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# Cell wall components and lignin biosynthesis in forages

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Iowa State University, 1990

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# Cell-wall components and lignin biosynthesis in forages

by

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A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Agronomy
Major: Plant Physiology

Approved:

Signature was redacted for privacy.

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa
1990

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# GENERAL INTRODUCTION

Current agricultural trends emphasize sustainable agriculture and use of molecular techniques to improve productivity and circumvent problems associated with monoculture systems. Renewed interest in forage production has transpired as a result of monetary and conservational benefits associated with sustainable crops as well as potential improvement of forage quality through selective breeding and new molecular techniques (Lundvall, 1989).

Better understanding of forage quality is needed to facilitate manipulation of cultivars for improved ruminant nutrition. A key aspect of forage research is cell-wall (CW) chemistry because components of CWs form barriers to digestion of forage tissue. Among CW components, lignin is the major factor impeding digestibility within a species. Research is needed to decrease lignin concentration and alter its composition so that forages are more easily digested by ruminants. As forage quality improves with research, producers will perhaps be inclined to include forages in their cropping systems.

The object of this research was to acquire a better understanding of developmental and physiological events that affect forage quality. Lignin biosynthesis was the focus of

this study because it is the most effective cell-wall component known to decrease forage quality and hence, ruminant nutrition. Concentration, content, deposition, and change in concentration of lignin were examined in relation to measurements of CW, cellulose, and hemicellulose, as well as activity of phenylalanine ammonia lyase (PAL), an enzyme involved in lignin biosynthesis.

## Explanation of Dissertation Format

Organization of this dissertation directs the reader through general background information followed by two research sections. The general background is outlined with a complete literature review and mini-reviews are provided in introductions of each of the following sections.

Section I addresses CW and CW component content, concentration, deposition, and change in concentration. Section II focuses on CW and lignin concentration and change in concentration in relation to PAL activity. Both sections were completed with assistance from the Forage Physiology Crew at Iowa State University.

# LITERATURE REVIEW

Chemical composition and biosynthesis of forage cell walls need further study to enable plant breeding manipulation and post-harvest treatment to achieve improved digestibility. Lignin is probably the most complex ingredient of cell walls that impedes ruminant nutrition. Specific information concerning relationships between cell-wall chemistry and biochemistry is needed to direct future work on chemical and molecular manipulation of enzymes involved in lignification. This review focuses on cell-wall constituents and their relation to digestibility, lignin biosynthesis, and chemical and molecular aspects of lignin biosynthesis.

## Cell-wall Constituents in Relation to Digestibility

Forage plant cells can be divided into two components: 1) cellular contents that constitute the highly digestible fraction and 2) cell-wall constituents that are usually viewed as having limited digestibility (Van Soest, 1985). Limitations that cell-wall materials impose on digestion justify research directed towards improving cell-wall digestibility. It is from the need to acquire a better understanding of this cell-wall digestibility that

researchers have devised the scheme of proximate analysis for separating constituents of plant fiber (Van Soest, 1967 and 1982).

Studies using the proximate system indicate that cell-wall concentration estimated by neutral detergent fiber (NDF) is the best predictor of ruminant animal intake potential (Van Soest and Robertson, 1980; Waldo, 1985 and 1986). Thus, those cell-wall constituents isolated by neutral detergent should be the factors that are most important and easily related to digestibility of total dry matter.

Neutral detergent isolates fibrous materials including cellulose, hemicellulose, lignin, cutin, and tannin. Of these components, cellulose, hemicellulose, and lignin constitute major fractions of secondary walls. Their concentration in cell walls can be calculated from subsequent steps in proximate analysis. Cellulose is calculated from the difference between acid detergent fiber (ADF) and acid detergent lignin (ADL) concentration; hemicellulose is calculated from the difference between NDF and ADF; and lignin is reported as ADL after removing ash.

Plant cell walls (CWs) are structurally described as cellulose microfibrils embedded in a hemicellulose and lignin matrix (Buxton et al., 1987; Jung, 1989). Secondary walls are derived from primary walls after thickening and inclusion of lignin into the matrix (Theander and Aman, 1984).

Specific secondary wall structures have yet to be described, although a generalized structure has been diagrammed by Jung (1989). Detailed secondary CW structure depicted in Figure 1 (Bidlack and Bidlack, 1990) modifies Jung's structure with additional information from the literature and integrates knowledge with an artistic interpretation of CW component interaction. Formation and structural integrity of the CW matrix are derived from interrelationships among these CW components (Hatfield, 1989).

In Figure 1, cellulose microfibrils are represented as long fibers consisting of cellulose chains. Hydrogen bonds connect cellulose microfibrils to a xylose chain of hemicellulose shown in the figure as a molecular box. Hydrogen bonds between cellulose and hemicellulose, rather than covalent bonds, have been postulated by Morrison (1979). Covalent linkages via ester bonds from arabinose or xylose to lignin are shown. Ferulic acid and p-coumaric acid are shown as principle non-core components ester-linked to core lignin (Hartley, 1972; Mueller-Harvey et al., 1986). Ether linkages, which are shown in the figure (Scalbert et al., 1985), as well as ester-linked diferulic, sinapic, cinnamic, and benzoic acid derivatives (not shown) have been described as part of the CW matrix (Hartley and Jones, 1976; Newby et al., 1980). While some of these phenylpropanoid derivatives provide linkage between hemicellulose and core lignin,

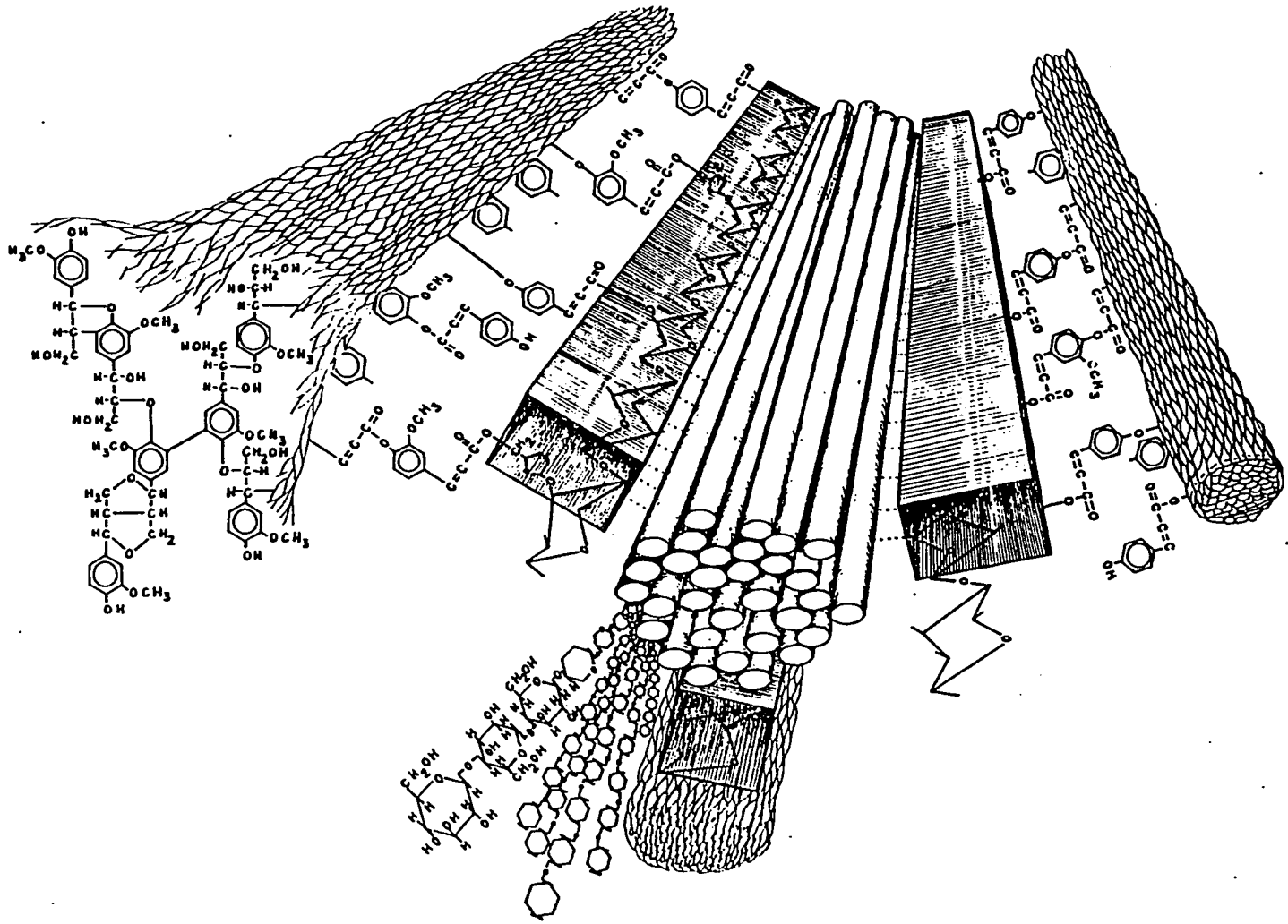


Figure 1. Secondary cell-wall structure (Bidlack and Bidlack, 1990)

molecules such as p-coumaric acid may be independently associated with core lignin (Jung, 1989). In the figure, non-core lignin is shown as p-coumaric acid and ferulic acid molecules and core lignin is shown as the outside core with a representative lignin molecule in the left portion of the foreground.

Of the three principal constituents of cell walls, lignin is most important in limiting digestibility. Lignin concentration demonstrates a very high, negative correlation to digestibility and usually is the best single predictor of digestibility within a species (Van Soest and Robertson, 1980; Van Soest, 1982).

Whether lignin content reduces digestibility by decreasing rate of digestion or extent of digestion is an important question when considering its function in reducing ruminant nutrition. Investigations generally have shown that lignin has a greater influence on extent of digestion than it does on digestion rate (Waldo et al., 1972; Lechtenberg et al., 1974). This implies that lignin composition, as well as lignin concentration, is an important factor in limiting forage digestibility. In accordance, Buxton (1989a) reported that low nitrobenzene oxidation:lignin ratio and low p-coumaric acid concentration reduced lignin's inhibitory influence on digestion kinetics.

Recent efforts focus on characterizing amounts and kinds of lignin among species (Adler, 1977; Himmelsbach and Barton, 1980; Antel and Crawford, 1981; Jung et al., 1983; Buxton and Fritz, 1985; Buxton and Russell, 1988), in different plant parts within the same species (Harkin, 1973; Buxton et al., 1985; Buxton and Hornstein, 1986), and under different environmental conditions (Wilson, 1982; Buxton, 1989b; Halim et al., 1989). These studies have inspired other investigators to evaluate reasons underlying differences in lignin concentration and composition among and within forage species. Forage scientists need to study lignification closely, determine how this process varies with age and source of tissue, and evaluate how variability in this process can affect digestibility.

Lignin's inhibitory influence increases as lignin concentration increases in maturing tissues (Harkin, 1973; Buxton et al., 1985; Buxton and Hornstein, 1986; Buxton and Russell, 1988). As forages mature, both quality and quantity of lignin have an effect on cell-wall digestibility (Buxton and Russell, 1988).

Although lignin concentration is higher in legumes than in grasses, grass lignin is more inhibitory to cell-wall digestion (Buxton et al., 1987). Differences between grass and legume lignin indicate that grass lignin has a greater effect on cellulose and hemicellulose digestion than does

legume lignin (Buxton and Russell, 1988). This could be related to monomeric constituents in that, the ratio of coniferyl to sinapyl alcohol decreases with maturity in grasses to a greater extent than in legumes (Buxton and Russell, 1988). Moreover, higher amounts of ester linked p-coumaric and ferulic acids in grasses compared with legumes (Hartley and Jones, 1977) could reduce digestibility by their toxic effect on rumination and by cross linking with carbohydrates (Buxton and Russell, 1988).

Information about lignin quality gives scientists insight about reasons for differences between legume and grass digestibility. More research is needed to explain how lignin is deposited in different tissues. Specific information on fiber deposition as a function of maturity has been reported for cotton (Schubert et al., 1973), but similar deposition studies have not been conducted for cell-wall components in forages. It is likely that differences in rate of lignin deposition in legumes and grasses contribute to species-specific differences in digestibility as tissues mature.

Because lignin may be envisioned as the principal cell-wall component affecting digestibility, its relation to other CW components and the effect of non-lignin components on digestibility cannot be ignored. Within secondary walls there is a complex matrix of polysaccharides, proteins,

phenolics, water, and minerals (Hatfield, 1989). Limitations to cell-wall degradation have been attributed to interaction of polyphenolics with cell-wall carbohydrates (Van Soest, 1981). Differences in extent of digestion of these complexes depends on degree of hydrogen bonding, branching patterns, and association with wall constituents (Hatfield, 1989).

Hemicellulose contains a large fraction of carbohydrates found in secondary walls. Xylans (containing arabinose, glucuronic acid, or combinations thereof), $\beta$-glucans, xyloglucans, and mannans are principal carbohydrates that make up the hemicellulosic polysaccharides of the cell wall as it rearranges and reorganizes with development (Hatfield, 1989).

Cell-wall digestibility can be affected by polysaccharide composition, particularly if lignin concentration is low (Buxton et al., 1987). Among cell-wall monosaccharides, galactose and arabinose seem to be more digestible than glucose or xylose (Albrecht et al., 1987). However, extent of branched xylans, measured by the arabinose:xylose ratio, seems to play a more important role in limiting digestibility of cell-wall carbohydrates (Brice and Morrison, 1982). Negative relationships have been shown between arabinose:xylose ratios and digestibility (Burritt et al., 1985; Buxton et al., 1987; Hornstein et al., 1989).

Lignin, structural carbohydrates, and interactions, which occur between these components and the cell-wall matrix, play determinant roles in cell-wall digestibility. Whereas some understanding of cell-wall digestibility can be obtained from a knowledge of its chemical composition, complete understanding can only be acquired with knowledge of cell-wall biosynthesis. Lignin biochemistry and its relation to cell-wall digestibility is probably the least studied aspect of forage quality.

## Lignin Biosynthesis

Lignification can be approached by following biochemical pathways that precede lignin monomeric formation. An understanding of these biochemical pathways is important to conceptualize lignification in growing plants. From knowledge of enzymatic and other regulatory factors of lignin biosynthesis, it may be possible to specify reactions responsible for lignin quantity and quality differences.

Lignin biosynthesis starts with the shikimic acid pathway. Steps leading to synthesis of lignin monomers from this pathway and other reactions have been reviewed (Hahlbrock and Grisebach, 1979; Bolwell, 1988). The shikimic acid pathway begins with merging of d-erythrose 4-phosphate and phosphoenolpyruvate to form shikimic acid which, in turn, follows a path via tyrosine or phenylalanine to trans-p-

coumaric acid (Figure 2). Trans-p-coumaric acid is converted to three lignin precursors: p-coumaryl, coniferyl, and sinapyl alcohol. Reactions of interest to forage physiologists may include: L-phenylalanine to trans-cinnamic acid, trans-cinnamic acid to trans-p-coumaric acid, L-tyrosine to trans-p-coumaric acid, trans-p-coumaric acid to the three lignin precursors, and polymerization of these precursors to form lignin. Reactions occurring at or after phenylalanine or tyrosine are most accurately described as being part of "general phenylpropanoid metabolism" (Ebel et al., 1974) and can be more closely associated with lignin biosynthesis than earlier reactions of the pathway.

Studying phenylpropanoid metabolism is important because enzymes involved regulate reactions that lead to lignin products. Understanding the nature and action of phenylpropanoid enzymes provides physiologists with a foundation of knowledge for advantageous manipulation of lignin.

Formation of cinnamic acid or coumaric acid from phenylalanine or tyrosine is regulated by phenylalanine ammonia lyase (PAL), E.C. 4.3.1.5, or tyrosine ammonia lyase (TAL), E.C. not defined, respectively. Grasses usually follow the TAL and PAL route, whereas legumes primarily follow the PAL route to coumaric acid (Goodwin and Mercer, 1983). Another enzyme that distinguishes legumes from

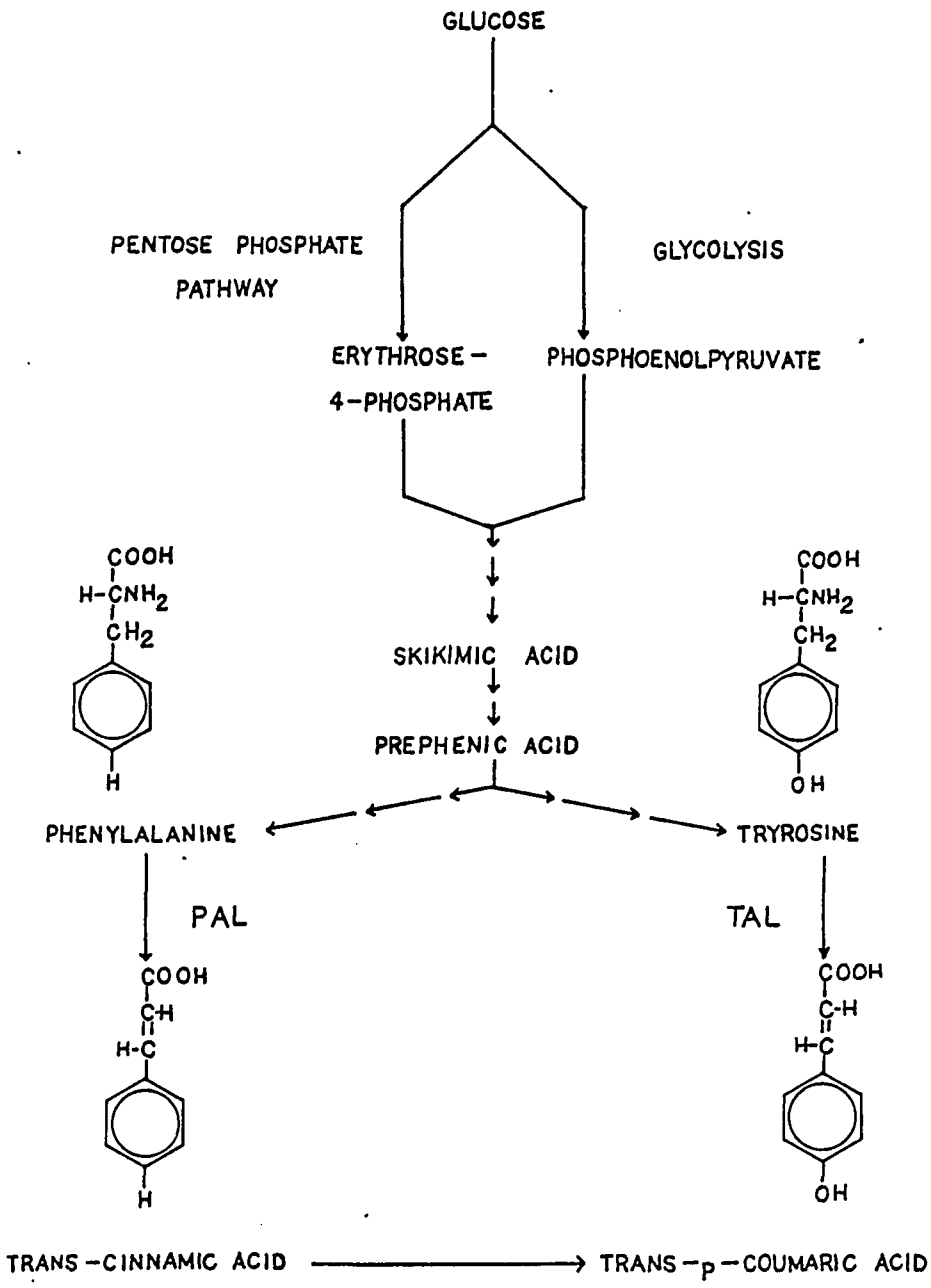



Figure 2. Lignin biosynthesis

grasses is cinnamate 4-hydroxylase, E.C. 1.14.13.11. Catalyzing conversion of cinnamic acid to coumaric acid, cinnamate 4-hydroxylase plays a key role in linking short routes via TAL with long routes via PAL to lignin monomers.

Although PAL, TAL, and cinnamate 4-hydroxylase play key roles in lignin biosynthesis, enzymes further down the pathway may be more closely related to final products. Three enzymes, 4-coumarate:CoA ligase, E.C. 6.2.1.12, cinnamoyl-CoA:NADPH oxidoreductase, E.C. 1.2.1.44, and cinnamyl alcohol:NADP oxidoreductase, E.C. 1.1.1.2, catalyze conversion of coumaric acid to lignin precursors. From studies of these three enzyme systems, it may be possible for physiologists to have a better understanding of what processes regulate committed steps of lignin biosynthesis.

Methods for assaying 4-coumarate:CoA ligase (Knobloch and Hahlbrock, 1975; Lüderitz et al., 1982), cinnamoyl-CoA:NADPH oxidoreductase (Lüderitz and Grisebach, 1981; Sarni et al., 1984), and cinnamyl alcohol:NADP oxidoreductase (Wyrambik and Grisebach, 1975; Grand et al., 1985) have been developed. Procedures for these enzyme assays were developed for soybean [*Glycine max* (L.) Merr.] suspension cultures and tree species rather than forage species. These procedures are complex because they usually involve sophisticated enzyme purification and synthesis of CoA esters as enzyme substrates. Lack of sufficient information for these enzymes

in forages and complexity of procedures make these enzymes unattractive for simple studies of forage lignification.

Cell-wall peroxidases, E.C. 1.11.1.7, catalyze final stages of lignification and are involved in oxidative polymerization of cinnamyl alcohols (Elstner and Heupel, 1976) and $H_2O_2$ formation (Elstner and Heupel, 1976; Mäder and Schloss, 1979; Pedreño et al., 1989). Peroxidases increase cell-wall cross-links (Lamport and Catt, 1981), which resist cell-wall degradation. Although these peroxidases are closely related to lignification, their diversity makes specific enzyme studies difficult.

Only one specific peroxidase, syringaldazine oxidase, has been linked directly to lignification (Harkin and Obst, 1973). This enzyme catalyzes the same free radicle formation encountered with laccase (p-diphenol:$O_2$ oxidoreductase, E.C. 1.10.3.2). Limited information on syringaldazine oxidase is available and procedures for this enzyme have not been developed for forage species. Forage physiologists will eventually need to develop procedures and study behavior of enzymes like syringaldazine oxidase before lignification is completely understood.

Among enzymes discussed in phenylpropanoid metabolism, ease of measurement of PAL activity makes this enzyme a good candidate for studying lignification. Simple spectrophotometric procedures have been developed for

assaying this enzyme (Koukol and Conn, 1961; Zucker, 1968; Camm and Towers, 1969; Guerra et al., 1985; Abell and Shen, 1988), and suitability of assaying at 290 nm has been established (Saunders and McClure, 1974).

Crude purification of PAL by acetone powder (Zucker, 1968; Koukol and Conn, 1961) or stepwise purification (Brödenfeldt and Mohr, 1988; Belunis and Hrazdina, 1988) is assumed to isolate the enzyme associated with lignin synthesis. Although PAL may be associated with chloroplasts, studies have shown that PAL linked to lignin exists in the cytoplasm or cytoplasmic surface of endoplasmic reticulum (Wagner and Hrazdina, 1984). Acetone powders containing PAL contain little chlorophyll and probably do not represent PAL associated with chloroplasts.

A simple procedure has been employed to study PAL and TAL activity in relation to lignin content in barley (Hordeum vulgare L.) as a function of irradiance (Guerra et al., 1985). Increased enzyme activity and lignification induced by light treatment may be a result of PAL's association with phytochrome (Brödenfeldt and Mohr, 1988) as well as its association with lignin formation. Other PAL interactions of interest include its association with pathogen attack (Vance and Sherwood, 1976; Fritzemeier et al., 1987) and shoot inversion (Prasad and Cline, 1987).

The role of PAL in this review focuses on lignification and its potential manipulation, as suggested in early studies by Zucker (1965 and 1968) and in numerous recent studies (Guerra et al., 1985; Fritzemeier et al., 1987; Prasad and Cline, 1987; Brödenfeldt and Mohr, 1988; Kishor, 1989).

Koukol and Conn have (1961) have shown that barley, alfalfa (Medicago sativa L.), rice (Oryza sativa L.), and pea (Pisum sativum L.) have higher PAL activity than sweet clover (Melilotus alba Med.) and lupine (Lupinus albus L.). Studies, which relate differences in enzyme activity to differences in digestibility among species, such as those investigated by Koukol and Conn, may begin to explain what regulates the quantity of lignin in forage plants. It would be interesting to detect and manipulate differences in PAL activity for optimization of forage quality.

## Chemical and Molecular Aspects of Lignin Biosynthesis

Successful improvement of forage quality has been demonstrated by plant breeders using divergent selection for high in vitro dry matter digestibility (Casler and Carpenter, 1989) and low herbage lignin concentration (Kephart, et al., 1990). With evidence of great heterogeneity in concentration and composition of lignin (Grand et al., 1982), it is likely that further improvement of forage quality by manipulating lignin is possible. Potential for manipulation of lignin in

plants is indicated by its variability in response to light (Wardrop, 1971; Guerra et al., 1985), temperature (Tomimura et al., 1979), pathogen attack (Vance and Sherwood, 1976; Vance et al., 1980), mineral nutrition (Wardrop, 1971; Acerbo et al., 1979), hormonal (Minocha and Halperin, 1974; Tomimura et al., 1979) and chemical (Armrhein and Gödeke, 1977) treatment, and natural variation (Sarkanen and Hergert, 1971; Grand et al., 1979). Targets for control include PAL and other key enzymes involved in lignification. Knowledge from mechanisms underlying these variations could eventually lead to techniques for precise control of lignin composition and concentration.

Closely regulated control of PAL has been accomplished through: light modification (Brödenfeldt and Mohr, 1988; Guerra et al., 1985); inoculation with pathogens (Vance et al., 1980; Gustine et al., 1978); and treatment with cinnamate (Khan and Vaidyanathan, 1987), sulfinamoyl tertiobutyl acetate (De Jaegher and Boyer, 1987), and o-benzylhydroxylamine (Hoagland, 1985). High irradiance and pathogen inoculants tend to increase PAL activity, but this increase can be reversed by heat-shock treatment (Walter, 1989). Most currently studied chemical treatments retard PAL activity and lignin biosynthesis. Because forage physiologists desire reduced lignification, chemical treatment has potential in allowing these scientists to reach

their goals. However, cost and inconvenience of chemical sprays to forage crops necessitates the development of alternative and more permanent means of manipulating lignin.

Molecular biology has potential for permanent manipulation of PAL. Fortunately, this enzyme is probably the most extensively studied enzyme of secondary metabolism (Bolwell, 1988). Abundant information on PAL's structure, catalytic action, and gene sequence is available in the literature. Physiologists have potential to compile this information and use current biotechnology for genetic alteration of genes responsible for lignin formation in forages.

Phenylalanine ammonia lyase is a tetrameric enzyme composed of four subunits, which range in molecular weight from 77,000 to 83,000 (Zimmerman and Hahlbrock, 1975; Bolwell, 1988). Two dehydroalanine-containing active sites are present in each PAL molecule from most tissue sources (Bolwell, 1988).

Catalytic activity of PAL may be rate-limiting for the formation of flavonoid glycosides (Hahlbrock and Grisebach, 1979), but it does not always follow Michaelis-Menten kinetics for the formation of cinnamic acid (Havir and Hanson, 1968). Recent findings, which indicate regulation of PAL activity by substrate supply (de Cunha, 1988), may result from circumvention of an inactivation system (Zimmerman and

Hahlbrock, 1975; Tanaka et al., 1977) that decreases PAL activity. Reports indicate that different forms of PAL exist and that its action is a subject of speculation (de Cunha, 1988; Tanaka et al., 1989). It would be no surprise if these different forms were regulated by more than one gene.

