STUDIES ON THE FEEDING AND NUTRITION OF *TUBEROLACHNUS SALIGNUS* (GMELIN) (HOMOPTERA, APHIDIDAE)

III. THE NITROGEN ECONOMY

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Since Tóth (1940) questioned that the dietary nitrogen supply of aphids is adequate for their growth and reproduction, considerable controversy has existed regarding the necessity and ability of aphids to supplement their dietary nitrogen supply with atmospheric nitrogen through the agency of their symbiotic micro-organisms. Few data are available, however, on the amounts of nitrogen actually ingested, excreted, and assimilated by aphids. The aim of the present investigation was to determine these data for *Tuberolachnus salignus* (Gmelin), and to resolve the controversy for this species.

MATERIAL AND METHODS

T. salignus was reared, handled, and caged as previously described (Mittler, 1957, 1958*a*). Potted, 2- to 4-year-old, *Salix acutifolia* Willd. trees, in various stages of leaf development, were used as host plants. The experiments were carried out in a greenhouse at a mean temperature of 20° C. under a photo-period of 16 hr.

Nitrogen assimilation of apterous and alate T. salignus. The following techniques were used to study the assimilation of nitrogen by a generation of apterous *T. salignus*: Twenty apterous adult aphids were confined in each of ten cages set up on the upright main stem (5–6 ft. long) of a single host plant. After 48 hr. the cages were opened, the adult aphids destroyed and 200 of their progeny of 800–1000 first-instar nymphs collected at random from all the cage sections. The average nitrogen content of the 200 first-instar nymphs was determined, after vacuum desiccation, by the standard micro-Kjeldhal steam distillation technique. The average nitrogen content of aphids in subsequent samples and that of cast skins were similarly determined.

The plant was then placed on its side on a bench and the pot wrapped in damp sacking to prevent the soil from spilling and drying out. In order to trap the skins cast by the nymphs which were allowed to remain and develop further on the stem a crystallizing dish containing water was placed under each of the ten cage sections. A daily count and collection of the cast skins established the percentage of all the nymphs on the stem which had moulted every 24 hr., and permitted the average nitrogen content of the skins cast by each instar to be determined. On the completion

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of each moult by all the nymphs 50–100 aphids were collected at random from the stem and their average nitrogen content determined. As the nitrogen assimilation of the apterous form of *T. salignus* was to be investigated exclusively in this experiment, all nymphs with wing pads (termed alate nymphs) were destroyed when the nymphs had completed their third ecdysis. The sample of fourth-instar nymphs therefore consisted entirely of apterous nymphs. The sample of third-instar nymphs may, however, have included a few alate nymphs as the aphids in this sample were not examined microscopically. External differences between potential apterous and alate individuals in the first and second instars are not apparent.

When the apterous nymphs remaining on the stem had reached the adult stage, the plant was set upright again and ten of the young apterous adults were confined in each of ten stem cages. Daily thereafter the entire nymphal progeny of the adult aphids was counted and collected; the average nitrogen content of the nymphs born each day subsequently being determined. Periodic samples of ten adult aphids were collected at random from the ten cages in order to determine the average nitrogen content of the adult aphids throughout their life.

The daily amount of nitrogen expended per adult aphid through reproduction (termed the *daily nymphage*) was estimated by multiplying the number of nymphs produced per adult per day with the average nitrogen content of the nymphs born per day. The total amount of nitrogen expended in reproduction per adult aphid since the onset of reproduction (termed the *total nymphage*) was calculated each day by summing the daily nymphage values. The total amount of nitrogen assimilated per adult aphid was periodically established during the aphids' adult life by adding the appropriate total nymphage value to each available value of the aphids' average nitrogen content.

In order to compare the nitrogen assimilation of apterous and alate *T. salignus* the above techniques were also used. The period between which the first and last nymph in each instar moults was reduced, however, by allowing the twenty apterous adults initially confined in each of the ten cages to remain on the stem for 24 hr. only. Samples of first- and second-instar nymphs were consequently omitted so that a sufficiently large number of aphids should be available for sampling later during their development. Nymphs in third- and fourth-instar samples were examined under a binocular microscope in order to separate alate and apterous individuals. Nitrogen determinations were then made of the two forms in each instar. When all the nymphs had reached the fourth instar they were collected and the alate nymphs separated from the apterous. Twelve of the alate nymphs were then confined in each of five cages and seventeen of the apterous nymphs in each of five other cages, set up on the stem in alternate positions. It was readily possible thereafter to compare the further development of the two forms and to establish their daily nymphage and total nymphage under identical environmental conditions.

