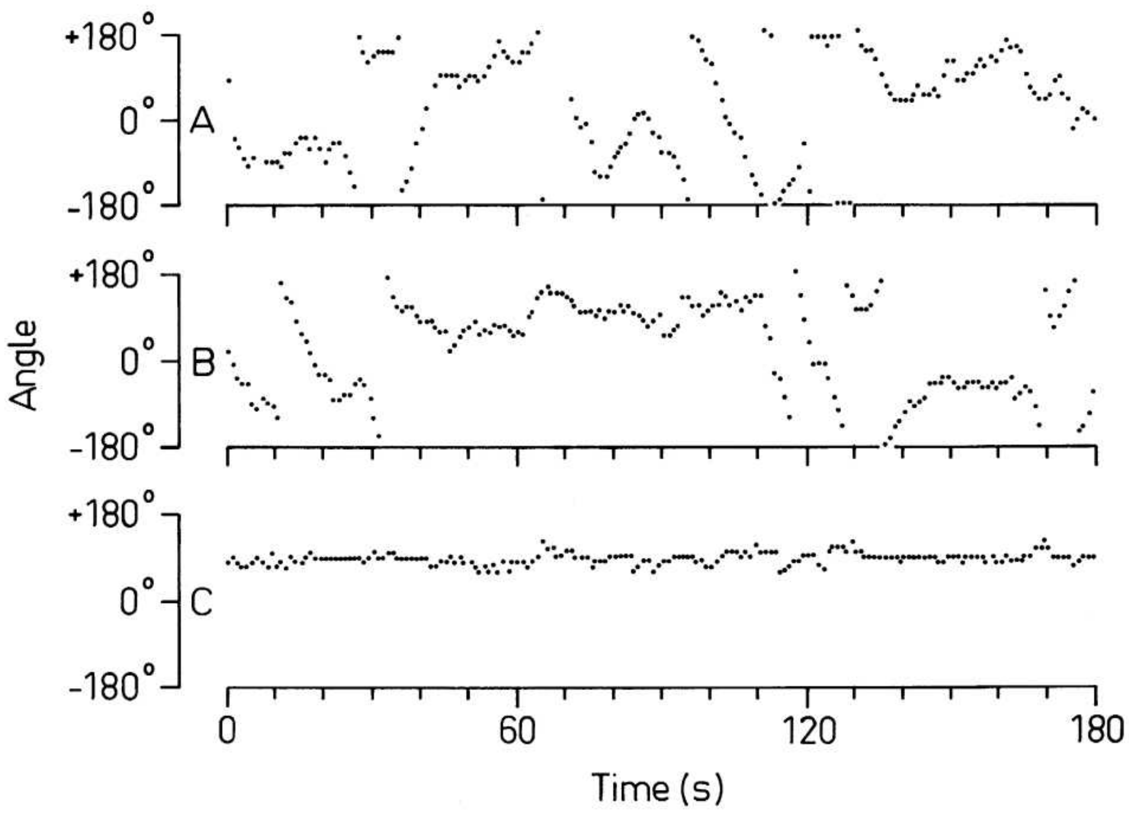
Beetle tracks for 3 minutes

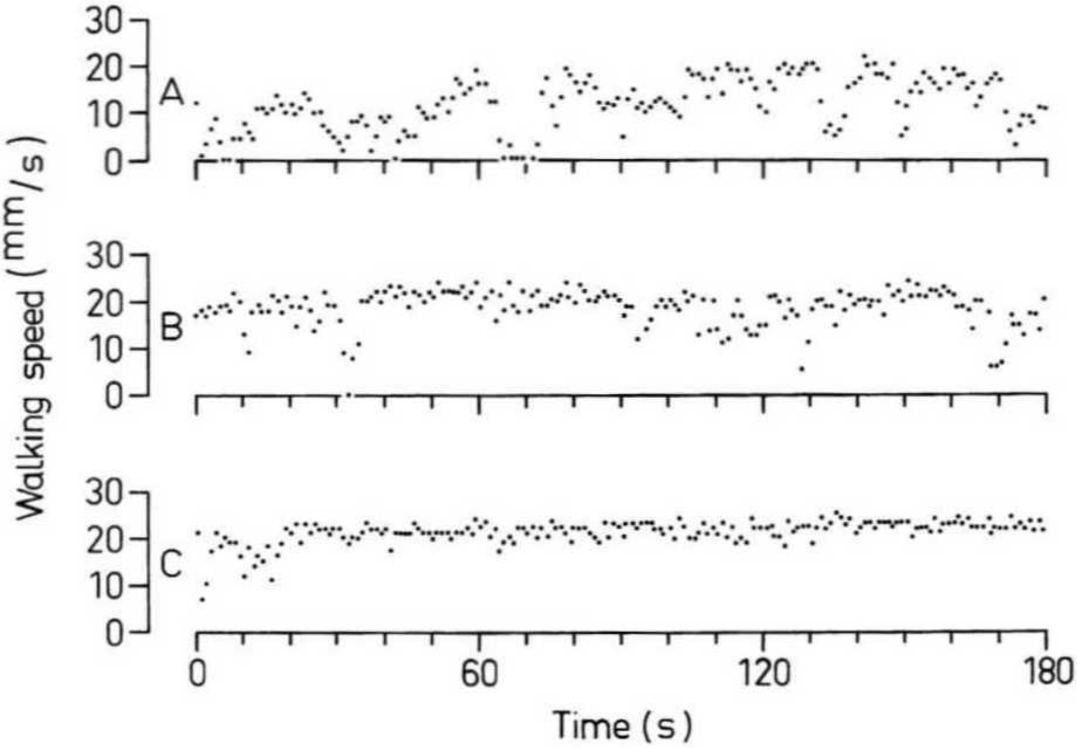


***Legend:** three-minutes walking tracks of one female Colorado potato beetle are shown. In control conditions (a) the beetle moves in circles around the starting position. Wind (b) activates the beetle which now walks further away. But, occasionally the beetle still walks in circles. In wind carrying potato plant odour (c) the beetle moves straight in the upwind direction. The beetle detects the wind direction and odour stimulates the beetle to walk upwind (which is called an odour-conditioned or odour-induced positive anemotaxis). In the figure below the walking tracks are analyzed for every second. In control conditions (A) a lot of circling, and, therefore, low walking speeds since the beetle has to slow down while turning. In wind (B) the beetle is activated, moves faster. Periods of keeping direction are alternated with loops. In wind carrying potato plant odour, when olfactory receptors activate (C) the beetle walks straight upwind with a regular walking speed, in a true positive odour conditioned anemotaxis.*

Tracks of a female Colorado potato beetle walking for 3 minutes in (a) control conditions – no wind -, (b) wind of 80 cm/s, and (c) wind carrying potato plant odour. Arrow indicates wind direction.