Recently acquired cDNA clones from sweet potato (Ipomoea batatas Lam.) (Tanaka et al., 1989), bean (Phaseolus vulgaris L.) (Edwards et al., 1985), and parsley [Petroselinum crispum (Mill.) Nym.] (Kuhn et al., 1984) will eventually enable availability of PAL cDNA clones for forages. These clones can be used by forage physiologists to select DNA sequences encoding reduced PAL activity.

Potato, bean, or parsley cDNA could be used directly as a heterologous probe to screen a library of DNA from plants demonstrating low PAL activity and low lignin. Once isolated, a selected vector could be used to clone this sequence for forage plant incorporation.

Methods for introducing cloned genes into plant species have been developed (Caplan et al., 1983; Horsch et al., 1985). Recently reported results indicate ease of genetic transformation into legume (as well and grass) species using Agrobacterium (White and Greenwood, 1987) or Ri plasmids (Swkhapinda et al., 1987). Successful incorporation of PAL sequences encoding reduced activity will depend on sequence characteristics as well as techniques used and compatibility

of foreign sequences in target plants.

Synthetic PAL genes may change more than just lignin concentration and content. It is difficult to predict PAL activity after successful transformation because the enzyme is also involved in flavonoid, alkaloid, xanthone, chalcone, and other product synthesis (Goodwin and Mercer, 1983). Little is known about reactions which control other products of phenylpropanoid metabolism (Rhodes, 1989). Moreover, enzymes of individual phenylpropanoid pathways can be induced up to over 100-fold by light, hormones, pathogens, and other stimuli (Hahlbrock and Grisebach, 1979). Thus, even if successful, incorporation of a synthetic PAL gene may not necessarily benefit the quality of forage tissues.

# SECTION I. CELL-WALL COMPONENT DEPOSITION IN STEM BASES OF FORAGE GRASSES AND LEGUMES

## Abstract

Differences in cell wall (CW) and CW component content and concentration exist among forage species. These differences vary as forages mature and are influenced by the dynamic nature of the CW matrix. This investigation examined differences in CW and CW component content, concentration, deposition, and concentration change in greenhouse-grown alfalfa (Medicago sativa L.), birdsfoot trefoil (Lotus corniculatus L.), red clover (Trifolium pratense L.), orchardgrass (Dactylis glomerata L.), smooth bromegrass (Bromus inermis Leyss.), and switchgrass (Panicum virgatum L.). Sheaths from orchardgrass and stems of the other five species from the basal 10 cm of forage were cut at 3 to 5 cm above the soil level. Samples were harvested weekly during 3 to 9 weeks of regrowth in 1987 and biweekly during 2 to 10 weeks of regrowth in 1988. Dried samples were weighed and analyzed for CW, cellulose, hemicellulose, and lignin. Results presented are content (yield) on a per pot basis and concentration on a dry weight and CW basis. Cell wall and all CW component content increased in most species with

development. Content deposition plots indicated that the order of maximum deposition in legumes and especially grasses was hemicellulose and cellulose followed by lignin. Graphical representations indicated that CW, cellulose, and lignin concentration increased with age in most species. Changes in concentration of these components were generally greater and decreased faster in legumes compared to grasses. Hemicellulose concentration remained the same or decreased with age in all species. Change in hemicellulose concentration decreased faster in grasses compared with legumes, particularly at later regrowth days.

## Introduction

Cellulose microfibrils embedded in a matrix of hemicellulose and lignin constitute plant cell walls (CW) (Buxton et al., 1987; Jung, 1989). Secondary walls are derived from primary walls after thickening and inclusion of lignin into the matrix (Theander and Aman, 1984). Lignin is the major factor contributing to lowered forage digestibility (Buxton and Russell, 1988), and its monomeric constituents seem to affect both rate and extent of CW digestion (Buxton, 1989). Whereas lignin concentration may affect digestibility because of its inert nature, lignin's interaction with hemicellulosic polysaccharides, pectic polysaccharides, and the remaining cellulosic fraction also seems to be important

in further limiting CW degradability (Hatfield, 1989).

Recent studies with old world bluestem (Bothriochloa spp.) stems indicate that CW and lignin concentration on a dry weight (DW) basis increase rapidly in young tissues and reach a plateau as the forage matures (Dabo et al., 1988). Attention should be focused on rapid changes in lignin concentration of young tissues because small increases in lignification of young forages may have greater negative effects on digestibility than subsequent increases in lignification of nearly mature forages (Jung and Vogel, 1986; Jung, 1989). Additional research is needed to explain why mature grass stems have faster rates of CW lignification than mature legume stems (Buxton and Russell, 1988).

Although CW lignin concentration is generally lower in grass stems than in those of legumes, apparent resistance to digestion of grass lignin is 60% greater than that of legume lignin (Buxton and Russell, 1988). Van Soest (1964) states that factors in addition to differences in lignin concentration are responsible for grass and legume digestibility differences. Evidence indicates that these differences are related to composition of lignin monomeric constituents (Hartley and Jones, 1977; Buxton and Russell, 1988).

Limited information is available regarding differences in CW and CW component content (yield) and concentration of

legumes and grasses as a function of regrowth after harvest and essentially no information is available to describe deposition and concentration changes of CW and CW components in forage sheaths or stems as a function of regrowth. This information is important because differences in CW deposition and concentration changes in legumes and grasses may contribute towards family- and species-specific digestibility differences.

Improved understanding of CW and CW component growth in orchardgrass sheaths, and grass and legume stems is approached in this investigation through the following objectives: 1) to study trends in grass and legume CW and CW component content and deposition as a function of regrowth after harvest, 2) to examine relationships between CW and CW component concentration and changes in concentration 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, concentration, deposition, and change in concentration as a function of regrowth.

## Materials and Methods

Three legume species studied were 'Arrow' alfalfa, 'Viking' birdsfoot trefoil, 'Arlington' red clover, and the three grass species studied were 'Napier' orchardgrass,

'Barton' smooth bromegrass, and 'Trailblazer' switchgrass. These were established in a greenhouse in 1987 in 25-cm 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. In 1987, seven pots of each species per replicate were used for seven weekly harvests. In 1988, five pots of each species per replicate were used for five biweekly harvests over a ten-week period. Thus, regrowth was sampled in 1987 at 21, 28, 35, 42, 56, and 63 days and in 1988 at 14, 28, 42, 56, and 70 days.

Experiments were conducted September through December of both years when greenhouse temperatures ranged from 20 to 37°C, and high pressure sodium lamps supplemented sunlight with a 14 h day and 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, 1981), as well as plant height from soil level, were recorded 1 or 2 days before sampling. Plants were cut at 3 to 5 cm above the soil level and the basal 10 cm of harvested stem material, or sheaths of orchardgrass, was dried at 55°C for 48 h. Remaining plant material from each pot was also dried and weighed to enable

calculation of the total plant DW. Dried sheath or stem 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, cellulose, hemicellulose, and lignin in the ground sheath or stem samples.

Dry weight, CW, cellulose, hemicellulose, and lignin were expressed on a content (per pot) or concentration (DW and CW) basis. Graphical representation of components as a function of regrowth days was performed by fitting data to the Gompertz function, $Y = a*\exp[(-b)*\exp(-ct)]$, 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 computer program and values for a were entered as the highest number from data within each species. Among estimated coefficients, the Gompertz b coefficient was viewed as the most important in expressing growth rate for this study. 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. Use of 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*exp(-ct)*exp[(-b)*exp(-ct)]. Data analyses for mean squares and curve fitting were performed by PROC GLM and PROC NLIN, respectively (SAS Institute, 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

### Plant height, stage of plant development, and total dry weight

Plant height increased significantly for all species both years (Tables 1 and 2). Except for the first samples of red clover and switchgrass in 1987, sufficient stem or orchardgrass sheath material was available from all pots to span the 10.0 cm sample height. All stem or sheath material was used for fiber analyses for those samples that did not exceed 10.0 cm. General trends from plant height indicated that legumes were often taller than grasses and that early regrowth of plants in 1988 was about the same or greater than that of early regrowth of plants in 1987.

Except for orchardgrass, stages of plant development in 1988 spanned a wider range than stages of plant development

Table 1. Plant height per pot for greenhouse-grown forage species in 1987

| Harvest day | Alfalfa | Birdsfoot trefoil | Red clover | Orchard-grass | Brome-grass | Switch-grass |
|---|---|---|---|---|---|---|
| | Species | | | | | |
| | mm | | | | | |
| 21 | 174[a] | 240 | 89 | 397 | 126 | 67 |
| 28 | 994 | 297 | 285 | 420 | 248 | 110 |
| 35 | 1276 | 386 | 585 | 475 | 340 | 160 |
| 42 | 1336 | 529 | 819 | 438 | 328 | 255 |
| 49 | 1578 | 595 | 858 | 468 | 355 | 318 |
| 56 | 1694 | 818 | 853 | 590 | 438 | 480 |
| 63 | 1623 | 932 | 933 | 590 | 498 | 615 |

[a]LSD for species X regrowth period equals 87 at P = 0.05.

Table 2. Plant height per pot for greenhouse-grown forage species in 1988

| Regrowth day | Alfalfa | Birdsfoot trefoil | Red clover | Orchard-grass | Brome-grass | Switch-grass |
|---|---|---|---|---|---|---|
| | Species | | | | | |
| | mm | | | | | |
| 14 | 410[a] | 162 | 170 | 295 | 198 | 185 |
| 28 | 962 | 332 | 420 | 428 | 302 | 392 |
| 42 | 1052 | 392 | 470 | 502 | 415 | 495 |
| 56 | 1112 | 462 | 528 | 565 | 470 | 828 |
| 70 | 1070 | 565 | 598 | 642 | 530 | 760 |

[a]LSD for species X regrowth period equals 67 at P = 0.05.

in 1987 (Tables 3 and 4). The wider range of plant stages in 1988 seems logical because the sampling period included days 14 through 70 as opposed to days 21 through 63 in 1987. Of special interest in these data is the narrow range of plant stages for greenhouse-grown orchardgrass both years and the narrow range of plant stages for alfalfa in 1987. Almost constant plant stages for orchardgrass were expected since this species requires vernalization before reproductive stalks are produced. Alfalfa plant stages probably did not vary widely in 1987 because budding, or "inflorescence visible" as termed by Ohlsson and Wedin (1989), was already observed on the first day of sampling.

Total plant mass increased with regrowth days in all species both years, although some pot-to-pot variability indicated negative growth during some regrowth periods (Tables 5 and 6). In 1987, order of highest to lowest yield at day 63 was alfalfa > red clover > orchardgrass > birdsfoot trefoil > bromegrass > switchgrass and in 1988 at day 70 the order of highest to lowest yield was switchgrass > orchardgrass > alfalfa > red clover > bromegrass > orchardgrass.

Content data

Dry weight of sheaths or stem bases and their content of CW and CW components differed significantly for the main effects of species and age and for the species X age

Table 3. Stages of plant development of potted forage species grown in the greenhouse in 1987

| Regrowth day | Alfalfa | Birdsfoot trefoil | Red clover | Orchard-grass | Brome-grass | Switch-grass |
|---|---|---|---|---|---|---|
| | Species | | | | | |
| | maturity index | | | | | |
| 21 | 51[a] | 38 | 35 | 21 | 32 | 31 |
| 28 | 59 | 60 | 41 | 22 | 33 | 32 |
| 35 | 67 | 55 | 56 | 23 | 40 | 33 |
| 42 | 66 | 63 | 62 | 23 | 35 | 34 |
| 49 | 68 | 62 | 66 | 23 | 38 | 38 |
| 56 | 66 | 65 | 68 | 23 | 38 | 45 |
| 63 | 69 | 64 | 68 | 24 | 43 | 56 |

[a]Maturities were 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. Inflorescence visible (legumes) or inflorescence emergence (grasses) is estimated as 50 in all schemes.

Table 4. Stages of plant development of potted forage species grown in the greenhouse in 1988

| Regrowth day | Alfalfa | Birdsfoot trefoil | Red clover | Orchard-grass | Brome-grass | Switch-grass |
|---|---|---|---|---|---|---|
| | Species | | | | | |
| | maturity index | | | | | |
| 14 | 37[a] | 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 |

[a]Maturities were 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. Inflorescence visible (legumes) or inflorescence emergence (grasses) is estimated as 50 in all schemes.

Table 5. Total dry weight yield per pot for greenhouse-grown forage species in 1987

| Regrowth day | Alfalfa | Birdsfoot trefoil | Red clover | Orchard-grass | Brome-grass | Switch-grass |
|---|---|---|---|---|---|---|
| | Species | | | | | |
| | g $pot^{-1}$ | | | | | |
| 21 | 10.9[a] | 6.8 | 12.5 | 8.4 | 11.7 | 5.0 |
| 28 | 21.9 | 9.1 | 11.7 | 18.5 | 14.6 | 6.8 |
| 35 | 36.3 | 8.2 | 22.2 | 20.1 | 16.0 | 5.2 |
| 42 | 40.4 | 16.8 | 26.7 | 27.7 | 23.3 | 11.7 |
| 49 | 45.9 | 19.2 | 36.3 | 31.0 | 27.9 | 20.2 |
| 56 | 49.6 | 29.8 | 44.0 | 40.2 | 27.5 | 24.5 |
| 63 | 64.6 | 32.8 | 52.6 | 43.5 | 31.9 | 28.9 |

[a]LSD for species X regrowth period equals 5.4 at P = 0.05.

Table 6. Total dry weight yield per pot for greenhouse-grown forage species in 1988

| Regrowth day | Alfalfa | Birdsfoot trefoil | Red clover | Orchard-grass | Brome-grass | Switch-grass |
|---|---|---|---|---|---|---|
| | Species | | | | | |
| | g $pot^{-1}$ | | | | | |
| 14 | 18.5[a] | 13.6 | 14.8 | 22.1 | 10.6 | 9.5 |
| 28 | 37.4 | 16.2 | 18.6 | 27.1 | 20.4 | 18.0 |
| 42 | 52.1 | 21.1 | 23.8 | 31.6 | 28.1 | 23.0 |
| 56 | 64.7 | 36.5 | 44.7 | 56.6 | 48.1 | 54.8 |
| 70 | 62.5 | 49.3 | 52.3 | 64.6 | 52.0 | 69.6 |

[a]LSD for species X regrowth period equals 6.0 at P = 0.05.

interaction both years except for lignin in 1987 (Tables 7 and 8). Significant differences in CW and CW component concentration with age were anticipated because their amounts increased with increasing plant mass.

Gompertz fitting of stem base DW per pot demonstrated a satisfactory representation of growth responses for grasses, but fitting of legume data was not very satisfactory (Figures 1 and 2). Fittings were viewed as "acceptable" in these analyses and those that follow when at least 50% (one-half) of the data variability could be attributed to regrowth days. For instance, in Figures 1 and 2, acceptable fittings were observed because $R^2$ values indicated that 71 to 99% and 84 to 98% variability in stem base dry weight could be attributed to regrowth days in 1987 and 1988, respectively. From the top two graphs of each figure, it is apparent that stem base DW increased sigmoidally with regrowth days, for both years, when directed through the origin. Deposition plots from these graphs showed a bell-shaped curve response with age in all species. Smooth bell curves in 1988, compared with saddle curves in 1987, indicate that Gompertz fitting was more appropriate when data collection began at day 14 (1988) instead of day 21 (1987). These plots show that the maximum rate of stem base deposition often occurred earlier in legumes than grasses. However the greater Gompertz a coefficient, more persistent maximum deposition rates shown

Table 7. Mean squares of 1987 stem base dry weights and their fiber analyses on a per pot basis

| Source | Dry weight | Cell wall | Cellulose | Hemicellulose | Lignin[a] |
|---|---|---|---|---|---|
| Species | 107.85** | 33.32** | 8.49** | 8.27** | 0.79** |
| Error a | 0.33 | 0.12 | 0.04 | 0.01 | 0.06 |
| Age | 15.54** | 7.08** | 2.46** | 0.65** | 0.86** |
| Species*Age | 2.57** | 0.93** | 0.27** | 0.18** | 0.05 |
| Error b | 0.93 | 0.40 | 0.13 | 0.06 | 0.03 |

[a]Values shown in table have been multiplied by 10.

**Significant at the 0.01 level of probability.

Table 8. Mean squares of 1988 stem base dry weights and their fiber analyses on a per pot basis

| Source | Dry weight | Cell wall | Cellulose | Hemicellulose | Lignin[a] |
|---|---|---|---|---|---|
| Species | 105.23** | 30.36** | 6.74** | 1.13** | 7.92** |
| Error a | 2.20 | 0.73 | 0.11 | 0.06 | 0.24 |
| Age | 53.73** | 21.51** | 1.95** | 2.37** | 7.57** |
| Species*Age | 4.77** | 2.12** | 0.35** | 0.17** | 0.63** |
| Error b | 1.24 | 0.48 | 0.08 | 0.04 | 0.14 |

[a]Values shown in table have been multiplied by 10.

**Significant at the 0.01 level of probability.

Figure 1. Per pot dry weight [LSD (0.05) = 1.35] and dry weight deposition in stem bases of legumes and grasses in 1987. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 2.58 | 1.87 | 0.0470 | 0.76 |
| Birdsft. trefoil | 2.34 | 2.28 | 0.0439 | 0.72 |
| Red clover | 2.87 | 2.20 | 0.0500 | 0.71 |
| Orchardgrass | 9.07 | 6.23 | 0.0860 | 0.93 |
| Bromegrass | 4.27 | 4.19 | 0.0603 | 0.99 |
| Switchgrass | 3.19 | 9.61 | 0.0713 | 0.85 |

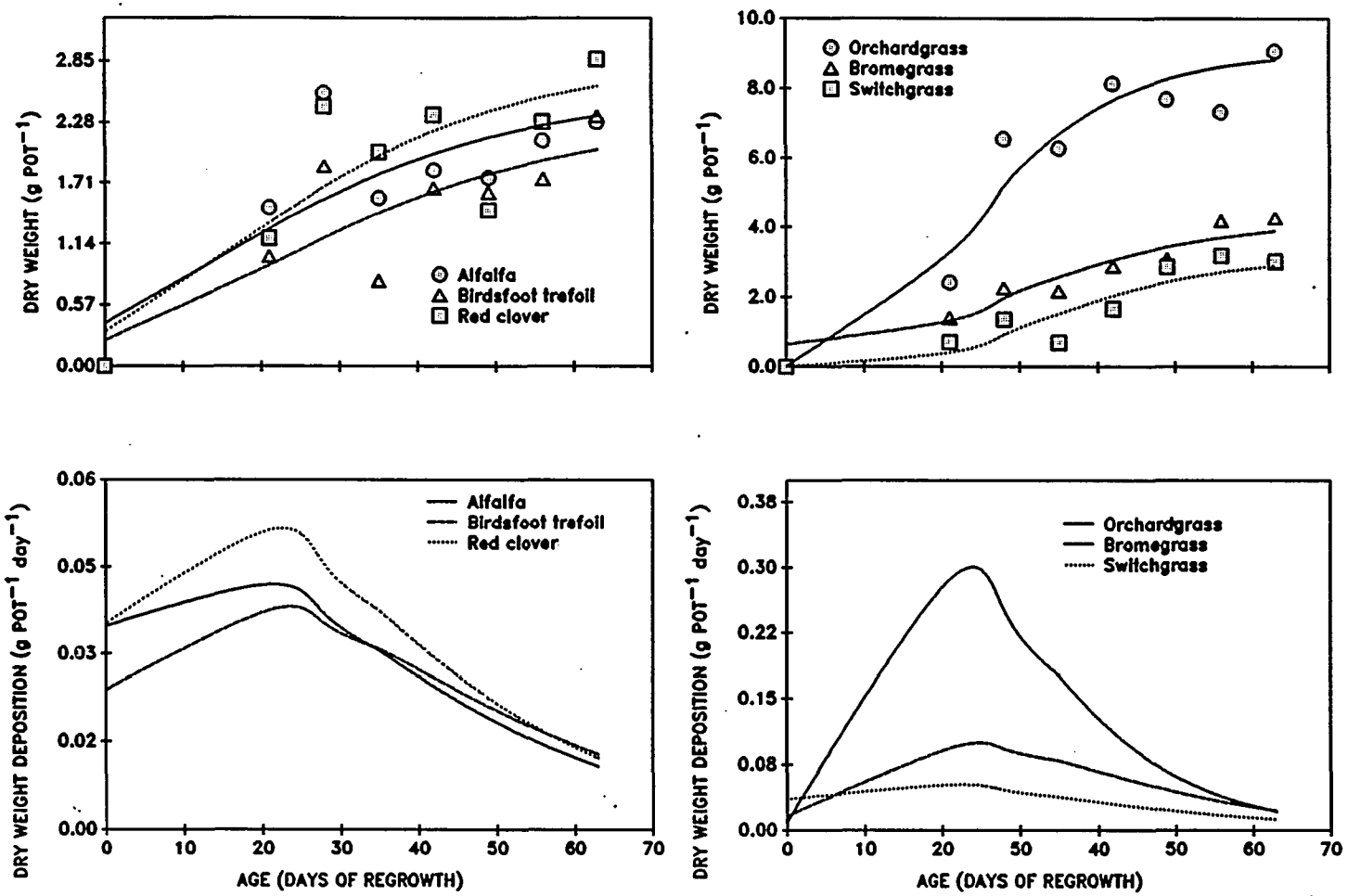
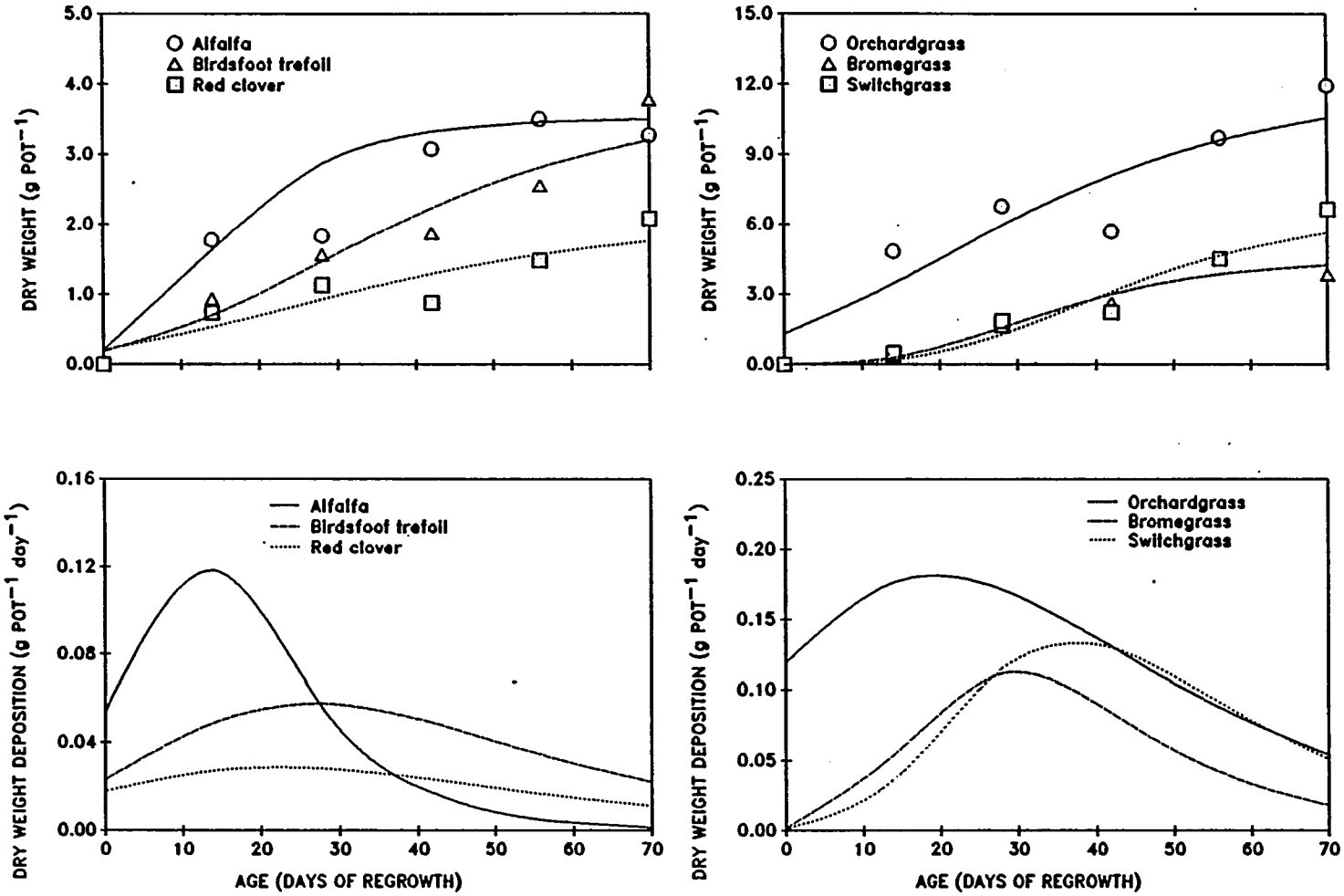

DRY WEIGHT (g POT$^{-1}$)
2.85
2.28
1.71
1.14
0.57
0.00
Alfalfa
Birdsfoot trefoil
Red clover
10.0
8.0
6.0
4.0
2.0
0.0
Orchardgrass
Bromegrass
Switchgrass
DRY WEIGHT DEPOSITION (g POT$^{-1}$ day$^{-1}$)
0.06
0.05
0.03
0.02
0.00
0.38
0.30
0.22
0.15
0.08
0
10
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

Figure 2. Per pot dry weight [LSD (0.05) = 1.56] and dry weight deposition in stem bases of legumes and grasses in 1988. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 3.51 | 2.87 | 0.0947 | 0.98 |
| Birdsft. trefoil | 3.78 | 3.02 | 0.0415 | 0.93 |
| Red clover | 2.08 | 2.32 | 0.0376 | 0.84 |
| Orchardgrass | 11.94 | 2.21 | 0.0412 | 0.86 |
| Bromegrass | 4.51 | 7.00 | 0.0675 | 0.96 |
| Switchgrass | 6.63 | 7.66 | 0.0550 | 0.93 |


Alfalfa
Birdsfoot trefoil
Red clover
Orchardgrass
Bromegrass
Switchgrass
DRY WEIGHT (g POT$^{-1}$)
DRY WEIGHT DEPOSITION (g POT$^{-1}$ day$^{-1}$)
AGE (DAYS OF REGROWTH)
0.0
1.0
2.0
3.0
4.0
5.0
3.0
6.0
9.0
12.0
15.0
0.00
0.04
0.08
0.12
0.16
0.05
0.10
0.15
0.20
0.25
0
10
20
30
40
50
60
70

by graphs, and generally greater b coefficient in grasses, are probably a result of new tillers encountered in pots of these plants.

Sigmoidal curves and high $R^2$ values for grasses both years and for legumes in 1988 also were observed for CW content as a function of regrowth days (Figures 3 and 4). Among the six species, deposition of CW material attained a maximum first in alfalfa at about day 14 and last in switchgrass at about day 40. Except for these two species, the maximum rate of CW deposition occurred at days 21 to 35 in both grasses and legumes. High Gompertz b values for switchgrass were coincident with delayed increases in CW content as a function of regrowth days, indicating that the relative growth rate of switchgrass is unique from the other five species studied.

Maximum values of cellulose deposition and $R^2$ values demonstrated parallel relationships to those of CW deposition in all species (Figures 5 and 6). This close association was expected since cellulose constituted 50% or more of the CW throughout most of the sampling period (see section on concentration data).

