

# Content and deposition rates of cellulose, hemicellulose, and lignin during regrowth of forage grasses and legumes

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Bidlack, J. E. and Buxton, D. R. 1992. Content and deposition rates of cellulose, hemicellulose, and lignin during regrowth of forage grasses and legumes. *Can. J. Plant Sci.* 72: 809–818. Cell-wall (CW) components greatly influence digestibility of forages as they mature. There is only limited information on the timing of rates of deposition of CW components. This investigation examined differences in CW and CW component deposition in greenhouse-grown alfalfa (*Medicago sativa* L.), birds-foot trefoil (*Lotus corniculatus* L.), red clover (*Trifolium pratense* L.), orchardgrass (*Dactylis glomerata* L.), smooth brome (*Bromus inermis* Leyss.), and switchgrass (*Panicum virgatum* L.). Plant material was sampled from the basal 10 cm of forage after harvesting at 3–5 cm above the soil level. Samples analyzed included sheaths from orchardgrass, sheaths and stems from brome and switchgrass, and stems from all legumes. After establishment and herbage removal, samples were collected biweekly between 2 and 10 wk of regrowth. Results indicated that, except for orchardgrass, maximum rates of CW and CW component deposition usually occurred earlier in legumes compared with those of grasses. Maximum CW deposition for all species occurred within 1–3 d of maximum dry weight deposition. Among CW components in grasses and legumes, maximum deposition of hemicellulose occurred first, followed by that of cellulose (1–6 d later), and then lignin (up to 14 d after maximum hemicellulose deposition). Maximum cellulose deposition in all species occurred at the same time as maximum CW deposition.

Key words: Cell wall, deposition, cellulose, hemicellulose, lignin, forage

Bidlack, J. E. et Buxton, D. R. 1992. Teneur et cadence d'accumulation de la cellulose, des hémicelluloses et de la lignine au cours de la repousse des graminées et des légumineuses fourragères. *Can. J. Plant Sci.* 72: 809–818. Les constituants pariétaux influent grandement sur la digestibilité des fourrages au fur et à mesure qu'ils avancent en maturité. On ne possède toutefois que peu de connaissances sur la cadence de formation de ces substances. On a examiné en serre les différences éventuelles dans la formation des membranes cellulaires et dans l'accumulation des constituants pariétaux entre la luzerne (*Medicago sativa* L.), le lotier corniculé (*Lotus corniculatus* L.), le trèfle rouge (*Trifolium pratense* L.), le dactyle pelotonné (*Dactylis glomerata* L.), le brome inerme (*Bromus inermis* Leyss.) et le panic raide (*Panicum virgatum* L.). Le matériel végétal était prélevé des 10 cm inférieurs des plantes coupées de 3 à 5 cm de la surface du sol. Les parties analysées étaient, pour le dactyle, les gaines foliaires, pour le brome et le panic, les gaines foliaires et les tiges et pour les légumineuses, les tiges. Les échantillons étaient prélevés toutes les deux semaines dans les deux à dix semaines suivant la première coupe. Les résultats montrent que, à part pour le dactyle, les cadences maximales d'accumulation des membranes cellulaires et de leurs divers constituants se produisent d'ordinaire plus tôt chez les légumineuses que chez les graminées. Chez toutes les espèces cependant, le taux maximal de formation

des membranes survenait dans les 1 à 3 jours précédant la production maximale de matière sèche. Parmi les constituants pariétaux, l'accumulation maximale de l'hémicellulose venait en premier, puis celle de la cellulose (1 à 6 j plus tard) et enfin celle de la lignine (jusqu'à 14 j après celle de l'hémicellulose). Chez toutes les espèces, l'accumulation maximale de la cellulose coïncidait avec le pic de formation des membranes cellulaires entières.

Mots clés: Membrane cellulaire, accumulation, cellulose, hémicellulose, lignine, fourrages

Cellulose microfibrils embedded in a matrix of hemicellulose and lignin constitute plant cell walls (CW) (Hatfield 1989; Jung 1989). Secondary walls are deposited within primary walls during thickening and inclusion of lignin into the matrix (Theander and Aman 1984). Lignin is thought to be the major chemical constituent contributing to lowered digestibility as forages mature (Buxton and Russell 1988; Jung 1989), and its monomeric constituents seem to affect both rate and extent of CW digestion (Buxton 1989). Lignin concentrations may affect digestibility because of its inert nature; but also seems to interact with hemicellulosic polysaccharides, pectic polysaccharides, and the remaining cellulosic fraction to limit CW degradability (Hatfield 1989).

