

FREE FLIGHT OF APHIDS IN THE LABORATORY

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INTRODUCTION

Much has been learned of the kinematics and aerodynamics, the metabolism and even the sensory and nervous physiology of insect flight from observations on tethered specimens, preferably in a wind tunnel or on a roundabout to provide a relative wind simulating that created by free flight (Pringle, 1957). Such devices were developed to a high pitch of refinement by Weis-Fogh (1956) for locusts and have proved invaluable for studying what he called 'the basic flight process itself' in a number of insects. Cockbain (1961*a*) mounted aphids (*Aphis fabae* Scop.) on pins in a wind tunnel and showed that they resembled locusts (Weis-Fogh, 1952*a*) in consuming carbohydrate initially but fat mainly, during a long flight, in accordance with Weis-Fogh's (1952*b*) theoretical expectation for migrant insects in general. Tethered flight, however, whether in still air ('tethered flapping': Weis-Fogh, 1956) or in an appropriate relative wind, does not provide an adequate approximation to free flight when some other aspects of it are under study. Thus Cockbain (1961*b*) noted that his fixed aphids continued 'flying' for considerably longer than free aphids were estimated to fly in the field. When flight movements are more or less forced, by deprivation of tarsal contact and other devices, then fuel can be studied because it has become the limiting factor, but the factors limiting free flights naturally cannot be studied.

Again, when the purpose is to study not the flight process in itself but the interaction between flight and alternative behaviour patterns, the use of tethered insects may distort the results owing to abnormal sensory inputs both during the fixed flapping and when the insects are transferred from the suspension to some test situation. Moericke (1941, 1955), Müller & Unger (1951, 1952) and Müller (1953) deduced from general observations in cages and in the field that a long flight must intervene before young winged adult aphids will respond to a host plant by settling down, feeding and larvipositing upon it. Correspondingly, most specimens of *Aphis fabae* and other species alighting on host plants, and virtually all alighting on non-hosts, in the field, have been observed to take off again very promptly (references in Kennedy, Booth & Kershaw, 1959, p. 438). B. Johnson (1958) remarked that it was very unlikely that such field alighters had been air-borne for less than 30 sec.; nevertheless, he found that 20-30 sec. of tethered flapping were enough to make *A. fabae* respond well when placed on leaves of both hosts and non-hosts. He obtained a similar effect from brief flights by aphids that were not tethered but mechanically interfered with in various ways. It seemed that the interference may have encouraged premature settling in some unknown manner.

The present work on aphids was started with that possibility in mind. When preliminary experiments seemed to confirm it as described below, further work was conducted entirely on aphids allowed to fly freely and alight freely without handling. The functional principles and design of an apparatus developed for that purpose are described here, together with some of the results obtained with it. Brief descriptions of the chamber, with summaries and discussions of the results obtained, have already been published elsewhere (Kennedy & Booth, 1956; Kennedy, 1958; Kennedy, Booth & Kershaw, 1961) and a derived model has been described by Legge (1962).

FUNCTIONAL PRINCIPLES

(1) *Non-interference*

One of the techniques used by B. Johnson (1958) was to allow an aphid to take off from a leaf surface and fly freely up to the glass-house roof, whence it was recaptured and returned to the leaf with a capillary suction tube applied to the thorax or wings. The single flights lasted around 6 sec. each and when an aphid had made enough of them in succession to reach a total of about 20 sec. flying, it showed the 'settling response' of wandering about on the leaf and going below to probe on the shady lower surface, instead of taking off again after a single brief probe on the upper surface. The effect of such handling with a suction tube was tested in the earlier version of the flight chamber described by Kennedy *et al.* (1961). For the first experiment, each aphid was allowed to take off from a detached mature bean (*Vicia faba*) leaf held in a corked tube with the petiole in water, and to fly up toward a bank of fluorescent lights, and was kept flying just out of reach of the bolting silk screen below the lights by suitable adjustment of a down-current of air, for a period that was lengthened slightly with each succeeding flight, starting with 1 sec. and working up to a maximum of 50 sec. Each flight was terminated by stopping the down-current so that the aphid reached and landed on the screen. One of two methods was then used to return the aphid to its original take-off position on the upper surface of the leaf at 30 cm. below the screen. Either (i) the leaf was brought up and its blade laid flat on the screen close in front of the newly alighted aphid, and as soon as the aphid walked on to the leaf this was set down again in the take-off position; or (ii) the aphid was picked off the screen with the sucker, brought down and deposited carefully on the leaf which remained at the take-off position. The two methods were alternated from aphid to aphid, one method only being used on each aphid throughout its series of flights. Given a combined total of 117 flights, twenty aphids which were not handled (method (i)) showed the 'settling response' twice altogether. Given a combined total of ninety-one flights, twenty-one aphids which were handled with the sucker (method (ii)) showed the 'settling response' twenty times.

In a second experiment, both sets of aphids were allowed, after each flight, to walk freely from the screen on to the leaf which was then returned to the take-off position as in method (i) above, but one set was thereupon picked up off the leaf with the sucker, held in the air for 3 sec. and put down on the leaf again; while the other set were not touched. Given a combined total of 108 flights, fourteen untouched aphids never showed a 'settling response'. Given a combined total of 114 flights, eighteen picked-up aphids showed the 'settling response' eleven times altogether.

For the third experiment, a number of fresh aphids were released together in the flight chamber and left to fly freely as a 'swarm' for 10 min. Twenty-two individuals were then collected one at a time out of the air on to detached leaves held in tubes as above. Collection was effected simply by presenting the leaf in the path of the chosen flier until it alighted. Immediately after it had alighted, every alternate individual was picked up and put down again with the sucker as in the previous experiment. The other individuals were not touched. Each leaf with its aphid was then isolated in a jar and left in the dark for 24 hr. By the end of that time the eleven untouched aphids had deposited an average of 1.9 larvae each (range 0-5) and nine of them (the mothers) had walked off their leaves; the corresponding figures for the handled aphids were 5.5 (0-10) larvae and four mothers walked off. This experiment was repeated with ten aphids in each set, and the untouched aphids deposited an average of 3.0 (range 0-6) larvae and six walked off, as against 6.1 (1-9) larvae and two mothers walked off among the handled aphids. The average numbers of larvae deposited (with ranges in brackets) by those aphids that remained on the leaves for the full 24 hr. were as follows, in the two experiments: 1.0 and 2.7 (0-2 and 0-5) from the untouched aphids, and 6.7 and 7.2 (4-10 and 4-9) from the handled aphids.