Trends in deposition rates of hemicellulose also demonstrated bell curve responses with regrowth days (Figures 7 and 8). Red clover both years and birdsfoot trefoil and bromegrass in 1987 did not show high $R^2$ values compared with

Figure 3. Per pot cell wall [LSD (0.05) = 0.89] and cell-wall deposition in stem bases of legumes and grasses in 1987. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 1.64 | 2.17 | 0.0628 | 0.81 |
| Birdsft. trefoil | 1.59 | 2.55 | 0.0439 | 0.74 |
| Red clover | 1.61 | 2.54 | 0.0474 | 0.78 |
| Orchardgrass | 5.41 | 5.49 | 0.0773 | 0.94 |
| Bromegrass | 2.73 | 4.52 | 0.0612 | 0.95 |
| Switchgrass | 2.36 | 19.84 | 0.0863 | 0.86 |

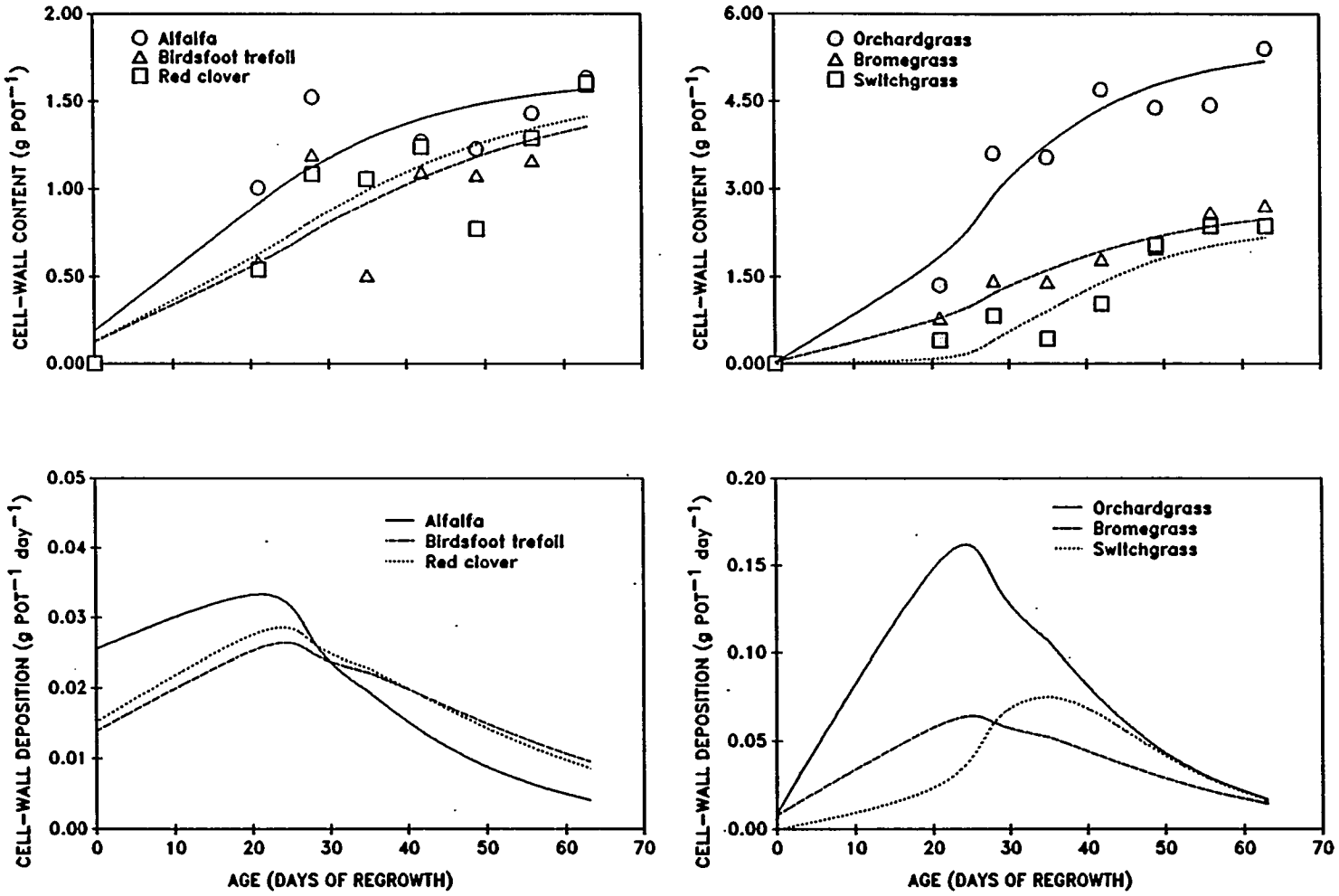

Alfalfa
Birdsfoot trefoil
Red clover
Orchardgrass
Bromegrass
Switchgrass
CELL-WALL CONTENT (g POT$^{-1}$)
CELL-WALL DEPOSITION (g POT$^{-1}$ day$^{-1}$)
AGE (DAYS OF REGROWTH)
0.00
0.50
1.00
1.50
2.00
1.50
3.00
4.50
6.00
0.01
0.02
0.03
0.04
0.05
0.05
0.10
0.15
0.20
0
10
20
30
40
50
60
70

Figure 4. Per pot cell wall [LSD (0.05) = 0.98] and cell-wall deposition in stem bases of legumes and grasses in 1988. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 2.05 | 3.76 | 0.1001 | 0.99 |
| Birdsft. trefoil | 2.15 | 3.39 | 0.0446 | 0.94 |
| Red clover | 1.02 | 2.80 | 0.0421 | 0.90 |
| Orchardgrass | 6.83 | 2.42 | 0.0409 | 0.88 |
| Bromegrass | 2.68 | 8.04 | 0.0700 | 0.95 |
| Switchgrass | 5.02 | 11.58 | 0.0627 | 0.94 |

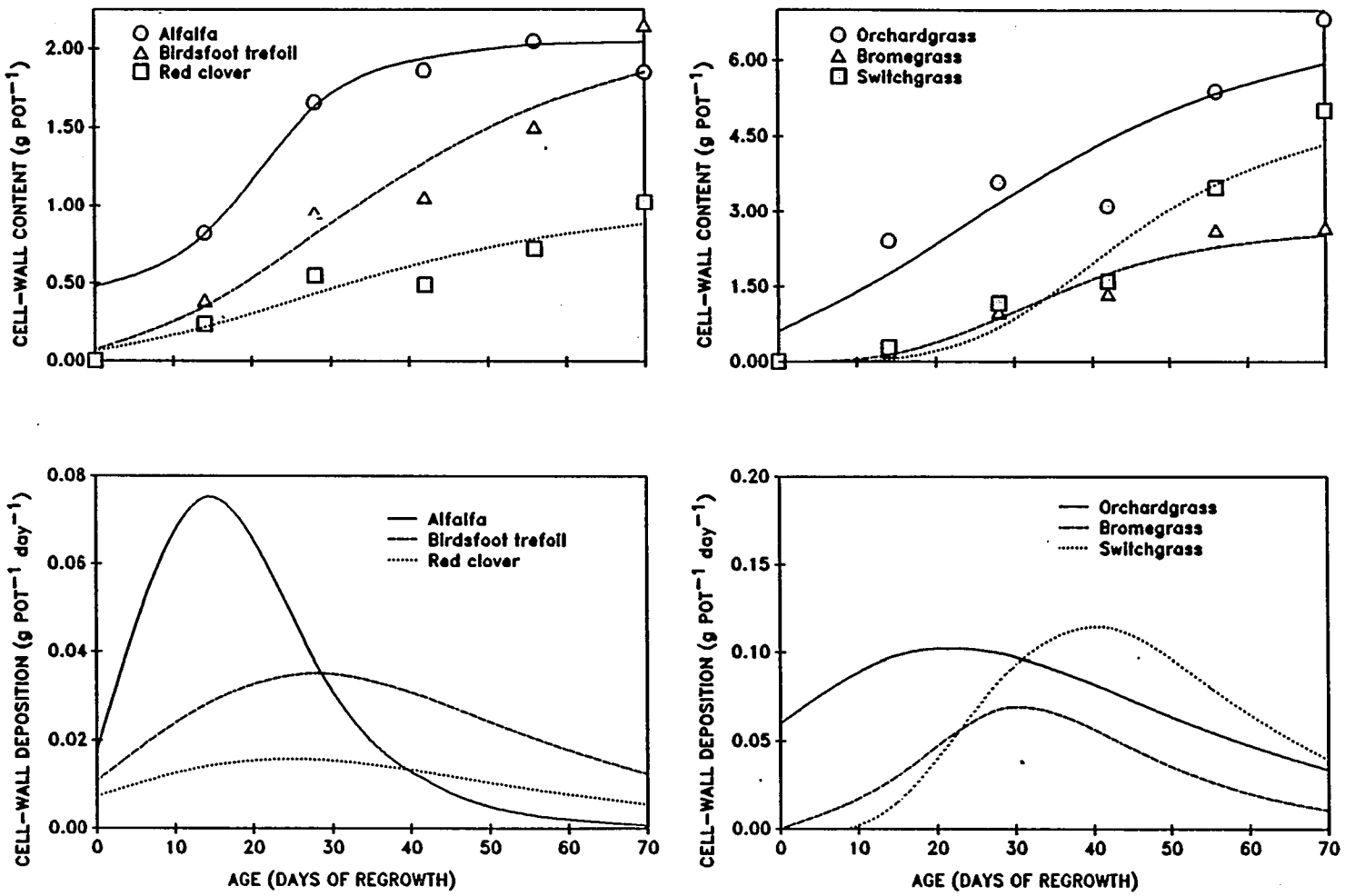

Alfalfa
Birdsfoot trefoil
Red clover
Orchardgrass
Bromegrass
Switchgrass
CELL-WALL CONTENT (g POT$^{-1}$)
CELL-WALL DEPOSITION (g POT$^{-1}$ day$^{-1}$)
AGE (DAYS OF REGROWTH)
0.00
0.50
1.00
1.50
2.00
3.00
4.50
6.00
0.02
0.04
0.06
0.08
0.05
0.10
0.15
0.20
0
10
20
30
40
50
60
70

Figure 5. Per pot cellulose [LSD (0.05) = 0.50] and cellulose deposition in stem bases of legumes and grasses in 1987. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 1.11 | 2.08 | 0.0614 | 0.79 |
| Birdsft. trefoil | 1.04 | 2.62 | 0.0442 | 0.76 |
| Red clover | 1.16 | 2.49 | 0.0447 | 0.79 |
| Orchardgrass | 2.92 | 5.95 | 0.0810 | 0.95 |
| Bromegrass | 1.53 | 5.12 | 0.0619 | 0.97 |
| Switchgrass | 1.33 | 823.35 | 0.1668 | 0.88 |

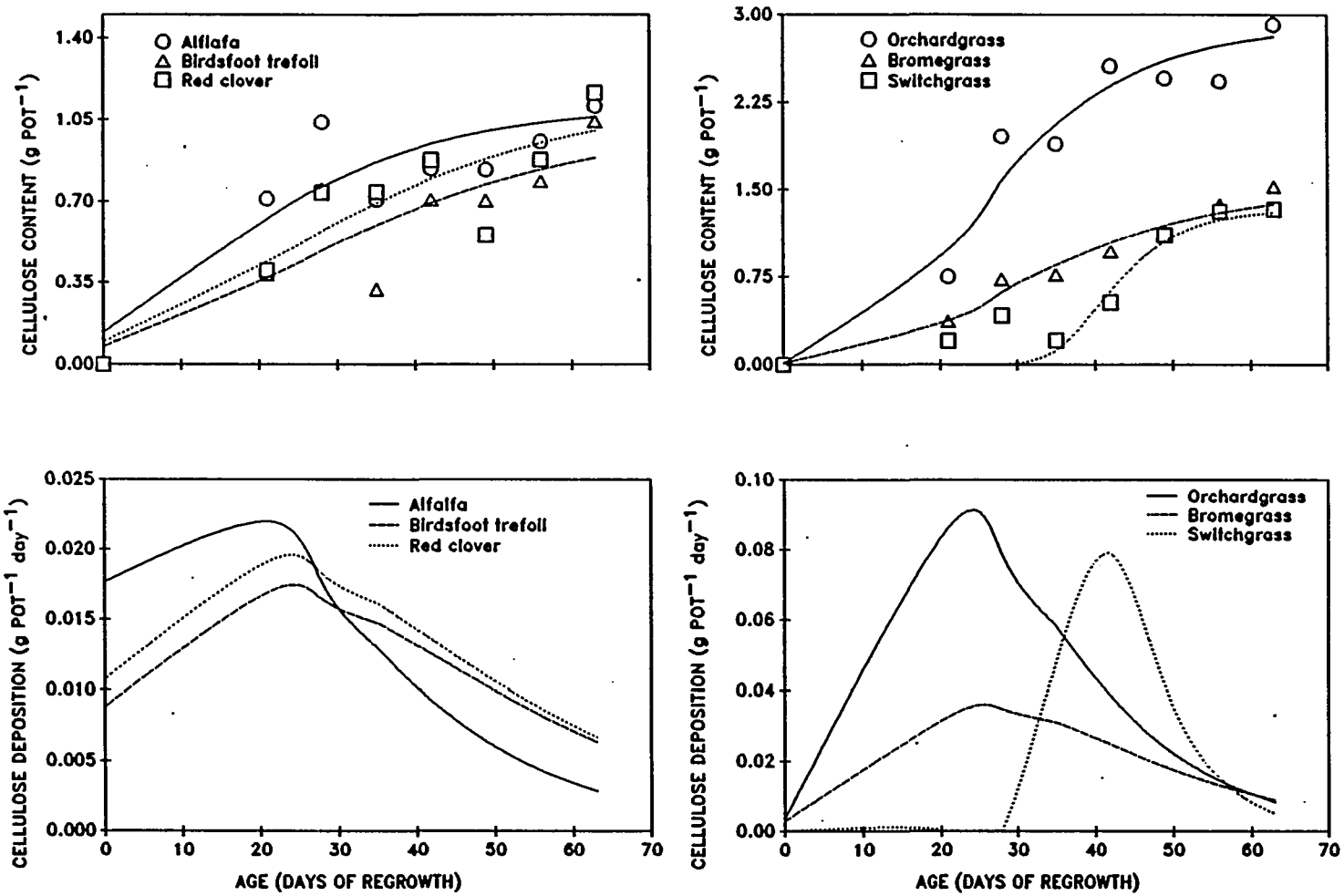

Alfalfa
Birdsfoot trefoil
Red clover
Orchardgrass
Bromegrass
Switchgrass
CELLULOSE CONTENT (g POT$^{-1}$)
CELLULOSE DEPOSITION (g POT$^{-1}$ day$^{-1}$)
AGE (DAYS OF REGROWTH)
0.00
0.35
0.70
1.05
1.40
0.75
1.50
2.25
3.00
0.000
0.005
0.010
0.015
0.020
0.025
0.02
0.04
0.06
0.08
0.10
0
10
20
30
40
50
60
70

Figure 6. Per pot cellulose [LSD (0.05) = 0.53] and cellulose deposition in stem bases of legumes and grasses in 1988. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 1.33 | 4.17 | 0.1106 | 1.00 |
| Birdsft. trefoil | 1.42 | 3.59 | 0.0468 | 0.95 |
| Red clover | 0.71 | 3.14 | 0.0459 | 0.94 |
| Orchardgrass | 3.76 | 2.41 | 0.0399 | 0.87 |
| Bromegrass | 1.48 | 8.32 | 0.0692 | 0.97 |
| Switchgrass | 2.91 | 16.55 | 0.0691 | 0.95 |

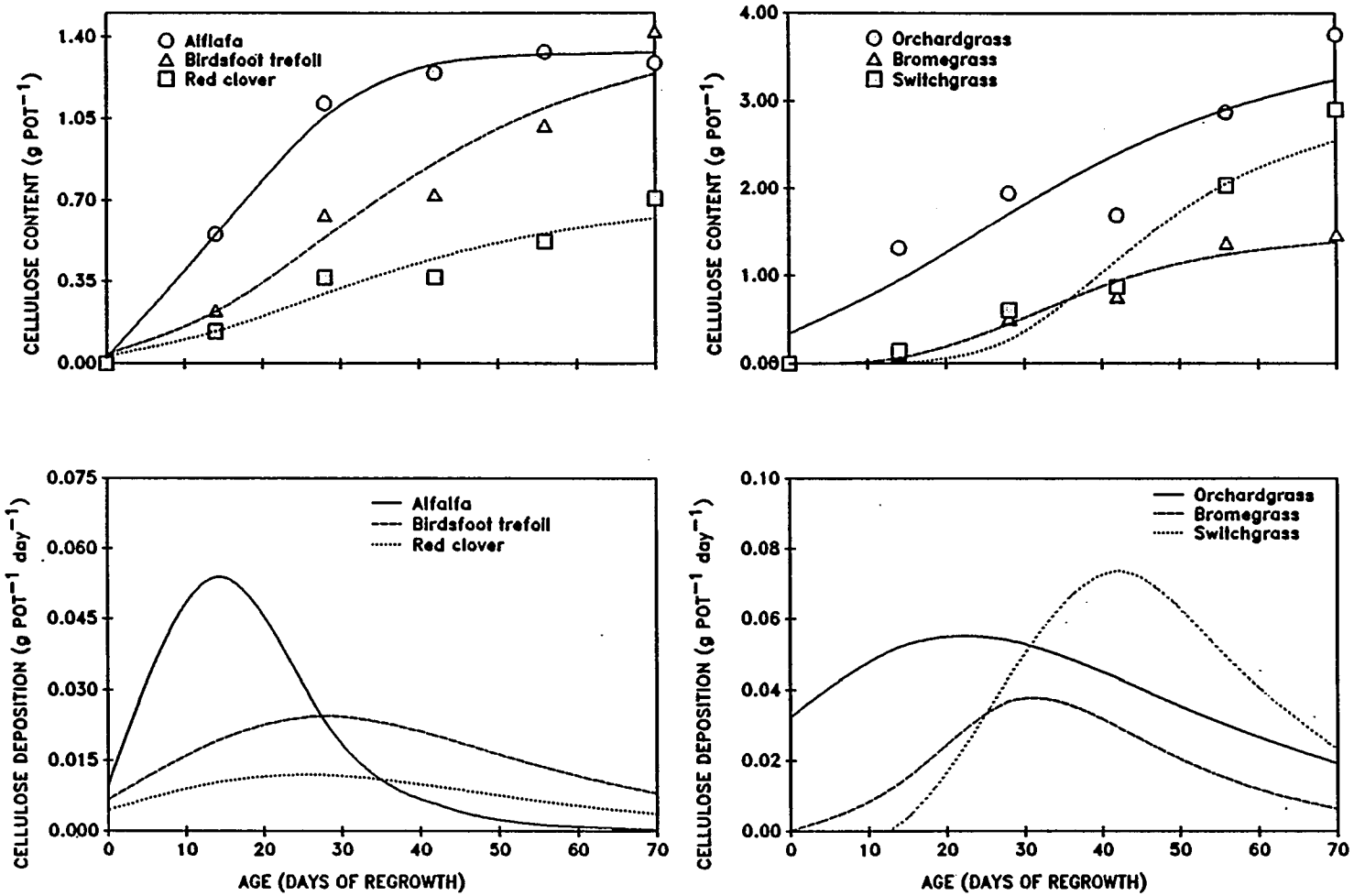

Alfalfa
Birdsfoot trefoil
Red clover
CELLULOSE CONTENT (g POT$^{-1}$)
1.40
1.05
0.70
0.35
0.00
Orchardgrass
Bromegrass
Switchgrass
CELLULOSE CONTENT (g POT$^{-1}$)
4.00
3.00
2.00
1.00
0.00
Alfalfa
Birdsfoot trefoil
Red clover
CELLULOSE DEPOSITION (g POT$^{-1}$ day$^{-1}$)
0.075
0.060
0.045
0.030
0.015
0.000
0
10
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)
Orchardgrass
Bromegrass
Switchgrass
CELLULOSE DEPOSITION (g POT$^{-1}$ day$^{-1}$)
0.10
0.08
0.06
0.04
0.02
0.00
0
10
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

Figure 7. Per pot hemicellulose [LSD (0.05) = 0.34] and hemicellulose deposition in stem bases of legumes and grasses in 1987. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 0.231 | 2.473 | 0.0755 | 0.83 |
| Birdsft. trefoil | 0.231 | 2.018 | 0.0406 | 0.57 |
| Red clover | 0.216 | 2.570 | 0.0673 | 0.68 |
| Orchardgrass | 2.199 | 5.108 | 0.0746 | 0.93 |
| Bromegrass | 1.002 | 1.655 | 0.0249 | 0.24 |
| Switchgrass | 0.871 | 12.470 | 0.0807 | 0.85 |

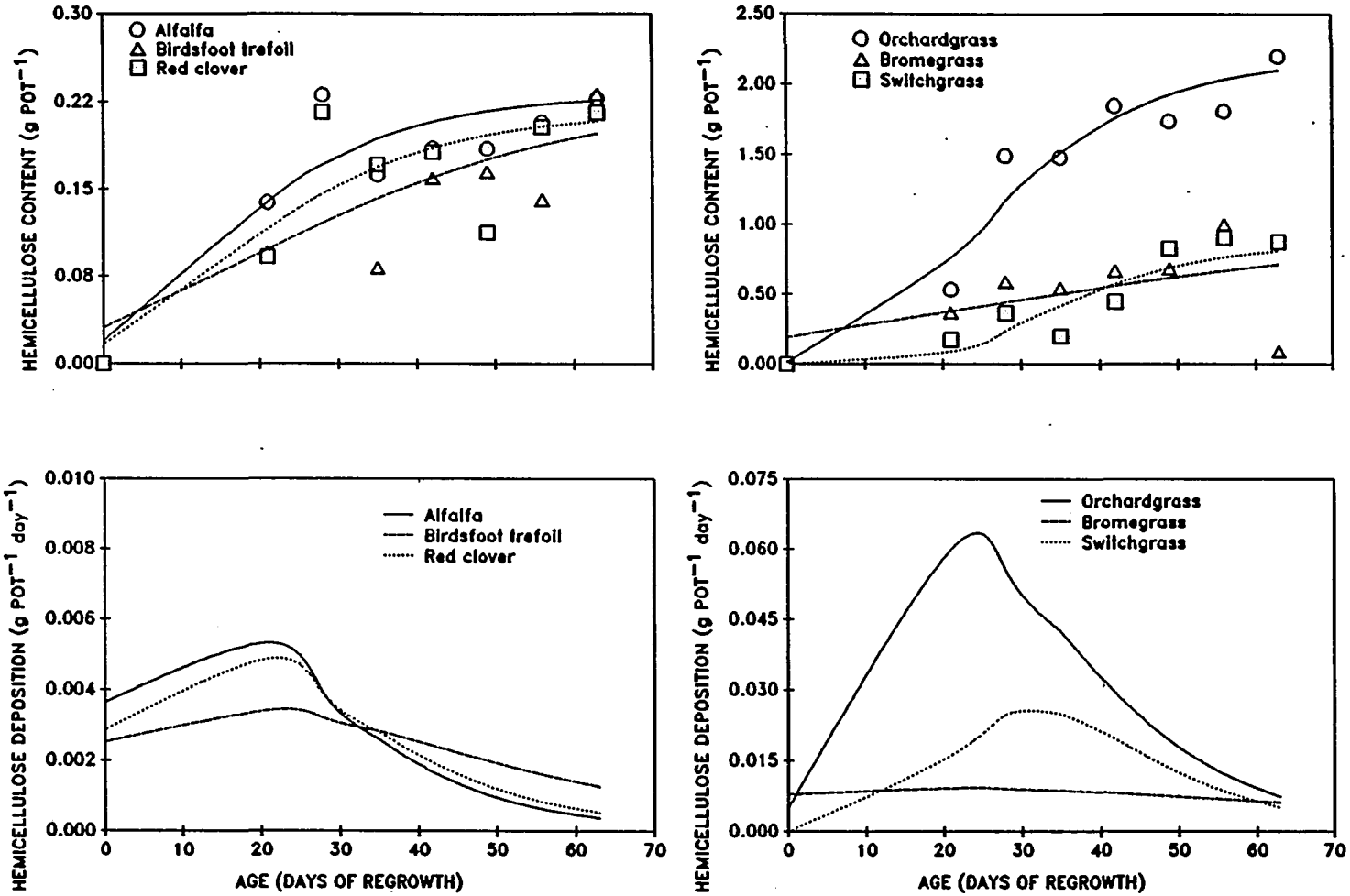
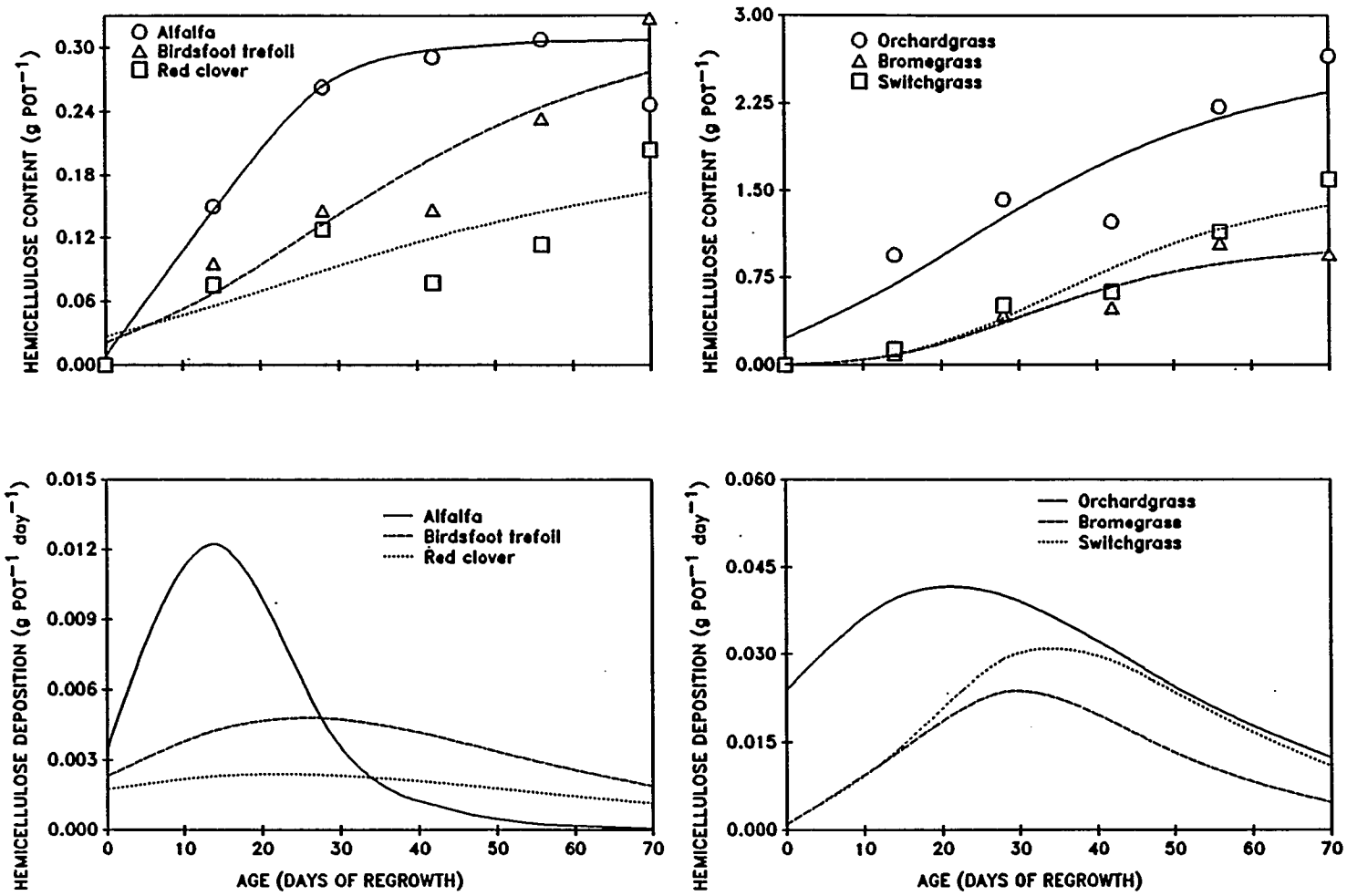

HEMICELLULOSE CONTENT (g POT$^{-1}$)
0.30
0.22
0.15
0.08
0.00
Alfalfa
Birdsfoot trefoil
Red clover
2.50
2.00
1.50
1.00
0.50
0.00
Orchardgrass
Bromegrass
Switchgrass
HEMICELLULOSE DEPOSITION (g POT$^{-1}$ day$^{-1}$)
0.010
0.008
0.006
0.004
0.002
0.000
0.075
0.060
0.045
0.030
0.015
0.000
0
10
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

Figure 8. Per pot hemicellulose [LSD (0.05) = 0.40] and hemicellulose deposition in stem bases of legumes and grasses in 1988. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 0.307 | 3.59 | 0.1126 | 0.94 |
| Birdsft. trefoil | 0.328 | 2.75 | 0.0399 | 0.90 |
| Red clover | 0.204 | 2.02 | 0.0318 | 0.69 |
| Orchardgrass | 2.654 | 2.45 | 0.0427 | 0.88 |
| Bromegrass | 1.044 | 5.96 | 0.0618 | 0.92 |
| Switchgrass | 1.596 | 6.07 | 0.0529 | 0.94 |


Alfalfa
Birdsfoot trefoil
Red clover
HEMICELLULOSE CONTENT (g POT$^{-1}$)
0.00
0.06
0.12
0.18
0.24
0.30
Orchardgrass
Bromegrass
Switchgrass
HEMICELLULOSE CONTENT (g POT$^{-1}$)
0.00
0.75
1.50
2.25
3.00
Alfalfa
Birdsfoot trefoil
Red clover
HEMICELLULOSE DEPOSITION (g POT$^{-1}$ day$^{-1}$)
0.000
0.003
0.006
0.009
0.012
0.015
0
10
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)
Orchardgrass
Bromegrass
Switchgrass
HEMICELLULOSE DEPOSITION (g POT$^{-1}$ day$^{-1}$)
0.000
0.015
0.030
0.045
0.060
0
10
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

other species both years. Compared with cellulose deposition, maximum rate of hemicellulose deposition occurred at about the same time in legumes, but preceded the maximum rate of cellulose deposition in grasses. Grass hemicellulose deposition may decline 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, deposition, and $R^2$ values followed the same trend as that of CW and cellulose in both years (Figures 9 and 10). Close observation of bell curve plots indicate that maximum rates of lignin deposition may occur slightly after maximum rates of cellulose and hemicellulose deposition, especially in grasses. Increased lignin deposition at late regrowth days concurs with the idea that secondary wall thickening occurs with inclusion of lignin in the CW matrix (Theander and Aman, 1984).