Nitrogen balance. It has previously been shown (Mittler, 1953, 1958*a*) that the total nitrogen (amino-acid and amide) concentration of the phloem sap of willows varies considerably with the developmental stage of their foliage. The hypothesis, that *the dietary nitrogen-supply received by* T. salignus *is adequate to support the*

aphid's nitrogenous requirements, was therefore tested in five separate experiments in which willows having a mature foliage and a phloem sap poor in nitrogen were used in three of the experiments. A willow with a foliage in an active stage of growth and another with a foliage in an advanced stage of senescence were used in the other two experiments, both these willows having a phloem sap relatively rich in nitrogen.

Three cages, each 1 in. apart, were set up on the upright stem of each willow and twenty apterous adults confined in each cage. After 24 hr. the cages were opened, the adult aphids destroyed and their progeny collected from the uppermost and lowermost (later termed the two 'outer') of the three cage sections. The average nitrogen content of these 0–24 hr. old nymphs was subsequently determined. The willow was then placed on its side on a bench, the usual precautions being taken to maintain the soil moist within the pot. In order to collect all the honeydew droplets excreted by the nymphs remaining on the stem in the central cage section a 3 × 4 in. mica or glass plate was placed 1–2 in. under the aphids. Flies and other insects were denied access to the aphids or to the honeydew on the plate by enclosing the central cage section and the plate in a glass and plywood box, 6 in. cube, having a removable glass front panel. On each of the 7–21 days of an experiment the nymphs were counted and the plate carefully removed and a clean one put in its place. The honeydew on each plate was rapidly dried in a vacuum desiccator, in which the plates were also stored. The daily amount of sugar excreted per nymph was subsequently estimated by dissolving the honeydew on each plate in water and determining the total sugar concentration of the solution by the method of Morris (1948).

The method of Morris (1948) was also used to determine the total sugar concentrations of daily samples of freshly excreted honeydew. These samples, 5–10 mm.^{3} in volume, were collected by means of a pipette from the surface of a waxed glass plate placed in the box for a few minutes each day. The methods of Tomkins & Kirk (1942) and Shaw & Beadle (1949) were used to determine the nitrogen concentrations of honeydew samples similarly collected and of phloem sap samples collected by the method previously described in detail (Mittler, 1958*a*). The amount of sugar excreted per nymph per day was divided by the corresponding concentration of sugar in the honeydew to give the volume of honeydew excreted per day; the total volume of honeydew excreted per nymph for a complete experiment was obtained by summing the daily values. The average nitrogen concentration of the phloem sap ingested by the nymphs during their development was determined by analysing periodic samples of the phloem sap ingested within 2–3 in. of the nymphs by some adult apterous aphids; the latter being allowed to feed for some hours per day on the stem in each of the two 'outer' cage sections.

At the end of the experiment the box was removed, the nymphs collected and their average nitrogen content determined. By subtracting the average nitrogen content of the 0–24 hr. old nymphs from this value, the amount of nitrogen assimilated per nymph was established; a small addition being made for the nitrogen in the skins cast by the nymphs. By multiplying the total volume of honeydew excreted per nymph by the difference in the average nitrogen concentrations of the phloem

sap and honeydew the total amount of dietary nitrogen absorbed per nymph throughout the experimental period was established.

Nitrogen assimilation in relation to nitrogen level of phloem sap. In an experiment to determine simultaneously the nitrogen assimilation of *T. salignus* nymphs ingesting phloem sap of different nitrogen concentrations, two willows (*A* and *B*) with an actively growing foliage and a phloem sap 'rich' in nitrogen (greater than 0·1%, w/v), one willow (*C*) with a foliage in an advanced state of growth and a phloem sap 'medium' in nitrogen concentration (0·05–0·1%, w/v), and three willows (*D*, *E* and *F*) with a mature foliage and a phloem sap 'poor' in nitrogen (less than 0·05%, w/v) were used as host plants. Three cages were set up on each willow and twenty apterous adults confined in each cage. After 24 hr. the cages were opened and the adult aphids destroyed. When the nymphal progeny, which remained on the stems, were 7–8 days old their form, instar and average nitrogen content were determined.

