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Chemical Composition and Enzymatic Degradability of Xylem and Nonxylem Walls Isolated from Alfalfa Internodes

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During plant maturation, degradability of alfalfa (*Medicago sativa* L.) stems declines due to accumulation of highly lignified xylary tissue. Xylem and nonxylem tissues dissected from lower alfalfa internodes were analyzed for cell wall constituents and degradability. Cell walls comprised 740 mg g^{-1} of xylem and 533 mg g^{-1} of nonxylem tissues. Xylem tissues contributed about 60% of the cell wall mass in internodes. Xylem walls contained 28% lignin, 4% pectin, 29% hemicellulose, and 39% cellulose as compared to 15% lignin, 25% pectin, 30% hemicellulose, and 30% cellulose in nonxylem walls. Fungal enzymes hydrolyzed 22 and 73% of the structural carbohydrates in xylem and nonxylem walls, respectively. In both cell wall fractions, the release of xylose was 56−90% lower than that of other sugars, indicating that lignin preferentially restricted xylan degradation in secondary walls and xyloglucan degradation in primary walls. Elucidation of lignin−xylose interactions may reveal strategies for improving fiber degradability of alfalfa.

KEYWORDS: Medicago sativa; xylem; cell wall; fiber; pectin; cellulose; xylan; xyloglucan; lignin; degradability; digestibility

INTRODUCTION

The enzymatic degradability of forages declines significantly during plant maturation, due to decreasing degradability of cell walls in stems and their increasing contribution to plant dry matter (*1*−*3*). Declining cell wall degradability has been associated with the deposition of lignin and xylose-containing polysaccharides in maturing stems (*4*−*7*). These associations weaken, however, in forages of similar maturity, indicating that additional factors influence degradability. Studies with forage grasses—using microscopic, tissue isolation, and model system approaches—indicate that a variety of chemical and anatomical factors influence cell wall degradability (*8*−*16*). Mechanisms limiting legume degradability are less understood, although declining degradability of stems is associated with the formation of a poorly degraded ring of highly lignified xylary tissues (*17*−*20*) composed of primary xylem, secondary xylem, and xylem fibers (**Figure 1A**,**B**). In contrast, nonxylary tissues, including pith parenchyma, chlorenchyma, secondary phloem, phloem fibers, collenchyma, and epidermis, have moderate to high degradability even in mature stems. Published work on isolated cell types from forage legumes is limited to an analysis of lignin concentration and composition in "wood" and "bark" of alfalfa internodes (*18*). Investigations of the carbohydrate chemistry and degradability of isolated cell types in forage legumes have not been reported; thus far, work on isolated tissues from herbacious dicots is limited to *Brassica* species (*21*, *22*). These studies revealed significant compositional and degradability differences between xylem and nonxylem tissues. In the current study, xylem and nonxylem tissues were isolated from alfalfa internodes and analyzed for cell wall constituents and carbohydrate degradability to enhance our understanding of factors limiting the degradability of cell walls in legume stems.

MATERIALS AND METHODS

Whole internode and tissue fractions used in this study were a subset of samples selected from a larger study examining relationships between the cross-sectional area and dry weight of tissues in alfalfa stems (M. T. Panciera and W. J. Wiebold, unpublished results). Field grown "Vernal" alfalfa plants, at the late bud to early flower growth stage, were cut at the soil surface on 17 July 1986 from an established stand at the Ohio State University Farm in Columbus, OH. Internode 1 (basal) to internode 6 were cut from the lower half of 15 stems and split longitudinally. A scalpel blade was used to peel and scrape outer nonxylem tissues (epidermis, chlorenchyma, collenchyma, phloem fibers, secondary phloem, and cambium) from xylem tissues (primary xylem, secondary xylem, and xylem fibers). The more easily removed inner-pith parenchyma was then scraped away from xylem tissues, using a scalpel handle, and combined with other nonxylem tissues. Xylem tissues were examined with a dissecting microscope to ensure that all nonxylem tissues were removed. Tissues from individual plants were combined, placed in prefolded filter papers, dried at 60 °C for 48 h, weighed, and ground with UDY cyclone mill to pass a 0.5 mm screen.

