

CO-ORDINATION OF SUCCESSIVE ACTIVITIES IN AN APHID. THE EFFECT OF FLIGHT ON THE SETTLING RESPONSES

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INTRODUCTION

In a quantitative study of a phenomenon first noted by Moericke (1941, 1955), Johnson (1958) showed that fresh-winged parthenogenetic females of *Aphis fabae* Scopoli would not settle down when placed on a host plant but became progressively more ready to stay, feed and larviposit there the longer they had flown. Graham (1959) found that flight had a similar effect in the scolytid beetle *Trypodendron lineatum* Olivier. He assumed that the unflown beetle's dominating attraction to light explained its failure to respond to a suitable log. Flight eventually suppressed this photopositive behaviour, as it does in the aphid (Kennedy & Booth, 1963). But Johnson (1958, 1959) (also Kennedy, 1958*a*) found that the unflown aphid failed to settle and larviposit on a host even when kept on it in the dark. In the aphid, therefore, flight lowers the threshold of the settling responses in addition to, and not merely because of, its effect on responsiveness to light. Johnson and others showed that locomotion by walking also promoted settling after a longer time.

Blest (1960) has pointed out that this aphid phenomenon is only one among a number of documented cases of walking or flying by insects having the effect of lowering the threshold of some quite different activity, notably feeding in the blowfly and in the honey-bee, and 'rocking', 'static display' and oviposition in various moths. Such a linking together in orderly succession of two different activities has attracted physiological study because it offers a convenient unit of higher-order integration. Both peripheral and central hypotheses of the link between the two activities have already been advanced, just as they were earlier for the lower-order linking of successive muscle contractions within a single activity, locomotion.

The 'peripheral' hypotheses are versions of the old chain-reflex theory of behaviour. They assume that the link lies outside the central nervous system in some cumulative physiological consequence of the locomotor activity, which provides 'feedback'. Thus it has been suggested for the aphid (Müller & Unger, 1951; Müller, 1962), for the blowfly and for the honey-bee (Evans & Dethier, 1957; Hudson, 1958) that flight is physiologically equivalent to starvation in its effect on feeding: the energy consumption in flight is supposedly monitored in some way which acts via the C.N.S. to lower feeding thresholds. The extensive experimental evidence from the blowfly contradicts any idea that falling blood sugar or other measures of the general nutritional state act in that way; there is some evidence that falling sugar concentration in the foregut may

do so (Evans & Dethier, 1957; Dethier & Bodenstein, 1958; Hudson, 1958; Evans & Browne, 1960). In the aphid, Johnson (1958) found an increased readiness to settle after only 20 sec. flight or brief anaesthesia with carbon dioxide (but not ether). He therefore advanced different 'peripheral' hypotheses based on respiratory metabolism.

On the other hand Blest (1959, 1960, 1961) has obtained, by experimentally excluding normal sensory inputs during flight, considerable presumptive evidence against any peripheral link being necessary between flight and the commensurate 'rocking' performance that follows it in the saturniine moth *Automeris aurantiaca* Weym. He therefore put forward a central hypothesis, 'that it is the length of time during which the central nervous mechanism mediating flight has been in a state of effective excitation which is registered and expressed quantitatively in subsequent behaviour' (Blest, 1960, p. 283).

Meanwhile, further work on lower-order, short-term, rhythmic co-ordinations has shown that peripheral and central links need not be regarded as mutually exclusive alternatives, as indeed Pringle (1940) and Gray (1950, p. 125) anticipated. Wilson (1961) carried out even more drastic ablation experiments than those of Blest and showed that the regular succession of up-and-down wing movements in the flying locust did not require correspondingly phased feedback. He concluded that the basic co-ordination was inherent in the C.N.S. although on top of this there acted the peripheral feedback loops which had once been thought sufficient in themselves (Weis-Fogh, 1956; Pringle, 1957). Bullock (1961) gives further such examples in his review. However, these lower-order, rhythmic co-ordinations have been regarded (e.g. by Bullock, 1957) as quite different in kind, as well as in time-scale, from higher-order co-ordinations like the flight-feeding sequence. The common practice of studying these latter as one-way sequences only, may have made simple hypotheses of cumulative peripheral feedback seem at first sight quite adequate to explain the long-term effects. And surgical treatments designed to test their adequacy tend to preclude two-way interaction.

In intact *A. fabae*, it has been shown that the marked changes of responsiveness to light that occur with prolonged flying are readily reversed by immediate external stimuli and hence governed, not directly by feedback (as apparently envisaged in some similar cases: Graham, 1961; Clegg & Evans, 1961), but through a central nervous integrating mechanism (Kennedy & Booth, 1963). As between flight and settling, Johnson (1958) has shown that the short-term and long-term effects of flight on settling by *A. fabae* are likewise reversible. Locomotion and settling were described by Müller (1953) as conflicting 'drives', and by Johnson (1958) as opposing, mutually exclusive responses. And it has been shown by Ibbotson & Kennedy (1959) in the apterous form of this aphid that the immediate reactions of locomotion by walking, and of probing the substrate (the first act in feeding), do interact in the manner of antagonistic reflexes and, in particular, that an alternation or oscillation between them can be set going by suitable non-oscillatory inputs in a manner reminiscent of the locomotor rhythms mentioned above. The present work deals with this two-way interaction on an extended time-scale, including prolonged flight and the other settling responses. Although the experiments themselves were not so divided, this account of them is conveniently divided into two parts, the first (this paper) being concerned with the one-way effects of flight on the settling responses and the second part with reverse effects and alternations

MATERIALS AND METHODS

The apparatus and procedures used for the rearing, selection and free-flying of individual fresh adult alate alienicolous female *A. fabae* were described by Kennedy & Booth (1963), together with the method for continuous recording of the photokinetic flight response of each aphid as expressed in its rate of climb toward the lights in the central roof window of the flight chamber. The responses of the same aphids to plant stimuli were tested on single detached leaves in the same chamber. The cut petiole of each leaf was passed through and held firmly in a hole in the blackened cork of a 2 × 1 in. glass vial containing water. The tube itself was hidden and firmly held in a metal sleeve on a ball-and-socket joint and articulated arm, all painted matt black like the chamber walls. The disposition of this leaf holder in the chamber was adjusted manually by the black-gowned operator wearing a black glove.

