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THE LACK OF MOVEMENT OF [^{14}C]-PHENYLALANINE AND [^{14}C]-CINNAMATE AFTER ADMINISTRATION TO LEAVES OF *POLYGONUM* AND WHEAT

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Key Word Index—*Polygonum bistorta*; Polygonaceae; *Triticum aestivum*; wheat; Gramineae; phenolic compounds; metabolism; mobility; 3-^{14}C-cinnamate; U-^{14}C-phenylalanine.

Abstract—U-^{14}C-phenylalanine and 3-^{14}C-cinnamic acid were fed to detached *Polygonum* leaves through the cut petioles and to the bases of detached wheat leaves. After feeding, the leaves were divided into basal, middle and terminal segments; for each treatment of each plant more than 80% of the total radioactivity incorporated remained in the basal segment. The distribution of radioactivity between ethanol-soluble and insoluble fractions in each segment was examined. The basal segments contained more insoluble radioactivity than the terminal ones; the differences were far more marked for both plants when cinnamate rather than phenylalanine was administered. In view of the gross differences in distribution of radioactivity between the basal and terminal segments of each leaf, it is concluded that basal infusion of precursors is not the most suitable technique for studies of phenolic biosynthesis.

INTRODUCTION

Bate-Smith [1,2] has shown that there is a widespread distribution of cinnamic acid esters in vascular plants, and investigations using ^{14}C tracers [3–5] have revealed the importance of both alcohol soluble and alcohol insoluble cinnamic acid esters in plant metabolism. Hanson [6] and also Taylor and Zucker [4,7] have emphasized the role of the "alcohol soluble" esters in lignification in *Xanthium* and potato. In contrast, other workers [3,5,8] have shown a rapid incorporation and turnover of labelled substrate in the "alcohol insoluble" ester pools of both healthy and rust-infected wheat and *Mentha*; they propose that, in these instances, the "alcohol insoluble" esters are more important in lignin biosynthesis.

A tracer technique which has been frequently employed to study both the biosynthesis of lignin [9] and the metabolism of phenolic esters [3,5,8,10] has been the infusion of ^{14}C-labelled compounds into the base of excised leaves or shoots for various periods of time. However, there has been little published evidence of the mobility of the phenolic compounds used in such experiments. MacLeod and Pridham [11] have indicated in *Vicia* and *Salix* that phenolics introduced via the main laminar vein can move into the phloem and down the plant by translocation, but Taylor [7], after feeding [^{14}C]-chlorogenic acid to mature leaves of otherwise defoliated *Xanthium* plants, found that very little activity had in fact moved beyond the treated leaf, even after a 26 hr infusion period.

The following experiments were designed to examine the incorporation and movement of phenylpropanoid precursors into detached leaves of both dicotyledonous and monocotyledonous plants and the distribution of this activity after infusion had occurred.

RESULTS AND DISCUSSION

Movement of ^{14}*C-tracer in* Polygonum *and wheat*

The first foliage leaves of both wheat (cv Cappelle–Desprez) and the fully expanded leaves of *Polygonum bistorta* cv Superba are both narrow and approximately 12 cm long when excised at the auricles or the petiolar base respectively. These leaves provide, therefore, a reasonably standard situation for the comparison of ^{14}C-tracer mobility in monocotyledonous and dicotyledonous leaves during basal infusion experiments.

After infusion with 0·1 μCi of either U-[^{14}C]-phenylalanine or 3-[^{14}C]-cinnamic acid for 20 hr, leaves (of *Polygonum* or wheat) were grouped together at random into blocks of four leaves to be

Table 1. The infusion of 3-[^{14}C]-cinnamic acid and U-[^{14}C]-phenylalanine into leaves of *Triticum aestivum* cv Cappelle-Desprez

Leaf section	d.p.m. × 10^{-3} 100% EtOH extract	50% EtOH extract	Insoluble esters	Cell wall	Total
(a) 3-[^{14}C]-cinnamic acid					
Terminal	13·9	4·6	2·6	3·7	24·8
Middle	23·6	3·8	8·5	3·6	39·5
Basal	79·4	28·2	107·6	138·0	353·2
Total	116·9	36·6	118·7	145·3	417·5
(b) U-[^{14}C]-phenylalanine					
Terminal	5·1	0·7	1·1	3·8	10·7
Middle	8·9	3·4	6·2	16·4	34·9
Basal	37·6	25·6	74·6	103·7	241·5
Total	51·6	29·7	81·9	123·9	287·1

Figures in the table represent the mean d.p.m. ($\times 10^{-3}$) recovered from three blocks of four leaves.

assayed. The leaves in each block were cut into three sections of equal length from the basal site of infusion (labelled terminal, middle and basal). Each of these sections was now ground in liquid N_2 and extracted successively with 100% ethanol, 50% ethanol, and 3N-NaOH (at room temperature under N_2 for 24 hr) and, including the residue, four fractions were obtained. The total activity in the 100 and 50% ethanol extracts and the residue was determined. However, the 3N-NaOH extract was acidified and extracted with ether before counting; the activity in this fraction therefore represents phenolic acids which were present in the plant as insoluble esters.

