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MOULTING RHYTHM IN THE ALIENICOLAE OF *APHIS FABAE* SCOP. (HEMIPTERA: APHIDIDAE) IN THE FIELD

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(With 1 Text-figure)

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In a natural population of alienicolae of *Aphis fabae* Scop. on field beans in July 1952, the frequency of moulting of the last larval instar into the alate form was usually relatively high between about 04.00 and 08.00 hr. G.M.T. This high rate was followed by a decline to a lower fluctuating rate for the rest of the day. The moulting rate at night was usually very low.

The high rate of moulting in the morning, just as the temperature is rising, may be due to acceleration of nymphal development which leads to a synchronization of moulting, much as teneral development of alatae, if accelerated, produces flight peaks during the day (Johnson, Taylor & Haine 1957): data on the relations of moulting and nymphal development to temperature are, however, insufficient for making a complete analysis along these lines.

Changes in moulting rate during the rest of the day are correlated with temperature and with time, both independently. A rise of 1° C. ambient temperature is associated with an 11.6% rise in moulting rate: a fall of 1° C. with a drop of 10.5% and a lapse of 1 hr. in time by a 5.4% drop in the rate. Sunshine and humidity show no simple correlation with moulting rate.

The migratory flight of alienicolae of *Aphis fabae* away from the beans on which they have bred shows a diurnal periodicity which is often bimodal. The alatae in this flight are produced some hours before by the moulting of the last larval instar and this moulting rate is also periodic (Johnson, 1954).

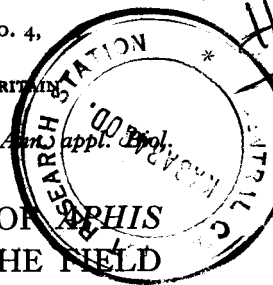
An analysis of the association between moulting and flight was therefore undertaken and data on flight and on moulting rates during the day were collected. In dealing with the whole problem it is more convenient and less confusing if the data on moulting, and the methods used to obtain them, are treated separately. This has been done in the present paper; the relationship between the moulting and flight rhythms is the subject of a companion paper (Johnson, Taylor & Haine, 1957).

METHODS

Counting the numbers moulting

Observations were limited to the moulting of the last instar of *A. fabae* into the alate form in a natural infestation of alienicolae on field beans in July 1952. Forty-eight leaves were taken, more or less at random, along the south and west sides of a small plot of beans (40 × 30 ft.) grown in a sheltered garden at Rothamsted Lodge.

Each leaf was tagged and numbered. As the nymphs on the leaves moulted and



flew away, fresh batches of leaves with aphids were taken: this was done twice during the period of observation—on 8 and 13 July.

Starting in the early morning, all the leaves were examined and alatae counted and carefully removed. At the first count each morning some of the alatae were more or less milky in appearance and had moulted recently: others, obviously older, were presumed to have moulted overnight, probably soon after the last inspection the day before. The leaves were examined subsequently during the day, at approximately hourly intervals, and newly moulted alatae were again counted and removed. The time taken for a complete examination of all leaves varied from 15 to 75 min.: usually each inspection lasted from 20 to 30 min.

Calculation of moulting rates

The present work deals with the last moult, after which the insects would have flown away had they not been removed: successive counts were thus always of different batches of individuals and so in a changing population level.

Had it been possible to count all the aphids in 5 or 10 min., all the forty-eight leaves could have been inspected exactly on the hour and moulting could have been regarded as having occurred at a mean time between the two inspections. Unfortunately inspection times were variable and often lengthy; this introduces some cumbersome arithmetic and accounts for certain discrepancies from the preliminary analysis in Johnson (1954). For example: suppose one inspection lasted from 05.00 to 06.00 hr. and the next from 07.00 to 07.30 hr. Insects newly moulted on the first leaf examined at 07.00 hr. moulted since its last inspection at 05.00 hr.: while those on the last leaf examined moulted between 06.00 and 07.30 hr. This gives mean moulting periods of 2 and $1\frac{1}{2}$ hr. respectively for first and last leaves with a mean *period* of $(2 + 1\frac{1}{2})/2$ hr. for all 48 leaves. From this, the mean rate of moulting (aphids per hour per 48 leaves) is calculated. The mean *time* of this moulting is midway between the mean times for first and last inspection periods—namely between 05.30 and 07.15 hr. or at 06.23 hr. \pm 53 min. and therefore appears in Table 1 (see later) in the interval 06.00–06.30 hr.

The first moults counted in the morning could never be assigned to a known period and thus there was no possibility of estimating confidently the mean time or the rate of moulting for a known period *before* the first morning count. All that could be done was to separate those insects which appeared to be freshly moulted that morning from those, obviously older, which presumably had moulted overnight. This was usually straightforward, the difficulty was to estimate the mean age and so to estimate the hourly moulting rate of the newly moulted alatae in the first count.