The other untethered flight technique by means of which B. Johnson (1958) induced settling was to allow the aphids to fly up to the under surface of a horizontal glass plate, on which they did not readily obtain a grip and so continued 'buzzing' for some time while touching it with antennae and forelegs. Most of his *A. fabae* that flew in this manner for over 20 sec. showed the 'settling response' when transferred to a leaf with the sucker, whereas most that flew so for less time did not. This technique was also compared with free unimpeded flight in the flight chamber, by inserting a glass plate under the bolting silk screen under the lights. The sucker was used to transfer the aphids to leaves immediately after measured flights of both kinds: against the glass in still air, or free in the down-current. All the aphids had been flying freely in the down-current for a few minutes before being given a test flight of either kind. Of twenty-eight aphids given a further free flight of rather more than 30 sec., twenty had taken off again within 15 min. of being placed on a leaf in the light. Of twenty flown against the glass for rather less than 30 sec. each, only three had taken off again in 15 min.

These preliminary results remain unexplained but sufficed to indicate the desirability of arranging for free flying and alighting in such work.

(2) Reactions to light

Light is required to initiate and maintain aphid flight, and also orients it. There is both field and laboratory evidence of the former, *photokinetic* reaction (Broadbent, 1949; Taylor, 1957). In order to avoid uncontrolled variations in the photokinetic stimulation during flight, it was desirable to minimize the changes of light intensity experienced by the flying aphid as a result of its own movements. This meant using a large light source, and keeping the aphid's position relative to the source as constant as possible. Good control of the flying aphid's movements was in any case essential to keep it out of contact with the walls of the flight chamber. The aphid's second type of reaction to light, the directed reaction or positive *phototaxis*, provided one component of an effective control mechanism.

There has been no systematic study of phototaxis in aphids but many authors have invoked positive phototaxis to account for the orientation of fresh-winged aphids just before and after take-off toward the main source of light (when a large source such as white clouds may be a more attractive than a more intense small one such as the sun) (Müller & Unger, 1951, 1952; Moericke, 1955). Moericke (1955) found no evidence of any tendency to fly upwards in response to stimuli other than light and reported that aphids would fly horizontally or downwards to a light. We can confirm that *A. fabae* alatae will fly upwards, horizontally or obliquely downwards to an artificial light in a dark room, but not that they do so with equal facility. Preliminary observations suggested that top light had a flight-stabilizing effect (dorsal light reflex?) which was required for controlled flight in any direction. It was therefore decided to set the lights above an opening in the roof of the chamber. This was the simplest system inasmuch as the three directional components of the flying aphid's environment—light, gravity and the air current (see below)—were then acting along one common axis.

(3) *The 'air treadmill'*

The second essential component of the flight control mechanism was a current of air blowing away from the lights so as to prevent the flying insect from reaching them: an 'air treadmill'. This principle was first tried in the form of a simple vertical wind tunnel with the air passing downwards through the light window and continuing at constant velocity to an outlet at the bottom of the chamber. But the rate of climb of many flying aphids toward the lights was found to undergo rapid changes from time to time. With a pre-set, constant air speed they could then rise to reach the window, or alternatively were swept down to the outlet, before the operator had had time to prevent either event by adjusting the air speed. This difficulty was overcome by allowing the down-current to emerge from the light window into a larger open space so that its speed decreased rapidly from the lights downwards, forming a jet instead of a stream. The flying aphid's vertical displacements, due to changes in its rate of climb, were then automatically damped. If it spurted upward toward the lights it encountered increased resistance to further ascent; if it began to sink away from them owing to a reduced rate of climb, its further descent was soon slowed. This damping gave the operator time to adjust the overall air speed, if necessary, to prevent the aphid reaching the roof or the floor.

Many aphids could be kept flying for long periods without this additional, coarse adjustment of the air speed by hand, and for some purposes this may be adequate (Legge, 1962). Nevertheless, hand adjustment, in such a way as to keep the flier within a narrow range of levels below the light window, was desirable for two reasons. First, with a very near source of light as in this apparatus, gross changes of incident light intensity and thus of photokinetic stimulation are brought about by small changes of distance from the source, a situation quite unlike that for fliers in the field. Secondly, if the flier is held at about the same distance below the lights, continuously balancing the downward air current at that level against its rate of climb, then a record of the air speed adjustments required to keep it there becomes a record of its rate of climb. The rate of climb depends on the photokinetic responsiveness of the insect and was one of the behavioural elements which it was desired to study.

DESCRIPTION AND OPERATION

The defects of the first model of flight chamber (described by Kennedy *et al.* 1961) were the slow and clumsy method of changing the speed of the down-current of air (by sliding rheostat), and the mosquito-netting curtains hanging from the margins of the light window where flying aphids too easily encountered them. The second model

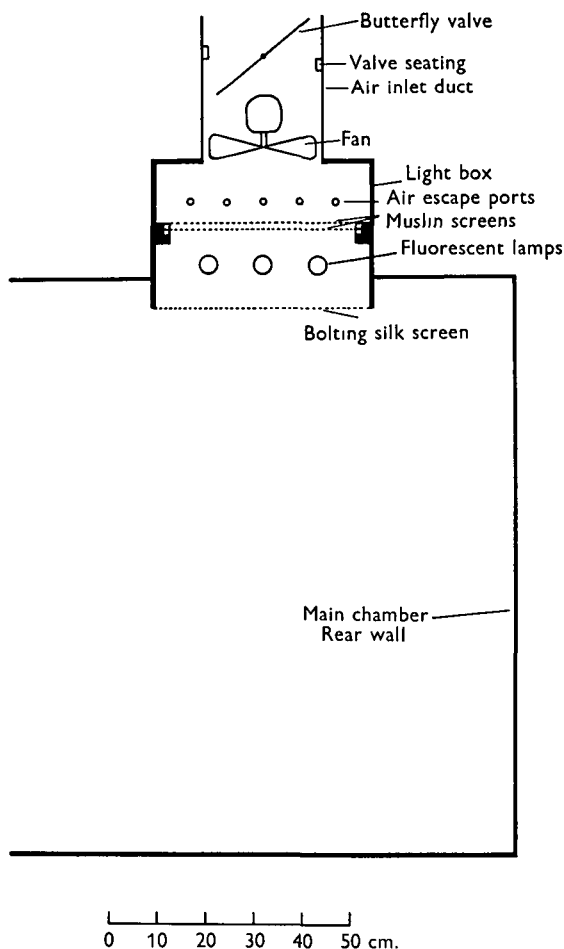


Fig. 1. Diagrammatic vertical section of flight chamber (third model) with front opening facing left. The expression 'light window' in the text refers to the opening covered by the bolting silk screen.

was illustrated by Kennedy & Booth (1956) and the experiments described by them, by Kennedy (1958), and in this and subsequent papers, were done with that second model or the very similar third model.