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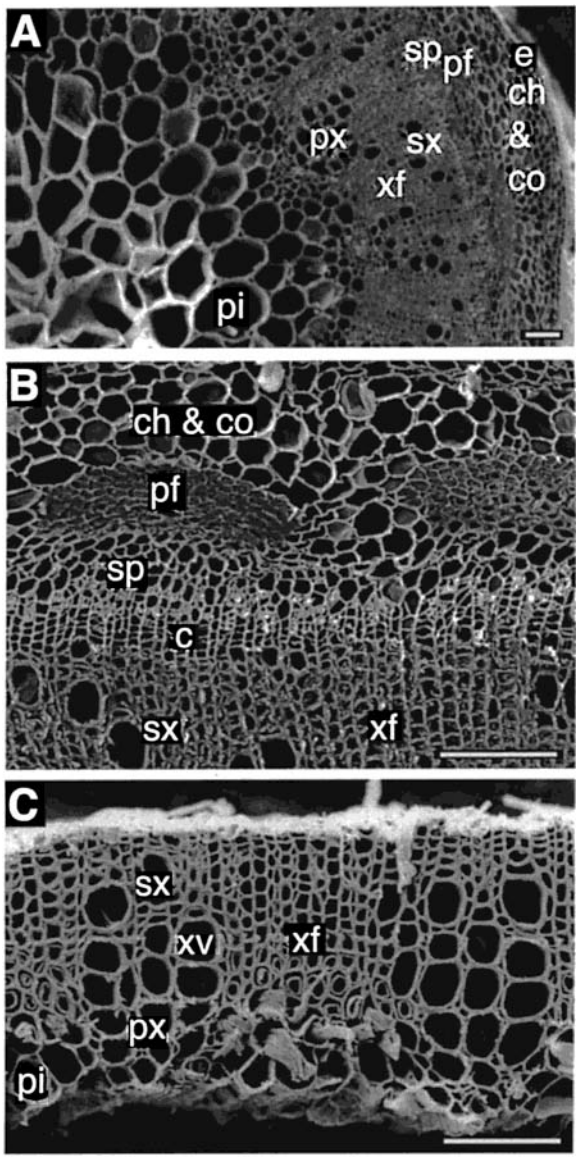

Figure 1. SEM cross-sections of lower internodes of alfalfa: tissue distribution in intact stems (**A**); organization of tissues around the meristematic cambial layer (**B**); and isolated xylary tissues (**C**). Bar = 100 μm; c, cambium; ch, chlorenchyma; co, collenchyma; e, epidermis; pf, phloem fiber; pi, pith parenchyma; px, primary xylem; sp, secondary phloem; sx, secondary xylem; xf, xylem fiber; xv, xylem vessel.

Unfortunately, xylem and nonxylem tissues collected from the third internode were not available for the current study. Free-hand cross-sections for scanning electron microscopy were dried, mounted on specimen stubs, coated with gold, and viewed with a JEOL 35CM (JEOL, Inc., Peabody, MA) at an accelerating voltage of 10 kV.

