

INTERACTION BETWEEN WALKING AND PROBING IN *APHIS FABAE* SCOP.

BY A. IBBOTSON* AND J. S. KENNEDY

*Agricultural Research Council Unit of Insect Physiology,
Zoology Department, University of Cambridge*

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INTRODUCTION

In a previous paper (Ibbotson & Kennedy, 1951) it was shown that the clustered distribution of the black bean aphid, *Aphis fabae* Scopoli, on its host plants was due partly to the gregariousness of the aphids. In a preliminary analysis of the stimuli mediating this behaviour the distribution of probes made by aphids walking on leaf surfaces was examined. Probes were grouped close to stationary live aphids, close to black models of aphids, where the divergence of a side vein impeded progress along a main vein, and where the aphids first encountered the leaf surface on debouching from a card 'cat-walk' up which they had climbed. Probes made close to other aphids were of longer duration than those made close to inert models, at the divergence of side veins or elsewhere on the leaf.

The act of probing is an elementary behaviour pattern universal among aphids whether they are gregarious or not. Further work was concerned primarily with the probe-evoking stimuli provided by the aphids themselves; but inevitably it touched upon other factors governing probing but not immediately concerned in aggregation. These experiments having a more general bearing are considered below, and the rest in a separate paper (Ibbotson, in preparation).

In order to avoid the irregularities of a leaf surface which themselves elicited probes, most of these experiments were done with aphids off the plant entirely. Typically, the aphids were made to walk along uniform cat-walks of card or paraffin wax about 2 mm. wide. These simulated leaf veins in that an aphid, once astride such a ridge, had a tendency to continue walking along it. Each experimental arrangement was enclosed in a white muslin tent set directly under a 60 W. tungsten lamp 45 cm. above the test aphids, so that they were uniformly illuminated and the observer sitting outside the tent was concealed from them. Further details of methods are given in the appropriate sections below. Only apterous virginoparous adults were used, bred in the mass culture described by Kennedy & Booth (1950) and selected as detailed below.

* Now at: School of Agriculture, King's College, Newcastle-upon-Tyne.

RESULTS

The act of probing

The series of movements making up a probe on the inert surfaces was the same as on a leaf (Ibbotson & Kennedy, 1951). The aphid checked its forward movement, protracted its rostrum, and, with the antennae pointing forwards and still vibrating, made a number of decisive 'jabs' at the surface beneath it. The legs were then disposed for centripetal traction and the antennae laid back as the aphid finally came to rest. Sometimes the rostrum was half raised for a short distance before the aphid stopped completely; occasionally, when the foothold seemed uneven, the aphid moved over the substrate in a more or less crab-wise movement, trailing the tip of the rostrum along the surface beneath it before jabbing; on yet other occasions the jabbing movements were interrupted and the aphid moved to a fresh position before completing the probe.

When the aphid's stylets penetrate deeply the labium becomes shortened and elbowed as the head approaches the surface. Aphids were commonly seen in this condition on the plants in the culture and could then be induced to withdraw their stylets only by prolonged stimulation. If such an aphid was forcibly dislodged the stylets were pulled out of the plant tissue and were obvious to the naked eye. On wax surfaces deep insertion of the stylets did not seem to occur as the rostrum was not elbowed, and when the aphid was dislodged the stylets were not similarly visible. Slight penetration by the stylets may have occurred because probes made on wax lasted longer, before locomotion was resumed, than they did on glass into which penetration was of course impossible. Moericke (1950) noted that *Myzus persicae* (Sulz.) left a minute drop of saliva at the point of each probe made on glass.

Elicitation of probes

Aphids kept in a glass container for some hours in the dark were found to be stationary with the rostrum retracted against the body. They began protracting the rostrum and probing only on exposure to light, which soon induced walking instead. On the other hand, when they were walking any considerable restraint imposed upon their progression led sooner or later to probing in situations which differed widely as far as the type of sensory input was concerned.

