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Tissue-specific developmental changes in cell-wall ferulate and dehydrodiferulates in sugar beet

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Abstract

Sugar beet (*Beta vulgaris* L.) seedlings were grown for 8–14 weeks, and then separated into leaf, petiole, inner and outer storage root and absorptive root fractions. Cell-wall ferulate and dehydrodiferulate esters were analysed by HPLC. In leaves, ferulate dimers were mostly 8–8 linked, while 8–O–4 and sometimes 8–5 linkages were most abundant in all other tissues. The total dimer content and percentage of dimerisation were much higher in the absorptive root than in other tissues. These results indicated varying patterns of ferulate and dehydrodiferulate ester content in different tissues, suggesting corresponding variations in the biosynthetic processes. When [^{14}C]-cinnamate was applied to the leaves at 4 weeks, and [^{14}C]-dimers measured in root cell walls at 8 and 14 weeks, a much higher proportion of 8–5 linkages was found in the [^{14}C]-dimers than in total (non-radioactive) dimers in all parts of the root, especially at 14 weeks, indicating further complexity in the metabolism of cell-wall phenolics.

Keywords: *Beta vulgaris* L.; *Chenopodiaceae*; Sugar beet; HPLC; Cell wall; Ferulate; Dehydrodiferulate; Leaf; Petiole; Storage root; Absorptive root

1. Introduction

Plant cell walls contain hydroxycinnamic acids, chiefly ferulic and coumaric acids, ester-linked to pectins in dicots and to arabinoxylans in grasses (Ishii, 1997; Waldron et al., 1997b). Some of the ferulic acid moieties are coupled by oxidative reactions to form a variety of dehydrodiferulate (DFA) dimers, which thus cross-link the polysaccharides (Ralph et al., 1994; Waldron et al., 1996). Such cross-links may have a number of important roles, such as decreasing cell-wall extensibility during cell maturation (Kamisaka et al., 1990; Sanchez et al., 1996) and when dark-grown tissues are exposed to light (Miyamoto et al., 1994). Dehydrodiferulate cross-links may also promote cell-cell adhesion (Ng et al., 1998) and maintain tissue texture during cooking (Waldron et al., 1997a,b). They may be nucleation sites for lignin synthesis (Ralph et al., 1995), and limit cell-wall degradability in forage grasses (Grabber et al., 1998).

Six different ferulate dimers have been isolated from extracts obtained from cell walls (Rombouts and Thibault, 1986; Ralph et al., 1994; Parr et al., 1996; Ishii, 1997; Waldron et al., 1997b). These were thought to be derived from four dimers present in the wall (8–O–4, 8–5, 8–8 and 5–5), alkaline extraction from the wall giving rise to two 8–8-linked products [the open, uncyclised form, and the cyclised, aryltetralin form (8–8A)] and two 8–5-linked products [the open, uncyclised form and the cyclised, benzofuran form (8–5B)] (Ralph et al., 1994). In most angiosperm tissues, the 8–O–4 and 8–5 dimers are the most abundant (Ralph et al., 1994; Parr et al., 1996; Waldron et al., 1996, 1997a), while in the one gymnosperm studied, the 8–8 dimers were the most abundant (Sanchez et al., 1996).

Little work has been carried out on the variations in dimer content in different tissues in the same plant or on tissues at different stages of development. In cocksfoot and switchgrass, differences in the absolute and relative

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abundance of the dimers between parenchyma and schlerenchyma were reported, with the 8–O–4 and 8–5 dimers generally being the most abundant (Ralph et al., 1994). In pine hypocotyls, dimer content increased greatly with increasing hypocotyl maturation, with the 8–8 dimers always being the most abundant (Sanchez et al., 1996). In oat (*Avena sativa*) coleoptiles, both ferulic acid and 5–5 dehydrodiferulic acid content increased during cell maturation (Kamisaka et al., 1990) and when dark-grown tissues were exposed to light (Miyamoto et al., 1994), and similar results were reported for rice (*Oryza sativa*) coleptiles (Tan et al., 1991, 1992). However, in neither of these tissues have analyses of the other dehydrodimers been reported.

In common with other members of the *Chenopodiaceae*, beet (*Beta vulgaris* L.) tissues contain greater amounts of wall-bound ferulate and dehydrodiferulates than other dicots (Rombouts and Thibault, 1986; Colquhoun et al., 1994; Waldron et al., 1997a). We have reported that dimer content is much higher (as a percentage of total ferulates) in the root than in the shoot in beet (Wende et al., 1999). The difference is due to increased proportions of the 8–O–4, 8–5 and 8–5B dimers in the root, the proportions of the other dimers changing relatively little. In both shoot and root, 8–O–4 DFA was the major dimer obtained (though the 8–5 and 8–5B dimers combined were present in similar amounts to the 8–O–4). Both ferulate and DFA content remained approximately constant during shoot development, but declined during root development.

