EFFECTS OF THE ANT, *LASIUS NIGER* L., ON THE FEEDING AND EXCRETION OF THE BEAN APHID, *APHIS FABAE* SCOP.

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Banks (1958) confirmed that colonies of *Aphis fabae*, attended by the ant *Lasius niger*, multiply more than ant-free colonies on bean plants, in the absence of the aphids' insect enemies. He showed that, when the aphids were ant-attended, they changed their excretion behaviour and that the normal dispersal of adult apterae from young apical growth of bean plants was delayed. No significant differences were found between the reproduction rates of individual ant-attended and ant-free aphids living on leaves of the same age, but the reproduction rates were significantly affected by the age of the plant tissue on which the aphids fed. The increased multiplication of ant-attended colonies was attributed to the delay in dispersal of adult apterae from the young growth, where they reproduced more, to the old growth, where they reproduced less.

Herzig (1937) considered that ant-attended aphids multiplied more than unattended ones because they fed more. He found that *Aphis fabae*, attended by *Lasius fuliginosus* Latreille, and *Aphis sambuci* L., attended by *Lasius niger*, each excreted more honeydew than when unattended, and assumed that the attended aphids therefore absorbed more plant sap. He further assumed that this supposed increase in feeding was the cause of the observed increase in multiplication of ant-attended aphids on a bean plot, and claimed that the stimulation of being fed upon could double or treble aphid numbers.

The experiments described below were made during the summer of 1957 to test the idea that the excretion and feeding rates of *Aphis fabae* on beans are stimulated by the attendant ant *Lasius niger*.

METHODS

Bean plants on which aphids were feeding were made radioactive with ^{32}P so that the aphids took up the isotope and excreted it in their honeydew. The radioactivity of the honeydew taken from them by attendant ants was then compared with that of the honeydew emitted concurrently by unattended control aphids on separate plants.

^{32}P was selected as the most suitable isotope because earlier work had shown that it is relatively simple to grow plants with leaves of the high specific radioactivity required for work of this kind (Watson & Nixon, 1953; Day & Irzykiewicz, 1953). Preliminary experiments showed that ^{32}P is excreted in considerable quantities by *Aphis fabae*

living under our experimental conditions. Because ^{32}P is concentrated in the reproductive organs of adult aphids (Watson & Nixon, 1953), losses of radioactivity due to the birth of nymphs would be considerable; to overcome this difficulty nymphs of apterous virginoparae of *A. fabae* were used in all these experiments.

Broad beans (*Vicia faba*) were germinated in wet sand and the seedlings transferred when about 10 cm. high into bottles containing 160 ml. of Hoagland's water-culture solution deficient in phosphorus. Several alate adult aphids were confined to one leaf of each plant in a small plastic box-cage fitted around the whole leaf, and allowed to settle and reproduce for 2 days. The cage was then removed and the alatae and excess nymphs picked off to leave one small group of nymphs on the undersurface of the leaf. ^{32}P, as H_3PO_4 with a small amount of 'carrier' phosphate to facilitate transfer, was introduced into the culture solution at the rate of 300 μc./l. of solution. To prevent contamination of the surroundings with radioactive honeydew, a black filter-paper was arranged below the aphids. Black paper was used because preliminary work showed that light reflected from white paper disturbed the aphids, causing them to move about the leaf. The plants were left for 12–48 hr. to allow the leaves and aphids to take up the isotope. When the plants and aphids were ready for use, the black filter-paper below the aphids was replaced with a wire frame supporting a filter-paper stained with bromocresol green, an indicator which changes from yellow to blue when honeydew falls on it (Smith, 1937; Broadbent, 1951), so that the drops could be seen and counted.

Two plants were then placed in a large raised cage standing in a garden and containing bean plants infested with aphids already being attended by ants (*Lasius niger*) which were able to enter and leave the cage through a small hole in the floor. The experiment was begun by trapping ants as they entered the cage and placing one of them on the aphid-bearing leaf of one of the radioactive plants. At the same time the indicator papers were put under both groups of aphids and a stop watch started to time the experiment. The ant was confined to the leaf by a band of grease on the petiole. When it was replete and ready to leave the aphids, it was removed, killed, put aside and immediately replaced by another ant and the whole process repeated. At the end of the experiment the ants, indicator papers, aphids and weighed samples from both leaves were taken for assay. Each sample was dissolved in hot nitric acid, diluted as necessary, and counted in a jacketed G.M. tube attached to a conventional scaling unit.

