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TEMPERATURE RELATIONS OF TENERAL DEVELOPMENT AND BEHAVIOUR IN *APHIS FABAE* SCOP.

By L. R. TAYLOR

Department of Entomology, Rothamsted Experimental Station, Harpenden, Herts

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INTRODUCTION

The period of relative quiescence which follows each ecdysis in arthropods varies greatly in duration in different stadia, in different individuals and in different species. It is associated, amongst other things, with hardening of the exoskeleton and also, at eclosion of the adult in alate insects, particularly with expanding and hardening of the wings; this must be completed before flight can take place. Thus the interval between eclosion and flight involves developmental processes which may be expected to respond to temperature in the way typical of other stages in insect development.

The word 'teneral' has been used to describe alate insects at about this time, although its usage varies between different orders. It has already been applied to the aphids with which this paper is mainly concerned, and it is convenient to adopt the name here and discuss its several meanings later. When teneral development is complete, flight may still be prevented, or delayed, by behaviour in response to physical factors such as temperature and light, and to biological factors such as the presence of adjacent insects. 'Teneral' is therefore used here to denote that stage between final ecdysis and the time when the alate aphid is capable of flight, external factors permitting.

Inhibition of flight, e.g. by low temperature, is an individual response; an insect will either take flight or not take flight at a given temperature on a particular occasion. Seen in a natural population this has the form of a frequency distribution against temperature. For example, in *Aphis fabae* the response curve rises from zero at 15.5° C. to a maximum at 17.3° C. and falls again to zero at 20° C. (see C. G. Johnson & Taylor, 1957).

As a consequence any attempt to measure the length of the teneral period at *constant* temperatures in the laboratory, without a previous knowledge of the response curves for behaviour inhibition and development, would probably lead to erroneous results below 20° C. At these temperatures flight-mature but flight-inhibited insects would not be distinguishable from flight-immature insects, and the resultant curve for time against temperature would go off to infinity at about 15.5° C.—the development velocity being apparently zero when there is no take-off. The concept of separate functions for behaviour and for the teneral period as a development stage would not emerge.

The independent effects of temperature on development and on behaviour were, in fact, found and segregated by analysis of the data obtained in field experiments during which the temperature was constantly changing over a wide range (*c.* 7–34° C.), along with other climatic factors. By this analysis it is possible to show that the variation in teneral time in *A. fabae* in a bean crop can be largely accounted for by four simple assumptions.

(1) The rate of development during the teneral period is logistically related to temperature between 10 and 30° C. irrespective of whether the temperature is constant or varies about a mean value.

(2) Inherent individual variations are imposed upon this trend.

(3) Take-off is inhibited at temperatures below *c.* 16° C. as measured at 1 ft. in the crop.

(4) Take-off is inhibited at light intensities below *c.* 100 ft.-candles.

This analysis alone does not, of course, prove that these are the direct causative factors, but once the development and behaviour factors have been segregated it is possible to confirm the results at constant temperatures in the laboratory.

The logistic formulation of the rate of development has been tested in the laboratory under conditions of constant and variable temperature and found to describe adequately the temperature relation up to the optimum response at about 28° C. Above this value neither were the field data sufficient to give accurate results, nor is the type of curve fitted appropriate to data showing a falling rate. Nevertheless, the range of temperatures, over which the constant-temperature treatment and the highly variable field treatment yielded almost identical developmental rates, is adequate evidence that temperature variations *per se*, and other climatic factors correlated with temperature (at least of the order found in nature), have little or no effect on developmental rates.

There are consequently four main issues in the present paper. First, that it is possible to distinguish between the effects of development and of behaviour in quantitative studies on populations in the field as well as in investigations of the behaviour of individuals. Secondly, that the teneral period, between eclosion and flight, can be treated largely as a developmental stage. Thirdly, as mentioned above, that this stage is controlled in its duration mainly by a quantity of heat, and that other climatic factors, especially rate of change of temperature, have negligible effect. Fourthly, that such temperature relationships can be analysed from field data obtained in highly variable climatic conditions with an accuracy closely approaching that obtained in the laboratory, without the attendant restrictions imposed by arbitrary control of environment and the consequent difficulty of application to natural conditions. Considerable attention has therefore been given to the method of analysis.

GENERAL METHOD

The material has been extracted from the tabulated raw data in C. G. Johnson, Taylor & Haine (1957), wherein the experimental site and procedure are described in detail, and where a full temperature table in half-hour means for the whole

experimental period is given. Briefly, alienicolae of *A. fabae* were inspected at frequent intervals *in situ* on the leaves of a naturally infested field-bean crop in a walled garden, and were marked with paint in batches as they moulted. The time of departure of these alatae (flight) was also recorded, again in batches between two successive inspections. All the insects marked in one batch did not necessarily depart together, and hence the history of an individual cannot be traced. Each experiment consisted of marking several successive batches with the same colour over a period of about 4 hr. (a 'colour group'), and it is the mean time of such a colour group from moulting to flight that is considered. Variation in individual behaviour is an additional factor eliminated by this treatment, but it has been studied independently in laboratory experiments (p. 203).

The data for each experiment were thus reduced to a single record, the mean teneral period, from moulting to flight, which was plotted against the arithmetic mean temperature for the interval. This gave a hollow curve with wide scatter, but sufficient to show that temperature was a major factor in determining the teneral period. From this stage, by a succession of approximations described later, the reciprocal curve (i.e. the rate of development curve) was gradually arrived at: batches which were estimated to have completed maturation in darkness or low temperature, and consequently to have been delayed by behaviour inhibition, were eliminated; until, finally, almost the whole variation in teneral time was accounted for. Inhibiting light and temperature levels were obtained, independently of these data, from trap catches in the same crop.

CLIMATIC FACTORS INHIBITING TAKE-OFF

Take-off is inhibited during the night as can clearly be seen from suction trap catches (C. G. Johnson, 1952). This spuriously lengthens the teneral period of aphids maturing during the night, and such insects cannot be used in the formulation of the temperature-velocity curve for teneral development. It is therefore necessary to determine the climatic limits beyond which take-off inhibition occurs. Such limits will of necessity be arbitrary, depending upon the means available for their measurement.

Temperature

The known temperature relations of aphid flight activity recently reviewed by Moericke (1955) are of little use for this purpose, because the threshold chosen must be a single temperature level measured at a specific place in relation to the aphids.

A suction trap (Taylor, 1951) was operated in the centre of the experimental crop with the collecting inlet just below the crop level. Such a trap may be used to give a direct density estimate (C. G. Johnson & Taylor, 1955) of flying insects per 9000 cu.ft. ($\pm 5\%$) of air per half hour (Taylor, 1955). Trap catches consist of insects just leaving the crop on their initial flight (see later).

Air temperature records for 1 and 4 ft. levels in the crop were also available, and from these the maximum temperature reached in any half-hour could be read.

