Feeding Determinants in Aphids

with Special Reference to the Rose Aphid *Macrosiphum rosae* (L.)

by

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Chapter 1.
General introduction and a review of aphid feeding determinants

Host specificity

Aphids are important plant pests. In the course of evolution, plants have developed various defences against aphids, many chemical, *i.e.* feeding deterrents or toxins. Aphid species have survived, nevertheless, because each has simultaneously developed adaptations to the resistance of particular parts of plants, or particular species or particular genera. These adaptations vary in their specificity, some polyphagous species feeding on up to six families of plants (Eastop 1973). On the other hand, the characteristic defensive chemicals of plants can become a distinctive signal for aphids for recognition of particular host plants. This means that such allelochemicals have become phagostimulants for certain species of aphids. An example is the glucoside phlorizin which can be extracted from the superficial tissues of apple leaves. The chemical deters the pea aphid *Acyrthosiphum pisum* (Klingauf 1971) and two other non-apple feeding aphids, *Myzus persicae* and *Amphorophora agathonica* (Montgomery and Arn, 1974), but it stimulates the probing of the apple aphids *Aphis pomi* and *Rhopalosiphum insertum* (Klingauf 1971).

The host specificity of aphids is more developed than that of many other insects. The range of host plants of 97% aphid species is limited within one genus of plants. By comparison, only 53% of Aleyrodidae, 54% of phytophagous Thysanoptera, and 95% of Psyllidae species choose their feeding sites on plants of one genus (Eastop 1973). A suitable host plant for an aphid species must provide the aphid with all the phagostimulants necessary at an effective level to induce feeding, while effective levels of deterrents must be absent. Lack of any one of the phagostimulants, or addition of any one of the deterrents, whether caused genetically,

phenologically, or environmentally, may lead to the failure of feeding by the aphid. In short, the host plants of aphids must possess allelochemicals that function as aphid feeding determinants.

The study of such allelochemicals is drawing more attention from geneticists and plant breeders who are working on the development of aphid-resistant hybrids. If genetic engineering techniques are to be adopted in breeding programs, a knowledge of the interactions underlying resistance is imperative.

Reported feeding determinants

Nutrients

Efforts to ascertain the role of nutrients in the determination of aphid feeding have been made for decades (Thorsteinson 1960, Auclair 1969, van Emden 1972, von Hook *et al.* 1980). Some evidence has been achieved, such as: (1) single nutrients, such as sucrose and methionine, were shown to stimulate feeding (Mittler 1967); (2) apart from the need for vitamins and trace elements, lack of some critical nutritional elements, usually amino acids, in artificial diets retards aphid growth and development (Auclair 1963); (3) the suitability of hosts is positively related to the nitrogen concentration, the content of free amino acids, or the ratio of amino acids to sugars in plants (McClure 1980, Auclair *et al.* 1957, Maltais and Auclair 1957, Febvay *et al.* 1988).

Many authors, however, showed contradictory evidence and there have been strong assertions that nutrients as such, whatever their effects on growth and development, have little or no influence on the initial selection of their host plants (Fraenkel 1969, Whittaker & Feeny 1971, Dreyer and Campbell 1987).

Waxes

There is a growing number of reports on the influence of chemicals present in the surface waxes on the responses of insects to the plant. Klingauf and his associates (1978) have demonstrated the importance of alkanes, among the commonest constituents of all plant waxes, in settling and feeding by *Acyrthosiphon pisum*. *Myzus persicae* adults are deterred from settling on membranes coated with solutions of monocarboxylic acids of chain length C_8 to C_{13}, but longer chain length acids do not have this effect (Greenway *et al.* 1978).

Phenols and tannins

Phenols occur naturally in higher plants. Tannins, a group of polyphenols with the ability to precipitate proteins in aqueous media, comprise two structurally different substances: condensed tannins and hydrolysable tannins. There have long been arguments about the role of tannins in the insect-plant relationship. Feeny (1970, 1976) reported the defensive function of leaf tannins against oak-insects and suggested that, by reducing digestibility, tannins functioned as quantitative defences for the longlived, widespread, obvious plants, which he called "apparent plants". However, Bernays (1978, 1981) rejected a generalized defensive effect of tannins in plants against insects, although she found definite phagostimulatory effects as well as true toxic effects with hydrolysable tannic acid, but not with condensed tannin.