Whole internode, xylem, and nonxylem tissues (350 mg) were weighed into 50 mL centrifuge tubes and moistened overnight at 5 °C with 1.5 mL of water. The following morning, tissues were suspended in ice-cold 50 mM NaCl (30 mL) and then alternately incubated on ice (30 min) and sonicated (10 min) over a 2 h period. Tissues were then pelleted by centrifugation (10 min, 2500*g*) and reextracted with fresh 50 mM NaCl as before. Next, tissues were resuspended, sonicated (10 min), and pelleted (10 min, 2500*g*) four times with 80% aqueous ethanol (20 mL) and once with water (30 mL). Finally, tissues were suspended in hot KH_2PO_4 buffer with α-amylase and amyloglucosidase, washed with water, and freeze-dried according to published methods (*23*) to yield crude cell walls. The amylase/amyloglucosidase extracts and water washes were combined, dialyzed against water for 48 h, and freeze-dried to recover extracted pectins (*23*). Crude cell walls and extracted pectins were analyzed for neutral sugars by high-performance liquid chromatography and for uronosyls by a colorimetric method (*23*). Ester- and ether-linked hydroxycinnamic acids were determined by gas chromatography-flame ionization detection (*24*). Acetyl bromide lignin and methanol were determined by colorimetric methods (*25*, *26*). Uronosyls were also determined on xylem and nonxylem tissues extracted with only 80% aqueous ethanol. Cell walls were suspended (0.7% w/v) in 20 mM acetate buffer (pH 4.8, 40 °C) and hydrolyzed with a mixture of Celluclast 1.5 L and Viscozyme L (Novo Nordisk), each added at 0.3 μL mg^{-1} of cell wall. This mixture contains the full complement of hydrolase activities required to degrade cell walls (*28*). After 3 and 48 h of enzymatic hydrolysis, wall residues were pelleted by centrifugation (2 min, 10 000*g*) and an aliquot of the supernatant was analyzed for uronosyls (*27*) and neutral sugars (*23*). All analyses were run in duplicate or triplicate. Changes in cell wall composition and degradability across internodes were described by linear or quadratic regression. Xylem and nonxylem data from individual internodes were considered as paired observations; means were compared using a paired *t*-test.

RESULTS AND DISCUSSION

Tissue Isolation and Cell Wall Content. Tissues were collected from the basal six internodes in which primary xylem, secondary xylem, xylem fibers and adjacent pith parenchyma had formed a rigid ring of xylary tissue. Tissues were peeled and scraped away from the inner and outer surfaces of the xylary ring to produce xylem and nonxylem fractions (**Figure 1C**). On the basis of histological studies (*18*, *20*, *29*), the xylem fraction would consist mainly of highly lignified cells with thin primary walls and thick secondary walls that are poorly degraded by rumen microflora. The nonxylem fraction would consist of nonlignified and highly degradable chlorenchyma, secondary phloem, collenchyma, and cambial cells with primary walls. This fraction would also contain tissues with moderate degradability—cutinized epidermis and partially lignified pith parenchyma with primary walls and phloem fibers that have partially lignified primary walls and nonlignified secondary walls. The proportion of internode dry matter comprised by xylem tissues averaged 527 mg g^{-1}, declining linearly by 130 mg g^{-1} from the first internode at ground level to the sixth internode, located midway up the stem (**Figure 2A**).

Mature alfalfa internodes contain about 100 mg g^{-1} protein (*1*), which may interfere with degradability estimates by fungal enzymes. Alfalfa stems also contain about 50 mg g^{-1} of starch (*30*), which can inflate estimates of cell wall glucose, and pigments, which interfere with colorimetric estimates of lignin (*31*). Unfortunately, hot detergent solutions and other solvents typically used to prepare cell walls often remove considerable amounts of pectin (*32*, *33*). Therefore, oven-dried whole internodes and isolated tissue fractions were sequentially treated with ice-cold 50 mM NaCl solution, aqueous ethanol, and hot buffer with amylase/amyloglucosidase in an attempt to remove starch, pigments, and some protein and yet retain structural polysaccharides, particularly pectins. Pectins extracted by the amylase/amyloglucosidase step were recovered by dialysis for analysis. This approach worked well for recovering pectic sugars from whole internodes, xylem, and most nonxylem fractions. Uronosyl concentrations were, however, unexpectedly low in crude cell walls and amylase−buffer extracts from nonxylem fractions isolated from the fifth and sixth internodes. Subsequent analysis of tissues, extracted by only 80% aqueous ethanol, revealed that preextraction with a weak NaCl solution removed about 23 mg g^{-1} of uronosyls from these nonxylem fractions. Presumably, pectin rich in polygalacturonic acid was solubilized from these intermediate internodes by the salt solution. Although not examined in our study, we suspect that upper internodes of alfalfa would be even more susceptible to pectin extraction by

