AN INVESTIGATION
OF THE BEHAVIOUR AND BIOLOGY
OF THE
CITRUS MUSSEL SCALE,
LEPIDOSAPHES BECKII (NEWM.)

by

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Resumé

1. The economic importance of the Diaspid Scale Insects is briefly discussed, and the desirability of an investigation of the crawler stage stressed.

2. A brief summary of previous work is given and the objects of the present investigation outlined.

3. Some details of the biology of the Scale Insect, *Lepidosaphes beckii* (Newm.) are given and the crawler is described. The life of the crawler falls naturally into three parts; hatching, the wandering period, and settling.

4. The general experimental technique is described.

5. The effects of light and temperature on the hatching of crawlers is investigated. A rhythm of hatching is described. It is concluded that, while either temperature or light changes will maintain the rhythm, both acting together produce a more marked rhythm.

6. The duration of the wandering period, and the factors possibly influencing it, are discussed.

7. The first work on the wandering period is concerned with the part played by the long anal filaments. Experiments show that the long anal filaments do not, as was thought possible, appear to serve any anchoring function in a wind. The possibility that the filaments might help a

crawler to right itself by their independent movements, is discussed, and the suggestion put forward that they might further be concerned in keeping the crawler air-borne during dispersal by wind.

8. Experiments designed to determine whether crawlers respond to the force of gravity, are described. The experiments show that no tendency to congregate at either the upper or lower edge of a vertical surface exists.

9. The influence of light on crawlers is next dealt with. The lack of success with small choice chambers is described, together with the development of a satisfactory apparatus. Experiments are described which show that crawlers exhibit a photopositive response.

10. An attempt to find the threshold of the response to light is described. This is found to vary widely in individual crawlers. However, the range of intensities over which a 0-100 per cent response occurs, is found.

11. The mechanism of the response to light is then investigated. Experiments show the response very probably to be either a klino- or a tropotaxis. The difficulty encountered in attempting to distinguish experimentally between these is mentioned. However, reasons are given for the belief that the latter response is the one operating in crawlers of L. beckii.

12. These experiments are discussed in the light of previous work, some of which conflicts with the above conclusions. Experiments show that the smell of the host plant does not appear to be responsible for the lack of response to light reported by one worker experimenting with crawlers on oranges. Further possible causes are discussed.

13. Experiments are then described which show that the failure of the small choice chambers is due probably to the action of the interior angles as releasers of settling behaviour.

14. The next sections contain observations on the settling of crawlers.

15. Apparatus which makes possible continuous observation of a crawler, walking naturally on a leaf, is described. The settling behaviour pattern is described.

16. Factors influencing settling are then investigated. The first of these is surface dust, which is found to hasten settling.

17. A series of experiments is described which first points to the conclusion that a crawler is stimulated to settle sooner by the presence of other crawlers, but later shows this to be untrue.

18. The high variability of the walking time is discussed, and experiments described which show that storage of the

infested material after collection in the field, and the time of day at which the experiments are carried out, are not causes of the variability.

19. Experiments then show that crawlers settle sooner on lemon leaves than on orange. No difference in settling, however, is detected between crawlers released on navel orange and valencia orange leaves.

20. Further experiments concerning the effects of such factors as light intensity, temperature, leaf maturity, and the age of the tree, are described. All of these have no great effect on walking time; but the possibility exists, however, that a real difference, too small to show significance in any but very large samples, might occur.

21. Conclusion.

I. General Introduction

The citrus industry is subject to a number of serious insect pests. Of these, the most important is a sedentary group known as the Armoured Scale Insects (Diaspididae). Ebeling (1950) states that they are of greater economic importance to the industry than all the other pests put together.

A great deal has been published on various aspects of the biology, ecology and control of the Diaspididae, much of the work appearing in the books of Quayle (1938), Ebeling (1950) and Bodenheimer (1951). It is very noticeable, however, that the study of the active larvae, or "crawlers", of these insects has been relatively neglected.

This lack of work on crawlers is surprising, since they are the only distributive stage in the life cycle of the species. The adult male is also free-living during its brief life span, but takes no part in the actual distribution of the species. It is the position of the female Scale Insect which is important in determining the further spread of the species, since this is the point from which subsequent crawlers will start out on their wanderings. The stage in the life cycle responsible for the position of the female is, of course, the crawler. The crawler is also responsible for the parallel distribution of the male Scales, so that the female can be fertilised. The rôle of the adult male is confined to the maintenance of the gene flow.

From an economic point of view it seems desirable that a complete study be made of the biology of this distributive stage of these important pests. Information on factors influencing the hatching (or birth of ovoviviparous species), and emergence from beneath the scale-covering of the female, would be useful. This information might enable forecasts of population increases to be made. The expected age composition of the population could also be calculated and appropriate control measures instituted. A knowledge of the effects of temperature, relative humidity, light intensity, gravity and wind force on emerged crawlers might open up new possibilities in the control of these insects.

Most existing knowledge of crawlers is limited to experiments on those of the Citrus Red Scale, Aonidiella aurantii (Mask.), the Citrus Mussel Scale, Lepidosaphes beckii (Newm.) and the San José Scale, Quadraspidiotus perniciosus Comst. The latter is a very minor pest of citrus although important in other sections of the fruit industry. Some details of the behaviour of crawlers of the San José Scale are given by Mathys (1953) and Gentile and Summers (1958).

This previous work has been concerned with such matters as the rates of locomotion of crawlers on different substrates at various temperatures, the duration of the wandering period, and responses to light, gravity and the smell of citrus.

The crawler is, then, the free-living larva of an essentially sessile animal. Adaptations for ensuring an efficient distribution of the species, and for increasing the probability of survival during this critical period, might be expected. It was hoped that the present study might show how the crawler is adapted to take full advantage of its free-living period, while a later comparison with the behaviour of a Soft Scale Insect, which retains the ability to change position through several instars, would be interesting.

It was decided to begin the study with crawlers of L. beckii, which is the most plentiful Scale Insect of citrus near Grahamstown.

Since many avenues opened up for study, however, work was confined to this one Scale Insect.

The mature female Mussel Scale lays her eggs under the scale-covering. The body of the insect shrinks as the eggs are laid, leaving behind it an egg-filled cavity (Quayle, 1938). The number of eggs laid by a female Mussel Scale in California varies from 40 to 80, according to Quayle (1938) and Ebeling (1950). Bodenheimer (1951), in Israel, obtained an average of 172 eggs per female. In South Africa, the number may exceed 100 (Stofberg, 1937).

The posterior-most eggs are the first to hatch. The crawlers may remain under the female scale-covering for some time, as several are often exposed when the scale-covering is lifted. Crawlers emerge at the posterior end of the scale-covering, where there is a gap due to the slight upward curl of its margin. The crawler then wanders on the host plant for a variable period, after which it settles.

The crawler of L. beckii (Figure 1) is of a pale yellowish colour, and is strongly dorso-ventrally flattened. It has two very long, fine anal filaments. Except for an illustration by Skaife (1953), only the thicker proximal part of the anal filament appears to have been figured previously. The lengths of five crawlers which were measured varied from 0.39 to 0.47 mm., with a mean value of 0.43 mm. This includes the anal filaments. The mean length of the body alone was 0.28 mm. This measurement was made in mid-January, 1960. The length of the crawler, as recorded by various workers, is very variable: 0.78 mm. in California (Quayle, 1912); 0.35 mm. in Israel (Bodenheimer, 1951); and in South Africa 0.29 - 0.32 mm. (Carnegie, 1955). Another measurement from South Africa (judged from a figured crawler) is 0.65 mm.

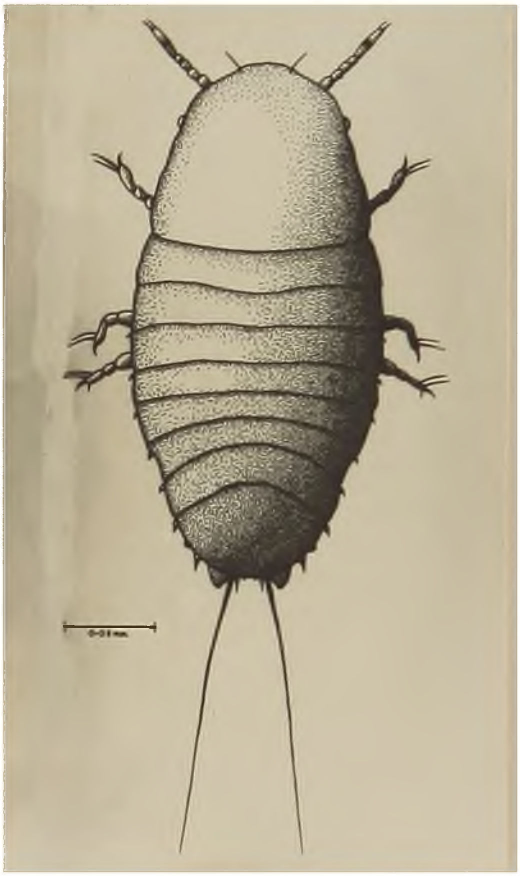

Figure 1. A crawler of L. beckii.

(Stofberg, 1937).

Further measurements of crawlers are needed. If this large size variation does exist, a study of its cause might prove interesting. Seasonal or environmentally induced effects such as temperature or nutritional status, might provide the answer. The lighting conditions under which the measurements were made, and thus the proportion of anal filament included in the measured value, might also account for some of this variability.

An investigation of the biology and behaviour of the crawlers of L. beckii falls conveniently into three parts, hatching, the wandering period, and settling. The following investigation will be described under these heads.

The crawlers used in the investigation were obtained from scale-infested navel orange leaves collected from a farm situated in the Belmont Valley, near Grahamstown.

The infested leaves were stored in polythene bags. Leaves stored in this way lasted for up to a week. Crawlers were obtained by overturning the female Scale Insect with a mounted needle. Only crawlers showing activity were used. All crawlers used in experiments were thus freshly removed from under the female, except where otherwise stated. Crawlers were transferred from the leaf by means of a small paintbrush which had been trimmed down to a few bristles.

II. Hatching

The first stage to be considered in the activities of a crawler is its hatching. Some experiments were carried out to determine the factors influencing this occurrence. Three obvious factors are light, relative humidity and temperature. It is unlikely, however, that humidity in a sheltered enclosure on a leaf surface would vary to any extent. The experiments were thus confined to testing the effects of temperature and light.

The following method was used. Females, together with their eggs, were transferred to petri dishes. This was necessary in order that actual hatching, and not emergence from under the intact female Scale Insect, could be observed. All or most of the eggs remained in the scale-covering and could be transferred to the dishes in this way. Only females with at least 20 eggs were taken. Fifty females were placed in each dish. The insects were placed in the dishes in rotation as they were removed from the leaves, in order to obtain similar samples in all the dishes. They were then overturned as a further precaution against measuring emergence from beneath the female, as crawlers might have waited under the scale-coverings in the dish. The Scale Insects were obtained from freshly collected infested leaves.

Each petri dish (Figure 2) was floored with black paper (C), which was sealed to the glass around the perimeter by means of a strip of paraffin wax (B). The paper acted as a suitable background against which to see the white eggs and light-coloured crawlers, while the wax served to prevent the crawlers from walking on to the underside of the paper. The petri dish (D) was covered by a larger petri dish (A).

In the first experiment, three pairs of covered dishes were

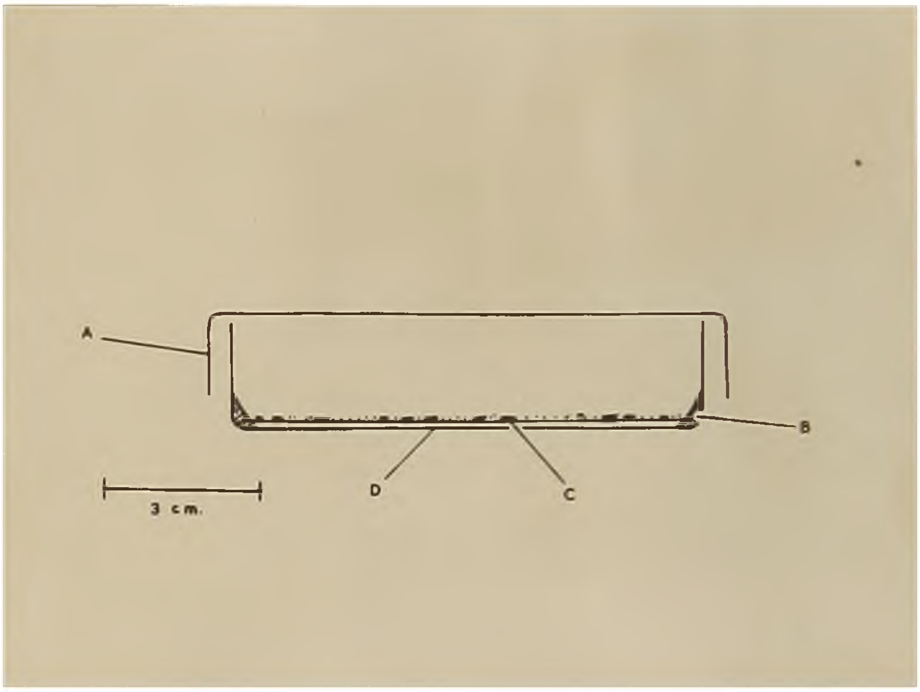

Figure 2. Container used in experiments on hatching. A, lid; B, paraffin wax; C, black paper floor; D, petri dish.

used. One pair of dishes was placed in the laboratory, just below a window, where external light and temperature changes would best be experienced. Two pairs of dishes were placed in a constant temperature room. One pair was exposed to continuous light from a 100 Watt electric light bulb, 100 cm. above, and one pair was exposed during the day only, being covered at night with a black-lined box. The dishes in the laboratory were also covered at night, since artificial lighting was in use during the evenings. When the light was switched off, the covering box was removed. The boxes were, in both cases, placed over the dishes at 6 p.m. The box covering the dishes in the constant temperature room was removed at 6 a.m.

Thus two dishes were kept at constant temperature in continuous light, two at constant temperature with alternating 12-hour periods of light and darkness, while the last two dishes were kept in the laboratory where temperature and light varied with external conditions, except that, at dusk, sudden darkness occurred when the box was placed over the dishes.

The eggs were left for 48 hr. to become acclimatised to the various conditions. All hatched crawlers were removed at midnight following the 48-hour period, and readings of the numbers of crawlers hatched were then made every 3 hr. for the next 48 hr. Temperature readings were taken at the same time. The dishes in the constant temperature room were kept under large bell jars, except when the one pair was under the box. The temperature remained at 26°C. The temperature next to the dishes in the laboratory showed minima of 17.5°C. and 17.2°C. at 6 a.m. on the two days of readings and maxima of 20.2°C. and 21.1°C. at noon - 3 p.m. and noon.

The count of crawlers was made under a binocular microscope, each crawler being removed as it was counted. The method was standardised as follows:- the dish was moved back and forth under the microscope so that a new strip of the bottom of the dish was searched at each sweep. Next, the perimeter was searched by revolving the dish once. The dish was then shaken to move the females, and any previously hidden crawlers counted. The majority of crawlers were stopped by the wax and deflected back into the dish or around the perimeter. Crawlers on the wax were not easily visible and difficult to remove as they often became stuck. For this reason no crawlers on the wax were counted. Any crawlers which were moving freely on the wax were, however, removed, so that they could not later find their way back into the dish.

The results are shown in Figure 3. The value for any 3-hour period represents the number of crawlers counted at the end of that period. Each value is the mean for two dishes for two 24-hour periods.

It can be seen that eggs kept at constant temperature with light and dark periods (B) and at regularly varying temperature with light and dark periods (A), appear to show peaks of hatching. Eggs kept at constant temperature in continuous light (C), appear to show a steady rate of hatching. The low values in (A) compared with (B) and (C) are due to the fact that the temperature in the laboratory was lower then that in the constant temperature room.

A Chi^2 test was carried out on the three sets of results. The (C) results did not show a significant departure from homogeneity (p greater than 0.1). The (A) and (B) results, however, showed a significant departure from homogeneity (p less than 0.001 in both cases). Thus the peaks in (A) and (B) are reflections of a real outburst of

Figure 3. Rates of hatching of eggs of L. beckii under the conditions of light and temperature shown above in the small graphs.

hatching during the "morning". It will be noticed that, in (B), the peak begins during the period before the light was switched on. This may be due to stimulation by the microscope light when the previous readings were taken, but implies some "readiness" on the part of this group of eggs to respond to a light at this time rather than any other.

A second experiment was conducted to find whether fluctuations in temperature alone, in continuous light, were sufficient stimulus to bring about the hatching rhythm. Two dishes were used; one was kept in a constant temperature room at 31°C. and in continuous light, and the other was kept, also in continuous light, in a second room, but at regularly varying temperature. The temperature was held at 31°C. from 6 a.m. until 6 p.m. and at 25°C. for the balance of the day.

The results of this experiment are shown in Figure 4. Here each value is the mean for two 24-hour periods for one dish. The regularly fluctuating temperature produced a significant peak (p less than 0.025) of hatching (A). The low values in (B), kept at constant temperature in continuous light, were due to a breakdown in the temperature control unit. The temperature during the initial 48-hour acclimatisation period rose to 33°C. and most crawlers had hatched before readings commenced. There appeared, however, to be no peak of hatching.