Recent studies with old world bluestem (*Bothriochloa* spp.) stems indicate that CW and lignin increase rapidly in dry matter of young tissues and reach a plateau as forage matures (Dabo et al. 1988). Attention should be focused on the early rapid change in lignin concentration before the plateau because small increases in lignification at this time may have greater negative effects on digestibility than subsequent increases in lignification of nearly mature forages (Jung and Vogel 1986; Jung 1989). There is limited information regarding the timing of deposition of lignin and other CW components in forage species. In this investigation, we report deposition rates of these CW components in sheath, sheath and stem, or stem bases of several forage grasses and legumes.

Although CW lignin concentration is generally lower in grass basal growth than in that of legumes, apparent resistance to digestion of grass lignin is 60% greater than that of legume lignin (Buxton and Russell 1988).

Limited information is available on the differences in CW and CW component content (yield) of legumes and grasses as forages grow, and essentially no information is available to describe deposition of CW and CW components in forages as a function of growth. This information is important because differences in CW deposition in legumes and grasses may contribute towards family- and species-specific digestibility differences.

Cell-wall and CW component depositions in bases of orchardgrass sheaths, bromegrass and switchgrass stems and sheaths, and alfalfa, birdsfoot trefoil, and red clover stems were studied to better understand factors affecting quality of basal tissue in forage canopies. Basal tissues were used instead of total herbage to attain uniformity in plant sampling, reduce protein contamination of CW constituents, and take advantage of the large variability of CW constituents that exist in mature basal tissues (Buxton and Russell 1988). With a focus on understanding CW growth in basal plant tissue the following objectives were addressed: (1) to study trends in grass and legume CW component content and deposition as a function of regrowth after harvest, (2) to examine timing of maximum CW component deposition of grasses and legumes as a function of regrowth, and (3) to detect generalized similarities and differences between grass and legume CW and CW component content and deposition as a function of regrowth.

#### MATERIALS AND METHODS

Three legume species studied were Arrow alfalfa (*Medicago sativa* L.), Viking birdsfoot trefoil (*Lotus corniculatus* L.), Arlington red clover (*Trifolium pratense*), and the three grass species were Napier orchardgrass (*Dactylis glomerata* L.),

Barton smooth brome grass (*Bromus inermis* Leys.), and Trailblazer switchgrass (*Panicum virgatum* L.). These were established in a greenhouse in 1987 in 25-cm-diameter pots with a capacity of 3.8 L. Plants were thinned to three legume seedlings or five to ten grass seedlings after establishment. Pots were arranged in a randomized complete block design with the six species in four replicates. A split-plot arrangement was employed with species as the whole plot and sample age as the subplot. Five pots of each species per replicate were used for five biweekly harvests over a 10-wk period to include samples from 14, 28, 42, 56, and 70 d of regrowth herbage.

Experiments described here were conducted from September through December of 1988 when greenhouse temperatures ranged from 20 to 37°C. High-pressure sodium lamps supplemented sunlight with a 14-h day and a 10-h night throughout the growth period. Stages of plant development for alfalfa and birdsfoot trefoil (Hedlund and Höglund 1983), red clover (Ohlsson and Wedin 1989), and grasses (Simon and Park 1983) were recorded 1 or 2 d before sampling. Plants were cut at 3–5 cm above soil level and the basal 10 cm of harvested sheaths (orchardgrass), sheaths and stems (brome grass and switchgrass), or stems (all legumes) was dried at 55°C for 48 h. Dried samples were weighed and ground to pass a 1-mm screen of a Udy Mill. Sequential fiber analyses according to Van Soest and Robertson (1980), with an amylase modification (Sigma No. A-1278), were used to estimate concentrations of CW (from neutral detergent fiber), cellulose, hemicellulose, and lignin in the ground plant samples.

Dry weight (DW), CW, cellulose, hemicellulose, and lignin were expressed on a content (per pot) basis. Graphical representation of components as a function of regrowth days was performed by fitting data to the Gompertz function,

$$Y = a \cdot \exp[(-b) \cdot \exp(-ct)]$$

where  $Y$  = component measured,  $a$  = maximum value of component,  $b$  = relative growth rate as affected by  $t$ ,  $c$  = estimated constant, and  $t$  = time in days (Hunt 1982). Values for  $b$  and  $c$  were estimated by the SAS PROC NLIN (Statistical Analysis System Institute, Inc. 1985) and values for  $a$  were entered as the highest number from data for each species. Components expressed on a per-pot basis were directed through the origin by adding the data point 0, 0 for graphical representation of each species. This function was justified because it optimizes growth response curves and has been used in similar studies (Pegelow et al. 1977; Hattendorf et al. 1988).