(1) Total restraint of locomotion

Aphids were taken at random from among those walking on the cage walls of the mass culture and each was suspended by a wire attached to the dorsum with spirit gum. When held without tarsal contact they waved their legs irregularly in the manner which usually leads to the righting of a free aphid fallen on its back. The leg movements were spasmodic, and combined irregularly with them were protractions and retractions of the rostrum. As the leg movements gradually subsided the aphid often came to rest with the rostrum protracted (Table 1).

When the tarsi were given contact with solid surfaces of four different kinds these were gripped by the claws or plantulae (the inflatable adhesive sacs at the tibio-

tarsal joint) so that the leg movements now took a locomotory form, with prolonged straining in one direction or another, until this again gradually ceased. Protraction of the rostrum rarely occurred in this situation until the phase of leg activity was over, apparently as the result of fatigue, and the preliminary jabbing with the rostrum seen in free aphids was never observed. When the aphids came finally to rest the rostrum was protracted about equally often on the four different surfaces, as with no tarsal contact at all (Table 1).

Table 1. *Numbers of suspended aphids, out of five per trial, which had protracted the rostrum into the probing position after 1 hr.*

Tarsi in contact with	Trial no.					Total out of 25
	1	2	3	4	5	
Air only	1	2	2	3	2	10
Filter-paper	3	2	3	2	3	13
Muslin	1	2	3	3	2	11
Glass	3	2	2	2	3	12
Wax	2	3	2	2	1	10

(2) *Texture of the substrate*

As reported in the previous paper, aphids walking astride a cardboard cat-walk often probed when they passed from this on to a leaf. Other 'neutral' surfaces were tested in the same way. Twin cardboard cat-walks each 2 mm. wide were arranged to lead almost vertically upwards, each to the centre of a circular test surface 2 cm. in diameter pressed against a window in a flat metal plate. In order to magnify the differences in the quality of the foot-hold that these surfaces provided, the plates were inclined over the cat-walks so that the aphids on the test surfaces were supporting their own weight in an upside-down position. Young adults taken from the walls of the culture cages were starved in glass tubes for 24 hr. before testing. They were made to walk one at a time up the cat-walks on to each surface in turn, the order being reversed from aphid to aphid.

Table 2. *Numbers of aphids (out of n) that probed when walking upside-down across various flat surfaces*

n	Filter-paper		Clean glass	Smooth wax	Leaf (<i>Vicia faba</i>)
	Rough	Rough waxed			
10	1	3	—	—	—
10	1	—	9	—	—
20	0	—	—	—	18
20	—	1	—	11	—
20	—	—	4	11	—
20	—	—	6	14	—
20	—	—	—	11	12
20	—	—	—	14	15

On rough filter-paper and rough wax the aphids obtained a secure grip with their claws and continued to move forward freely as on the cat-walk, and very few probes were made (Table 2). On the glass and still more on the smooth wax the aphid's tarsal claws seldom engaged and the plantulae were brought into play giving a poor grip against gravity. Some aphids lost all foothold and fell and were discarded. The progress of the others was halting and clumsy, for obtaining and releasing each foothold took longer with the plantulae than with the claws, and probes often ensued. Probes occurred on smooth wax almost as often as on a host leaf.

These results may be contrasted with those obtained from tethered aphids on horizontal surfaces of diverse texture as above. The tethered aphids gained a sufficient grip on all those surfaces to be stimulated by the resistance they offered (due to the tethering) into making strong locomotory traction responses with the legs. The eventual inhibition of the locomotor reflexes that was accompanied by rostrum protraction could reasonably be attributed to fatigue. There was little resistance to traction for free aphids upside-down on the surfaces offering an insecure grip, and the leg strokes were curtailed. These aphids often protracted the rostrum and began jabbing before leg movements had ceased, and fatigue alone could hardly account for the final complete inhibition of locomotory movements as probing proper began. Here it appeared to be the onset of probing itself which completed the process of inhibition of the locomotory reflexes begun by the substrate.