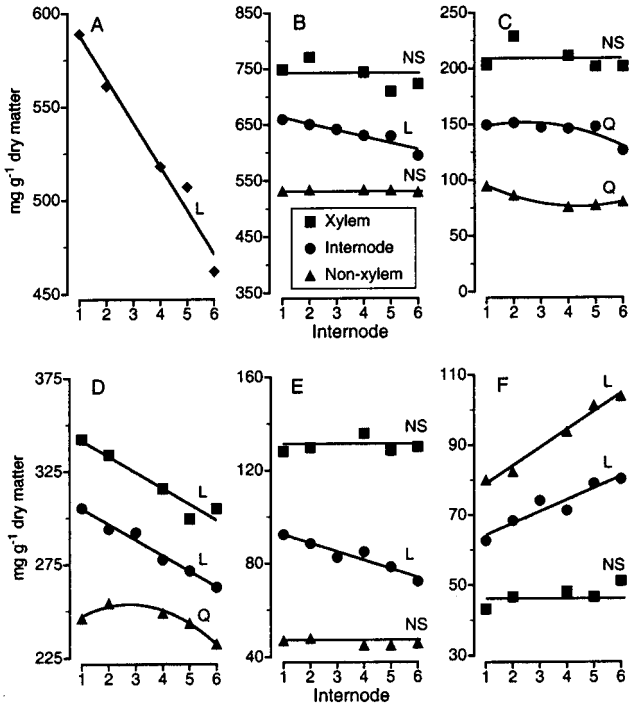

Figure 2. Proportion of xylem tissue in alfalfa internodes (**A**) and concentrations of cell wall (**B**), lignin (**C**), cell wall glucose (**D**), cell wall xylose (**E**), and cell wall uronosyls (**F**) in alfalfa internodes, xylem tissues, and nonxylem tissues. Nonsignificant (NS) and significant ($P < 0.05$) linear (L) and quadratic (Q) regressions are indicated for each curve.

weak salt solutions. All cell wall composition and degradability data were adjusted to account for pectic sugars extracted by salt and amylase/amyloglucosidase solutions.

Cell wall contributions to dry matter, estimated by summing the concentrations of total sugars and lignin, averaged 634 mg g^{-1} for whole internodes, 739 mg g^{-1} for xylem, and 533 mg g^{-1} for nonxylem fractions. These values include 31, 11, and 40 mg g^{-1} of pectic sugars extracted by salt and amylase/amyloglucosidase solutions from whole internodes, xylem, and nonxylem fractions, respectively. These estimates do not include about 25 mg g^{-1} of structural protein and 10 mg g^{-1} of minerals, most of which would probably be in primary walled tissues of the nonxylem fraction (*32*, *34*−*36*). On the basis of these estimates, the xylem fraction contributed an average of 60% of the cell wall mass in lower alfalfa internodes. The cell wall content of xylem and nonxylem fractions was not influenced by internode location (**Figure 2B**). Cell wall concentrations of whole internodes, however, increased toward the base of alfalfa stems, due to the increasing proportion of xylary tissue.

