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# OBSERVATIONS ON THE TRANSLOCATION OF PHENOLIC COMPOUNDS*

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**Abstract**—The rates of translocation of phenolic compounds introduced into the apical leaves of *Vicia faba* have been estimated. Some evidence for the occurrence of phenolic compounds in the sieve tubes of *Salix* and *Vicia* has been obtained using "aphid techniques".

## INTRODUCTION

THERE is now a substantial amount of information regarding the biosynthetic pathways involved in the formation of phenolic compounds in plants.[1] Precursors such as acetate and the cinnamic acids can be produced in photosynthesizing tissues and there be further elaborated to flavonoids and higher molecular weight phenolics. Whether phenolic compounds can actually move through the tissues and, in particular, be translocated in the phloem is debatable at the present time. In the case of woody plants, it would be interesting to know whether the flavonoids, tannins and lignins found in the bark and wood arise from phenolic compounds formed in the leaves or from more common metabolites such as acetate and/or carbohydrates.

Hillis and his associates[2–4] favour the theory that the formation of *Eucalyptus* wood polyphenolics occurs *in situ* from carbohydrates. They demonstrated the translocation of $^{14}$C-labelled D-glucose from the phloem to the heartwood–sapwood boundary followed by the incorporation of label into the phenolics. The histological studies of Wardrop and Cronshaw[5] using *Eucalyptus* also suggested that the tannins were produced from carbohydrate; in this case starch. Hathway[6] on the basis of ringing experiments with *Quercus pedunculata* claimed that (+)-gallocatechin and leucodelphinidin were translocated down from the leaves to the trunk and there oxidized to phlobotannins. In the case of phenolic glycosides, Miller[7] believed that they accumulated at sites of low metabolic activity and remained in these tissues until the death of the plant. His conclusions were mainly based on the treatment of gladiolus corms with *o*-chlorophenol which was converted to the $\beta$-gentiobioside; this was not translocated to the shoots when these corms were germinated or to

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[1] J. B. HARBORNE (Ed.) *Biochemistry of Phenolic Compounds*. Academic Press, New York (1964).
[2] W. E. HILLIS and A. CARLE, *Biochem. J.* **74**, 607 (1960).
[3] W. E. HILLIS and A. CARLE, *Biochem. J.* **82**, 435 (1963).
[4] W. E. HILLIS and M. HASEGAWA, *Phytochem.* **2**, 195 (1963).
[5] A. B. WARDROP and J. CRONSHAW, *Nature* **193**, 90 (1962).
[6] D. E. HATHWAY, *Biochem. J.* **71**, 533 (1959).
[7] L. P. MILLER, *Contrib. Boyce Thompson Inst.* **11**, 271 (1940).

resulting daughter corms. Macleod and Pridham[8] were unable to confirm this using quinol-treated *Vicia faba* seeds. The young shoots from the germinating seeds did contain arbutin. No movement of natural flavonoids from scions to stocks could be detected when Delavean[9] examined grafts of *Tropaeolum* spp. Gorz and Haskins,[10] however, were able to show that small amounts of coumarin could be translocated across graft unions.

The experiments we now wish to describe support the idea that phenolic compounds can and are translocated in the phloem of higher plants.

## RESULTS AND DISCUSSION

Initial experiments with *Vicia faba* plants (~30 cm high) showed that resorcinol, when introduced into the main vein of an apical leaf, became distributed over the whole plant, including the roots, within 30 min. A similar, although somewhat slower distribution, was effected by placing small volumes of resorcinol and other phenols dissolved in a dilute solution of "Tween 40" on the epidermal surfaces of the laminae. The minimum rates of movement of a number of foreign and naturally occurring phenols, introduced into the vein, are given in Table 1.

TABLE 1. PHENOL TRANSLOCATION RATES IN *Vicia faba*

| Phenol | Rate of movement down stem (cm/hr) |
|---|---|
| *m*-Hydroxyphenyl-β-D-glucoside | 108 |
| Arbutin | 90 |
| Aesculin | 84 |
| Salicin | 66 |
| Resorcinol | 60 |
| Catechol | 54 |
| Phloroglucinol | 54 |
| Quinol | 48 |
| Saligenin | 42 |
| Caffeic acid | 42 |
| Ferulic acid | 30 |
| Kaempferol | 18 |
| Quercetin | 12 |

The general translocation rates for phenols (with the exception of the flavonoids) appear to fall within the range found by others for $^{14}C$-assimilates (see Kursanov[11]). There is also fairly good evidence that glycosidic derivatives migrate more rapidly than the phenolic aglycones. The low rates observed with the flavonoids may be due to poor penetration and/or insolubility factors. Roberts[12] has suggested that the planar structure of these compounds may adversely affect their translocation. When resorcinol, catechol, quinol and saligenin were fed the corresponding mono β-D-glucosides were also observed in the tissues.