Ants sometimes investigated with their mouth parts the leaf surface from which aphids had recently been removed. This suggested that the ants might possibly become contaminated with ^{32}P by absorbing some sap directly from the plants. To test this possibility, nine ants were placed on leaves of high radioactivity from which aphids had just been removed. The ants, which were confined three at a time to three leaves in muslin bags, were seen to behave as described at the leaf surfaces. After 1 hr. they were removed, killed and assayed, but gave no detectable increase in count over the natural background rate.

For an average group of about thirty apterous nymphs used in these experiments, the rate of excretion was ascertained by recording the number of drops of honeydew

discharged in 30 min. by 668 nymphs on twenty-four separate plants. On the average, a nymph excreted $0{\cdot}75 \pm 0{\cdot}05$ drop of honeydew in 30 min.; large nymphs, like adult apterae, excreted less often but produced larger drops than small nymphs.

Over periods of 2–3 hr., the excretion rate of a group of apterous nymphs feeding on bean plants in water culture was found to be fairly constant from one $\frac{1}{2}$ hr. to the next, so long as conditions did not vary greatly. Thus, the number of drops of honeydew excreted by eight groups of apterous nymphs (mean, 22 nymphs per group) in successive periods of 30 min. were: 0·51, 0·44, 0·56, 0·47, 0·52, 0·57 (mean, $0{\cdot}51 \pm 0{\cdot}05$ drop per 30 min.).

RESULTS

Series I

The radioactivity of the honeydew collected by a number of ants from a group of aphids (*A*) was compared with that of the honeydew excreted concurrently by a similar group of unattended aphids (*B*) on a separate plant.

Preliminary observations showed a wide variation in the radioactivities of leaves and of aphids feeding on them; many experiments were, therefore, considered necessary to cover the expected range of variation.

In each of twenty-eight experiments, at least three ants (average, four per experiment) were used successively so that the aphids were continuously attended. The duration of the experiments, which varied from 19 min. to 158 min., averaged 58 min. and the average numbers of aphids were 34 ± 3 (ant-attended) and 32 ± 2 (ant-free). The time an ant spent attending depended on the number and size of the aphids of the group; on the average it was 20 min., although in some experiments it was as short as 10 min. Nearly all the ants attended assiduously until their crops were filled.

The radioactivities of the aphids were not correlated with the duration of the experiments but, as expected, were closely dependent on the radioactivities of the leaves on which they fed. In turn, the radioactivities of the honeydew samples were correlated with the radioactivities of the aphids from which they came.

The distributions and means of the radioactivities of the leaf samples, aphids and honeydew samples (expressed in counts per minute) are shown on logarithmic scales in Fig. 1. The distributions for the leaf samples are very similar and their means (*A*, 2·44, *B*, 2·38) are almost identical; those of the aphid radioactivities are also closely similar to each other and the mean radioactivities (*A*, 2·66, *B*, 2·51) also do not differ significantly.

But the distributions of the radioactivities of the two sets of honeydew samples are conspicuously different; that of the ant-free aphids is skew to the left, that of the ant-attended aphids to the right. The two means (*A*, 1·46, *B*, 1·06) are significantly different ($P < 0{\cdot}01$).

The radioactivity of the honeydew from the ant-attended aphids was apparently double that from the ant-free aphids. It is possible that both groups of aphids over a long period give off the same amount of active honeydew, the ant-attended aphids

producing it quickly to the ants and then becoming exhausted, the ant-free aphids giving off the same amount more slowly. To test this possibility, other experiments were made.