It has previously been shown that the rate of excretion of *T. salignus* is a good index of the aphid's feeding rate (Mittler, 1957). In order, therefore, to compare the feeding rates of nymphs developing for 7–8 days on willow *B* with those of nymphs developing for the same period on willow *D* the rates of excretion of the nymphs on the two willows were estimated daily by determining the average number of honeydew droplets excreted by them per hour and the average volume of their honeydew droplets (Mittler, 1958*b*). The nitrogen concentrations of the honeydew excreted by the nymphs on the two willows were compared by matching the colour intensities produced by various dilutions of their honeydew spotted on filter-paper treated with ninhydrin. As in the previous experiment, the form, instar and average nitrogen content of the nymphs were determined when they were 7–8 days old.

RESULTS

Nitrogen assimilation of apterous and alate Tuberolachnus salignus

Fig. 1 shows the percentage of the nymphs in each instar which had moulted on each day of the experiment. The arrows *A–E* indicate when the four nymphal samples and the first apterous adult sample were taken for nitrogen determinations. The average nitrogen content of these samples is plotted in Fig. 2. The points *E–F* represent the average nitrogen content of the apterous aphids during their adult life. Points *X–Y* represent the total nymphage values. The total amount of nitrogen assimilated per apterous aphid throughout its nymphal and adult life is indicated by the points *A–E–G*. It may be noted that there is no abrupt change in the rate of nitrogen assimilation of apterous *T. salignus* at the onset of reproduction. After 9–10 days of reproduction the apterous aphids had given birth to thirty to thirty-five young per aphid and their total nymphage was equal to the average nitrogen content of their bodies.

Fig. 3 shows the percentage of apterous and alate nymphs in the third and fourth instars which had moulted on each day of the experiment. The arrows *C* and *D* indicate when nymphal samples of each form were taken, and the arrow *E* indicates

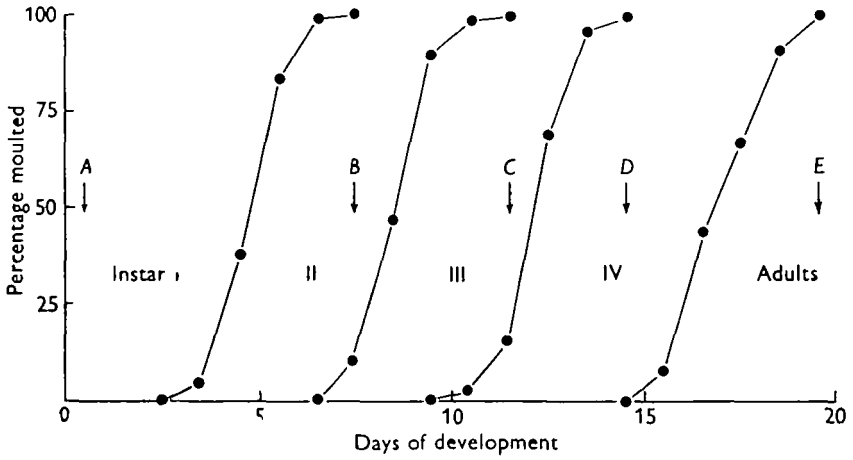

Fig. 1. Percentage of apterous nymphs moulting in first to fourth instars. Arrows indicate times of sampling.