Species-specific maximum CW and CW component deposition can be inferred from graphical representation of these data. However, since many of the bell curve deposition responses have been derived in this study by directing curves through the origin and because many of the maximum rates occur at or prior to the first sampling date, particularly in 1987, emphasis of maximum deposition for specific species has been avoided. Contributions of data from this study to current knowledge include evidence for general order of CW component

Figure 9. Per pot lignin [LSD (0.05) = 0.08] and lignin deposition in stem bases of legumes and grasses in 1987. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 0.299 | 2.43 | 0.0616 | 0.86 |
| Birdsft. trefoil | 0.318 | 2.95 | 0.0471 | 0.79 |
| Red clover | 0.227 | 3.98 | 0.0580 | 0.80 |
| Orchardgrass | 0.299 | 4.12 | 0.0621 | 0.80 |
| Bromegrass | 0.285 | 9.48 | 0.0660 | 0.96 |
| Switchgrass | 0.156 | 440.84 | 0.0022 | 0.91 |

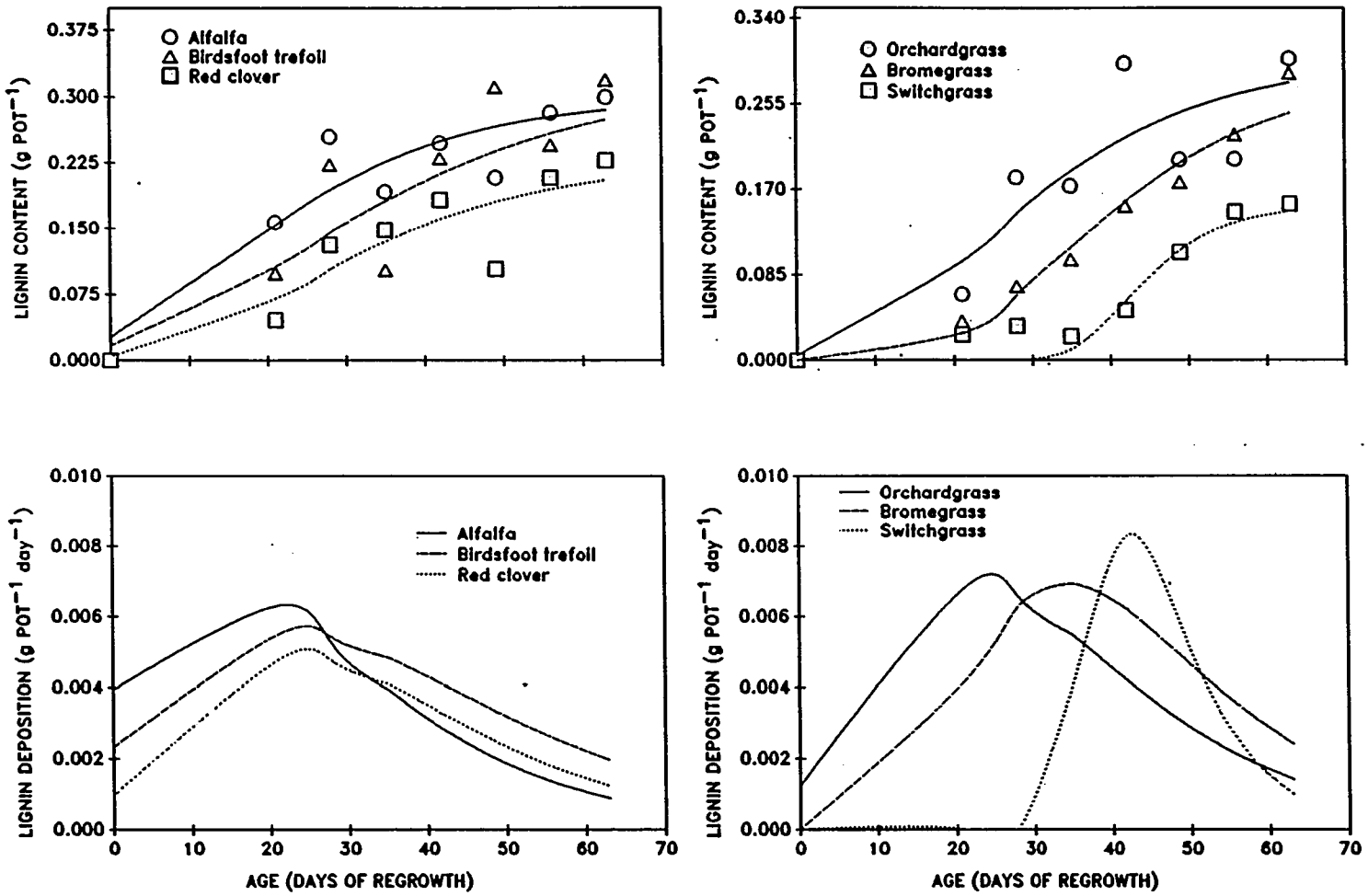
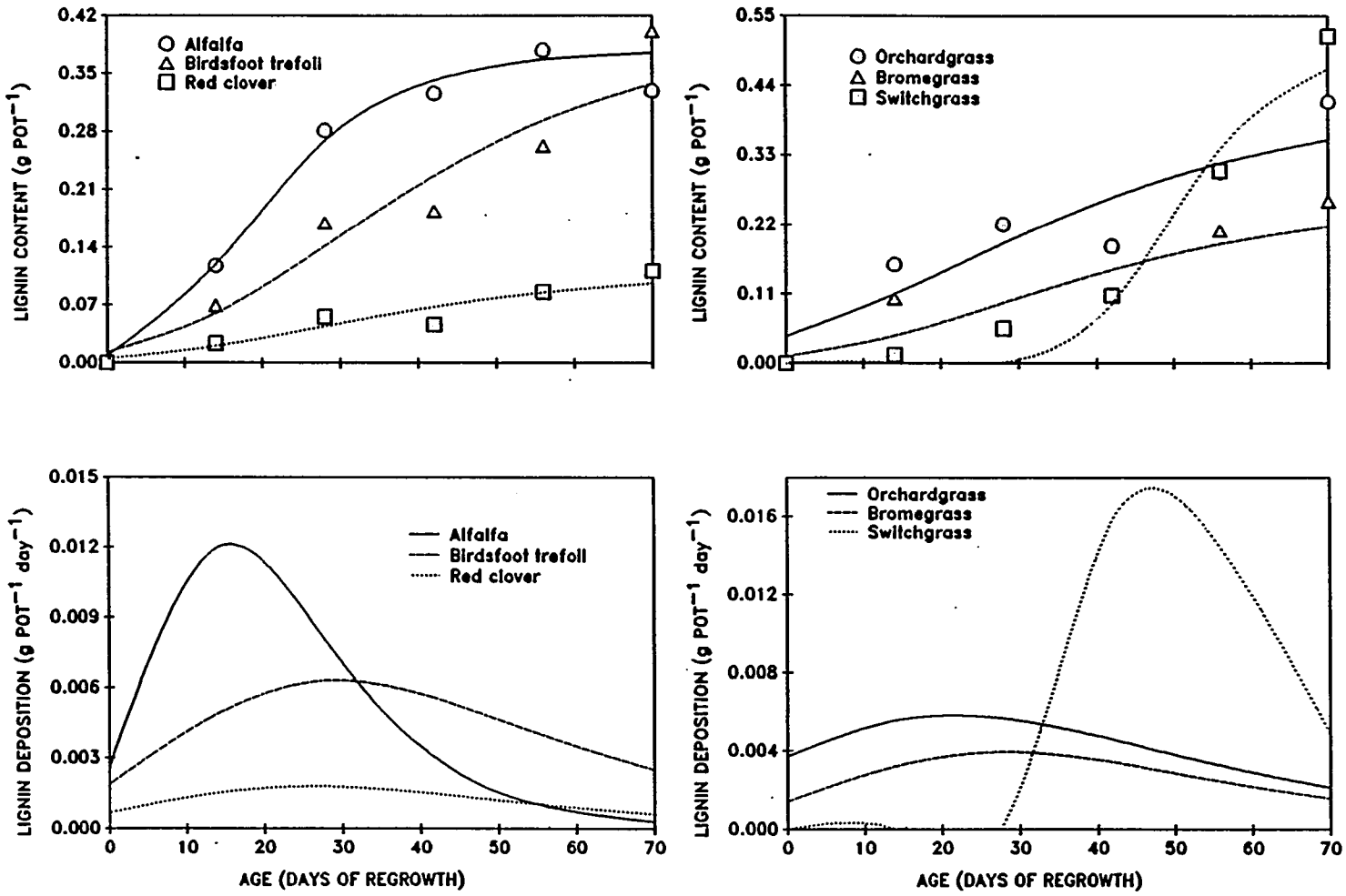

Alfalfa
Birdsfoot trefoil
Red clover
Orchardgrass
Bromegrass
Switchgrass
LIGNIN CONTENT (g POT$^{-1}$)
LIGNIN DEPOSITION (g POT$^{-1}$ day$^{-1}$)
AGE (DAYS OF REGROWTH)
0.000
0.075
0.150
0.225
0.300
0.375
0.085
0.170
0.255
0.340
0.002
0.004
0.006
0.008
0.010
0
10
20
30
40
50
60
70

Figure 10. Per pot lignin [LSD (0.05) = 0.10] and lignin deposition in stem bases of legumes and grasses in 1988. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 0.378 | 3.89 | 0.0870 | 0.98 |
| Birdsft. trefoil | 0.402 | 3.46 | 0.0428 | 0.92 |
| Red clover | 0.111 | 3.14 | 0.0439 | 0.90 |
| Orchardgrass | 0.414 | 2.27 | 0.0382 | 0.85 |
| Bromegrass | 0.258 | 3.19 | 0.0417 | 0.81 |
| Switchgrass | 0.518 | 111.87 | 0.0996 | 0.97 |


LIGNIN CONTENT (g POT$^{-1}$)
0.42
0.35
0.28
0.21
0.14
0.07
0.00
Alfalfa
Birdsfoot trefoil
Red clover
LIGNIN CONTENT (g POT$^{-1}$)
0.55
0.44
0.33
0.22
0.11
0.00
Orchardgrass
Bromegrass
Switchgrass
LIGNIN DEPOSITION (g POT$^{-1}$ day$^{-1}$)
0.015
0.012
0.009
0.006
0.003
0.000
Alfalfa
Birdsfoot trefoil
Red clover
0
10
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)
LIGNIN DEPOSITION (g POT$^{-1}$ day$^{-1}$)
0.016
0.012
0.008
0.004
0.000
Orchardgrass
Bromegrass
Switchgrass
0
10
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

deposition in grass and legume stems and orchardgrass sheaths as well as evidence indicating species-specific timing and amount of CW component deposition.

Relationship between stem base yield and CW composition

Implied differences in timing of CW and CW component deposition necessitated closer evaluation of dry weight and CW component content in relation to the dynamic nature of the CW matrix. Regression analysis was used to determine whether or not most of the variability in CW and CW component content could be linearly attributed to increasing plant mass as a function of regrowth days. If most of the CW and CW component content variability could be linearly attributed to increasing plant mass, then an additional study would be needed to provide more information about changes in the CW matrix.

Relationships between CW and CW component content and stem base DW and CW yield were evaluated by linear and quadratic regression analyses. From Tables 9 and 10, it was evident that, with exception of bromegrass, 64 to 100% of CW and CW component variability could be attributed to stem base DW and CW content. Higher $R^2$ values obtained from linear regression, and little or no improvement of regression by quadratic fitting in most species for both years, indicated that CW and CW component content increased proportionately with stem base DW and CW content. Good linear $R^2$ values

Table 9. Linear and quadratic $R^2$ values for cell wall (CW) and CW components vs. stem base weight (SW) and CW content on a per pot basis in 1987 for alfalfa (AL), birdsfoot trefoil (BT), red clover (RC), orchardgrass (OG), bromegrass (BG), and switchgrass (SG)

| | Species | | | | | |
|---|---|---|---|---|---|---|
| Regression components | AL | BT | RC | OG | BG | SG |
| CW vs. SW | | | | | | |
| Linear | 0.982 | 0.992 | 0.923 | 0.991 | 0.996 | 0.990 |
| Quadratic | 0.982 | 0.993 | 0.924 | 0.993 | 0.997 | 0.995 |
| Cellulose vs SW | | | | | | |
| Linear | 0.970 | 0.986 | 0.896 | 0.989 | 0.986 | 0.984 |
| Quadratic | 0.975 | 0.986 | 0.906 | 0.991 | 0.986 | 0.992 |
| Hemicellulose vs. SW | | | | | | |
| Linear | 0.968 | 0.884 | 0.922 | 0.986 | 0.016 | 0.998 |
| Quadratic | 0.975 | 0.885 | 0.959 | 0.986 | 0.173 | 0.999 |
| Lignin vs. SW | | | | | | |
| Linear | 0.857 | 0.954 | 0.797 | 0.882 | 0.948 | 0.927 |
| Quadratic | 0.901 | 0.954 | 0.807 | 0.924 | 0.948 | 0.967 |
| Cellulose vs. CW | | | | | | |
| Linear | 0.989 | 0.996 | 0.991 | 0.998 | 0.992 | 0.999 |
| Quadratic | 0.993 | 0.996 | 0.997 | 0.998 | 0.993 | 1.000 |
| Hemicellulose vs. CW | | | | | | |
| Linear | 0.952 | 0.859 | 0.795 | 0.995 | 0.013 | 0.994 |
| Quadratic | 0.966 | 0.859 | 0.868 | 0.996 | 0.211 | 0.998 |
| Lignin vs. CW | | | | | | |
| Linear | 0.878 | 0.980 | 0.948 | 0.882 | 0.950 | 0.967 |
| Quadratic | 0.900 | 0.980 | 0.956 | 0.901 | 0.956 | 0.992 |

Table 10. Linear and quadratic $R^2$ values for cell wall (CW) and CW components vs. stem base weight (SW) and CW content on a per pot basis in 1988 for alfalfa (AL), birdsfoot trefoil (BT), red clover (RC), orchardgrass (OG), bromegrass (BG), and switchgrass (SG)

| | Species | | | | | |
|---|---|---|---|---|---|---|
| Regression components | AL | BT | RC | OG | BG | SG |
| CW vs. SW | | | | | | |
| Linear | 0.981 | 0.988 | 0.952 | 0.998 | 0.962 | 0.998 |
| Quadratic | 0.992 | 0.996 | 0.960 | 0.998 | 0.962 | 0.998 |
| Cellulose vs SW | | | | | | |
| Linear | 0.978 | 0.978 | 0.909 | 0.996 | 0.952 | 0.995 |
| Quadratic | 0.998 | 0.996 | 0.926 | 0.997 | 0.952 | 0.995 |
| Hemicellulose vs. SW | | | | | | |
| Linear | 0.845 | 0.983 | 0.885 | 0.995 | 0.969 | 0.996 |
| Quadratic | 0.871 | 0.985 | 0.901 | 0.997 | 0.972 | 1.000 |
| Lignin vs. SW | | | | | | |
| Linear | 0.990 | 0.993 | 0.962 | 0.987 | 0.639 | 0.980 |
| Quadratic | 0.992 | 0.994 | 0.983 | 0.995 | 0.747 | 0.995 |
| Cellulose vs. CW | | | | | | |
| Linear | 0.992 | 0.998 | 0.989 | 0.998 | 0.997 | 0.999 |
| Quadratic | 0.994 | 1.000 | 0.994 | 0.999 | 0.997 | 0.999 |
| Hemicellulose vs. CW | | | | | | |
| Linear | 0.918 | 0.971 | 0.816 | 0.995 | 0.985 | 0.994 |
| Quadratic | 0.919 | 0.990 | 0.888 | 0.998 | 0.986 | 0.997 |
| Lignin vs. CW | | | | | | |
| Linear | 0.996 | 0.997 | 0.978 | 0.989 | 0.753 | 0.984 |
| Quadratic | 1.000 | 0.999 | 0.979 | 0.996 | 0.940 | 0.999 |

justified claims that CW and CW component content varied as a result of changes in stem base dry weight. Thus, an additional study was needed to explain the dynamic nature of the CW matrix and to better understand timing of maximum CW component deposition. One approach used to better conceptualize CW growth focused on CW and CW component concentration on a DW and CW basis.

Concentration data

Cell-wall and CW component concentration were studied to determine whether or not the CW matrix changes on a DW or CW basis as forage stems mature. Changes in CW and CW component concentration were also evaluated as a function of regrowth days to detect general similarities and differences between grass and legume CW growth.

With exception of hemicellulose, significant differences in CW and CW component concentrations (DW and CW basis) were observed for the main species and age effects as well as the species X age interaction in 1987 and 1988 (Tables 11 and 12).

With exception of alfalfa in 1987 and bromegrass in 1988, CW concentration increased and acceptable $R^2$ values were observed for all species as a function of regrowth days (Figures 11 and 12). Poor fit of alfalfa data to the Gompertz function in 1987 may be attributable to its narrow range of plant stages during sampling (Table 3). Rapid

Table 11. Mean squares of 1987 fiber analyses on a dry weight (DW) and cell-wall (CW) basis

| | Cell wall | Cellulose[a] | | Hemicellulose[a] | | Lignin[b] | |
|---|---|---|---|---|---|---|---|
| Source | DW | DW | CW | DW | CW | DW | CW |
| Species | 1237** | 927** | 1671** | 1926** | 5006** | 4963** | 10621** |
| Error a | 13 | 5 | 3 | 1 | 2 | 12 | 24 |
| Age | 339** | 187** | 33** | 7** | 76** | 259** | 352** |
| Species*Age | 50** | 31** | 15** | 5** | 19** | 264** | 51** |
| Error b | 9 | 5 | 4 | 2 | 4 | 10 | 17 |

[a]Values shown have been divided by 100.

[b]Values shown have been divided by 10.

**Significant at the 0.01 level of probability.

Table 12. Mean squares of 1988 fiber analyses on a dry weight (DW) and cell-wall (CW) basis

| | Cell wall | Cellulose[a] | | Hemicellulose[a] | | Lignin[b] | |
|---|---|---|---|---|---|---|---|
| Source | DW | DW | CW | DW | CW | DW | CW |
| Species | 1119** | 250** | 862** | 1224** | 2504** | 1471** | 5007** |
| Error a | 13 | 4 | 20 | 8 | 24 | 16 | 43 |
| Age | 580** | 450** | 198** | 16* | 291** | 256** | 293** |
| Species*Age | 50** | 44** | 38** | 7 | 45** | 34** | 53** |
| Error b | 11 | 7 | 11 | 5 | 10 | 9 | 20 |

[a]Values shown have been divided by 100.

[b]Values shown have been divided by 10.

**,*Significant at the 0.01 and 0.05 levels of probability, respectively.

Figure 11. Cell-wall concentration [LSD (0.05) = 42.3] and change in cell-wall concentration in stem bases of legumes and grasses in 1987. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 714 | 0.103 | 0.0245 | 0.44 |
| Birdsft. trefoil | 681 | 2.048 | 0.1109 | 0.94 |
| Red clover | 561 | 5.486 | 0.1071 | 0.94 |
| Orchardgrass | 594 | 0.252 | 0.0526 | 0.73 |
| Bromegrass | 648 | 22.437 | 0.2379 | 0.81 |
| Switchgrass | 761 | 0.756 | 0.0417 | 0.79 |

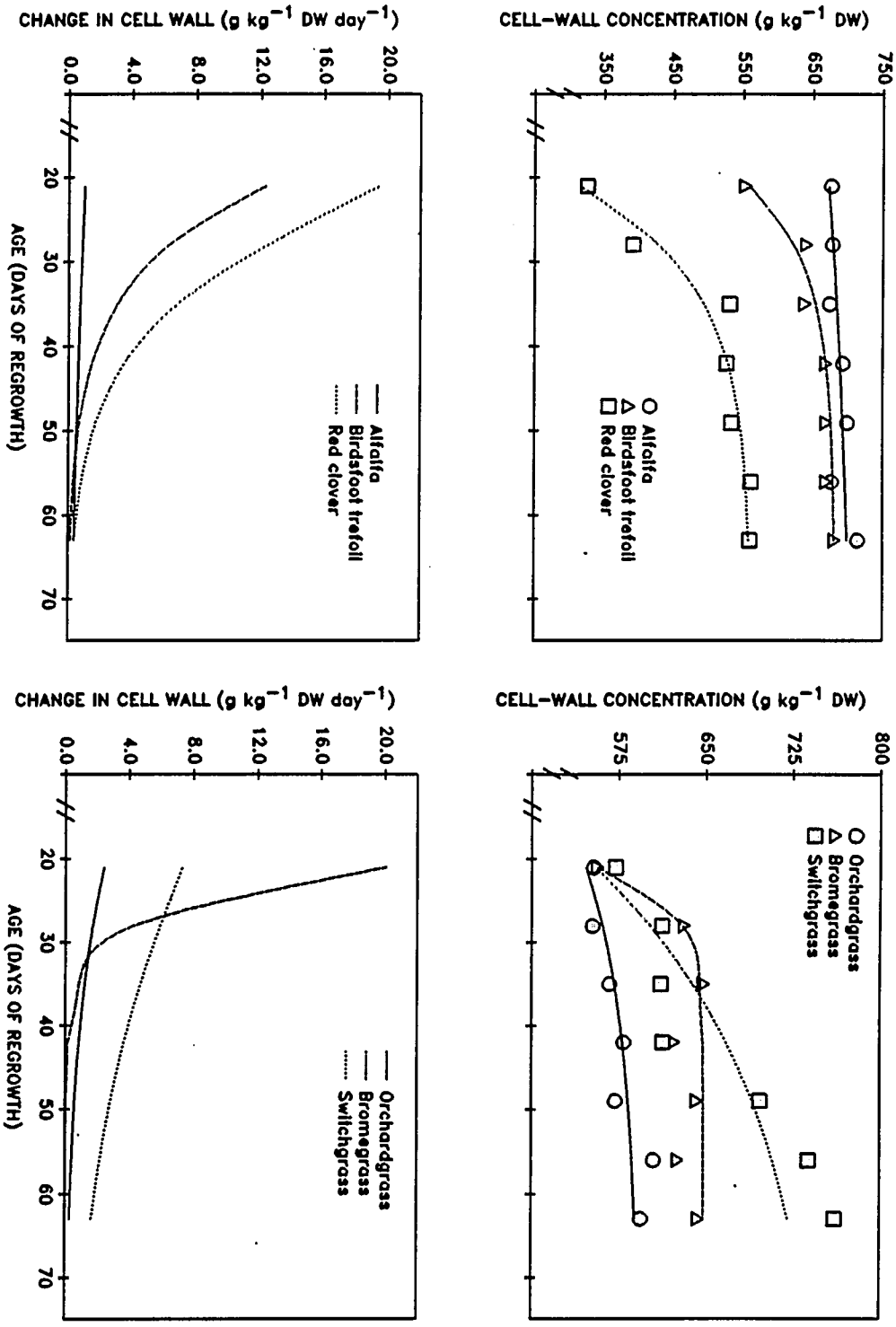
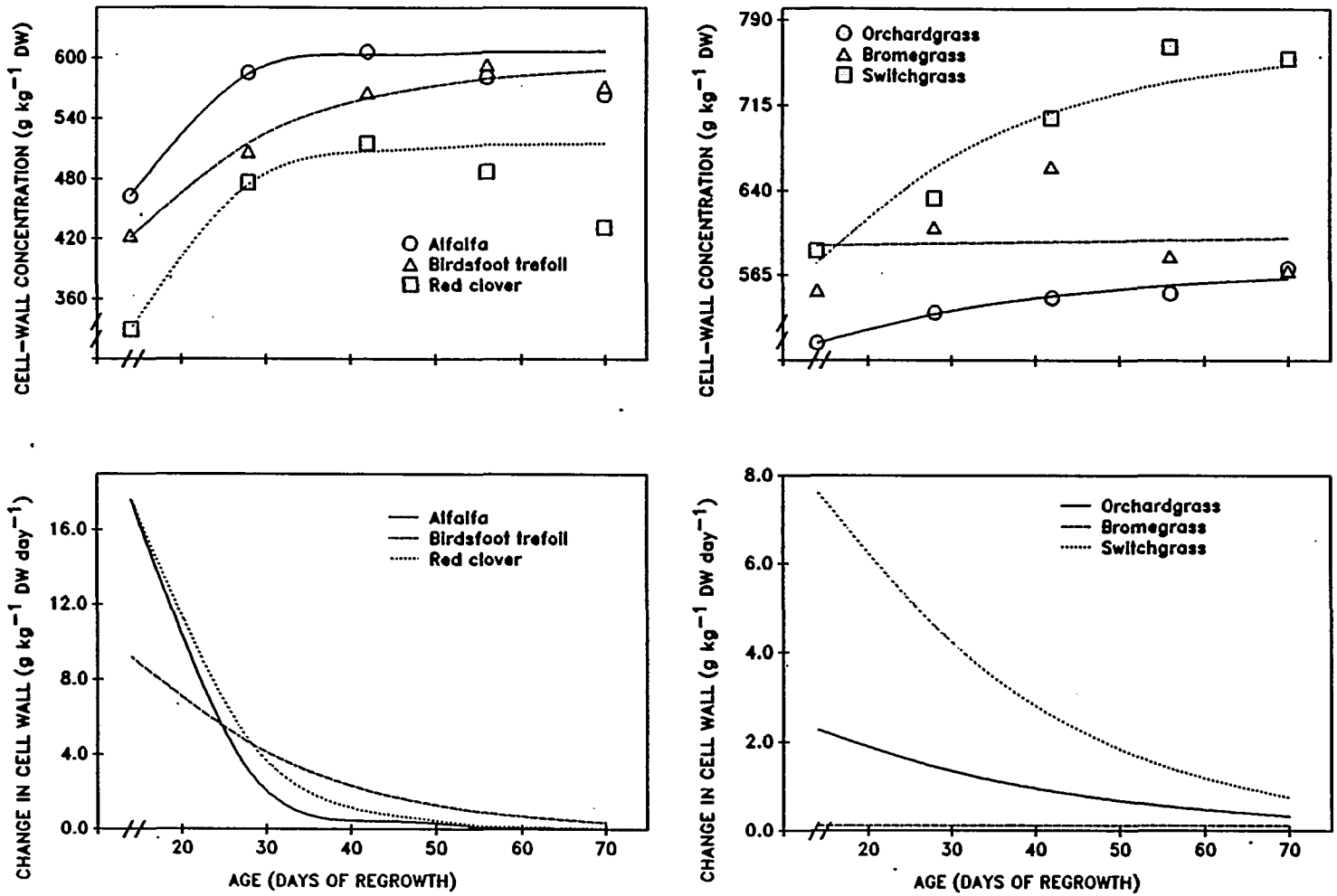