The test leaves had been picked within a few hours before use from pot plants raised in a glasshouse with supplementary heating and lighting in winter. Mature leaves, that is leaves which had ceased growing some time before and had not yet begun visibly to senesce, were selected for uniform quality and size from well-grown plants of broad bean (*Vicia faba*, var. Seville Longpod) as a favoured host of *A. fabae* and from potato (*Solanum tuberosum*, var. Majestic) and *Fuchsia* as non-hosts. The compound leaves of bean and potato were reduced to single leaflets of the right size, which will be called 'leaves' in this account. In addition, single young terminal leaflets from seedling bean plants, less than half as large as the mature leaves, were used as maximally favourable host material for this aphid. These young 'leaves' were used when the two halves of the lamina were still folded together so that the aphids could not crawl between them, for such plant crevices are powerfully arresting.

The standard procedure for stopping flight in order to measure the aphid's responsiveness on a mature leaf was as follows. The test leaf on its supporting arm, which had been kept out of the way in deep shadow during the flight, was now swung into place under the lights in the central roof window. The arm had been pre-adjusted so that the leaf blade would now stand tip uppermost about 10 cm. to one side of the centre of the light window at the same level as the flying aphid (5–12 cm. below the window screen). The flier would have hit the leaf in this position within seconds if it had not 'dodged'. As a rule it did 'dodge' one or more times before approaching the leaf and alighting; it alighted more promptly once it had entered upon the 'ranging' phase of its flight (Kennedy & Booth, 1963).

Up to the moment of alightment the leaf blade was held at a slight angle out of the vertical with the morphologically lower or abaxial surface uppermost. This created a difference between the two sides of the leaf as seen from the level of the flier. The abaxial surface appeared whiter, owing to the larger proportion of reflected light coming from it, than the partially shaded adaxial surface which appeared greener owing to the larger proportion of transmitted light from it. As intended, the result of this was that the flier almost invariably alighted on the morphologically upper or adaxial surface of the leaf. As soon as it did so, and before it started to probe the surface, the leaf holder was quickly but smoothly turned over and shifted until the leaf lay about 15 cm. beneath one margin of the light window with its adaxial surface uppermost and its tip pointing up toward the far side of the window at about 20° from

the horizontal, so that only transmitted light reached the now shaded abaxial surface. The aphid was thus fully exposed to the lights again, dorsum upwards on the adaxial surface, exactly as it had been at its previous take-off. The effects of different flight periods and other pre-treatments could then be compared repeatedly in the same situation. As this was the natural orientation of a leaf with respect to light and gravity, the several types of stimulus, from the leaf itself and from those external sources, that guide an aphid's behaviour on a leaf (Kennedy, 1958*b*; Johnson, 1958; Wensler, 1962, and unpublished) were acting in normal relationship without conflict.

The young folded terminal leaves of bean were handled in exactly the same way throughout, although here of course no adaxial surface was exposed so that both the lit and shaded sides were made up of abaxial surface.

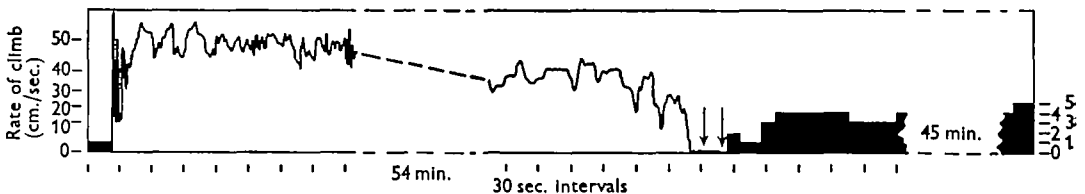


Fig. 1. Record of one individual's flight and subsequent activity on a young bean leaf, reading from left to right. Irregular line: approximate rate of climb of flying aphid according to scale at left. First arrow marks the moment when the leaf was presented and second arrow the moment of alightment on it. Height of black blocks thereafter indicates behaviour on the leaf according to scale at right—on leaf upper surface: 0, stationary, not probing; 1, walking; 2, probing. On leaf lower surface: 3, walking; 4, probing; 5, larvipositing.

A continuous record or 'ethogram' was kept of the behaviour of each aphid while it was on a leaf. The same hand-controlled pen as was used to record the rate of climb of the aphid while it was flying was now used at certain arbitrary settings to indicate whether the aphid was stationary, walking or probing on the upper or lower leaf surfaces, as shown in Fig. 1. In addition, the onset of parturition (larviposition) and take-off were noted on the record tape.

'Probing' here refers simply to the stationary attitude so described by Ibbotson & Kennedy (1959) when the rostrum (labium enclosing the mandibular and maxillary stylets) is protracted from its rest position lying between the coxae, and its tip is applied to the leaf surface, while the antennae are laid back. Aphids holding this posture for some seconds have been found to have their stylets thrust into the leaf to some extent; if they have held it for many minutes they are likely to be feeding.

Unless otherwise stated the results given below were obtained from two lengthy experiments, referred to as I and II and described in more detail below. Each experiment involved a number of different treatments replicated on a number of separate individuals, each individual receiving one treatment only. Some treatments lasted several hours so that each whole experiment lasted many weeks, with the different treatments being given in rotation to minimize any day-to-day or week-to-week variations in the aphids or other factors. The size, behaviour, fecundity and longevity of aphids are much affected by variations in the condition of the host plants on which they have been reared. The aphids were generally smaller, and less vigorous and persistent as fliers in Expt. I than in Expt. II, and this may have been due to seasonal variation in the quality of the rearing plants.

RESULTS

The sequence of settling responses

Fig. 1 illustrates the behaviour observed on a leaf when larviposition occurred there and the sequence of acts intervening between alightment and larviposition showed a minimum of reversions. So progressive and complete a sequence could normally be obtained only when the aphid landed on a most-favoured host plant organ after a long uninterrupted first flight, as will appear later. The immediate effect of gaining a foothold on any solid surface was the cessation of wing beating, followed by lowering and closing the wings and a few seconds spent quite motionless. This particular alighter now probed the leaf surface for about 15 sec., withdrew and walked to and over the edge of the leaf and probed again on the underside for more than 1 min. It then wandered slowly about on the lower surface until it arrived at the midrib into which it made its third probe. Larviposition began while the aphid was still inserted there, 45 min. later. Larviposition seldom occurred until the aphid had remained inserted at one place for tens of minutes. Such long (feeding) probes almost always occurred in the midrib or (less often) in one of the major side veins, and with the aphid's head directed proximally along the vein system (toward the midrib if on a side vein, toward the petiole if on the midrib). As soon as an aphid was seen to take up this position, a long stay and larviposition could be predicted with considerable confidence. The earlier probes made by a wandering alighter seemed to be distributed quite at random over the leaf lamina, but the impression was gained that, on the under surface, the wanderer eventually reached a stage when it probed preferentially on large veins.