Pulse-chase experiments in which [^{14}C]-cinnamate was supplied to leaves, and then radioactivity monitored during tissue development in root and shoot, indicated that the proportion of radioactivity in [^{14}C]-dehydrodimers increased with time in the shoot (Wende et al., 1999). This indicated an increase in diferulate cross-linking as tissues matured. Amongst the radioactive dimers in the root, the 5–5 dimer was much more prominent than in the non-radioactive dimers, and the proportion of the radioactive 5–5 dimer increased during root maturation. The reasons for this difference between radioactive and non-radioactive dimers in the same tissue were not clear.

In these pulse-chase experiments (Wende et al., 1999), autoradiography indicated that radioactive phenolics were present in both petioles and leaf laminae, and in both storage root and absorptive root. However, within the storage root, radioactive phenolics were localised in the epidermis and central vascular tissue, with relatively little radioactivity in the cortex. We have now studied the ferulate and dehydrodiferulate content of the petioles, leaf laminae, absorptive root and the inner and outer tissues of the storage root, and investigated how these change during seedling development. These analyses have been accompanied by pulse-chase studies in which [^{14}C]-cinnamate was supplied to leaves, and then radioactivity monitored during tissue development in the three root tissues.

2. Results

Sugar beet seedlings were grown for up to 14 weeks, and tissues harvested after 8, 10, 12 and 14 weeks of growth. At each harvest, between two and four plants were analysed. Tissues from each plant were divided into leaf laminae, petioles, inner and outer storage root, and absorptive root. Changes in dry weight of cell-wall material (alcohol-insoluble residue, "AIR") during development in each part of the plant are shown in Fig. 1. For pulse-chase experiments, [^{14}C]-cinnamate was applied to the two youngest leaves at 4 weeks old, and radioactively-labelled plants were then harvested at 8 and 14 weeks. Alkali-labile cell-wall phenolics were extracted from the tissue fractions and analysed by HPLC for both phenolic content (non-radioactive measurements) and for radioactively-labelled phenolics.

2.1. Ferulate and dehydrodiferulate (dimer) content in shoot tissues during development (non-radioactive measurements)

Total ferulates (monomers plus dimers) were generally more abundant in petiole tissue than in leaf tissue at all stages of development (Fig. 2). In both tissues, there were some changes in total ferulates content over the course of the experiment, but these changes were barely significant given the observed variability between different plants. The total dimer content was quite similar in leaves and petioles (Fig. 3), with little variation over time. Percentage dimerisation (total dimers expressed as a percentage of total ferulates) was, therefore, lower in petioles than in leaves (Table 1).

2.2. Ferulate and dehydrodiferulate (dimer) content in root tissues during development (non-radioactive measurements)

Total ferulates (monomers plus dimers; Fig. 4) increased slowly in all parts of the root during the experiment. From 10 weeks onward, total ferulates were present in approximately the same amounts in the inner and outer storage root tissues, with slightly lower amounts being present in the absorptive root (Fig. 4). Total dimer content also increased slowly over time in all parts of the root, but the total dimer content in the absorptive root was consistently almost twice as high as in the outer part of the storage root, and approximately four times as high as in the inner part of the storage root (Fig. 5). At all time points, the percentage dimerisation was, therefore, much higher in the absorptive root than in the storage root (Table 1).