Deposition graphs were constructed from the first derivative of the Gompertz function with respect to regrowth days,

$$dY/dt = abc \cdot \exp(-ct) \cdot \exp[(-b) \cdot \exp(-ct)].$$

Times of maximum deposition rate were determined from average values over replicates by setting the second derivative of the Gompertz function,

$$d^2Y/dt^2 = ab^2c^2 \cdot [\exp(-ct)]^2 \cdot \exp[(-b) \cdot \exp(-ct)] - abc^2 \cdot \exp[(-b) \cdot \exp(-ct)],$$

equal to 0 and solving to  $t$ . Data analyses for mean squares and curve fitting were performed by PROC GLM and PROC NLIN, respectively (SAS Institute Inc., 1985). Nonlinear regression  $R^2$  values for the Gompertz function were calculated by dividing the residual sum of squares by the corrected total sum of squares and subtracting from one (Hattendorf et al. 1988).

## RESULTS AND DISCUSSION

Except for orchardgrass, plant development during this study spanned early vegetative

Table 1. Stages of plant development of potted forage species grown in the greenhouse

Regrowth day	Species					
	Alfalfa	Birdsfoot trefoil	Red clover	Orchard grass	Brome grass	Switch grass
	Maturity index					
14	37 <sup>2</sup>	35	23	21	31	32
28	56	52	53	21	43	36
42	63	58	63	23	42	47
56	68	60	67	23	53	62
70	75	72	75	23	64	73

<sup>2</sup>Maturities are described according to Hedlund and Höglund (1983), Ohlsson and Wedin (1989), and Simon and Park (1981) for alfalfa and birdsfoot trefoil, red clover, and grasses, respectively. Flowers visible (legumes) or inflorescence emergence (grasses) is estimated as 50 in all schemes.

through advanced reproductive stages for all species (Table 1). Almost constant plant stages for orchardgrass were expected because this species was not vernalized, which resulted in non-elongated vegetative stems. Flowers appeared within 28 d in legumes, but inflorescences were not observed until about 50 d in grasses.

Significant differences in plant base DW of sheath (orchardgrass), sheath and stem (bromegrass and switchgrass), and stem (legumes), as well as their content (yield) of CW and CW components were encountered as a function of regrowth days (Figs. 1-5). Significant differences in CW and CW component content with age were anticipated because their amounts increased with increasing plant mass.

Gompertz fitting of basal tissue DW per pot demonstrated a good representation of data for regrowth responses of grasses ( $0.86 < R^2 < 0.96$ ) and legumes ( $0.84 < R^2 < 0.98$ ) (Fig. 1). From the top two graphs of this figure, it is apparent that basal tissue DW increased sigmoidally with regrowth days. Deposition plots shown in the bottom two graphs exhibited a bell-shaped curve response with age in all species. These deposition plots show that, with the exception of orchardgrass, the maximum rate of plant base DW deposition often occurred earlier in legumes than in grasses. Higher and later maximum DW deposition rates in grasses compared with legumes may be due, in part, to growth of new tillers in potted grasses.

Sigmoidal curves also were observed for CW content as a function of regrowth days in all species ( $0.88 < R^2 < 0.99$ ) (Fig. 2). Among the six species, deposition of CW material reached a maximum, first in alfalfa at about day 15, and last in switchgrass at about day 41. Except for these two species, the maximum rate of CW deposition occurred at days 23-30 in both grasses and legumes. Maximum daily rates for CW deposition occurred within 1-3 d of maximum DW deposition (Table 2). Delayed increases in CW content of switchgrass (Fig. 2), as a function of regrowth days, indicated that the relative growth rate of switchgrass is unique from the other five species studied.

Derivation of deposition plots from content plots of cellulose ( $0.87 < R^2 < 1.00$ ) revealed maximum deposition values which were within 1-2 d of CW deposition in all species (Fig. 3). This close association was expected because cellulose constituted 50% or more of the CW throughout most of the sampling period.

Hemicellulose deposition plots derived from content plots ( $0.69 < R^2 < 0.94$ ) also demonstrated bell curve responses with regrowth days (Fig. 4). Compared with maximum cellulose deposition, maximum hemicellulose deposition occurred 2-6 d earlier in all species except for that of orchardgrass which occurred about the same day (Table 2). Grass hemicellulose deposition, if expressed on a concentration basis, may decrease early because it is quickly diluted by increases in other CW components or redistributed to other cellular components (Hatfield 1989) as the forage matures.