Thus settling down on a leaf involved: (i) cessation of wing-beating, (ii) and (iii) probing and walking alternately, (iv) going under the leaf, (v) feeding and (vi) larviposition. All these components appeared in response to stimuli from the host leaf; but for analytical purposes it should be noted that the specific motor patterns concerned were not all excited by the leaf. The first observed response, cessation of wing-beating, was patently inhibitory, and, among the new motor patterns that appeared on the leaf, walking was also in a special category. Johnson (1958) classed walking together with probing, feeding and larviposition as different degrees of one 'settling response', on the grounds that 'each tends to sedentary as opposed to locomotory behaviour'. In experiments involving flight, walking may appear to be a settling response (if by that is meant that it is excited by stimuli from the host leaf as are probing, feeding and larviposition) in as much as more walking is observed on a host than on a non-host. This difference is illustrated by comparison of Figs. 1 or 2D with 2E, and again in Figs. 3 and 4. But in all these cases it can be seen that what cut walking short on the non-host was take-off, and that was due to the lights, not the plant. For when the flight response to light is excluded, as for example in reduced lighting, or in apterous aphids, then the non-host plant stimulates more walking than the host. This is the mechanism of host selection which has become familiar from many laboratory experiments (e.g. Wensler, 1962). The eventual settling on a host plant always necessarily involved inhibition of walking as well as of flight. It is inferred that walking was not excited by host stimuli and appeared only because they inhibited flight before their effect became strong enough to inhibit walking.

Hence walking, taking off and flying (with the associated orientation reactions) are

hereinafter classed together because they tend to be inhibited by host stimuli and excited by light whereas probing, feeding and larviposition (with their associated orientations) tend on the contrary to be excited by host stimuli and inhibited by light. This latter group, the 'settling responses' are therefore opposed or *antagonistic* to the former, locomotory responses.

That this mutual exclusiveness between the locomotory and settling responses is a central nervous antagonism and not due to mere physical incompatibility was shown by exceptional observations of the normally opposed motor patterns occurring as simultaneous mixtures. Partial overlap of walking and probing as reported by Ibbotson & Kennedy (1959) in apterous *A. fabae*, has been seen also in alates. Simultaneous

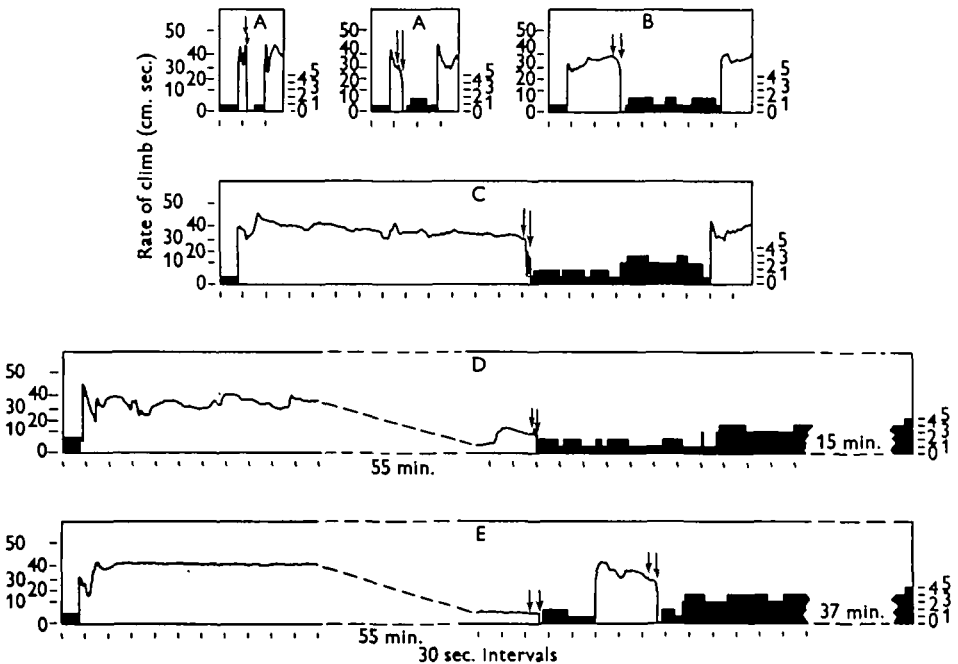


Fig. 2. Sample records, presented as in Fig. 1, showing the effect of varying the duration of flight on subsequent responses to a leaf. A-D, mature bean leaf; E, *Fuchsia* leaf followed by mature bean leaf after a further brief flight.

mixture of the motor patterns of flying and deep probing (as when feeding) has even been observed. On several occasions aphids that had been feeding normally were found hanging from the plant by their embedded mouthparts while the antennae, wings and legs were thrown into a flight-like attitude (wings extended laterally in mid-stroke position, antennae and forelegs extended forwards, hind legs extended backwards, middle legs drawn up fully flexed against the thorax), sometimes even with small-amplitude vibration of the wings. On one occasion hundreds of aphids in the stock culture were observed hanging so, with vibrating wings. In the course of the next several minutes they ceased vibrating their wings but still held the motionless flight posture, finally closing their wings and lowering their legs into the normal feeding posture again. The requisite conditions for such abnormal behaviour have not been identified.

Settling after a single first flight

As a consequence of the method of collecting the aphids for each flight treatment (Kennedy & Booth, 1963) their settling responses, beyond probing, were at the start unexcitable even by stimuli from the most-favoured organ of a host plant like the young growing bean leaf. On the other hand, the aphids were highly excitable locomotorily, and took flight when exposed to light. The two main experiments, I and II, were both designed, *inter alia*, to compare the behaviour of aphids when they landed on leaves after uninterrupted first flights of different durations. Four flight durations were compared in Expt. I and the behaviour on a mature host (bean) leaf of representative individuals from each of the four flight-duration groups is shown in Fig. 2 A-D.