Orientation angles walking speeds





***Legend:** Continuous recordings of orientation angles (up) and walking speeds (down) of a female Colorado potato beetle in (A) control conditions – no wind – , (B) wind of 80 cm/s, and (C) wind carrying potato plant odour. Upwind direction is at +90°.*

Track parameters.

The beetles' movement was recorded by two pulse generators, which both had a resolution of 0.25 mm/pulse. A ten-minutes walking period produced a large data array of 600 x,y positions, and for every second walking speed, orientation angle and angle deviation (direction change/s) were calculated. It should be noted that for accuracy reasons orientation angles were solely calculated in cases when walking speeds exceeded 1 mm/s. Various track parameters were evaluated for their effectiveness to describe the characteristics of tracks and the dynamics of walking. The following are quantified here:

(1) the vector length is the net displacement from the origin,

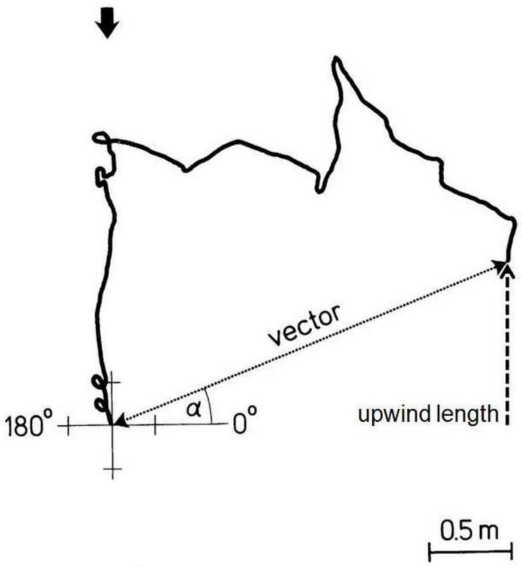
(2) the upwind length is the net upwind displacement,