It can be seen from Figures 3 and 4 that the peak appears to be more pronounced when light and temperature are acting together. Whether this peak was, in fact, more pronounced than those produced by either light or temperature acting alone, was tested statistically. It was found that the two factors working together produced a statistically greater peak than either working alone (p less than 0.01 in

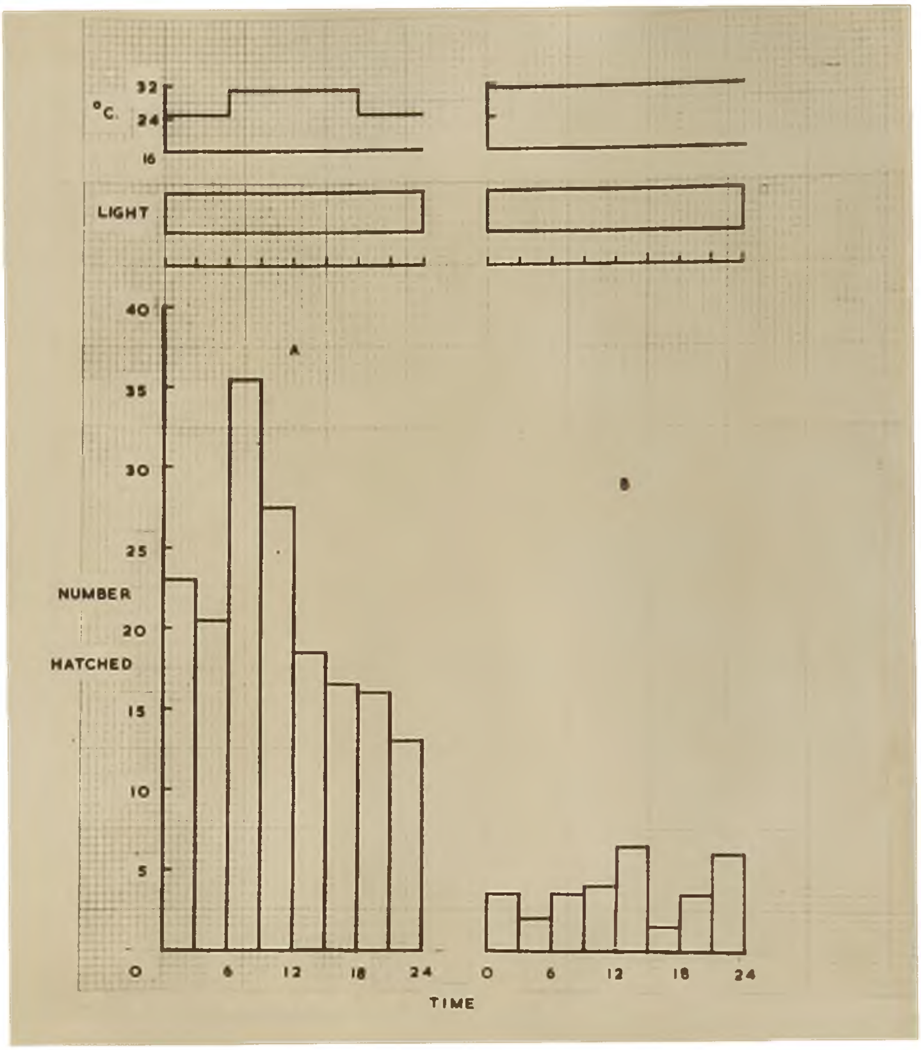

Figure 4. Rates of hatching of eggs of *L. beckii* under the conditions of light and temperature shown above in the small graphs.

either case). The effect of temperature might, however, be due only to a speeding up of the metabolism of the eggs, and not to a response to it as a specific stimulus. It seems likely, however, that a simple increase in the rate of metabolism on temperature increase, would result in a sustained higher hatching rate, and not in the peak and subsequent decline which actually occurs (Figure 4A).

An attempt was made to clarify this point, using a technique similar to that employed by Ewer and Ewer (1942) in an investigation of the effect of relative humidity on hatching in Ptinus tectus Boie. L. beckii eggs were kept at a low temperature for a long period, and then transferred to a higher temperature. If the temperature increase is the stimulus for actual eclosion, but metabolism proceeds fairly fast, a sudden hatching of all the eggs would be expected.

Fifty females and their eggs were kept in a petri dish at 14°C. in continuous light. This temperature is well above the lowest, 7.8–8.8°C., at which Bodenheimer (1951) found development to occur. A control dish was kept at 26°C. in continuous light. After a 48-hour equilibration period, the number of crawlers hatched in each dish was counted daily at 9 a.m. and 9 p.m. for a week (Table I). After four weeks at 14°C. the experimental eggs were transferred to 26°C. and the rate of hatching recorded as before for a further week (Table II).

There can be seen to be no sudden outburst of hatching, and the rate of hatching follows a similar pattern to that of the eggs originally kept at the higher temperature. Unfortunately, this negative result cannot be regarded as evidence that temperature increases do not act as specific stimuli, and that it is increased metabolic rate which is responsible for the hatching peak. It is possible that

Table I. Numbers of crawlers hatching per day at 14°C. and 26°C.

Day	1	2	3	4	5	6	7	Total
14°C.	1	1	1	1	2	2	2	10
26°C.	3	5	6	13	12	7	9	55

Table II. Numbers of crawlers hatching per day at 26°C. after four weeks at 14°C.

Day	1	2	3	4	5	6	7	Total
Number	1	4	6	7	8	9	11	46

four weeks was not long enough for complete development of all the eggs to occur at 14°C. The possibility remains that a positive result would be obtained if the eggs were kept for a longer period.

Light, like temperature increase, is not essential to hatching. This was shown by keeping 100 eggs in total darkness at a constant temperature of 26°C. At the end of this period, every egg was found to have hatched.

III. The wandering period

Once the crawler has emerged from under the scale-covering of the female, it may wander over the surface of the host plant for a variable period. According to Quayle (1938), crawlers may wander for up to three days, but the majority settle within one day. Carnegie (1955), however, states that crawlers seldom wandered for more than a few hours. Some were released on orange leaves in an attempt to obtain further information about the wandering period.

Sixty crawlers were released on dusty orange leaves and watched until they settled and produced wax threads. The average time spent before settling at 28°C. was 63.8 ± 3.9 min. The mean wandering period on leaves cleaned by means of a soft cloth was 91.0 ± 8.8 min. It was observed that crawlers which walked for periods of over 2 - 3 hr. often died without settling. Heavily infested leaves very often bear an extensive growth of lichen with much adhering dust, and often even uninfested leaves have dust adhering to the surface. It is thus probable that the lower value obtained is a better reflection of the normal walking time in field conditions.

During this period of wandering, the crawler will be exposed to various stimuli from its environment, and to other factors such as gravity. Of these, the factors most likely to influence the crawler in its wandering, are wind, gravity, light, temperature, and smell of the host plant.

Wind

The long anal filaments of the crawlers of *L. beckii* have already been mentioned. The surprising length of the filaments, a third

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Wind

The long anal filaments of the crawlers of L. beckii have already been mentioned. The surprising length of the filaments, a third of the total length, suggested some experiments in which an attempt was made to determine whether they might have some anchoring function. It was noticed that a crawler walked with difficulty on glass. Although the tarsi appear to be unable to gain sufficient purchase on the smooth surface, the difficulty is in fact largely due to the filaments anchoring the animal by adhering to the glass. This can be shown by cutting off the filaments, for then the crawler is able to walk a great deal more easily on the glass surface. On paper, which gave adequate purchase for the tarsi of the crawlers, the effect, if any, is not noticeable. Experiments were designed to determine whether this anchoring action of the filaments might keep the crawlers, walking on the host plant, from being blown away by wind.

The general procedure of an experiment was as follows. The anal filamnets of several crawlers were cut as short as possible; these crawlers were then placed on a leaf together with an equal number of intact crawlers. They were then subjected to air blasts until about half of them had blown away. The remaining crawlers were inspected to determine the numbers of operated and intact crawlers remaining.

The cutting of the anal filamnets was accomplished as follows. A section of razor blade mounted on a matchstick was used. The amputation was carried out under a binocular microscope by drawing the blade across the longitudinal axis of the crawler as it walked, allowing the blade to pass just behind the tip of the abdomen. It is not possible to keep the crawler still without damaging it. With the exception of a few instances where the tip of the abdomen was cut off, the above method was satisfactory. It was possible to cut off all the trailing portion of the filaments, leaving only the downward-directed stumps, which just made contact with the substrate. The extent of the amputation was then checked by inspection under the compound microscope. If the amputation was not satisfactory, the process was repeated. An equal number of crawlers was then subjected to the same process, except that the operation was not performed. The crawlers were then transferred to the arena. At first this was a small piece of black paper. Later, cleaned or scale-infested orange leaves were used. Any mature female Scales were removed, so that emerging crawlers would not give rise to misleading results.

A jet of compressed air was then played over the arena surface, until about half of the crawlers had been dislodged. The remaining crawlers were examined under the compound microscope and the number of intact and operated crawlers counted. The results of the experiments are given in Table III.

It appears that the presence or absence of the anal filaments has no effect on the ability of crawlers to withstand sharp air movement.

During this experiment it was found that crawlers were able to withstand a very powerful jet of air if it was of constant force and direction. A steady jet of air that moved a full matchbox to a distance of one yard along the bench did not dislodge any crawlers. A much lower pressure was effective if the jet was played back and forth across the arena.

During the course of observations on crawlers walking on leaves, a possible function of the anal filaments was noted. On a dusty leaf, it was seen that a crawler sometimes lost its footing and overturned. Dusty leaves occur commonly in the field. Very often the filaments would be caught under the animal. The body of the crawler

Table III Numbers of operated and intact crawlers remaining on arena after airblast.

Arena	Total number transferred	Number remaining	
		Operated	Intact
Paper	40	7	10
Clean leaf	30	7	7
Infested leaf	20	3	2
Total	90	17	19

would then lift spasmodically on its posterior tip and quite often fall over on to its ventral side again. This lifting of the body could have been due only to upward bending of the tip of the abdomen with the filament bases held rigidly or to independent movement on the part of the filaments. It seems unlikely that the tip of the abdomen would be capable of bending far enough to lift the body to the vertical without some independent movement on the part of the bases of the filaments.

How much overturning occurs in the field is another matter. If it does occur, the action of the anal filaments might have considerable survival value, for the flattened crawler has little chance of righting itself by means of leg movements alone.

Another possible function of the anal filaments might be that of a parachute. It is generally stated that crawlers are carried by air movement from tree to tree (Stofberg, 1937; Ebeling, 1950; Bodenheimer, 1951). The long anal filaments may assist in keeping the crawler airborne in the same manner as is said to occur in certain newly hatched spiders (Grassé, 1949).

Gravity

Another factor which might be expected to influence the wandering of crawlers is the force of gravity. Some experiments were carried out to investigate this possibility. Crawlers were released on a vertical surface and their movements observed.

The first apparatus to be used is shown in Figure 5(1). The vertical surface consisted of a 20 cm. long rod with diameter 6 mm.(C). This was painted with black poster paint so that the crawlers would be visible. A long entomological pin (E) was inserted into the rod at

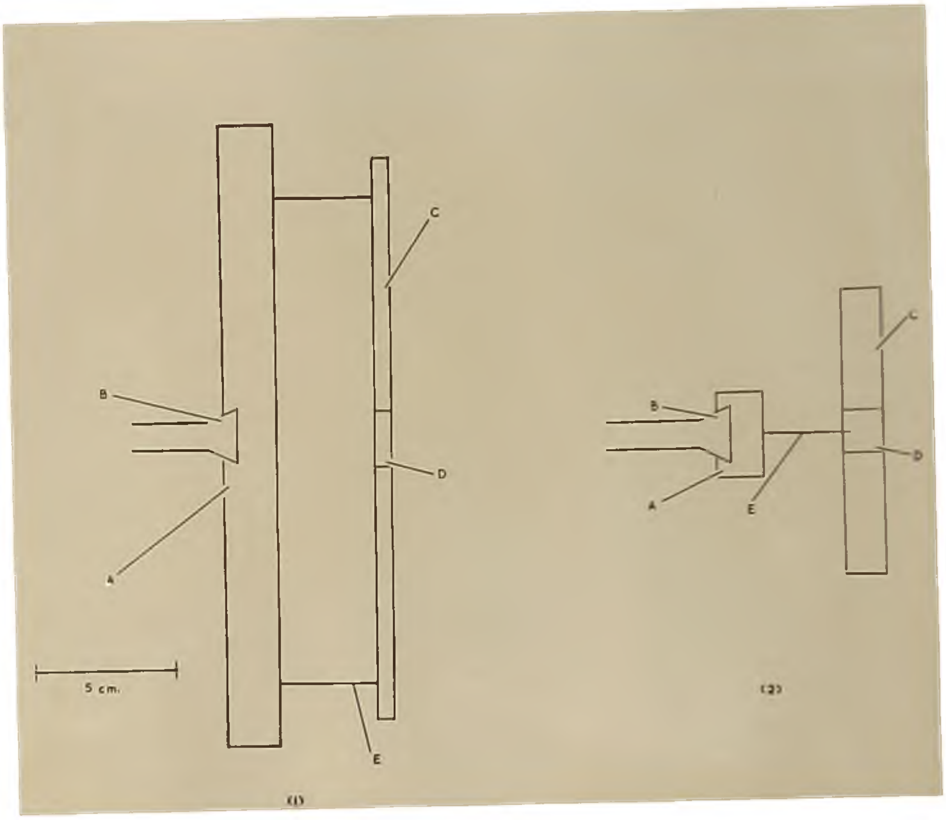


Figure 5. Apparatus used in testing for response to gravity. A, wooden support; B, retort stand clamp; C, wooden rod in (1) and piece of black paper in (2); D, release area; E, pin.

either end and fastened to an upright (A), which was held in a retort stand clamp (B). The pins gave a small area of contact with the rod and reduced the possibility of the escape of crawlers via the support. An exactly similar, but horizontally clamped control, was used.

A modification, shown in Figure 5(2), was later used. In this case the vertical surface was a strip of paper (C), 10 cm. long and 1.5 cm. wide. It was thought that crawlers might move more freely on the paper than on the paint. The paper was supported by a single pin (E), to which it was attached by a drop of paraffin wax.

The crawlers were released on a central band (D), 2 cm. wide in (1) and 1.5 cm. wide in (2). Crawlers were not regarded as having moved up or down until they had left this band.

The first experiments were carried out at 19°C. For the next experiments the temperature was increased to 25°C. in order to produce greater activity among the crawlers. Experiments were carried out in both light and darkness. The light originated from a 60 Watt electric light bulb on a level with the central band and 50 cm. away.

The results of experiments using apparatus (1) are given in Table IV and those of apparatus (2) in Table V. In both experiments the crawlers were found to be scattered over the vertical surface, no concentrations being formed at either high or low points. The results given in Tables IV and V support this observation. It would appear that there is no tendency on the part of L. beckii crawlers to move either upwards or downwards in response to the force of gravity.

Light

Light is an environmental factor which plays a very important part in the lives of many animals. This was the next factor whose effect

Table IV Numbers of crawlers on different zones of the rods at intervals. A, above; C, central zone; B, below; L, left; R, right.

In darkness

Experiment		1		2		
Time (hours)		16	24	4	8	24
Vertical rod	A	3	5	6	5	3
	C	4	1	5	5	2
	B	1	2	4	4	3
Horizontal rod	L	3	3	7	4	3
	C	4	3	5	4	4
	R	0	0	2	1	2

In light

Experiment		3	
Time (hours)		6	18
Vertical rod	A	9	6
	C	3	2
	B	5	4
Horizontal rod	L	7	5
	C	3	2
	R	7	3

Table V. Numbers of crawlers on different zones of vertical piece of paper at intervals.
A, C, etc. as in Table IV.

In darkness

Experiment	1
Time (hours)	5
A	2
C	3
B	7

In light

Experiment	2	3
Time (hours)	14	4
A	6	4
C	2	2
B	0	5

on the wandering of crawlers was investigated.

Some difficulty was experienced in finding a technique suitabl for use with such a small animal. The first attempt to study the light response of crawlers was made with a small choice chamber. This was designed to offer a choice between a light and a dark half.

This chamber is illustrated in Figure 6(1). It consisted of a bakelite ring (C), of internal diameter 1.5 cm., cemented to a microscopical slide (D). In order to keep the crawlers from the small gaps between ring and slide, a floor (E) was devised. This was formed by pushing a slightly oversize circular piece of paper into the ring and down to the surface of the slide. The lid (A), consisted of a coverslip mounted in a shallow cylinder of black paper (B). It was intended to darken one side by blackening one half of the coverslip. Observations were made by means of a binocular microscope. In trial runs, however, this apparatus proved unsatisfactory. Some crawlers escaped from the chamber by walking out between the coverslip and the upper rim of the ring. A second snag was the lack of continued movement by the crawlers. Crawlers soon flattened, or began to walk slowly with a great deal of stopping and turning. Flattening and slow walking commonly occurred at the angle of walls and floor.

To avoid such a junction, a cup-shaped glass chamber of internal diameter 1 cm. was obtained (Figure 6(2), B). The walls of the chamber were sloped outwards to facilitate observation. On vertical walls the slightest inequality serves to hide a crawler. The chamber was roofed by means of a coverslip (A), the top rim of the chamber having been ground to give a flush fit with the coverslip. It was necessary to paint the inner surface black for two reasons.

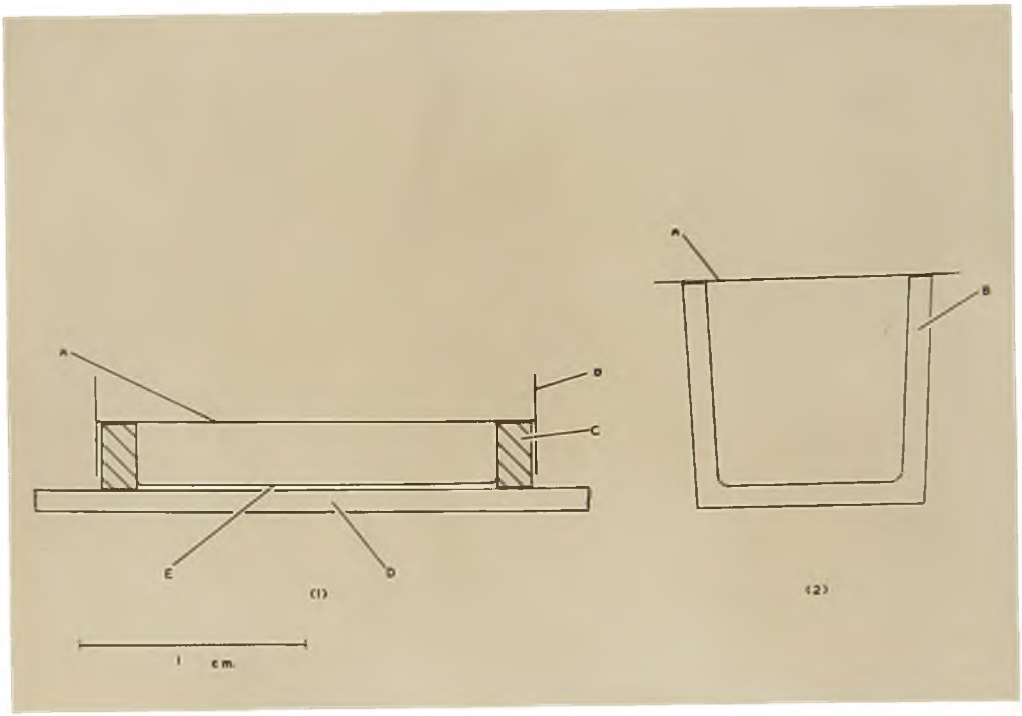


Figure 6. Miniature choice chamber. (1) A, cover slip; B, black paper cylinder; C, whole mount ring; D, microscopical slide; E, black paper floor.