After laboratory tests at different temperatures it was considered safe to regard all aphids with a milky appearance or with the legs still not fully pigmented, as having moulted within 4 hr. of the first inspection. Probably the period was much less than this—perhaps 2 hr.—with most of the insects. However, this count is treated separately in the analysis.

Meteorological records

Two bulbs of a mercury-in-steel distance thermograph were kept within the crop at 1 and 4 ft. above the ground: they were shaded by the crop and each also by a double roof several inches above the bulb. Wet- and dry-bulb readings were taken each hour with a whirling psychrometer at about 2 ft. above the ground within the crop and as near as possible to the experimental leaves. The temperatures of the upper thermograph bulb were, in general, about 2°C. higher than those of the lower bulb. A full record of these temperatures is given in the paper on flight rhythm (Johnson *et al.* 1957).

THE CHANGING MOULTING RATE

The validity of the first morning peak

Mean numbers moulted per hr. on forty-eight leaves, calculated as described above, are shown in Tables 1 and 2. In general, the moulting rate was relatively high early in the morning, usually followed by a lower but variable rate for the rest of the day. Sometimes the rate of moulting rose towards the end of the day; moulting rates at night were usually very low. A typical day is shown in Fig. 1.

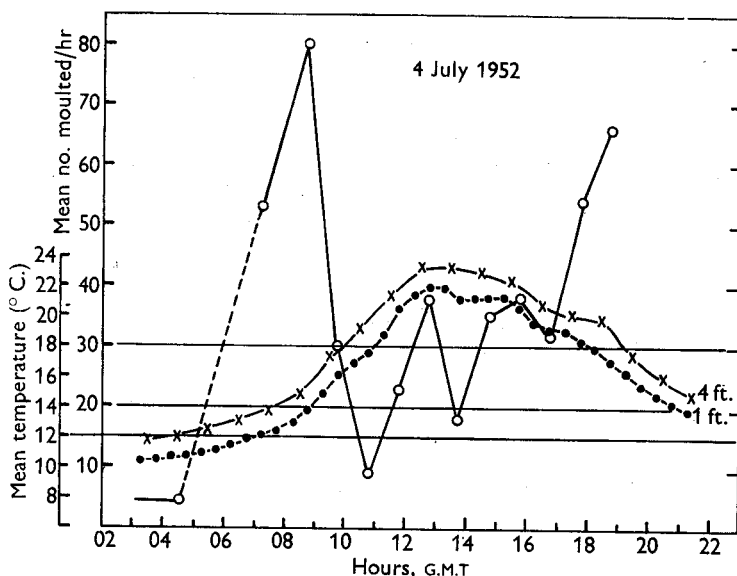


Fig. 1. Typical curve of the moulting rate of the last nymphal instar into the alate adult during the day. *A. fabae* Scop. (*alienicolae*) on beans, 4 July 1952. Data for other curves in Tables 1 and 2.

In view of the variation within days and from day to day and of the uncertainty of the first count in the morning, an analysis of variance was made to test the significance of changes in moulting rates after the first count. The original data for each day from 4 to 18 July (excluding the first morning count) was divided into the

periods 06.00-10.00, 10.00-14.00, 14.00-18.00 and 18.00-19.00, 20.00 or 21.00 hr. and the mean log (no. moulted per hour) was obtained for each period. The analysis is summarized in Table 3.

Numbers moulting change significantly from one period to another during the day. The mean values show that the morning decline was significant and therefore appears to be a real daily characteristic. There was no significant rise, however, in the evening owing to the irregularity both in the appearance and in the timing of the peak (see Table 1).

TABLE 1. *The mean rate of moulting (aphids/hr./48 leaves) of Aphis fabae, at Rothamsted Lodge, July 1952, excluding the first count in the morning*