Cell Wall Composition. The structural carbohydrate composition of lower alfalfa internodes (**Table 1**) was comparable to that reported previously for alfalfa (*5*, *7*). Structural carbohydrates in all tissues were composed primarily of glucose (55−60%). In alfalfa internodes, 80−90% of cell wall glucose is derived from cellulose and the balance is derived from hemicelluloses, mainly xyloglucans and glucomannans (*6*, *37*, *38*). On the basis of detailed carbohydrate analyses of similar tissue fractions from *Brassica* stems (*21*), most of the noncellulosic glucose in alfalfa probably originates from xyloglucans in primary walled nonxylem tissues. Xylose, derived mostly from xylans and xyloglucans, was the most abundant hemicellulosic sugar followed by mannose, derived primarily from glucomannans (*6*, *38*). Xylose concentrations, as a proportion of total sugars, were 2.5 times greater in xylem than in nonxylem walls, reflecting extensive deposition of xylans in secondary cell walls of xylary tissues. In the nonxylem fraction, most xylose would originate from xyloglucans in primary walled tissues and some would originate from xylans deposited in secondary walls of phloem fibers (*21*, *39*). Nonxylem and xylem walls contained small amounts of mannose, indicating that glucomannans were a minor component of primary and secondary walled tissues. In alfalfa stems, about two-thirds of the uronosyls are galacturonic acid, derived from pectin, and one-third is glucuronic acid derived from xylans (*6*, *38*). Uronosyl concentrations in nonxylem walls, as a proportion of total sugars, were 2.3 times greater than that in the xylem walls. About 25% of the uronosyls in both cell wall fractions was methyl-esterified. On the basis of other work with alfalfa stems (*23*) and tissue fractions from *Brassica* (*21*), most uronosyls in nonxylem walls probably originate from pectin. In xylem walls, uronosyls are likely a mixture of galacturonic acid from pectin and glucuronic acid from xylans. The proportions of other sugars, derived primarily from pectin, differed considerably between tissue fractions, with nonxylem walls having greater amounts of rhamnose, galactose, and especially arabinose than xylem walls. Although cell walls were not subjected to chemical fractionation, our compositional data suggest a polysaccharide distribution of roughly 5% pectin, 40% hemicellulose, and 55% cellulose in xylem walls and 30% pectin, 35% hemicellulose, and 35% cellulose in nonxylem walls.

On a cell wall basis, the lignin content of the xylem fraction (280 mg g^{-1}) was about 2-fold greater than that of the nonxylem fraction (150 mg g^{-1}). Vallet et al. (*18*) found comparable lignin concentrations in wood (xylem tissues and pith parenchyma) and bark (chlorenchyma, phloem, phloem fibers, collenchyma, and epidermis) dissected from alfalfa internodes. The high concentration of lignin in nonxylem walls was unexpected, since histological studies indicate the majority of such tissues are nonlignified (*18*, *20*, *29*). This discrepancy is probably due in part to the inclusion of partially lignified phloem fibers and pith parenchyma in our nonxylem fraction and to errors in detecting low amounts of lignin by microscopic and colorimetric methods (*25*, *40*). Low levels ($\leq$0.3 mg g^{-1}) of both ester- and ether-linked ferulic and *p*-coumaric acids were released from xylem and nonxylem fractions by alkaline hydrolysis. Apparently, hydroxycinnamates play an insignificant structural role in cell wall cross-linking and lignin formation in alfalfa as compared to that demonstrated for grasses (*15*, *24*, *41*).

Concentrations of lignin, xylose, and uronosyls were similar among xylem fractions isolated from alfalfa internodes (**Figure 2**). Glucose concentrations in the xylem fraction declined linearly from the first to the sixth internode, probably reflecting greater deposition of secondary cell wall cellulose in more mature basal internodes. In the nonxylem fraction, concentrations of xylose were similar, lignin and glucose decreased, and uronosyls increased from the first to the sixth internode. In whole internodes, concentrations of lignin, glucose, and xylose decreased and uronosyls increased from the first to the sixth internode due to changes in the composition and/or proportion of xylem and nonxylem tissues.