Lignin content ( $0.81 < R^2 < 0.98$ ) and deposition followed the same trend as that of CW and cellulose (Fig. 5). Maximum rate of lignin deposition occurred 2-13 d after maximum rate of hemicellulose deposition in legumes, 6-10 d after maximum rate of hemicellulose deposition in bromegrass and switchgrass, and at about the same time as maximum rate of hemicellulose deposition in orchardgrass (Table 2). Maximum rate of lignin deposition generally occurring after maximum rate of hemicellulose and cellulose deposition concurs with the concept that secondary thickening occurs with inclusion into the CW matrix (Theander and Aman 1984).

Species-specific maximum CW and CW component deposition can be inferred from graphical representation of these data. Maximum CW deposition occurred 1-3 d within maximum DW deposition. These data generally demonstrate that, among CW components, maximum deposition of hemicellulose occurs first, followed by that of cellulose (1-6 d later), and then lignin (up to 14 d after maximum hemicellulose deposition). Maximum cellulose deposition occurred at the same time as maximum CW deposition.

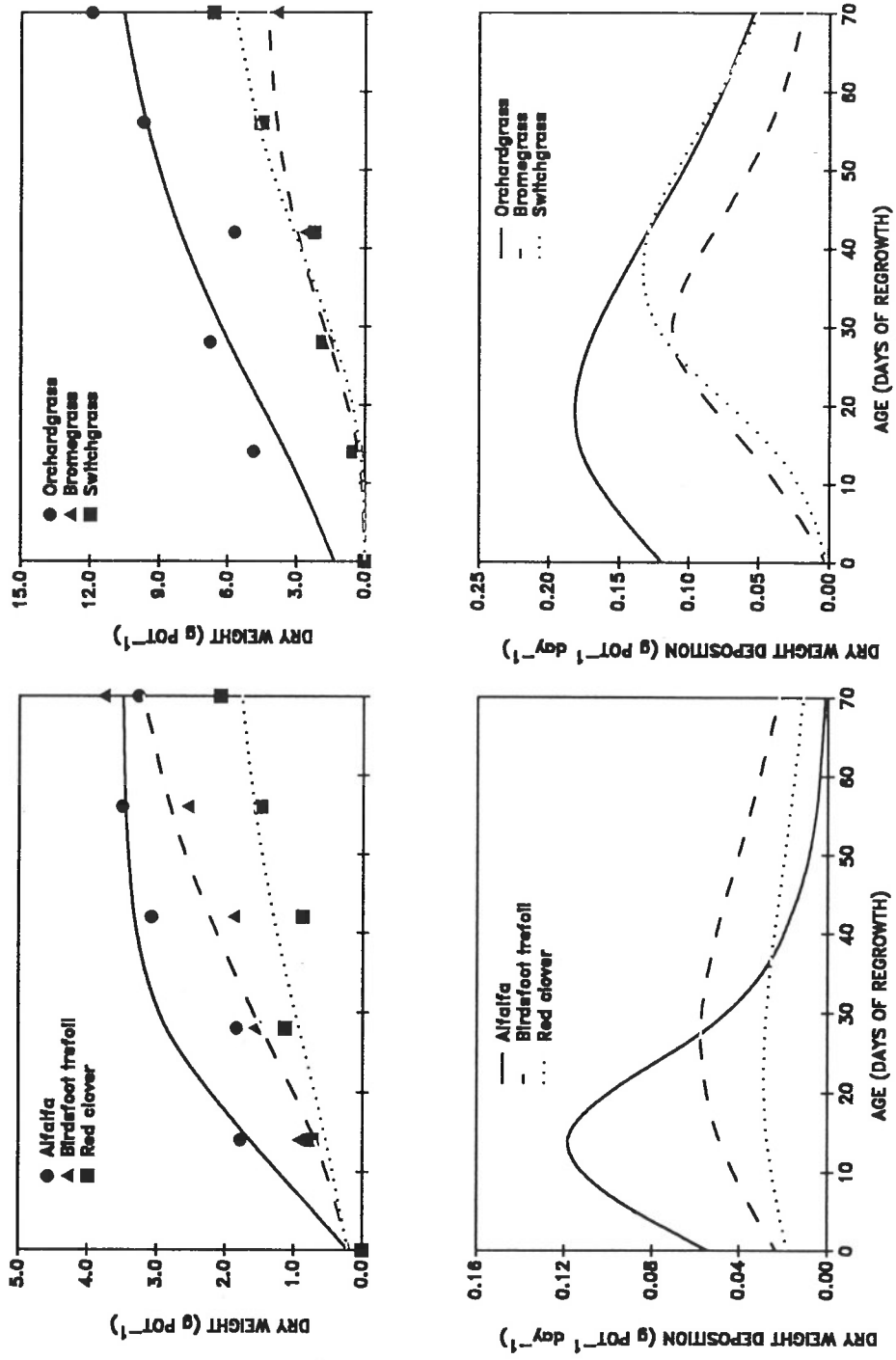


Fig. 1. Dry weight per pot [LSD (0.05) = 1.19] and dry weight deposition [LSD (0.05) = 0.054] in basal tissue of legumes and grasses.