(3) *Form of the substrate*

Some substrates stimulated locomotory movements at the expense of probing. On a vertical cardboard strip, the width of which exactly fitted the span of the legs, the aphid grasped the two edges with the tarsal claws, advanced rapidly and un-deviatingly when illuminated from the dorsal side, and probed very rarely. On a strip which was either too wide or too narrow for its edges to be gripped symmetrically the aphid's progress was irregular and slower, but leg movements were nevertheless strongly stimulated by the good grip obtained by some of the tarsi. The aphids made frequent changes of posture and grip, sometimes lurching over one edge and even sidling round to the opposite unlit face of the strip. Probes were more frequent than on a strip of the correct width, but still infrequent compared with those made on wax and glass where the claws seldom engaged. The elicitation of this 'locomotor struggling' at the expense of probing recalls the behaviour of the aphids tethered on various surfaces (see above) before fatigue reduced their locomotory excitation.

With wax it was possible to compare uniform substrates with the same, correct, width but different shapes. Three 2 mm. ridges were made by drawing metal templates along blocks of paraffin wax 10 cm. long (Fig. 1). A model aphid (black stone) was fixed at one end of each ridge as a standard probe-evoking object. In two duplicate experiments twenty-five fresh young adults were set to walk along each horizontal ridge in turn toward the model. When the ridge was square in cross-section (Fig. 1*a*) the aphids gripped its two edges firmly with the claws, walked

rapidly from one end to the other, and climbed over the model without probing at all (Table 3). When the ridge was almost flat (*c*) they advanced stably with moderate speed but did not maintain a straight course and usually left the ridge before reaching the model. When the ridge was smoothly semicircular in section (*b*) they stayed on it to the end but moved forward more slowly and waveringly, and more than half of them probed when they reached the model. In addition to the probes made in the immediate vicinity of the model, a number of the aphids probed elsewhere along this semi-circular ridge. These probes were not localized but distributed at random, like the probes made intermittently on the waxed Petri-dish rim (see below).

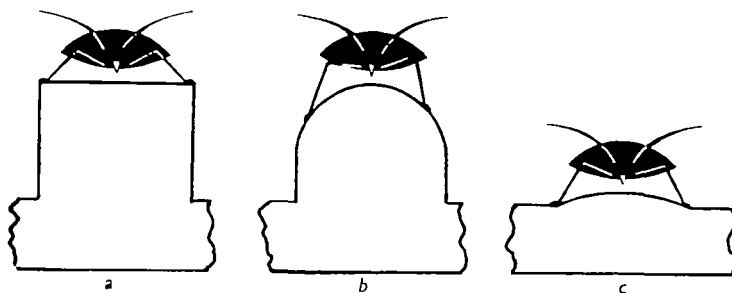


Fig. 1. Diagrammatic frontal view of aphids walking along wax ridges of equal width but different cross-sections, affecting the quality of foothold and gait (see text).

Table 3. Numbers of probes made within 5 mm. of model aphids set at the far end of straight wax ridges 10 cm. long, of different cross-section (see Fig. 1); and numbers of probes made at random along each ridge

(Two batches of twenty-five aphids, each aphid walking individually along each ridge in turn.)

Situation of probes	Shape of ridge in cross-section		
	Almost flat (<i>c</i>)	Semi-circular (<i>b</i>)	Square (<i>a</i>)
Localized, within 5 mm. of model	0	12	0
At random	0	9	1
	2	5	0

When watched through a medium-power binocular microscope, the aphids walking along the square ridge were seen to make firm contact with the tarsi at every step, after about the same amount of leg extension, and this even length of stride set up a regular walking rhythm which carried the aphid forward at a good pace. On the semicircular ridge the forward-swinging tarsi made contact with the ridge again at varying levels up and down its curved sides, so that the amount of leg extension was irregular from step to step and leg to leg. The aphid lurched slightly from side to side, continually making compensatory changes in the amount of leg extension and flexion in such a way as to keep the body oriented more or less

along the ridge. That the aphids on this ridge were being repeatedly disoriented but correcting their orientation in this manner was confirmed by removing the overhead light. They then walked steadily along the square ridge as before but wandered off the semicircular one after a few seconds. Stimuli from the semicircular ridge were not symmetrical enough to hold the aphids astride it without the addition of further symmetrical stimulation from the overhead light, although the former stimuli had more orienting effect than those from the ridge that was almost flat. The locomotory movements of the aphids on the semicircular ridge suffered, in the result, more impediment than on either of the other two ridges.