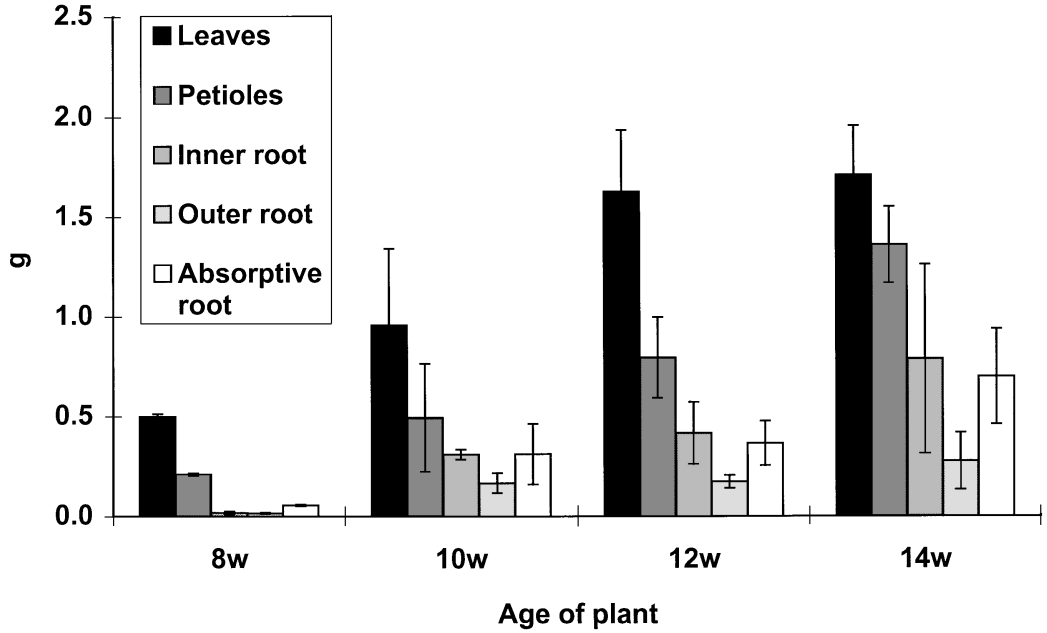

Fig 1. Changes in dry weight of tissue AIR's (cell-wall material) during development. Beet tissues were homogenised, extracted with 80% and 100% EtOH and Me_2CO, dried at 50°C, and weighed.

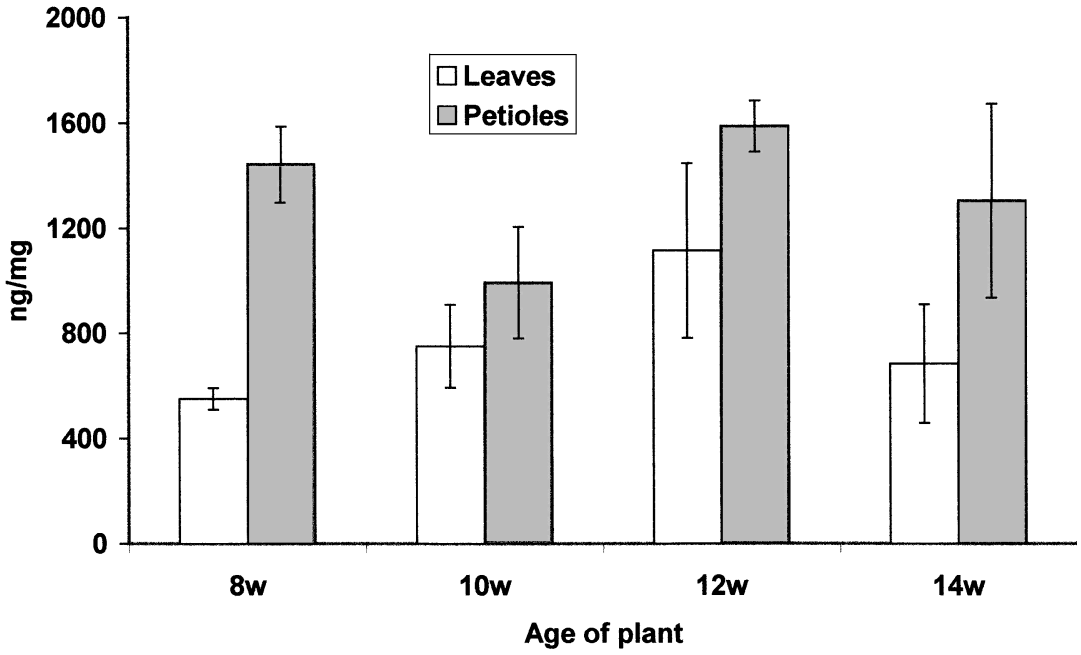

Fig. 2. Total ferulates in shoot tissues. Leaves and petioles were harvested from seedlings of different ages, and alkali-labile ferulates (monomers plus dimers) in the cell walls (AIR's) were measured by HPLC.

2.3. *Relative abundance of the different dimers in leaf and petiole during development (non-radioactive measurements)*

The relative amounts of the different dimers in leaf and petiole did not change greatly during development (Table 2). However, a major difference was observed between the two tissues. In petioles, the 8–O–4 dimer was the major dimer throughout, with all the other dimers being present in much lower amounts. In leaf laminae, the 8–8A dimer was the major dimer throughout, with the 8–8 and 8–O–4 dimers also being present in relatively large amounts. The 8–8 and 8–8A forms combined accounted for between half and two-thirds of the total dimers.