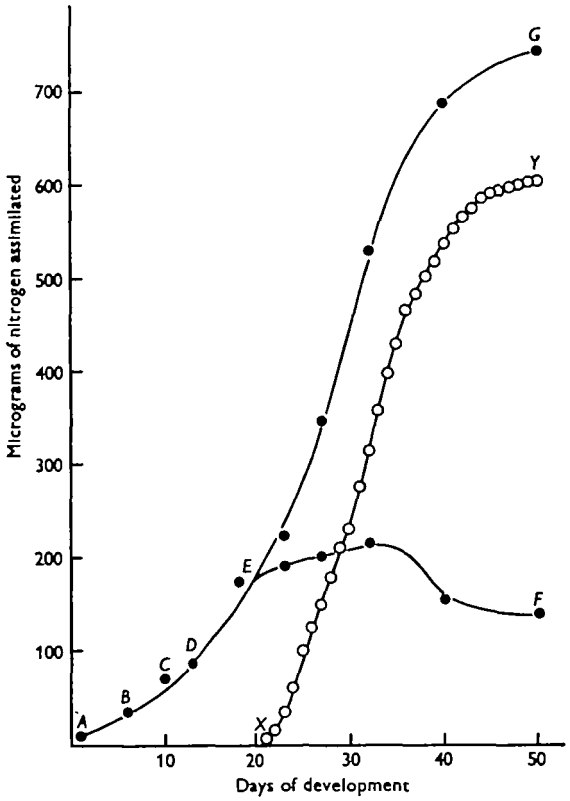

Fig. 2. Nitrogen assimilation of apterous *Tuberolachnus salignus*. For explanation see text.

when the first apterous adult sample was collected. In Fig. 4 the values of the average nitrogen content of the apterous nymphs and adults are represented by the points *C–E–F*, the total nymphage values of the apterous adults by the points *X–Y* and the total amount of nitrogen assimilated by the apterous nymphs and adults by the points *C–E–G*. The corresponding values for the alate nymphs and adults are represented by the points *C–P–Q*, *S–T* and *C–P–R*.

It is apparent from Fig. 3 that the alate third-instar nymphs moulted approximately 1 day after the apterous third-instar nymphs, and that the alate fourth-instar nymphs took 2–3 days longer to reach the adult stage than the apterous fourth-instar nymphs. As in the previous experiment, apterous adults began to bear young

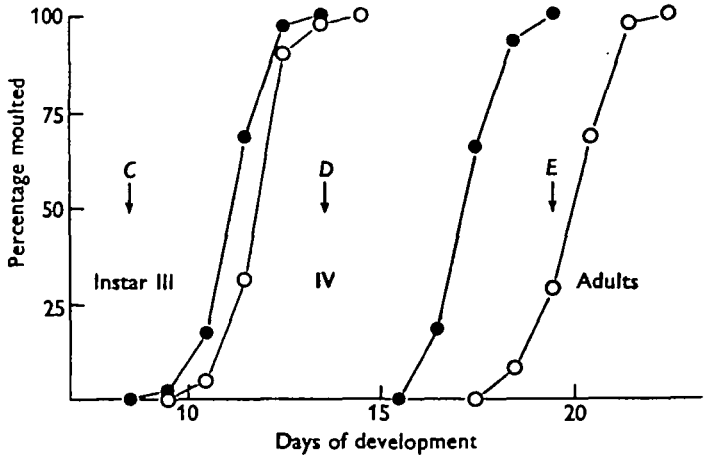

Fig. 3. Percentage of apterous (—●—) and alate (—○—) nymphs moulting in third and fourth instars. Arrows indicate times of sampling (see text).

approximately 2–3 days after their final moult; alate adults, however, did not start to bear young until 6–7 days after their final moult. This period of adult development may be called the *pre-reproductive* period. Whereas the average nitrogen content of apterous and alate nymphs in the third and fourth instars differed only slightly, the nitrogen content of the apterous adults at the onset of their reproduction was almost twice as great as that of the alate adults when they began to bear young. The following observations may throw some light on these differences.

The apterous adults tended to wander about in the cages for several hours on the first day of their adult life, but had settled to feed by the second day. This was indicated by honeydew droplets which began to accumulate on the cage walls at this time. The abdomen of these young apterous adults appeared swollen, and on dissecting some of them as many as seventy to eighty embryos in all stages of development were found in each aphid. Some dozen of the embryos in each aphid apparently were ready for birth.