The first was to avoid internal reflections and the second to provide crawlers with a surface upon which they could walk easily. The unsuitability of glass as substrate for a walking crawler has already been described.

Once again it was found that the crawlers escaped from the chamber, but far more important than this was that there was again a lack of sustained activity. The average duration of walking of 22 crawlers before a stop of at least 1 min. was 16 min. This did not include the slowing down and turning which occurred also in this chamber, and is reflected in the low rate of locomotion, 4.9 mm./min., determined at 30°C. using ten crawlers. Quayle (1912) had obtained an average value of 23.2 mm./min. at 31.7°C. for crawlers walking on a sheet of paper. Neither duration or speed of walking are satisfactory for a choice chamber experiment. The low rate of locomotion, compared with that obtained by Quayle,indicated that something about the enclosed space might be producing this effect.

To check this, four crawlers were released individually on a large sheet of black paper. It was found that only one crawler could be released at a time, since, even against the black background, finding the crawlers was difficult unless the progress of an individual was followed closely. Two of the crawlers were watched for 2 hr. and two for 1 hr. All had been walking continuously, and were still walking at the end of this period. These observations confirmed the suggestions put forward above that the behaviour of crawlers in a confined space is "abnormal" and the miniature choice chambers were abandoned as unsuitable. All further experiments were conducted on open arenas.

It was decided to record the response of each crawler by plotting its track on graph paper. It was necessary to have some sort of reference grid, which should not, however, have had any effect on the crawler.

A grid drawn in pencil on the arena did not show up, especially at lower light intensities. Crawlers were then released on a sheet of polythene, on the underside of which a grid had been drawn. A background of black paper under the polythene sheet enabled the observer to distinguish the crawler. This system was discarded due to the slow progress of the crawlers on the smooth polythene surface. The average speed of 12 crawlers at 25-28°C. was only 9 mm./min. The speed was found by measurement of the tracks with an opisometer.

An apparatus which made it possible for the crawler to walk on an untreated sheet of black paper is shown in Figure 7. A grid was drawn on a large sheet of glass (B), which was supported above the arena (G) on blocks (A), covered with black paper. The glass had the subsidiary function of shielding the arena from the air draught caused by the fan of the constant temperature unit. A screen (D), 20 cm. high, and consisting of black paper mounted on a wooden framework, surrounded the arena. It excluded extraneous light and also shielded the arena from the air draught.

For studying the response to directional light, a light source (C), consisting of a 60 Watt electric light bulb, was placed at one end of the arena. This provided oblique directional light, striking the release point at 30° from the horizontal. A museum jar (E), full of water, served as a heat filter and was placed between the light and the arena. The light intensity, with this source, and measured at the centre of the arena, varied between 800 and 1400 m.c. as the mains voltage

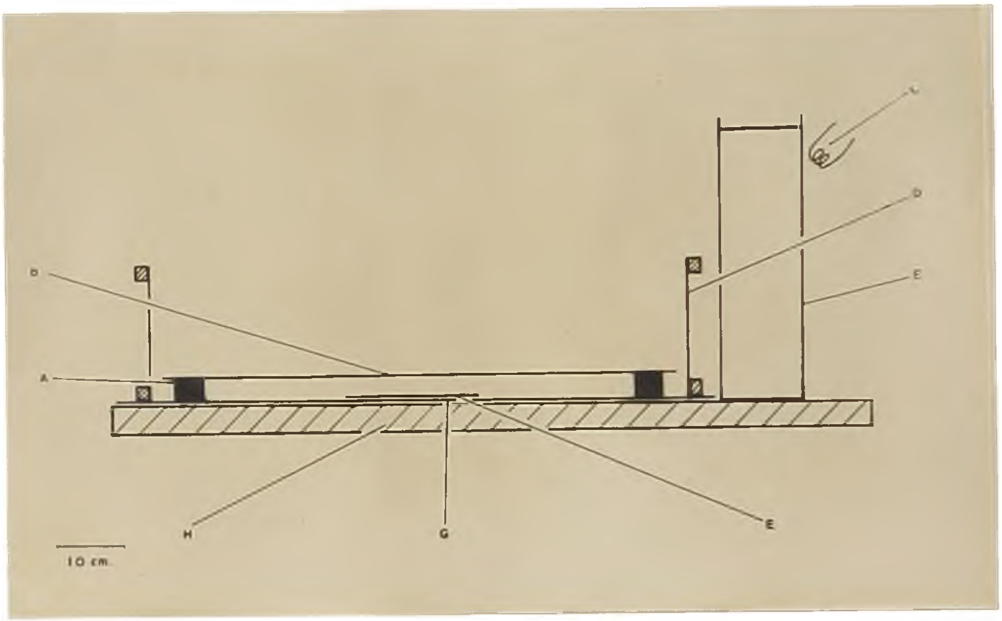


Figure 7. Apparatus which was used to investigate the response to light. A, support for glass grid; B, glass grid; C, light source; D, black paper screen; E, heat filter of museum jar full of water; F, replaceable arena; G, main arena of black paper; H, wooden board.

varied.

In the earliest experiments the temperature was kept fairly constant between 25° and 28°C. by means of a "Sunvic" relay. Later, constant temperature rooms became available. The temperature was then held at 25.5°C. Two thermometers, one at either end of the arena, showed that no temperature gradient existed.

On the paper arena the average speed of 11 crawlers was 18 mm./min. at 25.5°C. Crawlers observed on a small piece of black paper under a binocular microscope appeared to be walking without difficulty. The black paper arena thus appeared to be suitable for testing the responses of crawlers.

Some experiments were also conducted with a light source directly above the crawler. The same arrangement was used except for the light source. This was a 100 Watt argenta electric light bulb, housed in a light-tight box, which was open below. The box was supported 80 cm. above the arena on four legs which rested on the glass grid. Both box and supports were painted with flat black paint. The light could be moved so that it was always above the crawler.

(i) Nature of the response.

The tracks of crawlers were plotted under various conditions. For clarity, the more involved tracks are on a larger scale than the others (Figures 14 and 16).

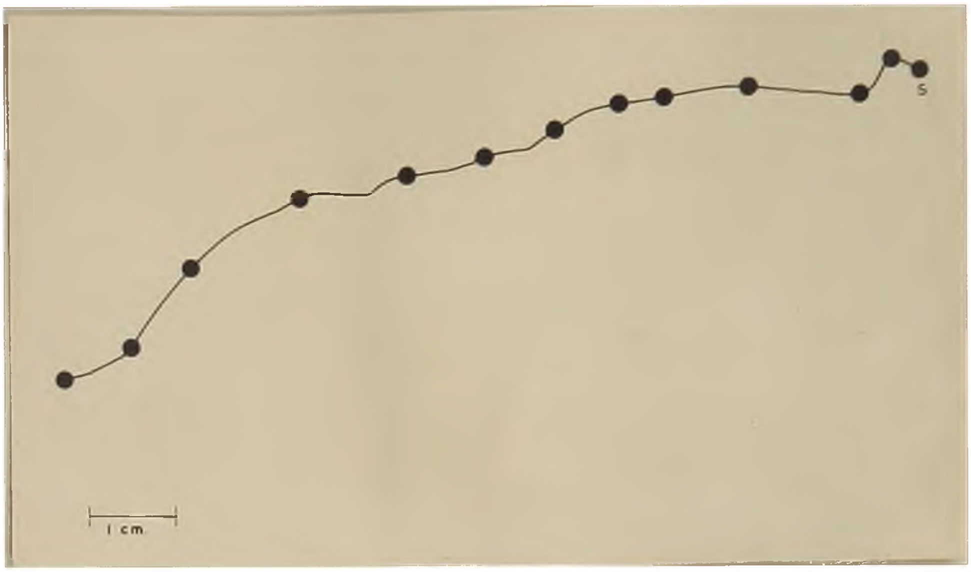
The handling of crawlers does not appear to change their response to light. Two experiments were conducted using crawlers which were not handled. This was done by waiting for a crawler to emerge from under a female, then cutting away the leaf on all sides. The small remaining section of leaf was gently placed on the arena and the crawler allowed to find its own way off. These crawlers showed the same response as crawlers transferred by means of a brush.

A definite response is shown when the light source is placed on one side, as illustrated in Figure 7. Typical tracks are shown in Figures 8 and 9. In Figures 10 and 11 the tracks after the first 10 min. (solid circles) were also obtained in this way. In some cases the track was fairly convoluted, or at a considerable angle to the light source, but in almost every case there was a displacement towards the source. In a group of 16 of the total of 45 experiments carried out, the light source was placed at the other end of the arena. The fact that the response still occurred shows that it was the light source and not some other feature of the apparatus to which the crawlers were responding.

Two parameters may be used in measuring a response. The first to be described provides a measure of the accuracy of the orientation while the second allows the directness of the track to be measured.

The accuracy of orientation can be arrived at by measuring the angle made by the track with the straight line joining the release point and a point vertically below the light source. The numbers of animals walking at given angles to left and right of this straight line (taken as zero) may be plotted in the form of a histogram. If the majority of animals walk towards the light with a fair degree of accuracy, a peak would be expected about 0°. The height of this peak gives an idea of the accuracy with which the group of animals have orientated.

With crawlers of L. beckii the accuracy of orientation is not very great (Figure 12). However, the size of the source should be taken into consideration, especially as the response may have been to the whole brightly illuminated museum jar full of water. The release point



Figures 8 and 9. Tracks of crawlers responding to oblique light from a single source (S = start, filled circles represent minute intervals). Light source on the left.

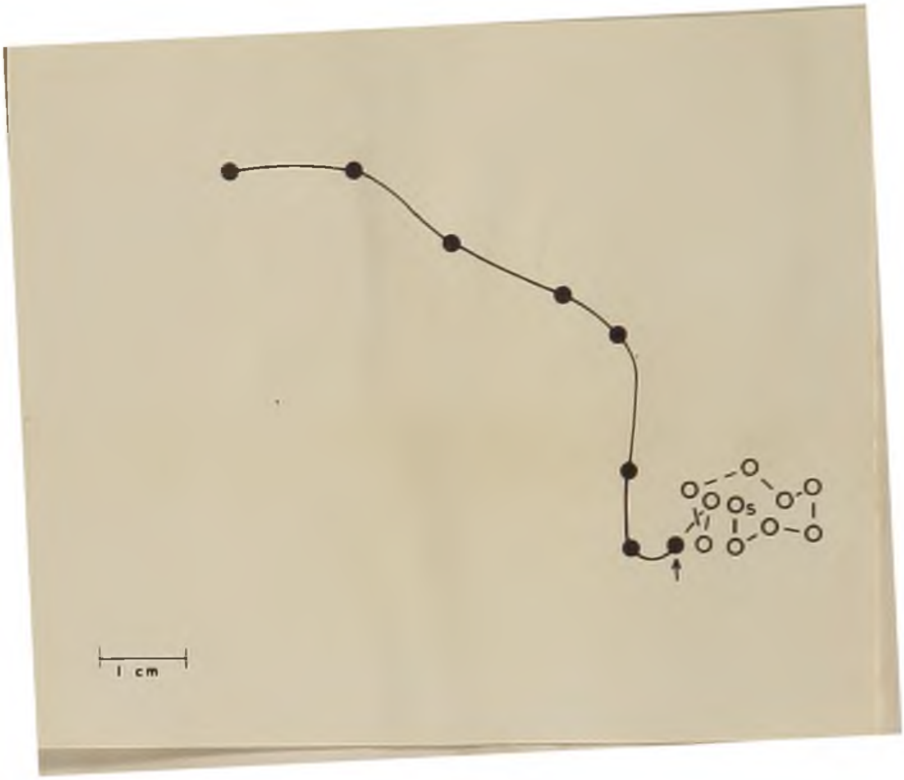


Figure 10. Position at minute intervals (represented by open circles, s = start) of a crawler walking in darkness, followed by the track resulting when oblique light was switched on (minute intervals represented by filled circles). Light source on the left.

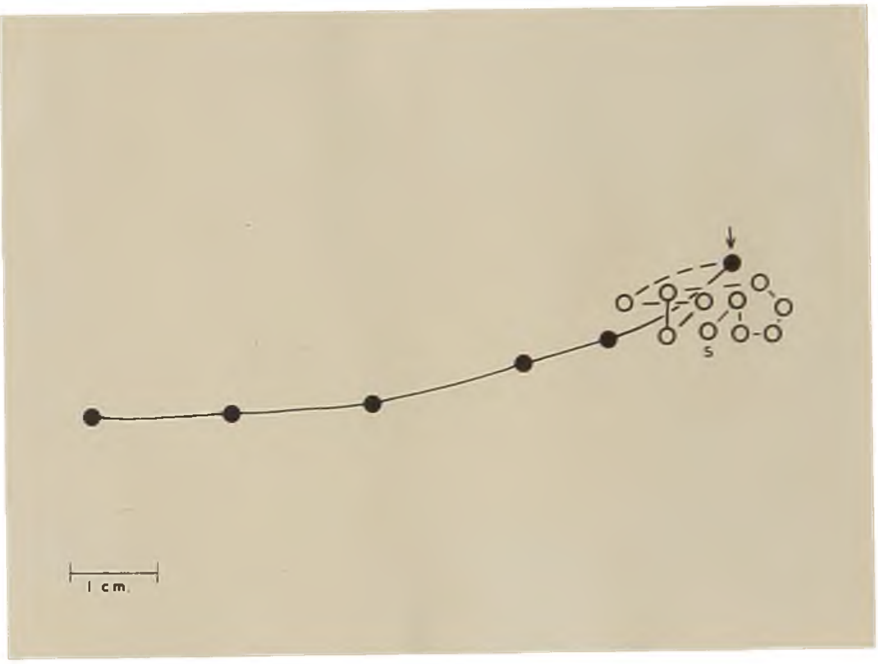


Figure 11. Position at minute intervals (represented by open circles, s = start) of a crawler walking in subthreshold oblique light, followed by the track resulting when the intensity of the source was raised above threshold (filled circles). The light source was on the left.

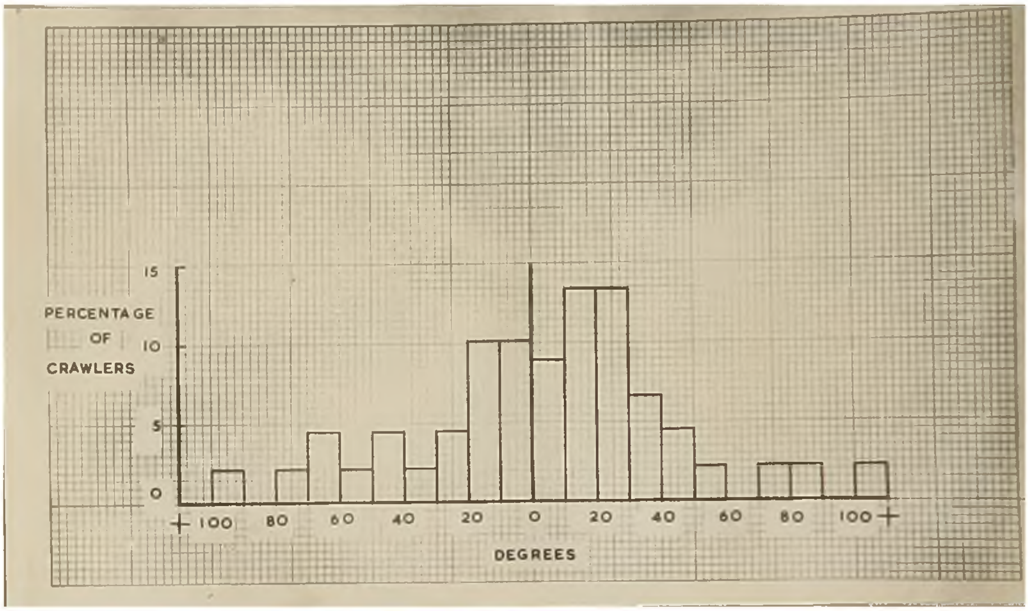


Figure 12. The accuracy of orientation of crawlers to a single light source, i.e. the angle formed by the source, and the position of each crawler after 5 min., with the release point.

made an angle of 40° with the lines joining it to the two sides of the jar. However, it can be seen that 45% of the tracks were within ±20° of the 0° line, and only 5% at angles greater than 90°.

A measure of the directness of the track is given by

$$\frac{\text{Length of track}}{\text{Length of straight line joining ends of track}}\text{; the directness ratio.}$$

A straight path would thus give a value of 1.0. Figure 13 shows the distribution of the values calculated from the tracks obtained above. Most of the crawlers can be seen to have followed a fairly direct course.

Control experiments were carried out with the light source directly above the crawler. Figure 15 shows a track obtained under these conditions. The tracks appear very much more convoluted and limited than is the case in oblique light. This is borne out by the high values of the directness ration calculated for tracks made in vertical light. The angle of orientation appears, as would be expected, to be random. Both sets of values are shown in Table VI.

The response to light of crawlers freshly removed from beneath the female Scale may be summarised as follows. Crawlers in non-directional (from above) light walked in such a way that the actual displacement from the release point was small. In directional (from the side) light there was a definite displacement towards the source. The actual path followed, however, was sometimes convoluted and at an angle to the light source.

(ii) Threshold of the response

An attempt was made to find the threshold of the response to light, using the tracking technique described above. A tungsten filament electric light bulb was used as light source, together with a voltage regulating system. A variable resistance, together with a voltmeter,

Table VI. Values of directness ratio and angle of orientation of crawlers in vertical light.