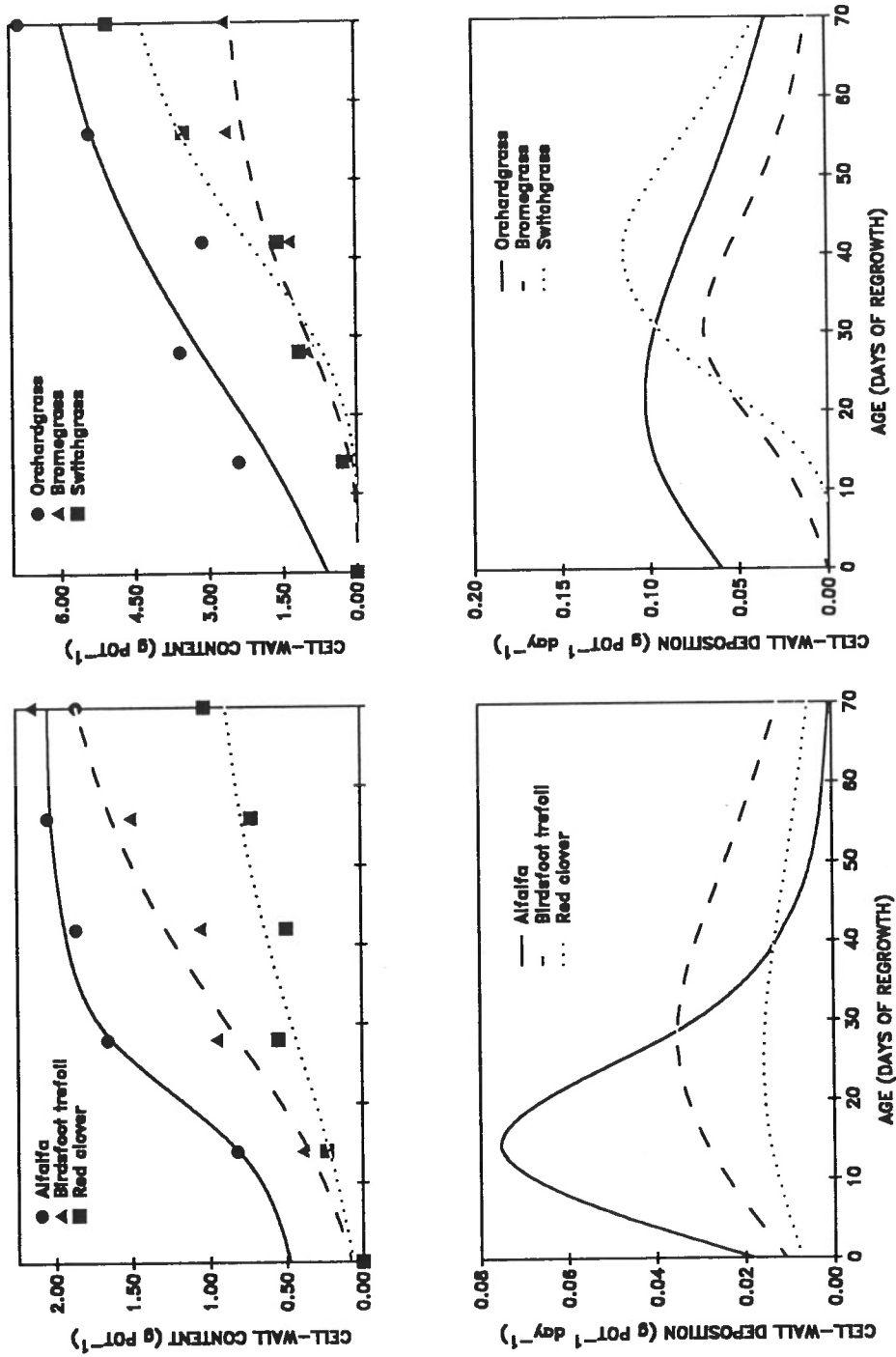


Fig. 2. Cell wall per pot [LSD (0.05) = 0.93] and cell-wall deposition [LSD (0.05) = 0.086] in basal tissue of legumes and grasses.