The alate adults spent the first 3–4 days of their adult life sitting on the stem or cage walls. During this period few honeydew droplets were observed in the cages

and only a few of the aphids were observed to have their stylets inserted into the stem, indicating that they were not ingesting any appreciable amount of food. The abdomen of each of the aphids was slender and contained only about thirty small embryos. On the fourth day of their adult life the aphids began to excrete as much honeydew as was being excreted by the apterous adults. After 2–3 days of feeding the abdomen of the alate adults had become distended and the aphids gave birth to their first young. Within 5 days of reproduction the aphids gave birth to an average of fifteen nymphs each and their total nymphage was equal to the average

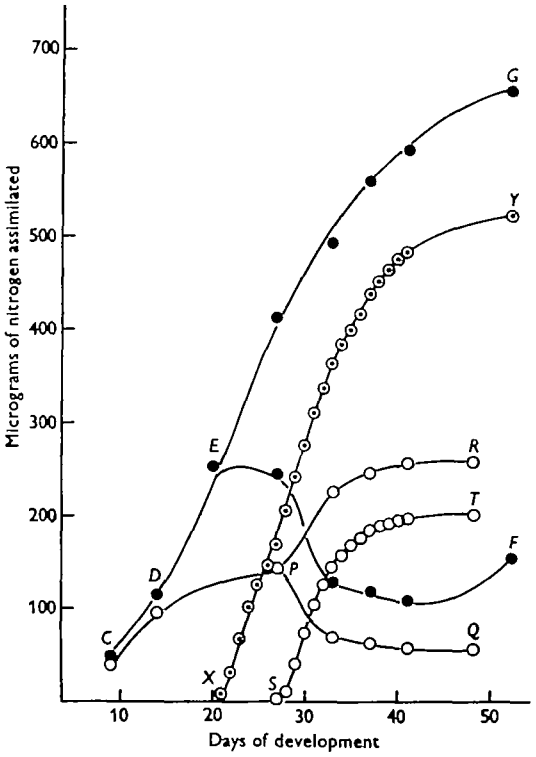

Fig. 4. Nitrogen assimilation of apterous and alate *Tuberolachnus salignus*. For explanation see text.

nitrogen content of their bodies. It is apparent from Fig. 4 that the total nymphage rate of the alate adults during the first 8 days of their reproduction does not differ greatly from the total nymphage rate which the apterous adults maintained during more than twice that period. The total number of young born per alate adult was forty-two compared to an average of eighty young to which the apterous adults gave birth. The reproductive life of the apterous adults was 22 days, however, compared to 15 days of reproduction by the alate adults.

During the last week of their adult life the apterous adults gave birth to very few young and their average nitrogen content increased somewhat. It was noted that

they continued to excrete large amounts of honeydew during this (*post-reproductive*) period and that their abdomens remained distended. However, not more than four embryos were found in each aphid dissected. Instead, their abdomen was found to be packed with thousands of deeply pigmented globules. The abdomen of alate adults appeared shrunken at the end of their reproductive period and was found to be filled with large wax-like concretions.

Nitrogen balance

Table 1 summarizes the results obtained in each of the five nitrogen balance experiments. It may be noted that the estimated amounts of nitrogen which the nymphs absorbed from the phloem sap they ingested during their development on each of the five willows do not differ greatly from the amounts of nitrogen actually

Table 1. *Balance sheet of nitrogen ingested, excreted and assimilated by* Tuberolachnus salignus *nymphs*

Experiment ...	1	2	3	4	5
Foliage of willow ...	Senescent	Actively growing	Mature	Mature	Mature
Duration of experiment (days)	22	9	7	11	11
Amount of nitrogen assimilated per nymph (μg.)	87·50	173·2	46·96	73·89	58·82
Total volume of honeydew excreted per nymph (mm.^{3})	102·4	261·6	130·1	200	300
Nitrogen conc. of phloem sap (w/v)	0·128	0·122	0·052	0·039	0·032
Nitrogen conc. of honeydew (w/v)	0·050	0·055	0·016	0·0–0·01	0·0–0·01
Amount of nitrogen absorbed per nymph (μg.)	78·9	175·3	46·8	58–78	66–99
% nitrogen absorbed from phloem sap	61	55	69·3	74–100	69–100
Volume of honeydew excreted per μg. of nitrogen assimilated (mm.^{3})	1·17	1·52	2·78	2·7	5·1

assimilated by the aphids, the discrepencies falling within the 10–20% error to which the estimations were subject. From the volumes of honeydew excreted it appears that (per μg. of nitrogen assimilated by them) *T. salignus* nymphs ingest a relatively larger volume of sap poor in nitrogen than of sap rich in nitrogen. It is also clear that the nitrogen lost by excretion is not necessarily negligible compared to that ingested and cannot therefore be neglected when computing the nitrogen balance (cf. Smith, 1948).