In a review of the chemical defence of plants, it is convenient to include tannins in a discussion of phenols generally. Zucker (1982) reported that the suitability of a tree, leaf, or a section of leaf of *Populus anqustifolia* for a galling aphid *Pemphigus betae,* a parenchyma-feeder, was inversely correlated with the concentration of total phenols (including tannins). In a bioassay of phenolic compounds in chemically defined diets, Todd *et al* (1971) found that, towards aphids *Schizaphis graminum,* most of the test phenols were toxic, including the two tannins, tannic acid and quercetin. Dreyer *et al.* (1980) also demonstrated the deterrency of

phenolic isolates from sorghum, such as hydroxybenzaldehyde, dhurrin, procyanidin, to *S. graminum,* and 4-hydroxycoumarins to *Acyrthosiphum pisum* (Dreyer *et al.* 1987). However, when examining the performance of the black bean aphid *Aphis fabae* on diets that incorporated a low concentration (<0.1 mg/ml) of individual phenolic compound, Jordens (1979) found that all the phenols tested (including some condensed tannins, such as quercetin) encouraged the aphid to probe for longer periods. The work of Miles (1985) on rose aphids provided an example of a phenolic, catechin, that was apparently phagostimulant towards aphids at low concentration, but deterrent at a high concentration.

The danger of generalizing the allelochemical functions of phenols, including tannins, was further indicated in the recent report of Jones and Klocke (1987), who demonstrated that ellagitannins, while strongly toxic to *Schizaphis graminum* and *Myzus persicae,* had no effect on other aphid species.

From an evolutionary point of view, phenols are "older" than alkaloids. Phenols such as flavonoids are widely distributed in plants, including mosses (bryophytes) and ferns (Heywood 1971). Alkaloids have never been reported from mosses (bryophytes) but are sometimes found in ferns (Robinson 1979), and occur most frequently in herbaceous plants (McNair 1935). In past evolutionary time, insects had more opportunities to develope counter-defences to phenols, the "older" defence system of plants. Possibly this is why aphids appear to have a general adaptation to low concentrations of phenolics. Of the various phenolic compounds, condensed tannins are "older" than the hydrolysable tannins and occur universally among both gymnosperms and woody angiosperms, while the latter occur only in woody angiosperms. It seems likely that this explains how aphids have been able to develop more effective means for the detoxification of condensed tannins than of hydrolysable tannins.

Miles (1969) presented a hypothesis that phenol-phenolase reactions between plants and sap-sucking insects, mediated by salivary enzymes, played a major

role in the adaptation of these insects including aphids to phenolic allelochemicals in their host plants.

Alkaloids

The occurrence of alkaloids in plants closely follows their taxonomic affinities: the alkaloids within any one genus are usually structurally similar; when different alkaloids are met with in the same family, they are generally each confined to a single genus (McNair 1935). This means that alkaloids could function as chemical markers of a single plant genus. Considering that most aphids confine their host plant range within one genus (Eastop 1973), we conclude that alkaloids would be cues available for aphids to recognise their particular host plants. Conversely alkaloids could also function as "non-host" indicators for most aphids, *i.e.* those that do not normally feed on plants containing the substance. How can we, however, explain host selection in the few polyphagous aphids? To some extent, it seems possible that apparent polyphagy could be associated with a phagostimulant function of the small number of alkaloids that are found in more than one family (McNair 1935).

Sinigrin, a characteristic alkaloid in Cruciferae is phagostimulant to the oligophagous aphid *Brevicoryne brassicae*, the host range of which is restricted to the Cruciferae. It is also tolerated, to a lesser degree, but not preferred by the polyphagous aphid *Myzus persicae,* the hosts of which include cabbage (van Emden, 1972); otherwise, sinigrin is strongly deterrent to aphids such as *Aphis fabae, Acyrthosiphon solani*, and *A. pisum* (Wensler 1962, Nault and Styer 1972). Sparteine, a quinolizidine alkaloid in broom plants, has been found to stimulate feeding by the broom aphid *Acyrthosiphon spartii* (Smith 1966), although it is deterrent to other aphids. This adaptation of aphids to a particular alkaloid appears to be the consequence of evolutionary adaptation. The aphid *Aphis cytisolum* prefers plants with a low concentration of quinolizidine. It is, presumably, on the way to developing its power to sequester and metabolize the quinolizidine alkaloids (Wink *et al.* 1982).