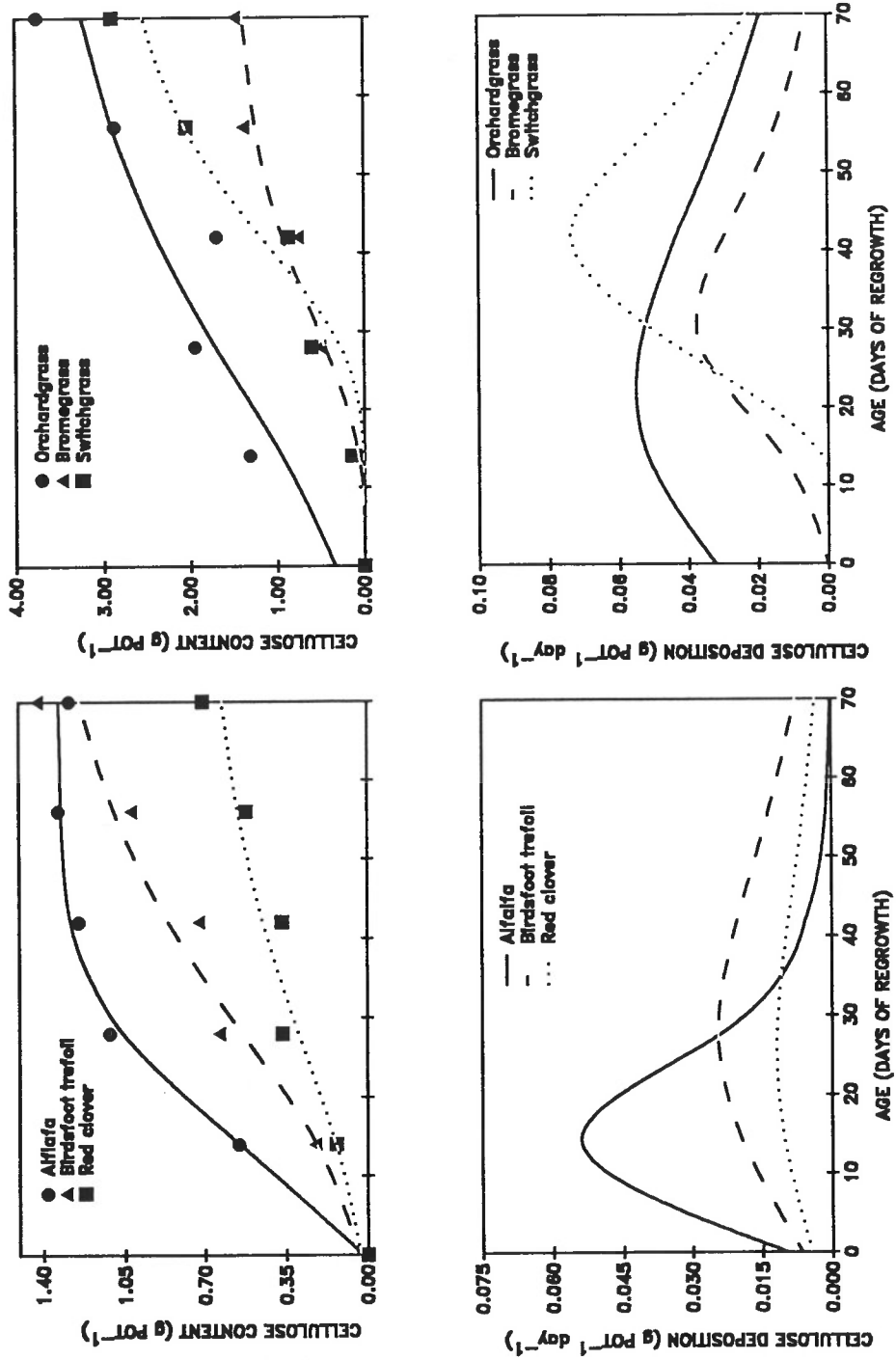


Fig. 3. Cellulose per pot [LSD (0.05) = 0.50] and cellulose deposition [LSD (0.05) = 0.016] in basal tissue of legumes and grasses.