Crawler	Directness ratio	Angles of orientation in degrees	
		to left	to right
1	3.9	54	
2	6.0	19	
3	6.3		81
4	3.5	30	
5	3.6	118	
6	7.8	168	
7	9.4		171

Table VII. Percentage of crawlers responding to light

Source	% response	Total Number
Fresh leaves	39	13
Stored leaves	29	14

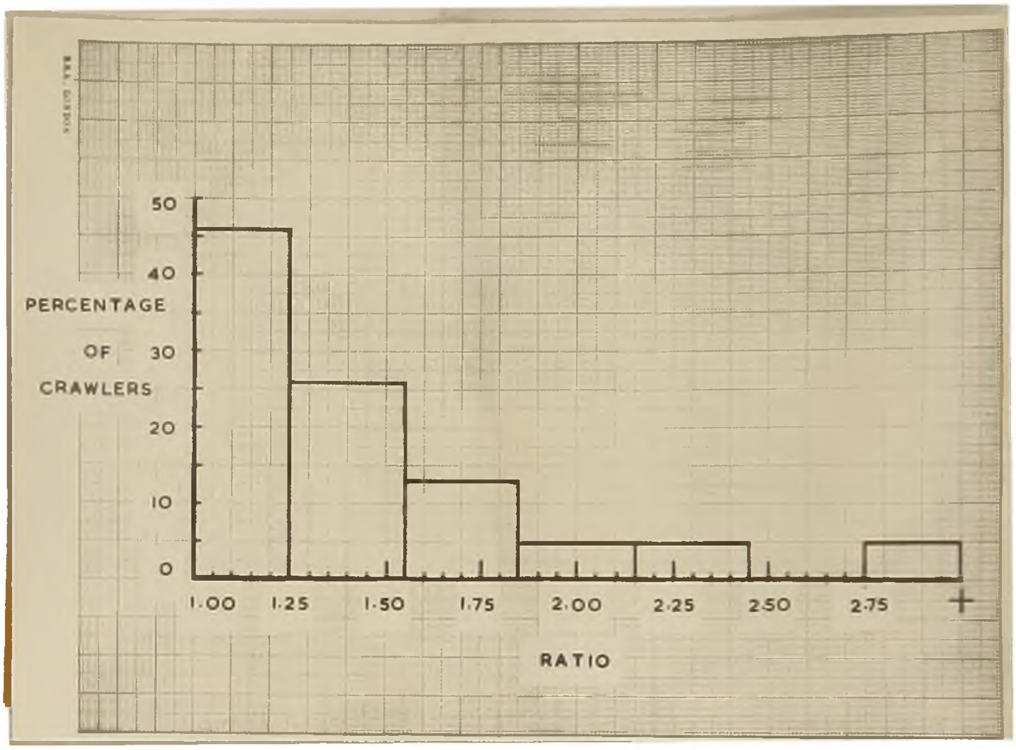


Figure 13. Directness of orientation of crawlers to a single light source; calculated by ratio $\frac{\text{length of track after 15 min.}}{\text{straight line joining ends of track}}$

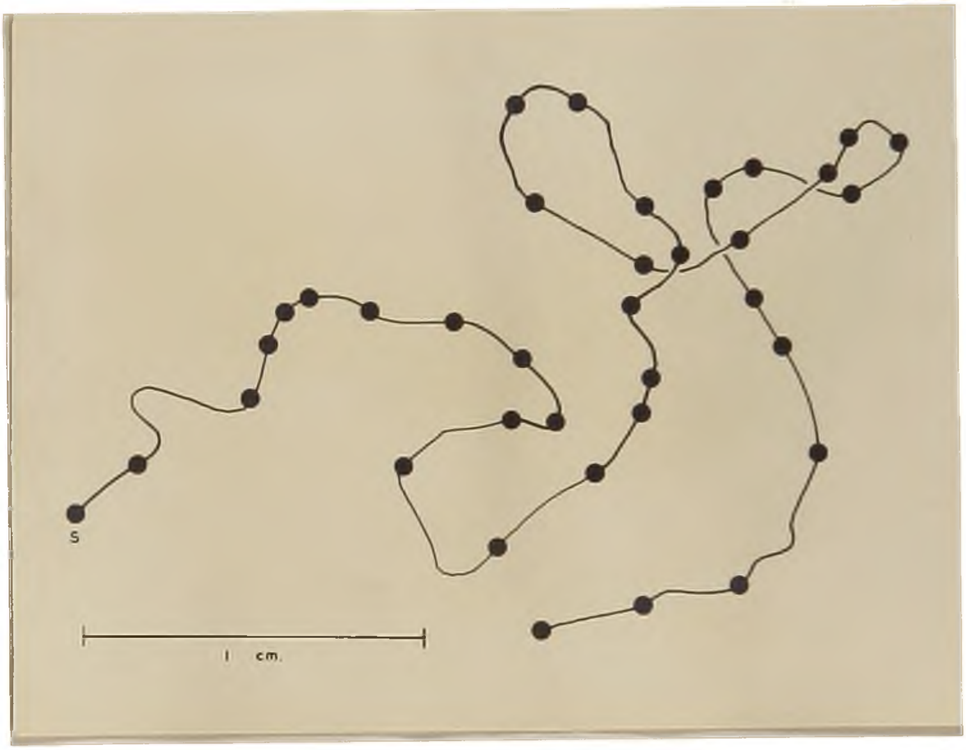


Figure 14. A track of a crawler walking in vertical light. s = start, filled circles represent minute int[illegible]ls.

permitted controlled variations in voltage (and thus in light intensity) to be made.

The light intensities corresponding to the voltages set by means of the variable resistance were measured. An attempt to do this by using a phototransistor coupled with a sensitive ammeter was unsuccessful with the low intensity, unfocussed light used for these experiments. The light intensity was finally measured by means of a waxblock photometer together with a Tungsten standard lamp.

An electric torch was used for plotting the tracks at low intensities as the crawlers were not visible under these conditions. In order to standardise results the torch was used at higher intensities as well, even though it was not necessary in the upper range. The tracks obtained, then, consisted only of the plotted series of positions occupied by the crawler at the end of each minute interval.

Two criteria were used in analysing these tracks. The first was to judge by the appearance of the track whether the animal was responding to the light, or doing the restricted wandering characteristic of locomotion in non-directional light. The second criterion was as follows: if, at any time during the first 15 min. the crawler was found outside a circle of radius 5 cm. with centre the release point, it was considered to have responded to the light.

The results, analysed by the two methods described, are shown in Figure 15. The numbers of observations were not sufficient for any definite conclusion to be drawn, as can be seen from the vertical lines, which represent one S. E. on either side of the mean. The percentage responding at 2.6 m.c. (using the measured results) did not differ significantly from the value at zero intensity, i.e. in darkness. The

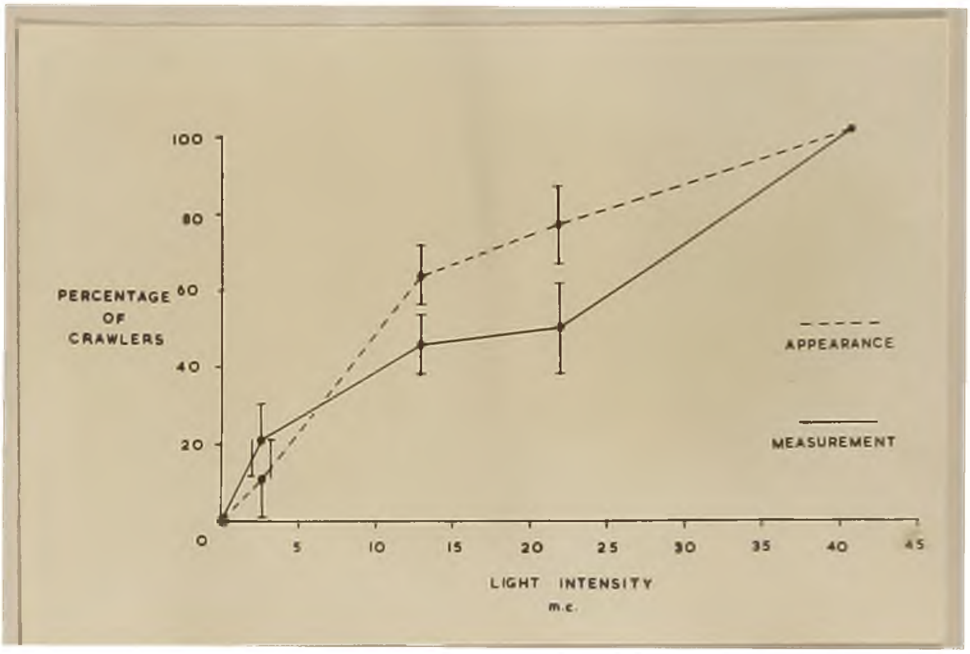


Figure 15. Percentage of crawlers responding to oblique light of different intensities

difference between the 2.6 m.c. readings and the summed readings for 13 and 22 m.c. was, however, significant (p less than 0.05). It can be seen from Figure 15 that the same qualitative conclusions apply to the results judged by appearance.

It is clear that an increasing number of crawlers respond as the light intensity is increased from zero to approximately 42 m.c., when a 100% response is obtained. The track of a crawler which was released at an intensity below 2.6 m.c. for 10 min., after which the intensity was increased to 70 m.c. (both oblique light), is shown in Figure 11. The response following the increase in light intensity is very clear.

It was thought that the lack of a sharply defined threshold might be due to the storage of the infested leaves in polythene bags. This period of storage might have affected the light response of the crawlers, causing a variation and thus obscuring the sharpness of the threshold effect. It was possible to test whether this had in fact occurred.

The numbers of crawlers responding to a 13 m.c. light source among 13 crawlers from freshly collected leaves and 14 from leaves stored for at least 48 hr. were compared. The results, analysed by measurement as described above, are shown in Table VII. Storage of infested leaves obviously has no effect on the light responses of the ensuing crawlers. Either the threshold is not sharp, or is affected by some other factor.

(iii) Mechanism of the response

There are a number of different mechanisms by means of which an animal may respond to a stimulus (Fraenkel and Gunn, 1940). These fall ~~into two groups.~~ In the first the animal responds only to changes of intensity of stimulus; these are known as kineses. In the second group the response is to the direction of the stimulus and these are known as taxes.

Animals which respond to intensity changes do so in two different ways: the first consists of a lowering in either the rate of locomotion or the activity level as the preferred or eccritic intensity for the particular animal is approached. This is known as orthokinesis and leads to an aggregation of animals in the eccritic region. This aggregation is not static, however, but due to an accumulation of animals in the region of slowest movement or lowest activity. The second type of response is due to changes in the rate of change of direction (r.c.d.) of the animal. The r.c.d. may be described as the sum, in degrees, of all the turns to either side made by the animal in unit time. Animals may aggregate in the eccritic region simply by an increase in r.c.d. in that region; this is known as klinokinesis without adaptation (Ewer and Bursell, 1950). Klinokinesis may also operate in a second manner. Here, the essential factor is adaptation occurring after an increase in r.c.d. This may best be explained by considering a hypothetical animal which, in constant conditions, makes a 180° turn at regular intervals. When the animal walks away from the eccritic region, the r.c.d. increases and a 180° turn soon occurs. The animal then walks back toward the eccritic region. By this time, however, adaptation has occurred and, as a result, the r.c.d. has decreased so that this section of path towards the eccritic region is longer than that leading away from it. A turn will, of course, eventually occur, and the animal will again walk away from the eccritic region. The whole process then occurs again. Since all those sections of the path leading towards the eccritic region are longer than those leading away from it, there is an overall displacement towards the eccritic region. A similar but far more complicated argument shows difference between the 2.6 m.c. readings and the summed readings for 13 and 22 m.c. was, however, significant (p less than 0.05). It can be seen from Figure 15 that the same qualitative conclusions apply to the results judged by appearance.

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Animals which respond to the direction of the stimulus may do so in several different ways, three of which are relevant.

The first involves regular deviations from side to side of that part of the animal bearing the receptor. During each deviation the intensity of stimulus is compared with that during the previous deviation. A balance can thus be struck, and the animal is able to move towards or away from the source of stimulation. This is known as klinotaxis, and, in common with the two reactions already described, can be effected with only one receptor.

The second type is known as tropotaxis. In this case a balance is also struck; now, however, of stimuli received by one receptor in two successive positions, but of stimuli received simultaneously by two receptors. If one of these is put out of action, the response cannot occur.

The remaining taxis, telotaxis, is a response which does not depend on the balancing of stimuli. In this case it is the particular portion of the receptor stimulated which determines the direction of locomotion. Inhibition of information received from other sources of stimulation is essential if telotaxis is to operate successfully in any but the simplest conditions.

The above account is over-simplified to some extent, as both klino- and tropotaxis can occur when there is no directional quality in the stimulus. This can only be the case, however, in a very steep gradient.

A number of tests exist which enable one to determine which

Animals which show a kinetic response are able to move up (or down) a non-directional gradient of (say) light intensity. This is not true of animals which show a tactic response, except when, as stated above, the gradient is very steep. The tracks produced in a photokinesis are random in direction, while in a phototaxis they are directed towards or away from the light source. Considerable error may occur, however, and a wavy path may result.

An animal orientating by orthokinesis moves at different speeds at different intensities of light. In klinokinesis, an animal experiencing a sudden change in light intensity will show a change in r.c.d. which may or may not return to the previous value, depending upon whether adaptation occurs or not.

Animals responding klino- or tropotactically to two lights will take the path between the two, and more nearly in line with the stronger. Animals responding telotacticly will respond to only one light at a time, usually finally orientating to one.

Klino- and tropotaxis may be distinguished by the fact that circus movements occur only in the latter. Circus movements are the circling movements made by tropotacticly behaving animals when unilaterally blinded.

Some of these tests have been applied to investigate the mechanism of the photopositive response of crawlers of L. beckii.

(iii.a) Kinetic responses

The orientation could be accomplished, in part at least, by an orthokinosis. A scrutiny of the tracks made by crawlers in oblique light shows that in most cases the response is too direct to be brought about by an orthokinesis acting alone. Nevertheless, an orthokinetic element might be present, and to test this possibility, observations were made upon the rate of locomotion at different light intensities. No specific study was made upon activity as there had been no indication that an activity orthokinesis could be involved, since the crawlers are almost invariably active until they finally settle. The usual tracking technique, and the apparatus shown in Figure 7, was used.

Observations were made at two different light intensities. Each crawler was released and left for 5 min. and then its track was plotted for 10 min. The tracks were measured with an opisometer and the speeds of the crawlers calculated.

In the first experiment, the two intensities used were 45 m.c. and 1500 m.c. Six crawlers were released at each intensity. Table VIII shows the results obtained. There appears to be no marked difference in speed at the two light intensities.

In the second experiment, some modifications were made. The intensities were more widely spaced so that any difference not brought out in the first experiment would be shown. It can further be seen from Table VIII that the results are very variable. For this reason paired readings were taken in the second experiment. The crawler was allowed 5 min. in darkness before being exposed to the second intensity. Four crawlers were used. Two were released at the low intensity first, and two at the high intensity first, to avoid bias due to the animals tiring. The intensities used were 45 m.c. and approximately 4000 m.c. The results are shown in Table IX.

It can be seen that it is unlikely that any difference in speed exists at different light intensities. There is certainly no difference of sufficient magnitude to suggest that any orthokinetic element is involved in the response to light.

Table VIII. Speeds of crawlers at two different light intensities

Speed in mm./min. at 45 m.c.	Speed in mm./min. at 1500 m.c.
12.0	16.0
22.0	10.4
16.6	11.4
16.4	17.4
17.0	16.0
14.8	10.0
Mean 16.5	13.5

Table IX. Speeds of crawlers at two different light intensities.

Crawler	Speed in mm./min. at 45 m.c.	Speed in mm./min. at 4000 m.c.
1	20.6	20.0
2	14.6	16.2
3	14.0	15.4
4	16.2	12.8
Mean	16.4	16.1

Another possibility is that klinokinesis is responsible for the photopositive response. This method of orientation is unlikely in view of the directness in many cases of the crawlers' response (Figures 8 and 9). However, a more quantitative test is required than this. This is done by an analysis of the tracks for changes of r.c.d. Any change may be expected to show if the animal is allowed to make a track for a period during which it is subjected to a change of light intensity.

If the change in intensity is to or from darkness, the change in r.c.d. is likely to be more pronounced. The smallness of the crawler leads to difficulty. Attempts to get the crawlers to leave a track on a sooty surface failed, because the crawler soon became coated in soot particles. Crawlers show a response to a Philips neon bulb. This excludes the possibility of using this source for observations in light imperceptible to the crawler. It is, however, possible to obtain a map of the crawler's progress, using an electric torch in the manner described for low light intensity observations. Such a track is shown in Figure 16. Another is shown in Figure 10 (circles), where a crawler was exposed to oblique light after 10 min. of tracking in darkness. From these tracks all that can be concluded is that they are very similar to those produced in non-directional light. Crawlers left uninterrupted in darkness for the same period showed a similar displacement at the end of that time, so that it is unlikely that the short flashes of torchlight had any effect on the behaviour of the crawler.

It was possible, however, to measure the r.c.d. of crawlers at two different light intensities. In a photopositive animal, klinokinesis with adaptation would depend on an increase in r.c.d. with a decrease in light intensity, while klinokinesis without adaptation would require a

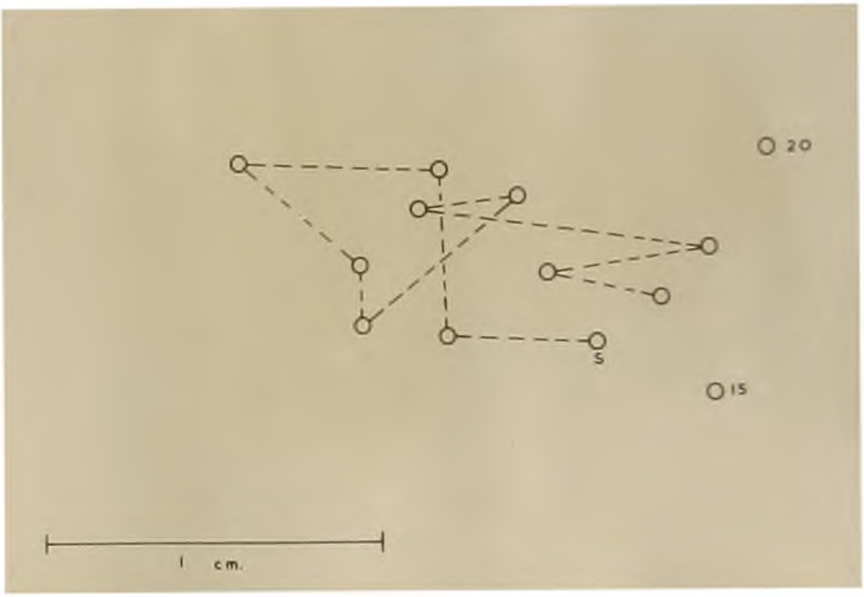


Figure 16. Positions at minute intervals (represented by open circles) of a crawler walking in darkness for 10 min. and positions after 15 and 20 min. S = start.

decrease in r.c.d. Both possibilities were investigated by measuring the r.c.d. of tracks of crawlers before and after a decrease in intensity of non-directional light.