There is no doubt, however, that alkaloids contribute much to aphid-resistance of crops. Those in the Gramineae, such as hydroxamic acid and gramine, are effective defences in wheat, corn, barley, and rye against aphids (Argandoña *et al.* 1980, Zúñiga *et al.* 1985). In Leguminosae, some pyrrolizidine, indolizidine, and quinolizidine alkaloids showed deterrent effects towards the pea aphid *Acyrthosiphon pisum* in a series of bioassays with alkaloid-supplemented diets (Dreyer *et al.* 1985). The alkaloids of Solanaceae, such as nicotine, nornicotine, and anabasine, have been used as aphicides (Thurston *et al.* 1966).

The concentration of alkaloids in plants usually varies with the development of the plant, and is consistent with changes in suitability to aphid infestion. Normally, older barley plants are more susceptible to aphids, because they have less gramine in their tissues (Zúñiga *et al.* 1985, 1986). The older plants of wheat and rye, with lower concentrations of hydroxamic acid, are similarly more suitable for the aphid *Schizaphis graminum* and *M. dirhidum* (Argandoña *et al.* 1980, 1981).

Polysaccharides

Pectin, a polysaccharide, is an important component in plant cell wall and the chief intercellular material. Because the probing of aphids was considered to be largely intercellular (Pollard 1973), the quality and quantity of pectin was assessed as playing an important role in aphid feeding (Dreyer and Campbell 1987)

In several early reports, the penetration of aphid stylets was considered to be assisted by their injection of salivary pectinase into the spaces between the plant cells (Adams & McAllen 1958; McAllen & Adams 1961). The use of the bioregulator Cycocel was reported to significantly increase pectin content in susceptible sorghum, and greatly decreased the reproductive rate of the green bug *Schizaphis graminum*. It is claimed that the increase in the content of the methyl ester of pectin in the sorghum variety hindered aphids in penetrating host-plant tissue; whereas a new biotype of green bugs with reinforced pectin methylesterase activity was said to show an ability to

overcome this phytochemical barrier (Dreyer and Campbell 1984). A higher degree of susceptibility of a sorghum variety was associated with a greater rate of hydrolysis of sorghum pectic substances by extracts of a greenbug biotype according to Campbell and Dreyer (1985).

The actual process by which pectin composition of tissues related to aphid feeding was thrown in doubt when it was found that tethered aphids eventually penetrated resistant tissues as fast as non-resistant. For this reason, Dreyer *et al.* (1986) in further experiments found a variation of stimulatory or inhibitory behavioral responses in aphids towards polysaccharides which are structurally related to the pectin fragments generated from plant matrix polysaccharides by the aphids' polysaccharase. Whether plant pectin has any universal significance in aphid host selection, will remain uncertain pending more representative evidence.

Examples of reported aphid feeding determinants are listed in Table 1.1

Table 1.1
A list of reported aphid feeding determinants

Stimulant	Host plant	Aphid	Data
Nutrients			
Total nitrogen	Elongate hemlock	*Fiorinia externa*	McClure 1980
Higher concentration of amino acids	Alfalfa	*Acyrthosiphum pisum*	Auclair *et al.*1957
High ratio of amino acids /sugars	Alfalfa	*Acyrthosiphum pisum*	Maltais *et al.* 1957 Febvay *et al.* 1988

Table 1.1 (cont.)

Stimulant	Host plant	Aphid	Data
Asparagine Glutamine	Brussels sprouts	*Brevicoryne brassicae* *Myzus persicae*	van Emden *et al.*1971
Lower concentration of γ-amino butyric acid	Brussels sprouts	*Brevicoryne brassicae*	van Emdem *et al.* 1971
Waxes			
Alkane	Cabbage	*Brevicoryne brassicae*	Thompson 1963
Alkane $C_{32}H_{66}$	Broad bean	*Acyrthosiphon pisum*	Klingauf *et al.* 1971
Phenols			
Coumestrol	Alfalfa	*Acyrthosiphon pisum* *Therioaphis maculata*	Loper 1968
Phlorizin glucoside	Apple	*Rhopalosiphum insertum* *Aphis pomi*	Klingauf 1971
DOPA	Broad bean	*Aphis fabae*	Jordens & Klingauf 1977
Catechin (low concentration)	Rose	*Macrosiphum rosae*	Miles 1985

Table 1.1 (cont.)

