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

I. THE UPTAKE OF PHLOEM SAP

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It has generally been supposed that the uptake of large quantities of plant sap by aphids is the direct result of suction. Zweigelt (1914) has questioned this concept, and Kennedy & Mittler (1953) have thrown further doubt on the supposition that aphids are typical 'sucking' insects. The present paper sets out results, obtained in a study of the uptake of phloem sap by *Tuberolachnus salignus* (Gmelin), which may help to clarify this question.

MATERIALS AND METHODS

The experimental aphid, *T. salignus*, is only known to occur in the apterous and alate parthenogenetic viviparous forms. The species was reared throughout the year on stem-cuttings of *Salix alba* L. 2–3 ft. long. These were planted in sandy soil in 7 in. clay pots and were kept in a greenhouse at a mean temperature of 20° C. under a photoperiod of 16 hr. Non-dormant stems readily rooted and produced leafy shoots within 1 or 2 weeks. Dormant stems were stimulated into growth by treating them with a rooting hormone (Seradix 3) and by applying some bottom heat to the pots. The culture was started by placing some naturally infested willow branches over rooted stem-cuttings in three pots. After a week three further pots were set up in close proximity to these, and new aphid colonies rapidly established themselves. A weekly production of several hundred apterous adult aphids was maintained by adding three pots of willow stems to the culture each week and discarding these after every 4 or 5 weeks. A working culture consisted of 12–15 pots. As needed, a hundred or more apterous adults 1–2 days old were obtained from the stems and from the rim of the pots where they tended to wander. The aphids were collected individually by means of a camel-hair brush or by the use of forceps with flattened tips.

Several species of *Salix* were used as experimental host plants for *T. salignus*. Unless otherwise stated 2–4-year-old trees of *S. acutifolia* Willd. (also known as *Salix daphnoides* Vill.), with straight main stems 5–6 ft. long, were used throughout these studies. This species was found to support the heaviest naturally occurring infestations of *T. salignus*, and was readily obtainable for potting.

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To determine the course taken by the stylet bundles of *T. salignus* in the stems of *S. fragilis* L. the proboscides of feeding aphids were severed according to the method outlined by Kennedy & Mittler (1953). Pieces of the stem containing the stylet bundles were fixed in Carnoy's fluid, cut into transverse and longitudinal tangential sections, and stained according to the method of Rawitscher (1933).

The relative positions of the tips of the mandibular and maxillary stylets of *T. salignus* at different stages of their insertion into willow stems were determined by withdrawing, by means of forceps, severed embedded stylet bundles and examining these microscopically.

To assess the contribution made by the pressure of the phloem sap in willow stems to the rate of sap uptake by normally feeding *T. salignus*, the rate at which aphids excrete honeydew and the rate at which sap exudes from the cut ends of their embedded stylet bundles were simultaneously estimated. This was achieved by collecting and measuring the volume of honeydew excreted in an hour by a colony of aphids of the same age, and by collecting and measuring the volume of sap exuded in 5–8 hr. from the severed stylets of one or more of the aphids in the colony. Measurements of the radius of the food canal within the stylets of adult *T. salignus* were obtained from electron-microscope photographs taken of the stylets. The length of the stylets was measured under a binocular microscope. The viscosity of phloem sap passing through the stylet food canal was estimated by measuring the total sugar concentration of the sap by the method of Morris (1948). An estimate of the osmotic pressure of the phloem sap was also obtained from a determination of the total sugar concentration.

In order to determine whether the ingestion of sap by *T. salignus* is an active process, colonies of feeding aphids were rapidly anaesthetized so that the aphids had no time to withdraw their inserted stylets. This was achieved by directing a gentle stream of carbon dioxide gas on to the colonies. Aphids were maintained anaesthetized by this means for 24 hr.

To test the effect of wilting and loss of turgor of the host plant on aphid feeding, the foliage of some potted willows was induced to wilt by allowing the soil to dry out over a period of several days. Wilting and loss of turgor were also induced by making deep incisions into the bark of *S. alba* stems about small colonies of *T. salignus*, and gently prising the bark away at its cambial layer. This procedure did not disturb the feeding aphids directly.