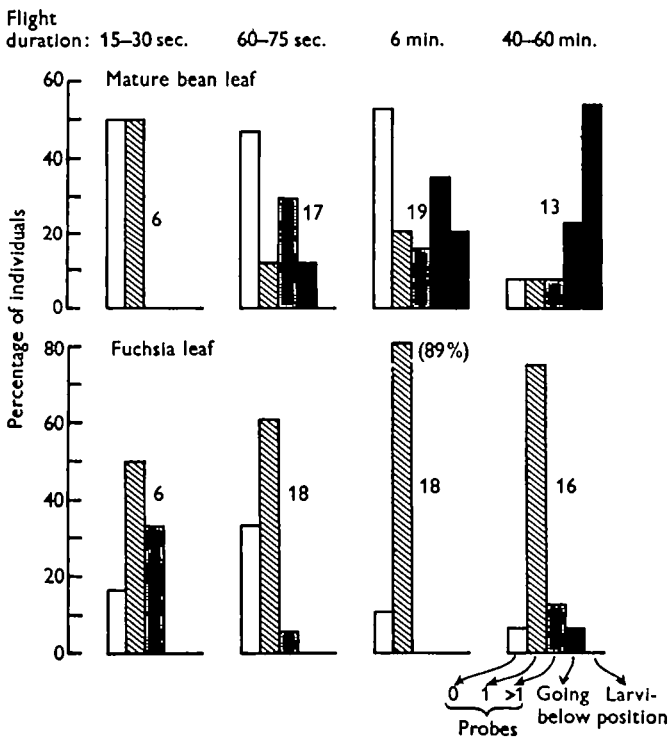


Fig. 3. Behaviour of aphids alighting on two types of leaf after first flights of four different durations (Expt. I). Each of the eight histograms shows the percentage frequency distribution of the individuals within one treatment group according to the stage they reached in the sequence of settling responses. Key to responses at bottom right applies to all groups, and the figure against each histogram is the total number of individuals in the group.

The behaviour of the individuals varied greatly within each group, and Fig. 3 (upper row) summarizes their behaviour group by group as frequency distributions obtained after placing each individual in one of five behavioural classes, representing the furthest stage to which it advanced through the settling sequence which is described in the preceding section and illustrated in its complete form in Fig. 1. The behaviour of a second set of aphids treated in the same way but landing on a non-host leaf, *Fuchsia*, is similarly summarized in the lower row in Fig. 3. In both sets, the individuals in the

last flight-duration group (40–60 min.) were kept flying until they began 'full ranging' or had flown for 1 hr., whichever was the shorter. 'Full ranging' is a stage in the progressive weakening of the flier's photokinesis and positive phototaxis, which is marked by the horizontal excursions reaching beyond the margins of the light window instead of remaining well within them (Kennedy & Booth, 1963).

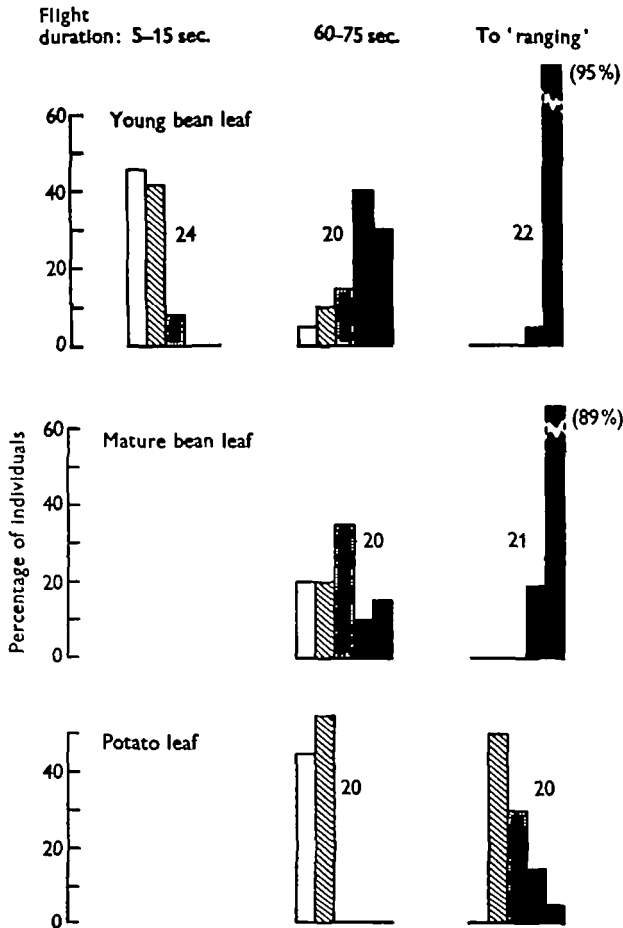


Fig. 4. Behaviour of aphids alighting on three types of leaf after first flights of two or three different durations (Expt. II). Details as in Fig. 3.

Fig. 4 summarizes in the same way the results of the first flights in Expt. II, in which three types of leaf and either two or three flight durations were used. The aphid in the longest flight-duration group were all kept flying until they began 'full ranging's. This ensured greater behavioural uniformity among them just prior to landing, at the price of wide diversity in the actual periods flown (7–235 min.—see fig. 6 of Kennedy & Booth, 1963, which refers to these aphids).

As expected from previous experiments in which certain kinds of gentle handling were found to favour settling (Kennedy & Booth, 1963), the settling responses after free flying and alighting were weaker, especially on non-hosts, in the present experiments than in Johnson's (1958) experiments where such handling was practised

Nevertheless, Figs. 2-4 confirm his main finding of a direct relationship between flight duration and the subsequent excitability of the settling responses. The longer flying continued before it was inhibited by contact with a leaf, the more lasting this inhibition was (as shown by the lengthening time before re-take-off) and the more likely to appear were the successive settling responses: probing once on the upper surface, probing again there, going below and probing there, and finally, larviposition. The responses to any one kind of leaf increased with the duration of the preceding flight in the same way as they increased, after any one flight duration, with the 'host status' of the leaf then alighted upon, from non-host (*Fuchsia* or potato) through mature host leaf to young host leaf. Moreover, Figs. 3 and 4 show a distinct increase in average readiness to settle on host leaves following a very small increase in flying time, from 5-30 to 60-75 sec. This is not very different from Johnson's figure of 20-30 sec. as the minimum duration of flight required for an increase of settling readiness in handled aphids (without handling it was not possible to obtain a strictly comparable figure, starting with an entirely unflown aphid).