Nitrogen assimilation in relation to nitrogen level of phloem sap

Table 2 sets out for comparison the average nitrogen content of nymphs which had developed for the first 7–8 days of their lives on each of six willows differing in the nitrogen concentration of their phloem sap. The number of nymphs on each

willow in the second, third and fourth instars is stated, as well as the number and percentage of alate individuals amongst the third- and fourth-instar nymphs on each willow. It may be noted that the average nitrogen content of the 7- to 8-day-old nymphs on the 'nitrogen rich' willows *A* and *B* was almost ten times greater than that of the nymphs on the 'nitrogen poor' willows, *D*, *E* and *F*. The nymphs on plants *D*, *E* and *F* were, moreover, not much bigger than they were at birth and many of them were still in the second instar. The nymphs on plants *A* and *B*, on the other hand, were of 'normal' size, had all reached the third instar and several of them had already reached the fourth instar. It is also interesting to note that the percentage of alate nymphs is lowest on willows *A* and *B*.

Table 2. *Development and nitrogen assimilation of nymphs in relation to nitrogen level of phloem sap*

Phloem sap nitrogen level ...	Rich		Medium	Poor		
Plant	*A*	*B*	*C*	*D*	*E*	*F*
Average nitrogen content of 7- to 8-day-old nymphs (μg.)	109·0	116·0	46·8	14·5	11·7	11·5
No. of nymphs in each instar	40, 3rd; 9, 4th	141, 3rd; 32, 4th	166, 3rd	63, 3rd	35, 2nd; 14, 3rd	78, 2nd; 6, 3rd
No. of alate 3rd and 4th instar nymphs	5	34	83	26	17	8
% alate 3rd and 4th instar nymphs	10·2	19·7	50	41·3	54·8	57·1

Table 3. *Development and nitrogen assimilation of nymphs in relation to nitrogen level of phloem sap*

Phloem sap nitrogen level	Plant	Instar reached	Ratio of apterae to alatae	% alate	Total vol. of honeydew excreted (mm.³)	Nitrogen assimilated (μg.)	Vol. of honeydew excreted per μg. of nitrogen assimilated (mm.³)
Rich	*B*	3rd–4th	32:13	23·6	110	104·9	1·06
Poor	*D*	3rd	15:46	66·8	140	5·7	24·4

Table 3 sets out the results obtained in another experiment in which nymphs were again allowed to develop for 7–8 days after their birth on willows *B* and *D*. The amino-acid concentration of the honeydew excreted by the aphids feeding on willow *B* was found to be twenty-five times greater than that excreted by the aphids on willow *D*. The nymphs on willow *D* had all reached the third instar, although they were not much bigger than they were at birth and had assimilated almost no nitrogen during the 7–8 days of their development. The average nitrogen content of the nymphs on willow *B*, on the other hand, had increased twenty-fold since their birth and a few of them had already reached the fourth instar.

It may also be noted that the average volume of honeydew excreted per μg. of nitrogen assimilated by the nymphs on willow *D* was considerably larger than that

excreted by the nymphs developing on willow *B*. Table 4 summarizes the honeydew excretion data for the nymphs in the first, second and third instars on each willow. It is immediately apparent that the excretory activity and hence also the feeding activity of the nymphs on willow *D* is considerably greater than that of the nymphs on willow *B*; the nymphs on willow *D* having a higher rate of excretion and a higher frequency of excretion, but a lower droplet volume (in consequence of their small size), than the nymphs on willow *B*.