The aphid density, plotted against the maximum temperature for half-hour periods in the mornings and covering a range of 12–20° C., is shown in Fig. 1 (4 ft.) and Fig. 2 (1 ft.). The diagrams are interpreted thus; as the sun rises and the tops of the plants are warmed, the aphids in the crown of the plants take flight sporadically, as temperature permits. This temperature is recorded by the thermometer at 4 ft., and the threshold is the temperature at which the first few aphids are caught, i.e. at 15–17° C. But full-scale catches cannot be expected until the whole crop is raised above the threshold temperature as recorded by the thermometer at 1 ft. Con-

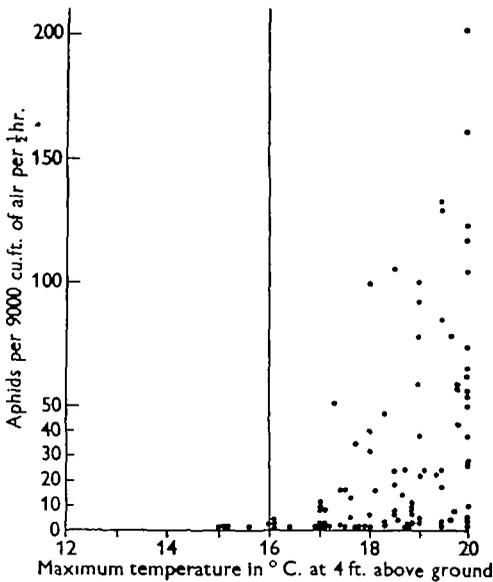


Fig. 1

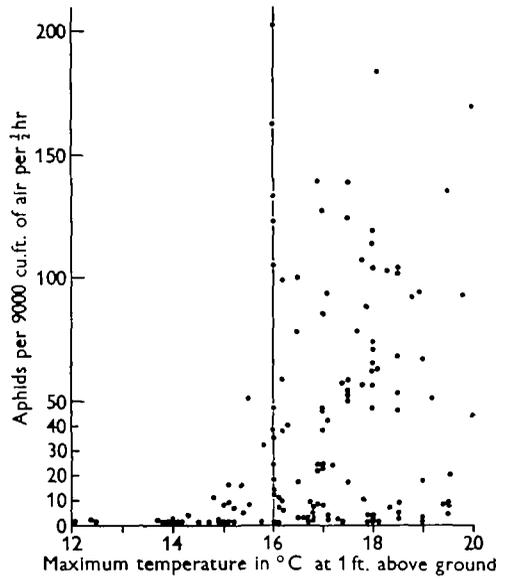


Fig. 2

Figs. 1, 2. Each point represents the mean aerial density for $\frac{1}{2}$ hr. plotted against the maximum temperature during that $\frac{1}{2}$ hr. Samples taken each morning for 20 days.

sequently, in Fig. 2, the temperature at which large catches (20–30) begin may be taken as the threshold. Considered together these two diagrams give an operational threshold of 16° C. at 1 ft.; the four largest catches below 16° C. fall on the same day, an occasion when the thermometer at 1 ft. registered 15–16° C. for several hours and some parts of the crop must have had temperatures above 16° C.

This is not the mean of the take-off threshold temperature distribution as measured in the laboratory (C. G. Johnson & Taylor, 1957); that gives the actual temperatures to which the aphids respond. The temperature threshold for take-off used here is 16° C. at 1 ft. in a bean crop; no appreciable take-off occurs below this temperature.

Light

Laboratory experiments by Broadbent (1949) indicated that flight is completely inhibited in *Myzus persicae* Sulz. at just below 1 f.c. and in *Brevicoryne brassicae* L. at about 1 f.c. (fluorescent lamp); full activity is approached at intensities above

100 f.c. There was no information about *Aphis fabae*. A method similar to that employed for temperature was therefore used to find the light threshold. The aphid density was plotted against the time (15.00–24.00 hr., G.M.T.), at the end of the day when light was failing but temperature still high (Fig. 3); no records at temperatures below 16° C. were used. Only in a general way can light be associated with time; dull days will reach the same light value earlier in the day. The fall-off in catch at 19.00–19.30 hr. G.M.T. is, however, fairly clear, although it must occur earlier than this on some occasions. The experimental crop was surrounded on the south and west by trees and the light intensity would be slightly lower than standard, i.e. 10–20 f.c. at sunset (c. 20.10 hr.) instead of 33 f.c. and about normal, i.e. 0.4 f.c. at the end of civil twilight (c. 21.00 hr.).

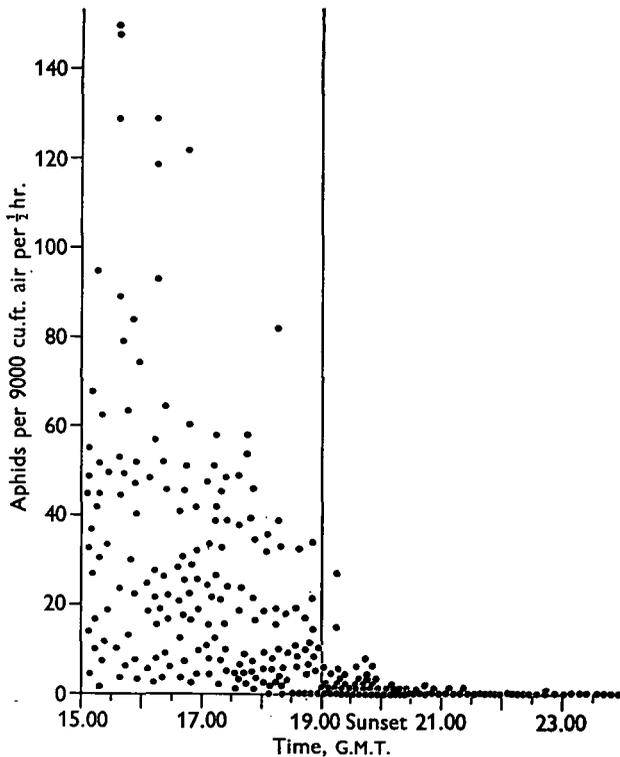


Fig. 3. Each point represents the mean aerial density for $\frac{1}{2}$ hr. at the mean time shown. Samples taken each evening for 20 days, at temperatures above 16° C. Sunset indicated at a mean position.

Thus the values are of the same order as for Broadbent's *Brevicoryne brassicae* and *Myzus persicae*: a reduction in activity commencing about $3\frac{1}{2}$ hr. before sunset (c. 1000 f.c.) and falling with increasing steepness to reach negligible proportions at about 1 hr. before sunset (c. 10 f.c.), with complete cessation by the end of civil twilight (0.4 f.c.). The light threshold used is 1 hr. before sunset.

There may be a slight excess in the numbers of aphids recorded in the last few catches of the day owing to the escape of insects from one disk of the trap to the next (C. G. Johnson & Taylor, unpublished), but the effect must be very small, as can be seen by inspection of the catches in C. G. Johnson *et al.* (1957).

Wind and humidity

Wind speed is a significant factor in delaying take-off but, unless combined with low temperature, probably rarely prevents it (Haine, 1956). In these experiments the wind rarely exceeded 4 m.p.h. and was usually below 2 m.p.h.

Humidity is probably not a factor in take-off behaviour, although sudden changes may have a temporary effect on activity (Broadbent, 1949). There are apparently no such sudden changes in the humidity in the present record, as measured by a whirling hygrometer between the plants, and this must reflect the condition at the leaf surface to a large degree.