(4) *Individual objects*

The relation between probes made at random along the uniform ridges and what we may call 'localized' or simply 'local' probes made in the immediate vicinity of newly encountered objects was examined further by setting aphids to walk round the waxed rim of a 9 cm. Petri dish (Fig. 2). The rim was coated by repeated dipping in molten paraffin wax and then smoothed until semicircular in cross-section and about 2 mm. wide. The dish was surrounded by a 5 cm. high wall of cartridge paper 2.5 cm. away, all within the white muslin tent. In this situation the aphids remained walking round the rim for long periods. Five of them left there overnight on one occasion were still walking around it next morning. If the overhead light was turned off, or the perimeter wall or muslin sides were removed, thus destroying the uniformity of the visual field, the aphids soon climbed down from the rim.

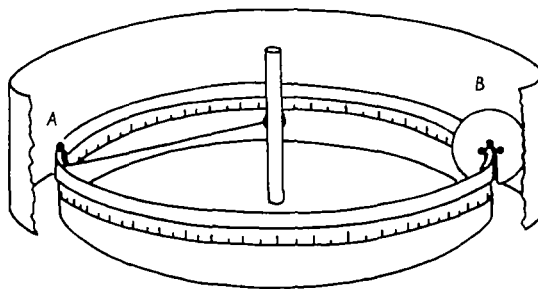


Fig. 2. Sketch of Petri dish with waxed rim, and (cut away) perimeter wall, showing arrangement of live aphid fixed on wire, (A) and transparent, black-spotted disk (B) used for testing probing responses of aphids walking round the rim.

The first objects used to provide localized stimulation for aphids walking on the rim were other live aphids attached with spirit gum by the ventral surface of the abdomen to the flattened ends of thin copper wire, arranged as shown in Fig. 2A. Walking aphids that began to climb over one of these fixed ones, or caught up with the walking aphid next in front, were immediately lifted over with a capillary suction tube applied to the dorsum and set down on the rim beyond, where they continued walking. By this means five aphids could be kept circling the rim and encountering the fixed objects with only rare encounters among themselves. A

batch of five aphids was taken from the culture on three separate occasions, and allowed to circle the rim together until each had completed five circuits in three consecutive trials: (i) with one live aphid fixed on the rim, (ii) with two live aphids fixed at diametrically opposite points on the rim, and (iii) with none. The three trials were conducted in a different order with each batch, and the distribution of probes was recorded by reference to a millimetre scale beneath the rim.

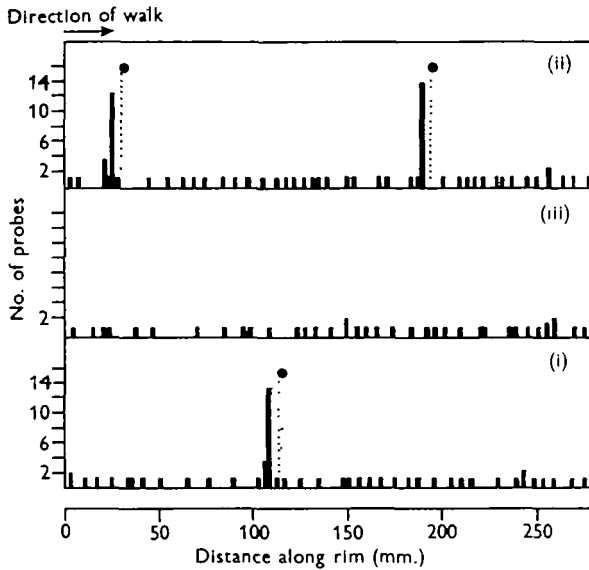


Fig. 3. Distribution of probes made by aphids walking round waxed Petri-dish rims and encountering one, two, and no live aphids fixed upon it at the positions indicated by black circles and dotted lines. Total random probes made more than 5 mm. from a fixed aphid: 36 with arrangement (i), 37 with (ii) and 38 with (iii).