The third model (Fig. 1) consists of a large open-fronted box, 106 cm. square in horizontal section and 123 cm. high, with a central opening in the roof into which fits a smaller open-bottomed box 43 cm. square internally and containing the lights. The standard lighting is three evenly spaced 40 W. 2 ft. Mazda 'Daylight' fluorescent

lamps. The light box in turn has a central opening in its roof, into which fits a cylindrical duct (cushion-seated to prevent vibration of the main chamber) containing a 9 in. G.E.C. propellor fan (1350 rev./min.) blowing downwards into the light box and thence into the main chamber. The slight projection of the light box into the chamber helps to reduce turbulence at the margins of the window and keeps the roof (and the observer's face outside the chamber) in deep shadow. Between the fan and the lights are two layers of muslin (*ca.* 19 threads/cm.) to smooth out the air flow, and the bottom opening of the light box into the flight chamber proper is covered with bolting silk (*ca.* 12 threads/cm.) to prevent accidental entry of fliers. Small pluggable air escape ports in the walls of the light box above the muslin screens can be used to alter the maximum speed of the down-draught (hereinafter called simply the *draught speed*). All three screens are removable to permit the frequent cleaning that is necessary.

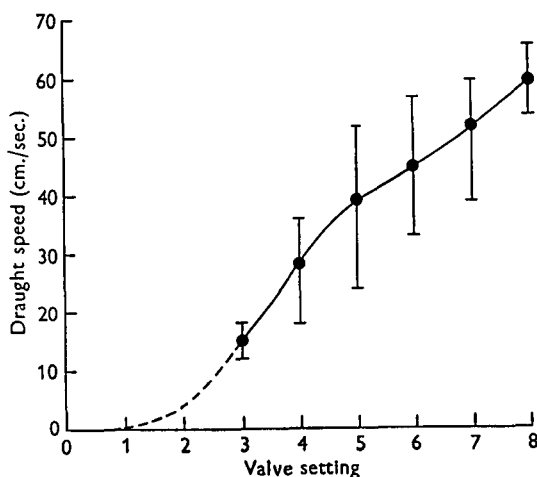


Fig. 2. Relation between butterfly valve setting (arbitrary units) and speed of resulting down-draught 8 cm. below the light window of flight chamber. Each point represents the mean of readings at nine positions covering the central area 20×20 cm., and the vertical lines show the maximum range of variation among positions.

The draught speed in the chamber is regulated, during an insect's flight, not by altering the fan speed but by regulating the admission of air to the fan by means of a butterfly valve in the duct just above it. The valve spindle is linked by chain drive to a control knob outside the left-hand wall of the chamber where it can be manipulated comfortably for long periods by the observer standing or sitting before the open front of the chamber and watching the flying insect. This control also actuates the pen of a recorder with the paper band usually moving at 2.5 cm./min., a second pen making 30 sec. time signals and a third pen for the operator to mark occasional events as required. This recorder and its dim light are invisible from inside the flight chamber; the chamber walls, floor and roof are painted matt black and the operator wears a black gown and black gloves and keeps his face in the shadow. These precautions are needed to prevent the flying aphid from being drawn away from the central light window prematurely. A black-painted adjustable arm (not shown in Fig. 1) serves

to hold up the detached leaf or other object used as a take-off and alighting platform for the insect.

The setting of the butterfly valve is adjusted repeatedly during a flight in such a way as to keep the insect somewhere between 5 and 12 cm. below the screen of the light box. No attempt is made to counteract its rising and sinking within those limits, or even beyond them if the excursion lasts only a second or two. Attempts at finer adjustment produce not a more but a less faithful recording of the insect's behaviour.

The draught speed was measured with a Simmons shielded hot-wire anemometer at 25 positions 10 cm. apart at each of two levels under the light screen, for different settings of the butterfly valve as far as the sensitivity of the instrument would permit (down to *ca.* 15 cm./sec.). With the valve fully open the mean draught speed over the whole area of the screen was about 53 cm./sec. (107 ft./min. or 1.2 m.p.h.) at 8 cm. below the screen, and about 25 cm./sec. at 15 cm. below it. The flying aphids, however, generally turned back toward the centre of the screen well before they reached its edge, confining their horizontal to and fro movements to the central area about 20 cm. square (see Fig. 5). Since the draught speeds tended to be lower near the edges, the nine measuring positions distributed over that central area only are used to calculate the mean draught speeds incorporated in the calibration curve (Fig. 2) for converting the records of the butterfly valve settings to very approximate rates of climb by the flying insects.

With any one valve setting, the draught speed was found to vary widely and irregularly from position to position even within that central area, as indicated in Fig. 2. This is a defect of the present apparatus, but the flying aphid is exposed only momentarily, in practice, to the draught speed at any one position. From time-exposure photographs of aphids flying in the chamber (e.g. Fig. 5) the horizontal air speed was estimated at around 20 cm./sec. At this speed the flying aphid would pass from one measuring position to the next (10 cm.) in $\frac{1}{2}$ sec. and would complete a whole circuit of the central area in 4 sec. In the course of such an 80 cm. circuit passing through eight measuring positions at the 8 cm. level, with a mean draught speed of 39 cm./sec. (when there was the greatest variation between positions: Fig. 2), the maximum change of level attributable to the variation of draught speed would be about 7 cm. This calculation ignores the increase of draught speed on rising and the decrease on sinking, which would further reduce the amplitude of flight level changes attributable to the horizontal variations of draught speed. Thus, changes of flight level of a few cm. up or down in a few sec. would often be due only to the irregular air flow, and any recorded attempts to compensate such changes by adjusting the butterfly valve were ignored. But a change of several cm. lasting more than 5 sec. could generally be attributed to a real change in the flying insect's rate of climb.

It will be clear from the foregoing that the records provide a better measure of *changes* in the rate of climb, than of the actual rate of climb at any moment.