(3) the sine of the vector angle ranges from +1.000 (upwind) to –1.000 (downwind),

(4) the total straightness is the quotient of vector length and track length. The alternation of straight walks with occasional turns is quantified by

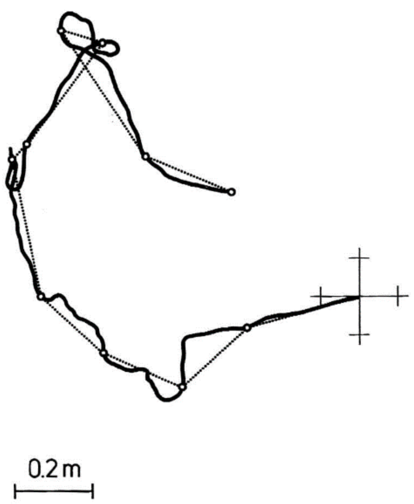
(5) the mean straightness of 30-seconds track intervals. In addition (6) circling is defined as the percentage of the tracking period when absolute angle deviations per 5 s > 50o. See illustrations below for parameters 1-5.

Vector length



Legend: *vector length is the shortest distance between start and finish of the track. Vector angle and upwind length are illustrated. Total straightness is the quotient of vector length (dotted line) and track length (solid line) at the end of the tracking record. Arrow indicates wind direction.*

Mean straightness



L

L*egend:* *Example of average straightness is calculated for 30-seconds track intervals: the quotient of vectors (dotted lines) and tracks (solid lines) measured every 30 s (track parts between dots).*

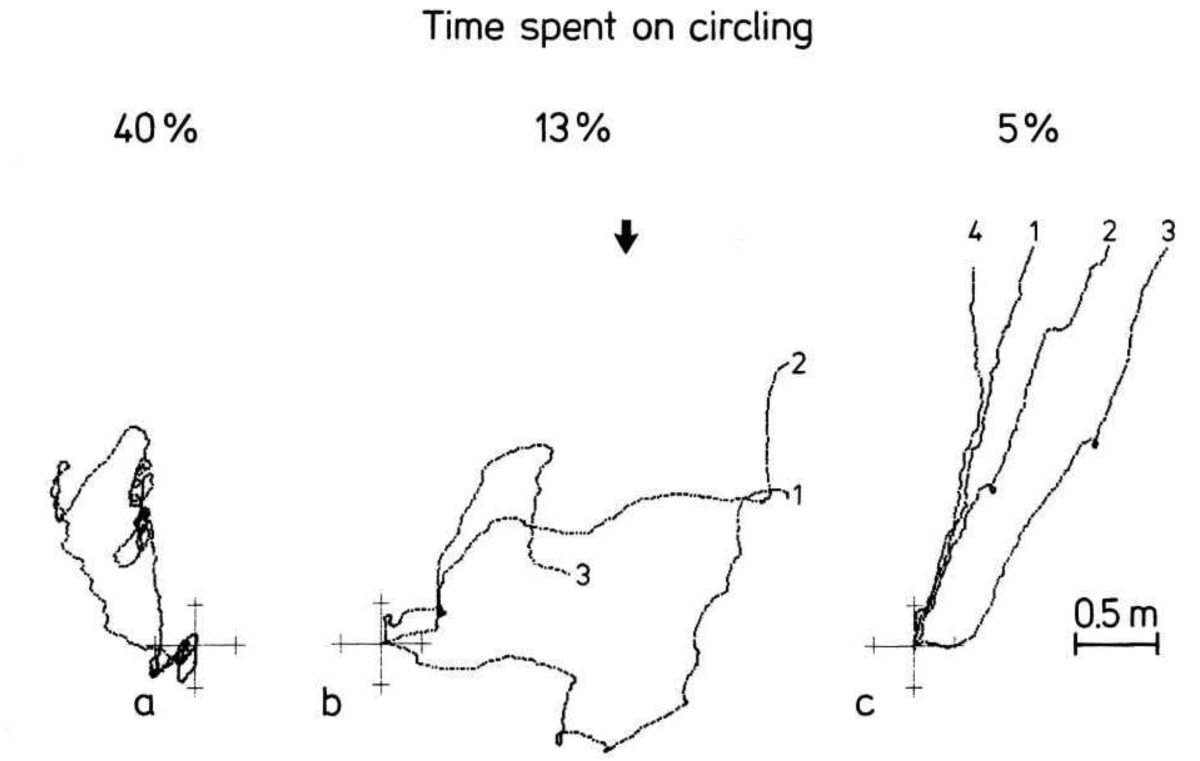
For the studies presented in the table (see below) we used newly-emerged female Colorado potato beetles from the stock culture of the Department of Entomology in Wageningen. Individual beetles were isolated and starved overnight for at least 17 hours. From emergence to the experiments these beetles never had contact with potato foliage, so were inexperienced. The ten-minutes walking tracks of beetles were recorded on the servosphere under three experimental conditions, namely (a) control condition, the lights are on (light intensity is 1750 Lux, 2500 Hz, at 24oC) and no wind, (b) wind condition, the same as control condition but now with wind coming from the outlet of the wind tunnel (80 cm/s), and (c) wind+odour condition, the same as wind condition but now the wind carrying the odour of six fully-grown potato plants (cultivar Eigenheimer) standing upwind in the wind tunnel.