The total number of probes made at random round the rim was about the same in (i), (ii) and (iii), although the total number of local probes varied with the number of fixed aphids present (Fig. 3). The number of local probes made within 5 mm. of each fixed live aphid was about the same in (i) and (ii). Within the limits of the experiment, readiness to stop and probe was thus unaffected by the total distance walked since last encountering an object, or by the number of probes recently made. These experiments also showed that the probe-evoking effects of two objects at opposite points on the rim could be regarded as independent and thus compared.

The first objects compared by this means were fresh, young aphids (smooth, glossy and dark green) and older ones (rugose, matt and nearly black), both collected while wandering in the culture cages. Free aphids of these same two age-groups were tested in batches of five against the two ages of fixed live aphids. Table 4 shows that young walking aphids were more responsive than older ones and that fixed young aphids evoked fewer responses than did fixed old ones.

Table 4. *Numbers of localized and random probes made per twenty-five circuits of the circular waxed rim by successive batches of five old or five young test aphids, when similar old or young aphids were fixed at opposite points on the rim*

Free aphids under test	Localized probes		Random probes
	At fixed, live old aphid	At fixed, live young aphid	
Old	3, 1, 0, 0	0, 0, 1, 0	2, 0, 0, 0
Young	14, 11, 10, 10	7, 4, 6, 3	4, 4, 0, 0

Further comparisons were designed to separate and identify those features of a fixed live aphid which stimulate another approaching aphid to stop and probe. Objects embodying particular aphid-like features, and control objects resembling them except for the lack of the chosen features, were both tested against the common standard of a live older aphid fixed on the opposite side of the rim. The objects are listed below:

Aphid-simulating	Controls
1. Aphid model (visual): black stone of aphid size	1 a. White stone of aphid size
2. Aphid group silhouette: transparent disk of talc 2 cm. in diameter set astride the rim by a radial slot, and painted with 3 black spots 2 mm. diam. and 2 mm. apart grouped round the apex of the slot on the rim (Fig. 2)	2 a. Transparent talc disk without black spots
3. Aphid odour: white (stone) model smeared with the fresh reddish brown wax extruded from the siphunculi of adult aphids as a reflex response to rough handling—in this case pinching the thorax with forceps	3 a. White aphid models smeared (i) with a pigment suspension simulating the appearance of the models smeared with siphuncle wax, and (ii) with honeydew
4. Mechanical impediment: a loose meshwork of cotton fibres lying across the rim	4 a. Three horse-shoe-shaped copper wires 0.5 mm. diam. and c. 2 mm. apart, lying across rim
5. Mechanical block: a thin glass rod drawn to a point and set above and parallel to the rim in such a way that the point struck the walking aphid squarely on the frons	(5 a. = 2 a, i.e. less abrupt block)

Each object was tested with three separate batches of five free aphids on the rim. Tests were conducted only with those batches which, in a preliminary check, made ten or more local probes in every twenty-five encounters with the fixed aphid. At this level of responsiveness the free aphids differentiated clearly between each object which embodied a chosen aphid-like feature and the control object which lacked it, consistently making ten or more local probes in every twenty-five encounters with the former, and less than five in every twenty-five encounters with the latter.

Aphids approaching the black model (1) and the siphuncle-wax-smeared model (3) stopped abruptly and probed at once, usually just before their forward-pointing antennae had touched it. They reacted to the broader group silhouette (2) in the same way except that some aphids moved crab-wise on the rim with the rostrum

protracted either before or during jabbing. The failure of the control objects 1*a*, 2*a* and 3*a* showed that the visual and olfactory stimuli given by other aphids were both effective in causing walking aphids to stop and probe, while honeydew was not. Mechanical obstruction was most immediately effective when encountered in the most sudden and complete form, as the glass point (5). This usually passed between the forward-pointing antennae without touching either and the impact it then made on the frons induced a temporary complete inhibition of leg movements. On the other hand, the mechanical obstruction provided by the cotton fibres (4), which interrupted the leg movements directly and occasionally caught the claws, was not effective until after a few seconds, and the wires (4*a*) over which the aphids clambered with the minimum of constraint were ineffective with aphids at the given level of responsiveness.