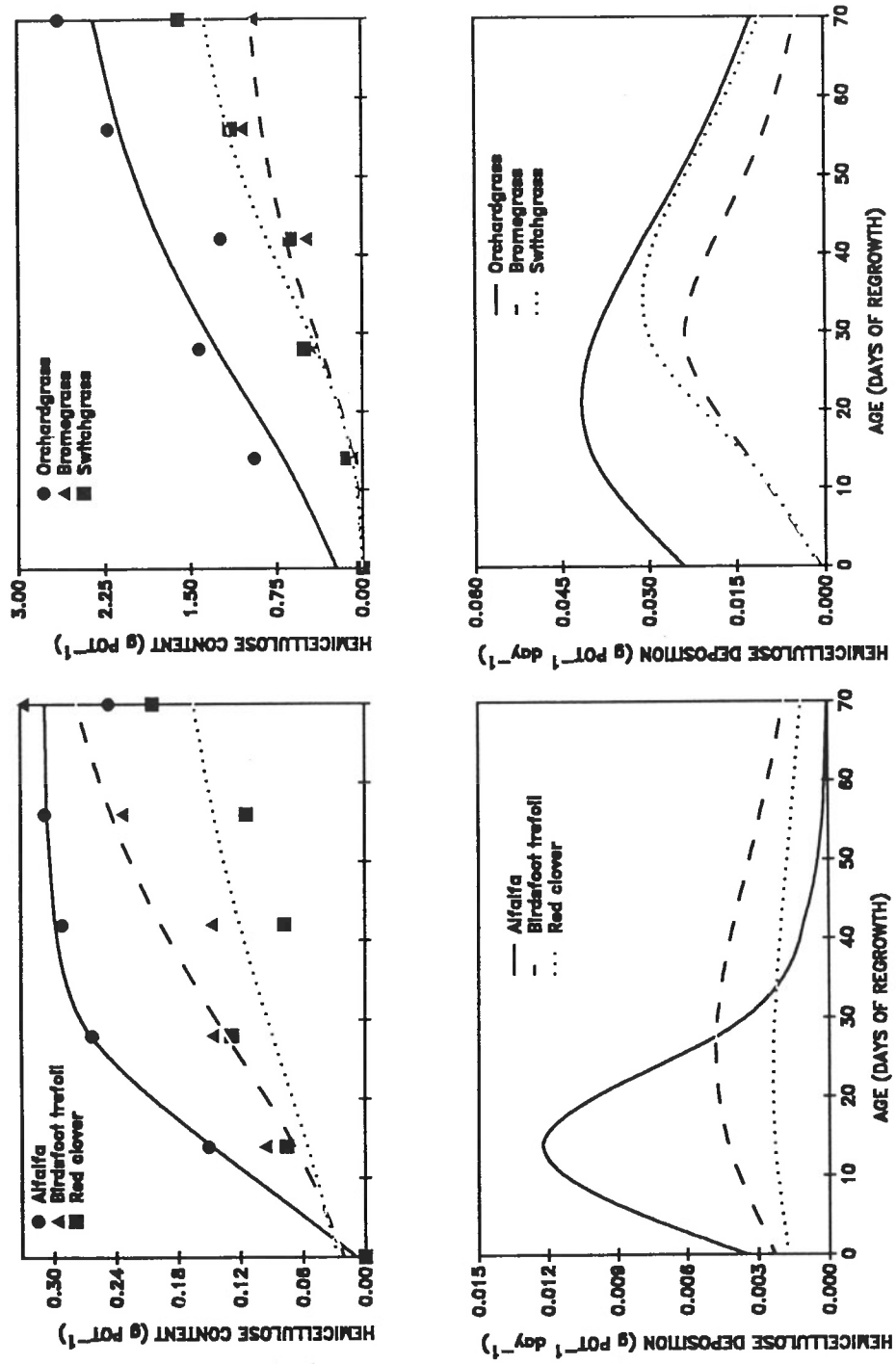


Fig. 4. Hemicellulose per pot [LSD (0.05) = 0.37] and hemicellulose deposition [LSD (0.05) = 0.011] in basal tissue of legumes and grasses.