CELL-WALL CONCENTRATION (g kg$^{-1}$ DW)
750
650
550
450
350
Alfalfa
Birdsfoot trefoil
Red clover
CHANGE IN CELL WALL (g kg$^{-1}$ DW day$^{-1}$)
20.0
16.0
12.0
8.0
4.0
0.0
Alfalfa
Birdsfoot trefoil
Red clover
AGE (DAYS OF REGROWTH)
20
30
40
50
60
70
CELL-WALL CONCENTRATION (g kg$^{-1}$ DW)
800
725
650
575
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN CELL WALL (g kg$^{-1}$ DW day$^{-1}$)
20.0
16.0
12.0
8.0
4.0
0.0
Orchardgrass
Bromegrass
Switchgrass
AGE (DAYS OF REGROWTH)
20
30
40
50
60
70

Figure 12. Cell-wall concentration [LSD (0.05) = 47.5] and change in cell-wall concentration in stem bases of legumes and grasses in 1988. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 606 | 1.949 | 0.1414 | 0.81 |
| Birdsft. trefoil | 594 | 0.833 | 0.6360 | 0.97 |
| Red clover | 516 | 2.400 | 0.1202 | 0.64 |
| Orchardgrass | 572 | 0.206 | 0.0364 | 0.94 |
| Bromegrass | 662 | 0.115 | 0.0018 | 0.00 |
| Switchgrass | 767 | 0.548 | 0.0463 | 0.92 |


CELL-WALL CONCENTRATION (g kg$^{-1}$ DW)
600
540
480
420
360
Alfalfa
Birdsfoot trefoil
Red clover
790
715
640
565
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN CELL WALL (g kg$^{-1}$ DW day$^{-1}$)
16.0
12.0
8.0
4.0
0.0
8.0
6.0
4.0
2.0
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

increase in bromegrass CW concentration in 1987 and very poor fit in 1988 may be attributable to uniqueness of bromegrass stem base CWs. Similar results from Buxton and Russell (1988) demonstrated essentially no difference in CW concentration of immature and mature bromegrass stems. Smooth bromegrass may exhibit little or no increase in CW concentration because stem bases can be diluted by storage fructosans (Smith and Nelson, 1985). Derivative plots depicting change in CW concentration with age provided evidence that initial values and rates of change in CW concentration were greater and decreased faster during early regrowth days in legumes compared with grasses. This general trend is especially evident when the anomalous 1987 alfalfa and 1988 bromegrass data are excluded.

Cellulose concentration and its rate of change on a DW (Figures 13 and 14) and CW (Figures 15 and 16) basis followed the same trend with age as did CW concentration. Regression $R^2$ values for the Gompertz fit were usually better for cellulose concentration expressed on a DW basis compared with a CW basis. Alfalfa and orchardgrass in 1987 (Figure 13) and bromegrass in 1988 (Figure 14) demonstrated poor $R^2$ values for cellulose on a DW basis, whereas all legumes and orchardgrass in 1987 (Figure 15) and alfalfa and orchardgrass in 1988 (Figure 16) demonstrated poor $R^2$ values for cellulose on a CW basis. Increases in cellulose concentration and

Figure 13. Dry weight cellulose concentration [LSD (0.05) = 30.7] and change in cellulose concentration in stem bases of legumes and grasses in 1987. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 486 | 0.050 | 0.0051 | 0.02 |
| Birdsft. trefoil | 448 | 0.892 | 0.0744 | 0.95 |
| Red clover | 407 | 3.390 | 0.0848 | 0.92 |
| Orchardgrass | 331 | 0.177 | 0.0309 | 0.54 |
| Bromegrass | 365 | 2.702 | 0.1038 | 0.82 |
| Switchgrass | 426 | 1.102 | 0.0422 | 0.75 |

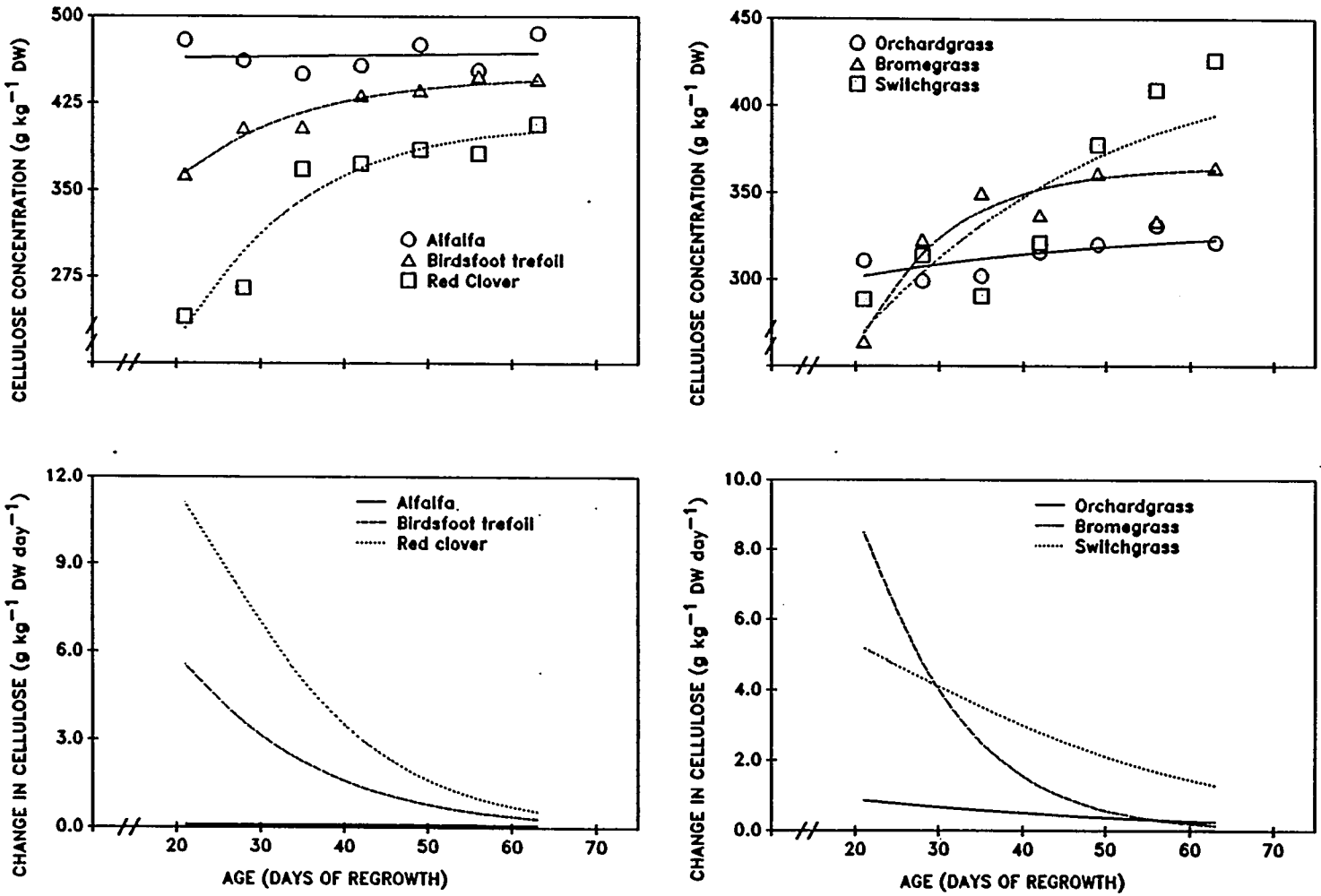

CELLULOSE CONCENTRATION (g kg$^{-1}$ DW)
500
425
350
275
Alfalfa
Birdsfoot trefoil
Red Clover
450
400
350
300
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN CELLULOSE (g kg$^{-1}$ DW day$^{-1}$)
12.0
9.0
6.0
3.0
0.0
Alfalfa
Birdsfoot trefoil
Red clover
10.0
8.0
6.0
4.0
2.0
0.0
Orchardgrass
Bromegrass
Switchgrass
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

Figure 14. Dry weight cellulose concentration [LSD (0.05) = 36.3] and change in cellulose concentration in stem bases of legumes and grasses in 1988. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 405 | 2.116 | 0.1498 | 0.85 |
| Birdsft. trefoil | 402 | 1.599 | 0.0819 | 0.97 |
| Red clover | 386 | 2.512 | 0.0890 | 0.64 |
| Orchardgrass | 315 | 0.195 | 0.0290 | 0.81 |
| Bromegrass | 366 | 0.279 | 0.0149 | 0.16 |
| Switchgrass | 452 | 0.887 | 0.0453 | 0.91 |

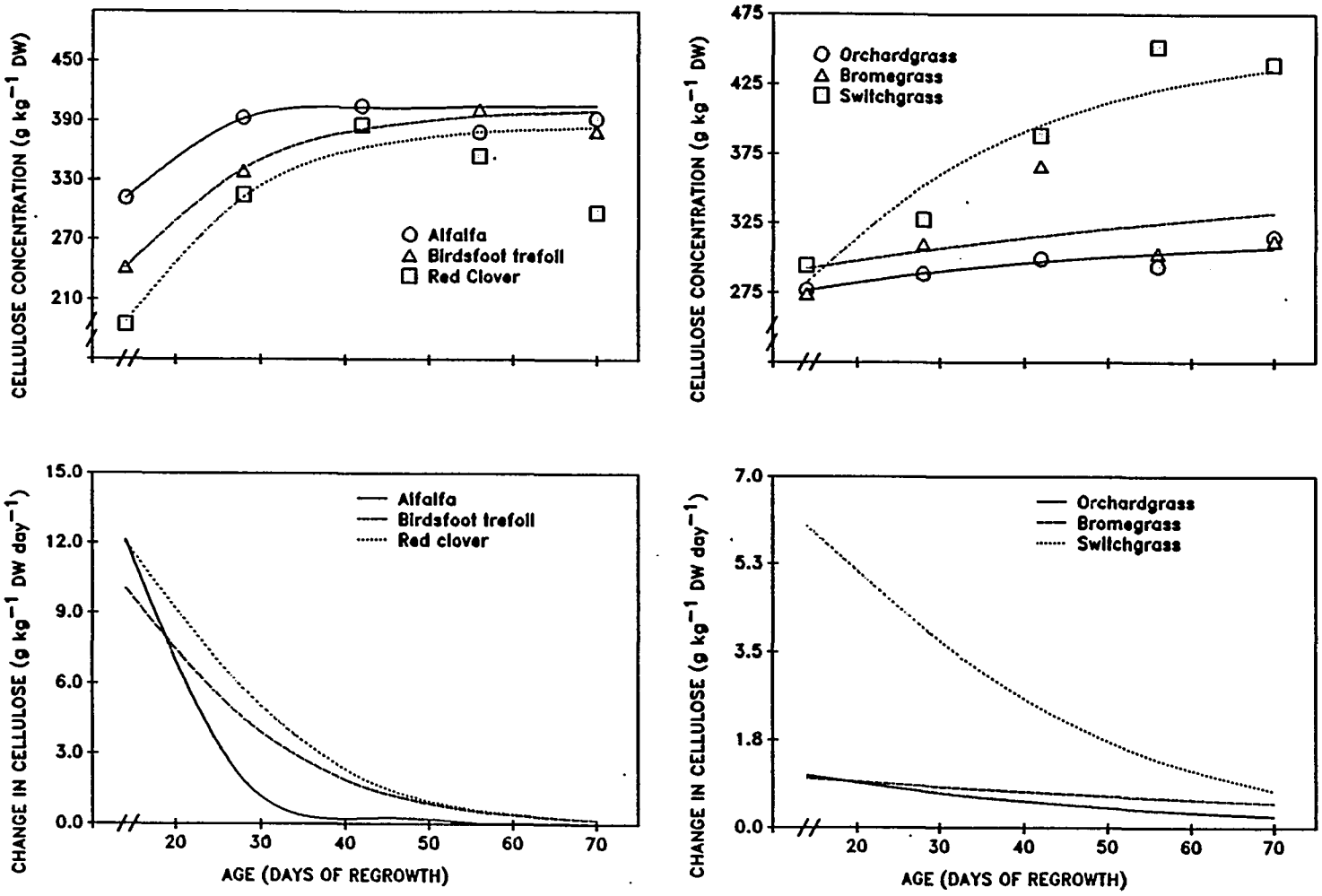

CELLULOSE CONCENTRATION (g kg$^{-1}$ DW)
450
390
330
270
210
Alfalfa
Birdsfoot trefoil
Red Clover
475
425
375
325
275
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN CELLULOSE (g kg$^{-1}$ DW day$^{-1}$)
15.0
12.0
9.0
6.0
3.0
0.0
Alfalfa
Birdsfoot trefoil
Red clover
7.0
5.3
3.5
1.8
0.0
Orchardgrass
Bromegrass
Switchgrass
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

Figure 15. Cell-wall cellulose concentration [LSD (0.05) = 28.3] and change in cellulose concentration in stem bases of legumes and grasses in 1987. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 706 | 0.023 | -0.0125 | 0.16 |
| Birdsft. trefoil | 673 | 0.071 | 0.0172 | 0.21 |
| Red clover | 738 | 0.044 | 0.0007 | 0.00 |
| Orchardgrass | 560 | 0.019 | -0.0058 | 0.03 |
| Bromegrass | 566 | 0.702 | 0.0706 | 0.84 |
| Switchgrass | 559 | 0.284 | 0.0372 | 0.54 |

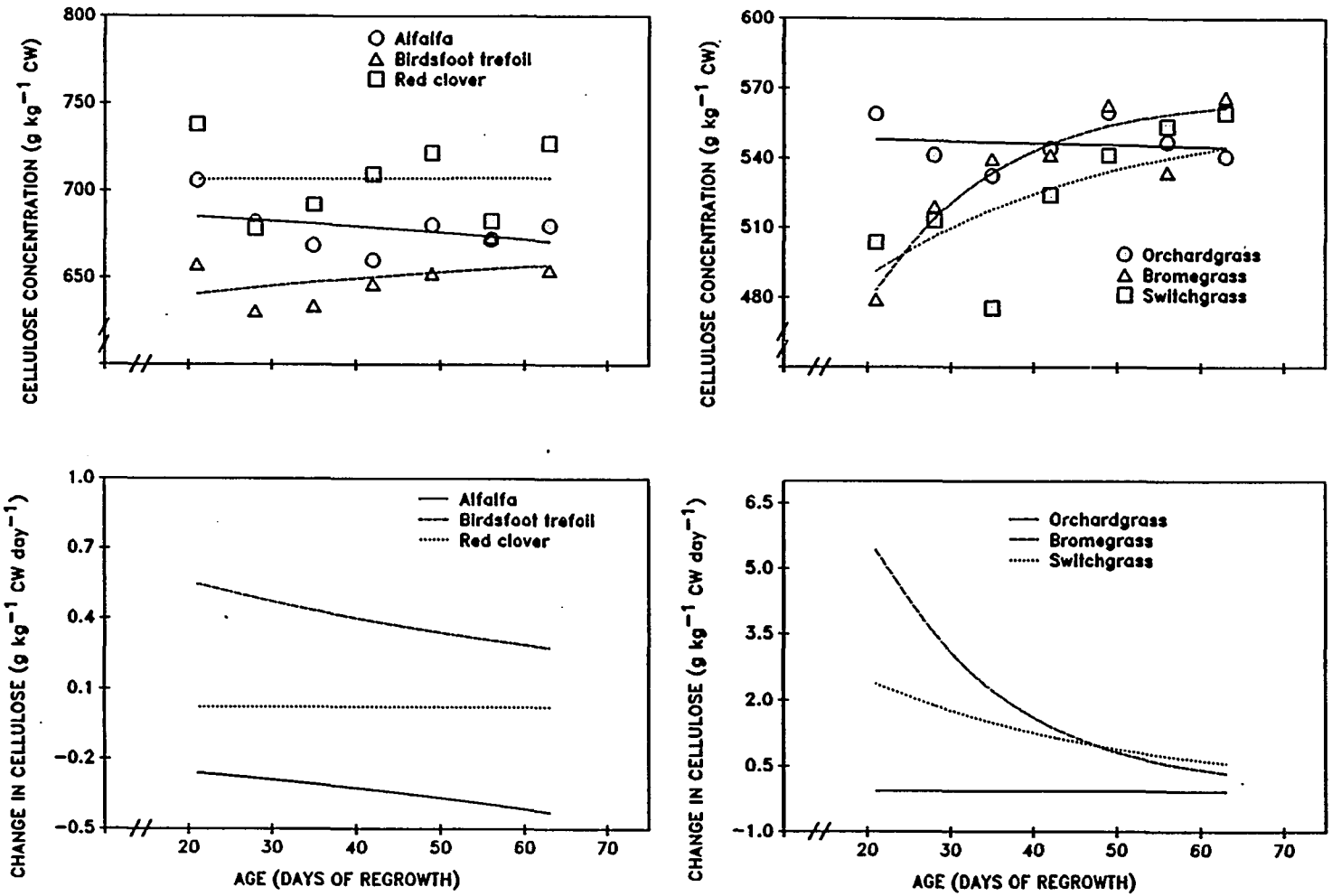
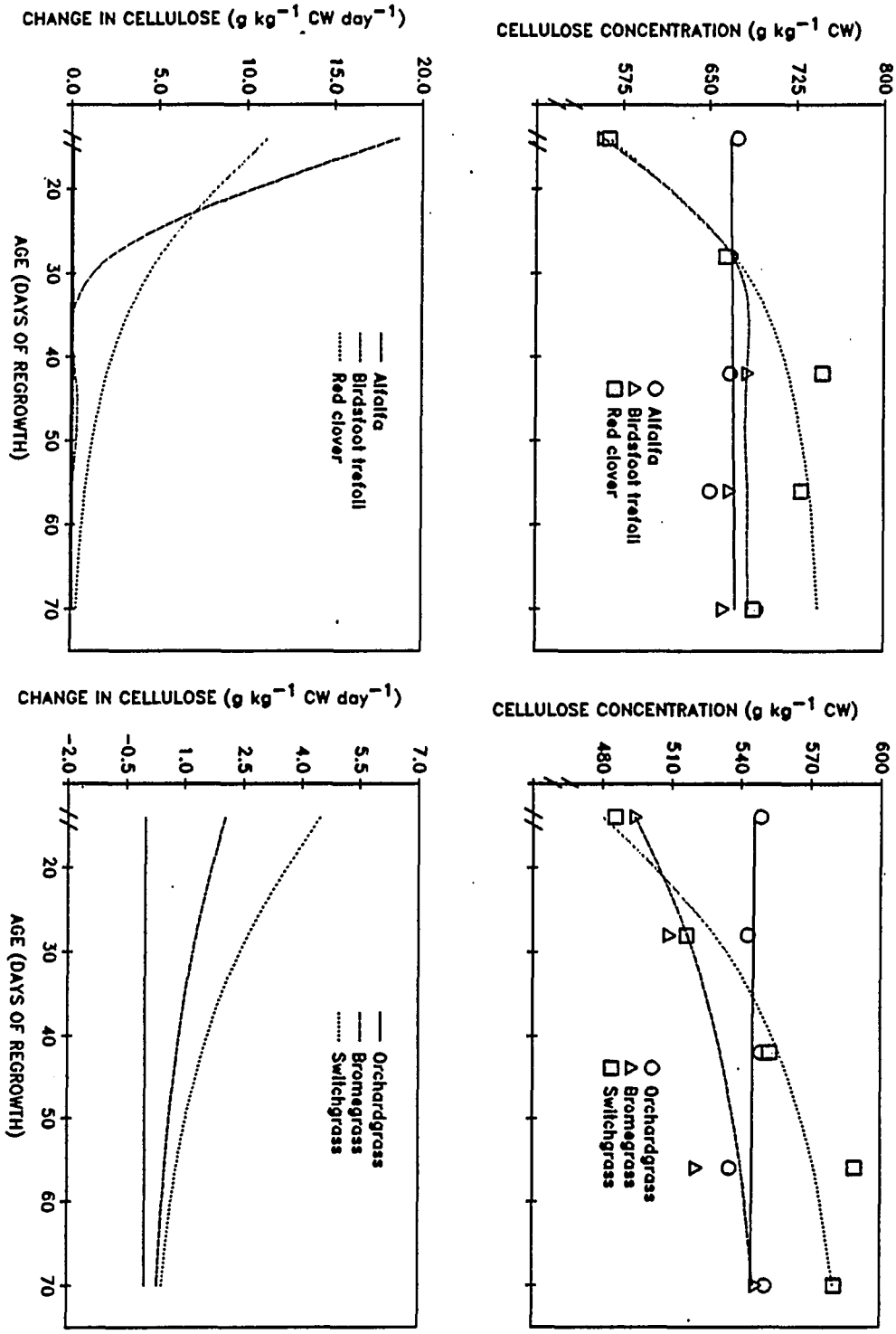

CELLULOSE CONCENTRATION (g kg$^{-1}$ CW)
800
750
700
650
Alfalfa
Birdsfoot trefoil
Red clover
600
570
540
510
480
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN CELLULOSE (g kg$^{-1}$ CW day$^{-1}$)
1.0
0.7
0.4
0.1
−0.2
−0.5
6.5
5.0
3.5
2.0
0.5
−1.0
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

Figure 16. Cell-wall cellulose concentration [LSD (0.05) = 47.5] and change in cellulose concentration in stem bases of legumes and grasses in 1988. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 691 | 0.036 | 0.0044 | 0.03 |
| Birdsft. trefoil | 684 | 2.038 | 0.1646 | 0.94 |
| Red clover | 748 | 0.752 | 0.0700 | 0.81 |
| Orchardgrass | 551 | 0.009 | -0.0032 | 0.01 |
| Bromegrass | 554 | 0.190 | 0.0359 | 0.63 |
| Switchgrass | 589 | 0.386 | 0.0454 | 0.93 |


CELLULOSE CONCENTRATION (g kg$^{-1}$ CW)
800
725
650
575
Alfalfa
Birdsfoot trefoil
Red clover
CHANGE IN CELLULOSE (g kg$^{-1}$ CW day$^{-1}$)
20.0
15.0
10.0
5.0
0.0
Alfalfa
Birdsfoot trefoil
Red clover
AGE (DAYS OF REGROWTH)
20
30
40
50
60
70
CELLULOSE CONCENTRATION (g kg$^{-1}$ CW)
600
570
540
510
480
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN CELLULOSE (g kg$^{-1}$ CW day$^{-1}$)
7.0
5.5
4.0
2.5
1.0
-0.5
-2.0
Orchardgrass
Bromegrass
Switchgrass
AGE (DAYS OF REGROWTH)
20
30
40
50
60
70

changes in concentration were more consistent across years when expressed on a DW basis compared with a CW basis. This may be due to compounded analytical and experimental error that results from expression of cellulose on a CW basis, but it may also reflect conditions where cellulose is increasing in the same proportion as are CWs, as indicated by the 1987 legume and orchardgrass and 1988 alfalfa and orchardgrass data. In such case the Gompertz fit is poor and the derivative of that line is unreliable.