Effects of pre-treatment on probing

In addition to the variation in responsiveness mentioned above as associated with age in aphids taken from the walls of the culture cages in the glasshouse, where they were free to leave and return to the plants, some of the variation among these aphids appeared to be governed by the weather in the preceding hours. In selecting the batches of five aphids for testing, as above, it was found that those collected and tested in the morning usually responded well to probe-evoking objects on the rim; whereas if the day had been bright and warm since the early hours, aphids of similar appearance were relatively unresponsive. Similarly, those collected and tested in the afternoon usually responded poorly, but if the day had been particularly dull and cool they often responded well. These weather differences also affected the amount of wandering seen in the cages. When bright, warm conditions prevailed all day wandering activity showed one peak in the earlier part of the morning and a second peak in the late afternoon, with a lull around midday when most of the morning wanderers were back on the plants. This sequence recalls the diurnal variations in the aerial population of alate aphids (Johnson, 1954). In dull weather there was less wandering altogether and little sign of a midday lull. Prior activity off the plant was thus associated with lesser readiness to probe on encountering an aphid-like object. These effects were investigated further as follows.

Aphids were reared on young bean plants in two dark rooms maintained at 19 and 24° C. Within 24 hr. of the last moult the adult apterae were separated into batches of 50 and given one of the treatments listed in Table 5. Aphids from each treatment were then tested in turn on a waxed Petri dish rim in the same two dark rooms, with two diametrically placed transparent talc disks astride the rim, one with and the other without three black spots (objects 2 and 2*a* in the previous experiment). Each treatment was applied to two batches of aphids and timed so that the second batch was ready for testing the day after the first. All the treatments, and the series of probing trials, were started at about 9 a.m. each day. Each trial lasted about 30 min. so that the batch tested last in a given day's series had been undergoing its treatment for several hours longer than the batch that was tested first. The different treatments were equalized in this respect by testing in a different

Table 5. *Mean numbers of localized probes near the talc disks, and of random probes, made per twenty-five circuits of the circular waxed rim, by four separate batches of test aphids from each treatment*

(All treatments except E carried out in the dark. Starved aphids kept in a clean Petri dish. All experiments conducted after the aphids had been exposed to the light for 15 min. at the temperature at which the experiment was done.)

Treatment	Localized probes		Random probes
	At spotted disk	At plain disk	
At 19° C.			
A. Fresh from rearing plants	18·00	6·25	8·25
B. Starved for 24 hr.	13·00	3·75	21·50
C. Starved for 24 hr., tested at 24° C.	8·25	2·00	17·75
D. Starved for 48 hr.	4·50	0·25	48·25
E. Starved for 24 hr., in daylight	7·00	1·00	14·50
F. On young bean shoots for 48 hr.	24·25	23·75	34·25
At 24° C.			
G. Fresh from rearing plants	17·00	6·00	9·25
H. Starved for 24 hr.	11·75	2·75	45·50
J. Starved for 24 hr., tested at 19° C.	20·00	8·00	30·00

Analysis of Variance

	S.S.	D.F.	M.S.	F
Total	17643·55	107	—	—
Probes	6937·85	2	3468·92	466·19
Treatments	4006·46	8	500·81	67·30
I.A.	6096·49	16	381·03	51·21
Error	602·72	81	7·44	—

Minimum significant difference between treatment means within probe type: at 5%, 6·18; at 1%, 7·25.

order each day: 1st day—A, B, C, E, G, H, J; 2nd day—D, F, J, H, G, E, C, B, A; 3rd day—F, D.

Two sets of five aphids were taken at random in succession from each treatment-batch each day and set to walk round the rim as described above until each single aphid had completed five circuits. The total number of random probes, and of local probes made within 5 mm. of each talc disk, were recorded separately for each set. The mean numbers of probes per set (25 circuits) are given in Table 5 with a simple analysis of variance. The within-probe between-treatment minimum significant differences were computed by Tukey's method for comparison of treatment means (Snedecor, 1956).