The apparatus already described for obtaining tracks in vertical light was again used (See (i)). A variac transformer was used to control the voltage across the electric light bulb and so alter the light intensity to which the crawler was exposed. The 100 Watt bulb was first used at normal voltage (250 volts) and then at 150 volts. The latter voltage gave just enough illumination for the crawler to be visible. The crawler was allowed to walk for 8 min. at the higher intensity, after which the intensity was decreased and the crawler allowed to walk for a further 8 min. The tracks plotted during these periods were then analysed and the r.c.d. calculated for the two parts of the track. These values are given in Table X.

There appears to be no difference in r.c.d. at the two light intensities. It therefore appears that there is no klinokinetic element in the response.

(iii.b) Tactic responses

The above experiments exclude the possibility that there is a kinetic element in the response. The three possible remaining mechanisms by means of which the response might work are the taxes already described.

A two light experiment was carried out to distinguish between telotaxis and the other two taxes which depend on balance of stimuli. Two lights of equal intensity were set up to make an angle of 90° with the release point. A circular hole, 5 cm. in diameter, was cut low down on either side of the screen (D), so that nearly horizontal light could be obtained. Each lamp was screened and light could reach the

Table X. Values of r.c.d. at two different light intensities (degrees/min.).

Crawler	Bright light	Dim light
1	115.1	104.0
2	175.1	208.6
3	412.8	370.8
4	191.8	288.4
Mean	223.7	243.0

Table XI. Percentage of crawlers responding to light.

Analysed by:	Treatment	% response	Total Number
Measurement	Smell	42	19
	Untreated	50	22
Appearance	Smell	67	21
	Untreated	65	20

arena only through the two holes. A small museum jar full of water was placed at each hole. Each served both as a heat filter and to keep any draught from blowing through the holes. The glass grid was raised so that light would not be reflected from its undersurface.

The experimental procedure was the same as in the experiments in (i), where a single light was used. The tracks of 44 crawlers were plotted. These were analysed, as in (i), for accuracy of orientation. In this case, however, the line passing midway between the two lights was taken as 0°.

If klino- or tropotaxis occurred, the results would be expected to have the same distribution about the 0° line as is the case with one light (Figure 12), i.e. the crawlers walk between the lights. Figure 17 shows the results of this experiment compared with those obtained using one light. The angles of deviation to right and left of the 0° line were in each case lumped. The results from the experiment in which a single light was used may legitimately be treated in this way since no bias to left or right occurred, as can be seen from Figure 12. This is true also of the results obtained in the two light experiment, where 21 crawlers went to the left of the 0° line and 23 to the right. A Chi^2 test for homogeneity was applied to these lumped results. This showed that there was no significant difference between the two distributions shown in Figure 17 (p greater than 0.1).

It can be seen that either klino- or tropotaxis does in fact occur, as many crawlers walk between the lights. Some tracks which were obtained are illustrated in Figure 18; tracks 1, 2, 3 and 5 show how the crawler orientates between the two lights. The curving of tracks into one or other of the two lights in two light experiments has been

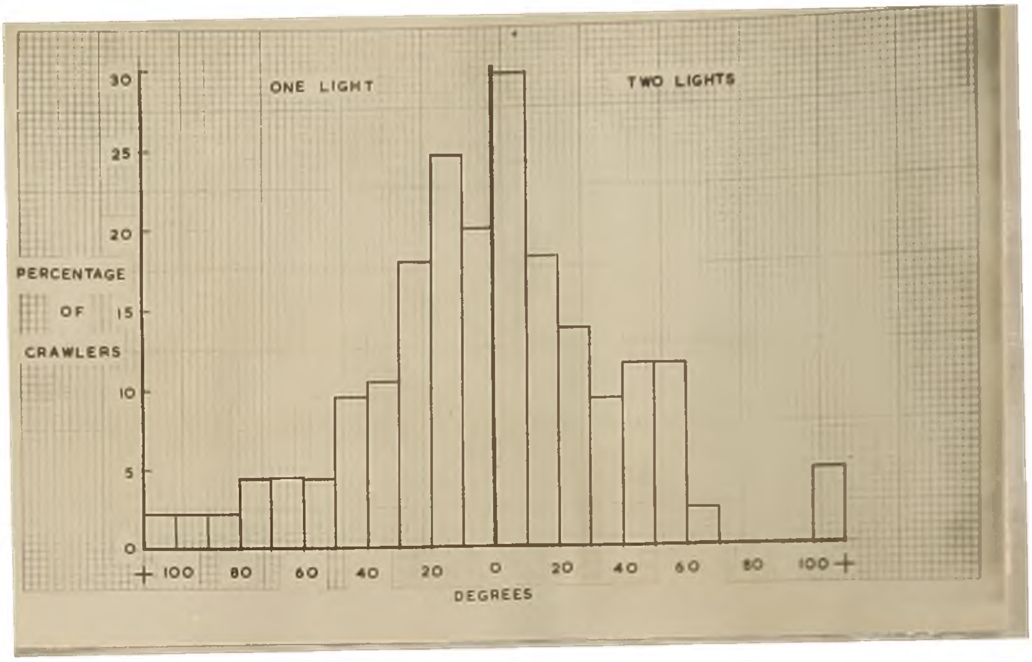


Figure 17. Comparison of accuracy of orientation of crawlers to oblique light from one source and from two equal sources making an angle of 45° with the release point. The one light results were derived from the data shown in Figure 12 by summing the two sides. In the two-light experiment, similar figures were obtained by taking the line passing midway between the two lights as 0°.

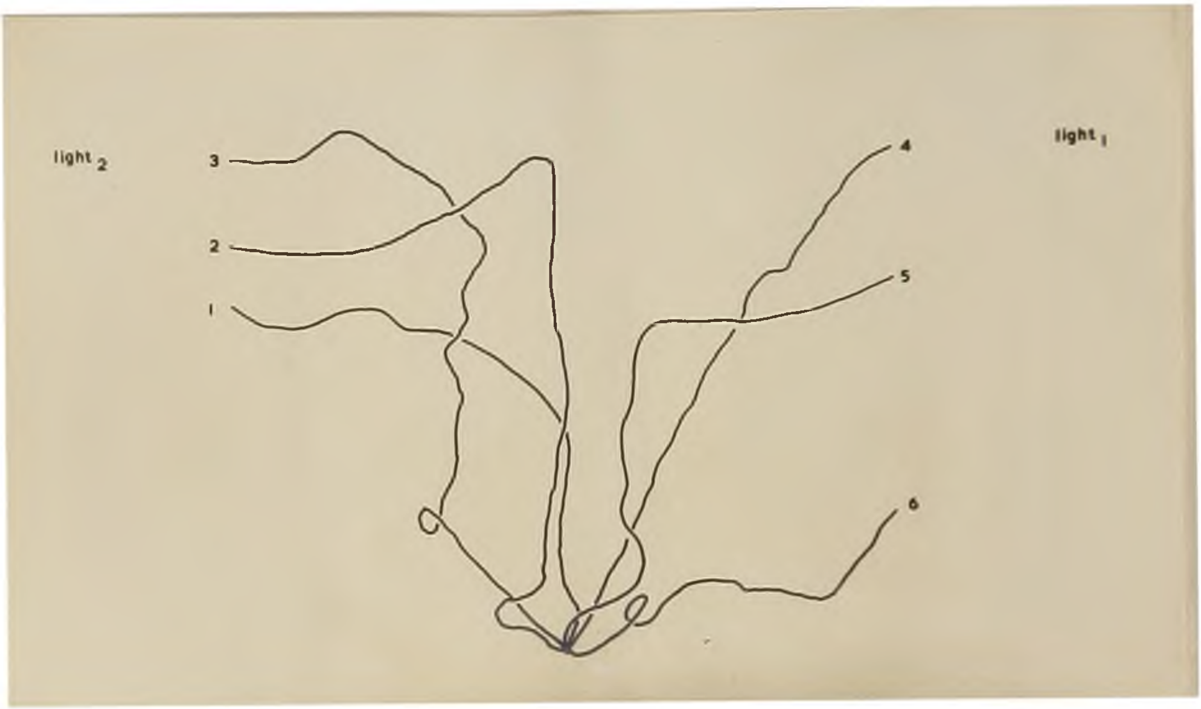


Figure 18. Tracks of crawlers walking in the presence of oblique light from two sources, l_1 and l_2, making an angle of 45° with the release point.

explained by Fraenkel and Gunn (1940). When the animal is nearly between the two lights, any accidental error of orientation will bring the animal nearer to one of the two lights, with the result that the effect on the eyes of this light is increased. This cumulative effect results in a path curving in towards one of the lights.

A certain number of crawlers, however, orientate to one light or the other fairly directly. This can be seen in tracks 4 and 6 in Figure 18, and is reflected in the small peak in the 40-60° region in Figure 17. This might be regarded as evidence for the occurrence under certain conditions of telotaxis. Nevertheless, it is felt that these are more likely to be irregularities such as might be expected with a not very efficient klino- or tropotaxis.

Crawlers can be seen from Figure 12 to be fairly bad at orientation, even to a single light. A crawler which, due to poor initial orientation, walks in the general direction of one of the lights instead of between them, would tend to go directly towards that light. This would only be counteracted if the power to initiate turning of the rear part of the eye was relatively greater than that of the front part. The eyes of a Diaspid crawler consist, according to Essig (1942), of a pair of simple, single lens ocellanae. My personal observations suggest that this is true of L. beckii crawlers. This simplicity makes it improbable that any differentiation occurs in the eye, and could explain the inaccuracy of orientation. The lateral position of the eyes is probably of no great importance, since the animal is fairly transparent. It appears from comparison of the histograms in Figure 17 that the peak in the 40 - 60° region for the two light experiment might be due to the "gathering" by the lights, of crawlers walking at angles greater than 60°.

Further support for this idea may be found in the tracks of the crawlers (Figure 18). Tracks 3 and 5 show that a crawler may orientate first to one light, and then between the two lights. The appearance of the track suggests that a further error in orientation gave the crawler a new start, so that it then orientated successfully between the two lights. Track 2, which goes between the two lights, was produced by a crawler which had been returned to the release point after walking almost directly towards one of the lights (Track 6). It seems unlikely that such a changeover to telotactic behaviour would occur for no apparent reason. It is moreover improbable that, with the simple type of eye present in crawlers, telotaxis could occur.

These results, then, can be explained simply in terms of a klinotactic or a tropotactic response.

It was not possible to find out whether the response was klinotactic or tropotactic. With an animal as small as a crawler, unilateral blinding is awkward and doubtful, if not impossible. The fact that there are two receptors, and the direct nature of the tracks, suggests, however, that the mechanism which is responsible for the orientation is tropotaxis.

(iv) Discussion

The above results are in general agreement with those of Bodenheimer (1951) but conflict with those of Carnegie.(1955, 1957) and Ebeling (1950).

Bodenheimer carried out experiments using some type of light/dark boundary apparatus which, however, he does not describe. A significantly higher proportion of crawlers was found on the light side, which was lit by "dispersed" light.

Carnegie observed the distribution of crawlers settling on two oranges, in contact, but under different conditions of illumination.

In essence, his results are as follows: crawlers do not migrate from a brightly illuminated fruit to a fruit in contact with it, but in darkness. Crawlers also do not migrate from a fruit in darkness to an adjacent brightly illuminated fruit. Thus it was concluded that crawlers are indifferent to light. An explanation of this result might be that some factor over-rides the response to light. This could be the smell of the host plant, in the presence of which the light response did not occur.

It was decided to test this possibility by noting the effect on the light response of impregnating the substrate with the smell of the host plant. The technique and apparatus were the same as those used for the investigation of the threshold of the light response. The only modification was the use of a 20 cm. square sheet of black paper (F, Figure 7) as a renewable starting point. This paper was laid at the centre of the arena. When the crawler reached the edge of this paper, the experiment was terminated. It was found that the paper was of sufficient size for a track to be made which could be analysed.

A circle was drawn with centre the middle of each of these sheets of paper. These circular areas, of diameter 5 cm. were in some cases treated with the smell of orange and in others left untreated as controls. Orange smell was applied as follows:- the peel of an orange was abraded and this abraded part rubbed on the circular area. Next, a deep cut was made in the orange and the juice rubbed on to the arena. During this treatment the rest of the piece of paper was protected by a mask with a circular hole. The treated area was then allowed to dry. Applied in this way, the smell of orange was easily detectable on the paper by myself.

After each experiment, the brush used to transfer the crawler was rinsed in absolute alcohol and left to dry. This was to avoid any contamination of the clean circular areas by the brush while the crawler was being transferred.

The light intensity chosen was 13 m.c. being one at which not all crawlers respond to the light source. It was thought that any inhibiting effect of smell on the light response would be most likely to show under these conditions. The two criteria already described were used in deciding whether a crawler had responded or not. The temperature was kept constant at 25.5°C. An electric torch was used as before to observe and plot the positions of the crawlers.

Tracks were plotted of the paths of 41 crawlers. These were, as before, only maps of the successive positions of crawlers at minute intervals. Of these crawlers, 19 were released on the treated paper and the remainder on the untreated paper. The results obtained are summarised in Table XI. All those which were found to have responded to the light by either criterion were found also to have shown a displacement towards it.

There is no statistical difference between the numbers responding in the experimental and control groups (Chi^2 less than 1). Thus it appears that the smell does not prevent the response to light. The smell of the host plant was thus unlikely to have been the cause of the lack of response to light observed by Carnegie.

Another possible reason for these differing results might be that a change in response to light occurs as the crawler grows older. The experiments described above were carried out with crawlers freshly removed from beneath the female Scale, while the results of Carnegie may depend largely on the response of crawlers at the end of the wandering period.

The conclusions of Carnegie, however, appear to depend on a series of negative results. His controls, consisting of two oranges both illuminated, also showed that no migration had occurred. An occasional crawler reached the second orange, but this animal was the exception. It appears that these results show not that crawlers are indifferent to light, but that crawlers do not migrate from one orange to another. Some reason for this lack of migration might be found. It seems likely that the difference in illumination of the two oranges would break down at the point of contact. A crawler which had reached this point would show no special tendency to move on to the second orange, because the only part of it visible would be under the same conditions of illumination as the first. Moreover, the point ot contact of the two oranges might actually serve as a trap for crawlers, which tend to settle in any inequality or irregularity of the substrate (See Part IV).

No explanation can be offered for Ebeling's statement since no description of his method is given.

<u>The small choice chamber</u>

The slow and hesitating movement and rapid settling of crawlers in small choice chambers (see "Light") suggested some experiments to investigate why this occurred. This behaviour does not occur as soon on an open arena of the type used in the light experiments.

It was thought that the accumulated smell of an animal confined to a relatively small area of substrate might possibly be the cause. Some experiments were carried out to test whether this was so.

Scale insects were scraped off some leaves and left overnight under a watchglass on some black paper. The Scale insects were then brushed off and a crawler released in the centre of the treated area. Five experiments were carried out, together with five controls on untreated paper. It was found that there was no tendency for crawlers to remain or settle on the treated areas. Both experimental and control crawlers walked immediately towards the light used for the observations. Two further experiments were carried out where crawlers were confined overnight on the paper. These produced similar results to the first experiments. Next, crawlers and Scale Insects were crushed on the surface of the paper. In three experiments, no tendency was shown by crawlers to walk slowly on the treated area or remain within it.

These results and the observation that the first crawler in a newly made choice chamber starts walking slowly almost immediately, i.e. before any smell can have accumulated, make it seem unlikely that smell is the factor concerned.

Changes in temperature and relative humidity due to the heating effect of the microscope light used, might have been responsible for the slow walking and rapid settling. However, when the light was passed through a heat filter, consisting of a small jar full of water, these effects still persisted, and so could not be ascribed to these causes. Nor is the effect due to the vertical light, as the average speed of movement of four crawlers walking in an open area in vertical light was 19mm./min. at 25.5°C. which agrees closely with the values for crawlers in directional light.

Air movement is a factor present on a large open arena which is not present in a small closed choice chamber. It was decided to investigate whether lack of air movement might be the cause of the unusual behaviour shown in the small choice chambers.

Crawlers were released on two arenas on a sheet of black paper, one covered by a watch glass, and one open to the air. The arenas consisted of two circular areas surrounded by paraffin wax. The wax stopped or delayed the escape of crawlers. Observations were made under a binocular microscope. The numbers of crawlers flattened were noted at 10-minute intervals. After each observation, straying crawlers were returned to the arena. Flattened crawlers were not disturbed.

In four experiments a total of 84 crawlers was used. These were obtained from under the female Scales in the usual way. As they were removed from the leaves, the crawlers were placed alternately in the two arenas, so as to ensure that similar samples were obtained. This took about 10 min. No special draught was played on the arena; the only air movement was due to air circulation in the room. A window was left open to ensure that some air movement did occur.

The temperature varied between 18°C. and 26°C., but a variation of 2.5°C. was the greatest to occur during any single experiment.

The results of the experiments are shown in Figure 19. It can be seen that the general appearance of the graphs indicates that the proportion flattened may be higher in crawlers in the enclosed arena. An analysis of variance was carried out on the values for the first two hours from all four experiments.