Table 4. *Honeydew excretion of nymphs in relation to nitrogen level of phloem sap*

Instar	Phloem sap nitrogen level	Plant	Frequency of excretion (drops/hr.)	Droplet size (mm.^{3})	Rate of excretion (mm.^{3}/hr.)
1st	Rich	*B*	1·5	0·071	0·106
	Poor	*D*	11·3	0·055	0·622
2nd	Rich	*B*	2·68	0·136	0·364
	Poor	*D*	9·86	0·077	0·759
3rd	Rich	*B*	3·18	0·305	0·969
	Poor	*D*	6·2	0·173	1·073

DISCUSSION

In 1912 Peklo examined the mycetocytes of aphids and concluded that they contain symbiotic micro-organisms resembling *Azotobacter* bacteria, a group of bacteria known to be responsible for the fixation of atmospheric nitrogen in the root nodules of leguminous plants. This conclusion has recently been upheld by Peklo (1953) to counter claims made by Lanham (1952*a*, *b*) that the particles contained in the mycetocytes of aphids are not symbiotic bacteria but cell particulates. Assuming, however, that mycetocytes do contain symbiotic *Azobacter* bacteria Tóth (1940, 1946, 1952) claimed that these micro-organisms also provide aphids with reduced atmospheric nitrogen. The evidence for and against the fixation of atmospheric nitrogen by aphids may be discussed under following headings.

In vitro *fixation.* Tóth (1946, 1952) reported that the nitrogen content of nutrient media in which breis of various aphid species were incubated, increased by 100–150% in 24 hr., the only source of nitrogen being the air. Smith (1948) repeated these experiments in detail but could not detect any *in vitro* fixation of atmospheric nitrogen. Koch (1952) showed, however, that a pure culture of the symbiotic bacteria of a coccid (*Pseudococcus citri*) reduces atmospheric nitrogen and synthesizes six of the eight amino-acids which occur in the coccid's blood.

In vivo *fixation.* Smith (1948) exposed young feeding colonies of *Myzus persicae* and *Doralis* (*Aphis*) *fabae* to an atmosphere of oxygen and nitrogen containing 31·5% of the stable isotope ^{15}N. No trace of the isotope was detected in the aphids after they had been exposed to such an atmosphere for 2–3 days. It might be supposed, however, that under the conditions of the experiment the aphids were receiving an abundant dietary nitrogen supply and hence not forced to draw upon atmospheric nitrogen, or that they are only forced to do so during reproduction.

As *in vitro* nitrogen fixation does not occur unless the nitrogen content of the culture medium is very low, Fraenkel (in a discussion of Tóth, 1952) questioned the ability of symbionts to fix atmospheric nitrogen in the nitrogen rich milieu of an insect cell.

Respiratory quotient. Tóth & Wolsky (1941) demonstrated that living *Pterocallis juglandis* have a R.Q. of 0·86. This value was claimed to indicate that protein is practically the sole substrate being oxidized by the aphids; the implication being that reduced atmospheric nitrogen is available to the aphids in large enough quantities for them to oxidize it. It may be noted, however, that a R.Q. of 0·86 may also indicate an oxidation by fasting aphids of a nitrogen-free substrate composed of carbohydrates and fats in the proportion 7:3.

Nitrogen content of sap and honeydew. Data on honeydew and phloem sap from divers sources led Tóth (1940, 1946) to suppose that the nitrogen concentration of the honeydew excreted by aphids is greater than that of the phloem sap they ingest; this supposition implying that reduced atmospheric nitrogen is available to aphids in such quantities that they have to excrete some of it. Michel (1942) indicated that the honeydew excreted by *Lachnus roboris* contains twice as much nitrogen per dry weight as the phloem sap of the aphid's host plant. These results may be questioned, however, on the grounds that the phloem sap was collected directly from oak stems, whereas the honeydew was collected from the aphids colonizing severed oak branches. Lindemann (1948) has since clearly demonstrated that the honeydew excreted by *Cryptomyzus ribis* has a consistently lower nitrogen content than the phloem sap of *Ribes rubrum* branches colonized by the aphids. Such a relationship between phloem sap and honeydew has also been amply confirmed for *T. salignus* with respect to each amino-acid and amide as well as with respect to the total nitrogen contained by these fluids (Mittler, 1953, 1958*a*). If some of the amino-acids in honeydew were the products of atmospheric nitrogen fixation one might expect a few of them at least to differ from the amino-acids found in the phloem sap. This was not found to be the case for *T. salignus* honeydew and *Salix* phloem sap. It is interesting to note, however, that Gray (1952) found six to seven amino-acids in the honeydew of *Pseudococcus brevipes* which were not present in sap expressed from the coccid's host plant. The possibility that these amino-acids are general metabolic products of the coccid cannot be ruled out.