APHID SUPPLY

The aphids used for the flight experiments came from the long-established clonal culture of *A. fabae* described by Kennedy & Booth (1954). Preliminary flight experiments were done with alatae of unknown age and experience, taken from the walls of

the culture cabinets, but the work reported in this and subsequent papers was done entirely on alatae taken at the moment when they took wing for the first time after completing the teneral period (Taylor, 1957) under controlled conditions. Clumps of young beans (*Vicia faba*) bearing the first crop of alate nymphs produced by crowding apterous mothers, were placed level with and 30 cm. away from two 80 W. 4-ft. 'Daylight' fluorescent lamps in a glass-fronted box (Fig. 3). As soon as they become ready to take off the fresh adult alatae flew towards the lamps and were arrested by the glass. The slope of the glass towards the lights induced the alatae to walk straight up it and over its open top edge into the box where they were trapped by their attraction to

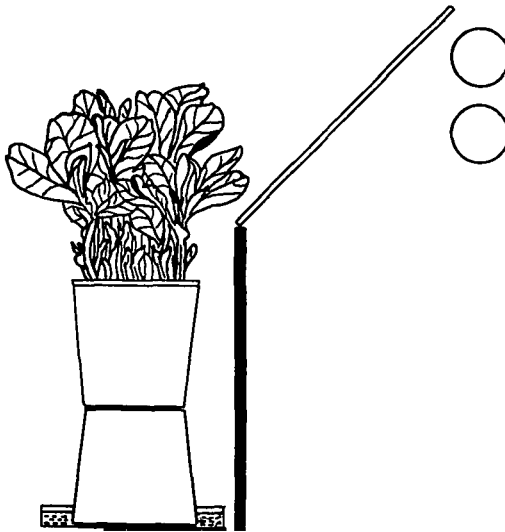


Fig. 3. Sectional diagram of non-return arrangement for collecting alatae at their first take-off. Particulars in text.

the light and eventually died. It could therefore be assumed that any alata seen taking off from the plants was flying for the first time. Periodical checks showed that none of the alatae did in fact stay to reproduce on the plants.

When an alata was required for experiment, the clumps were inspected until an alata was seen to take off and it was at once collected from the glass with a camel-hair brush. After a quick check that the wings were perfectly formed and free of honey-dew droplets, and that the insect measured not less than 5 mm. from frons to wing tips, it was transferred to a detached leaf with the petiole in water in a corked tube, in the flight chamber. The rearing plants were kept in the culture room at about 13° C., while the flight chamber was in a separate, dark room at about 19° C.

BEHAVIOUR IN FREE FLIGHT

(1) *Changes during one flight*

For the start of a flight, the leaf with the aphid on its upper side was held at a slight angle with the tip pointing up toward the centre of the light window from 15 cm. below one margin of it. The aphid walked to the tip and took off from there, whereupon the leaf was swung away out of sight on the movable arm. Not surprisingly, the unrestrained flight behaviour even of uniformly fresh alatae from the same rearing plants was highly variable in almost every respect, but it followed the same general course.

When the first flight of an individual was allowed to continue as long as it would without interruption by the operator, three phases could be distinguished, albeit merging one into the other. The first part of Fig. 4, from *A* to *B*, illustrates this typical

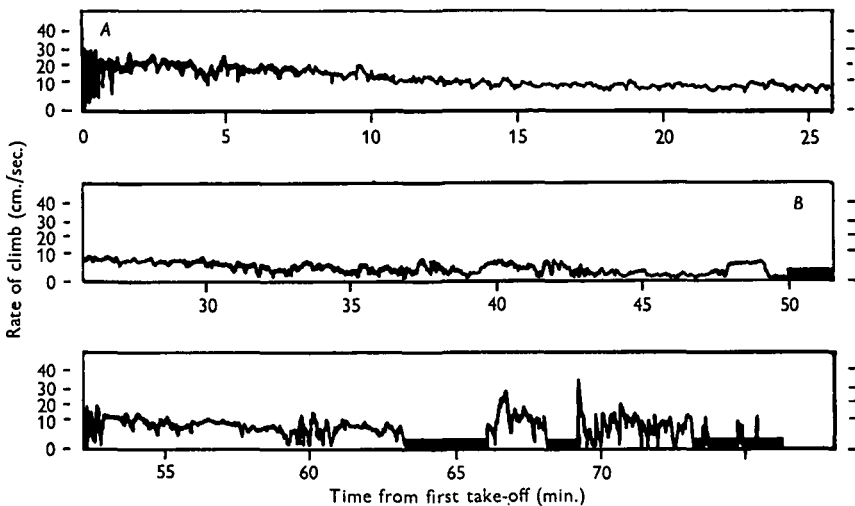


Fig. 4. Complete record of the rate of climb of one aphid allowed repeated flights until three flights in succession had lasted less than one min. Thick strips along time base represent periods spent on a non-host leaf between flights.

sequence in the complete rate-of-climb record of one individual, necessarily somewhat simplified during re-drawing on a contracted time-scale (see also Fig. 2 in Kennedy & Booth, 1956). Fig. 5 shows the phases of uninterrupted flight in more detail, by means of excerpts from the rate-of-climb record of another individual of which the flight path was periodically photographed by time exposure to produce a partial record of its horizontal displacements as well.

Although aerodynamically stable, the fliers behaved in an unstable, sometimes quite 'wild' manner at the start. For a short period, from a few tens of seconds to several minutes, there were gross and irregular variations in the flight both vertically and horizontally (Fig. 5 A), betraying a wide-amplitude oscillation in the strength of both types of locomotor response to the light. The rate of climb, which can be taken as a direct measure of the photokinetic response, fluctuated widely at the beginning of this phase, and the maximum rate for the whole flight was usually reached within the first