EXPERIMENTAL ANALYSIS

Extraction of the data

The raw data are given in Table 2 of C. G. Johnson *et al.* (1957), and Tables 1 and 3 of this paper show the data after condensation. The method of construction of these tables must be described, for it involves an arbitrary step in the analysis.

In the original table the time of marking of each experimental batch of colour-marked aphids is given. Moulting, which occurred during the interval between marking and the previous inspection, is assumed to have taken place midway between these two inspections. The mean moulting time (Table 1, col. 4) for the whole experiment is a weighted mean of all such batches.

Departure times (Table 1, col. 7) are treated in the same way when the rate of departure plotted against time gives a fairly normal curve, as it generally does. When the departures are split into two groups by nightfall however, departure curves are usually very skew. All experiments were therefore drawn as histograms, against time, and when these were skew an attempt was made to find the mode. Assessment of the mode was then, of necessity, arbitrary and was found from a curve fitted by eye; the experiments in which this was done are indicated in the table. The possible errors introduced are not large, but obviously cannot be assessed.* For this reason and also because the history only of batches not of individual aphids can be followed, statistical analysis of the means is not possible. Exps. 45 and 46 were added together to facilitate the assessment of means, for this is more critical when the teneral time is short as it was here.

When the departures extended over 2 days and the two departure groups were of equivalent size it was assumed that, in general, the first to moult were the first to depart, and the experiment is accordingly divided in two (see Johnson *et al.* 1957). This leads to a slight bias in rate of development between the two groups owing to the non-randomness of the segregation, the first group maturing rather more rapidly than normal, and the second group more slowly. Where either of these two groups

was too small to be used alone it was included in the gross mean, but the inhibiting hours of darkness and cold were eliminated to maintain a balanced distribution.

Table 1. *Condensed data for field experiments used in estimating general time*

Exp. no.	Moulting times, G.M.T.			Flying times, G.M.T.			No. aphids	General time	Thermal summation	
	Distribution limits		Mean	Distribution limits		Mean			Arith- metic	Logistic
	From	To		From	To					
1	12.00	18.00	15.7/2	05.00	08.00	11.3/4	9	43.6	145	90.9
2	13.30	19.30	17.4/5	06.15	15.00	10.3/6	23	16.9	118	95.0
3	04.15	10.15	06.9/6	13.00	19.00	15.9/6	40	9.0	139	101.2
6	04.15	10.15	06.6/8	12.30	11.15	18.7/8	34	12.1	106	96.0
7*	05.15	07.15	06.3/9	13.30	06.15	16.5/9	39	10.2	112	98.7
9*	06.15	08.15	07.3/9	11.00	19.00	17.0/9	42	9.7	107	99.1
12	11.30	19.30	14.4/9	06.30	15.30	09.6/10	44	19.2	130	104.8
13	15.30	19.30	16.9/9	07.00	14.30	11.7/10	32	18.8	120	99.0
14	05.15	08.15	06.7/10	13.30	18.30	16.2/10	42	9.5	123	101.4
16*	06.15	09.00	07.7/10	11.30	18.30	16.5/10	59	8.8	123	102.3
17	10.15	17.30	14.5/10	06.15	17.30	11.0/11	55	20.5	151	112.7
20	09.45	13.00	11.4/11	09.30	15.00	12.2/12	29	24.8	132	106.0
21	05.15	09.45	06.7/12	18.00	16.45	11.3/13	70	28.6	152	103.2
22	09.45	16.00	13.3/12	18.00	19.15	14.2/13	42	24.9	144	102.9
23*	13.30	18.30	16.0/12	06.15	18.30	14.8/13	29	22.8	138	96.2
24*	13.30	18.30	16.0/12	08.00	16.45	14.4/13	22	22.4	138	90.8
26	09.45	16.30	12.5/13	07.30	15.30	11.2/14	39	22.7	138	102.6
27	09.45	13.00	12.3/14	09.00	18.30	14.4/15	30	26.1	107	99.8
28	05.15	12.30	07.8/15	08.00	16.15	10.9/16	49	27.1	119	105.7
29	07.15	11.00	09.1/15	19.00	16.15	10.0/16	51	24.9	105	94.3
30	09.15	16.30	12.7/15	16.30	19.15	13.1/16	61	24.4	101	94.7
32	07.15	11.00	09.1/16	17.40	11.45	09.6/17	20	24.5	135	104.3
33	09.15	13.30	11.8/16	18.30	18.30	12.6/17	33	24.8	130	104.0
35	09.15	16.30	11.9/17	07.00	16.45	12.1/18	26	24.2	145	103.0
36	16.30	18.00	17.3/17	12.30	07.30	15.8/18	25	22.5	145	96.8
39	10.15	18.30	13.9/18	19.00	16.45	10.7/19	37	20.8	134	105.9
40*	14.30	18.00	16.3/18	07.30	19.15	12.4/19	32	20.1	142	107.4
41	15.00	19.00	17.2/18	10.00	19.15	12.2/19	29	19.0	130	97.9
43	12.30	19.00	14.8/19	06.30	15.00	08.8/20	45	18.0	125	107.4
44	13.30	18.00	15.7/19	06.30	19.15	09.3/20	33	17.6	123	96.4
45 + 46	06.15	11.00	08.5/20	11.00	09.00	16.8/20	57	8.3	137	101.5
47	15.30	18.00	16.6/20	18.00	18.00	08.5/21	18	15.9	133	105.9

* Mode used; not mean.

In columns 4 and 7 the mean times are given as: time (G.M.T.) to the first decimal of an hour/date (July 1952).

Small isolated departures, dissociated from the main mass, are not included in the assessment of means, as it is believed that those departing soon after marking were disturbed either by the marking or by movement of other aphids, and those remaining very late may have been damaged. In Table 1 this is indicated by the limits of time for the mean (cols. 2 and 3, and cols. 5 and 6), which exclude such odd aphids, and by the slight reduction in numbers (col. 8) as compared with this value in the original table. The total number of aphids is reduced from 1248 to 1196.

Table 1 does not include those experiments estimated to be delayed by behaviour pattern. These are presented in Table 3 (see p. 201), but it should be emphasized that at each stage in the analysis these experiments were considered individually before they were discarded.

Method of curve-fitting

During the analysis the process of curve-fitting, which takes place in several stages, runs parallel with that of segregating behaviour from development; they are in fact complementary. It is simpler, however, to describe curve-fitting first, dealing only with those experiments in which flight was not evidently delayed by low temperature or light intensity.

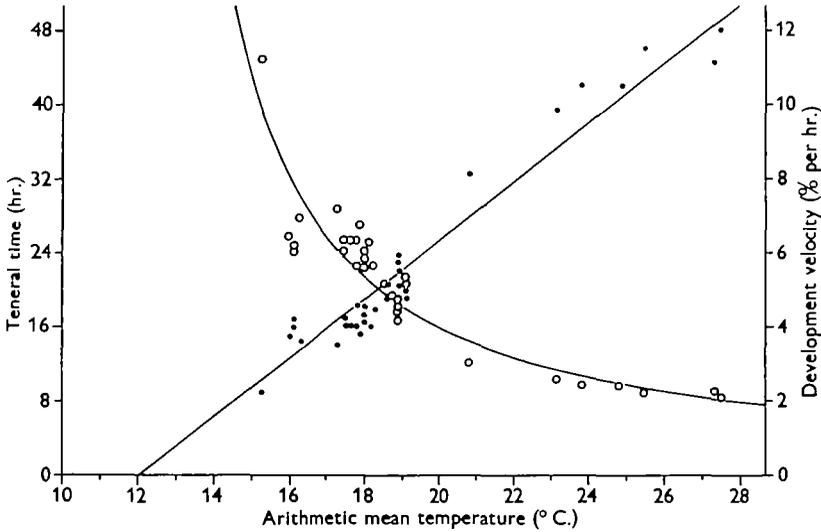


Fig. 4. Teneral time and development rate in nature using arithmetic means of variable temperatures.