The analysis showed that the difference is significant (p less than 0.001). Thus a crawler is more likely to flatten in a closed arena than in an open one. The magnitude of this effect, however, does not appear to be great enough to account for the very decided slowness

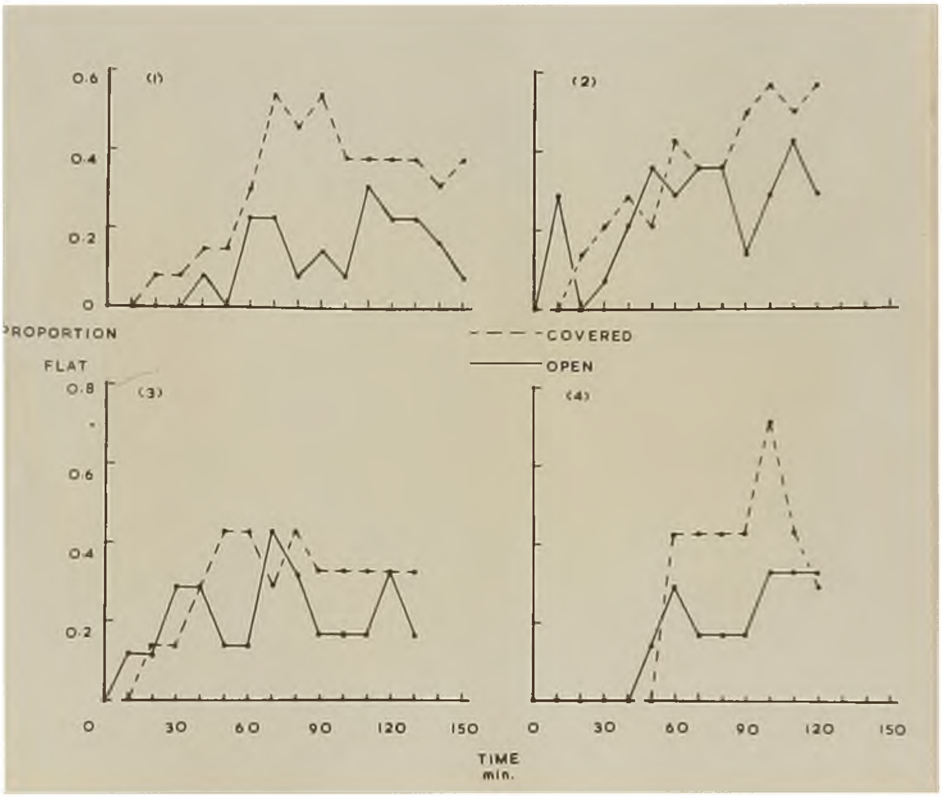


Figure 19. Proportion of crawlers flattened at intervals in open and closed arenas.

and stopping which occurs in the small choice chambers. It is conceivable that it might be greater in the choice chamber, which had a diameter of 1 cm. than under a watchglass of diameter 6.4 cm. This, it is felt, would however still be an unsatisfactory explanation.

If a crawler is put into a small choice chamber and watched under a binocular microscope, a clue to the possible reason for the early slow walking and flattening is obtained. A crawler walking around the perimeter of the floor keeps coming into contact with the wall. Each time this occurs, the crawler hesitates and may stop and butt the wall in an apparent attempt to go underneath it. These stops may be momentary or last for several minutes. The same occurs if the crawler is walking on the wall and comes into contact with either floor or coverslip. Mechanical interruption of locomotion causes probing in _Aphis fabae_ Scop. (Ibbotson and Kennedy, 1959). A similar phenomenon may occur in the crawlers of _L. beckii_, as interference with locomotion causes more rapid settling (see Part IV (ii)). The failure of the choice chambers may thus be due to their action as releasers of the settling behaviour pattern. Some experiments were carried out to test this hypothesis.

The first apparatus tried was a small glass bulb; this had none of the angles of the small choice chamber, yet still provided an enclosed space. If the above hypothesis were true, crawlers would be expected to walk for long periods in the bulb without slowing or stopping. Crawlers with anal filaments amputated were used, so that they would be able to walk more easily on the glass. It was found, however, that the speed of these crawlers was still low, about 4 mm./min., and that they walked with some difficulty in spite of the absence of the anal filaments. A crawler walking on a smooth surface, which interfered with locomotion, would be expected both to walk slowly and, because of the interference, to stop sooner. This appeared to be so, but the difficulty in distinguishing between normal slow walking and slow walking due to the smooth surface, made measurements of the time until the commencement of the former impossible.

An open arena which supplied some angles was next tried. Folded black paper was considered, but the folding caused a "wooliness" of the surface which obstructed the crawler. The next arena tried was a thin, flat 5 cm. square piece of wood with small grooves in the surface. This was prepared as follows. Two pieces of wood were smoothed on both surfaces with fine emery cloth. One was used as control, while grooves approximately 1 mm. deep and 1 mm. wide, and 4 mm. apart were filed in both sides of the second. A second set of grooves was then filed at right-angles to the first. The grooves were then smoothed with fine emery cloth.

Crawlers were released and watched on the two pieces of wood. The time until the first slow walking (Stage II behaviour of Part IV) of at least 5 min. duration, was noted. Experiments were terminated after 30 min. The results are given in Table XII.

It can be seen that the grooves cause slow walking in some cases, yet fail to do so in others. This failure may be due to the fact that the grooves were unavoidably rounded in cross section, and not square. The obstruction caused to locomotion by a rounded groove is less than that caused by one with vertical walls and horizontal floor. The condition in the choice chamber approximates the latter. It was decided to approach the problem from another angle.

Table XII. Time in minutes from release until a 5-min. long period of Stage II behaviour

Released on wooden platform with grooves	Released on smooth wooden platform
13	30 +
9	30 +
30 +	30 +
3	
30 +	

Table XIII. Time in minutes from release until a 5-min. long period of Stage II behaviour

Bakelite ring chamber with roof	Bakelite ring chamber without roof
9	19
28	10
5	23
5	8
9	14
21	30 +
27	

Further experiments were carried out using the original bakelite ring choice chamber, but without the roof. This gave an open arena without loss of the floor to wall angle. The complete choice chamber was used for control experiments. The time until at least 5 min. of slow walking was noted, as before. Crawlers which reached the rim of the open chamber were replaced on the floor. The results are shown in Table XIII.

It can be seen that slow walking occurs even in the open choice chamber; it thus appears that its probable cause is the action of the angle between walls and roof and floor, as a releaser of settling behaviour. There is, however, the remaining possibility that the bakelite surface itself, or the paint surface in the glass choice chamber was the cause of the slow walking.

This was tested with an open choice chamber made entirely of black paper. This chamber consisted of a shallow cylinder of black paper with a circular black paper floor forced into it. Seven crawlers were watched individually in this chamber. The results are given in Table XIV.

In this case also the slow walking soon occurred. It appears, therefore, that the factor responsible for the failure of the small choice chambers must be the action by the angle between walls, and floor and roof, as a releaser of settling behaviour.

Table XIV. Time in minutes from release until a 5-min. long period of Stage II behaviour in all-paper chamber.

30 +
20
6
16
18
9
3

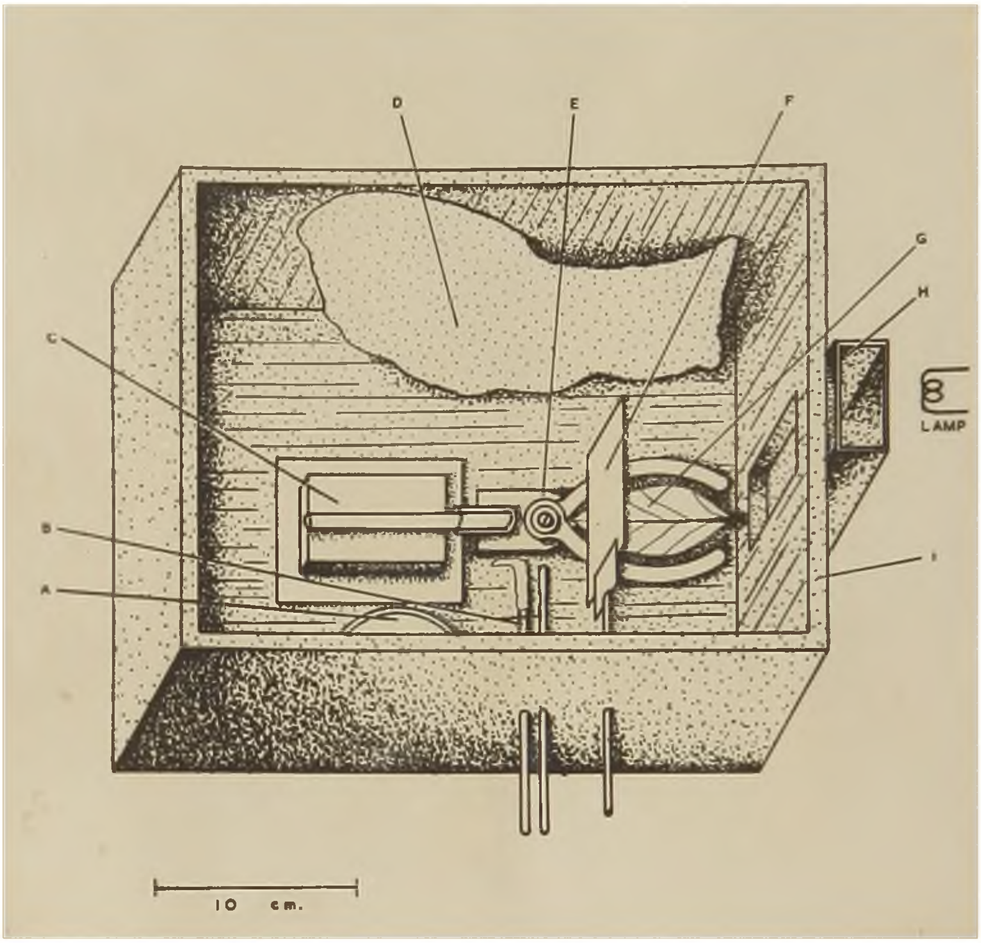


Figure 20. The apparatus used in the investigation of factors influencing settling of crawlers. A, "Edney" paper hygrometer; B, wet and dry bulb thermometers; C, moving element; D, polythene "glove"; E, perspex cradle; F, mirror; G, arena leaf; H, heat filter of museum jar full of water; I, wooden box.

fine control and the lack of vibration permitted the observation of naturally walking crawlers at magnifications of up to 100X.

The apparatus was enclosed in a wooden box (I) from which the lid had been removed. It was lined inside with black paper. The box was covered by a sheet of polythene (not shown) which preserved a stable environment inside the box and also kept the observer's breathing from the crawlers. A hole was cut in the polythene, and the cut edge tied around the objective of the microscope. A hole was cut in the side of the box so that the movable element could be manipulated. A glove (D) consisting of a polythene bag into which the observer's hand could be inserted, was fastened over the hole. This made it possible to move the leaf without disturbing the conditions in the box.

Light from a Watson microscope lamp (after being filtered through water in a museum jar (H))entered the box through a hole cut in its side. The jar also closed up the hole. The horizontal beam of light was reflected downwards on to the surface of the leaf by means of a small mirror (F), made from a microscopical slide. The light obtained in this way fell on the leaf at 15° from the vertical. This was done to avoid the possibility of the crawlers responding to a lateral light source. The mirror was held in a retort clamp just below the binocular microscope (not shown).

Two small holes in the box permitted the introduction of the bulbs of wet and dry bulb thermometers (B). These were used periodically to re-calibrate an "Edney" paper hygrometer used for measuring relative humidity. The relative humidity was kept at 70 - 80% by means of a tray full of water under the fan of the temperature control direction, occurs.

After a short period of such behaviour the crawler often comes to a complete halt, making only slight pawing movements (Stage IV). It is thought that penetration of the substrate by the rostrum begins at this time. Stage V is the final one: the antennae are folded back against the body and at the same time the crawler flattens against the surface of the leaf. Then the legs are withdrawn under the body, and the crawler remains motionless, having settled. The insertion of the beak progresses rapidly, as crawlers removed after 20 min. often have the beak quite deeply inserted. Within 50 min. to 3 hr. the characteristic wax threads begin to appear, short at first, but lengthening quite rapidly. Very few crawlers were watched for longer than this, but the scale-covering in some cases was seen to be partly formed within 24 hr.

This sequence of behaviour pattern is not only shown by crawlers artificially removed from the parent Scale, but has twice been observed in individuals which emerged naturally from beneath a female: there is therefore good reason to believe that this represents the natural sequence of events, and that it is not affected by removing the crawlers from their parents for the purpose of experiment.

The sequence of events described above is the most usual one, but in many cases it may be broken at some point, with reversion to an earlier stage. Stage II especially may occur a number of times, with reversion to Stage I between. It was found, however, that the folding back of the antennae was a reliable indication that settling would occur: in only a very few cases was there a reversion once this had occurred. Even after flattening, however, the crawler was sometimes seen to move off again. Here the reversion was usually to Stage III, with a new flattening following soon, in almost the same place. A reversion to fast walking was seen to occur, even at this stage. Crawlers were also seen behaving in a manner which suggested strongly that the beak could be retracted, in the first stage of insertion at least. A strained attitude was adopted by such a crawler, with legs pawing actively until, with a sudden jerk, just as if the beak had suddenly pulled free, the animal moved off. The great majority of crawlers do not move after the first flattening.

Crawlers settle mainly along the midrib, the next most frequent place being the hollow under the edge of the leaf. This appears to be due to the fact that Stage II behaviour is often elicited by an obstruction such as the midrib or the edge of the leaf. It was further noticed that patches of dust were especially effective for producing this behaviour, as also were other scale insects.

(ii) The effect of dust

Some experiments were carried out to test whether dust did, in fact, cause crawlers to settle sooner. The apparatus shown in Figure 20 was used. Crawlers were released on cleaned or dusty valencia orange leaves. The clean leaves had been wiped with a soft cloth. Dust was applied to the other leaves as follows. The leaf was first cleaned with a soft cloth, and then soil from a sample collected at the start of the experiments was poured over the leaf. Excess dust was then blown off, leaving only that which adhered firmly to the leaf.

Crawlers were obtained from under the female scale-covering in the usual manner. Only one crawler was taken from a leaf, since a leaf might have been subjected to conditions producing abnormal behaviour in the ensuing crawlers. Each crawler was watched either until the time it first flattened, or until it finally flattened and produced threads. These two intervals will be referred to below as first flattening and final settling times. The production of wax threads was regarded as a sign that final settling had occurred, since no crawler was ever seen to move off once this had begun. All readings of over 200 min. were discarded (see (iv)). Sixty crawlers settled successfully on dusty leaves and thirteen on clean leaves.

The mean values for the final settling were 63.8 ± 3.9 min. on dusty leaves and 91.0 ± 8.8 min. on clean leaves. The corresponding means for the first flattening were 57.0 ± 3.5 min. and 81.0 ± 7.9 min.

These means are both significantly different (p less than 0.01 in both cases). Thus the effect of dust is to cause the crawler to settle sooner, in agreement with the subjective impression already mentioned, that crawlers were stimulated to pass to Stage II of the settling behaviour pattern by patches of dust. Ibbotson and Kennedy (1959) found that any obstruction that interrupted locomotion in *Aphis fabae* Scop. caused probing to occur. It is not surprising that a somewhat similar state of affairs is found in a crawler of a Coccid.

(iii) The effect of numbers

(iiia) Initial experiments

Ibbotson and Kennedy (1959) further found that visual stimulation provided by other aphids or models would cause a walking aphid to stop and probe. Some experiments were carried out to test whether there might be a similar effect in crawlers of L. beckii. Crawlers would be expected to settle sooner in groups than when isolated. These experiments were carried out in the same manner as those described above, except that groups of eight crawlers were released on a leaf at once. Controls were conducted with isolated crawlers. Dusty orange leaves were used so that the experiment would occupy less time. In the group experiments it was necessary to remove each crawler as soon as it flattened with antennae back, in order to prevent any that might start walking again from confusing results. The control crawlers also, were timed to the first flattening. Each group of crawlers was timed from the moment when half of the crawlers had been transferred to the leaf. 28 of the grouped and 24 of the isolated ones flattened.

The mean time of walking of the grouped crawlers until flattening was 34.1 ± 3.1 min. and that for the isolated crawlers 46.0 ± 4.3 min. All readings of over 90 min. were discarded (see iv). Since there were only two readings over 90 min. in this case, both from isolated crawlers, the effect of excluding them was to lessen the gap between the means. In spite of this, the difference between the means was just significant (p less than 0.05). It thus appeared that groups of crawlers settled more rapidly than isolated crawlers.

This effect may be expressed in another way. A graph, Figure 21, was drawn to show the time at which flattening occurred in all the grouped experiments which had been carried out (76), and in 60 experiments with isolated crawlers. It can be seen that the results from individuals and groups differ considerably. (A) shows the distribution of the individual results and (B) the distribution of the group results. Both sets of values are those for the first flattening. It was found, however, that the results of the isolated experiments taken until the final settling, had the same type of distribution as the isolated first flattening results.

It can be seen from Figure 21 that, while the distribution of the results from isolated crawlers was 'normal', that from grouped crawlers was decidedly skew. It was thought that this skew distribution might be due to some mutual stimulation causing numbers of crawlers to settle sooner than they would have done if isolated. As a result, the mean of the group results is not a good reflection of the mode (mean, 44.2 ± 4.2 min.) This skewness results, however, in a reduction of the difference between the means of the grouped and the isolated results and will therefore not invalidate the conclusion reached that there is a statistically significant difference between the flattening times of the isolated and grouped individuals.

(iiib) Experiments to investigate mechanism of the group effect

To study the mechanism of this suspected mutual stimulation, groups of crawlers were left undisturbed on clean leaves for 1 hr. when the numbers which had flattened were noted. Each leaf was impaled on a long pin, which in turn was supported by a base consisting of a piece of cork. This precaution was necessary because crawlers walked off a leaf unless it was suspended above the substratum.

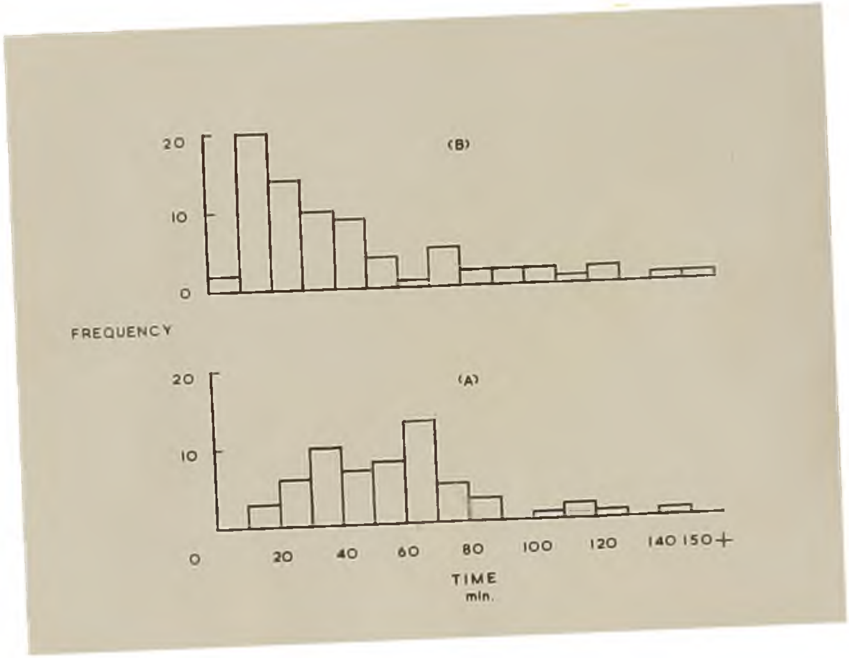


Figure 21. The distribution of first flattening times of crawlers released (A) individually, and (B) in groups, on dusty orange leaves.