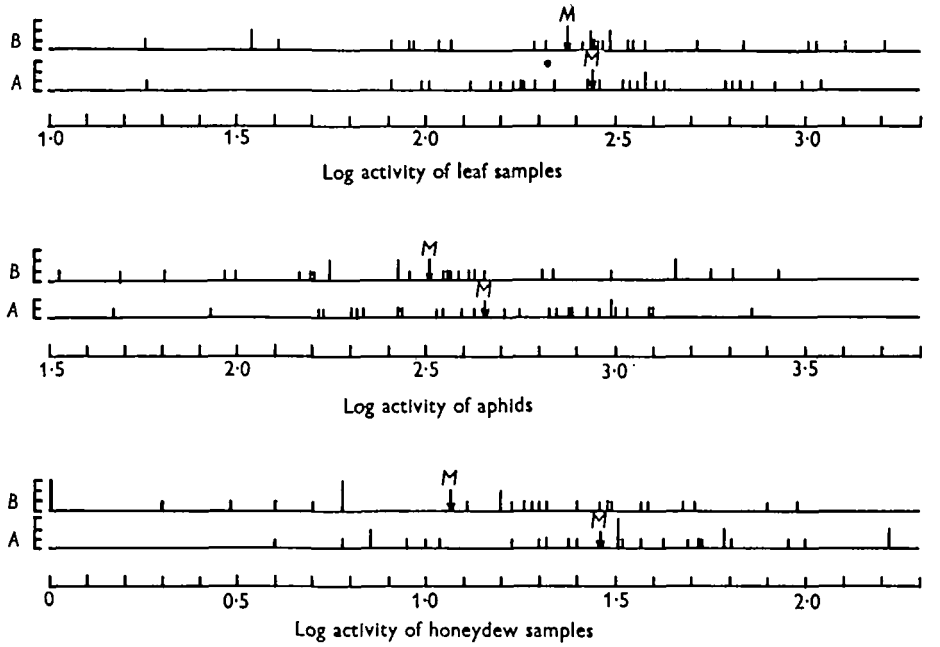

Fig. 1. Logarithmic values of radioactivity of leaf samples, in counts per minute per milligram fresh weight; of aphids, (*A*) ant attended, (*B*) ant free, in counts per minute per aphid; and of honeydew samples in counts per minute per drop. (*M*) mean logarithm. The vertical scales show the number of observations at each value.

Series II and III

In five experiments (Series II), the radioactivity of the honeydew from a group of aphids (*A*), attended by a single ant, was compared with that from a similar group of ant-free aphids (*B*); this time, each experiment was divided into five consecutive periods as follows:

*T*1—a period to establish the 'normal' rate of excretion of the two groups of aphids; it lasted 30 min. and was followed immediately by *T*2.

*T*2—the time which a ***single*** ant spent attending the aphids on the experimental plant *A*; it lasted 32 min. on the average. When the ant was replete with honeydew and showed signs of leaving the aphids, it was removed and killed.

*T*3—followed immediately after the removal of the ant. During this period the attended aphids did not emit any honeydew for an average time of 19 min. (range, 15–25 min.). They remained motionless for some time, but then became more and more restless, waving their abdomens and legs in the manner of unattended aphids about to excrete. The ant-free aphids continued to excrete at their normal rate during *T*3.

*T*4—began as soon as the first drop of honeydew was excreted by the attended aphids, and lasted for 30 min. During this period, the rate of excretion of the

attended aphids was half the normal rate and the drops of honeydew were conspicuously larger than those produced before the ants attended the aphids.

$T5$—followed immediately on $T4$ and also lasted for 30 min. The rate of excretion of the attended aphids tended to return to normal during this period.

Throughout the experiments of Series II, each of which lasted about 2½ hr., the ant-free control aphids excreted at their normal rate. The relative changes in activity of the honeydew from both groups of aphids are shown in Table 1.

During $T1$, both sets of aphids of the Series II experiments produced honeydew whose relative radioactivities were identical; but, as expected from the Series I results, the activity of the honeydew collected by the ants during $T2$ was double that from the ant-free aphids.

Table 1. *Relative changes in radioactivity of honeydew from ant-attended (A) and ant-free (B) aphids. Series II. Results of five experiments with* 151 *(A) and* 183 *(B) aphids. Series III. Results of six experiments with* 177 *ant-attended aphids*

Series II

Period	Radioactivity of honeydew per aphid		Mean radio-activity per drop		Rate of excretion per 30 min.		Mean duration (min.)
	A	*B*	*A*	*B*	*A*	*B*	
$T1$	1·00	1·00	1·00	1·00	1·00	1·00	30
$T2$	2·08	1·03	—	1·04	—	1·08	32
$T3$	0	1·05	0	1·07	0	0·64	19
$T4$	1·06	1·08	1·96	1·11	0·49	1·00	30
$T5$	1·69	1·11	2·27	1·15	0·63	1·01	30

Series III

Period	Radioactivity of honeydew per aphid	Mean radio-activity per drop	Rate of excretion per 30 min.	Mean duration (min.)
$T1$	1·00	1·00	1·00	30
$T2$	2·60	—	—	28
$T3$	0	0	0	18
$T4$	0·88	0·84	0·50	30
$T5$	1·94	2·50	1·00	30

Although the ant-attended aphids excreted nothing during $T3$, the ant-free aphids still excreted honeydew whose radioactivity added to that they had already produced during $T2$ equalled that collected by the ants.