The dormancy of potted *S. acutifolia* trees, which in winter were kept outdoors, was broken within 2–3 weeks by bringing the plants into the greenhouse.

RESULTS

(*a*) *The disposition of the stylets in the host plant*

The stylet bundles of feeding *T. salignus* were found to pass inter- and intracellularly through the cortical tissues of *S. fragilis* stems and to end in the newly differentiated phloem sieve-tubes adjacent to the cambium. It may be noted that

investigations by Horsfall (1923), Davidson (1923), Smith (1926), Rawitscher (1933), Romell (1935), Grossmann (1937), Tate (1937), Roberts (1940), Leonhardt (1940) and Lindemann (1948) have shown that a large number of other aphid species also insert their stylet tips into the phloem sieve-tubes of their host plant.

The stainable sheath of saliva, which surrounds the stylet bundles within a willow stem, was found to extend beyond the surface of the willow bark in the form of a small cone about the stylet bundles. Within cells the sheath had a smooth outline, but within intercellular spaces it had a beaded appearance. A small accumulation of stainable saliva was occasionally found where fibre bundles diverted stylets from their radial course to the phloem. Salivary sheaths in the phloem were occasionally observed to branch. An accumulation of stainable saliva was never detected beyond the innermost limit of a stylet bundle in the phloem.

Microscopic examination of the stylet bundles of feeding aphids showed the curved tips of the maxillary stylets to be apposed to one another, and the serrated tips of the mandibular stylets to be staggered and a short way behind the former. The observation by Leonhardt (1940), that the food and salivary canals do not terminate at the extreme tips of the maxillary stylets, was confirmed. The canals appeared to open into a lenticular cleft a short way behind their apposed tips. Stylet bundles in various stages of insertion showed the tips of the maxillary and mandibular stylets to be staggered.

The extent to which the stylets of feeding *T. salignus* are inserted into the stems of several species of *Salix* was found to be related to the age of the stems. Whereas only the distal end of a stylet bundle is inserted by an aphid feeding on 1-year-old stems, the entire length is inserted into 5–6-year-old stems. In 2–4-year-old stems stylet bundles are inserted for one-third to one-half of their length. It may be noted that the distance between the surface of a willow stem and the newly differentiated phloem elements adjacent to the cambium increases each year due to the secondary thickening of the stems.

(*b*) *The mechanism of sap uptake*

The radius of the stylet food canal of adult *T. salignus* was found to be 1·8μ near the aphid's head and 0·6μ at the distal end. The stylets of adult *T. salignus* were found to be 1·8 mm. long, and the viscosity of the phloem sap passing through the stylet food canal was estimated to be close to 1 centipoise. Measurements of the rate of sap exudation from severed stylets and of the rate of honeydew excretion from feeding aphids showed the normal rate of flow of sap through the stylet food canal of adult aphids to lie between 1 and 2 cu. mm. per hour. Applying Poiseuille's relationship to the flow of phloem sap through the stylet food canal of aphids, it was calculated that the pressure required to maintain a rate of flow of 1–2 cu. mm. per hour through the stylet food canal of a feeding adult *T. salignus* is 20–40 atm.

Measurements of the total sugar concentration of exudates from severed stylets indicate that the osmotic pressure of the phloem sap of *S. acutifolia* is at least

5 atm. and probably considerably greater than 10 atm. One may note that Münch (1930) and Dixon (1933) have shown the phloem sap of *Quercus*, *Robina* and *Fraxinus* spp. to have an osmotic pressure of 20·9 to 37·3 atm.

T. salignus, which were feeding in colonies on the main stem of *S. acutifolia* trees, dispersed and made repeated efforts to reinsert their stylets into the stems as soon as the foliage of the trees showed signs of wilting. The leaf-feeding aphid *Cavariella aegopodii* (Scop.), which was also present on these trees, dispersed at the same time.