Settling after repeated flights

Aphids that did not larviposit at their first alightment on a leaf in Expt. II were allowed to fly and alight repeatedly on the same leaf as the one on which they had alighted at first (Fig. 4), until they either (i) settled down and larviposited on it, or (ii) were lost somewhere on the dark walls of the flight chamber, or (iii) remained airborne for less than 1 min. at three take-offs in succession, or (iv) spread and flicked the wings up and down once but failed to continue beating them so failing to become air-borne at all, also three times in succession. The aphids which had been presented with a leaf for alightment 1 min. after their first take-off were presented with it 1 min. after all subsequent take-offs, or as soon as full ranging began if that was in less than 1 min. The total number of such 'interrupted' flights made by these aphids was often considerably less than the total number of minutes they spent flying because on later flights they often failed to alight promptly when the leaf was presented. The aphids that had not been presented with a leaf on their first flight until they had begun 'full ranging' were treated in this same way on all subsequent flights. The aphids that were allowed these 'uninterrupted' flights alighted promptly when the leaf was presented but the total time they had spent flying after a given number of flights of course varied greatly between individuals. Any aphids that reached the point of behaving in ways (iii) and (iv), above, will be referred to purely descriptively as 'exhausted'. When they reached this point they were allowed to alight, or walk if they would not fly, on to a young bean leaf for a final test of their responsiveness to a good host. The weakened and changeable phototactic behaviour of aphids nearing exhaustion and their consequent tendency to fly into darkness (Kennedy & Booth, 1963) made it easy to lose them before they could be given this final test.

The behaviour of all the aphids at their successive alightments is summarized in Fig. 5. These histograms display as frequency distributions the number of flights and alightments that had been made when the individuals *first* showed each of the successive settling responses or, alternatively, became exhausted without ever showing the specified response (subsequent behaviour on a young bean leaf in these cases is excluded from this figure).

It is clear from Fig. 5 that the excitability of the settling responses rose further when the flight duration was added to piecemeal between successive alightments on all three types of leaf. How many flights and alightments the aphids required to reach any given stage in the sequence of settling responses varied with the duration of each flight ('interrupted' or 'uninterrupted') and the age and kind of the leaf, exactly as might have been predicted by extrapolation from the results of their first unbroken flights in Fig. 4, which showed a cumulative effect of flight on the settling responses. All but three of the total of eighty individuals that alighted repeatedly on host leaves

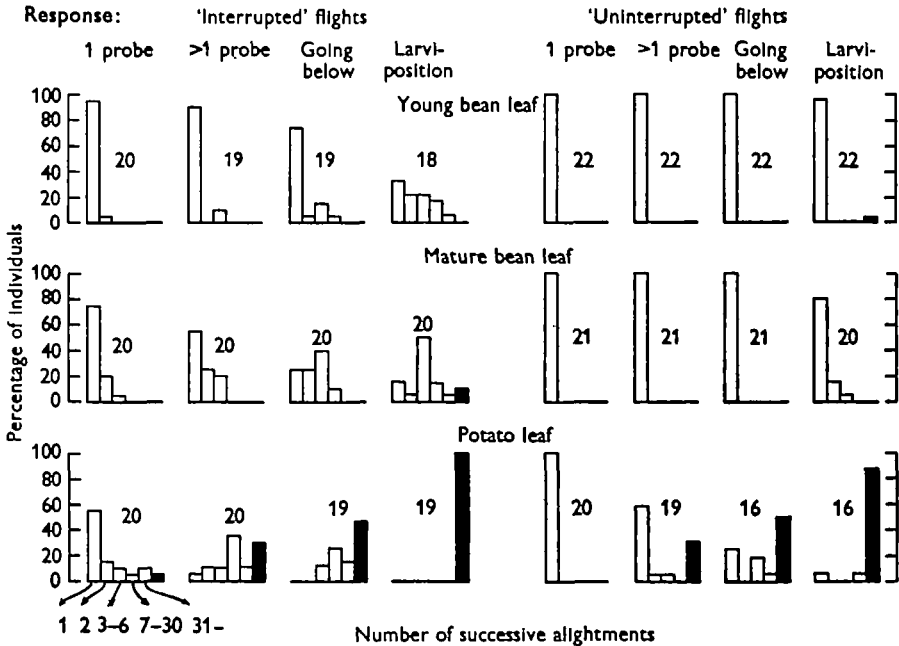


Fig. 5. Behaviour of aphids alighting repeatedly on three types of leaf between flights of short ('interrupted') or long ('uninterrupted') duration. Each row of four histograms refers to the same group of individuals, as they reached different stages in the sequence of settling responses, indicated above. Each histogram shows the percentage frequency distribution of the individuals according to the number of successive alightments they had made when they first gave the stated response. Key to numbers of alightments (open blocks) at bottom left applies to all twenty-four histograms, except that the '31-' group is omitted from the histograms for the 'uninterrupted' flights, since the maximum number of such flights made by any aphid was 21 in this experiment. Black blocks refer to individuals that became exhausted without having given the stated response. The figure with each histogram is the total number of individuals on which the percentages are based.

(young and mature) after 'interrupted' and 'uninterrupted' flights, passed sooner or later through the whole sequence of settling responses and reached the stage of larviposition before becoming exhausted. This statement does not of course refer to the individuals that were lost before either larviposition or exhaustion, although these are included in the histograms for the responses that they had shown before being lost. All of the nineteen alighters on the non-host, potato, that were given 'interrupted' flights, and fourteen of the sixteen given 'uninterrupted' flights, still had not settled down and larviposited on the potato leaf when they became exhausted. Some of the

potato alighters also failed to show one or more of the antecedent settling responses before exhaustion, and the proportion of such failures increased with each successive response through the sequence, again as expected.

Nineteen individuals which had become exhausted without settling down after 18–94 'interrupted' flights and alightments on potato, totalling 21–161 min. of flying, were then tested for responsiveness to a young bean leaf. Eighteen of them settled down and larviposited at their first encounter with the young bean leaf, and the 19th did so at its second encounter after another 2 min. of flight. This was a notably better performance than that of the aphids which encountered the young bean leaf after the first of their short 'interrupted' flights (Fig. 4). Eight individuals which had become