Fig. 4 shows the condensed data for teneral period given in Table 1, col. 9, plotted against the *arithmetic* mean temperature during the teneral period obtained from the table in C. G. Johnson *et al.* (1957). The reciprocals of teneral period, or development velocities, for each experiment are also plotted and to these has been fitted the straight regression line:

$$v = 0.78 (\theta - 12.0), \quad (1)$$

where v = development velocity (% per hour) and θ = empirical temperature (arithmetic mean) in ° C. This gives a fitted curve (rectangular hyperbola) for the teneral period:

$$t (\theta - 12.0) = 128, \quad (2)$$

where t = teneral period in hours. The so-called thermal constant, 128 degree-hours, implies that, from the time of eclosion, the sum of hourly mean temperatures in excess of 12° C. (thermal summations) reaches 128 upon completion of teneral development. The observed thermal summations for each separate experiment is given (Table 1, col. 10), and the estimated thermal constant is the mean of these summations. The thermal sums vary from 100 to 150, and an expected time for maturation in variable temperatures assessed from these equations would be liable to a correspondingly large error.

This is because the relationship of v to θ is not adequately accounted for by the straight-line regression; it is in fact better described by a sigmoid curve, but such a curve cannot be fitted to the points as they stand. Each point represents the mean period of time for a group of insects, plotted against the arithmetic mean temperature during that period, implying that development during that time is linearly related to temperature. The temperature may in fact vary over almost the whole range under consideration during the time expressed by a single point, and if the fitted line is curvilinear, then the individual arithmetic means become invalid, and the points move. In other words, a curve must first be estimated and any means taken must be allowed due weight for the development rate appropriate to each temperature along that curve, i.e. the developmental increment equivalent to each temperature, the temperature equivalent, must be used in assessing means, instead of the measured temperature.

The method of obtaining means by use of equivalent, or effective, temperatures may be clarified by a more straightforward example. If a process is linearly related to temperature, e.g. the observed values at 10:20:30° C. are 2:4:6 per hour; then $\frac{1}{2}$ hr. at 10° + $\frac{1}{2}$ hr. at 30° gives an observed value of $(1+3)=4$ for 1 hr. This is equivalent to 1 hr. at $(10+30)/2=20^\circ$ C., i.e. the arithmetic mean temperature gives the correct temperature for the observed value.

If the process is logarithmically related to temperature, e.g. the observed values at 10:20:30° C. are 2:8:11.5 per hour, then $\frac{1}{2}$ hr. at 10° + $\frac{1}{2}$ hr. at 30° C. gives an observed value of $(1+5.75)=6.75$ for 1 hr. This is *not* the value observed at 20° (the arithmetic mean temperature) but at the log mean (or geometric mean) temperature, i.e. at $\text{antilog} \left\{ \frac{1}{2}(\log 10 + \log 30) \right\} = 17.4^\circ$ C. In other words, the true mean temperature for a logarithmic relation is found by converting the actual temperature to temperature equivalents using logarithmic tables, taking a mean, and converting back.

Exactly the same process is necessary to find the true mean for a logistic relationship, except that the logistic tables are not published but must be made from the curve used. Even if the curve used is not exactly logistic, or is not fitted accurately, the results obtained will approach more closely to the truth than will arithmetic means. *The mere fact that the correct functional relationship is not known beforehand is no justification for using arithmetic means except as a purely provisional estimate.*

The errors introduced by using arithmetic means have been analysed, in an investigation similar to this, by Andrewartha (1944). The curve from which he obtained his temperature equivalents was based on laboratory data. In the present analysis a process of successive approximation was adopted.

As a first approximation an arbitrary sigmoid curve was drawn through those arithmetic means in Fig. 4 with the smallest temperature range, i.e. in which distortion was minimal. Using this curve a table of temperature equivalents was made by reading off the appropriate development rate, or temperature equivalent, at each temperature. The observed $\frac{1}{2}$ -hourly temperatures were then converted by this table to temperature equivalents, new means were taken and converted back to actual temperatures by the same table, and these new points plotted. To this

second estimate was then fitted a formulated (logistic) curve, and from this another table of logistic temperature equivalents obtained. Logistic means were taken and the process repeated successively, each time using a new logistic conversion table to obtain means from the original, observed, temperature record. By noting the movements of the points in relation to the line, a curve was eventually found which gave means equally distributed on each side of the line, and of minimum deviation. The final rate of development, or equivalent temperature conversion table for these aphids, is given in Table 2.

Table 2. *Conversion table for degrees Centigrade to equivalent semi-logistic developmental units for Aphis fabae*

Degrees Centigrade	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	Semi-logistic equivalent									
9	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
10	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2
11	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3
12	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.5
13	0.5	0.5	0.5	0.6	0.6	0.6	0.6	0.7	0.7	0.7
14	0.7	0.8	0.8	0.8	0.9	0.9	0.9	1.0	1.0	1.1
15	1.1	1.2	1.2	1.3	1.3	1.4	1.5	1.5	1.6	1.7
16	1.8	1.9	2.0	2.0	2.1	2.2	2.3	2.4	2.5	2.6
17	2.7	2.8	3.0	3.1	3.2	3.3	3.4	3.5	3.7	3.7
18	3.9	4.0	4.2	4.4	4.5	4.6	4.8	5.0	5.1	5.2
19	5.4	5.5	5.7	5.8	6.0	6.1	6.3	6.5	6.6	6.8
20	6.9	7.1	7.2	7.4	7.5	7.7	7.8	7.9	8.1	8.2
21	8.4	8.5	8.6	8.8	8.9	9.0	9.1	9.2	9.3	9.5
22	9.6	9.7	9.8	9.9	10.0	10.0	10.1	10.2	10.3	10.4
23	10.5	10.5	10.6	10.7	10.8	10.8	10.9	10.9	11.0	11.0
24	11.1	11.2	11.2	11.3	11.3	11.3	11.4	11.4	11.4	11.5
25	11.5	11.5	11.6	11.6	11.6	11.7	11.7	11.7	11.7	11.8
26	11.8	11.8	11.8	11.8	11.9	11.9	11.9	11.9	11.9	11.9
27	11.9	11.9	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
28	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	11.9
29	11.9	11.9	11.9	11.9	11.8	11.8	11.8	11.7	11.7	11.7
30	11.6	11.6	11.6	11.5	11.5	11.5	11.4	11.3	11.2	11.1
31	11.0	10.9	10.8	10.6	10.5	10.4	10.2	10.1	9.9	9.8
32	9.6	9.5	9.3	9.1	8.9	8.7	8.5	8.3	8.0	7.7
33	7.4	7.1	6.8	6.4	6.0	5.5	4.9	4.2	3.4	2.1
34	0	0	0	0	0	0	0	0	0	0

From 9 to 28° C. the equivalents are derived from the fitted logistic curve. Above 28° C. the equivalents are from the semi-logistic curve in Fig. 6.