Legend Table: *Means of orientation responses of 75 female Colorado potato beetles walking in three successive treatments for 10 minutes each. Different letters in a row indicate statistical differences between treatments at P ≤ 0.003 (2-tailed). Statistics applied: s means Sign Test and w means Wilcoxon matched-pairs, signed-ranks Test (see S. Siegel, 1956: Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill Kogakusha, Tokyo, 312 pp.). Speed deviation is the mean of standard deviations of walking speeds.*

	Control	Wind	Wind + Odour	Statistics
Walking speed (mm/s)	12.3 a *	14.8 b	15.1 b	w
Speed deviation (mm/s)	3.6 a	3.6 a	3.2 b	w
Mean straightness	0.57 a	0.72 b	0.84 c	s
Sine of vector angle	0.227 a	0.185 a	0.873 b	w
Vector length (cm)	182 a	360 b	687 c	w
Total straightness	0.25 a	0.41 b	0.75 c	s
Upwind length (cm)	41 a	71 a	627 b	w
Circling time (%)	33 a	22 b	10 c	s

The data presented in the table are very convincing. It is difficult to imagine that in the older literature the olfactory attraction of Colorado potato beetles towards potato plants has been doubted. It is seen from the data that beetles move in the upwind direction when stimulated by potato plant odour: see sine of the vector angle and upwind length. In addition, tracks of beetles in wind carrying odour are straighter: see total and mean straightness. Therefore, beetles move further from the starting position: see vector length.

The explanation of the tracks observed is straightforward: orientation is the resultant of circling (idiothetic control) and keeping direction (allothetic control). In control conditions circling dominates, when stimulated by wind circling decreases and the wind stimulates the beetle to keep direction, although the preference for a particular angle changes every few minutes (because of circling), and in wind carrying odour keeping direction now dominates and circling is of minor importance (see also walking tracks below). The explanation of insect orientation, being the resultant of idiothetic and allothetic control, is valid for all animals, from tiny insects upto elephants (see Visser, 1988 for further explanations).