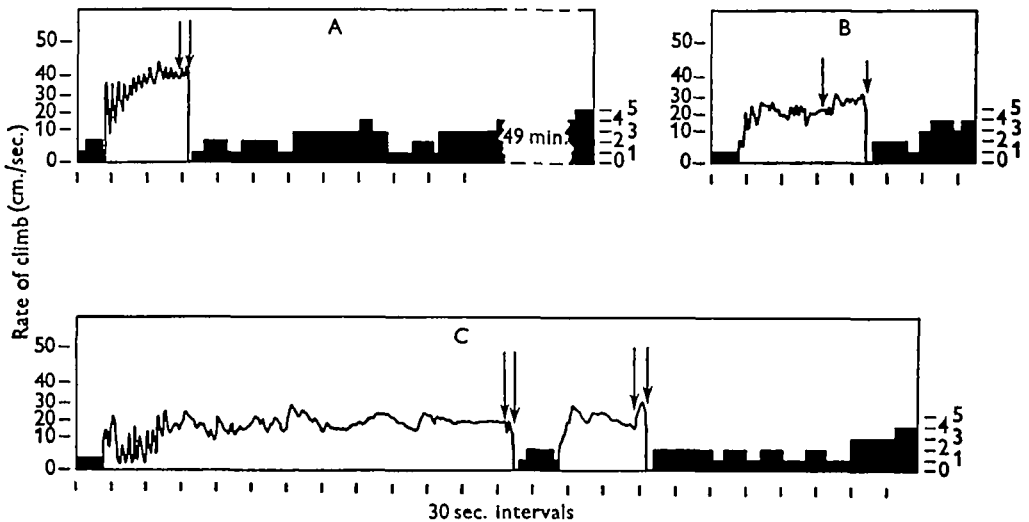


Fig. 6. Sample records, presented as in Fig. 1, showing that a rising rate of climb up to the moment of alightment may be followed by good settling responses to a suitable leaf. A, B, young bean leaf; C, *Fuchsia* leaf followed after a further brief flight by mature bean leaf.

exhausted without settling down after 4–13 'uninterrupted' flights and alightments on potato, totalling 27–260 min. of flying, were then tested on a young bean leaf and all of them settled down and larviposited on it at the first encounter. This was as good a performance as that of the aphids which encountered the young bean leaf after the first 'uninterrupted' flight (Fig. 4). Thus, multiple experiences of a non-host, with its visibly inhibitory effect on the settling responses initiated at each separate encounter, did not eliminate the increased excitability of those responses after flying, when the flying had been continued to the point of exhaustion.

Flight activity preceding settling

If there had always been a decline in the excitability of flight once it was set in train, then the concurrent rise in the excitability of the settling responses might have been a result of the weakening of inhibitory influence from the antagonistic flight response. This permissive or disinhibitory hypothesis of the effect of flying on settling could be checked from the records of the rate of climb of the flying aphids up to the moment of alightment.

Fig. 2 B and Fig. 6 illustrate the fact that the fliers' rate of climb toward the light was often actually rising when these short first flights were terminated and good settling responses ensued. Scrutiny of the flight records of aphids landing on host leaves after short first flights showed that, in Expt. I, the rates of climb of eight of the individuals that were flown for 6 min. had by then fallen below what their rates had been at 1 min. after take-off, while the rates of the eleven other individuals in this 6 min. group had either risen or remained the same. Yet both subgroups considered

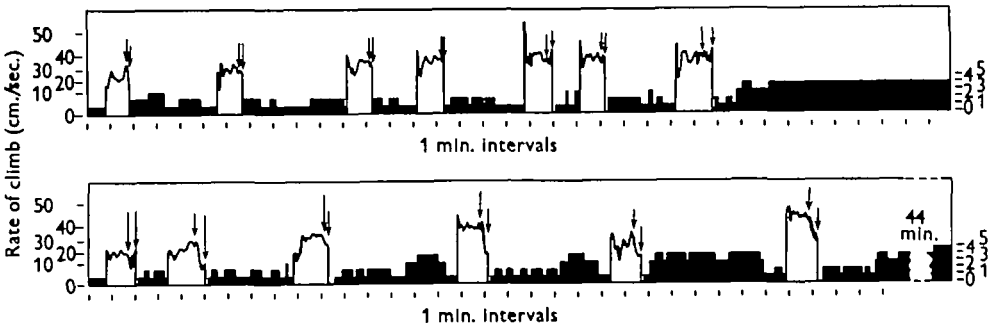


Fig. 7. Sample records, presented as in Fig. 1, showing that a rising rate of climb in the course of a succession of brief flights and alightments may be followed by good settling responses to a young (above) or mature (below) bean leaf.

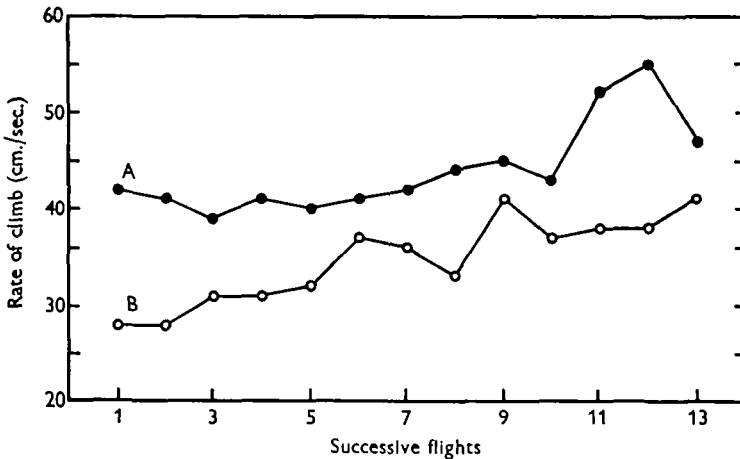


Fig. 8. Mean rates of climb of two aphids during each of a succession of brief (60-90 sec.) flights interrupted by alightments on a mature bean leaf. Aphid A went underneath the leaf for the first time after its 12th flight, aphid B after its 10th; both aphids settled and larviposited after the 13th flight.

separately showed a greater readiness to settle than the aphids that landed after only 1 min. of flight. In Expt. II, a total of twenty individuals were allowed a first flight of about 1 min. before landing on young growing host leaves. In fourteen of these individuals the rate of climb just before landing had risen or (in three cases) not changed since 10 sec. from take-off; they nevertheless showed greater settling readiness on landing than the twenty-four aphids that landed on similar leaves after flying 5-15 sec.

Many aphids that made further short flights and alightments after the first had likewise shown no decrease and even an increase in their rate of climb since their first flight, when they eventually responded well to the leaf. Four illustrations of such 'interrupted' fliers going underneath the leaf for the first time after a flight during which their rate of climb was greater than it had been at the start, are given in Figs. 7 and 8. Scrutiny of the flight records of all the aphids that made more than one 'interrupted' flight and alightment on a host leaf and eventually settled and larviposited in Expt. II, yielded the figures in Table 1. Altogether, seven out of twenty-seven aphids had a lower rate of climb just before settling down on the leaf than they had at the start, eight had about the same rate and twelve had a higher one.

Evidently the increased excitability of the settling responses after short unbroken flights, and even after moderately long total periods spent flying with interruptions, did not depend on a reduction in the excitability of the antagonistic flight response.