Stimulant	Host plant	Aphid	Data
Alkaloids			
Sinigrin (mustard oil glycoside)	Cabbage	*Brevicoryne brassicae*	Wensler 1962
	Cruciferae	*Hyadaphis erysimi*	Nault & Styer 1972
		Myzus persicae	
Sparteine (quinolizidine)	Broom	*Acyrthosiphon spartii*	Smith 1966
Quinolizidine (low concentration)	Broom	*Aphis cytisolum*	Wink *et al.* 1982

Deterrent	Host plant	Aphid	Data
Phenols			
Total phenols	Narrowleaf cottonwood	*Pemphigus betae*	Zucker 1982
Phlorizin glucoside	Apple	*Eriosoma lanigerum*	Sen Gupta & Miles 1975
	Apple	*Acyrthosiphum pisum*	Klingauf 1971
	Apple	*Myzus persicae*	Montgomery & Arn 1974
		Amphorophora agathonica	
Benzyl alcohol	Barley	*Schizaphis graminum*	Juneja *et al.* 1972
Quercetin	Cotton	*Schizaphis graminum*	Hedin *et al.* 1974

Table 1.1 (cont.)

Deterrent	Host plant	Aphid	Data
Coumarin	Leguminosae	*Aphis craccivora*	Mansour *et al.* 1982
p-Hydroxyl benzaldehyde	Sorghum	*Schizaphis graminum*	Dreyer *et al.* 1980
Dhurrin			
Procyanidin			
Catechin	Rose	*Macrosiphum rosae*	Miles 1985 Peng & Miles 1988a
Alkaloids			
Sinigrin glucoside	Crucifera	*Acyrthosiphun pisum*	Nault & Styer 1972
		A. sonani	
		Aphis fabae	
		Myzus persicae	van Emden 1972
Hydroxamic acid	Gramineae	*Schizaphis graminum.*	Zúñiga *et al.* 1983
	Wheat Barley	*Metopolophium dirhodum*	Argandoña *et al.*1980
	Corn	*Rhopalosiphum maidis*	Long *et al.* 1977
Gramine (indole alkaloid)	Barley	*Schizaphis graminum*	Zúñiga *et al.* 1985
	Barley	*Rhopalosiphum maidis*	Corcuera1984
	Barley	*Rhopalosiphum padi*	Zúñiga 1986

Table 1.1 (cont.)

Deterrent	Host plant	Aphid	Data
Quinolidine	Lupin	*Acyrthosiphum pisum*	Wegorek 1970
		Macrosiphum euphorbiae	Brusse 1962
	Broom	*Aphis cytisorum*	Wink *et al.* 1982
Nicotine	Nicotina	*Myzus persicae*	Thurston *et al.* 1966
Terpenoids			
Gossypol	Cotton	*Aphis gossypii*	Bottger *et al.* 1964
(E)-β-farnesene	Potato	*Myzus persicae*	Gibson & Pickett 1983
β-caryophyllene	Potato	*Myzus persicae*	Ave *et al.* 1987
Polysaccharides			
Pectin variants	Sorghum	*Schizaphis graminum*	Dreyer *et al.* 1983
Pectin methyl ester	Sorghum	*Schizaphis graminum*	Dreyer & Campbell 1983
Ketone			
Tridecanone	Tomato	*Aphis gossypii*	Williams *et al.* 1980

Location of feeding determinants

The most preferred feeding site of Aphididae is phloem, although there is evidence that aphids also suck the mesophyll sap (Pollard 1973). Before the aphid stylet reaches the phloem, a series of activities must occur, such as alighting, test probing and deep penetration. Any plant component located during one step of the process may exert a significant impact on eventual aphid feeding. This means that

aphid feeding determinants may be present on the plant surface, in the plant tissues penetrated by the stylet, and in the final feeding site, whether phloem or other tissue.

Trichome glands.

During the host finding stage, alate aphids visit numerous plants, both host and nonhosts. They are mainly attracted by the yellowish component of plant colours which helps them to distinguish plants from their surroundings (Kring 1969). Other factors, however, such as olfactory stimuli and deterrents may also affect host-choice. Sesquiterpene components exuded from the glandular trichomes of plant surface interrupt aphid landing. β-caryophyllene and (E)-β-farnesene (an aphid alarm pheromone) mainly released from type A trichomes of the wild potato species *Solanum berthaultii* and a resistant cultivated potato *S. tuberosum* repelled green peach aphids *Myzus persicae* (Gibson & Pickett 1983; Ave *et al.* 1987). Gossypol produced by gland cells in leaves of cotton was shown to be toxic to the cotton aphids *Aphis gossypii* (Bottger 1964), although it was attractive to the boll weevil (Maxwell *et al.* 1965). Nicotine, an alkaloid secreted by trichomes of *Nicotiana* species, killed the green peach aphid *Myzus persicae* by contact (Thurston *et al.* 1966).