Although these results show the feasibility of phenol translocation they can be criticized on the grounds that the compounds tested were foreign to the plant and/or, were introduced into the phloem in a "non-physiological" manner. The only certain way of showing that

[8] N. J. MACLEOD and J. B. PRIDHAM. Unpublished results.
[9] P. G. DELAVEAN, *Compt. Rend.* **258**, 318 (1964).
[10] H. J. GORZ and F. A. HASKINS, *Crop Sci.* **2**, 255 (1962).
[11] A. L. KURSANOV, *Advanc. Botan. Res.* **1**, 209 (1963).
[12] E. A. H. ROBERTS, *Nature* **185**, 536 (1960).

phenols are translocated is to demonstrate their natural occurrence as sieve-tube constituents. This can be achieved with little risk of contamination from phenols in surrounding tissues by the "aphid stylet" technique using *Tuberolachnus salignus*.[13] Like other aphids, this organism feeds directly from sieve-tubes. Our own attempts to use this procedure with *Salix* spp. were unsuccessful although Bate-Smith and Swain[14] report that phloem exudates obtained in this way from similar species did contain small amounts of fluorescent materials which appeared to be phenolic on paper chromatograms and which gave u.v. spectra characteristic of cinnamic acid derivatives.

*T. salignus* is a large aphid and therefore ideally suited for the "aphid-stylet" technique. Aphids feeding on herbaceous hosts are usually smaller and more delicate and hence cannot readily be dissected whilst feeding. The phloem constituents can, however, be examined by an analysis of the gut contents or the honeydew but this method does suffer from the disadvantage that the enzymes in the alimentary tract (cf. Auclair[15]) may modify the constituents as they pass through.

Therefore, before utilizing *Macrosiphum pisi* for an examination of the phloem of *Vicia faba*, buffered extracts of the aphid were examined for enzyme activity. Thus α- and β-D-glucoside:glucohydrolases, α-D-galactoside:galactohydrolase and an esterase capable of hydrolysing chlorogenic acid were all shown to be present (Table 2). The location of these

TABLE 2. ENZYME ACTIVITY IN *M. pisi* EXTRACTS

| Substrate | Products | Inference |
|---|---|---|
| Maltose | Glucose | α-D-Glucoside:glucohydrolase |
| Isomaltose | Glucose | |
| Methyl-α-D-glucoside | Glucose | |
| Raffinose | Galactose, sucrose | α-D-Galactoside:galactohydrolase |
| Cellobiose | — | |
| Methyl-β-D-glucoside | Glucose | β-D-Glucoside:glucohydrolase |
| Gentiobiose | Glucose | |
| Arbutin | Glucose, quinol | |
| Salicin | Glucose, saligenin | |
| Chlorogenic acid | Caffeic acid, quinic acid | Esterase |
| Catechol | "Melanin" | Phenolase |
| (+)-Catechin | "Melanin" | |

enzymes in the body was not determined but it is reasonable to assume that some, at least, must have been present in the alimentary tract. Phenolase activity was also apparent in the *M. pisi* extracts. This enzyme complex is common in insects but here again little is known about its location except that it occurs in some bloods and cuticles.[16] The enzymic modification of dietary phenolics was therefore considered to be a possibility but it seemed unlikely that this would be a rapid process and, more important, that the actual synthesis of phenols from non-phenolic compounds would occur.

An examination of the gut contents of *M. pisi* feeding on *V. faba* stems after introduction of foreign phenols into the apical leaves confirmed that there was little change in the compounds that were fed. Thus quinol, catechol, resorcinol, ferulic acid, caffeic acid and arbutin

[13] J. S. KENNEDY and T. E. MITTLER, *Nature* **171**, 528 (1953).
[14] E. C. BATE-SMITH and T. SWAIN. Unpublished results.
[15] J. L. AUCLAIR, *Ann. Rev. Entomol.* **8**, 439 (1963).
[16] C. B. COTTRELL, *Advances Insect Physiol.* **2**, 175 (1964).

were all present after 1 hr in aphids feeding at stem sites approximately 10–15 cm from the points of introduction of the phenols. Some slight hydrolysis of arbutin to quinol had occurred either in the plant or the alimentary tract and no glucosides appeared to have been synthesized. This suggests that the glucosylation observed in the earlier experiments did not occur in the sieve-tubes but in the adjacent tissues. The honeydew from *M. pisi* feeding on the stems of untreated *V. faba* plants contained β-(3,4-dihydroxy-phenyl)-L-alanine (DOPA), which occurs in relatively high concentrations in this plant (Guggenheim[17]; Andrews and Pridham[18]), and hence appears to be translocatory material. Other compounds giving azo dyes with diazotized *p*-nitroaniline/NaOH were also detected as sieve-tube components.

Paper chromatographic analysis of the honeydew from *T. salignus* feeding on branches of *Salix daphnoides* showed the presence of compounds with phenolic properties which were also detected in leaf and bark extracts of the plant (Table 3). One compound was positively identified as tyrosine.