T. salignus inserted their stylets for short periods into dormant willow stems, but did not excrete honeydew and presumably did not feed. As soon as the dormancy of the plants was broken and bud swelling occurred the aphids began to feed normally on the stems.

T. salignus ceased to excrete honeydew but maintained their stylets inserted for 2–3 hr. in pieces of bark after these had been stripped from the cambial layer of *S. alba* stems.

In a like manner, *T. salignus* feeding on a *S. fragilis* tree ceased to excrete 2–3 hr. after stems on which they were feeding were cut into 5–6 in. lengths. The aphids, however, maintained their stylets inserted for 5–6 hr. Exudation of sap from severed stylets also ceased 2–3 hr. after the stems were cut. This is in contrast to the unabated exudation of sap for more than 24 hr. frequently observed from severed stylets embedded in intact stems of various *Salix* spp. A large droplet of sap could be made to exude from severed stylets which had ceased to exude by pressing on the bark within 1 cm. of the stylets. On releasing the pressure the droplet was withdrawn into the plant through the stylets, showing that cessation of sap exudation was not due to blockage of the stylets but due to insufficient pressure within the plant.

Table 1 sets out the results of experiments in which the average rate of honeydew excretion of *T. salignus* in each instar, and the average rate of sap exudation from their severed stylets were simultaneously determined.

Table 1. *Average rate of excretion of honeydew by* Tuberolachnus salignus *in each instar and the average rate of sap exudation from their severed stylets at* 20° C.

Instar	Average rate of excretion (cu. mm./hr.)	Average rate of sap exudation (cu. mm./hr.)
First	0·45	0·64
Second	0·73	0·72
Third	0·94	1·05
Fourth	1·36	1·46
	1·43	1·47
Adult apterous	2·06	1·89
	1·87	1·75
	1·82	1·80
	2·08	1·82
	1·84	1·89
	1·71	1·79

Feeding aphids which were subjected to carbon dioxide anaesthesia for 24 hr. did not exude sap from any part of their body nor did they become distended. When their stylets were severed after this period sap exuded readily from the embedded stylet stumps showing that the pressure was maintained within the stylet food canal.

CONCLUSIONS AND DISCUSSION

The fact that *T. salignus* and other aphid species insert their stylet tips into the phloem sieve-tubes of their host plant suggests that the insects ingest sieve-tube sap in preference to other plant sap. A high nutrient value of the sieve-tube sap has generally been supposed to be the basis of the aphid's choice. The pH value of the sieve-tube sap has also been held responsible for its preferential selection by other plant-feeding Hemiptera (Fife & Frampton, 1936).

Although aphids may choose the sieve-tube sap as food because of its taste or nutrient value their choice may have a different basis. When an aphid inserts its stylets into a plant numerous cells are pierced in succession. The vacuolar sap of each of the cells is under a certain turgor pressure, and as the cells are pierced some of their sap is forced into the stylet food canal. As the cortical cells contain only a limited amount of sap under pressure they can only force a very small volume of nutrient sap up the stylet food canal of a feeding aphid. The phloem sieve-tubes, on the other hand, whose contents are in some form of organic continuity (Crafts, 1939; Esau, 1939), may be compared to an extensive system of anastomosing 'pipe-lines' which may supply an aphid with large amounts of nutrient sap under undiminishing pressure. The efficiency with which such a system supplies an aphid with the sap it ingests can be gauged from the fact that an uptake of 1 cu. mm. of sap per hour by one aphid from a single phloem sieve-tube cell indicates that a volume of sap 100,000 times greater than the capacity of the cell passes through it in 1 hr. This may involve a rate of sap flow of 500 cm. per hour within the phloem sieve-tubes of a willow stem which is comparable to the highest rates of translocation listed for other plants by Tammes (1952).

It therefore appears that aphids use the phloem as a feeding site mainly because this tissue has the greatest capacity for *maintaining* a supply of nutrient sap under pressure.