Hemicellulose concentration on a DW (Figures 17 and 18) and CW (Figures 19 and 20) basis showed the most unusual response as a function of regrowth days. Fit of hemicellulose concentration on a DW basis by the Gompertz function was generally acceptable for grasses and poor for legumes both years. On a CW basis, hemicellulose concentration data fit the Gompertz equation poorly and was inconsistent between years for many species. Dry weight and CW hemicellulose concentration increased slightly or decreased in both grasses and legumes. In 1987, negative changes in hemicellulose concentration observed in most species were usually accelerated at later regrowth days although their scale of change was not as great as that encountered in most other CW components. These results suggest that, dilution or transient loss of hemicellulose may occur in the CW matrix as forages mature, especially in

Figure 17. Dry weight hemicellulose concentration [LSD (0.05) = 18.3] and change in hemicellulose concentration in stem bases of legumes and grasses in 1987. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 104 | 0.180 | 0.0354 | 0.12 |
| Birdsft. trefoil | 116 | 0.065 | -0.0168 | 0.19 |
| Red clover | 89 | 1.016 | 0.0532 | 0.33 |
| Orchardgrass | 247 | 0.223 | 0.0329 | 0.54 |
| Bromegrass | 263 | 0.009 | -0.0512 | 0.77 |
| Switchgrass | 286 | 0.693 | 0.0789 | 0.64 |

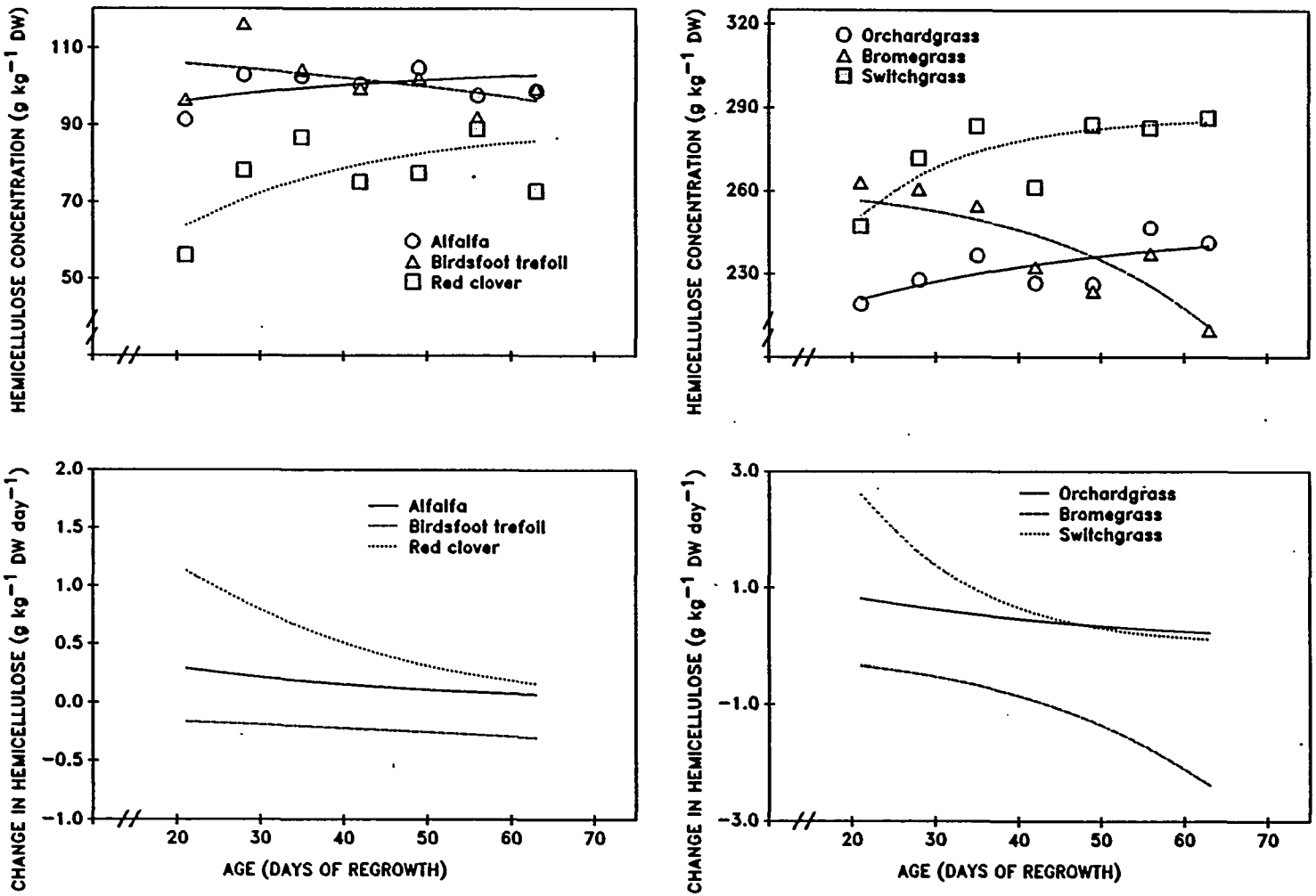

HEMICELLULOSE CONCENTRATION (g kg$^{-1}$ DW)
110
90
70
50
Alfalfa
Birdsfoot trefoil
Red clover
HEMICELLULOSE CONCENTRATION (g kg$^{-1}$ DW)
320
290
260
230
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN HEMICELLULOSE (g kg$^{-1}$ DW day$^{-1}$)
2.0
1.5
1.0
0.5
0.0
−0.5
−1.0
Alfalfa
Birdsfoot trefoil
Red clover
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)
CHANGE IN HEMICELLULOSE (g kg$^{-1}$ DW day$^{-1}$)
3.0
1.0
−1.0
−3.0
Orchardgrass
Bromegrass
Switchgrass
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

Figure 18. Dry weight hemicellulose concentration [LSD (0.05) = 30.3] and change in hemicellulose concentration in stem bases of legumes and grasses in 1988. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 95 | 0.0002 | -0.0981 | 0.57 |
| Birdsft. trefoil | 106 | 0.1251 | -0.0080 | 0.07 |
| Red clover | 116 | 0.0771 | -0.0228 | 0.40 |
| Orchardgrass | 226 | 0.2819 | 0.0494 | 0.95 |
| Bromegrass | 262 | 0.0110 | -0.0445 | 0.91 |
| Switchgrass | 276 | 0.0023 | -0.0597 | 0.92 |

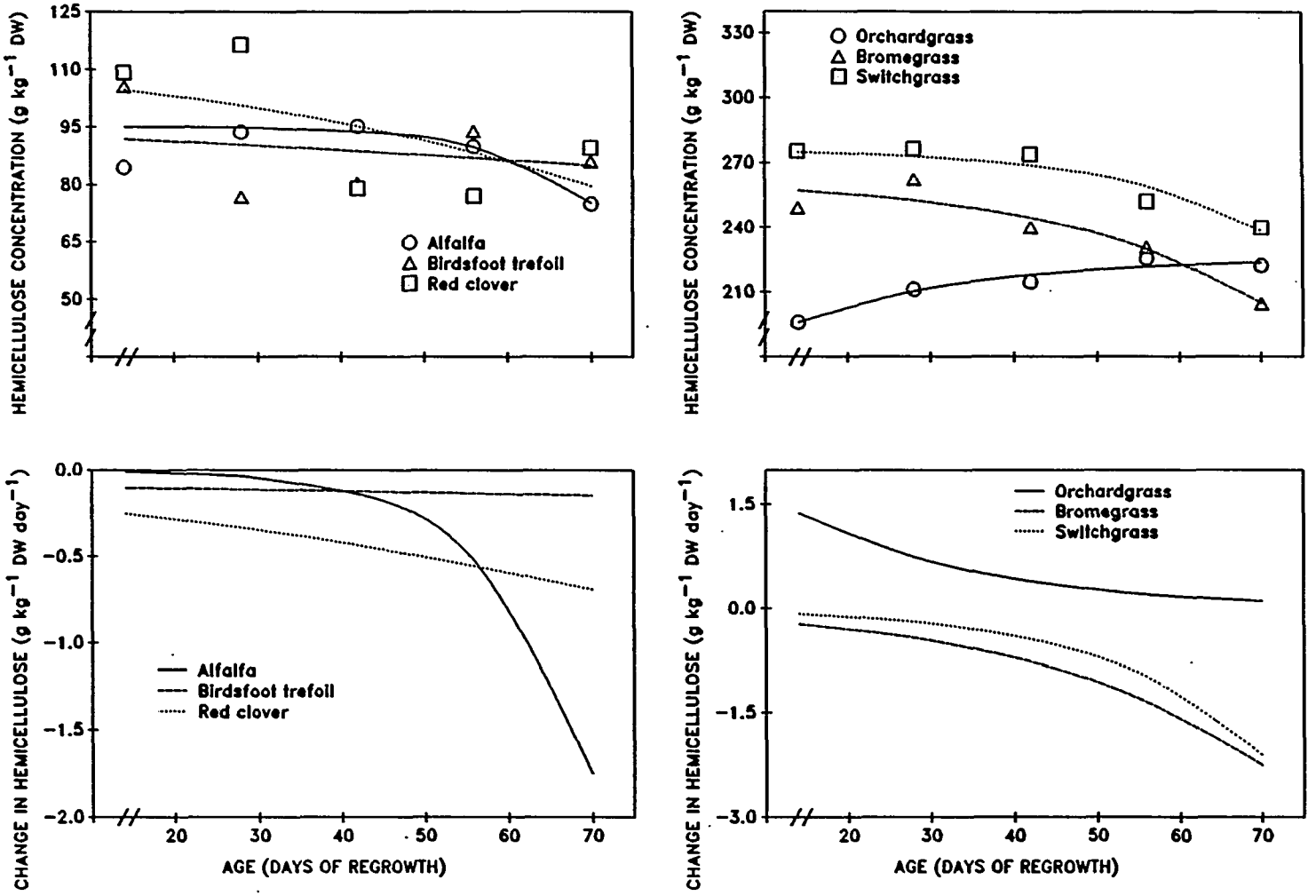

HEMICELLULOSE CONCENTRATION (g kg$^{-1}$ DW)
125
110
95
80
65
50
Alfalfa
Birdsfoot trefoil
Red clover
330
300
270
240
210
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN HEMICELLULOSE (g kg$^{-1}$ DW day$^{-1}$)
0.0
-0.5
-1.0
-1.5
-2.0
1.5
0.0
-1.5
-3.0
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

Figure 19. Cell-wall hemicellulose concentration [LSD (0.05) = 30.0] and change in hemicellulose concentration in stem bases of legumes and grasses in 1987. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 152 | 0.014 | -0.0266 | 0.09 |
| Birdsft. trefoil | 181 | 0.026 | -0.0372 | 0.71 |
| Red clover | 201 | 0.090 | -0.0237 | 0.37 |
| Orchardgrass | 417 | 0.035 | 0.0016 | 0.00 |
| Bromegrass | 476 | 0.044 | -0.0345 | 0.74 |
| Switchgrass | 464 | 0.011 | -0.0481 | 0.80 |

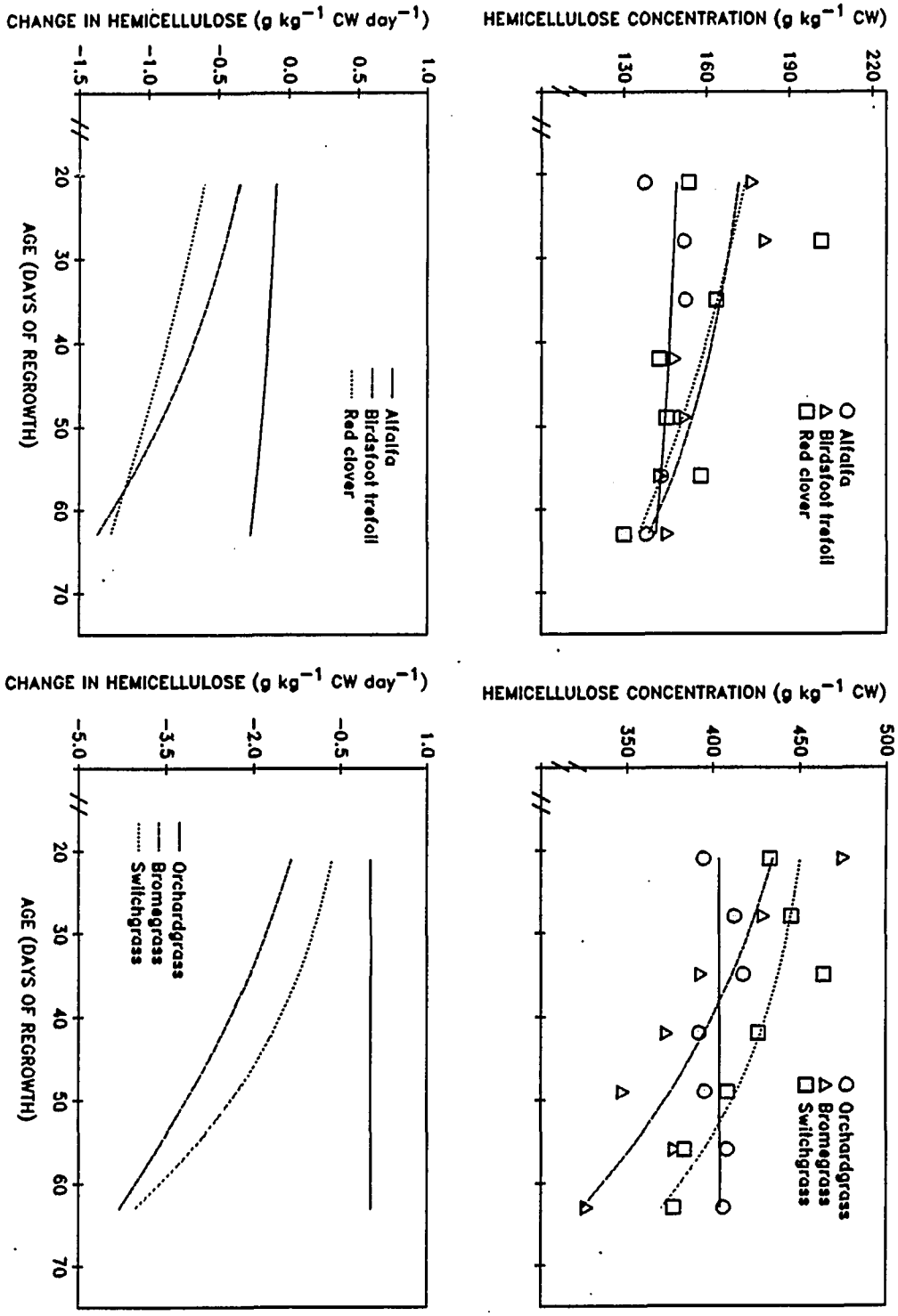
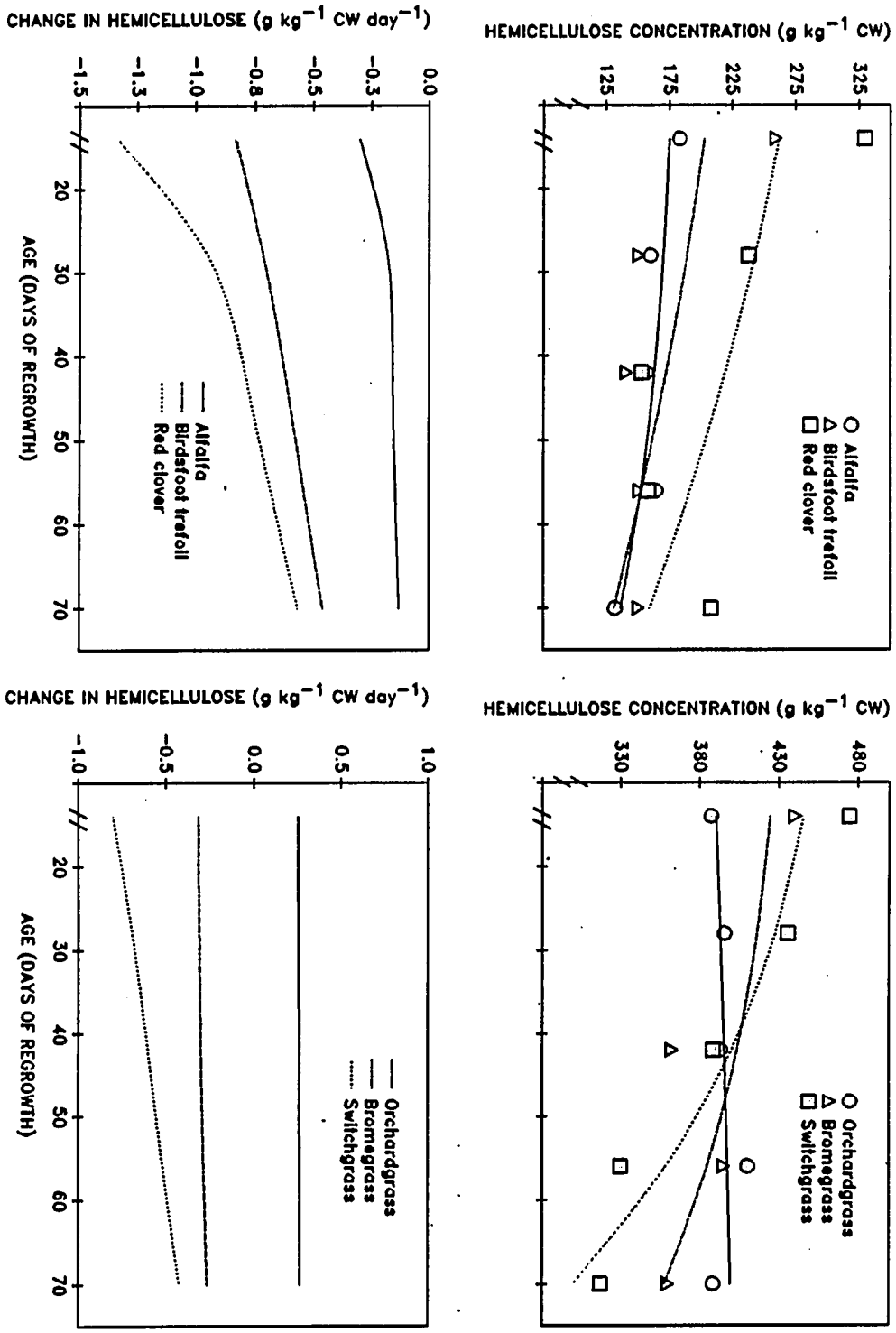

HEMICELLULOSE CONCENTRATION (g kg$^{-1}$ CW)
220
190
160
130
Alfalfa
Birdsfoot trefoil
Red clover
CHANGE IN HEMICELLULOSE (g kg$^{-1}$ CW day$^{-1}$)
1.0
0.5
0.0
-0.5
-1.0
-1.5
Alfalfa
Birdsfoot trefoil
Red clover
AGE (DAYS OF REGROWTH)
20
30
40
50
60
70
HEMICELLULOSE CONCENTRATION (g kg$^{-1}$ CW)
500
450
400
350
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN HEMICELLULOSE (g kg$^{-1}$ CW day$^{-1}$)
1.0
-0.5
-2.0
-3.5
-5.0
Orchardgrass
Bromegrass
Switchgrass
AGE (DAYS OF REGROWTH)
20
30
40
50
60
70

Figure 20. Cell-wall hemicellulose concentration [LSD (0.05) = 43.8] and change in hemicellulose concentration in stem bases of legumes and grasses in 1988. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 183 | 0.029 | -0.0333 | 0.71 |
| Birdsft. trefoil | 260 | 0.191 | -0.0182 | 0.40 |
| Red clover | 329 | 0.173 | -0.0205 | 0.40 |
| Orchardgrass | 411 | 0.060 | 0.0112 | 0.12 |
| Bromegrass | 440 | 0.024 | -0.3111 | 0.59 |
| Switchgrass | 475 | 0.039 | -0.0387 | 0.88 |


HEMICELLULOSE CONCENTRATION (g kg$^{-1}$ CW)
325
275
225
175
125
Alfalfa
Birdsfoot trefoil
Red clover
CHANGE IN HEMICELLULOSE (g kg$^{-1}$ CW day$^{-1}$)
0.0
-0.3
-0.5
-0.8
-1.0
-1.3
-1.5
Alfalfa
Birdsfoot trefoil
Red clover
AGE (DAYS OF REGROWTH)
20
30
40
50
60
70
HEMICELLULOSE CONCENTRATION (g kg$^{-1}$ CW)
480
430
380
330
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN HEMICELLULOSE (g kg$^{-1}$ CW day$^{-1}$)
1.0
0.5
0.0
-0.5
-1.0
Orchardgrass
Bromegrass
Switchgrass
AGE (DAYS OF REGROWTH)
20
30
40
50
60
70

grasses. Redistribution of hemicellulose in the CW matrix is consistent with that suggested by Hatfield (1989), although it is evident from Figures 7 and 8 that hemicellulose content increases with plant mass throughout plant growth.

Lignin concentration on a DW (Figures 21 and 22) and CW (Figures 23 and 24) basis increased hyperbolically with regrowth days in all species except orchardgrass. Orchardgrass lignin concentration on a DW and CW basis had very low $R^2$ values because the concentration of DW and CW lignin in orchardgrass sheaths was essentially constant and the regression equation provided little evidence that lignin concentration varied as a function of regrowth days. For other species except switchgrass in 1987, acceptable $R^2$ values indicated that the Gompertz function provides a reliable description of lignin concentration as a function of regrowth days. These results were similar to, and magnified, that of CW and cellulose concentration and concentration changes because lignin concentration was greater in young legume stems and its change in concentration decreased faster in legumes compared with grasses. These results confirm those implied by Kondo and coworkers (1987) who provided graphs which can be derivatized to show faster initial decreases in changes in lignin concentration of alfalfa compared with that of Italian ryegrass (Lolium multiflorum Lam.). Figures 23 and 24 show that bromegrass and

Figure 21. Dry weight lignin concentration [LSD (0.05) = 13.9] and change in lignin concentration in stem bases of legumes and grasses in 1987. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 135 | 0.885 | 0.0594 | 0.73 |
| Birdsft. trefoil | 140 | 4.908 | 0.1184 | 0.95 |
| Red clover | 90 | 7.220 | 0.0899 | 0.87 |
| Orchardgrass | 36 | 0.388 | 0.0118 | 0.08 |
| Bromegrass | 70 | 3.186 | 0.0558 | 0.90 |
| Switchgrass | 48 | 0.951 | 0.0311 | 0.42 |

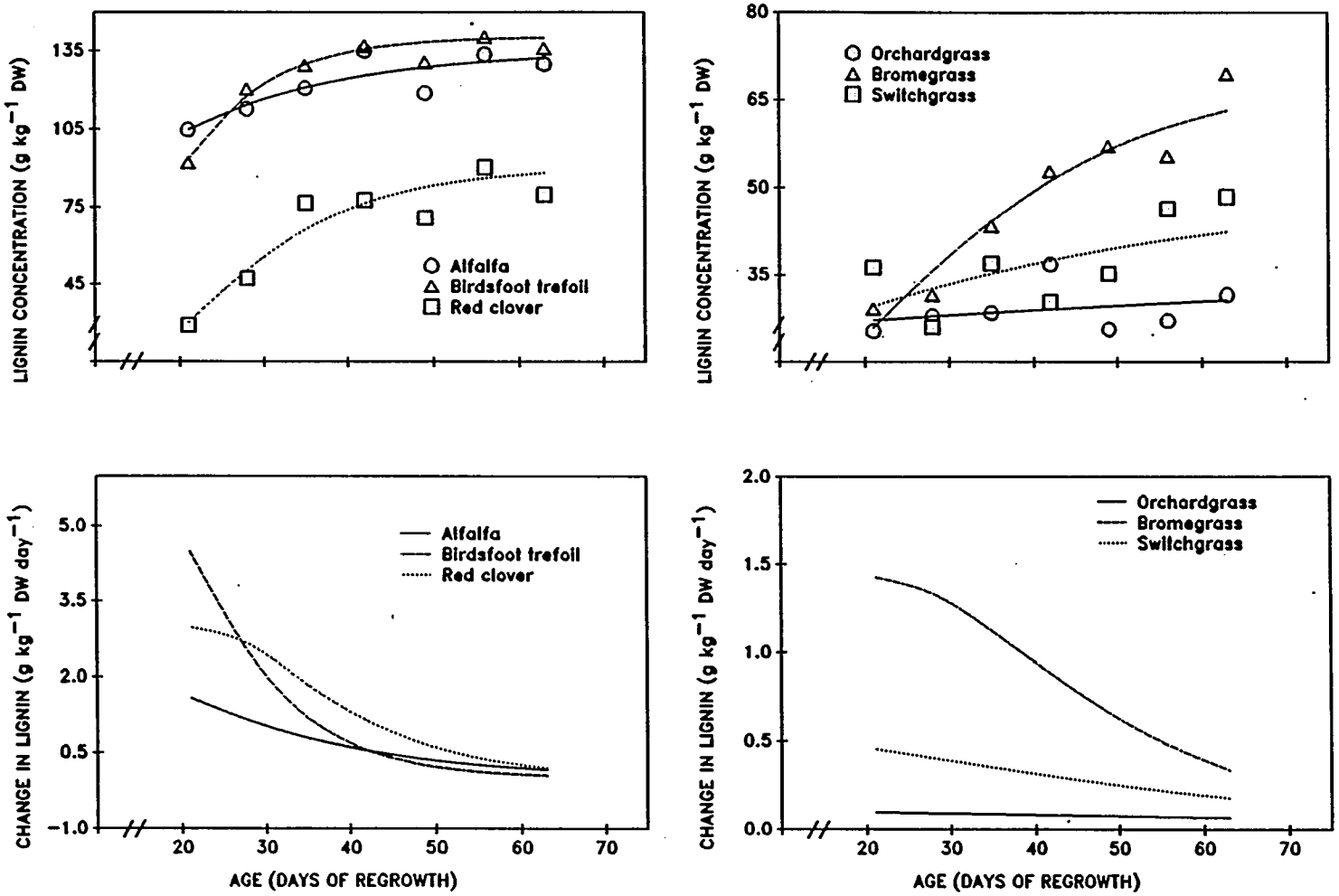

LIGNIN CONCENTRATION (g kg$^{-1}$ DW)
135
105
75
45
Alfalfa
Birdsfoot trefoil
Red clover
80
65
50
35
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN LIGNIN (g kg$^{-1}$ DW day$^{-1}$)
5.0
3.5
2.0
0.5
-1.0
2.0
1.5
1.0
0.5
0.0
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

Figure 22. Dry weight lignin concentration [LSD (0.05) = 13.5] and change in lignin concentration in stem bases of legumes and grasses in 1988. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 106 | 3.308 | 0.1335 | 0.96 |
| Birdsft. trefoil | 106 | 0.792 | 0.0598 | 1.00 |
| Red clover | 55 | 2.632 | 0.0873 | 0.81 |
| Orchardgrass | 34 | 0.080 | 0.0029 | 0.01 |
| Bromegrass | 55 | 1.527 | 0.0564 | 0.83 |
| Switchgrass | 78 | 1.742 | 0.0340 | 0.74 |

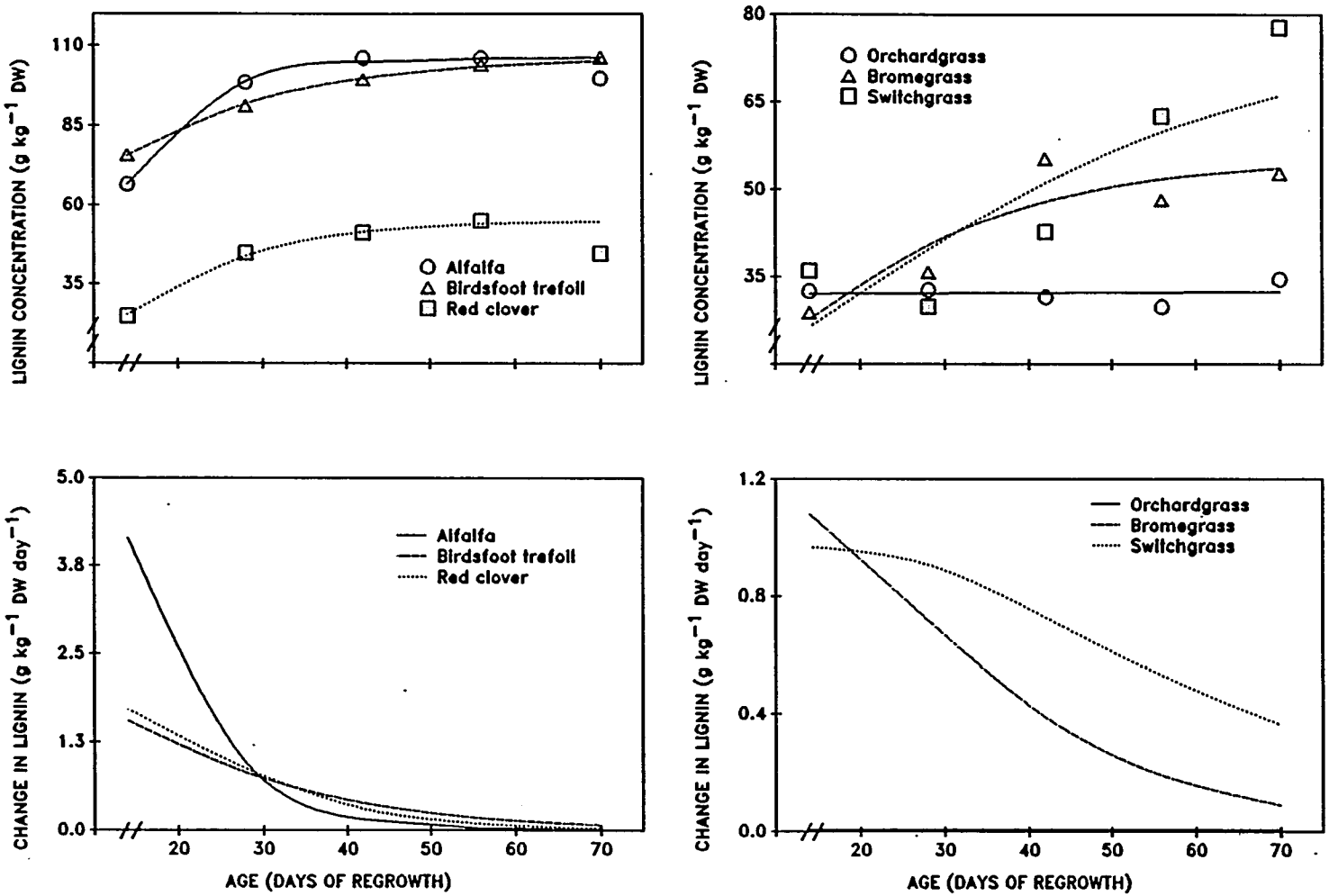

LIGNIN CONCENTRATION (g kg$^{-1}$ DW)
110
85
60
35
Alfalfa
Birdsfoot trefoil
Red clover
80
65
50
35
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN LIGNIN (g kg$^{-1}$ DW day$^{-1}$)
5.0
3.8
2.5
1.3
0.0
1.2
0.8
0.4
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