During $T4$, each group of aphids excreted honeydew of almost identical radioactivity, but by the end of $T5$, the honeydew radioactivity from the ant-attended aphids was 52% greater than that from the controls.

In interpreting these results, the first problem was to decide whether the attended aphids were exhausted of honeydew by the ants and therefore could excrete only at a reduced rate during $T4$ and $T5$; or whether they replenished the honeydew during $T3$ and were holding it back until an ant came to remove it.

The first of these suppositions must be rejected because of the following facts:

(1) the excretion rate of the attended aphids was increasing and tending to return to normal during $T4$ and $T5$, as it did in another set of six experiments (Series III; Table 1). (2) During $T3$, the attended aphids were raising and waving their abdomens as if ready to excrete. (3) The drops of honeydew which attended aphids eventually excreted during $T4$ and $T5$ were conspicuously larger than normal drops. (4) The radioactivity of these drops was double that of normal drops; that is, the rate of excretion is not so significant as the size and activity of each drop. (5) Finally, the experiments of Series I showed that successive ants were able to remove twice as much radioactivity from the aphids they attended as the controls produced freely. It follows, therefore, that the attended aphids had not been exhausted of honeydew for long, but had replenished it rapidly during $T3$; and that the few which excreted during $T4$ and $T5$ did so because they could not wait any longer, the others continuing for a while to hold back their honeydew. The experiments show, therefore, that when attended by ants, the aphids produced twice as much radioactivity in their honeydew as ant-free aphids did.

The next problem was to consider the source of the 'extra' radioactivity in the honeydew of the ant-attended aphids; it could come either from the aphids' tissues or from the plant, following an increased uptake of sap by the aphids.

If the radioactivity of the honeydew came from the aphids' tissues, there would be a heavy drain of phosphorus which would have to be replaced by an increased rate of intake and an even more rapid rate of assimilation of phosphorus into the tissues, for we know from the Series I experiments that the radioactivities of the two sets of aphids did not differ significantly at the end of the experiments. There would also be a rapid drain from the tissues of fluid carrying the extra radioactivity and a correspondingly rapid replacement. There is no mechanism in these aphids for the rapid removal of fluid from the gut. The elaborate 'filter chamber' of many Homoptera, whose function is thought to be the by-passing of excess fluid from the anterior to the posterior part of the gut (Wigglesworth, 1953; Waterhouse, 1957), does not occur in *Aphis fabae* and most other aphids, which have not even the simplest of these devices (Weber, 1930); nor have aphids any Malpighian tubes, which are the typical excretory organs of insects (Weber, 1930).

It must be concluded that the ant-attended aphids produced the 'extra' radioactivity in the honeydew by increasing the uptake of radioactive sap from the plants.

It follows, therefore, as Herzig supposed, that the ants directly stimulated both the excretion and feeding rates of the aphids.

DISCUSSION

It was long thought that aphids fed by actively sucking up plant sap, until Kennedy & Mittler (1953) and Mittler (1957) showed that the aphid *Tuberolachnus salignus* (Gmelin) during its normal feeding depends almost entirely on the pressure of sap within the phloem sieve tubes of the plant to force the sap up its stylet food canal.

Mittler concluded that the rate at which the plant forces the sap up the stylet food canal determines the rate of sap uptake and excretion by the aphids. He also

said that the aphids must 'actively swallow' the sap forced up the canal but does not suggest how this could happen. Other workers are under the impression that the aphid has little or no control over its feeding and excretion and that the uptake of sap is continuous. Thus, Waterhouse (1957) supposed that 'the turgor pressure in the plant tissue largely maintains the flow of sap through the stylets into the alimentary canal of the insect so that, once a suitable source of sap is tapped, ingestion is a relatively passive process'; again, Ewart & Metcalf (1956), referring to the article of Kennedy & Mittler, said that 'plant pressure forces large amounts of sap, in the form of honeydew, through the insect's alimentary system'; Bodenheimer & Swirski (1957) also understood that 'the sap is forced into the aphid by its own pressure (Mittler, unpublished data)'.