2.4. Relative abundance of the different dimers in root tissues during development (non-radioactive measurements)

The relative amounts of the different dimers were similar in all three root tissues studied, and did not change greatly during development (Table 3). In all cases, the 8–O–4 dimer was the major dimer. The 8–5B dimer was generally the second most abundant, except in the inner and outer storage root at 8 weeks, where the 8–8A dimer was the second most abundant. The 8–O–4 dimer was more abundant than both the combined 8–8 dimers and the combined 8–5 dimers, except in the

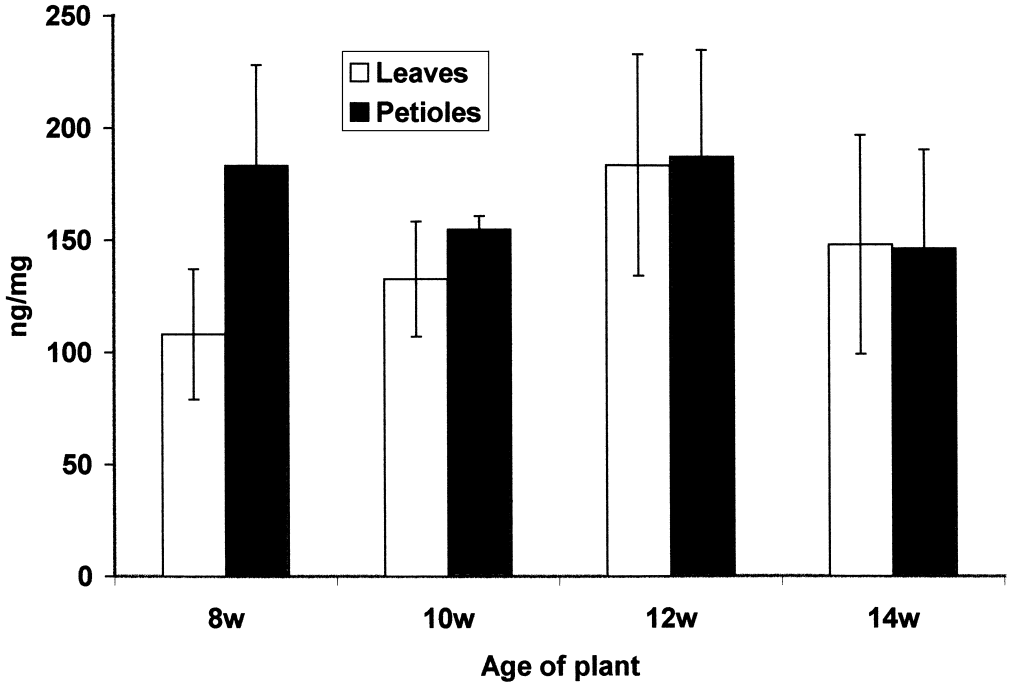

Fig. 3. Total dehydrodiferulates in shoot tissues. Leaves and petioles were harvested from seedlings of different ages, and alkali-labile ferulate dimers in the cell walls (AIR's) were measured by HPLC.

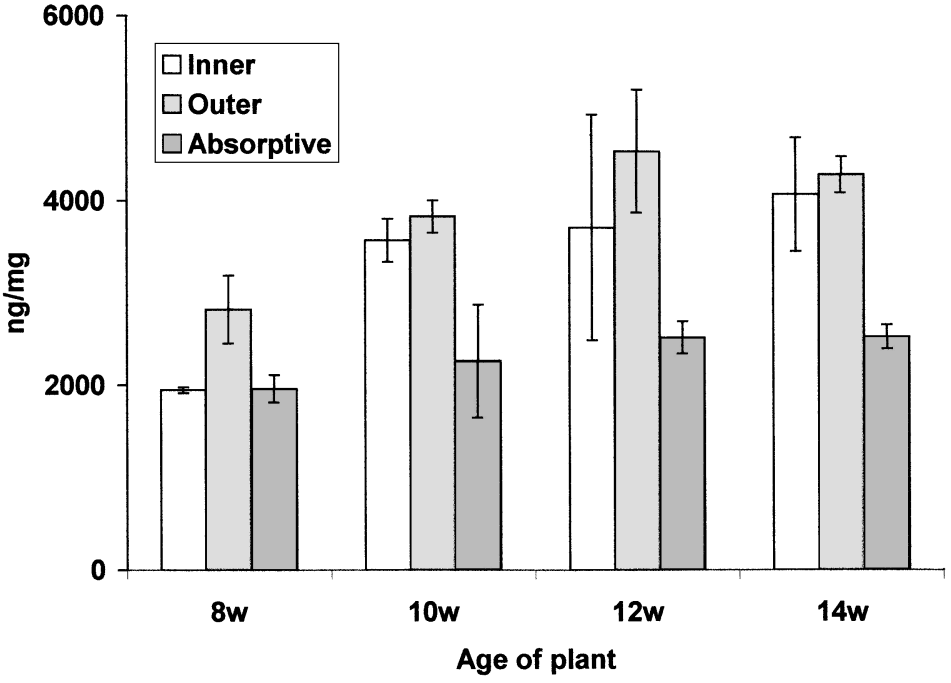

Fig. 4. Total ferulates in root tissues. Inner and outer storage roots and absorptive roots were harvested from seedlings of different ages, and alkali-labile ferulates (monomers plus dimers) in the cell walls (AIR's) were measured by HPLC.