Thermal sums from successive tables were used as indicators for the improvement of the curve used, and the final values are given in col. 11, Table 1. They may be compared with the arithmetic thermal sums (col. 10). Both have been halved to make the theoretical thermal constant 100 for col. 11, this being necessitated by the use of $\frac{1}{2}$ hr. periods; consequently col. 11 is also expressed directly as a percentage of the expected constant. As the accuracy of the fitted curve is judged by the closeness with which the thermal sums approach a mean (the thermal constant), the value

of this increased accuracy is perhaps best expressed by the standard deviations which are:

	Thermal constant	
	Mean	S.D.
Straight line (arithmetic means)	128.9	14.0
Sigmoid curve (logistic means)	100.9	5.1

This means that although expression (2) may be useful for comparison of the temperature relations of the teneral stage in different aphid species, or in different phases of the same species, it is of little value in forecasting the time of flight ($\pm 22\%$ for the 95% confidence limits); the second curve makes this possible, within reasonable ecological limits ($\pm 10\%$) (see C. G. Johnson *et al.* 1957).

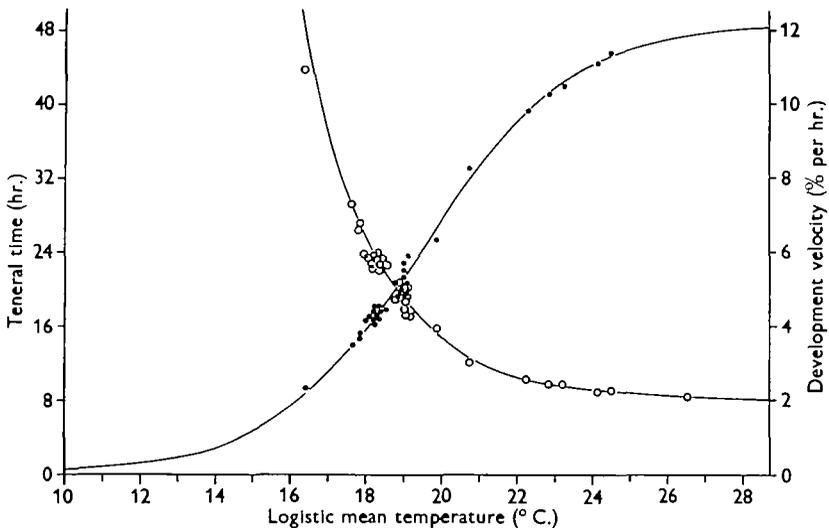


Fig. 5. Teneral time and development rate in nature fitted to logistic temperature-velocity curve.

The marked aphids were nearly all at the tops of plants on the edge of the crop and would therefore respond to air temperatures recorded at about 4 ft. But since the mass of aphids in the crop, and hence the trap catch, responded more clearly to the 1 ft. air temperature, it is this record which is reproduced (Table 1, Johnson *et al.* 1957). The present curve has been fitted to both records with identical results except that the absolute level differs by 2° C. Therefore the 1 ft. record was finally used, since it is published, and the resultant curve raised 2° C. to bring it to the 4 ft. level, to which these aphids were more nearly responding. Consequently the sums for col. 10, Table 1, should be considered in excess of 10° C. when dealing with the published temperature record.

The curve used

The development-temperature relationship follows a sigmoid curve from the cold death-point to an optimum and then falls quickly as first shown by Verworn (1894). This curve is very like the Pearson type 1 curve of negative skew distribution

(Elderton, 1953), and it seems reasonable to attribute the resultant temperature response to the statistical summation of individual responses of innumerable biochemical processes, each point on the curve being an index of the efficiency with which the processes contributing to speed of development operate at that temperature. The curve could therefore be considered as a distribution curve of relative efficiency with an optimum at the point at which the greatest number of processes are working with, and co-ordinated with, the greatest efficiency. The Pearson type I curve is, however, difficult to fit to such irregular data.

Many of the curves produced in the past to relate these two variables have been linear transformations, some based on hypothetical functional relationships, but the best description of the rising part of the curve is given by Davidson's (1942) logistic, which is purely empirical and closely approximates to the rising part of the negative skew distribution curve. It covers most of the range required here and is easy to fit; this is the curve used, fitted by the 3-point method of Davidson (1944).

The logistic expression used to produce Fig. 5 is

$$\frac{100}{t} = \frac{12.25}{1 + e^{1.934 - 0.5066t}} \quad (3)$$

The optimum occurs at *c.* 28° C. (see later), but there are so few experiments extending above 30° C. that any deviation above this would not affect the result. Similarly, below 10° C. the curve has almost reached zero but the records are too few to define a cut-off.

The segregation of development and behaviour

The time between moulting and flight may be influenced in two ways: either by factors affecting the rate of teneral development, or by factors affecting behaviour at the end of the teneral period.

If there are climatic factors other than temperature involved in *development* they should appear, not as additions to teneral time associated with the level of the factor at the time of flight, but as logarithmic deviations associated with the mean factor over the whole teneral period. No such relationship has been found with wind speed, or humidity or rate of change of temperature. It is therefore assumed that whatever other climatic factors control development during the teneral time, they are so closely associated with temperature that they appear in the logistic temperature regression.

Behaviour, on the other hand, operates as an inhibitor to flight, *adding* to the teneral time. This addition will not be dependent upon the mean level of the activating factor during the measured time; it will be evident as a release of activity at some *particular level* of the factor concerned at the moment of departure. Conversely if flight appears to take place at a particular temperature level, or level of light intensity, the teneral time apparently having continued longer than expected, and the expected termination of flight having occurred at an inhibiting level of the factor concerned, then behaviour could be postulated as the cause.

Behaviour is already taken into account to some extent in removing those experiments in which the teneral time theoretically terminated at temperatures below 16° C., or after 19.00 hr. G.M.T., this being done anew after each successive estimate of teneral time. Residual temperature effects above 16° C., effects due to wind speeds, and effects of rate of change of temperature at the time of departure were sought but not found; neither are the deviations from the expected values related to the teneral time itself, so that there do not appear to be any accumulative large errors nor any other behaviour factors seriously affecting time of flight. The experiments in which behaviour is believed to be a major factor are presented in Table 3. These cannot conveniently be classified and require individual examination.