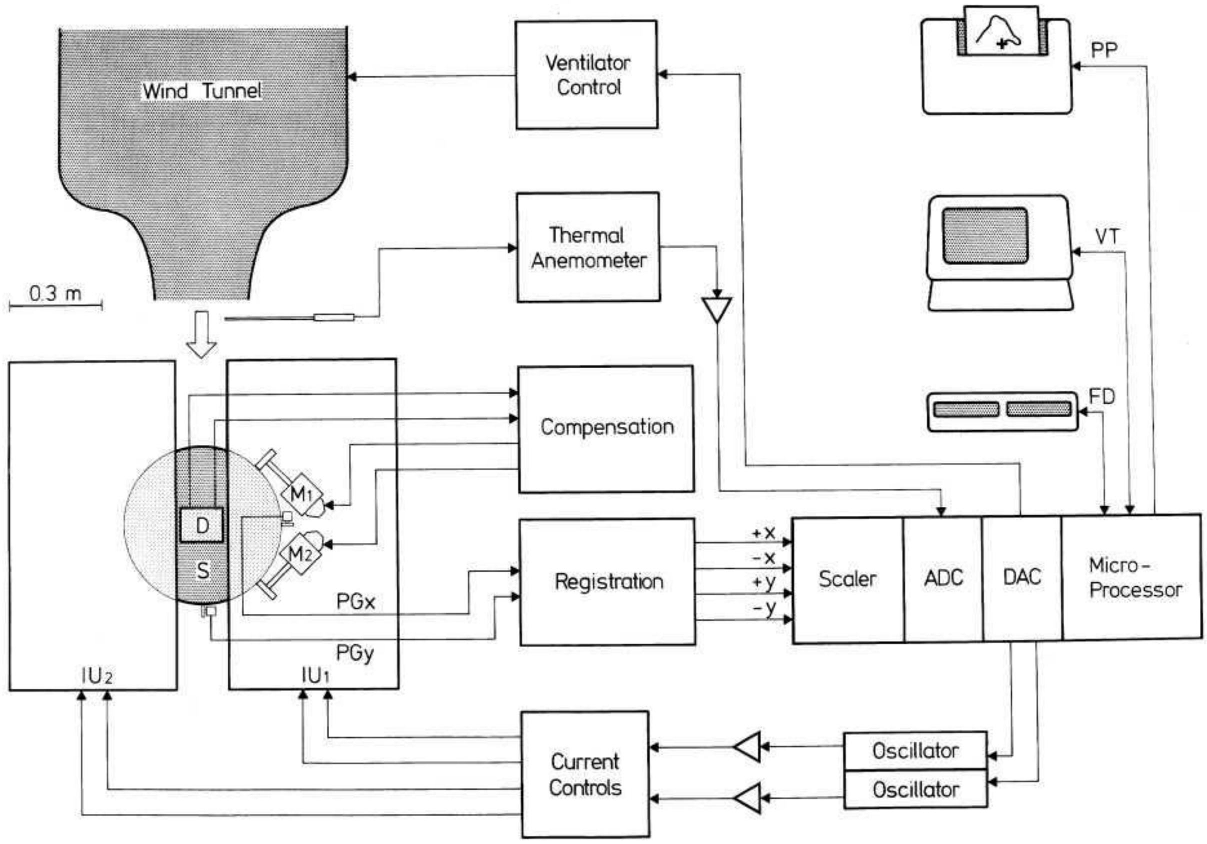
Legend: *Walking tracks of a female Colorado potato beetle under three conditions of 10 minutes each: (a) control – no wind -, (b) wind of 80 cm/s, and (c) wind carrying potato plant odour. Time spent on circling (%) is the percentage of the 10-minutes period when absolute angle deviations per 5 s > 50^o. Plotter was reset to the origin on reaching paper margin, and numbers show sequence of tracks. For each female tested, circles sizes are relatively constant, their frequency strongly reduces when progressively increasing the external stimulation. We could also identify right or left turning individuals, tendency maintained in each individual with no relation to morphometric traits. Arrow indicates wind direction for treatments b and c.*

Acknowledgments

This project was supported by a grant from BION-ZWO to J.H. Visser, J. de Wilde and L.M. Schoonhoven, and by the Wageningen Agricultural University. We express special thanks to J. de Wilde and L.M. Schoonhoven for constant intellectual support in this project.