Table 1. *Flight activity of 'interrupted' fliers when larviposition on host leaves ensued*

(Expressed as the number of aphids which, during their last flight, showed a rate of climb that was higher than, equal to or lower than the rate during their first flight. The number of flights and alightments and total time spent flying by these groups of aphids are also indicated.)

Rate of climb	Young leaves			Mature leaves		
	No. of aphids	No. of flights (range)	Min. flown (range)	No. of aphids	No. of flights (range)	Min. flown (range)
Higher	4	2-40	3-59	8	2-13	2-18
Equal	7	2-21	2-30	1	50	83
Lower	1	5	7	6	3-11	4-18

Host selection

Breaking off the sequence of settling responses at an earlier stage after alightment on one kind of leaf than on another constitutes the only known mechanism of host selection in aphids (Kennedy, Booth & Kershaw, 1959; Müller, 1962). It is particularly these differences that seem to have been minimized by Johnson's (1958) experimental techniques. He regularly obtained several minutes of probing and walking, including going underneath, on both host and non-host leaves after 30 sec. of flight. Figs. 3 and 4 show that after free first flights even exceeding 1 min., such behaviour was not obtained regularly except on the young host leaves.

When the first flight lasted a minute or less, Figs. 3 and 4 show that some alighters took off again without probing at all. Müller (1953, 1962) has recorded many more *A. fabae* doing that after alighting on non-host plants than on hosts in the field, thus demonstrating the occurrence of sensory discrimination of host plants by stimuli received from the intact external surface of the plants (cf. Kennedy *et al.* (1959) and Wensler (1962) on *Brevicoryne brassicae* (L.)). Some evidence tending to confirm this was obtained in the flight chamber Expt. II (Fig. 4), when more alighters took off without probing from the non-host leaves than from the host leaves; and, in addition, more such take-offs occurred from the mature host leaves than from the young ones. When further 'interrupted' flights and alightments were allowed (Fig. 5), all but one of the forty alighters on host leaves had probed at their first or second alightment and

the remaining one probed at its third. Five of the twenty alighters on potato did not probe until later: one probed for the first time at its fourth alightment, one at its 19th, one at its 49th and one at its 54th. The fifth of these individuals reached the point of exhaustion without having probed the potato leaf once, after making fifty-one flights and alightments totalling 95 min. of flying.

When a first flight had lasted for some minutes, alighters invariably probed the surface whether this was a host leaf, a non-host leaf or even a card. This initial probe was always brief, less than 1 min., and in that time the stylets are unlikely to have penetrated beyond the cell-wall of the leaf epidermis (Hennig, 1962). But it was after this probe that the behaviour of most alighters showed a difference between one kind of leaf and another. In the field, also, discrimination between plants after one brief probe has been observed as the typical behaviour of *A. fabae* (Müller, 1953, 1962; Kennedy *et al.* 1959). In apterous *B. brassicae*, Wensler (1962 and unpublished) has shown that, during such a first brief probe into a leaf, chemical stimuli are received via the stylets which determine whether or not the aphid will make more and longer probes and, when it walks to the edge of the leaf, will turn down on to the shaded underside owing to the changed 'sign' of its reactions to light and to gravity. Accordingly, it can be seen in Figs. 3 and 4 (illustrative examples in Fig. 2D and E) that the alighters' behaviour after the first probe differed, as between host and non-host, in every recorded respect: in the length of stay on the leaf upper surface before re-take-off, in the number of probes made there, in going underneath, and in larviposition having gone underneath. For example, in Expt. I, thirty-six out of the total of forty-nine aphids flown for 1 min. or more probed a second time on the host leaves, but only 3/52 did so on the non-host leaves (Fig. 3). In Expt. II, the figures were 37/40 on the young host leaves, 33/41 on the mature host leaves and 10/40 on the non-host (Fig. 4).

Table 2. *Larviposition on young and mature host plant organs during the first 24 hr. after flight*

Expt. no.	Young shoots		Mature leaves	
	Mothers remaining	Larvae per mother	Mothers remaining	Larvae per mother
1	15	6.5	15	2.7
2	16	6.2	16	2.0
3	18	7.9	20	3.5
4	6	12.2	16	5.6

Additional experiments were done solely on the final larviposition response, by placing the aphids in the dark after alightment to promote settling, in view of Johnson's (1954) unsupported statement that the larviposition rate of *A. fabae* alatae so treated is not influenced by the quality of the host as claimed by Kennedy & Booth (1951). A 'swarm' of aphids was left flying in the flight chamber for 30 min. and then single, detached, germinating bean shoots at the early 'hook' stage, and mature bean leaves from large plants, were introduced alternately and one at a time until each had collected one alighter. This was continued until twenty individuals had been collected on the young shoots and twenty on the mature leaves. All were placed in the dark as soon as they alighted and examined 24 hr. later. Some of the alatae had wandered

diff in that time, especially from the young shoots on which they had some evident difficulty in retaining a foothold, and only the larvae produced by mothers that had remained on the shoot or leaf were used to calculate larviposition rates. The same experiment was repeated four times and the results given in Table 2 show a substantially higher rate of larviposition on the young shoots ($P < 0.01$ in each separate experiment, even by the very insensitive Median Test of Siegel, 1956).

DISCUSSION

When Johnson (1959; cf. Chiang, 1960) found that wing muscle breakdown and embryo development, the normal sequels to settling, could be induced in *A. fabae* by mutilating the unflown insects or simply tying them down, he suggested that these treatments might have taken effect by interfering with normal locomotor behaviour, and went on to point out that his (1958) experiments on the induction of settling by flight had likewise involved artificial restraints upon flight. We have found that such restraints can promote settling (Kennedy & Booth, 1963) but the present experiments show they are not a pre-requisite for it.

The original problem remains, then, of how normal flight activity promotes settling. Moericke (1955) pictured the flight-settling sequence as a one-way succession of 'moods' actuated by 'drives', either unfolding internally or perhaps switched over by some stimulus. Müller & Unger (1951; also Müller, 1953, 1962) supposed that the flight drive gave way to the settling drive under the influence of fatigue and hunger—a theory of peripheral feedback. These ideas were disproved by the very quick and reversible effect of flight on settling readiness obtained by Johnson (1958) and here confirmed (Figs. 2 and 4). He used the term 'response' instead of 'mood' but with the same generalized and abstract meaning; and his modified hypotheses of peripheral feedback now seem implausible.