Table 3. *Condensed data for experiments in which aphids are delayed by behaviour*

Exp. no.	Moulting times, G.M.T.			No. aphids	Thermal constant reached at	Expected mean times of flight	Flying times, G.M.T.		
	Distribution limits		Mean				Distribution limits		Mean
	From	To					From	To	
4	06.15	11.00	07.9/7	41	17.00/7	04.30/8	14.30	10.30	05.3/8
5	16.30	19.00	17.9/7	24	11.00/8	11.00/8	06.15	16.45	12.6/8
8	07.15	09.15	08.3/9	13	18.00/9	07.00/10	07.00	10.00	08.5/10
10	08.15	11.15	09.3/9	33	18.30/9	07.00/10	06.15	11.45	08.4/10
11	09.15	11.30	10.4/9	21	19.30/9	07.00/10	17.30	10.00	07.8/10
15*	08.15	12.30	09.5/10	17	18.30/10	07.00/11	06.15	13.30	08.0/11
18	04.15	10.15	07.2/11	41	19.00/11	09.30/12	16.00	16.15	09.6/12
19	06.15	10.15	08.2/11	66	20.00/11	09.30/12	17.00	15.00	09.3/12
25	05.15	09.45	06.3/13	31	19.30/13	08.30/14	17.00	14.30	08.4/14
31*	05.15	09.15	07.1/16	46	05.30/17	08.30/17	08.00	13.15	08.6/17
34	05.15	09.15	07.1/17	69	17.00/17	08.30/18	08.30	19.15	09.0/18
37*	06.15	11.00	08.6/18	33	03.00/19	06.00/19	18.00	18.30	06.5/19
38*	06.15	11.00	08.6/18	32	03.00/19	06.00/19	14.30	14.30	07.4/19
42	07.15	14.30	09.8/19	29	19.00/19	07.00/20	17.00	11.30	06.7/20

* Mode used, not mean.

In Exp. 4 the thermal constant is reached at 17.00 hr., yet flight did not occur that evening. It may have been dark earlier than usual, but the temperature never fell below 16° C. all night. This is the only occasion when the cause of delay is not evident. Flight took place as soon as it was sufficiently light, at 05.30 hr., for the mean cannot be expected to occur immediately the inhibiting bar is passed.

In Exp. 5 the thermal constant is reached at 11.00 hr. and flight is delayed for 1½ hr. This may be hardly significantly different from the expected time, but inspection of the temperature table shows that temperature was low (c. 17° C.) during the morning, and those insects which should have matured and flown before the mean were probably slightly delayed, so that all went after the expected mean time. Exps. 4 and 5 both include aphids from four leaves on which the departure records are rather difficult to interpret (see original data).

Exps. 8, 10 and 15 are the second-day batch of experiments which departed on 2 days, and, as explained earlier (p. 194), these would be expected to have means slightly delayed. Delay of 1 hr. after the expected departure time would cause the

mean to be after 'dark', as are Exps. 11, 18, 19, 25, 37, 38 and 42. All these batches were delayed overnight by darkness and in the morning by low temperature. In col. 6 the expected time of flight is the end of the first half-hour period after the 16° C. bar is passed; they all departed within reasonably short intervals of this time.

Exp. 31 was delayed in the morning from 05.00 to 08.30 hr. by low temperature and departed almost immediately the temperature rose. Exp. 34 was expected to terminate at 17.00 hr. on an evening following rain at 15.00 hr. and of low temperature (*c.* 17° C.); it was also probably dark. The insects departed at the expected time next morning.

CONFIRMATION OF RESULTS

In the field

In addition to experiments given in full in Table 2 of Johnson *et al.* (1957), there was a short series of ten experiments, designed to find out when insects moulting during the night would fly. All alatae were removed from the experimental leaves at dusk, and those which appeared by the next morning were marked then and their fighting times recorded as in the other experiments. It is thus not possible to obtain a mean moulting time for these with any accuracy, for most moulting probably occurred towards the beginning or the end of the moulting period, not in the middle of the night where the mean would be. It is possible, however, to use these experiments as a check for the other data by working back from the mean departure time to find an expected moulting time; only in Exp. A is there any apparent delay due to behaviour. These results are presented in Table 4, from which it will be seen that, out of the ten experiments, in all save A the expected moulting mean falls within the recorded times. In Exp. A the error may not be more than an hour, and this is associated with the fact that the mean fighting time falls outside the known limits of light intensity for flight, *i.e.* at 21.00 hr., because of the equally balanced departure before and after nightfall.

Allowing a slight variation of the 16° C. bar to flight for individual aphids and some variation of temperature within the crop, it therefore seems that the conditions

Table 4. *Confirmatory experiments in the field*

Exp. no.	Flying times, G.M.T.			No. aphids	Expected mean moulting time	Moulting times, G.M.T.	
	Distribution limits		Mean			Distribution limits	
	From	To				From	To
A	17.15/8	11.30/9	21.0/8	21	07.30/8	19.00/7	07.10/8
B	11.45/9	17.00/9	14.5/9	48	19.00/8	17.30/8	06.30/9
C	10.00/10	17.00/10	13.3/10	50	19.00/9	17.00/9	06.30/10
D	09.25/13	11.05/14	18.5/13	68	01.00/13	18.30/12	06.15/13
E	19.45/14	19.00/15	09.7/15	32	22.00/13	19.05/13	07.00/15
F	07.15/15	14.15/15	09.5/15	30	22.00/14	19.30/14	07.30/15
G	12.00/19	18.40/19	14.6/19	21	23.00/18	18.30/18	06.00/19
H	14.30/19	19.15/19	16.7/19	52	06.30/19	19.10/18	07.30/19
I	10.45/20	15.30/20	13.1/20	20	19.00/19	18.15/19	07.00/20
J	09.15/21	18.00/21	12.0/21	32	20.30/20	19.15/20	07.00/21

stated in the introduction (p. 190) can account for almost all the variation in teneral period found, with the possibility that some occasional, unsuspected factor may produce a small deviation (Exps. 4, 5 and A).

In the laboratory

To find out if the absolute level of the temperature-velocity curve, with temperatures measured at 1 ft. from the ground, bore any relation to the direct response curve of aphids in relation to the temperature of their immediate surroundings, and also to check the shape of the curve against that obtained at constant temperatures, teneral period was measured at several constant (and some variable) temperatures in the laboratory using the experience gained in the field as a guide. A freehand curve of a shape similar to the logistic, but curving down to zero at the upper limit, was drawn through the constant-temperature means, and this curve was used to calculate the means for the variable temperatures. I have called this a semi-logistic curve.

For this purpose final instar nymphs of alienicolae from culture or from an infested crop were placed on separate leaves standing in water and, as they moulted, were transferred to the constant-temperature cabinet, where they were covered individually by transparent cages. The bulb of the same thermograph as had been used in the field experiments lay between the cages. The accuracy would therefore be of the same order as that obtained in the other experiments, i.e. errors of the order of up to $\pm 0.5^{\circ}$ C. were possible. Variable temperatures were obtained by keeping some of the aphids longer in the laboratory, where the air temperature adjacent to the cages was measured by thermometer, before placing them in the constant-temperature cabinet. The cabinet was illuminated by mercury vapour lamp giving about 200 f.c. at the level of the cages, and this also contributed to raising the temperature. The insects were inspected through a window in the cabinet to obtain the time of flight.

The insects in the lowest temperature group were kept at a temperature below flight threshold until they were estimated to have almost completed their development; then the light was switched on and the temperature raised to about 20° C. (i.e. above the take-off threshold). This is treated as a constant-temperature group as the time at the higher temperature was negligible. Owing to a premature raising of temperature most of the insects had flown before inspection on this occasion, and the plotted mean was obtained from the remaining insects assuming the frequency distribution to be the same as for other groups.