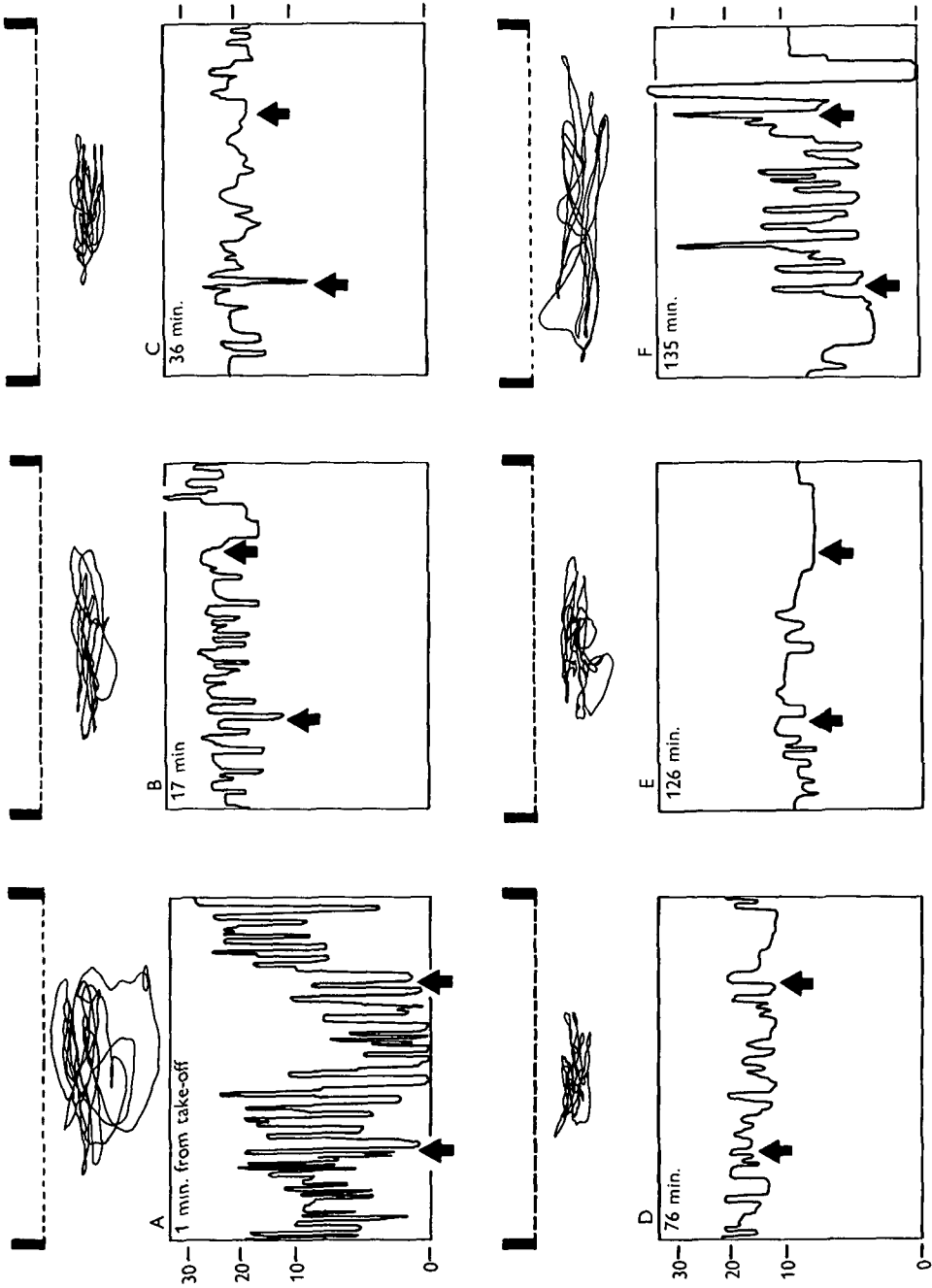


Fig. 5. Six excerpts from the record of one uninterrupted first flight of 137 min. In each case the lower tracing shows the rate of climb over 1 min.; during the 30 sec. period between the two arrowheads the flying aphid was being photographed simultaneously in side view to give the upper, skein-like tracing of its horizontal movements beneath the 43 cm. square light window which is indicated by the broken line. One min. after the end of record F the aphid flew right away from the light window and landed on the chamber wall. Rate of climb scale in cm./sec.

minute. In some aphids the maximum was not reached until up to several minutes later, when the flight was less 'wild', as in Fig. 5 B. The rate-of-climb fluctuations soon decreased, but some minutes usually passed before they were reduced to the minimum for that flight, which was itself very different from aphid to aphid. Concurrently there was a steadying of flight behaviour in the horizontal plane also, that is, a reduction in the amplitude of the outward excursions towards the margins of the light window before the flier turned back in response to the growing asymmetry of the incident light (Fig. 5 A-C). This amplitude provides a measure of the positively phototactic responsiveness.

In this way the insect passed over into the second phase, of relatively steady 'cruising' flight lasting many minutes and even hours during which the rate of climb gradually declined (Fig. 5 B-E). Occasionally during this phase, a flier might show a quite sudden and unaccountable change in the rate of climb. It was also easy for the operator to induce changes. For example, allowing the aphid to fly closer to the lights for a few minutes caused a lasting increase in the rate of climb when the flier was returned to the normal flight level. The type of horizontal movement—looping, criss-crossing, circling, etc.—changed frequently. Some fliers became extremely steady in this phase but most showed irregular short-period fluctuations in the rate of climb, as in Figs. 4 and 5. The rate of climb of some fliers (e.g. Fig. 4) fell close to zero many minutes before this phase ended, while the horizontal excursions were still small.

Eventually (after 49 min. in the Fig. 4 example; 126 min. in the Fig. 5 example) a short third phase opened which resembled the first phase in reverse, except that the rate-of-climb fluctuations were usually much smaller. The earliest indication of the onset of the third phase was generally an increase in the amplitude of the horizontal excursions from the centre of the light window (referred to as *ranging*). This amplitude usually decreased again one or more times before it increased further and lastingly, until the flier was going out to the very margin of the light window before each recoil (Fig. 5 F). During these minutes the rate of climb also began to fluctuate, and more downward than upward. Each outward excursion in the horizontal plane coincided with a decrease in the rate of climb, and each return toward the centre with an upward spurt. Horizontally, too, the aphid gave the impression of decelerating on each outward excursion and accelerating during each return. Aphids whose rate of climb was already near zero when the third phase opened (e.g. Fig. 4) also showed increased vertical excursions, here due to alternately losing and regaining height (hence not shown in Fig. 4).

The horizontal and vertical amplitudes continued to increase irregularly until—usually within a few minutes of the excursions first reaching the margin of the light window—the aphid's rate of climb fell to zero (if not already there) and its excursions passed beyond the margins of the light window. The stage thus initiated will be referred to as *full ranging*, and lasted seconds only, the aphid being now of course out of the operator's control. Many aphids made one or more wide, circling flights at any height around the peripheral space in the chamber before finally swinging out further to alight on a wall. A few sank or fluttered down to the floor without reaching the stage of full ranging.