Figure 23. Cell-wall lignin concentration [LSD (0.05) = 18.11] and change in lignin concentration in stem bases of legumes and grasses in 1987. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 194 | 0.491 | 0.0417 | 0.52 |
| Birdsft. trefoil | 210 | 1.540 | 0.0916 | 0.80 |
| Red clover | 159 | 9.570 | 0.1198 | 0.84 |
| Orchardgrass | 63 | 0.310 | 0.0058 | 0.02 |
| Bromegrass | 107 | 3.303 | 0.0587 | 0.94 |
| Switchgrass | 64 | 0.244 | 0.0153 | 0.05 |

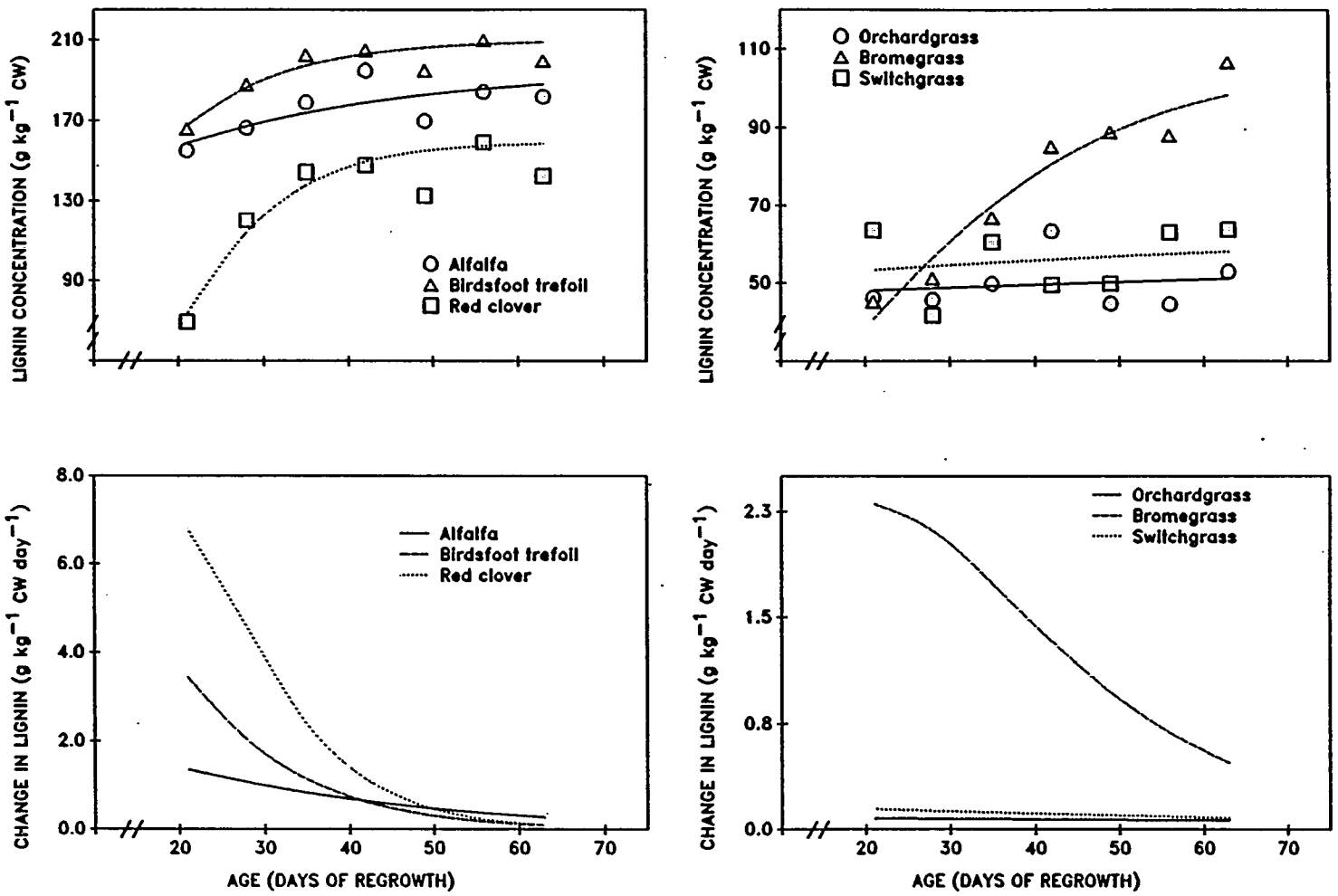
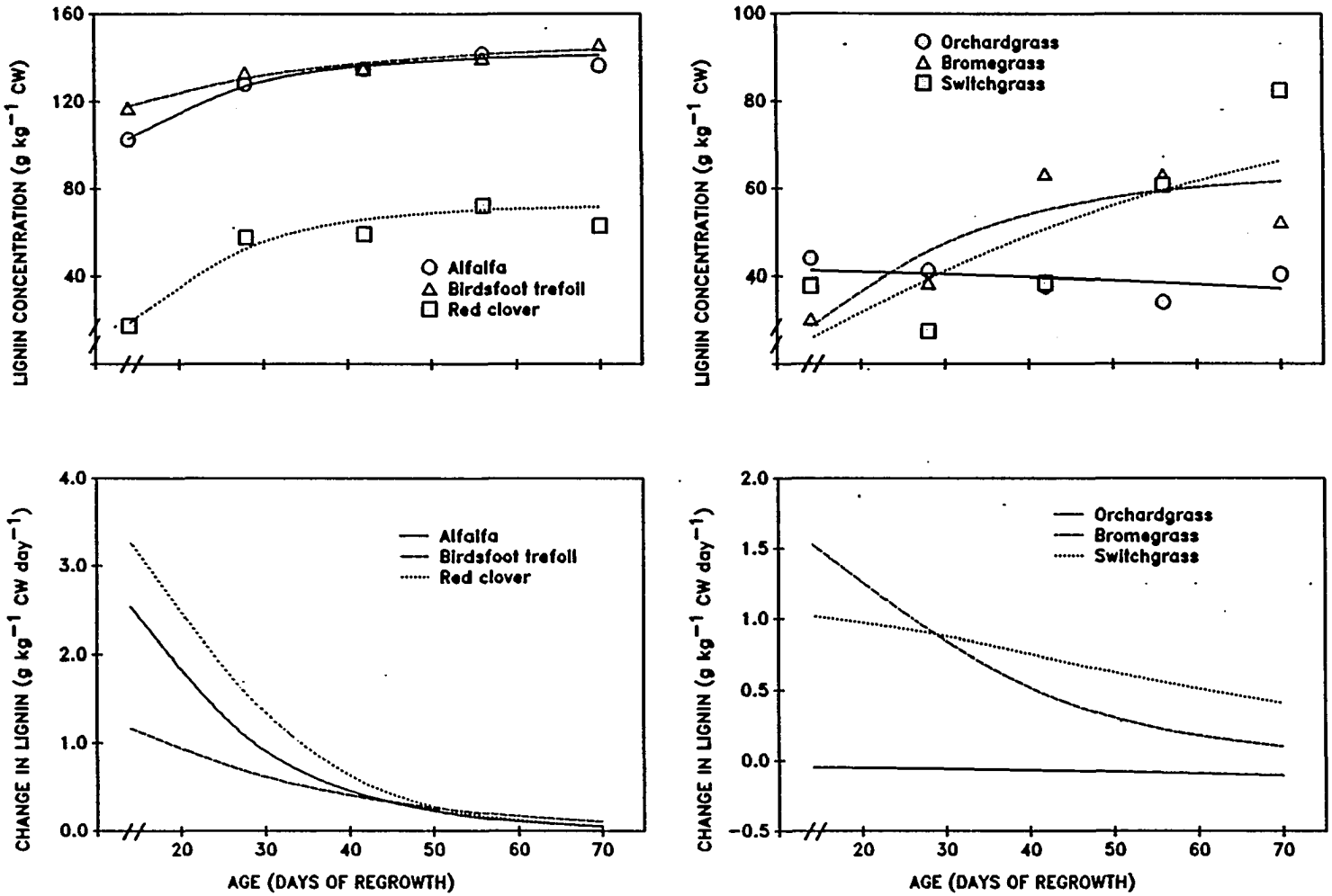

LIGNIN CONCENTRATION (g kg$^{-1}$ CW)
210
170
130
90
Alfalfa
Birdsfoot trefoil
Red clover
110
90
70
50
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN LIGNIN (g kg$^{-1}$ CW day$^{-1}$)
8.0
6.0
4.0
2.0
0.0
2.3
1.5
0.8
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

Figure 24. Cell-wall lignin concentration [LSD (0.05) = 19.9] and change in lignin concentration in stem bases of legumes and grasses in 1988. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 182 | 0.678 | 0.0742 | 0.97 |
| Birdsft. trefoil | 186 | 0.308 | 0.0450 | 0.95 |
| Red clover | 112 | 2.163 | 0.0862 | 0.92 |
| Orchardgrass | 64 | 0.037 | -0.0161 | 0.26 |
| Bromegrass | 83 | 1.223 | 0.0581 | 0.74 |
| Switchgrass | 102 | 1.187 | 0.0277 | 0.64 |


LIGNIN CONCENTRATION (g kg$^{-1}$ CW)
160
120
80
40
Alfalfa
Birdsfoot trefoil
Red clover
100
80
60
40
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN LIGNIN (g kg$^{-1}$ CW day$^{-1}$)
4.0
3.0
2.0
1.0
0.0
2.0
1.5
1.0
0.5
0.0
-0.5
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

switchgrass demonstrate a more persnt vnt value for change in lignin concentration at later regrdays days compared with the rapid decrease in lignin concentratchanchange of legumes. Orchardgrass probably did not demorte tlte this response because only sheaths were produced regi regrowth in this experiment.

## Conclusior

Content data indicated that tl of r of maximum deposition of CW components demonstrates an oiof heof hemicellulose and cellulose followed by lignin. Thiser seer seems particularly true in grasses, although in both ses, ies, net hemicellulose deposition may be influenced by diln frn from other CW components and redistribution in tl matı matrix. Evidence from these results show that relatiffeiifferences in timing of maximum CW component deposition existexist among the species studied.

Concentration of CW and CW comnts cnts differed among grasses and legumes. Legumes ofteronstionstrated greater and faster decreases in CW and lignin cntraintration changes at early days of regrowth compared witassesasses. This indicates that CW and lignin concentrations cgume gume stem bases quickly decline to a minimum value,reas reas those of grass stem bases undergo a more gradual cne wine with plant age. Concentration data also indicated themichemicellulose may be

diluted by other CW components or may experience transient loss in the CW matrix of stem bases as forages mature.

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# SECTION II. PHENYLALANINE AMMONIA LYASE ACTIVITY IN RELATION TO CHANGE IN LIGNIN CONCENTRATION OF FORAGE STEMS

## Abstract

Forage quality can be improved by decreasing cell wall (CW) and lignin concentration in stem tissue. Potential for improved forage quality might be possible through molecular manipulations that decrease activities of enzymes involved in lignification. Phenylalanine ammonia lyase (PAL) activity and change in CW and lignin concentration were studied in this investigation to better understand relationships among enzyme activity, CW growth, and lignification in maturing forage stems. Six forage species, including alfalfa (*Medicago sativa* L.), birdsfoot trefoil (*Lotus corniculatus* L.), red clover (*Trifolium pratense* L.), orchardgrass (*Dactylis glomerata* L.), smooth bromegrass (*Bromus inermis* Leyss.), and switchgrass (*Panicum virgatum* L.), were established in a greenhouse, and regrowth herbage was sampled at 14, 28, 42, 56, and 70 days. Sheaths of orchardgrass or stems from the other five species from the basal 10 cm of regrowth were subdivided and either dried for sequential fiber analysis or frozen in liquid nitrogen for spectrophotometric PAL assays. Cell wall and lignin

concentrations increased in all species with plant age. First derivative plots indicated that changes in legume CW and lignin concentration were often greater and decreased faster compared with grasses at early days of regrowth. In all species, decreasing lignin concentration changes showed a parallel relationship to decreasing PAL activity. Close relationships between PAL activity and change in lignin concentration were consistent within species. Changes in lignin concentration were overestimated by PAL, indicating that the enzyme is involved with additional metabolic reactions. On a per pot basis, graphical representations of PAL total units resembled lignin deposition as a function of regrowth days. These results indicate potential for manipulating forage quality through PAL, but only with concomitant changes in other phenylpropanoid reactions.

## Introduction

Ruminant intake potential is best indicated by CW concentration of forages (Van Soest and Robertson, 1980; Waldo, 1985). Of CW components, lignin is usually the major factor identified for the lowering of digestibility of forage tissues as they mature (Harkin, 1973; Buxton et al., 1985; Buxton and Russell, 1988; Jung, 1989). Differences that exist between grass and legume lignin concentration and chemical variability as tissues develop are thought to be

responsible for variation in forage quality (Buxton and Russell, 1988). Natural variation, which exists in lignin concentration and content of plants (Grand et al., 1982), has provided incentive for studies on inhibition of lignification through chemical applicants (Buck et al., 1989). Some inhibitors, such as N(O-aminophenyl)sulfinamoyl tertiobutyl acetate (Boudet and Grand, 1987) and O-benzylhydroxylamine (Hoagland, 1985) have decreased PAL activity and lignin concentration in plant tissues.

Selective breeding for decreased CW concentration (Kephart, et al., 1990) and increased in vitro digestible dry matter has been successful (Casler and Carpenter, 1989; Kephart et al., 1990). These breeding efforts may be accelerated by specific selection and alteration of enzymes leading to reduced lignification. Among enzymes in lignin biosynthesis, PAL has been characterized in greatest detail (Koukol and Conn, 1961; Havir and Hanson, 1968; Tanaka et al., 1989) and has potential for molecular manipulation (Fritzemeier et al., 1987). From studies of PAL and other lignin-related enzymes, molecular biologists and plant breeders will be able to direct efforts toward efficient means for improving forage quality.

Change in lignin concentration was studied in this investigation in relation to PAL activity in stem or sheath bases of grasses and legumes. The purpose of this experiment

was to determine whether or not lignification parallels PAL activity in developing forages and to study variation in CW and lignin concentration and PAL activity among grass and legume species.

## Materials and Methods

Three legume species studied included 'Arrow' alfalfa, 'Viking' birdsfoot trefoil, 'Arlington' red clover, and three grass species studied included 'Napier' orchardgrass, 'Barton' smooth bromegrass, and 'Trailblazer' switchgrass. These species were established in a greenhouse in 25-cm diameter pots with a capacity of 3.8 L. Pots were arranged in a randomized complete block design of the six species in four replicates. A split-plot arrangement was employed with species as the whole plot and sample age as the subplot.

### Sampling

Regrowth was sampled from two or three pots within each replicate at 14, 28, 42, 56, and 70 days of regrowth during September through December. Greenhouse temperatures ranged from 20 to 37°C and high pressure sodium lamps supplemented sunlight to provide a 14 h day and 10 h night. Stages of plant development for alfalfa and birdsfoot trefoil (Hedlund and Höglund, 1983), red clover (Ohlsson and Wedin, 1989), and grasses (Simon and Park, 1981), as well as plant height from soil level, were recorded 1 or 2 days prior to sampling.

Plants were cut at 3 to 5 cm above soil level and the basal 10 cm of stem material, or sheaths of orchardgrass, was weighed and subdivided for fiber and enzyme analyses.

Exactly 5.0 g of fresh weight material was put into liquid nitrogen for PAL analysis and remaining stem or sheath material was put into a paper bag for fiber analysis. After harvesting each replicate, materials were taken into a lab for enzyme extraction and drying of fiber materials.

Enzyme extraction and assay

Approximately 200 mL of dry $CO_2$-cooled acetone was mixed with 5.0 g of frozen stem or sheath material in a blender for 2 min. The mixture was transferred with an additional 50 mL of cooled acetone to a square jar and ground with a homogenizer for 3 min. Büchner filtration of the resulting mixture yielded an acetone powder, which was rinsed with 50 mL of cooled acetone to reveal a light green product. After air-drying for 1 h, the powder was stored in an ultra-low freezer at -100°C.

Complete replicates were removed from the freezer and 0.025 g of acetone powder from individual samples was mixed with 25 mL of 100 m*M* borate buffer, pH 8.7, at 0°C. Suspensions were shaken at least once every 5 min for 1 h and then filtered to give a clear, crude, enzyme extract. These extracts were assumed to contain PAL of endoplasmic reticulum origin and known to be associated with lignin biosynthesis

(Wagner and Hrazdina, 1984). Protein concentration (Bradford, 1976) and enzyme activity (Abell and Shen, 1988) were obtained from duplicate runs of each sample.

Protein concentration according to Bradford (1976) was obtained directly from stem or sheath extract and PAL was assayed spectrophotometrically from a mixture of 1.5 mL of extract and 4.5 mL of 6.68 m$\underline{M}$ phenylalanine in borate buffer over a period of 1 h at 30°C. Concentration of phenylalanine in the assay mixture was 5.01 m$\underline{M}$. Enzyme activity was obtained by calculating production of cinnamic acid (CA) ($\epsilon$ = 9000) and expressed on a protein, dry weight (DW), or per pot basis. Suitability of this procedure has been outlined for crop species (Saunders and McClure, 1974).

Fiber analysis

Material for fiber analysis was dried at 55°C for 48 h. Dried samples were weighed and ground to pass through a 1 mm screen of a Udy Mill. Sequential analysis according to Van Soest and Robertson (1980), with an amylase modification (Sigma No. A-1278), was used to determine CW and lignin components in the ground stem or sheath samples. Neutral detergent fiber was used as an estimate of CW concentration.

Graphical representation and statistical analysis

Dry weight, protein, CW, lignin, and PAL were expressed on a yield (per pot), concentration (g kg $DW^{-1}$ and g kg $CW^{-1}$), or activity ($\mu$mol g $protein^{-1}$ $h^{-1}$ and $\mu$mol g $DW^{-1}$

$h^{-1}$) basis. Graphical representation of components as a function of regrowth days was performed by fitting data to the Gompertz function, y = a*exp[(-b)*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 computer program and values for a were entered as the highest number from data within each species. Change in concentration graphs were constructed from the first derivative of the Gompertz function with respect to regrowth days, dy/dt = abc*exp(-ct)*exp[(-b)* exp(-ct)]. Protein and PAL were graphed as a function of regrowth days by using quadratic equations with coefficients estimated by the computer program.

Data analyses for mean squares and curve fitting were performed by PROC GLM and PROC NLIN, respectively (SAS Institute, 1985). Nonlinear regression $R^2$ values for Gompertz and quadratic equations 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

All components measured differed significantly for the species X age interaction (Table 1). Differences were encountered for species and age among all components measured

Table 1. Mean squares from analysis of variance of dry weight (DW), cell wall (CW), lignin (DW and CW basis), and PAL activity (protein and DW basis) of stems of greenhouse-grown forages

| Source | Dry weight (per pot) | Cell wall[a] (DW) | Protein (DW) | Lignin[b] (DW) | Lignin[b] (CW) | PAL activity (protein[a]) | PAL activity (DW) |
|---|---|---|---|---|---|---|---|
| Species | 105** | 1119** | 114 | 1471** | 5007** | 1480** | 166** |
| Error a | 2 | 13 | 72 | 16 | 43 | 56 | 5 |
| Age | 54** | 580** | 10 | 256** | 293** | 87** | 20** |
| Species X Age | 5** | 50** | 126* | 34** | 52** | 38** | 15** |
| Error b | 1 | 11 | 67 | 9 | 20 | 12 | 3 |

[a]Values shown in table have been divided by 100.

[b]Values shown in table have been divided by 10.

**,*Significant at the 0.01 and 0.05 levels of probability, respectively.

except for protein concentration. Trends in protein concentration varied among species because plant development (Table 2) and stem elongation, as indicated by height (Table 3), occurred at different times for different species.

Gompertz fitting of DW data allowed smoothing of the data with respect to plant mass as a function of regrowth days even though fluctuation occurred (Figure 1). Fittings were viewed as "acceptable" in these analyses and those that follow when at least 50% (one-half) of the data variability could be attributed to regrowth days. For instance, in Figure 1, acceptable fittings were observed because $R^2$ values indicated that 71 to 96% of the variability in stem or sheath base dry weight could be attributed to regrowth days. Grass DW and DW deposition was greater than that of legumes because more stem or sheath base material was available and new grass tillers increased sample mass. It is evident from these graphs that stem or sheath base DW increases hyperbolically with age. Coincident with increased plant mass and development, CW and CW component contents usually increased, although CW and CW component concentrations on a DW or CW basis were assumed to vary with the dynamic nature of the CW matrix (Hatfield, 1989).

Cell wall concentration in dry matter increased hyperbolically with regrowth days for all species except smooth bromegrass (Figure 2). Other studies with smooth

Table 2. Stages of plant development of potted forage species grown in the greenhouse in 1988

| Regrowth day | Species | | | | | |
|---|---|---|---|---|---|---|
| | Alfalfa | Birdsfoot trefoil | Red clover | Orchard-grass | Brome-grass | Switch-grass |
| | maturity index | | | | | |
| 14 | 37[a] | 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 |

[a]Maturities were 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. Inflorescence visible (legumes) or inflorescence emergence (grasses) is estimated as 50 in all schemes.

Table 3. Plant height per pot for greenhouse-grown forage species in 1988

| Regrowth day | Alfalfa | Birdsfoot trefoil | Red clover | Orchard-grass | Brome-grass | Switch-grass |
|---|---|---|---|---|---|---|
| | Species | | | | | |
| | mm | | | | | |
| 14 | 410[a] | 162 | 170 | 295 | 198 | 185 |
| 28 | 962 | 332 | 420 | 428 | 302 | 392 |
| 42 | 1052 | 392 | 470 | 502 | 415 | 495 |
| 56 | 1112 | 462 | 528 | 565 | 470 | 828 |
| 70 | 1070 | 565 | 598 | 642 | 530 | 760 |

[a]LSD for species X regrowth period equals 67 at P = 0.05.

Figure 1. Stem base dry weight (DW) [LSD (0.05) = 1.56] and stem base DW deposition legumes and grasses. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 3.50 | 1.80 | 0.0710 | 0.96 |
| Birdsft. trefoil | 3.78 | 2.76 | 0.0394 | 0.88 |
| Red clover | 2.08 | 1.81 | 0.0319 | 0.71 |
| Orchardgrass | 11.94 | 1.63 | 0.0337 | 0.74 |
| Bromegrass | 3.85 | 12.06 | 0.0943 | 0.91 |
| Switchgrass | 6.63 | 7.71 | 0.0551 | 0.91 |

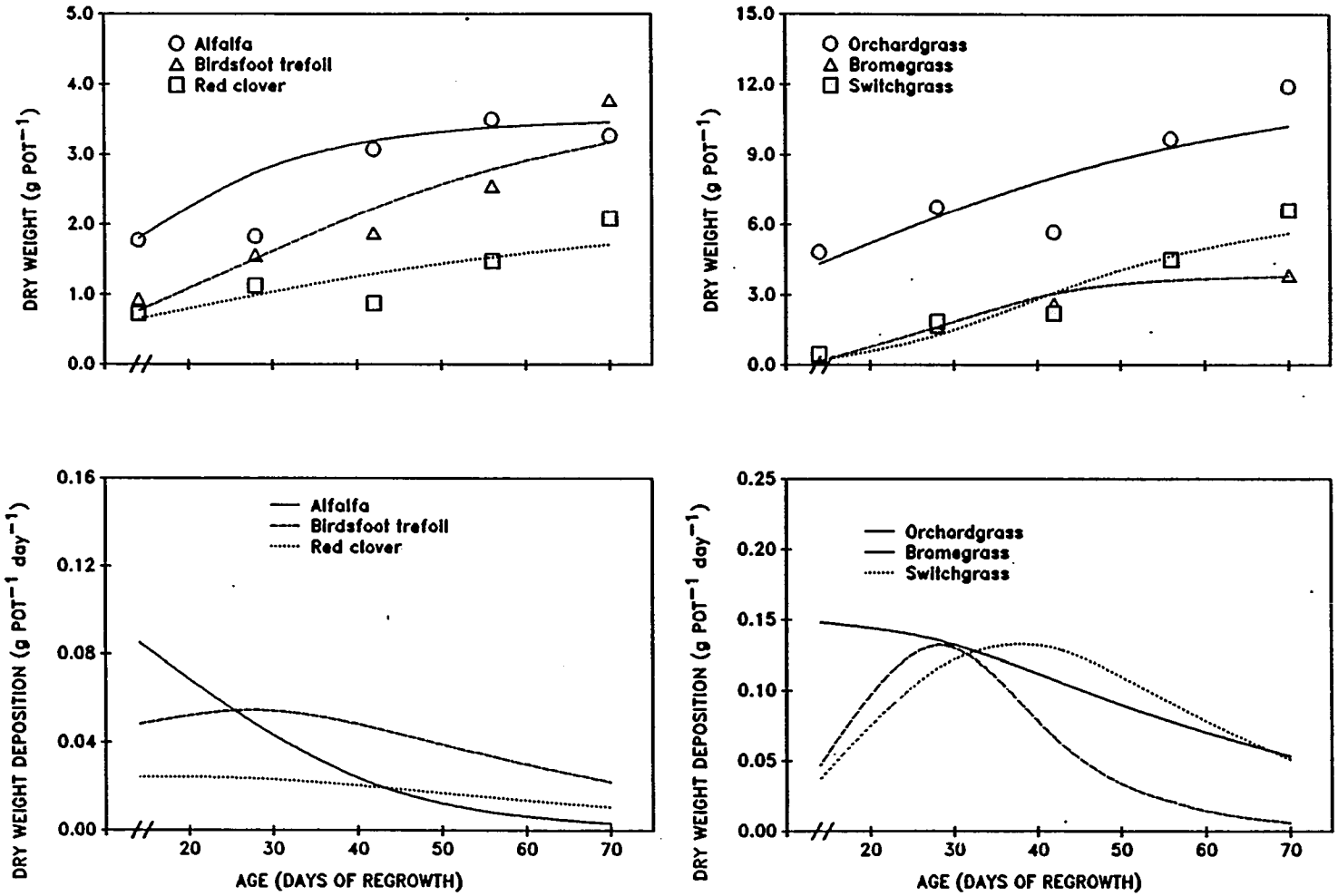
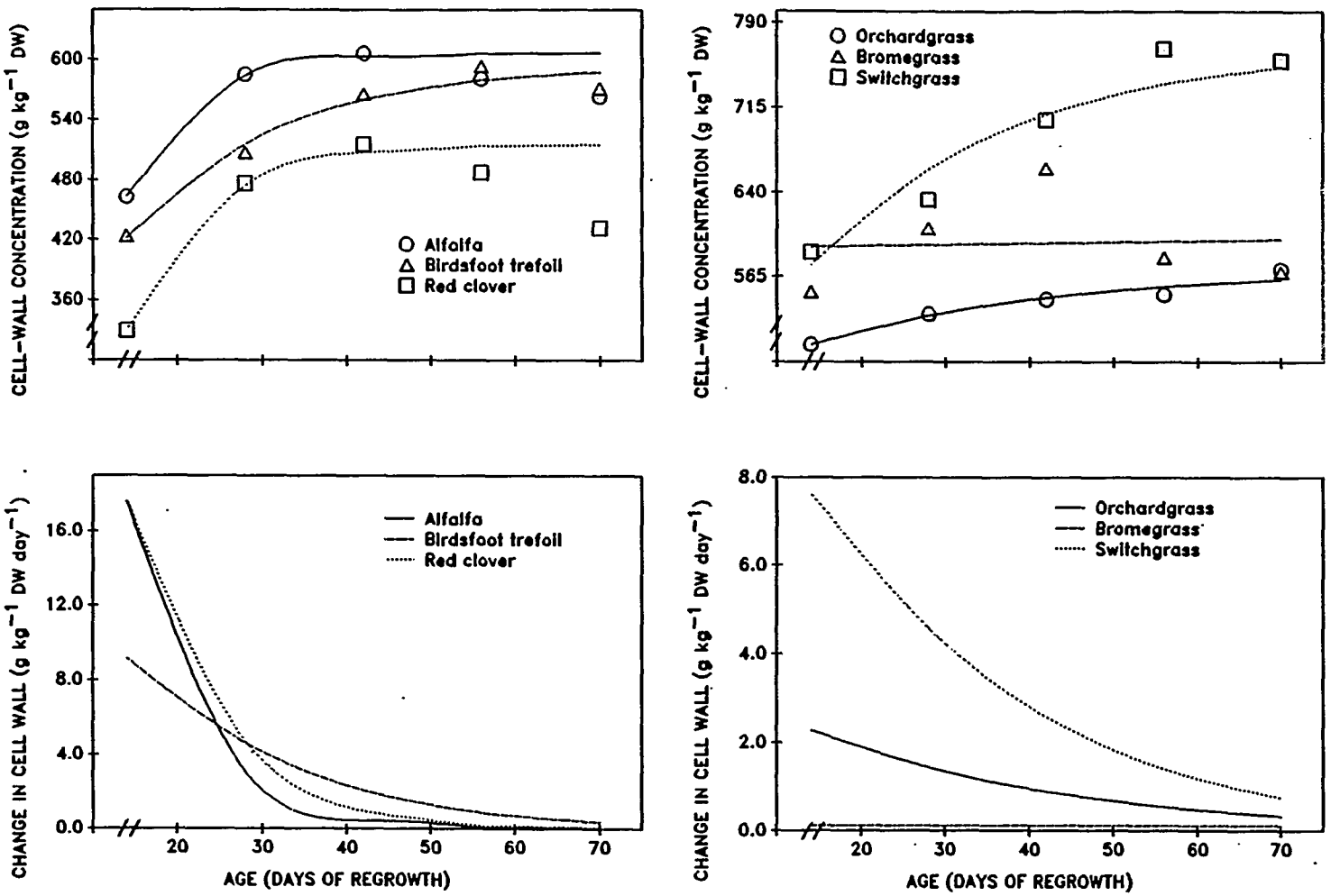