Assuming that as fresh adults all batches entered treatment in the same physiological condition, the effect of the treatments was to produce batches of aphids in an almost continuous series of degrees of readiness to probe. There was a linear

relationship between the number of local probes at the spotted disk and the number at the plain disk, with roughly three probes at the former for every one at the latter, except with the most responsive batch (F) which made equal numbers of probes at the two disks. The treatments did not, however, always affect local and random probing in the same way. The frequency of random probing was increased in varying degree by all the treatments, whereas local probes were sometimes increased and sometimes reduced by comparison with the behaviour of fresh aphids.

Keeping the aphids in the dark off the plant in a glass dish (where they apparently moved little and were found motionless at the end of the treatment) increased the frequency of random probes and at the same time reduced that of local probes, more or less in proportion to the duration of treatment or the temperature during treatment (A *v.* B *v.* D; G *v.* H; B *v.* H). A similar treatment in daylight where the aphids remained active (E) also increased random probing but significantly less than did darkness, and it reduced local probing still more. (Two batches given a tenth treatment not listed in Table 5, starvation in the dark at 24° C. for 48 hr., were all dead at the end of it.) 48 hr. spent at rest feeding and larvipositing on a fresh plant in the dark increased random probing less than did 48 hr. off the plant in the dark; and at the same time it increased local probes to the maximum instead of reducing time (F *v.* D). Transfer from a treatment temperature of 19° C. to a temperature of 24° C. for testing reduced random and local probes alike but not significantly (C *v.* B). On the other hand, transfer from a treatment temperature of 24° C. to a temperature of 19° C. for testing reduced random probing while substantially increasing local probes (J *v.* H). To sum up, keeping the aphids off a host plant lowered their threshold for locomotion-inhibiting stimuli from the substrate, but at the same time raised their threshold for stimuli received from other, incidental, aphid-like objects encountered while walking. The latter threshold was low in the fresh aphids which had long been subject, and responding, to stimuli from the host plant during their development, and it was lowered further only by continuing that same treatment.

DISCUSSION

So long as these parasitic insects are active but not on a host plant their activities are limited almost entirely to two motor patterns, walking and probing. As a rule only one of these occurs at one time. This is not simply because they are mechanically incompatible, for they occasionally overlap to some extent as when protraction of the rostrum and jabbing began before locomotion had ceased and when suspended aphids waved their legs and protracted the rostrum simultaneously. The mutual exclusiveness is evidently a central nervous interaction in the first place; the two alternative reflex systems are antagonists, excitation of either inhibiting the other. The clearest indication from the present work that they are antagonists in the full sense of Sherrington (1947) was the sustained alternation between them that was generated in the aphids by a particular external environment which was not itself alternating but constant. When an aphid was walking on the wax rim and lit from above, and the external inhibitory influence of the substrate upon locomotion

reached a certain level, probing as it were 'broke through' (in Sherrington's phrase) the inhibitory influence which was being exerted upon it centrally by the locomotory activity in progress. The substrate soon inhibited probing in its turn and locomotion then broke through the central inhibitory influence of probing upon it; and so on, cyclically.

In alate virginoparous *A. fabae* a similar alternation generated by a constant environment has been found between locomotion (here flying) and probing, as reported preliminarily by Kennedy (1958). This phenomenon, and other features of the relation between the alata's two systems of reflexes, serving locomotion on the one hand and host exploitation on the other, showed a detailed parallelism with the relation between antagonistic reflexes in the mammalian spinal cord (Sherrington, 1947). Either of the two reflex systems could be caused experimentally to 'rebound', through 'successive induction' by the other. The present experiments were not designed to test for rebound in the aptera, but Moericke (1950) has demonstrated something very like it, successive colour contrast, in probing by both alate and apterous *Myzus persicae* (Sulz.).

Given an antagonistic relationship between two reflex systems, it becomes difficult to determine from simple behavioural experiments whether an observed response has been brought about by direct excitation, or indirectly by first inhibiting the antagonistic system. There is in fact no evidence that any of the external stimuli used in these experiments stimulated probing directly. Very diverse types of stimulation evoked probing, and inhibition of locomotion may have been their one common feature.