Table 1
Percentage of ferulate cross-linking (total dimers as a percentage of ferulate monomers plus dimers) in developing tissues in young sugar beet plants

	8 Weeks	10 Weeks	12 Weeks	14 Weeks
Leaves	19.3±3.8	17.7±0.5	16.9±3.6	21.7±3.4
Petioles	12.5±1.9	16.1±3.0	12.6±2.0	11.2±1.0
Inner root	15.6±1.5	8.6±0.7	9.3±1.4	10.6±2.0
Outer root	26.7±3.5	21.4±2.5	20.6±2.1	25.8±1.3
Absorptive root	63.3±5.0	69.9±3.0	71.6±4.8	76.2±0.9

absorptive roots at the earlier time points, when the combined 8–5 dimers were of comparable abundance.

2.5. Changes in radioactively-labelled dehydrodiferulates in the root during growth

In our previous work (Wende et al., 1999), it was noted that the relative abundance of radioactively labelled dimers in the root was different to that of non-radioactive dimers when [^{14}C]-cinnamate was applied to the leaves and subsequently monitored in the root. In this work, we therefore sought to determine whether this was due to differences between different root tissues. As noted in the earlier publication, of the six dimers, only the 8–O–4, 8–5B and 5–5 dimers were sufficiently well resolved by HPLC for their radioactivity to be determined. Results are, therefore, quoted as a percentage of total alkali-labile radioactivity recovered from the cell wall material (AIR). [^{14}C]-Cinnamate was applied to the cotyledons at 4 weeks old and subsequently monitored in the different tissues of the root at 8 and 14 weeks. As previously, [^{14}C]-labelled dimers gave results that were significantly different from those of non-radioactive dimers (expressed as a percentage of total alkali-labile phenolics). Data for the three dimers whose radioactivity was resolved on HPLC are shown in Table 4. The [^{14}C]-5–5 dimer was present in relatively much greater abundance than in non-radioactive dimers, and this disparity increased as the tissues matured, so that in the absorptive root at 14 weeks, the [^{14}C]-5–5 dimer was present in greater amounts than the [^{14}C]-8–O–4 or the [^{14}C]-8–5B dimer. However, a similar pattern was seen in all three root tissues, so the difference

Table 2
Content of ferulate dimers as a percentage of total dimers in developing shoot tissues of sugar beet plants

Tissue	Dimer	8 Weeks	10 Weeks	12 Weeks	14 Weeks
Leaves	8–8A	32.9±1.7	32.1±2.9	35.8±2.7	30.7±8.0
	8–8	31.7±3.3	26.8±6.0	13.7±7.1	27.7±6.7
	8–5	6.3±0.1	8.2±1.2	11.4±1.5	7.4±0.8
	5–5	5.1±1.3	7.2±3.2	6.8±2.1	7.3±2.2
	8–O–4	18.5±2.1	20.4±2.0	24.3±2.8	21.2±4.2
	8–5B	5.4±1.4	5.3±1.0	8.0±2.7	5.6±1.9
Petioles	8–8A	15.1±0.8	12.7±3.8	9.7±3.7	13.3±5.8
	8–8	17.2±4.2	20.6±1.6	14.0±2.1	10.9±7.7
	8–5	8.0±1.1	10.4±1.4	10.1±2.8	12.7±5.8
	5–5	10.5±1.6	11.3±1.3	13.2±1.2	11.4±1.8
	8–O–4	34.6±0.2	33.3±2.2	36.8±4.7	36.5±1.9
	8–5B	14.6±2.5	11.8±1.6	16.2±2.6	15.2±1.6