(2) Duration and number of flights

In one series of experiments a total of eighty-three fresh alatae were kept flying in the chamber without interference until the stage of full ranging. The total time from take-off to that moment varied in different individuals from 7 to 235 min., with a frequency distribution as shown in Fig. 6. These times do not represent the flight

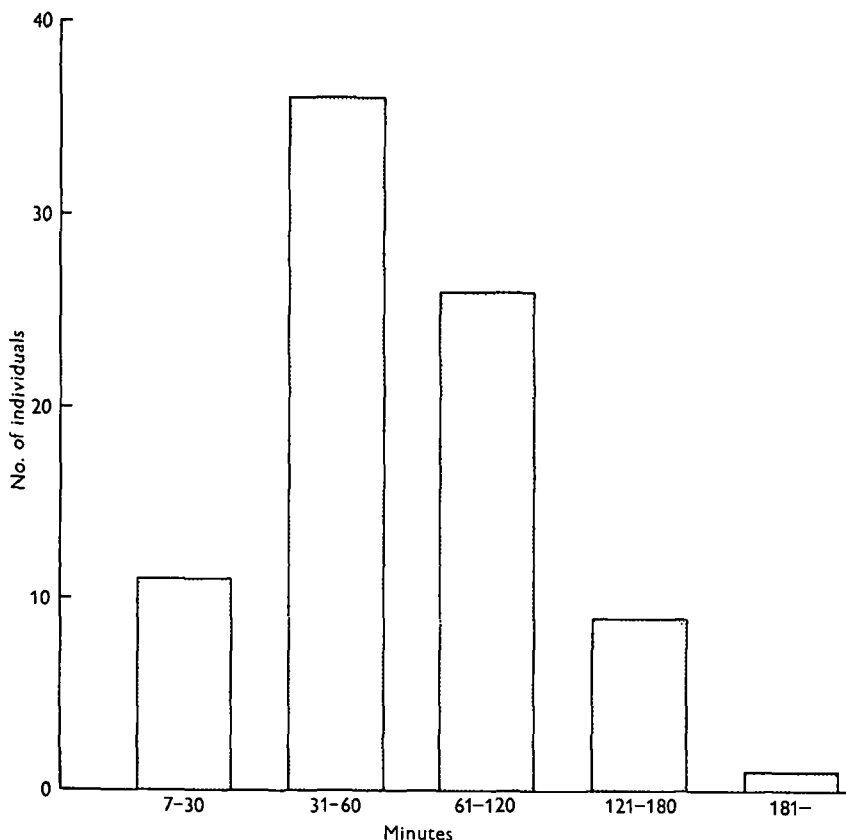


Fig. 6. Frequency distribution of durations of uninterrupted first flights.

durations of which most of these aphids were capable without 're-fuelling'. That is to say, the cessation of their climbing flight and alightment on the dark walls was not due to complete exhaustion of available reserves, which is the presumed cause of cessation of flight by tethered insects that are repeatedly stimulated to fly (Wigglesworth, 1949; Cockbain, 1961 *a*). This was shown by inducing aphids that had arrived at the stage of full ranging to land on a non-host leaf (potato) and at once placing the leaf in the starting position again. After a pause on the leaf of usually less than 1 min. (range $\frac{1}{4}$ –4 min.), with one or more probes of $\frac{1}{4}$ – $\frac{3}{4}$ min., when no sap is likely to have been imbibed (Müller, 1958), the aphids took off and passed again through the whole behaviour sequence described in the previous section, although usually in less time.

When this procedure was repeated, as in the case illustrated in Fig. 4, the initial rate of climb on second or later take-off was for several flights as high as or even higher

than it had been at the first take-off. But after the initial spurt the rate fell again more rapidly than before, until, after a number of flights, the steady 'cruising' phase of each flight sequence finally dropped out altogether and the flights were all brief and erratic both vertically and horizontally. Ten aphids were flown repeatedly to the stage of full ranging with alightments on a potato leaf in this way until, at three take-offs in succession, they either failed to become air-borne at all (spreading and flicking the wings but failing to vibrate them and so somersaulting on the leaf or tumbling off it to the floor) or became air-borne for less than 1 min. The number of flights of more than 1 min. by one individual averaged 6.4 and ranged from 1 to 17. The total duration of all an individual's flights of more than 1 min. averaged 105 min. (range 32-260), of which time the first flight accounted for an average of 75 % (range 24-100).

Even flights of only a few seconds may be of considerable importance in host finding and virus spreading in the field (Kennedy *et al.* 1959). Aphids that were allowed to make further take-offs from a potato leaf after the arbitrary end-point above often made a long series of brief flights. Many of these could not be kept under the operator's control because the aphid began full ranging almost at once; hence, still more and longer flights were theoretically possible. For example, one specimen made successive flights of 143, 25, 7 and 1 min. duration, then three flights of less than 1 min. each, followed by fourteen more flights varying in duration from 15 sec. to 5 min., and was then lost before it had become exhausted; another made a first flight of 17 min. followed by twenty more flights of from 20 sec. to 6 min. each, and was then lost before it had even reached the point of making three flights of less than 1 min. in succession.

(3) *Reactions to secondary light sources in the chamber*

As reported previously by Kennedy *et al.* (1961), light reaching the flying aphid horizontally, from one side, readily draws it across the space below the light window and even beyond, even when this light is merely a partial reflexion of that from the light window itself. That is why it was necessary to keep all reflexions to a minimum. With the walls painted matt black, it was at first supposed that the aphids' eventual departure from the central space under the light window was due to a complete reversal of the sign of their phototaxis from positive to negative. However, during the brief period of full ranging the aphids in fact made repeated half-turns back toward the window, while keeping at a distance from it, as already mentioned. Thus the dimmer light now being received from the distant window was still exerting a positively phototactic turning effect, when the bright light received by the aphid close to it had ceased to do so demonstrably.

Secondly, as Dr H. C. Chiang was the first to remark, when the aphids did finally turn away to a wall at the end of their *first* flight, they did not go to the darkest places visible (the corners of the chamber, or the deeply shaded peripheral parts of the roof, or the deep shade outside the open front of the chamber), but typically landed near the middle of a wall. The light reflected from there, neither the weakest nor the strongest reaching the flier, now evidently exerted the strongest 'pull' on it. At the end of subsequent, short flights when the aphids appeared very fatigued and had dropped out the 'cruising' phase of each flight bout altogether, then they did typically fly out toward the darkest place, the peripheral roof.