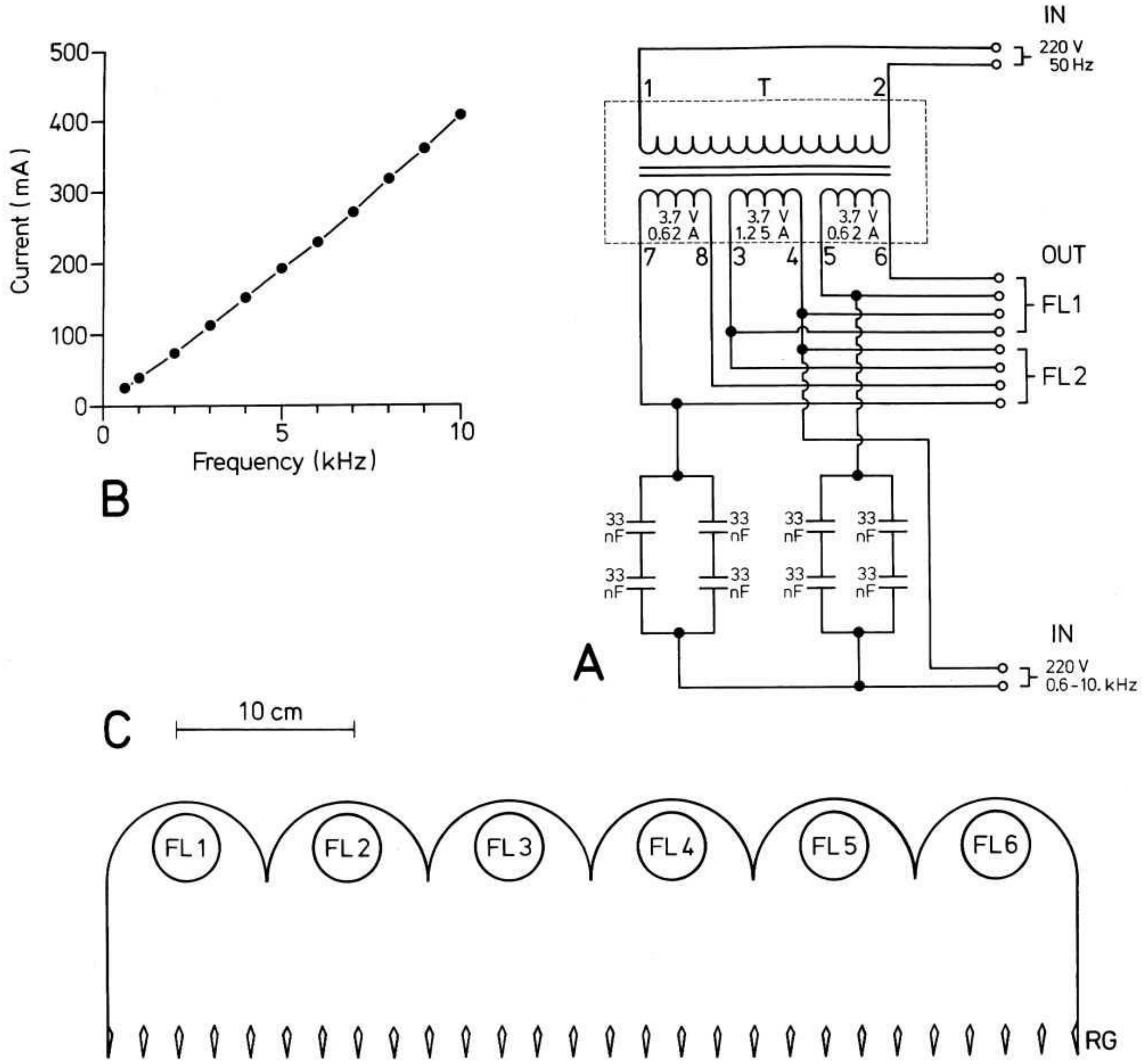
Quite a number of people were involved in realizing the servosphere equipment. The original designers E. Kramer and P. Heinecke (MPI Seewiesen) advised and the last one built the servosphere electronics. In order to study small insects like aphids, an amplifier and filter for the position-detector signal was included. The mechanical parts were constructed by our technicians G. van Surksum, G. van den Brink and G.G. Schuurman. The advice of T.H. Heijnsdijk and H.H. Perquin (Philips) and the assistance of our technicians A.H. Gerritsen and C.G.M. Derksen led to the development of the high-frequency illumination units. Valuable computer advice for a CAMAC MIC/11 system with EIS/FIS chip was given by J.G. de Swart (ITAL) and R.A.M. van Lopik (TFDL). Software was developed in RT11/Fortran by H. Wezel (TFDL), R. Stouthamer and H.A.J.M. Toussaint.