Date (July) Time (G.M.T.)	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
06.00-06.30	.	.	33
06.30-07.00	.	16	62	.	.	.	55
07.00-07.30	53	.	42	158	42	.	.	61	74	20	.	43	48	.	41
07.30-08.00	46	.	.	*	*	*	.	.	60	.	12	12	.	.
08.00-08.30	.	.	*	61	19	.	68	48	27	13	23	48	44	.	35
08.30-09.00	80	15	13	.	*	*	*	13	.
09.00-09.30	.	.	.	57	9	19	34	26	41	21	48	47	30	32	9	14	.	.	6
09.30-10.00	30	50	*	*	.	.	.	15	.	.
10.00-10.30	*	*	.	25	36	43	40	29	55	32	33	18	13	11	18	24	.	.	.
10.30-11.00	9	10	21	*	*	.	.	.	8	.
11.00-11.30	.	.	.	19	7	37	32	29	17	29	30	.	19	5	29	23	.	.	5
11.30-12.00	23	17	19	.	.	*	.	17	.	.
12.00-12.30	.	.	25	25	43	34	35	27	19	31	15	.	28	24	3	10	.	6	2
12.30-13.00	38	6	2
13.00-13.30	.	11	.	50	37	26	25	26	17	47	49	35	11	24	18	.	.	.	3
13.30-14.00	18	.	24
14.00-14.30	.	6	.	12	22	45	24	25	31	53	20	22	33	29	19	19	.	.	.
14.30-15.00	35	*
15.00-15.30	.	.	18	9	28	40	41	15	.	24	22	28	8	4	10	.	5	3	3
15.30-16.00	38	7	*	18	*	*	.	.	*
16.00-16.30	.	*	.	18	12	21	64	27	*	50	31	27	15	5	18
16.30-17.00	32	54	8	.	.	*	14	7	3	3
17.00-17.30	*	.	.	9	5	50	49	27	17	23	22	13	11	40	14
17.30-18.00	54	22	6	*	*	*	*	.	*
18.00-18.30	.	.	*	22	.	46	43	1	.	66	24	16	35	43	29
18.30-19.00	66	.	.	.	46	.	.	.	16	10	.	.	.
19.00-19.30	.	.	.	21	.	39	47	.	.	22
Total	476	208	190	486	306	446	564	341	332	431	372	316	295	277	243	126	62	29	20

Data for each day between the asterisks are used in the regression (Table 5).

Fluctuations in moulting rate after the first peak

It was obvious that changes in the moulting rate during the rise and fall of the first peak were not correlated simply with temperature, although during the rest of the day temperature clearly played a part. Therefore the rise and fall of the first peak and the apparent rise at the end of the day were excluded from the data for each day as shown by the asterisks in Table 1. The remaining data on hourly moulting rates, somewhat arbitrarily selected for the middle part of the day, were plotted against the hourly mean temperature. An extremely large scatter was shown,

a rise of 1° C. was accompanied by a rise of 11.6% in numbers moulted and a fall of 1° C. by a fall of 10.4% over a temperature range of 16–31° C. Similarly, a lapse of 1 hr. in time was accompanied by a decrease of 5.4% in numbers moulted.

Sunshine and atmospheric humidity

Correlations were sought between moulting rate and both atmospheric humidity and the duration of sunshine. Neither with 1-hourly periods nor with 4-hourly periods were such correlations found, and this applied whether the factor was measured during the current moulting period or in the previous period.

DISCUSSION

The moulting sequence from the final instar nymphs of *A. fabae* into alatae shows a characteristic daily periodicity. It consists of a high rate of moulting in the early morning, followed by a lower but variable rate during the day, sometimes rising again in the evening and falling again almost to zero during the night. Biological rhythms of this kind are usually classified as endogenous when they are maintained even under constant external conditions, and exogenous when they appear in response to external, usually climatic, factors.

There is little reason to believe that the moulting rhythm is endogenous. The irregularities in the timing of the early morning peak and the occasional appearance and irregularity of the evening peak do not suggest an inherent rhythm (see also Haine, 1957).

Of the possible exogenous factors meteorological conditions undoubtedly have a profound effect on the moulting rhythm. It was apparent that the rise and fall of numbers moulting during the first peak were not correlated simply with any single factor; the succeeding fluctuations were, however, significantly correlated with temperature such that an increase in temperature tended to be accompanied by an increase in the moulting rate and a decrease by a decrease in the moulting rate. No simple correlations were found between the moulting rate and other climatic factors.

In addition, a significant correlation was found with time of day, independently of any associated temperature changes. In the period under consideration, the regression shows a consistent trend towards a decrease in the numbers moulting during the day. This fall was not the result of a general reduction in the population from day to day, as there was no over-all decrease in the total number of moults per day during the experimental period. In order that the same general level of numbers moulting should be maintained, the numbers must increase again before the commencement of the succeeding period, an increase not involving the early morning peak. It is quite possible that, although independent of current temperature changes, the decrease reflects previous temperature changes in their effect on the development and moulting rates of the late instar nymphs. The two processes of development and moulting probably have different temperature coefficients and thus, within the population at any given time, either of these processes may be

proceeding more rapidly than the other, depending upon the general temperature level. With a fluctuating temperature therefore, fluctuations in the developmental rates of the nymphs, and also in the moulting rates of the nymphs, would occur, and these processes, interacting but not coinciding, would result in fluctuations in the supply of fully developed nymphs. This could account for the correlation of numbers with time, for in general, temperature fluctuates rhythmically with time.

It could also account for the early morning peak, which might result from the accumulation of unmoulted nymphs during the night, the temperature presenting a bar to moulting as with the first peak of flight (see case 1 in Johnson & Taylor, 1957). Alternatively, it could be caused by the slowing down of development during the night, complete development being accomplished rapidly in the morning as the temperature rises (see case 6 in Johnson & Taylor, 1957). But the temperature relations of rate of development in *A. fabae* are not yet sufficiently well known to be able to verify this.

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