Cell Wall Degradability. Crude cell walls were incubated with a cellulase, hemicellulase, and pectinase mixture for 3 and 48 h to study how readily and extensively various structural polysaccharides undergo enzymatic hydrolysis (**Table 2**). The loss of total sugars from internode cell walls, 27% after 3 h and 48% after 48 h of hydrolysis, was comparable to that reported by in vitro digestions with ruminal microflora (*23*, *43*). Pectic sugars—rhamnose, arabinose, and galactose—underwent rapid hydrolysis from internode cell walls and their final 48 h

Table 1. Concentrations of Cell Wall Monosaccharides (mg g^{-1} Total Sugar), Total Cell Wall Sugars (mg g^{-1} Dry Matter), and Lignin (mg g^{-1} Dry Matter) in Tissues of Alfalfa Stems[a]

	rhamnose	arabinose	galactose	uronosyls	mannose	xylose	glucose	total sugar	lignin
internode	13.2± 1.6	35.9 ± 5.3	26.6 ± 1.5	148.4 ± 19.7	29.4 ± 0.9	170.0 ± 11.2	576.6 ± 16.1	489.2 ± 16.4	144.3 ± 10.0
xylem	9.7 ± 1.8	11.7 ± 3.0	15.4 ± 0.6	89.1 ± 7.0	25.4 ± 1.2	246.4 ± 8.8	602.4 ± 18.6	529.8 ± 15.5	209.4 ± 11.6
nonxylem	16.8 ± 1.5	60.0 ± 2.2	36.2 ± 2.3	205.1 ± 21.8	33.9 ± 2.8	102.6 ± 4.4	545.3 ± 20.8	449.7 ± 8.0	83.0 ± 7.7

[a] Data are averaged across internodes, with ± standard deviations. Includes cell wall sugars extracted by 50 mM NaCl and amylase/amyloglucosidase solutions during preparation of crude cell walls. Xylem and nonxylem means within each constituent differ ($P < 0.01$) using a paired *t*-test.

Table 2. Cell Wall Sugars Released from Alfalfa Stem Tissues (%) by a Fungal Enzyme Mixture Containing Cellulase, Hemicellulase, and Pectinase Activities[a]

	rhamnose	arabinose	galactose	uronosyls	mannose	xylose	glucose	total sugar
				Released by 3 h Hydrolysis				
internode	66.9 ± 6.9	71.6 ± 8.5	62.4 ± 10.1	34.1 ± 18.4	35.9 ± 5.1	11.4 ± 3.2	26.1 ± 3.6	27.2 ± 6.7
xylem	29.0 ± 2.6	50.8 ± 5.5	47.4 ± 7.8	5.6 ± 2.6	21.1 ± 3.1	5.2 ± 1.1	15.5 ± 2.4	12.5 ± 2.2
nonxylem	91.3 ± 7.4	75.2 ± 10.2	74.4 ± 7.2	76.8 ± 20.9	44.6 ± 5.9	27.7 ± 6.0	43.1 ± 5.4	51.0 ± 9.2
				Released by 48 h Hydrolysis				
internode	75.4 ± 7.9	88.0 ± 10.6	73.3 ± 9.3	68.0 ± 10.4	51.0 ± 11.9	13.6 ± 2.1	47.6 ± 4.9	48.4 ± 6.1
xylem	37.0 ± 3.9	67.7 ± 6.4	48.0 ± 3.3	24.9 ± 6.7	26.3 ± 4.1	7.0 ± 1.5	24.9 ± 1.1	21.6 ± 1.3
nonxylem	91.0 ± 3.0	91.2 ± 10.0	86.1 ± 10.2	84.8 ± 8.9	67.0 ± 1.9	30.6 ± 4.2	70.2 ± 2.9	72.9 ± 5.2

[a] Data were averaged across internodes, with ± standard deviations. Includes cell wall sugars released by 50 mM NaCl and amylase/amyloglucosidase solutions during preparation of crude cell walls. Xylem and nonxylem means within each constituent and hydrolysis period differ ($P < 0.01$) using a paired *t*-test.

extent of hydrolysis ranged from 73 to 88%. Uronosyls, primarily from pectin, were also extensively released (68%) from internode cell walls but at a slower pace comparable to that of glucose and mannose, components of cellulose and mannans with a 48 h degradability of about 50%. Only 14% of xylose-containing polysaccharides was degraded after 48 h of hydrolysis. Eighty percent of the degradable xylose was, however, released within the first 3 h of hydrolysis, indicating that a small fraction of these polysaccharides was rapidly degraded.