Technical addendum for servosphere equipment



Legend: *The servosphere equipment consisted of five subsystems for: (1) compensation of locomotion, (2) registration of locomotion, (3) data storage and calculations, (4) illumination controls, and (5) wind speed control. Triangles represent amplifiers. Open arrow indicates wind direction. D is position detector, FD is dual floppy disk, IU1 & IU2 are high-frequency illumination units, M1 & M2 are motors for compensation, PGx & PGy are pulse generators for registration, PP is printer plotter, S is sphere, and VT is video terminal.*

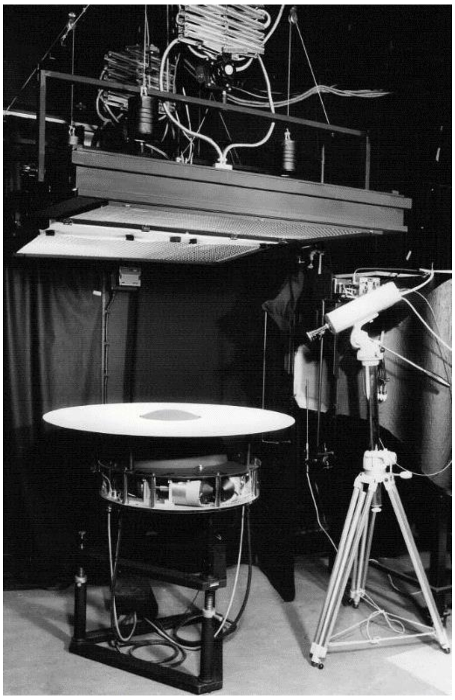
High-frequency illumination

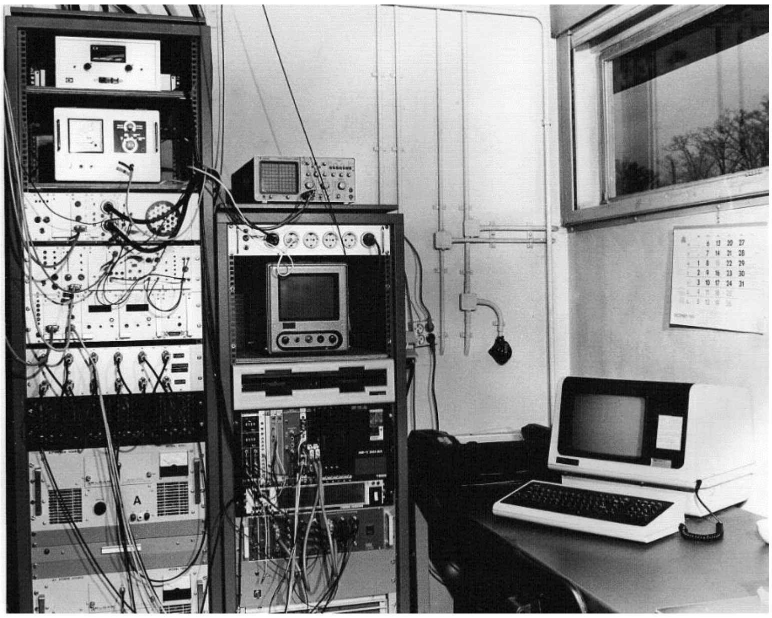


Legend: *Explanation of high-frequency illumination units. High frequency illumination was built to mimic the natural light and thus avoid interfering with the normal walking behavior.*

In A current control circuitry for 2 fluorescent lamps FL1 & FL2, T is transformer, variable frequency input came from oscillator (Krohn-hite function generator 1000) connected with AC power source (Elgar model 1001 C-103). In B frequency-current function for 1 TL-M fluorescent lamp 40W/220V. In C cross section of light frame, on the inside painted with white lead, FL1-FL6 are six fluorescent lamps, color 55 (daylight), RG is reflection grid. Since insect eyes possess high flicker-fusion frequencies insects may *observe the 100 Hz flicker from normal fluorescent lamps. For that reason high-frequency illumination was used.*

Global view of the experimental chamber. Servosphere in front of wind tunnel servosphere electronics





Legend: *the servosphere (the experimental arena) is physically separated from the observer the electronics and the wind tunnel. Right picture show the electronic control devices and data recording. The observer can follow the walking experiments via a video screen.*

Servosphere equipment from December 1982 in the Entomology lab Wageningen: "those were the days ……."

Illustrations and text adapted from:

https://olfacts.nl/

Related publications at:

https://www.researchgate.net/profile/Hans-Visser-2

https://www.researchgate.net/profile/Denis-Thiery-2