Nitrogen assimilation. Tóth (1940) claimed that the nitrogen expended per day in terms of young by an adult *Aphis sambuci* at the peak of its reproduction is equal to the aphid's own weight of nitrogen, and that the dietary nitrogen supply is inadequate for this. The results of the present study show that apterous *T. salignus* expend only about one-tenth of their own weight of nitrogen per day when maintaining a steady rate of reproduction. Although alate *T. salignus* expend one-fifth of their own weight of nitrogen per day during their shorter period of reproduction this is not due to a higher total nymphage rate, but due to the lower nitrogen content of the alate adults. The rate at which nitrogen is assimilated during reproduction by the alate adults is in fact less than that of the apterous adults. During reproduction both forms draw to some extent on nitrogen assimilated by them during their

nymphal life; the alate adults apparently doing so right at the onset of reproduction. This is readily understood when one considers that embryos are already being formed during the nymphal development of apterous and alate *T. salignus*, and that autolysis of the wing muscles of the alate adults normally sets in shortly before they begin to bear their first young (Johnson, 1957). A limited amount of 'stored' nitrogen is therefore available and used up during reproduction.

Tóth (1940) showed that mycetocytes increase in number and that symbionts get dissolved in the body cavity of an aphid when it reproduces. This was interpreted to indicate that the aphid calls on its symbionts to supply it with atmospheric nitrogen for reproduction. Although there is a gradual increase in the rate at which nitrogen is assimilated by apterous *T. salignus* during their development it was noted that at the onset of reproduction there was no marked change in the assimilation rate. This indicates that apterous *T. salignus* when reproducing do not call upon a process of nitrogen assimilation differing from that responsible for their nymphal nitrogen assimilation. The marked change in the assimilation rate of alate *T. salignus* at the onset of their reproduction has already been discussed. It was felt, therefore, that any conclusions which might be drawn from the results of the experiments on the assimilation of dietary nitrogen by *T. salignus* nymphs would also apply to *T. salignus* adults when reproducing.

Lindemann (1948) pointed out that if aphids were able to draw on atmospheric nitrogen their growth should be independent of the nitrogen level of the phloem sap of their host plant. Lindemann found, however, that the size of *Cryptomyzus ribis* was positively related to the nitrogen concentration of the phloem sap of the host plant. The results of the present investigation also indicate that the size and nitrogen content of *T. salignus* depend directly on the nitrogen concentration and volume of the phloem sap ingested. *T. salignus* assimilate only as much nitrogen as they are able to absorb from the phloem sap ingested by them. Although certain aphid species may have evolved the ability to assimilate atmospheric nitrogen, no evidence for this has been found from a study of *T. salignus*, which is a species belonging to the 'primitive' aphid family—the Lachnidae.

The results further indicate that a higher proportion of aphids develop wings on a diet poor in nitrogen than on one relatively rich in nitrogen. This is in agreement with the findings of Evans (1938). It may be speculated that apterous adult aphids, which may be considered to be neotenous individuals, are produced in response to a nitrogen-rich food supply from nymphs whose reproductive development is in advance of the differentiation of their adult characters.

SUMMARY

1. The investigation concerns the ingestion, excretion, and assimilation of dietary nitrogen by *Tuberolachnus salignus* (Gmelin) developing on *Salix* trees in various stages of leaf development.

2. Apterous and alate forms of *T. salignus* differ in their rates of development and nitrogen assimilation and in their behaviour.

3. The aphids assimilate considerably more nitrogen when developing on willows having a phloem sap rich in nitrogen than on willows having a phloem sap relatively poor in nitrogen.

4. For a given amount of nitrogen assimilated the aphids ingest a larger volume of a phloem sap poor in nitrogen than of a phloem sap rich in nitrogen.

5. Nitrogen balance experiments have shown that *T. salignus* nymphs assimilate only as much nitrogen as they are able to absorb from their food.

6. The evidence for and against the fixation of atmospheric nitrogen by aphids through the agency of their symbionts is briefly discussed. *T. salignus* do not supplement their dietary nitrogen supply by fixing atmospheric nitrogen.

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