When the stimulation arose from direct mechanical interference with the normal pattern of locomotory reflex actions it gave a strong impression of causing probing in that indirect way, especially since the more abrupt and complete the arrest of locomotion the more promptly probing broke through. The most effective form of mechanical obstruction, in causing probing, was the glass point. Without any 'warning' from the antennae (nor apparently from other distance receptors) this suddenly arrested the forward swing of the body pivoting on all the legs, and thus presumably created the same kind of proprioceptive input as obstructing the legs themselves all at once. A summation in time of such inhibitory stimulation of locomotion was indicated by the longer delay before probing broke through when the cotton fibres did obstruct the legs themselves but not all at once; or when the transparent disk barrier was encountered first by the antennae, or when the uniform substrate alone hindered stepping.

Even when the stimulation that evoked probing was not mechanically obstructive but took effect through distance receptors, visual or olfactory, it may have acted through inhibition of locomotion; and direct evidence that visual stimulation from black objects can induce probing by this means will be given in the subsequent paper. No matter by what central pathway they act, the visual and olfactory stimuli that induced probing would contribute most to gregarious behaviour, because these types of stimulation are the least likely to be provided by objects other than fellow aphids in nature. If there are any non-host stimuli that evoke

probing by exciting it directly, these more or less aphid-specific ones seem the most likely to do so, as a logical consequence of the evolution of the gregarious habit. But with present methods no more can be said on this point.

A further consequence of the antagonistic relationship between walking and probing is that the results of the pre-treatment experiment cannot be interpreted with any assurance in terms of the 'priming' of either activity. It seems reasonable to suppose that the excitabilities of walking and probing were both affected, in varying degree, by the different pre-treatments; but any distinction is difficult since any change in either would have some reciprocal effect on the other. Some clue is perhaps available from the paradoxical results of the treatments in which the aphids were denied access to a plant. All these treatments made the aphids probe more frequently at random along the ridge but less frequently when they encountered barriers across it. The increase in random probes implies some priming of probing (feeding 'centre') and the decrease of localized probes at barriers implies just the opposite, priming of locomotion. The results can be explained by assuming priming of both activities but not of either alone.

Thus in the *alata* (Kennedy, 1958) locomotory excitation, as measured by the rate of climb of the flying aphid, rose to a peak after each take-off following inhibition of probing on a non-host surface, and then declined more or less steadily. We may postulate that the same kind of sequence occurred during each bout of walking by the apterae between the random probes which they made on the wax ridge. Assuming also that the priming of locomotion raised the peak level of locomotory excitation attained within each walking bout, then locomotion-inhibiting stimuli encountered around the peak in each bout would become less effective, leading to the observed decrease in the number of probes at the obstacles. The priming of probing could nevertheless show itself in causing probing to break through sooner as locomotory excitation was declining again, thus reducing the average duration of each walking bout and leading to the observed increase in the frequency of random probes. Increased flight vigour combined with decreasing duration of flight bouts was recorded in the *alata*.

SUMMARY

1. In experiments with apterous adult aphids walking on non-plant surfaces, rostrum protraction and probing of the surface were elicited by a variety of situations having the common effect of interfering mechanically with the normal pattern of locomotory movements.
2. A uniform wax ridge which fitted the span of the aphid's legs but afforded a poor grip to the tarsi and broke up the stepping rhythm, combined with an overhead light, generated a cyclical alternation between walking and probing.
3. When the uniform substrate was interrupted by some form of obstacle to locomotion further probes were elicited, and the more abruptly and completely locomotion was arrested, the more promptly these appeared.
4. Visual stimulation (from black models of aphid size) and olfactory stimulation (from the aphids' siphuncle wax) also caused walking aphids to stop and probe.

5. Readiness to probe varied greatly with age and pretreatment. Aphids denied access to a host plant for 1-2 days probed more frequently at random in response to the substrate but less frequently at objects encountered.

6. Walking and probing interact in the manner of antagonistic reflex systems, and the diverse types of stimulus which elicited probing may all have done so indirectly by first inhibiting locomotion.

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