The semi-logistic curve is shown in Fig. 6 along with the means obtained; the logistic curve from the field data is also given for comparison. The close similarity over the rising part of the curve is obvious, and this confirms the conclusions drawn earlier in the paper. The variation of individuals is given in Table 5. The range is approximately $\pm 30\%$ (95% limits) and is a constant proportion at all temperatures, as would be expected if temperature levels merely act through the genetic structure of the population in relation to rate of development, other factors being randomly distributed.

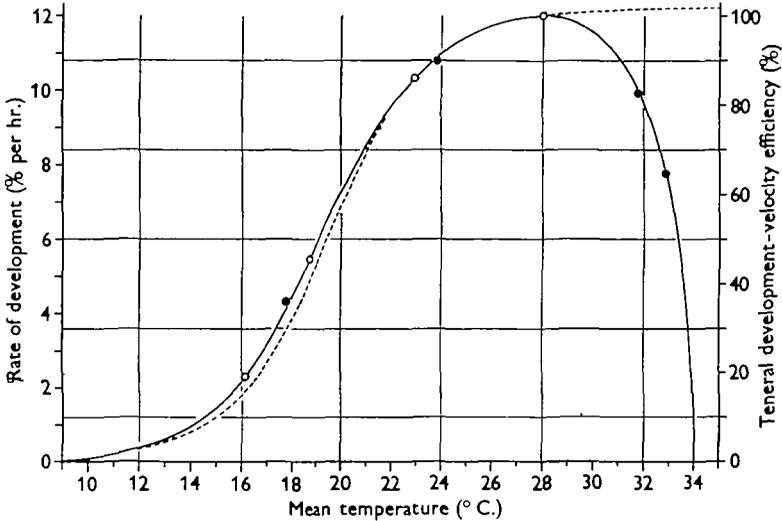


Fig. 6. Semi-logistic temperature relation measured in laboratory compared with logistic curve from nature. —, curve from laboratory data; - - -, curve from field data; ○, constant temperature means; ⊗, variable temperature means.

Table 5. *Teneral time of Aphis fabae in the laboratory*

No. of aphids	Mean temp. (° C.)	Temp. range (° C.)	Mean developmental rate	Mean teneral time, hours	Standard deviation of time	Standard deviation as % of mean (±)
19	32.9	28-36	7.94	12.61	1.90	15.08
22	31.7	17-32	9.90	10.10	1.89	18.71
31	28.0	Const.	12.10	8.26	1.63	19.73
19	23.8	17-31	10.80	9.26	1.03	11.12
44	22.9	Const.	10.23	9.78	1.22	12.47
25	18.8	Const.	5.62	17.81	2.43	13.64
35	17.7	15-32	4.33	23.12	3.03	13.10
(15)	16.1	Const.	(2.39)	(41.8)	—	—

THE TENERAL STAGE

Although the importance of flight to insects has been well emphasized, the time of acquisition of wings has received remarkably little attention. This may be attributed partly to the apparent rapidity of the process in some species, especially those with large wings in which the actual expansion of the wings is usually completed in a few minutes. Interest has also been directed toward the change in colour which often takes place; the time involved in this process, however, may be of quite a different order. For example, in Odonata complete coloration may take several weeks (Tillyard, 1917, p. 98). and the same may apply to Coleoptera, e.g. 25 days in *Leptinotarsa decemlineata* Say (Dunn, 1951). The actual process of cuticle reformation may take an equally long time, e.g. 2 weeks in adults of *Rhodnius prolixus* Stål (Wigglesworth & Gillett, 1936). But these insects have resumed active life before this.

There is, however, an interval succeeding any ecdysis during which the soft cuticle and relative inactivity of the insect are likely to render it particularly vulnerable. It is difficult to obtain evidence of this in nature although it justifies further examination, but Corbet (1956) ascribed a loss of 35.4% of a culture of *Anax imperator* Leach to cannibalism during ecdysis. It is this vulnerable, inactive stage which is of particular ecological interest, and which is terminated by resumption of active life, viz. by flight, after eclosion of the imago.

If flight normally occurs as soon as it is possible, then a state of flight maturity is implied and the stage preceding it is developmental. This is justified for *Aphis fabae* by the results presented here. The word 'teneral' (Latin *tener*) means literally 'soft' or 'immature' and aptly describes the stage up to flight maturity, although pigmentation maturity has been taken as the criterion in Odonata.

The changes in wing appearance and pigmentation after eclosion in *A. fabae* were examined in relation to flight by B. Johnson (1955), who found that the wings passed through five recognizable stages: opaque; transparent with a shiny white appearance; with a dull blue iridescence; with a reddish sheen and looking much harder in texture; finally, the formation of a pigmented bar along the post-axial border of the radial sector within 'several hours' of the end of the teneral period. The 'red' stage is reached 'at or shortly before reaching the end of the teneral period'; at this stage the aphid will take-off if disturbed, and he described them as 'flight mature'. He pointed out that they did not appear to fly immediately this condition was reached, and Moericke (1955) has emphasized this as a behaviour inhibition (*verhaltensmässige*), the insects not being in a 'mood' for flight. But from the results presented here it would appear that this delay is directly related to temperature, for it is included in the teneral time; it cannot therefore be in the nature of a behaviour inhibition unless it is included in the individual variation around the mean departure time, and in this respect it is so short as to be negligible.

That the time interval is short is shown by B. Johnson's own description of aphids caught in a suction trap on the same plot on which these experiments were carried out. Of 247 aphids 238 were freshly emerged with no pigmentation of the radial sector. This confirms the fact that *A. fabae* flies almost as soon as it is capable of flight, at least in the field, and that this is therefore a good criterion for termination of the teneral stage.

The behaviour of aphids in the laboratory during the teneral period is very consistent. Immediately after moulting they walk freely to a suitably sheltered place and settle down. At this time they are easily disturbed and quickly respond to visual and tactile stimuli. Once they have settled down the wings rapidly expand, the stylets are inserted and the aphids then remain in an almost torpid state. During this time light intensity and touch have a very much reduced effect in inducing movement. After sitting motionless for hours the insects suddenly withdraw their stylets, wave their antennae and walk up the leaf on which they have been sitting and possibly feeding. They then walk about, at a speed dependent upon the temperature, until they find a suitable take-off point on the leaf, and within a few seconds fly away. As an example, at 29° C. one aphid took 50 sec. from withdrawal of the stylets

to flight, and whilst this observation was being made another aphid had gone—in an even shorter interval. At lower temperatures the time taken is correspondingly greater.

It was at first thought that this immediate, unstimulated, flight response of *A. fabae* at the end of the teneral period might be a migratory act, and to confirm this, pupae of the hibernating, non-migratory, autumn phase of *Aglais urticae* L. (Lepidoptera), the Small Tortoiseshell butterfly, were timed and observed at eclosion in different temperatures. The results do not cover a sufficiently wide range to give a full semi-logistic response curve, but the times recorded in Table 6 show essentially the same picture as for aphids. The behaviour also conformed remarkably well to the description for *Aphis fabae* given above, except that the insect coils and uncoils its proboscis at the time when the aphid withdraws its stylets.