Plant surface.

Waxes are the commonest components on the plant epidermis. Plant wax composition has been considered as a "fingerprint" in plant taxonomy (Eglinton and Hamilton 1963), because the plant surface lipids give patterns characteristic of the particular species of plant (Purdy and Truter 1961). It is not surprising to find evidence that aphids use plant waxes as token stimuli to find their particular host plants. For instance, cabbage leaf wax stimulates the cabbage aphid *Brevicoryne brassicae* to probe and settle, whereas non-waxy varieties were rejected by the aphid (Thompson 1963). Plant waxes are mixtures. The composition of waxes extracted from the leaf cuticle of the host plants of the pea aphid *Acyrthosiphum pisum* showed an influence

on its behaviour. Alkanes are the major components in the waxes. The alkane fragments from broad bean *Vicia faba* stimulated *A. pisum* to move from the upper to the lower side of the leaf, whereas that from a nonhost had no such effect. Mechanical destruction of the wax layer elicited an increase in the time for such movement (Klingauf 1978). Furthermore, a specific $C_{32}H_{66}$ alkane, from broad bean caused *A. fabae* to probe for longer periods into a parafilm sachet (Klingauf *et al.* 1971). Phlorizin, a phenolic glucoside, which can be rapidly extracted in water from the surface of uninjured apple leaves has also been shown to affect the choice of host plant by aphids (Klingauf 1978).

Intercellular substances of parenchyma.

After alighting on the surface of host plants, aphids begin to penetrate the plant parenchyma tissue, mostly intercellularly, with the bristle-like mandibular and maxillary stylets. Chemical variants of pectins between cells were considered as inhibitors for the stylet penetration (Dreyer *et al.* 1983, Dreyer and Campbell 1983), but this idea requires reassessment (see discussion above of polysaccharides as feeding determinants).

Parenchyma.

Many plant toxic substances are said to be sequestered in vacuoles, but do not occur in vascular bundles (Matile 1984). Dreyer and Campbell (1987) asserted that the secondary plant metabolites in tissues other than phloem could not therefore contribute to aphid resistance, because phloem sap was the predominant food source of the aphids. Risebrow and Dixon (1987) argued, however, that aphids would still make contact with these chemicals during the superficial test probes before a deeper feeding probe. A strong deterrent, such as N HCl, could stop deep probe by *M. persicae* (Mittler and Dadd 1965). Gramine, an indole alkaloid in barley, was reported to be toxic to the aphid *Schizaphis graminum* (Corcuera 1984) and *Rhopalosiphum*

*padi (*Zúñiga and Corcuera 1986) when incorporated into synthetic diets. This was in agreement with negative correlation between the susceptibility of barley cultivars and the content of gramine in their tissues (Zúñiga *et al.* 1986). Since gramine was present only in the epidermal parenchyma but not in vascular bundles, Argandoña *et al.* (1987) suggested that its deterrent effects in protecting barley plants might occur during probing by the stylets and subsequent penetration before the phloem was reached. To test if aphids could locate phloem as a food source by avoiding the feeding deterrents within tissues through which their styles passed, Montgomery and Arn (1974) fed aphids with two-layered diets separated by a membrane and found that, for *Amphorophora agathonica*, the presence of phlorizin in the first layer decreased significantly the ingestion of a non-deterrent diet in the second layer.

Phloem.

Substances in phloem at an effective concentration are commonly accepted as aphid feeding deterrents (Dreyer & Campbell 1987). Unfortunately, only few published data relate to the occurrence of plant secondary products in phloem.