It can be seen that in each case (Tables 1 and 2) the bulk of the total activity recovered occurs in the two insoluble fractions, the "cell wall" plus the "insoluble ester" fraction. However, there is considerably more activity in the insoluble ester fraction in the wheat than in the *Polygonum* with both tracers. In all four cases there is more activity in the 100% than in the 50% ethanol soluble fraction but when cinnamic acid is fed there is relatively more activity in the 100% than in the 50% fraction than when phenylalanine is administered.

However, it should be emphasized that each tracer is relatively immobile in each plant since at least 84% of the total activity recovered is in the basal 4 cm and no more than 6% of the activity reaches the terminal 4 cm. The distribution of activity which has been described above therefore represents mainly the activity in the basal 4 cm. It is important to note that the distribution of activity between the individual fractions is different in the terminal 4 cm. When cinnamate is fed, more than 75% of the total activity in the terminal seg-

Table 2. The infusion of 3-[^{14}C]-cinnamic acid and U-[^{14}C]-phenylalanine into leaves of *Polygonum historta* cv Superba

Leaf section	d.p.m. × 10^{-3} 100% EtOH extract	50% EtOH extract	Insoluble esters	Cell wall	Total
(a) 3-[^{14}C]-cinnamic acid					
Terminal	14·0	2·5	0·3	2·0	18·8
Middle	17·1	5·6	1·9	15·3	39·9
Basal	86·2	50·7	32·4	182·1	351·4
Total	117·3	58·8	34·6	199·4	410·1
(b) U-[^{14}C]-phenylalanine					
Terminal	5·4	4·1	2·2	6·6	18·3
Middle	6·3	5·3	1·8	12·6	26·0
Basal	50·8	43·1	31·7	227·0	352·6
Total	62·5	52·5	35·7	246·2	396·9

Figures in the tables represent the mean d.p.m. ($\times 10^{-3}$) recovered from three blocks of four leaves.

ments is soluble in either 100 or 50% ethanol and when phenylalanine is fed between 50 and 55% of the activity is ethanol soluble. In the middle sections, the situation is intermediate between the terminal and basal sections.

Because the relative activities in all four fractions change so markedly between the basal and terminal portions of the leaf, especially when 3-[^{14}C]-cinnamic acid is administered it is not possible to draw any realistic conclusions about the role of the individual fractions in phenolic metabolism of the leaf as a whole.

The 100 and 50% ethanol-soluble fractions obtained after feeding 3-[^{14}C]-cinnamic acid to wheat were examined in more detail by fractionation into pigments (100% ethanol fraction only), sugars, organic acids, amino acids and phenolic acids. More than 94% of the recovered radioactivity was found in the phenolic acid fraction; one- and two-dimensional thin-layer chromatograms were examined by autoradiography and in a radiochromatogram-scanner; the bulk of the radioactivity was in ferulic acid, a little in *p*-coumaric acid and some in cinnamic acid, presumably in non-metabolized starting material.

In order to determine whether or not the immobility of the applied tracer was due to its metabolism in wound-healing reactions, two further experiments were carried out in which U-[^{14}C]-phenylalanine was fed to wheat. In the first case it was fed via one portion of the root, the remainder of which was in water. Radioactivity was determined in that part of the root which had been fed, in the seed plus remainder of the root, in the shoot plus second leaf, and in the basal, middle and terminal portions of the first leaf. In the second case radioactive phenylalanine was administered to excised shoots and the portions analysed for radioactivity were the shoot plus second leaf, and the basal, middle and terminal portions of the first leaf. In both cases (Table 3) radioactivity was mainly found in that part of the plant which had been fed. However, when the whole plant was fed, there was slightly more radioactivity in the terminal than in the middle or basal sections of the leaf. Since material hardly moved out of either the fed root, or the cut shoot, it was concluded that the radioactive compounds were being metabolized at the site of infusion, although not necessarily in a wound-healing reaction, and that they were therefore essentially immobile.

Table 3. Distribution of radioactivity after administration of U-[^{14}C]-phenylalanine to (a) intact wheat plants via one portion of the root, and (b) excised shoot via cut shoot base

Portion of plant analysed	% Total radioactivity in that portion
(a) Intact wheat plant	
First leaf	
Terminal section	1·0
Middle section	0·8
Basal section	0·9
Shoot plus second leaf	2·7
Seed plus remainder of root (not fed)	13·4
Fed root	81·2
(b) Excised shoot	
First leaf	
Terminal section	0·9
Middle section	2·0
Basal section	8·1
Cut shoot plus second leaf	89·0

It is our intention to examine the effects of infection with *Puccinia striiformis* on phenolic metabolism in wheat. Since the fungal lesions are confined to the terminal end of wheat leaves it is important to ensure that the phenolic precursors reach the site of fungal infection. It is obvious that it will not be possible to use the basal infusion technique, nor will it be possible to infuse the compounds via roots since they are immobile in that case also. In addition it seems that experiments using this technique in which others have attempted to measure phenolic metabolism in detached leaves and shoots of either monocotyledons or dicotyledons [3,5,8,10] have probably only measured phenolic metabolism at the leaf, petiole or shoot bases.