Mittler (1957) described an experiment in which he anaesthetized feeding *T. salignus* with CO_2 for 24 hr., during which time they did not excrete or become distended. When he severed the inserted stylets of these aphids, however, sap exuded from the cut ends showing that, although it was still under pressure, it had not continued to enter the anaesthetized aphids which had been able to shut off the flow. It has already been stated that *Aphis fabae* can control its rate of excretion by withdrawing the honeydew into the anus when ant-attended (Banks, 1958), and the experiments now described show that the aphids hold back the honeydew until an ant comes to remove it and that they alter their rate of feeding when ant-attended. The excretion and feeding rates are therefore directly controlled by the aphids themselves and are not determined solely by the forces within the plant; the plant merely provides sap under pressure which the aphids are able to tap as required.

The mechanism for controlling sap-intake is probably the so-called 'sucking pump' in the aphid's head (Weber, 1928). This dilatation of the anterior part of the gut lies between the proximal end of the stylet food canal and the oesophagus into which it leads. It is depressed from front to rear and its anterior wall, thinner than the posterior wall, is attached by muscles to the anterior wall of the head. When the muscles contract, they would dilate the lumen of the pump and admit sap under pressure from the stylet food canal; when they relax, the anterior wall would return to its normal position by elasticity and shut off the flow of sap; there are no retractor muscles to close the pump. If the flow of sap into the aphid were continuous, these muscles would be permanently contracted. We suggest that they are normally relaxed so that the pump remains closed; but that periodically they contract to admit sap. Thus, when Mittler anaesthetized aphids, the dilator muscles remained relaxed so that the aphids did not feed and therefore did not become distended.

If this is so sap which has entered the pump would no longer be under pressure and could not, therefore, enter the oesophagus and stomach unless forced. We suggest that the pump opens ventrally to admit sap and then closes; the sap is then forced into the stomach by the closure of the pump, starting at the ventral end. During normal feeding, periods of opening and closing of the pump probably occur at regular intervals, corresponding to the regular emissions of honeydew; when the aphid is ant-attended, the pump would operate more frequently so as to force sap into the stomach more often. But, as Mittler pointed out, the artificial feeding

experiments of Hamilton (1935) and Maltais (1952) showed that aphids can take up limited amounts of fluid which is not under pressure, possibly by suction.

Herzig (1937) supposed that the increased feeding of ant-attended aphids improved their nutrition and consequently increased their reproduction. Waterhouse & Day (1953) quoted Herzig and suggested that the increase in reproduction might be caused by an increased intake of protein. El-Ziady & Kennedy (1956) thought that *Lasius niger* might exercise some control over the physiology of *Aphis fabae* and suggested that the ant 'raises the plane of nutrition of the aphid, perhaps as a result of stimulating its excretion and thereby its feeding as Herzig (1937) thought'.

No differences were found between the reproduction rates of individual ant-attended and ant-free aphids living on leaves of the same age (Banks, 1958). It is concluded that the stimulation of feeding caused directly by attendant ants has little, if any, effect on the aphid's reproduction rate, which is significantly affected, however, by the age of the plant tissue on which it feeds. It seems, therefore, that the reproduction rate of the aphid is affected more by the nature, than by the quantity, of the nutrients which the aphid receives.

SUMMARY

1. To test the idea of Herzig (1937) that the excretion and feeding rates of aphids are stimulated by attendant ants, bean plants (*Vicia faba*), on which groups of nymphs of *Aphis fabae* were feeding, were made radioactive with ^{32}P in water culture, so that the aphids took up the isotope and excreted it in their honeydew. The radioactivity of the honeydew taken from them by attendant *Lasius niger* was then compared with that of the honeydew excreted concurrently by unattended control aphids on separate plants.

2. By increasing their uptake of plant sap the ant-attended aphids produced twice as much radioactivity in their excreta as did the ant-free aphids.

3. The aphids directly control their rates of excretion and feeding, which are not determined solely by forces within the plant.

4. The aphid apparently controls its feeding by the 'sucking pump' in its head. It is suggested that the pump is normally closed but that periodically it opens to admit sap into its lumen and then closes ventrally to force the ingested sap into the stomach. During normal feeding the pump probably opens and closes at regular intervals; but when the aphid is ant-attended it could operate more frequently so as to force sap into the stomach more often. The uptake of sap by normally feeding aphids is apparently not continuous.

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