The 48 h extent of total sugar hydrolysis from xylem walls was low (22%), and about one-half of these sugars was released within the first 3 h (**Table 2**). The degradation of xylose-containing polysaccharides was extremely poor after 48 h of hydrolysis (7%), but as noted earlier, small differences in xylose release between 3 and 48 h indicate a very small fraction degraded quite rapidly. The 3 and 48 h hydrolysis of cellulose and mannans in xylem walls, as indicated by the release of glucose and mannose, was about 3−4-fold greater than that of xylose-containing polysaccharides. The release of uronosyls was comparable to that of xylose after 3 h of hydrolysis, but after 48 h, their release was more comparable to that of glucose and mannose. As noted earlier, a large proportion of uronosyls in xylem walls would be associated with xylans that are essentially undegradable. Therefore, most uronosyls released from xylem walls probably originate from slowly degraded pectins. Other pectic sugars—arabinose, galactose, and rhamnose—had a greater 48 h extent of hydrolysis from xylem walls (37−68%) with 75% or more of their degradation occurring within the first 3 h of hydrolysis. Rhamnose, like uronosyls, was less degradable than arabinose or galactose, perhaps due to cross-linking of rhamnogalacturonans to other cell wall constituents (*23*). On the basis of other work (*6*, *20*, *29*), we suspect that most cell wall sugars released from the xylem fraction were derived from xyloglucans, glucomannans, cellulose, and pectins in non- or partially lignified primary walls of xylem vessels and pith parenchyma. Glucose, mannose, and small amounts of xylose may have also been hydrolyzed from cellulose, glucomannans, and xylans in portions of secondary walls of xylem fibers that undergo limited lignification. Cellulose, glucomannans, xylans, and small amounts of pectin deposited in highly lignified secondary walls of primary and secondary xylem and xylem fibers were probably nondegradable.

Nonxylem walls, with 42% less lignin than xylem walls, had a 3−4-fold greater release of cell wall sugars after 3 and 48 h of hydrolysis (**Table 2**), indicating that cell wall matrix interactions involving lignin differed between tissue fractions. The release of total sugars from nonxylem walls after 48 h of hydrolysis (73%) was somewhat lower than expected, based on histological reports that such tissues are often completely degraded (*18*, *20*, *29*). This discrepancy is due in part to the inclusion of moderately degradable phloem fibers and pith parenchyma in our nonxylem fraction. We also suspect that hydrolysis of pectins in the middle lamella of nonxylem walls may permit remaining portions of these walls to become dislodged from stems, thereby giving the appearance of complete cell wall degradation in microscopic studies. Most sugars, except for xylose, were rapidly and extensively released from the predominantly nonlignified primary walled tissues of the nonxylem fraction. About one-third of the xylose-containing polysaccharides was rapidly degraded within the first 3 h of hydrolysis, and little additional breakdown occurred up to 48 h of hydrolysis. Hydrolysis of pectic sugars—arabinose, galactose, uronosyls, and rhamnose—was nearly complete after 3 h of hydrolysis, and the final extent of hydrolysis at 48 h exceeded 85%. These results indicate that structural factors limiting the hydrolysis of pectins, particularly rhamnogalacturonans, in xylem cell walls were absent in nonxylem cell walls. The 48 h extent of glucose and mannose release was intermediate, indicating a degradability of cellulose and mannans of at least 65%; about two-thirds of these sugars were released within the first 3 h. On the basis of other work (*6*, *20*, *29*), the release of sugars from the nonxylem fraction would be limited by poor degradation of primary walls in pith parenchyma, phloem fibers, and epidermis. Therefore, the poor hydrolysis of xylose from nonxylem tissues may be due to restricted hydrolysis of xyloglucans in primary walls—not xylans in secondary walls, as was the case with the xylem fraction. Although poor degradability of xylans in secondary walls of legumes was