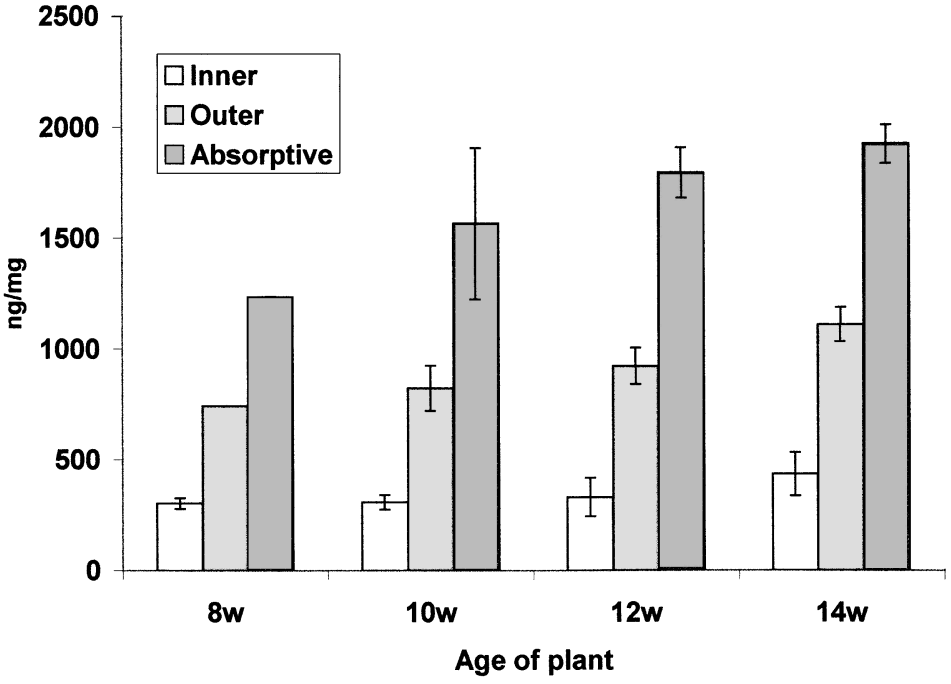

Fig. 5. Total dehydrodiferulates in root tissues. Inner and outer storage roots and absorptive roots were harvested from seedlings of different ages, and alkali-labile ferulate dimers in the cell walls (AIR's) were measured by HPLC.

Table 3
Content of ferulate dimers as a percentage of total dimers in developing root tissues of sugar beet plants

Tissue	Dimer	8 Weeks	10 Weeks	12 Weeks	14 Weeks
Inner root	8–8A	18.6±2.5	10.4±0.6	12.9±2.7	12.0±1.5
	8–8	12.7±9.4	11.3±1.9	9.5±1.6	2.6±1.0
	8–5	10.5±1.1	8.2±0.4	7.6±0.8	8.1±1.4
	5–5	8.5±1.2	14.5±1.9	12.0±1.4	16.3±1.4
	8–O–4	36.5±4.9	37.0±1.6	38.3±1.9	39.7±3.2
	8–5B	13.1±0.3	18.7±0.8	19.8±1.6	21.3±3.9
Outer root	8–8A	18.3±1.5	15.7±5.9	12.6±0.3	8.0±2.1
	8–8	11.4±8.4	2.5±1.5	6.3±0.7	5.7±0.3
	8–5	8.5±0.1	7.8±0.4	7.1±0.3	6.5±0.5
	5–5	8.4±1.9	9.2±0.5	8.9±0.7	10.8±0.9
	8–O–4	38.7±3.7	42.7±3.6	44.0±1.2	42.3±0.9
	8–5B	14.6±4.2	22.0±2.5	21.1±1.6	24.3±1.0
Absorptive root	8–8A	11.1±0.7	13.6±3.7	13.8±1.0	15.6±1.8
	8–8	0.8±0.1	4.0±0.4	4.0±0.5	3.8±0.5
	8–5	20.2±4.7	10.2±0.8	9.7±0.5	9.1±0.5
	5–5	6.8±0.2	8.1±0.7	6.8±1.7	6.1±2.0
	8–O–4	38.3±0.6	36.2±1.8	38.7±2.7	37.0±1.4
	8–5B	26.2±0.4	27.9±0.9	27.0±2.9	28.5±1.1

between radioactive and non-radioactive measurements was not attributable to tissue-specific variations.

3. Discussion

The analyses reported here have revealed several major differences in the alkali-labile ferulates in different beet tissues. In the shoot, total diferulate levels were similar in lamina and petiole, even though total ferulate levels were greater in the petiole. Hence the degree of polysaccharide cross-linking may be controlled directly at the level of dimerisation, as previously suggested for light-grown *Avena* coleoptiles (Miyamoto et al., 1994), rather than by the levels of monomers available for cross-linking. Moreover, the similarity in total diferulate levels in the two tissues is maintained even though the relative amounts of different dimers in the two tissues is remarkably different. This suggests an unexpected degree of sophistication in the mechanisms which control the degree of polysaccharide cross-linking. The reasons for the different relative abundance of dimers in the two tissues remain to be explored. The most obvious possibility is that different peroxidase isoenzymes, producing different ratios of dimers, are present in the two tissues. It is interesting that the dominance of the 8–8 dimers in beet leaves, not previously found in any other angiosperm tissue, is similar to the pattern found in hypocotyls of pine, a gymnosperm (Sanchez et al., 1996). Comparison of isoenzyme patterns in beet tissues and pine hypocotyls might prove very informative. Alternatively, 'dirigent proteins', analogous to those reported to bring about stereospecific coupling of coniferyl alcohols to give (+)-pinoresinol (Davin et al., 1997), might be involved. Whether different dimers might have different physiological roles also remains to be investigated.