It may be suggested that the stainable saliva which forms the stylet sheath is only injected into a pierced cell after the turgor pressure of its sap has been reduced by the entry of some of the sap into the stylet food canal. When stylets pierce a phloem sieve-tube and the pressure is maintained aphids do not pump (or are unable to pump) stainable saliva down the salivary canal of their stylets, although the stylet tips may remain in a sieve-tube for many hours. It seems probable that phloem sap enters the stylet food canal of a feeding aphid through the cleft between the curved tips of the maxillary stylets when they are apposed to one another.

The occurrence of branched salivary sheaths in the phloem of a plant has been interpreted by Zweigelt (1914) to indicate that an aphid may simultaneously obtain sap, in a haustorial-like manner, from all the branches its stylets have made in the

phloem. This phenomenon may, however, also be interpreted as an aphid's response to a local exhaustion of phloem sieve-tube sap, or lack of sufficient pressure for the aphid to be able to maintain its normal feeding rate. It appears to be a convenient method for an aphid to seek out and tap different parts of the phloem of its host plant successively, without it having to withdraw its stylets completely and to reinsert them elsewhere.

The observations that *T. salignus* do not feed on dormant willows and that they seek out the newly differentiated phloem sieve-tubes adjacent to the cambium of secondarily thickened willow stems, further support the view expressed by Kennedy, Ibbotson & Booth (1950) that aphids require to tap a fully functional 'pipe-line' in order to obtain sufficient sap.

As it is questionable whether an aphid could produce a suction pressure of even 1 atm., and as calculations have shown that a pressure of 20–40 atm. may be necessary to maintain the normal rate of flow of sap through the stylet food canal, one may suppose that an aphid would not be able to feed at its normal high rate by its own sucking efforts.

Although aphids may be able to suck up a limited amount of fluid when it is not under pressure, as artificial feeding experiments have indicated (Hamilton, 1935; Maltais, 1952), the results suggest that aphids depend on the natural turgor pressure of their host plant for their normal feeding. This pressure is responsible for causing the exudation of sap from severed embedded stylets and may be assumed to be operative in forcing sap up the stylet food canal into intact feeding aphids. It appears that the rate at which the host plant forces the sap up the stylet food canal determines the rate of sap uptake and excretion of the aphids. In order to ingest the sap aphids must, however, actively swallow the sap forced up their stylet food canal even though they do not have to exert, or are unable to exert, very much suction on it.

One may speculate that the differences in the rates of exudation from the severed embedded stylets of the various instars are due to differences in the dimensions of their stylet food canal.

Although a low concentration of certain organic constituents in phloem sap may necessitate the ingestion of as much sap as possible, the excretion of large quantities of amino-acids (Maltais & Auclair, 1952; Mittler, 1953) suggests that the host plant is forcing more nutrient sap into feeding aphids than the insects are able to absorb.

A theory of aphid feeding based on a supposed physico-chemical action (*exosmosis*) of aphid saliva on the cells of a plant, thereby making sap available under pressure to aphids, has been proposed by Zweigelt (1914). Although very little evidence exists to support this theory it is interesting to quote some of Zweigelt's conclusions: 'The complicated action attributed to the muscles of the pharyngeal suction pump overrates the efforts of the insect, which merely has to swallow and not to suck.' The present investigation supports these conclusions but rejects the concepts on which they are based. The generally accepted view that an aphid's normal mode of feeding is by suction is, however, being challenged.

SUMMARY

1. A study has been made of the factors involved in the uptake of phloem sap by *Tuberolachnus salignus* (Gmelin) feeding on the stems of various *Salix* spp.

2. A method has been developed for maintaining the parthenogenetic viviparous forms of *T. salignus* in culture throughout the year.

3. It has been established that during normal feeding *T. salignus* have the tips of their stylets inserted into the phloem sieve-tubes of the host plant.

4. The phloem sieve-tube sap of intact and turgid willow stems is under considerable pressure. This pressure forces the sieve-tube sap up the stylet food canal of feeding aphids, and also causes the sieve-tube sap to exude for many hours from the cut end of embedded stylet bundles.

5. Intact and feeding *T. salignus* rely almost entirely on this pressure to maintain their normal rate of sieve-tube sap uptake. The aphids must, however, swallow actively in order to ingest.

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