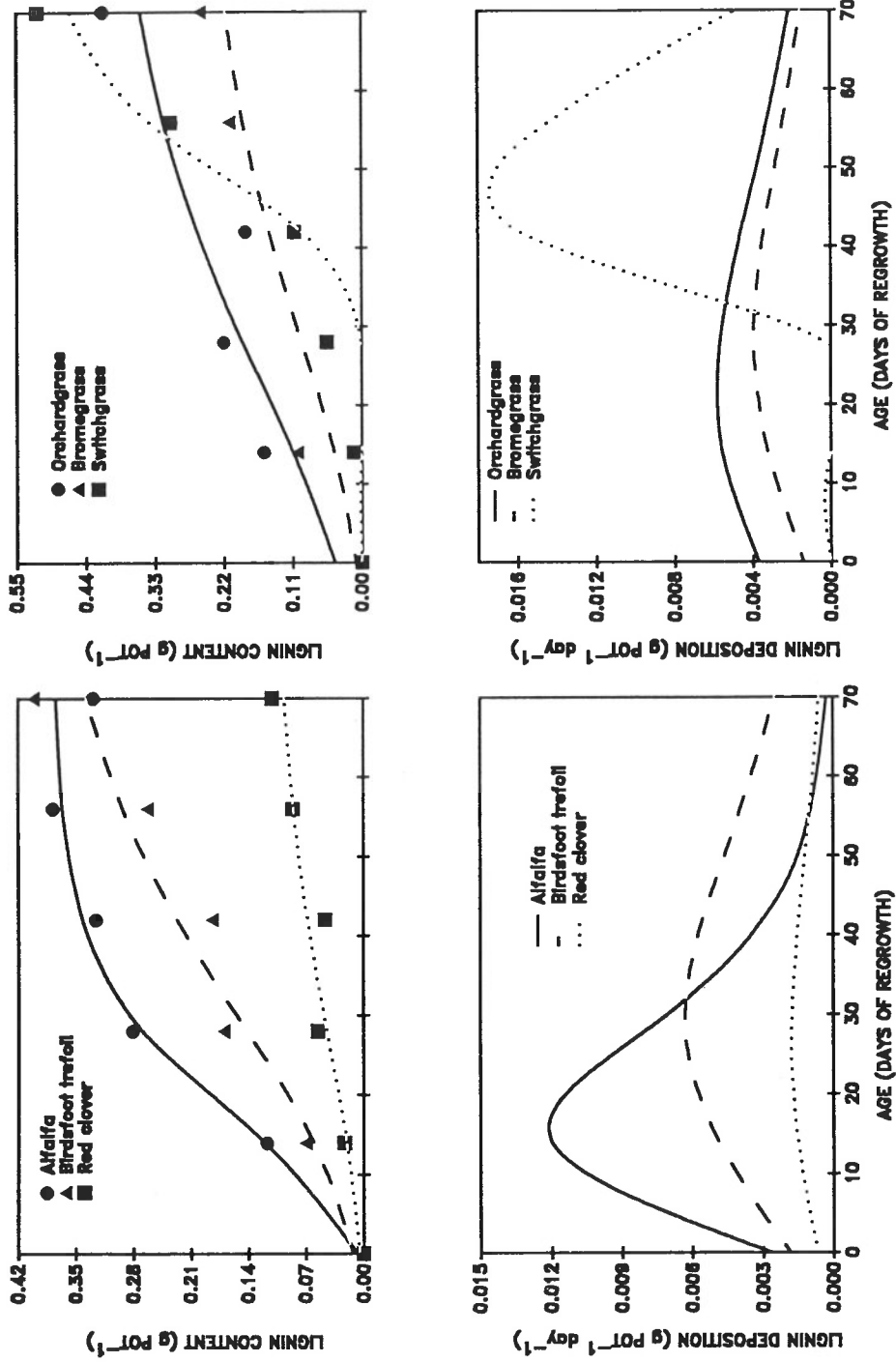


Fig. 5. Lignin per pot [LSD (0.05) = 0.09] and lignin deposition [LSD (0.05) = 0.006] in basal tissue of legumes and grasses.

Table 2. Estimated times of maximum deposition rates for per-pot components calculated by setting the second derivative of the Gompertz function equal to 0 and solving for  $t$ 

Component	Species					
	Alfalfa	Birdsfoot trefoil	Red clover	Orchard grass	Brome grass	Switch grass
	Days					
Stem base DW <sup>z</sup>	12.8	27.2	27.7	21.3	31.6	39.3
Cell wall	14.9	28.3	24.6	23.5	29.5	41.0
Cellulose	14.7	28.7	25.0	23.6	30.0	43.0
Hemicellulose	13.0	25.5	20.6	23.5	28.2	36.4
Lignin	17.4	30.0	34.0	21.3	34.7	46.1
LSD <sub>0.05</sub>	2.5	6.3	7.3	3.2	3.5	4.3

<sup>z</sup>DW = dry weight.

Except for orchardgrass, the last CW component that demonstrated maximum deposition was lignin.

### CONCLUSIONS

Legume DW, CW, and CW component content of basal tissue were generally lower and increased earlier and faster than those of grasses. This may be due, in part, to the appearance of new tillers in potted grasses. The timing of maximum CW component deposition usually followed the order, hemicellulose, cellulose, and lignin. Both plant families were similar, in that CW content increased with the DW of the plant and maximum CW component deposition usually followed the same order.

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