In the root, while the relative amounts of the different dimers were quite similar in the different parts of the root, the level of total dimers varied greatly. By far the highest dimer levels were found in the absorptive root, even though the total ferulate levels were generally lower in the absorptive root than in the rest of the root. The exceptionally high percentage of ferulate cross-linking in the absorptive root accounts for the higher overall percentage of cross-linking previously observed in the whole root when compared to the whole shoot (Wende et al., 1999). It is unclear what the physiological role of this localised high dimer content is. Given the high surface area of the absorptive root, and the fact that the next highest dimer content is found in the outer storage root, it may be that the dimers are present in high concentrations in epidermal cells. In that case they might be involved in protecting the cells against damage by pathogens and/or soil abrasion. They might also be involved in controlling the overall growth of the plant,

Table 4
Changes in radioactive and non-radioactive ferulate dimers as a percentage of total phenolics in developing root tissues

Tissue	Dimer	8 weeks		14 weeks	
		Radioactive	Non-radioactive	Radioactive	Non-radioactive
Inner root	5–5	3.6±0.1	1.4±0.4	4.2±0.2	1.7±0.3
	8–O–4	5.2±1.8	5.8±1.3	)	3.6±0.1
				) 7.7±1.2	
	8–5B	3.1±0.2	2.1±0.2	)	2.0±0.7
Outer root	5–5	6.8±2.5	2.2±0.2	22.5±0.2	2.7±0.3
	8–O–4	7.3±0.9	10.3±0.4	)	11.1±0.3
				) 26.5±0.6	
	8–5B	5.9±1.2	3.9±0.6	)	6.5±0.3
Absorptive root	5–5	11.9±2.4	4.3±0.2	24.9±1.7	5.1±1.8
	8–O–4	6.8±2.0	24.3±2.3	18.3±0.7	28.8±1.4
	8–5B	5.1±0.4	16.6±1.5	17.3±3.9	21.9±0.9

since the epidermis is thought to limit overall growth in some situations. It is possible that different degrees of cross-linking in different tissues might be related to differential incorporation of monomers and dimers into lignin, since they may act as nucleation sites for lignin synthesis (Ralph et al., 1995).

The relatively high abundance of [^{14}C]-5–5 dehydrodiferulate compared to other ^{14}C-dimers in all three root tissues, especially at 14 weeks, showed that this was a general phenomenon throughout the root. It seems likely that this represents a change during tissue maturation of cell-wall material that was laid down early in tissue development (Wende et al., 1999). This might be due to varying crosslinking or turnover patterns during maturation.

4. Experimental

4.1. Plant material

Seeds of sugar beet (*Beta vulgaris* L., var. Saxon) were soaked in vigorously aerated water at 22°C overnight. Seeds were then germinated in vermiculite and the seedlings transferred into 5× strength Hoaglands solution plus 0.5% ferrous sulfate and 0.4% tartaric acid. The plants were grown in the presence of white fluorescent light and in the dark (16 and 8 h, respectively) at 22°C.

4.2. Analysis

Tissues were harvested after 8, 10, 12 and 14 weeks of growth, and separated into leaf, petiole, inner and outer storage root and absorptive root. Outer tissues were cut from the storage root with a scalpel. Alcohol insoluble residue (AIR) was prepared separately from the separate tissues of each of 2–4 plants per time point. Plant tissue was homogenised in liquid nitrogen, suspended in 80% EtOH, centrifuged (1500 *g*, 5 min), and the supernatant discarded. The pellet was incubated twice in 80% EtOH at 80°C for 10 min, washed twice in 100% EtOH and finally twice in Me_2CO, and dried overnight at 50°C. Cold-alkali-labile cell-wall phenolics were extracted separately from each AIR with 2 M NaOH at room temperature for 18 h, acidified with HCl, and the phenolic material partitioned into EtOAc (three extractions, recovering at least 98% of the phenolic material) and analysed by HPLC (Waldron et al., 1996). This technique resolved *cis*- and *trans*-ferulic acid and the six dehydrodiferulic acids, together with other phenolics (Fig. 1 of Waldron et al., 1996).

4.3. Uptake of radiolabel into plant tissues

[Side-chain-3-^{14}C]*trans*-cinnamic acid (185 kBq; 529 Mbq.mmol^{-1}) in 10 μl 50% EtOH was applied to the two youngest leaves of the seedlings (2 per time-point) after 4 weeks of growth (5 μl per leaf, placed as a single spot at the centre of the leaf). Tissues were subsequently harvested at 8 and 14 weeks. AIR's were prepared, saponified, and the alkali-labile phenolics analysed by HPLC as above. However, for radioactive work it was necessary to collect fractions from the effluent from the HPLC column. This resulted in a loss of resolution, so that generally only the 8–O–4, 5–5 and the 8–5B dehydrodimers could clearly be resolved. Hence results of the radioactivity feeding experiments are reported as the percentage of radioactivity present in each of these dimers, relative to the total alkali-labile radioactivity recovered from the column.