The advantage of this method is that it permits experiments to be carried out in darkness, since continuous observation is not necessary. If stimulation were visual, the group effect would be expected to disappear in darkness. Experiments with sand grains and dead crawlers as substitutes were envisaged for further investigation.

Experiment 1

Sixty crawlers were released in the light, and ninety-six in darkness. At the end of the one-hour period, 23% had settled in the light, and 33% in darkness, indicating that the group effect was not visual. At this point, however, it was noticed that control experiments with isolated crawlers were yielding anomalous results. Fifty-five isolated crawlers had been released in the light and thirty in darkness. At the end of 1 hr. 27% had flattened in the light, and 37% in darkness. Thus a comparison of the grouped results from light and darkness was meaningless, since in this case settling times for each were no shorter than those of the corresponding isolated crawlers.

Experiment 2.

The experiments were then repeated on dusty leaves since the original experiments had given positive results on dusty leaves. The group effect was again not demonstrable. In the light 52% of 47 isolated crawlers, and 45% of 20 grouped crawlers had flattened, while in darkness 54% of 24 isolated crawlers and 45% of 20 grouped crawlers had flattened. Thus the absence of dust on the leaves in the previous experiments was not the reason for the failure to

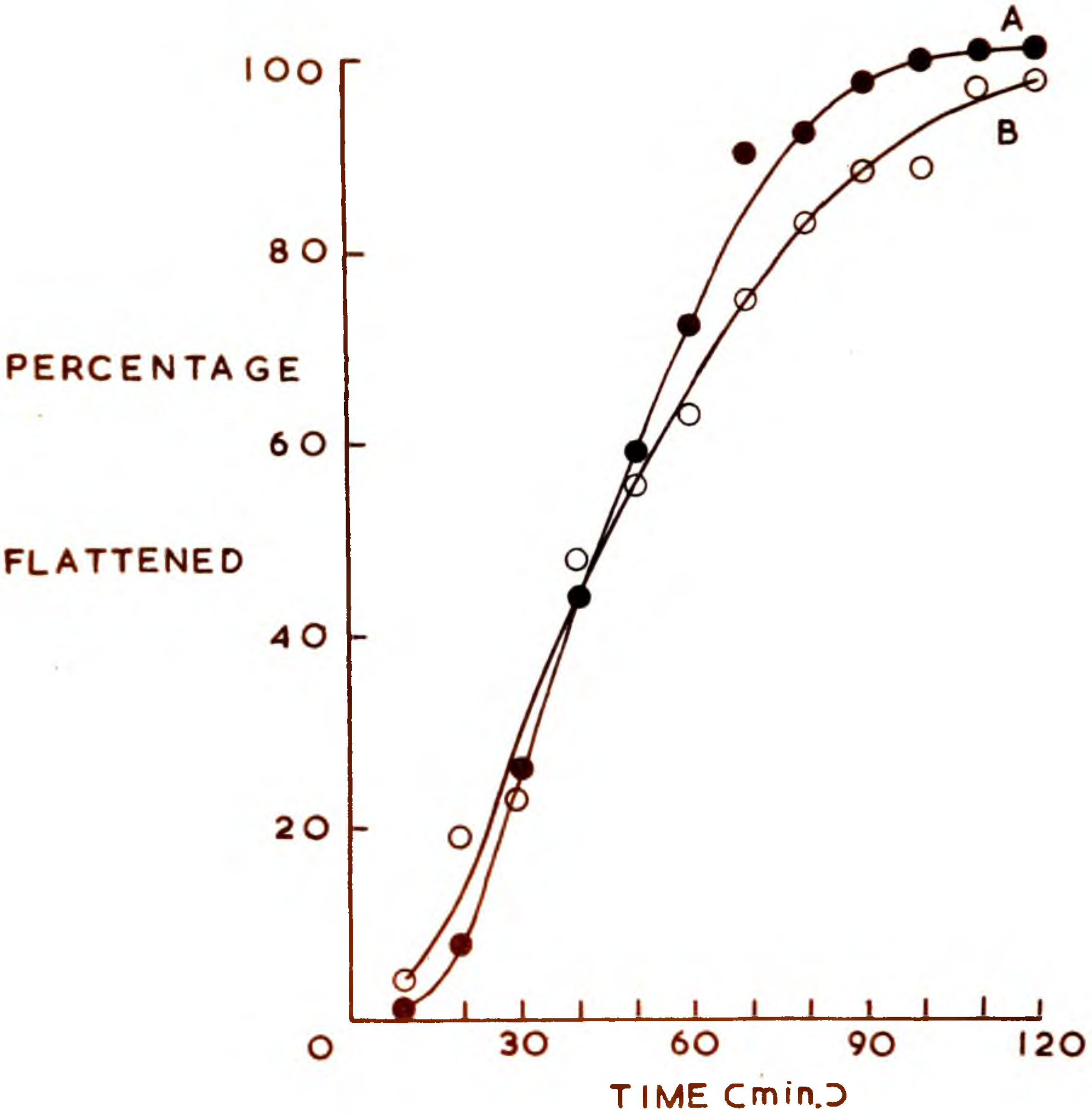


Fig 22. Crawlers flattened on orange leaves at 10-min. intervals. A. groups of crawlers.
B. isolated crawlers.

demonstrate a group effect.

(iiic) Reappraisal of group effect

Experiment 1

It was decided to test again for the group effect, using the new technique. A possibility existed that the one-hour period was unsuitable. The cumulative curves of the percentage of crawlers flattened at intervals in groups and alone might be expected from Figure 21 to be approximately sigmoid. The period during which the curves are widely separated might therefore be small and the choice of the period after which inspection occurs would then be critical.

Some experiments were carried out in an attempt to establish whether a favourable period existed. Isolated crawlers and groups of eight crawlers were released on dusty leaves as before, except that the number flattened was counted every 10 min. for 2 hr. Each leaf was cut in half, and as far as possible, a single individual and a group were released on the halves of each leaf. Ten experiments were carried out in this way, and then a further 16 individual experiments. It was hoped that these paired experiments would reduce any possible effects resulting from characteristics of individual leaves. The numbers of crawlers dwindled during the experiments from 80 and 26 to 50 and 24, due to crawlers falling from the leaves on to the cork and the bench surface. The percentage flattened was calculated from the actual total number of crawlers present on the leaf at the given time. The temperature varied from 21°C. to 25°C, while the relative humidity was held at 50-60% by means of an atomiser.

The results are shown in Figure 22. It can be seen that the curves are not widely separated at any point, and that the difference is not at any time large enough for the type of experiment attempted above. The mean walking times calculated from these results were 45 min. for groups and 48 min. for individual crawlers. This differences does not approach that observed in the initial experiments. No group effect was thus demonstrated.

Experiment 2

It was considered possible that the lower temperature and relative humidity, and possibly also some chemo-receptive effect resulting from the cut leaves, might have been responsible for the absence of the group effect in these experiments. In order to investigate this, the previous experiment was repeated under the same conditions as the initial experiments which had given the positive result. The relative humidity was held at 70-80% and the temperature at 28°C. Intact leaves were used. In these experiments, pieces of black paper were pushed on to the pins below the leaves. Many crawlers which fell off the leaves were then visible against the black background and could be replaced on the leaves. Numbers fell from 27 and 61 for isolated and grouped crawlers respectively to 26 and 56, during the 2 hr. of the experiment.

The cumulative frequency curves were again very close together and similar to those in Figure 22. The mean walking times calculated from these results were 50 min. for grouped crawlers and 53 min. for isolated crawlers. Thus a group effect is again absent; neither temperature, relative humidity, nor leaf smell having been responsible for its absence.

Experiment 3.

A further possibility was that some factor such as mutual interference might counteract the group effect once a critical number of crawlers per group is exceeded. Experiments were thus carried out with 2, 4 and 8 crawlers per group. A pair and a group of 4 or 8 crawlers were released on the halves of each leaf. Numbers fell from 64 and 124, for groups of 4 and 8 respectively, to 56 and 102 after 2 hr. No crawler of the 60 released two on a leaf was lost.

The results are shown in Figure 23. There appears to be no difference comparable with that noted in the initial experiments. The mean walking times for groups of 2, 4 and 8 crawlers were 51 min., 49 min. and 54 min. respectively. It can further be seen (Figures 22 and 23) that the distributions do not differ, as was the case in the initial experiments (Figure 21). All these latter distributions plotted in the same manner as Figure 21, are approximately normal.

It appears therefore, that the group effect noted in the initial experiments might have been an artifact. There is also the possibility of an effect so small that very large numbers of experiments would be required to demonstrate it.

(iv) Variability of settling time

Another point which emerges from a scrutiny of Figure 21 is that in both cases a small number of crawlers walked for much longer than the rest. It was for this reason that it was decided to discard all results over 90 min. In the earlier experiments comparing dusty and clean leaves, a limit of 200 min. has been used. This figure was arrived at from a scrutiny of results of experiments

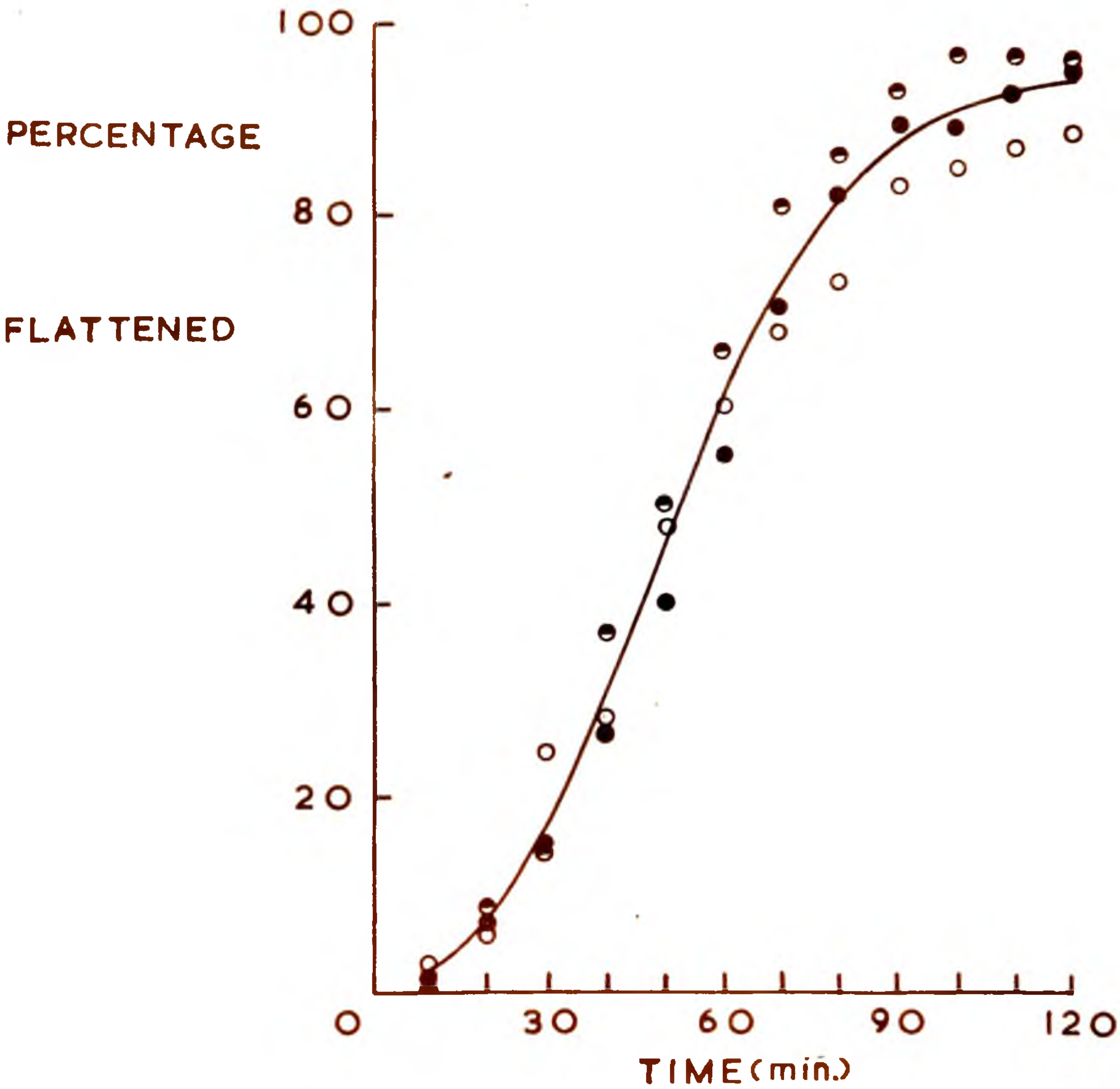


Fig 23. Crawlers flattened on orange leaves at 10-min. intervals. groups of: 2.. ○
4.. ◓
8.. ●

designed to check whether storage of the infested leaves in polythene bags had any effect on the behaviour of the crawlers.

The results of 55 experiments on dusty valencia orange leaves were divided into groups according to the time the infested leaves had been stored. The results for final settling, analysed in this way, are given in Table XV.

The above results plotted in graphical form are shown in Figure 24. It can be seen that three values are much greater than the rest. These high values may be excluded by a limit set at 200 min. After these atypically high values are excluded the results may be tested for any effect of storage time upon the behaviour of the crawlers. The correlation coefficient was calculated using both means and individual values. In each case, the value of the correlation coefficient corresponded to a probability of just more than 0.1, indicating that, at least in this particular series of experiments, storage had no effect on the behaviour of the crawlers.

It may be that the atypical values arise from damage to crawlers in transferring them to the arena. While such a point is open to an experimental check, it would be laborious and since it is the typical behaviour of crawlers which is primarily of interest, the question was not followed. Nevertheless, even after the exclusion of atypically high results, the results are very highly variable. Since storage cannot be a factor responsible for this high variability, it was decided to test whether the time of day at which the experiment was carried out, might have any effect on results.

The results obtained were divided into four groups, according

Table XV. Final settling times in minutes of crawlers after various periods of storage of source leaves.

Day							
1	2	3	4	5	6	7	8
70	33	107	23	76	86	82	41
40	70	68	58	67	82	(212)	80
37	71	45	58	66		86	68
45	(205)	31	25	89			
32	40	77	44				
75	44	(287)	113				
51	64		50				
133	134						
21	18						
17	101						
58	68						
69	97						
19							
37							
59							
36							
68							
49							
51	67	66	53	75	84	84	63 *

* Means (bracketed values excluded).

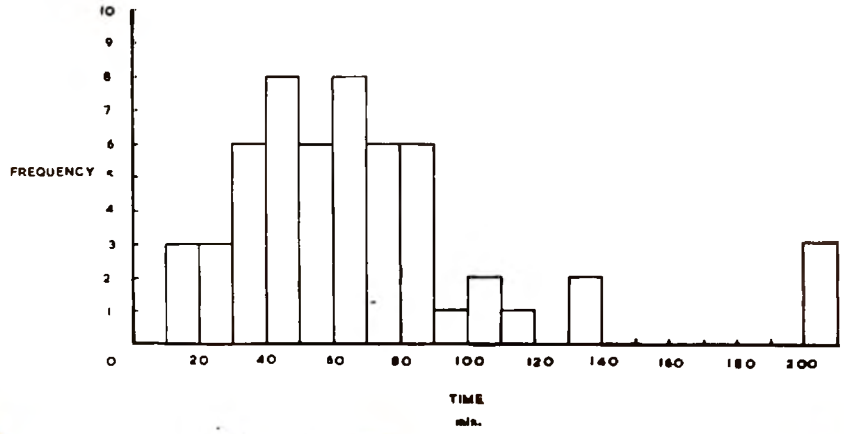


Figure 24. Distribution of final settling times of crawlers released individually on dusty orange leaves.

to the time of day at which an experiment was carried out; morning, 10 a.m. until 1 p.m.; early afternoon, 1 p.m. until 4 p.m.; late afternoon, 4 p.m. until 7 p.m. and night from 7 p.m. until midnight. Where overlap occurred, the reading was placed in the category in which the greater part of the walking had taken place. The readings from 58 experiments were treated in this way. The results are given in Table XVI. Both first and last flattenings are given in each category. This table also gives an idea of how many crawlers move off again after a flattening. Eight out of the 58 can be seen to have done so.

An analysis of variance showed that no significant difference occurs between the results from the four periods (p greater than 0.05 in both cases). It is possible that a small effect might not be detected by doing a statistical test on a small sample such as that above. This, however, is of no importance in the present case, as such a small difference could not give rise to such great variability as that found in these experiments.

Thus the reason for the great variability remains obscure.