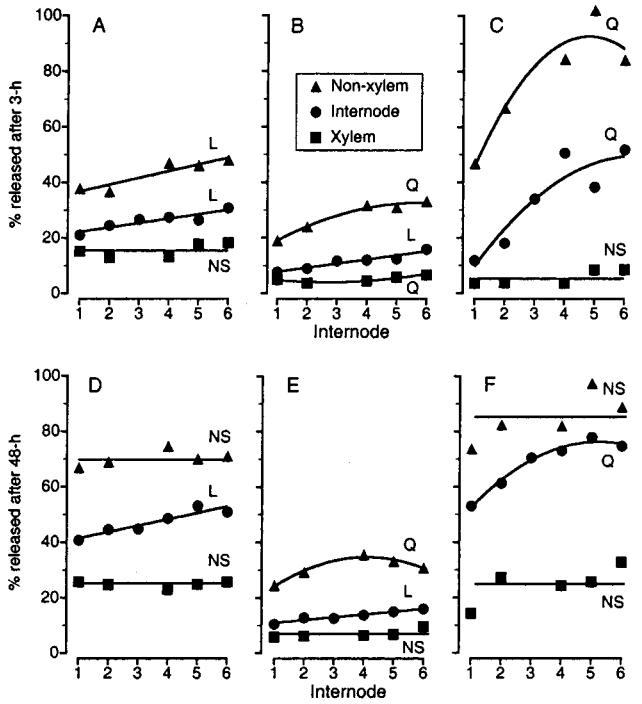

Figure 3. Release of glucose (**A**, **D**), xylose (**B**, **E**), and uronosyls (**C**, **F**) from cell walls of alfalfa internodes, xylem tissues, and nonxylem tissues after 3 and 48 h of hydrolysis with fungal enzymes. Nonsignificant (NS) and significant ($P < 0.05$) linear (L) and quadratic (Q) regressions are indicated for each curve.

reported long ago (*4*), our study is the first to indicate poor degradability of xyloglucans in primary walls. Proof of poor xyloglucan degradation in primary walls of legumes will require studies with nonxylem fractions less-contaminated with secondary walled tissues or studies with primary cell wall model systems such as that developed for grasses (*43*). The mechanism responsible for poor xylose degradation is unknown but commonly attributed to some type of interaction with lignin (*44*). Unlike grasses, interactions between xylose-containing polymers and lignin in alfalfa do not appear to involve ferulic acid. Further work is needed to identify mechanisms by which lignin restricts the degradation of xylans and xyloglucans in legume cell walls.

Cell wall sugars in nonxylem fractions were released more rapidly after 3 h but not more extensively after 48 h of hydrolysis from nonxylem fractions isolated from intermediate internodes than from basal internodes. Internode location did not affect the degradability of major cell wall sugars from xylem fractions (**Figure 3**). The degradability of major cell wall sugars in whole internodes increased from the first to the sixth internode. Increases in internode degradability were primarily due to a reduction in the proportion of less degradable highly lignified xylem tissue, and secondarily, to an increase in the degradability of the nonxylem walls. Indeed, direct or indirect selection of plants with low proportions of xylary tissues will improve alfalfa degradability (*45*) as will down-regulation of lignin biosynthesis or postharvest removal of lignin (*46*, *47*). We believe that further work to elucidate the nature of lignin−xylose interactions in alfalfa tissues will reveal additional strategies for improving fiber degradability. In the end, successful approaches to improving alfalfa degradability must be environmentally safe and cost-effective without adversely affecting forage yields, pest resistance, and plant longevity (*48*).

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