Thirdly, flying aphids would approach and alight on a leaf or other small light-

reflecting object in the chamber at any stage of a previously uninterrupted flight up to the onset of full ranging. For routine purposes the object was held vertically at the same level as the flier and 10 cm. from the centre of the light window. The records from a total of 160 aphids on their first flight show that a bean or potato leaf was alighted upon about equally promptly when so presented at any time from 1 min. after first take-off till ranging began: in 5–15 sec. in 119 cases, in less time in 8, and in more time (max. 95 sec.) in 33 cases. The leaf was presented to a further sixty aphids for the first time at the moment they began full ranging. Inevitably, many of these aphids failed to come within the range of the leaf at which the 'cruising' aphids had responded to it, and twenty-two of them landed on a wall first. Of the remaining thirty-eight which did land on the leaf, fourteen did so in less than 5 sec.—a notably higher proportion of quick alighters than among the earlier lot. A leaf was presented for the first time to a large number of other aphids at a still later stage (of their first or subsequent flights) when full ranging was more advanced and they were making peripheral sweeps round the chamber. At this time the aphid could not be expected to pass near the leaf if this was held in the standard position close to the light window. Instead, the leaf was moved to and held in any convenient position directly in the path of the flying aphid. As a rule, the aphid was now actually repelled by the leaf, 'dodging' it repeatedly and landing instead on a wall.

These three types of observation all show that the strongest light does not always evoke the strongest phototactic response. The optimal or 'preferred' light intensity for the positive phototaxis declines as locomotory activity (the photokinesis) wanes toward the end of a flight, until, as the end-point of this process, the aphids become negative phototactically even to the dimmest incident light.

Evidence that the phototactically optimal light intensity varies with the photokinetic state was obtained earlier in flight, also, from the detailed behaviour of 'cruising' aphids prior to their alightment on reflecting objects. Alightment on a leaf or similar object is due to a complex of responses ending with the cessation of wing-beating upon receipt of contact stimulation of the tarsi. It was shown previously (Kennedy *et al.* 1961) that a narrow light-reflecting object held vertically near a 'swarm' of flying aphids attracts them both phototactically and by virtue of the optomotor stimulation created. The optomotor stimulation had, in addition to its orienting effect, a marked slowing (akinetik) effect on forward and upward flight at close range, facilitating alightment. It was observed in the present work with single 'cruising' fliers that, during the interval between presentation of such an object and alightment upon it, the aphid made one or more horizontal movements clearly directed toward it (*approaches*) but then turned abruptly away again, each approach being accompanied by a drop in the rate of climb and each recoil by a rise. This effect was observed on innumerable occasions; and it was repeated many times in succession when alightment was delayed as a result of frequent alightments on a non-host beforehand, as in the cases illustrated in Figs. 7 and 8.

This behaviour resembled that of ranging aphids approaching and recoiling from the margin of the light window when no target was present. In approaching the target the aphid was subject to increasingly unequal illumination, which provoked a turn back so long as the stronger window light was exerting a greater phototactic 'pull' on the aphid than the reflected target light. But the target was at the same time exerting

an increasing akinetic effect on the aphid at each approach. This was visible in numerous cases typified by Fig. 8 as a step-wise series of drops in the rate of climb. Associated with this weakening of the photokinesis, there was a weakening of the phototactic 'pull' of the window light relative to the 'pull' of the target until at last the approaching aphid did not turn back from it but went on to alight.

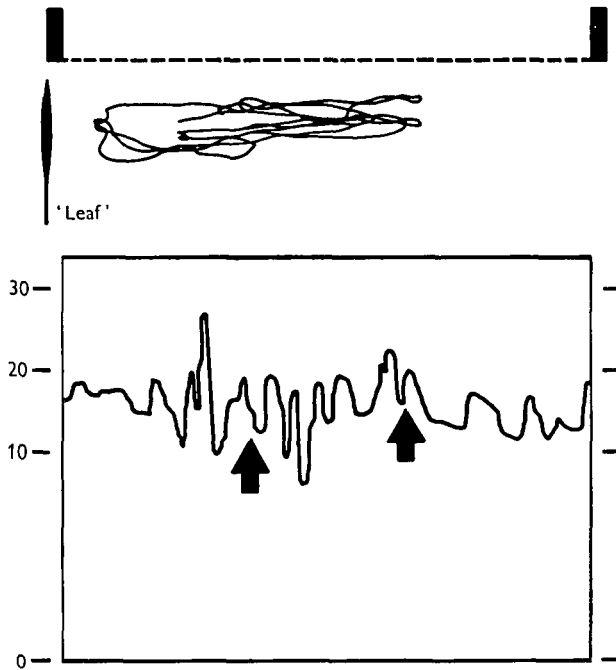


Fig. 7. Behaviour of flying aphid in the presence of a yellow card 'leaf' held upright under the left hand margin of the light window. Rate-of-climb tracing below, with arrowheads marking the 15 sec. period during which the aphid's horizontal movements were photographed to give the tracing above, as in Fig. 5.

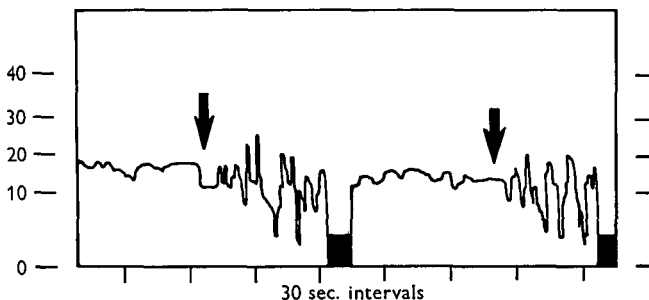


Fig. 8. Rate-of-climb record from an aphid making delayed landings on a non-host leaf held upright under the centre of the light window. Black blocks on time base indicate periods spent on the leaf. The leaf was removed immediately after take-off, replaced at the moments marked by the arrowheads and left in position until the aphid landed on it.

DISCUSSION

It should be stressed that the method described here for maintaining flight in the laboratory depends absolutely on the insect's persistent positive phototaxis, which even the aphid displays for only a limited time. This is a feature of the specialized and transient *migratory* behaviour pattern in many insects (C. G. Johnson, 1960; Kennedy, 1961; Southwood, 1962) especially those drifting forms that were often misleadingly called 'passive' migrants in the past; it is their very active method of embarking upon the free-circulating upper air. Thus we have been able to keep adult staphylinids, caught in drifting flight outdoors, flying for long periods in the flight chamber; but *Calliphora* or *Pieris*, caught 'flitting' about, flew at first toward the lights but soon ceased to do so.