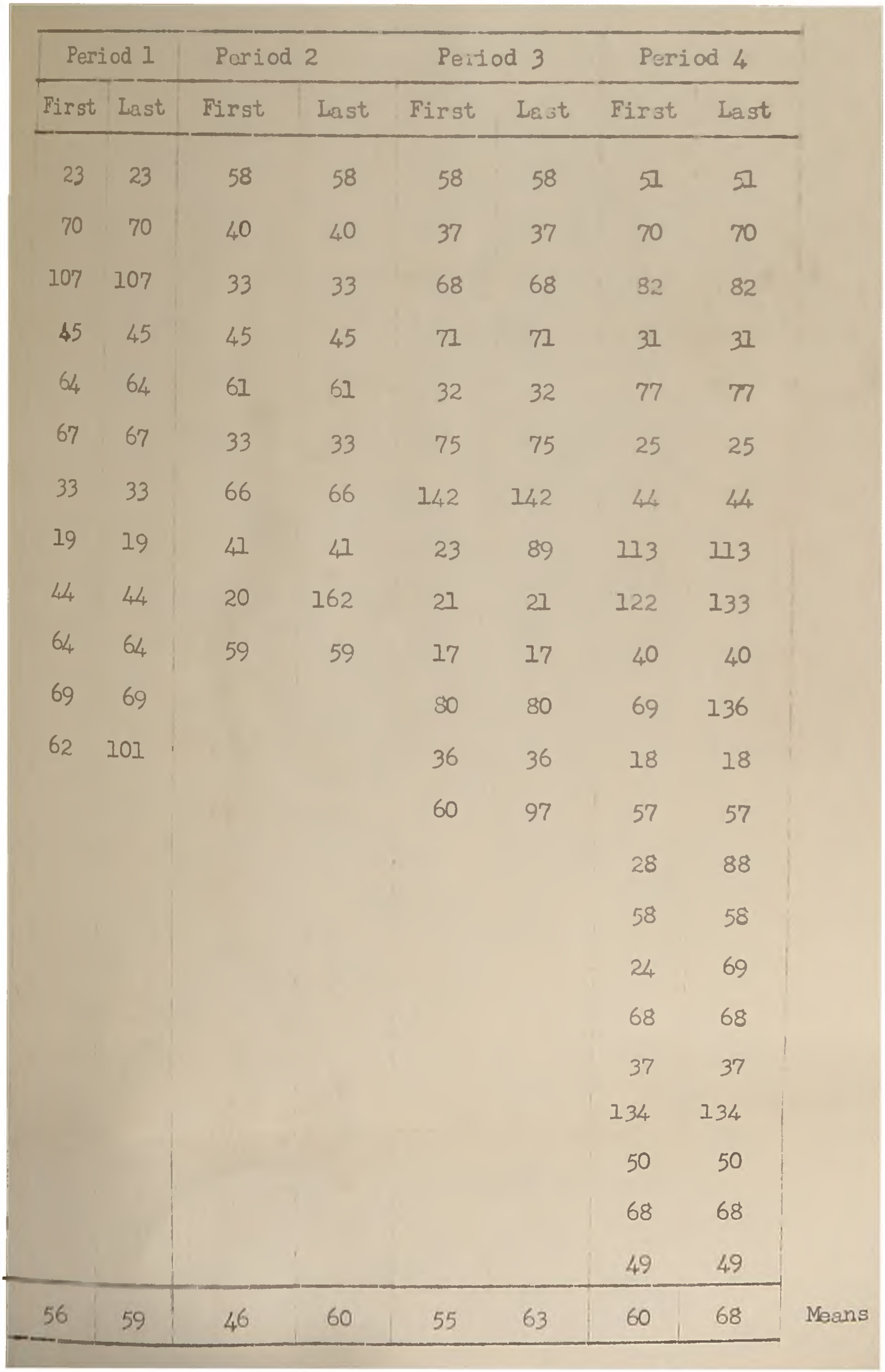
(v) The effect of the host plant

The next experiments concerned the effect of different varieties of citrus on the readiness with which the crawler settles. Navel and valencia oranges and lemon were those tested.

The same technique was employed. Single crawlers were released on the three types of leaves and the times from release until first and last flattenings noted.

Eight experiments were conducted on navel orange leaves

Table XVI. First flattening, and last settling times in minutes of crawlers during different periods of the day.



Period 1		Period 2		Period 3		Period 4		
First	Last	First	Last	First	Last	First	Last	
23	23	58	58	58	58	51	51	
70	70	40	40	37	37	70	70	
107	107	33	33	68	68	82	82	
45	45	45	45	71	71	31	31	
64	64	61	61	32	32	77	77	
67	67	33	33	75	75	25	25	
33	33	66	66	142	142	44	44	
19	19	41	41	23	89	113	113	
44	44	20	162	21	21	122	133	
64	64	59	59	17	17	40	40	
69	69			80	80	69	136	
62	101			36	36	18	18	
				60	97	57	57	
						28	88	
						58	58	
						24	69	
						68	68	
						37	37	
						134	134	
						50	50	
						68	68	
						49	49	
56	59	46	60	55	63	60	68	Means

and 52 on valencia. The mean times to the first flattening were 60.9 ± 13.0 min. and 56.4 ± 3.6 min. on navel and valencia leaves respectively. The corresponding times for the final settling were 76.8 ± 15.1 min. and 61.8 ± 3.9 min. Statistical tests failed to establish that the difference was significant. However, with the prevailing high variability of results only very large differences would be shown up by such a small sample of navel leaves.

For this reason, more experiments were carried out, using groups of crawlers. Thirty crawlers were released on navel and 95 on valencia leaves. The means (first flattening only) were 32.7 ± 3.1 min. for navel and 34.0 ± 1.9 min. for valencia leaves. There was, as might be expected from the closeness of the means, no statistical difference.

The results from single crawlers for navel and valencia orange leaves were summed and compared with 14 results from single crawlers walking on lemon leaves. The first flattening mean time for orange leaves was 57.0 ± 3.5 min. and for lemon 33.4 ± 3.5 min. The corresponding means for the last settling were 63.8 ± 3.6 min. and 36.6 ± 4.6 min. The difference between the means is significant (p less than 0.001 for both).

It appears from these results that crawlers of <u>L. beckii</u> flatten sooner on lemon leaves than on orange. If this is taken to indicate that lemon leaves are "preferred", the results are in conflict with field observations, which show that <u>L. beckii</u> infestations are less extensive on lemon than on orange or grapefruit trees (Carnegie, 1955). However, as already seen, chemoreception may play little part in the readiness with which a crawler settles (Part III, Light, (iv)). The explanation of this apparent conflict may be that no response to the leaf, as such, occurs, but that the crawler responds merely to the nature of the actual surface upon which it is walking. The importance of the surface has already been seen in the experiments on the effect of dust on a leaf (ii). The surface of a lemon leaf is rougher than that of an orange leaf. It is possible that the more rapid settling of crawlers on lemon leaves than orange may be due to this factor.

(vi) Other experiments

Other experiments which were carried out early in the series were aimed at discovering the effects of factors such as light intensity, temperature, relative humidity and age of the arena leaf on the walking time. Unfortunately, all these experiments were later discovered to be inconclusive due to the small size of the samples. The negative results obtained in these experiments might thus reflect a lack of difference between the means concerned, or simply that the variability was too high for any but a large difference to be shown statistically in such samples.

It was possible to estimate roughly the number of experiments which would be required so that the observed differences would become statistically significant. These were found to be large, i.e. the second part of (a), over 100 more group results, and (b), over 20 more individual experiments. It was decided to leave the experiments as they were since too much time would be required to produce conclusive results. On the other hand, however, the results do show that no large difference in walking time occurs, such as that caused by dust on the leaf, of lemon and orange leaves. The experiments are thus described below.

(a) Light Intensity.

Early experiments were conducted with the microscope lamp kept at low intensity. Seven experiments were then conducted with the lamp at maximum intensity and the results compared with the original 53 experiments.

The first flattening mean times were 56.9 ± 3.9 min. and 57.4 ± 4.2 min. for low and high intensities respectively, while the final settling mean times were 63.2 ± 4.2 min. and 68.3 ± 8.8 min. It seems very probable that further experiments would fail to establish a significant difference between the means.

Another series was designed to test the effect of light conditions prior to the emergence of the crawler. It is not known how much light reaches crawlers under the scale-covering. The rear edge of the covering curls up, however, and light probably enters here. These experiments were carried out by the group method. Some infested leaves were stored in continuous light for 48 hr. at 30°C. The readiness with which crawlers from these leaves flattened was compared with that of crawlers from freshly collected leaves. The freshly collected leaves had been exposed to normal light and dark periods, and also to lower and more variable temperatures.

The mean walking time of 10 crawlers from continuously illuminated leaves was 48.5 ± 14.2 min. and that of 14 crawlers from fresh leaves 43.4 ± 9.3 min.

(b) Age of leaf

In this series, dark green mature leaves and light green young leaves were used.

The mean times for 10 crawlers on mature leaves and 14 on young leaves were, for the first flattening, 51.6 ± 8.6 min. and 64.4 ± 7.3 min. respectively. The final settling mean times were 51.6 ± 8.6 min., no crawlers moving off after flattening, and 65.1 ± 7.8 min. for mature and young leaves respectively.

These differences are large, and more experiments might show that the difference is significant. This would be in agreement with the field observations of Bodenheimer (1951) that L. beckii is not found on new growth of citrus trees.

(c) Temperature

A series of experiments was conducted to test the effect of temperature on the readiness of crawlers to settle. Prior to the experiments, all crawlers had been kept in a constant temperature room at 30°C. Experiments were conducted at 30°C. and 34°C. using the group method. Another series was carried out to test whether the temperature at which the crawler had been kept before the experiment exerted any effect on readiness to settle. Some infested leaves were kept at 25°C. and others at 30°C. for 48 hr.

It can be seen that each series of experiments has one treatment in common, i.e. the crawlers kept at 30°C. and released at the same temperature. Under these conditions the mean walking time was 43.4 ± 9.3 min for 14 crawlers.

The mean walking time for the seven crawlers kept at 30°C. and walking at 34°C. was 56.7 ± 13.3 min. With a larger number of experiments it seems that temperature may be found to affect directly the time for which the crawler walks.

However, the temperature at which the crawler was kept before the experiment did not appear to affect the walking time, since the mean walking time of seven crawlers released at 30°C. but previousl kept at 25°C. was 43.8 ± 10.9 min.

(d) Age of tree

During the experiments on the effect of the variety of citrus leaf on the walking time of crawlers, the lemon leaves were obtained from two different trees. The one was small, about six years old, and the other an old tree of at least 20 years. The results obtained were divided into two groups in order to test whether the age of the tree had any effect on the readiness with which a crawler settled on its leaves.

Seven crawlers were released on leaves from each tree. The first flattening mean times for leaves from the old and young trees respectively were 36.1 ± 5.0 min. and 30.7 ± 5.2 min. The corresponding values for the final settling were 36.1 ± 5.0 min. and 37.1 ± 8.3 min.

It is unlikely that any difference does exist. This concludes the experiments on factors affecting settling in crawlers of <u>L. beckii.</u>

V. Conclusion

It is now possible to survey the foregoing laboratory experiments and to make an attempt to correlate the results with field observations and the mode of life of L. beckii

Light and temperature increase were found to stimulate hatching. The result of the combined action of these two factors is that crawlers emerge during the early warmth of morning, presumably when conditions are most favourable for survival. A similar situation, with regard to light, appears to exist in the mite, Metatetranychus ulmi Koch (Hueck, 1951) which is another small arthropod living on foliage. It is not unexpected that hatching or emergence during the morning are fairly widespread, if this is the most favourable period. The dungfly, Scopeuma stercoraria (L.), emerges from pupation during the morning, and according to Lewis and Bletchley (1943), light is at least a contributory factor in the onset of emergence. Drosophila has a persistent "clock" which is set during the larval stages by the coming of dawn (Brett, 1955). The flies later emerge at dawn when pupation is completed. A somewhat similar state of affairs appears also to occur in Dacus tryoni (Bateman, according to Andrewartha and Birch, 1954), although the time of emergence is more scattered, with peak about 2 p.m. Ephestia kuhniella Zell. also has a persistent rhythm of emergence, but here the peak occurs during the evening (Scott, 1936). However, this is to be expected, since E. kuhniella is nocturnal.

Crawlers of L. beckii respond during the wandering period to light, but not to the force of gravity. The lack of response to gravity may be readily comprehended. A crawler has very limited powers of locomotion in relation to the size of an orange tree, and even in relation to the size of a single branch. With this limitation, any attempt to move up or down on an irregular object such as a leafy twig would be restricted by the numerous dead-ends into which the crawler would be led by the response itself.

The photopositive response is, for the same reason, unlikely to affect the distribution of the insect on the tree as a whole. L. beckii, according to several authors (Quayle, 1938; Ebeling, 1950; Carnegie, 1955; De Bach, 1958) occurs mainly on the lower branches of the shady side of the tree and on the inside foliage (Bodenheimer, 1951; Muma, 1955). Personal observations are in agreement with this, although it should be emphasised that scattered infested leaves are to be found all over the exterior of the tree. An explanation given by Carnegie (1955) of this phenomenon is that it is due to the action of parasites and predators on the Scale Insects on the other, sunny parts of the tree. However, the uniform distribution on all sides of the tree (Ebeling, 1947; Bodenheimer, 1951) of the Red Scale, Aonidiella aurantii Mask., a species far more heavily preyed on and parasitised than L. beckii, makes this appear unlikely.

It is possible that the distribution of L. beckii on the tree may be determined by its inability to survive in unfavourable conditions, rather than by migration of the crawlers. Some preliminary observations indicate that relative humidity in the interior of an orange tree on the shady side is considerably higher than that in the interior on the sunny side. Air temperature appeared to show little variation through the tree. However, the body temperature of Red Scales on leaves exposed to sunlight has been shown to be considerably higher than that of the air (Munger, 1948). It is possible that inability to survive prolonged exposure to these high temperatures, or to low humidity, may be responsible for the absence of L. beckii on the sunny side of the tree. Indeed, it is known that L. beckii cannot be cultured successfully either at low humidities (Lindgren and Dickson, 1941; De Bach, 1958) or at high temperatures (De Bach, 1958). Further conclusions must await work on the survival of various stages of L. beckii under various conditions of temperature, humidity and possibly light.

The observations of Stofberg (1937) and Carnegie (1955) that Mussel Scale occurs mainly on the upper surfaces of leaves may, however, be accounted for by the photopositive response.

Another point of possible importance is that an animal responding by locomotion to a directional stimulus will very probably spend more time on the edges and tips of leaves than one which did not. It is possible that dislodgement and subsequent dispersal by wind would be more likely to occur under these conditions.

Unfortunately, for purposes of comparison, the position is not clear in other Armoured Scale Insects. Experiments appear to have been carried out using crawlers moving on fruits, and thus other factors such as orange smell and asymmetries such as the presence of the calyx were not excluded. Flanders (1951) found A. aurantii to be weakly photopositive and geonegative, while Carmin and Scheinken (1934) concluded that crawlers did not disperse in such a way as to suggest a response to light. Bodenheimer (1951) suggests that the factor to which crawlers of this species respond, may be heat rather than light. More critical experimentation is needed before any comparative studies can be made. The Soft Brown Scale, Coccus hesperidium L., is, however, also dispersed by wind and its crawlers are photopositive (Dingler, 1923; Bodenheimer, 1951) and geonegative (Bodenheimer, 1951)

Distribution on the tree is thus probably not attributable to any direct action on the part of the crawler. However, it is necessary to consider the factors actually concerned in the settling of the crawler. The final settling of a crawler may be regarded as having two requirements. The crawler must carry out the settling behaviour pattern, and once flattened, should remain without wandering away again. The initiation of the settling behaviour appears to be largely independent of all but mechanical factors. It has been seen that dust on a leaf produces settling sooner, and that the rough surface of a lemon leaf might be responsible for a similar effect. This is thought to be due to the interference with locomotion of dust or the rough surface. Further, flattening is not restricted to the leaves of citrus trees. A crawler released on a Sonchus leaf flattened in 36 min. and remained for over 3 hr. It eventually moved off when the leaf was already badly wilted. This walking time was well within the limits already observed on host leaves. Six crawlers released on a leaf of Quercus also settled. The crawlers were not watched continuously, but five had settled within 120 min. and 30 min. later the last had settled. This time the leaf was kept fresh by dipping the petiole into a specimen tube full of water. No crawler moved off again in this case. Crawlers released on black paper also flattened, but walked for longer before doing so. Nine crawlers were released on black paper. Four were flat within 90 min. and two more within 130 min.; the remaining three were still walking after periods of 130 min.

Thus crawlers will flatten on foreign surfaces, in some cases at least, before any "fatigue" is likely to have occurred. The evidence indicates that the length of the wandering period is determined only, or mainly, by mechanical factors which probably act by interfering with locomotion. The presence of other crawlers has no effect on the wandering period. The fact that crawlers will settle on black paper makes it unlikely that chemoreception plays any major part in eliciting settling behaviour.

Whether the crawler remains or not, may also depend on such mechanical properties, i.e. the substance being penetrated by the rostrum.

Crawlers on orange leaves sometimes desert the first-chosen flattening site, only to settle again in almost the same place. It is suggested that this might be due to the penetration of the rostrum into one of the numerous oil glands in citrus leaves. This may be a chemoreceptive response. Crawlers released on Quercus, as already stated, showed no tendency to move off once flattened. If a chemoreceptive response is concerned in "acceptance" after rostrum penetration, it must thus be of the most general type.

This general chemoreceptive factor, possibly acting in both flattening and remaining, once flattened, may be either relative humidity above the substrate or its moisture content. An indication of this was obtained in some experiments where crawlers were released on black paper at 100% R.H. and 60% R.H. Crawlers on black paper at saturation were found after 12 hr. to have their rostra deeply inserted, while those at 60% R.H., although motionless, had not inserted their rostra. Black paper normally lacks the necessary qualities to restrain a crawler from moving off, or from another viewpoint, has qualities which repel the crawler. Paper at saturation appears to be accepted, after long periods at least, and it seems likely that either the moisture in the paper, detected by the rostrum, or the saturated conditions above the surface, may be responsible. A high relative humidity, due to transpiration, appears to occur on the surfaces of leaves (Ramsay, Butler and Sang, 1938) so the latter hypothesis is physically possible.

Another factor, not investigated, but possible affecting the time of walking, is air movement. An animal such as L. beckii must strike a balance between repopulation of the host and dispersal to new hosts. A method by which this could be accomplished would be to have a short walking period in high winds, and a long one in gentle breezes. In this way spread to new hosts would be maintained during calmer weather, while in very windy weather the future of the host population would be safeguarded by prevention of the loss of all the emerging crawlers.

The lack of specificity in host choice is not at all surprising if the biology of the animal is considered from a teleonomic (Pittenbrigh, 1958) point of view. Many crawlers may reach non-host plants during airborne dispersal, but the chance of such a crawler getting back to a host plant by its own efforts is small. It thus makes little difference whether the new plant is accepted or not. Instead, large number of crawlers are produced per female, to ensure safe arrival of at least some crawlers on a host plant.

The similarity with respect to factors interrupting locomotion, of L. beckii and Aphis fabae has already been mentioned. Some preliminary experiments showed that crawlers aged for 12 hr. settled much faster than fresh ones. This, if shown not to be due to some factor such as dehydration, would be evidence for a reflex type of antagonism between locomotor and settling responses, similar to that postulated for A. fabae by Kennedy (1956). Further experiments would, however, be necessary to establish this fact.

The foregoing experiments were undertaken to elucidate some of the behaviour patterns shown by the young crawlers of L. beckii. They show that the crawlers display a marked photopositive response and that their tendency to settle is increased when leaves are dusty. These results correlate with field observations of the distribution of the more mature stages of L. beckii. They do not, however, tell the whole story, for the broad distribution of L. beckii on citrus trees points to some further factor or factors being at work. Whether the distribution is simply a matter of a failure of L. beckii to survive in certain conditions or is due to behaviour patterns which, by preventing them from settling, offer the crawlers "another chance" can only be resolved by further studies both of the movement of crawlers in the field and of their survival and behaviour in laboratory conditions.

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