Catechin, a phenol that is toxic towards *Macrosiphum rosae* (Miles 1985) and *Schizaphis graminum* (Todd *et al.* 1971), has been detected in phloem tissue (Hemingway *et al.* 1981). An aphid-deterrent, chlorogenic acid (Todd *et al.* 1971), was found to be a phloem component in *Prunus* trees (Feucht & Schmid 1979). Phlorizin, which has been known to affect aphid feeding, is present in the sieve tube sap of the apple genus, *Malus* (Montgomery and Arn 1974). Several toxic phenols, such as catechol, phloroglucinol, and quercetin (Todd *et al.* 1971) were able to be translocated in the sieve tubes of *Vicia faba* (Macleod and Pridham 1965). Also, DOPA, proanthocyanidins (condensed tannins), and phenolic acids were detected in the phloem of cherry and radish plants (Schmid and Feucht 1981, Hussain *et al.* 1974).

Some alkaloids with weak bases are phloem-mobile, including Papaveraceae alkaloids and ricinine (Waller and Nowacki 1978). Quinolizidines, a

group of alkaloids that are responsible for the resistance of some lupin varieties towards pea aphids (Wegorek *et al.* 1970), and function as general aphid-repellents in the plant (Wink *et al.* 1982), are also transported via phloem in the legumes (Wink and Witte 1984). Other alkaloids, such as hydroxamic acids, which are widely distributed in Gramineae and are toxic to the aphids *Rhopalosiphum maidis* (Long 1977), *Metopolophium dirhodum* (Argandoña 1980) and *Schizaphis graminum* (Argandoña 1983), were detected in the vascular bundle of maize and wheat at effective concentrations (Argandoña *et al.* 1985, 1987).

Methods of detection of feeding determinants

The classical method of research into aphid feeding determinants is to look for the correlation of aphid infestion or population with the concentration of a certain chemical in extracts of the host. A negative coefficient has been taken as evidence that the chemical is deterrent. Miles (1985) discussed the possible importance of catechin in the relationship between the aphid *Macrosiphum rosae* and rose buds in the light of an inverse correlation between the population density of *M. rosae* and the catechin concentration in the tissue. The variation of hydroxamic acid content in different species of cereals (Argandoña *et al.* 1980), or in different varieties of corn (Long *et al.* 1977) was found to be negatively correlated with the population growth of aphids. Such findings stimulated further investigations into the effect of hydroxamic acids on aphid feeding (Argandoña *et al.* 1980, 1981).

Bioassay with a pure chemical added to artificial diets is widely used in the estimation of the impact of a substance on aphid feeding behaviour. In this way, Schoonhoven and Derksen-koppers (1976) examined 24 secondary plant substances in a dual choice by experiment. Qin and Ke (1984) applied radioactive amino acids in the diet for an investigation into the influence of secondary plant products on food uptake by aphids. The average aphid weight or survival rate of progeny after feeding (Todd *et*

1971), or the duration of an aphid's first probe via parafilm membrane into the test solution (Jordens 1979) have been used for such assessment (Jordens 1979). The significance of gramine and hydroxamic acids in the resistance of cereals against aphids has also been tested in bioassay with synthetic diets (Long 1977, Corcuera 1984, Zúñiga and Corcuera 1986).

Components of the phloem have been considered of particular significance, since most aphids appear to feed mainly on phloem. Because of the difficulty of obtaining phloem sap, however, determination of aphid feeding determinants in phloem remains difficult. Following the discovery that sap exuded from the severed stylets of the willow aphids (Kennedy and Mittler 1953), stylet ablation has led to the successful obtaining of phloem sap from some graminaceous plants, *e.g.* by cutting the stylets of planthoppers with a laser beam while the insects were feeding (Chino *et al.* 1986). Attempts of other workers, however, to repeat this technique have not always been successful.

The selective excision of tissues has been used in the analysis of contents of the vascular bundle and other tissues of woody plants (Feucht and Schmid 1979) and of herbaceous plants (Argandoña *et al.* 1987), and there are reports of successful collection of phloem exudates from *Yucca* and some legumes by collection of exudates from cut seive tubes (Pate and Sharkey 1974, van Die and Tammes 1975). Recent versions of this method involve insertion of detached leaves or fruits in a solution containing a chelating agent such as ethylene diamine tetraacetate (EDTA). Satisfactory collection of phloem sap was reported in some non-woody plants, such as the Perilla (King & Zeevaart 1974), legumes (Fellows & Leggett 1978, Hoad 1980, and Urquhart & Joy 1981), the Caprifoliaceae, the Fabaceae (Bolsinger & Fluckiger 1987) and lucerne (Febvay *et al.* 1988).