The conclusion drawn from the aphids' behaviour in the chamber, to the effect that there is a phototactically optimal or 'preferred' light intensity which falls and rises together with the level of locomotor excitation (photokinesis), is perhaps a new idea and requires systematic experimental confirmation. Moericke (1941, 1955) was the first to recognize that changes of responsiveness must occur during flight in nature, and his inferential account of a brief initial unsettled (*unruhig*) flight phase followed by a long phase of *Distanzflug* and a final phase of *Befallsflug* is strikingly paralleled in the flight chamber. But the phase of *Befallsflug* ('alighting flight': used here as in Kennedy *et al.* (1961) to cover both the 'hovering' and 'attacking flight' of B. Johnson (1958)), when the aphids fly low and sometimes alight upon plants, yellow traps, etc., in the field, presumably begins much earlier than does the brief final 'ranging' phase in the chamber.

In the field, the sky light reaches the flier not from overhead only, but as from a dome, and the vegetation reflects it substantially. Hence the phototactic 'pull' of light from the sides and below will begin to rival that of the top light earlier in the field than in the black-walled chamber. The 'ranging' aphids that dodged leaves and landed on the dark walls of the chamber are not equivalent to the field low-fliers that 'dodge' any foliage they meet (Kennedy *et al.* 1959), for the latter do not then fly into the shadows but stay out in the open above and between plants. These field low-fliers that dodge leaves are evidently at an earlier stage in the process of photokinetic and phototactic decline than those that dodged leaves in the chamber, and earlier also than those that alight on leaves in the field or the chamber. The positive phototactic response of field low-fliers to the light reflected from vegetation has become strong enough to prevent them from soaring skyward; but many of them are still sufficiently positive to the sky light as well to be prevented from approaching foliage closely enough to alight; thus they seem to dodge. The prompt alightment on a proffered leaf by chamber fliers within a minute or so from first take-off may be an artifact due to the dark background and powerful optomotor stimulation.

By low-level flight here is meant flight within a very few metres of the ground, within what Taylor (1958) called the 'boundary layer' among and just above the vegetation. It is not suggested that the changing phototactic responsiveness of fliers controls their vertical distribution directly, through active, large-scale vertical movements on their part. Their major vertical displacements, upwards and downwards, are effected by the vertical movements of the air (C. G. Johnson, 1956, 1957), to which the aphids

abandon themselves, temporarily, by soaring skywards in the first place. The changing phototactic responsiveness while in the upper air can nevertheless lead to low-level 'alighting flight', and so control vertical distribution indirectly, if it prevents fliers from soaring skyward again when the circulating air brings them down.

With B. Johnson's (1958) work, the flight chamber experiments call for two modifications of Moericke's (1955) original scheme of aphid flight phases. First, the phases of *Distanzflug* and *Befallsflug* are only quantitatively different, with a gradual transition from one to the other. Secondly, the sequence of phases is not irreversible; and this incidentally invalidates the working assumption of C. G. Johnson (1954, 1956) that each aphid makes effectively one single flight. A period of strongly positive phototactic response to the main flights in the chamber, which represents *Distanzflug*, can recur after the aphid has passed on through the equivalent of *Befallsflug* and alighting. This whole cycle of changes can be repeated many times, if with diminishing amplitude. During one flight or over a succession of flights, there can even be a temporary strengthening of the positive response to bright light instead of the weakening typical of uninterrupted flights, depending upon the external stimuli received.

If the changes of responsiveness during flight were irreversible, then they might be attributed simply to some equally irreversible or slowly reversible physiological process accompanying flight. Thus 'fatigue' was suggested by Müller & Unger (1952) and oxygen debt by B. Johnson (1958). There may be some such cumulative process that meters flight in aphids and provides a 'feed-back' which in turn promotes the weakening of the kinetic and positively phototactic reactions to light. Graham (1961) has shown that the change from photo-positive to photo-negative behaviour which is induced by flight in the ambrosia beetle, *Trypodendron lineatum* Olivier, depends on distension of the gut by air swallowed while flying. Clegg & Evans (1961) have shown that the wing-beat frequency of a tethered blowfly, *Phormia regina* Meigen, declines together with the concentration of trehalose in the blood. Trehalose appears to be the main source of flight energy, and the authors conclude that the blood trehalose determines the wing-beat frequency. A comparable suggestion could well be made concerning the declining rate of climb of the flying aphid. But only one method was tried of reversing the flight-induced change in the beetle's phototaxis and the fly's wing-beat frequency. The method was to reverse the internal process which was believed to have brought on the behavioural change: by deflating the beetle's gut, and by feeding the fly. This succeeded strikingly, and the experiments might therefore leave the impression that those non-nervous processes of gut distension and blood-trehalose depletion determined the behavioural changes in some direct and unconditional fashion. Whatever feed-back process there may be in the flying aphid, we know that it does not so determine the behavioural changes. For they can be reversed readily by other inputs (e.g. from an unacceptable leaf), and without the supposed feed-back process itself being reversed, since subsequent flights show cumulative behavioural changes in the original direction. The determining changes during flight would seem to occur, therefore, not in any one peripheral process, but in a central nervous balance mechanism that is tipped one way or the other by different inputs. More evidence on this aspect of aphid flight (Kennedy, 1958) will be given in a subsequent paper.

SUMMARY

1. The working principles, design and operation of an apparatus for keeping aphids in free flight are described. It consists of a large black-walled chamber with a battery of lights above a central opening in the roof and a fan blowing air down past them into the chamber. The aphid flies up toward the lights and is held at a small, fixed distance from them by adjusting the downward air speed to balance the aphid's rate of climb, which can thus be recorded continuously.

2. The uninterrupted first flight of virginoparous *Aphis fabae* in this chamber comprises a brief erratic opening phase, a long 'cruising' phase during which the rate of climb declines, and a final brief erratic phase when it falls to zero and the aphid flies away from the lights. Contact with a non-host surface will then restore the previous behaviour and a number of flights, similar but of decreasing duration, ensue until exhaustion at $\frac{1}{2}$ –4 hr.

3. During one flight and over a series of flights there are changes in the aphid's responsiveness to the main lights and to light reflected from the chamber walls or from small objects such as leaves presented in the chamber. There appears to be an optimal ('preferred') light intensity for the positive phototaxis, which starts high but declines as the photokinesis (locomotor excitation, measured by the rate of climb) declines, until near exhaustion when the phototaxis becomes negative even to the lowest intensities.

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