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References

Colquhoun, I.J., Ralet, M-C., Thibault, J-F., Faulds, C.B., Williamson, G., 1994. Feruloylated oligosaccharides from cell wall polysaccharides. Part II. Structure identification of feruloylated oligosaccharides from sugar beet pulp. Carbohydrate Research 263, 243–256.

Davin, L.B., Wang, H.B., Crowell, A.L., Bedgar, D.L., Martin, D.M., Sarkanen, S. et al., 1997. Stereoselective bimolecular phenoxy radical coupling by an auxiliary (dirigent) protein without an active center. Science 275, 362–366.

Grabber, J.H., Hatfield, J.R., Ralph, J., 1998. Diferulate cross-links limit the enzymatic degradation of synthetically lignified primary walls of maize. Journal of the Science of Food and Agriculture 77, 193–200.

Ishii, T., 1997. Structure and function of feruloylated polysaccharides. Plant Science 127, 111–127.

Kamisaka, S., Takeda, S., Takahashi, K., Shibata, K., 1990. Diferulic acid and ferulic acid in the cell wall of *Avena* coleoptiles — their relationship to mechanical properties of the cell wall. Physiologia Plantarum 78, 1–7.

Miyamoto, K., Ueda, J., Takeda, S., Ida, K., Hoson, T., Masuda, Y., Kamisaka, S., 1994. Light-induced increase in the contents of ferulic and diferulic acids in cell-walls of *Avena* coleoptiles — its relationship to growth inhibition by light. Physiologia Plantarum 92, 350–355.

Ng, A., Harvey, A.J., Parker, M.L., Smith, A.C., Waldron, K.W., 1998. Effect of oxidative coupling on the thermal stability of texture and cell wall chemistry of beet root (*Beta vulgaris*.). Journal of the Science of Food and Agriculture 76, 3365–3370.

Parr, A.J., Waldron, K.J., Ng, A., Parker, M.L., 1996. The wall-bound phenolics of Chinese water chestnut (*Eleocharis dulcis*). Journal of the Science of Food and Agriculture 71, 501–507.

Ralph, J., Grabber, J.H., Hatfield, R.D., 1995. Lignin-ferulate cross-links in grasses: active incorporation of ferulate polysaccharide esters into ryegrass lignins. Carbohydrate Research 275, 167–178.

Ralph, J., Quideau, S., Grabber, J.H., Hatfield, R.D., 1994. Identification and synthesis of new ferulic acid dehydrodimers present in grass cell walls. Journal of the Chemical Society, Perkin Transactions 1, 3485–3498.

Rombouts, F.M., Thibault, J.-F., 1986. Feruloylated pectic substances from sugar-beet pulp. Carbohydrate Research 154, 177–188.

Sanchez, M., Pena, M.J., Revilla, G., Zarra, I., 1996. Changes in dehydrodiferulic acids and peroxidase activity against ferulic acid associated with cell walls during growth of *Pinus pinaster* hypocotyls. Plant Physiology 111, 941–946.

Tan, K.-S., Hoson, T., Masuda, Y., Kamisaka, S., 1991. Correlation between cell wall extensibility and content of diferulic acid and ferulic acid in cell walls of *Oryza sativa* coleoptiles grown under water and in air. Physiologia Plantarum 83, 397–403.

Tan, K.-S., Hoson, T., Masuda, Y., Kamisaka, S., 1992. Involvement of cell wall-bound diferulic acid in light-induced decrease in growth rate and cell wall extensibility of *Oryza coleoptiles*. Plant Cell. Physiology 33, 103–108.

Waldron, K.W., Parr, A.J., Ng, A., Ralph, J., 1996. Cell wall esterified phenolic dimers: identification and quantification by reverse phase high performance liquid chromatography and diode array detection. Phytochemical Analysis 7, 305–312.

Waldron, K.W., Ng, A., Parker, M.L., Parr, A.J., 1997a. Ferulic acid dehydrodimers in the cell walls of *Beta vulgaris* and their possible role in texture. Journal of the Science of Food and Agriculture 74, 221–228.

Waldron, K.W., Smith, A.C., Parr, A.J., Ng, A., Parker, M.L., 1997b. New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. Trends in Food Science and Technology 8, 213–221.

Wende, G., Waldron, K.W., Smith, A.C., Brett, C.T., 1999. Developmental changes in cell-wall ferulate and dehydrodiferulates in sugar beet. Phytochemistry 52, 819–827.