EXPERIMENTAL

Plant material. 1st foliage leaves of *Triticum aestivum* cv Cappelle-Desprez 12 cm long after excision at the auricles were obtained from seedlings grown under greenhouse conditions for 11 days. Fully expanded leaves of *Polygonum historta* cv Superba, 12 cm long after excision at the petiolar base, were obtained from plants grown at the University Botanic Gardens.

Radioactive tracers. U-[^{14}C]-phenylalanine 513 mCi/mmol was obtained from the Radiochemical Centre, Amersham, and 3-[^{14}C]-cinnamic acid 54·5 mCi/mmol, was obtained from Schwarz–Mann, Orangeburg, New York.

Tracer infusion (a) *Excised leaves.* The leaves (12 from each plant) were placed with their bases in vials containing 0·1 μCi (in 0·05 ml of deionized water) of radioactive material. The leaves were then left to infuse the labelled compound at a temp. of 24 $\pm$ 0·5° and a constant illumination of 2480 lx. After 1·00 hr and 4·45 hr, 0·1 ml H_2O was administered to each vial; after

8·45 hr a further 0·4 ml H_2O was added and the leaves left to infuse the label to a total of 20 hr. The leaves were then removed, the bases washed, and split at random into 3 blocks of 4 leaves.

(b) *Feeding into roots or cut shoots.* For feeding into roots a plant was placed with two roots in a small beaker of H_2O and the third root in a vial containing 0·2 μCi of U-[^{14}C]-phenylalanine in 0·2 ml H_2O. Cut shoots were placed in a vial containing 0·2 μCi of U-[^{14}C]-phenylalanine in 0·2 ml H_2O. In each case 0·15 ml H_2O was added to the vial after 3 hr and an additional 0·2 ml H_2O was added after a further 3 hr. Both the whole plant and the cut shoot were left for a total period of 20 hr then the fed portion was washed with water.

Assay of radioactivity. (a) *Extracts from fed leaves* (*Tables* 1 *and* 2). The leaves of each block were cut into 3 portions, 4 cm long, called the terminal, middle and basal, and weighed. Each portion was then frozen in liquid N_2 and ground with a pestle and mortar. The macerated leaf tissue was then washed into a flask with 30 ml EtOH and refluxed 30 min. The EtOH was then removed as a supernatant by a Pasteur pipette and the residue washed with 2 × 10 ml EtOH (the residue was by then colourless). The residue was then extracted with 30 ml of 50% (v/v) aq. EtOH as above to give the 50% EtOH extract; 15 ml of 3N-NaOH were then added to the residue, the flask flushed with N_2 and sealed, and left at 30° for 24 hr. The resultant hydrolysate was acidified to pH 3 with HCl and filtered through a Gelman Metricel filter (type GA-6), pore size 0·45 μm. The cell wall fraction collected on the filter was dried in a vacuum dessicator, cut into ± equal portions and counted in 10 ml of scintillation fluid (Cell wall) (formula: 6 g PPO, 0·3 g POPOP, 100 g naphthalene dissolved in 1 litre toluene to which was added 300 ml ethoxy ethanol). The acidified hydrolysate was extracted 3× with 75 ml Et_2O and this extract reduced to 2·0 ml *in vacuo*. 2 × 0·1 ml aliquots were counted in 10 ml of scintillation fluid (insoluble esters). The EtOH extract was a deep green colour and hence was treated to remove lipids and pigments by the method of El Basyouni *et al.* [3]; the fraction was reduced to dryness *in vacuo* and taken up in 3× 10 ml boiling H_2O and filtered whilst still hot through a bed of Celite 545. The pale yellow filtrate was reduced to 2·0 ml and 2 × 0·1 ml aliquots counted in 10 ml scintillation fluid (100% EtOH extract). The 50% EtOH extract which was lipid free and colourless was reduced to 2·0 ml and 2 × 0·1 ml aliquots counted in 10 ml scintillation fluid (50% EtOH extract).

(b) *Plant portions from root and shoot feeding experiments* (*Table* 3). The whole plant was divided into: (i) fed root; (ii) seed and remaining two roots; (iii) shoot plus second leaf; (iv) basal; (v) middle; and (vi) terminal portions of the first leaf. The cut shoot was divided into: (i) shoot plus second leaf; (ii) basal; (iii) middle; and (iv) terminal portion of the first leaf. In each case each portion of the plant was dried in an oven at 80°, ground in a small mortar and extracted with 100% ethanol. An aliquot of the extract was counted and the residue was dried and counted suspended in a thixotropic scintillation gel containing 4% Cab-O-Sil. The total counts in each portion of the plant were then calculated.

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