Table 6. *Teneral time at different temperatures in Aglais urticae* L. (Lepidoptera)

Temperature (° C.)	17.4	18.5	18.8	19.9	20.8	22.8	24.1	26.8	31.8
No. of insects	10	10	10	10	10	10	10	10	7
Mean teneral time in hours	4.7	4.5	3.8	2.9	2.6	2.6	2.3	1.8	1.3

It seems that this obligatory waiting period may be temperature-dependent in groups other than aphids, and that the use of the word 'teneral' in this connexion may have application outside the Aphididae.

Glossina spp., after eclosion, pass through an active, but wingless, 'spider' stage which lasts for up to an hour, and then after expansion of the wings may be described as 'inactive' for the first 2 days (Jackson, 1946). The 'teneral' stage is described as terminated by the closure of the ptilinal suture, except in *G. palpalis fuscipes* in which the suture never closes; but this depends upon the first blood meal to harden the cuticle (Jackson, 1945). Thus the 'teneral' stage would be extended by failure to find food and is not, in that case, development-limited. Buxton (1955) referred particularly to the 'spider' stage as vulnerable, but this probably applies to the inactive stage also, and this may be temperature-dependent as in *Aphis fabae* and *Aglais urticae*, the spider stage corresponding with the shorter interval which these species spend in finding a suitable site to settle down.

The term 'spider stage' could quite well be applied particularly to *Aglais urticae* at that time, and it may be that continued activity defers further development as in the 'walker' stage (Graham-Smith, 1916) of *Calliphora* (Diptera) forced to continue digging in the soil (Fraenkel, 1935). This will not be of general occurrence in wild populations, and in considering teneral time, whatever definition is used, temperature will be a major factor and should be stated.

Whether or not aphids feed during this time is not known, but insects passing their teneral stage on leaves did not move if the leaves wilted, nor did those few on the cage wall depart appreciably from the general pattern of response. Moericke (1955) found a slight reduction, 1-1½ hr, in the teneral time of 'hungry' *Myzus*

persicae Sulz. It would be difficult, without very large samples, to verify this, especially at temperatures other than optimal, since it is well within the standard deviation and it is not known if temperature was taken into consideration.

SUMMARY

1. The teneral stage in *Aphis fabae* Scop. is defined as the interval between eclosion of the alate imago and flight when flight is not extrinsically activated or inhibited.
2. In field marking experiments the teneral stage is shown to be usually development-limited, not behaviour-limited, by its relationship to temperature.
3. It is suggested that the logistic curve fits the rising temperature \times development-velocity curve because this is a statistical distribution like the Pearson type 1.
4. The logistic curve is fitted to teneral period in naturally varying temperatures by a method of successive approximation.
5. In trapping experiments take-off is inhibited by light below *c.* 100 f.c. and by temperature below *c.* 16° C. at 1 ft. in a bean crop.
6. These behaviour responses sometimes lengthen the apparent teneral period.
7. The results are confirmed in the laboratory at constant and at varying temperatures.
8. This also confirms that temperature changes *per se* even over a range of *c.* 7–34° C., and other climatic factors, have negligible effect on rate of development during the teneral period.
9. Individual variation is a constant proportion of teneral time at all temperatures.
10. In laboratory experiments the same criteria apply to *Aglais urticae* L.
11. This period of inactivity warrants further examination in an ecological context.

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REFERENCES

- ANDREWARTHA, H. G. (1944). Air temperature records as a guide to the date of hatching of the nymphs of *Austroicetes cruciata* Sauss. (Orthoptera). *Bull. Ent. Res.* 35, 31–41.
- BROADBENT, L. (1949). Factors affecting the activity of alatae of the aphids *Myzus persicae* Sulzer and *Brevicoryne brassicae* L. *Ann. Appl. Biol.* 36, 40–62.
- BUXTON, P. A. (1955). *The Natural History of Tsetse Flies*. London: H. K. Lewis.
- CORBET, P. S. (1956). Environmental factors influencing the induction and termination of diapause in the emperor dragonfly, *Anax imperator* Leach (Odonata: Aeshnidae). *J. Exp. Biol.* 33, 1–14.
- DAVIDSON, J. (1942). On the speed of development of eggs at constant temperatures. *Aust. J. Exp. Biol.* 20, 233–9.
- DAVIDSON, J. (1944). On the relationship between temperature and rate of development of insects at constant temperatures. *J. Anim. Ecol.* 13, 26–38.
- DUNN, E. (1951). Wing coloration as a means of determining the age of the Colorado beetle (*Leptinotarsa decemlineata* Say). *Ann. Appl. Biol.* 38, 433–4.
- ELDERTON, W. P. (1953). *Frequency Curves and Correlation*, 4th ed. Cambridge University Press.
- FRAENKEL, G. (1935). Observations and experiments on the blow-fly (*Calliphora erythrocephala*) during the first day after emergence. *Proc. Zool. Soc. Lond.* pp. 893–904.
- GRAHAM-SMITH, G. S. (1916). Observations on the habits and parasites of common flies. *Parasitology*, 8, 440–544.

- HAINÉ, E. (1956). Häutung, Abflug und Landung der Blattläuse in Wechselwirkung auf die Blattlauszahlen in der Luft. *Mitt. biol. Bundesanst. Berl.* **85**, 23-7.
- JACKSON, C. H. N. (1945). Comparative studies of the habitat requirements of tsetse fly species. *J. Anim. Ecol.* **14**, 46-51.
- JACKSON, C. H. N. (1946). An artificially isolated generation of tsetse flies (Diptera). *Bull. Ent. Res.* **37**, 291-9.
- JOHNSON, B. (1955). Studies in flight, host selection and reproduction in aphids. Thesis (Ph.D.), London University.
- JOHNSON, C. G. (1952). The changing numbers of *Aphis fabae* Scop. flying at crop level, in relation to current weather and to the population on the crop. *Ann. Appl. Biol.* **39**, 525-47.
- JOHNSON, C. G. & TAYLOR, L. R. (1955). The measurement of insect density in the air. *Lab. Pract.* **4**, 187-92, 235-9.
- JOHNSON, C. G. & TAYLOR, L. R. (1957). Periodism and energy summation with special reference to flight rhythms in aphids. *J. Exp. Biol.* **34**, 209-21.
- JOHNSON, C. G., TAYLOR, L. R. & HAINÉ, E. (1957). Analysis and reconstruction of diurnal flight curves in alienicolae of *Aphis fabae* Scop. *Ann. Appl. Biol.* (in the Press).
- MOERICKE, V. (1955). Über die Lebensgewohnheiten der geflügelten Blattläuse (*Aphidina*) unter besonderer Berücksichtigung des Verhaltens beim Landen. *Z. angew. Ent.* **37**, 29-91.
- TAYLOR, L. R. (1951). An improved suction trap for insects. *Ann. Appl. Biol.* **38**, 582-91.
- TAYLOR, L. R. (1955). The standardization of air-flow in insect suction traps. *Ann. Appl. Biol.* **43**, 390-408.
- TILLYARD, R. J. (1917). *The Biology of Dragonflies*. Cambridge Zoological Series.
- VERWORN, M. (1894). *Allgemeine Physiologie*. Jena.
- WIGGLESWORTH, V. B. & GILLETT, J. D. (1936). The loss of water during ecdysis in *Rhodnius prolixus* Stål (Hemiptera). *Proc. R. Ent. Soc. Lond.* A, **11**, 104-7.