TABLE 3. SOME COMPONENTS FOUND IN *T. salignus* HONEYDEW AND THE BARK (B) AND LEAVES (L) OF *S. daphnoides*

| $R_f$ values in EAW solvent | | | |
|---|---|---|---|
| B and L components | Honeydew components | Properties | Identity |
| 0·34 (B, L) | 0·34 | u.v., blue fluorescent | Cinnamic derivative? |
| 0·59 (B) | 0·59 | Diazo spray, red<br>Ninhydrin, blue | Tyrosine |
| 0·72 (L) | 0·72 | u.v., blue fluorescent | Cinnamic derivative? |
| 0·92 (B) | 0·92 | u.v. blue fluorescent<br>Diazo spray, red | |

The results of this study show clearly that phenols introduced into the leaves can move down the plant by the normal processes of translocation and there is definite evidence for the occurrence of phenolic compounds as natural constituents of the sieve-tubes. The presence of tyrosine in the sieve-tubes of *Salix* has been clearly established and this phenolic amino acid has been noted, together with phenylalanine, in honeydews from aphids feeding on a number of different hosts.[15] On the basis of this observation alone there is no reason to suppose that all complex phenolic compounds are biosynthesized *in situ* from non-aromatic derivatives. These two amino acids could be translocated to tissues possessing the necessary ammonia lyases[19, 20] and there be converted to polyphenolics via the corresponding cinnamic acids. The presence of DOPA in the sieve-tubes of *V. faba* is interesting in view of its probable metabolic importance in other plants in alkaloid,[21] betacyanin[22, 23] and, possibly, caffeic acid[24] formation.

[17] M. GUGGENHEIM, *Z. Physiol. Chem.* **88**, 276 (1913).
[18] R. S. ANDREWS and J. B. PRIDHAM. Unpublished results.
[19] J. KOUKOL and E. E. CONN, *J. Biol. Chem.* **236**, 2692 (1961).
[20] A. C. NEISH, *Phytochem.* **1**, 1 (1961).
[21] E. RAMSTAD and S. AGURELL, *Ann. Rev. Plant Physiol.* **15**, 143 (1964).
[22] L. HÖRHAMMER, H. WAGNER and W. FRITZSCHE, *Biochem. Z.* **339**, 398 (1964).
[23] L. MINALE, M. PIATTELLI and R. A. NICOLAUS, *Phytochem.* **4**, 593 (1965).
[24] N. J. MACLEOD and J. B. PRIDHAM, *Biochem. J.* **88**, 45P (1963).

## EXPERIMENTAL

*Vicia faba* (Var Johnson's Longpod) plants, approximately 30 cm high, were used in all the feeding and aphid experiments which were carried out at room temperature. Small branches of *Salix daphnoides* infected with *Tuberolachnus salignus* were obtained from the grounds of Royal Holloway College.

### *Paper Chromatography*

Phenolic compounds were examined on Whatman No. 3 paper using *n*-butanol–ethanol–water (40:11:19; BEW) and ethylacetate–acetic acid–water (9:2:2; EAW) solvents. Location was effected by u.v. light, diazotized *p*-nitroaniline/NaOH and, in the case of phenolic amino acids, ninhydrin. EAW solvent was used for sugars and quinic acid which were located with *p*-anisidine hydrochloride and $AgNO_3$–NaOH, respectively.

### *Phenol Translocation Rate*

Aqueous solutions (1%, w/v) of phenols were fed into apical leaves of *V. faba* plants for periods varying from 10 min to 3 hr. This was achieved by partially dissecting the main veins from the laminae, dipping these veins into the solutions contained in 5-ml beakers and then cutting off the ends of the submerged veins. After treatment, the stems and roots of the plants were quickly cut into 1 cm sections and these were extracted with aqueous methanol (80%, v/v) and examined on paper chromatograms. The rate of translocation in cm/hr could thus be calculated.

### *Enzyme Activity in* Macrosiphum pisi *Extracts*

Extracts were prepared by macerating feeding aphids with 0·05 M-sodium acetate buffer (pH 5·6; 5 aphids/2 ml buffer) and incubating (25°) the centrifuged preparations with various substrates at 0·5% (w/v) concentrations. The products were examined on chromatograms (see Table 2). Control reaction mixtures using boiled enzyme preparations were also examined.

### *Location of Phenols Fed to* V. faba *Using* M. pisi

All but the apical leaves were removed from *V. faba* plants and aphids then placed on the main stems 10–15 cm down from the leaves. The organisms were confined to these regions by cotton wool packed around the stems. Phenols were introduced into the veins, as previously described, and after 1 hr the aphids removed and aqueous extracts examined on paper chromatograms.

### *Collection of Honeydew*

This was achieved by allowing it to fall on to glass plates placed beneath the plants. The individual globules of syrup were then dissolved in small volumes of water and taken up in capillaries for chromatographic analysis. In other cases aphids were induced to excrete honeydew by anal stimulation with a needle. The droplets could then be collected directly with capillary tubes.

### *Extraction of Plant Tissues*

This was effected with cold aqueous methanol (80%, v/v). The solutions were filtered and concentrated in a rotary evaporator at 40°.