First, the graded settling responses produced by varying the duration of prior flight could be produced equally well by varying the type of immediate stimulation the aphid received after alightment, as if these were but two different ways of influencing the same central nervous mechanism. When flight was prolonged and the aphid's rate of climb fell progressively (Figs. 1 and 2 C-E; also Kennedy & Booth, 1963) there may well have been some cumulative physiological feedback such as has been demonstrated, or suggested, in other cases cited in the Introduction. But whatever feedback there was and however excitable settling became as a result of it, it did not determine the behavioural outcome directly, as already noted in connexion with the flight-induced changes in flight behaviour itself (Kennedy & Booth, 1963). For the outcome depended also on the external stimuli received when flight ceased, again implying control through a central nervous integrating mechanism. The excited settling responses were initiated on any surface but were soon broken off on non-hosts, with reversion to flight. And this was not because the effect of the first flight had been entirely eliminated by the non-host for further flying excited settling still further (Fig. 5).

Secondly (as already inferred from other, indirect evidence in the Introduction) the effect of prior flight on the settling responses was not in the first place an indirect, permissive, releasing or disinhibitory one such as Graham (1959, 1961) postulated in *Myrpyodendron*. When, as flight continued, the rate of climb began to fall, the lowering

of flight excitability thus revealed did presumably contribute to the further lengthening of the period of inhibition of flight after landing, and to the continued increase in the excitability of the antagonistic settling responses by disinhibition. The noteworthy point, however, is that this was a secondary, reinforcing effect merely, and not a necessary one. For some rise in the excitability of the settling responses was obtained before there was any sign of a lowering of flight excitability, and often while it was actually rising (Figs. 6–8, Table 1). The excitatory after-effect of flight on the settling responses was therefore in the first place a direct one, and this was quantitatively related to the antecedent, immediate effect which was on the contrary inhibitory. Flight inhibited the antagonistic settling responses entirely so long as it lasted and the result of this, when flight came to be inhibited in its turn, was a commensurate increase in the excitability of those settling responses. In short, it was as antagonistic responses that flight and settling were linked in succession.

This antagonistic relationship between locomotion and settling in aphids has been recognized previously, but in terms (conflicting, opposed, mutually exclusive—see Introduction) that emphasized their separation and unconnectedness; the connexion between them was sought elsewhere, in peripheral feedback, overlooking the fact that antagonism is a form of connexion. In a previous paper (Ibbotson & Kennedy, 1959) and again in this one, evidence has been given that the mutual exclusiveness of locomotion and settling is not physical (mechanical) but nervous. The antagonism is a central nervous phenomenon, and from this it can be inferred that the primary link between flight and the settling it induces is central, not peripheral.

This type of behavioural co-ordination may be called *antagonistic induction*. The same type of sequence is familiar at lower integrative levels. It has been described in rhythmic reflex co-ordinations under Sherrington's (1906) names, *successive induction* or *rebound*, not only in the spinal mammal and lower vertebrates but also in the insect thorax (Pringle, 1940). Bullock (1957) has described such rebound or *post-inhibitory excitation* (as well as the opposite kind, post-excitatory depression, and other features of reflex action) at a still lower level: within individual crustacean neurones. Blest (1960) made no mention of these other cases and he objected to Sherrington's terms being applied to insect behaviour (by Kennedy, 1958*a*) on the grounds that they were 'loaded with causal assumptions' derived from the mammal. The slightly modified term 'antagonistic (instead of "successive") induction' might meet this objection in part but its purpose is rather to stress the aspect most important in the study of behaviour, which is constantly concerned with successions anyway. Using similar terms for the same integrative principle at more than one level (as already done at the neuronal and reflex levels) does not assume the same causes or imply that no new principles arise at higher levels.

The term antagonistic induction is required to identify this principle in behaviour itself, regardless of underlying causes. For, as we have found in the particular aphid case, this principle has generally gone unrecognized behind vaguer descriptions, themselves loaded with causal assumptions. These assumptions are unproven, and call for new experiments once the principle of antagonistic induction is recognized. Thus Sherrington (1947, pp. xiii–xxiv), Bullock (1957, 1962), and other physiologists, have accepted as components of higher nervous activity 'the instincts actuated by "urges" and "drives"' (Sherrington) and 'the plausibly postulated energies, drives,

appetitive behaviour, releasers and other entities of behavioural science' (Bullock, 1957), which they regard as different in principle from any known lower mechanisms. Yet when the descriptive principle of antagonistic induction is identified at this level, then internal drive no longer seems something separable from or 'on top of' (Bullock, 1962) reflex action. What was put in the special class of *internal drives* can in reality be generated by external stimulation—but of an antagonistic reflex, and hence unremarked. Even 'vacuum activity', considered demonstrably spontaneous because without the usual stimuli, and attributed simply to their being withheld (Bullock, 1962), might similarly be produced by antagonistic induction. In just this way, extension of the spinal dog's leg occurs 'spontaneously' after prolonged stimulation of the flexion reflex (Sherrington, 1906). To discover what is new in higher nervous integration it is necessary to discern what is not.

It should be stressed that this work raises but leaves entirely open the question whether the neurophysiological mechanism underlying antagonistic induction at the behavioural level is like that at lower reflex levels. Further purely behavioural information having some indirect bearing on this question is given in the subsequent paper on reciprocal effects and alternations. But the main purpose is to contribute to that conceptual framework of behaviour, for the lack of which 'proper use of the neuro-physiological information already at hand as well as the planning of new experiments is hampered', as Roeder (1962) has said.

SUMMARY

1. Previous evidence that fresh-winged adult aphids become increasingly ready to settle down on a leaf the longer they have flown beforehand, and that this effect is appreciable after less than 1 min. flight, was confirmed with aphids allowed to fly and alight freely without interference.

2. The settling responses initiated after flight were quickly inhibited again by an unsuitable leaf, with reversion to flight, but repeated flights had a cumulative effect.

3. The excitability of the settling responses increased as an after-effect of flights in which the excitability of flight itself (measured by the rate of climb) was not falling and was even increasing.

4. It is concluded that settling is not released by flight but is positively 'primed' while it is inhibited by flight, these two activities having been shown to be centrally antagonistic. Settling may be further promoted by some peripheral physiological feedback during long flights when the rate of climb falls, but this is a secondary, merely reinforcing effect.

5. The primary effect is given the name 'antagonistic induction'. It resembles successive induction or rebound in lower reflex action. As a principle of nervous integration at the behavioural level it calls in question previous assumptions concerning internal drives in aphids and other animals.

6. Host plant selection occurred as a result of the sequence of settling responses being broken off at an earlier stage on one kind or age of leaf than another. Most commonly the difference of behaviour appeared only after a first probe, but following very short flights discrimination was sometimes observed without any probing.

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