Alfalfa
Birdsfoot trefoil
Red clover
DRY WEIGHT (g POT$^{-1}$)
5.0
4.0
3.0
2.0
1.0
0.0
Orchardgrass
Bromegrass
Switchgrass
DRY WEIGHT (g POT$^{-1}$)
15.0
12.0
9.0
6.0
3.0
0.0
Alfalfa
Birdsfoot trefoil
Red clover
DRY WEIGHT DEPOSITION (g POT$^{-1}$ day$^{-1}$)
0.16
0.12
0.08
0.04
0.00
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)
Orchardgrass
Bromegrass
Switchgrass
DRY WEIGHT DEPOSITION (g POT$^{-1}$ day$^{-1}$)
0.25
0.20
0.15
0.10
0.05
0.00
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

Figure 2. Cell wall (CW) concentration [LSD (0.05) = 47.5] and change in CW concentration on a dry weight basis in legume and grass stems. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 606 | 1.95 | 0.1414 | 0.81 |
| Birdsft. trefoil | 594 | 0.83 | 0.6360 | 0.97 |
| Red clover | 516 | 2.40 | 0.1202 | 0.64 |
| Orchardgrass | 572 | 0.21 | 0.0364 | 0.94 |
| Bromegrass | 662 | 0.12 | 0.0018 | 0.00 |
| Switchgrass | 767 | 0.55 | 0.0463 | 0.92 |


CELL-WALL CONCENTRATION (g kg$^{-1}$ DW)
600
540
480
420
360
Alfalfa
Birdsfoot trefoil
Red clover
790
715
640
565
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN CELL WALL (g kg$^{-1}$ DW day$^{-1}$)
16.0
12.0
8.0
4.0
0.0
8.0
6.0
4.0
2.0
0.0
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

bromegrass evinced essentially no difference in CW concentration between immature and mature stem bases (Buxton and Russell, 1988). Stem bases of smooth bromegrass may be diluted by storage fructosans and thereby demonstrate little change in CW concentration as a function of regrowth days (Buxton, 1990). General trends for overall CW results illustrated greater and faster concentration changes in legumes compared with grasses at early regrowth days.

Hyperbolic increases in lignin concentration on a CW basis resembled hyperbolic trends of CW increases in all species except orchardgrass (Figure 3). Orchardgrass demonstrated a poor fit to the Gompertz equation because little variability in lignin concentration was observed as a function of regrowth days. Differences in lignin concentration changes between grasses and legumes followed trends encountered with changes in CW concentration. Changes in legume lignin concentrations were generally twice those of grasses at early regrowth days although grasses demonstrated a much slower decrease in lignin concentration changes as a function of regrowth days.

Even though protein concentration varied differently among species with age, PAL activity on a protein basis decreased consistently for most species (Figure 4), and acceptable $R^2$ values indicated a reliable fit of data to second order quadratics, with exception of orchardgrass.

Figure 3. Cell wall (CW) lignin concentration [LSD (0.05) = 19.9] and change in CW lignin concentration in legume and grass stems. Gompertz coefficients and $R^2$ values for fit of data are as follows:

| | Gompertz coefficients | | | |
|---|---|---|---|---|
| Species | a | b | c | $R^2$ |
| Alfalfa | 182 | 0.678 | 0.0742 | 0.97 |
| Birdsft. trefoil | 186 | 0.308 | 0.0450 | 0.95 |
| Red clover | 112 | 2.163 | 0.0862 | 0.92 |
| Orchardgrass | 64 | 0.037 | -0.0161 | 0.26 |
| Bromegrass | 83 | 1.223 | 0.0581 | 0.74 |
| Switchgrass | 102 | 1.187 | 0.0277 | 0.64 |

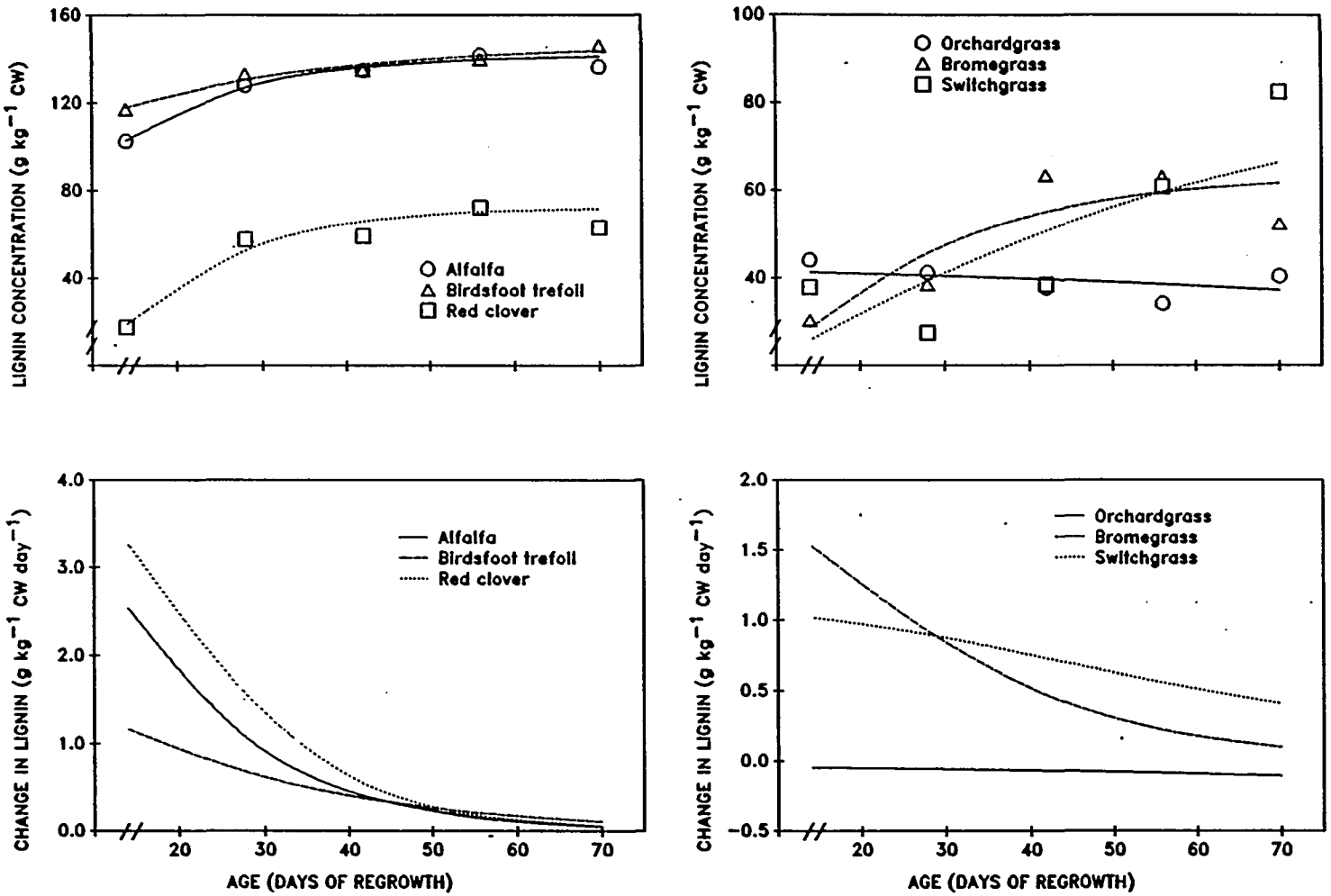

LIGNIN CONCENTRATION (g kg$^{-1}$ CW)
160
120
80
40
Alfalfa
Birdsfoot trefoil
Red clover
100
80
60
40
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN LIGNIN (g kg$^{-1}$ CW day$^{-1}$)
4.0
3.0
2.0
1.0
0.0
2.0
1.5
1.0
0.5
0.0
−0.5
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

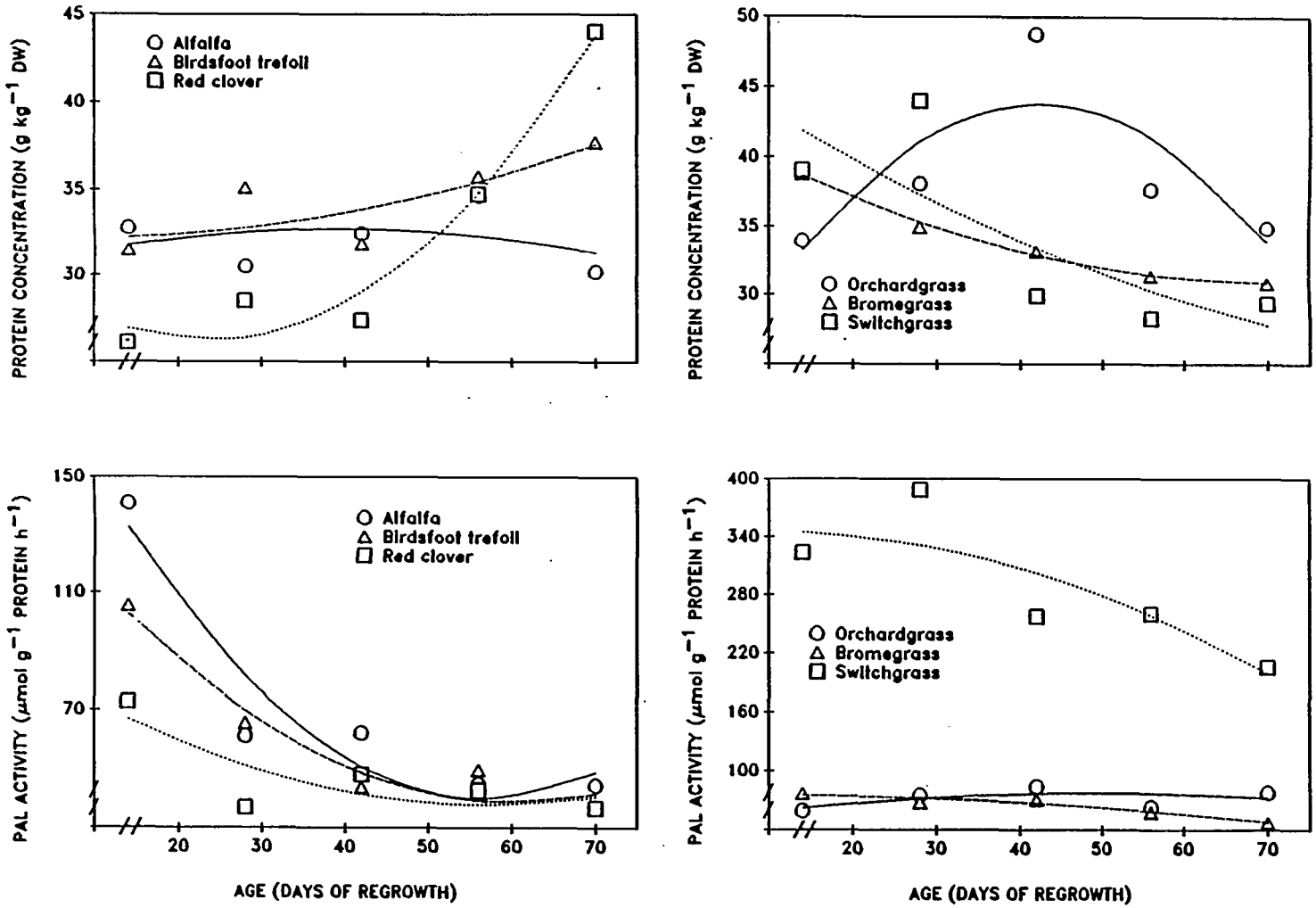
Figure 4. Protein concentration on a dry weight basis [LSD (0.05) = 11.5] and phenylalanine ammonia lyase activity on a protein basis [LSD (0.05) = 48.8] in legume and grass stems. Quadratic coefficients and $R^2$ values are as follows:

Quadratic coefficients for protein concentration

| Species | Quadratic | Linear | Intercept | $R^2$ |
|---|---|---|---|---|
| Alfalfa | -0.0015 | 0.1161 | 30.45 | 0.10 |
| Birdsft. trefoil | 0.0013 | -0.0164 | 32.21 | 0.65 |
| Red clover | 0.0081 | -0.3791 | 30.65 | 0.96 |
| Orchardgrass | -0.0130 | 1.1002 | 20.44 | 0.65 |
| Bromegrass | 0.0024 | -0.3373 | 42.91 | 0.99 |
| Switchgrass | 0.0017 | -0.3958 | 47.02 | 0.63 |

Quadratic coefficients for PAL per unit protein

| Species | Quadratic | Linear | Intercept | $R^2$ |
|---|---|---|---|---|
| Alfalfa | 0.0511 | -5.7869 | 203.61 | 0.90 |
| Birdsft. trefoil | 0.0300 | -3.6148 | 147.42 | 0.94 |
| Red clover | 0.0158 | -1.7977 | 88.97 | 0.64 |
| Orchardgrass | -0.0118 | 1.1746 | 48.08 | 0.32 |
| Bromegrass | -0.0066 | 0.0660 | 75.63 | 0.92 |
| Switchgrass | -0.0387 | 0.6571 | 343.07 | 0.71 |


PROTEIN CONCENTRATION (g kg$^{-1}$ DW)
Alfalfa
Birdsfoot trefoil
Red clover
Orchardgrass
Bromegrass
Switchgrass
PAL ACTIVITY (µmol g$^{-1}$ PROTEIN h$^{-1}$)
AGE (DAYS OF REGROWTH)

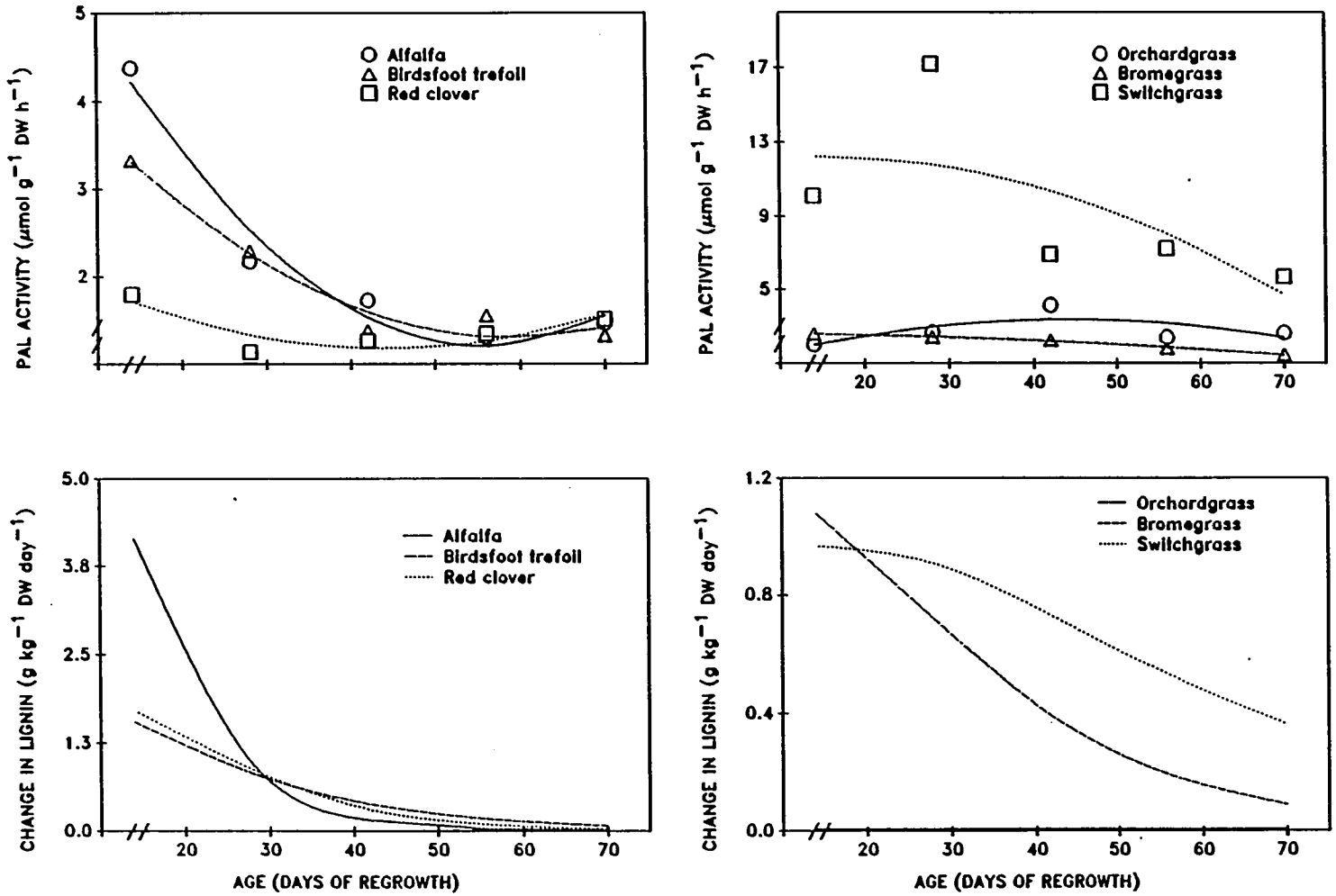
Protein concentration increased exponentially in red clover after internode elongation and varied widely in orchardgrass as might be expected since uniform sheath samples were difficult to collect. Other species demonstrated essentially no change or decrease in protein concentration as a function of regrowth days. Most interesting was the obvious decrease in PAL activity in legumes and the slower decrease in PAL activity in grasses as a function of regrowth days.

Both PAL activity and change in lignin concentration were expressed on a DW basis for relative comparison (Figure 5). These results show similar trends to those encountered with change in lignin concentration on a CW basis (Figure 3) and PAL activity on a protein basis (Figure 4). Trends from these figures indicate that PAL activity closely parallels lignin concentration changes as a function of stem or sheath age. Faster decreases in PAL activity and lignin concentration changes were observed in legumes compared with grasses at early days of regrowth. Close relationships between PAL and change in lignin concentration were demonstrated within a species as indicated by parallel rapid decreases in alfalfa and slower decreases in switchgrass.

Theoretical concentrations of lignin synthesized by PAL overestimated those that were actually observed. For instance, at the 14-day sample of alfalfa in Figure 5, PAL activity was 4.5 $\mu$mol g $DW^{-1}$ $h^{-1}$, which calculates to a

Figure 5. Phenylalanine ammonia lyase (PAL) activity [LSD (0.05) = 2.27] and change in lignin concentration [LSD (0.05) = 13.5] on a dry weight basis in legume and grass stems. Quadratic coefficients and $R^2$ values for PAL activity on a dry weight basis are as follows:

| | Quadratic coefficients | | | |
|---|---|---|---|---|
| Species | Quadratic | Linear | Intercept | $R^2$ |
| Alfalfa | 0.0017 | -0.1933 | 6.590 | 0.97 |
| Birdsft. trefoil | 0.0010 | -0.1165 | 4.748 | 0.96 |
| Red clover | 0.0006 | -0.0052 | 2.318 | 0.76 |
| Orchardgrass | -0.0015 | 0.1316 | 1.342 | 0.49 |
| Bromegrass | -0.0002 | -0.0020 | 2.670 | 0.99 |
| Switchgrass | -0.0024 | 0.0699 | 11.722 | 0.45 |


PAL ACTIVITY (μmol g⁻¹ DW h⁻¹)
Alfalfa
Birdsfoot trefoil
Red clover
Orchardgrass
Bromegrass
Switchgrass
CHANGE IN LIGNIN (g kg⁻¹ DW day⁻¹)
AGE (DAYS OF REGROWTH)

change in lignin content of 16.0 g lignin as cinnamic acid (CA) per kg $DW^{-1}$ $day^{-1}$:

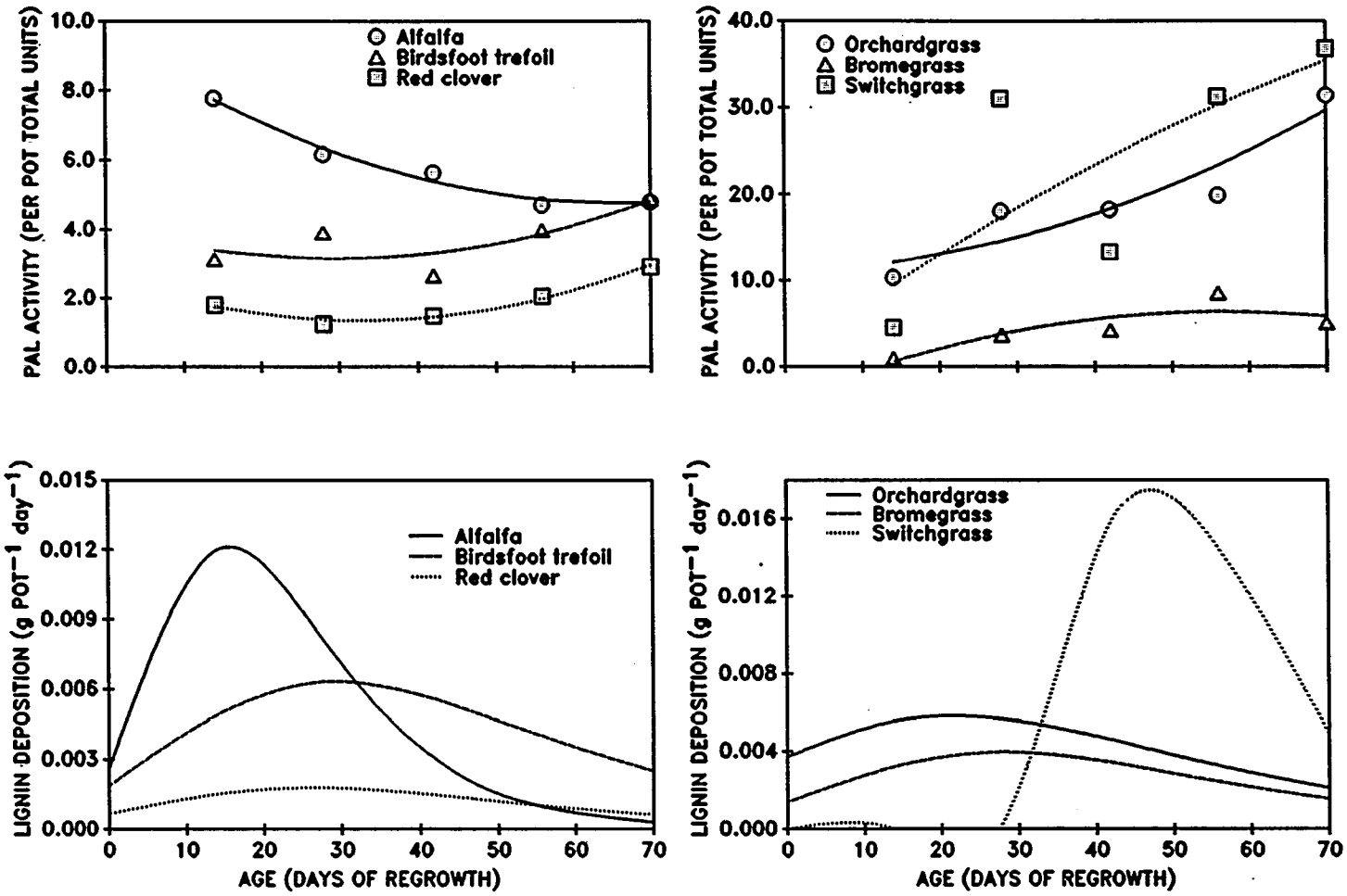
$$\frac{4.5\ \mu mol\ CA}{g\ DW\ h} \times \frac{1.48\ E\text{-}4\ g\ CA}{\mu mol\ CA} \times \frac{1000\ g}{kg} \times \frac{24\ h}{day} = \frac{16.0\ g\ lignin}{kg\ DW\ day}$$

The actual change in lignin of alfalfa stems, as shown in the bottom of Figure 5 at day 14 was only 4.4 g lignin kg $DW^{-1}$ $day^{-1}$. Overestimation of lignin concentration from enzyme activity may be attributable to additional roles of PAL in metabolism, elevated temperature, and substrate saturation used to run the assay. These results indicate that PAL contributes to lignin biosynthesis and that PAL probably has other roles, such as those documented for flavonoid, alkaloid, phenolic, and other phenylpropanoid product biosynthesis (Goodwin and Mercer, 1983). However, predicting PAL's effect on other metabolites is difficult because the enzymes involved can be induced over 100-fold by environmental stimuli (Hahlbrock and Grisebach, 1979), and little is known about mechanisms of control (Rhodes, 1989).

Comparison between lignin and PAL was also evaluated on a total yield or "per pot" basis to show how amount of lignin synthesized related to total PAL units. Multiplication of DW from Figure 1 by PAL activity and change in lignin from Figure 5 revealed estimates of PAL total units and lignin deposition in Figure 6. Lignin deposition plots were

Figure 6. Phenylalanine ammonia lyase (PAL) activity per pot (total enzyme units) [LSD (0.05) = 7.41] and lignin per pot deposition in legume and grass stems. Quadratic coefficients and $R^2$ values for PAL activity per pot are as follows:

| | Quadratic coefficients | | | |
|---|---|---|---|---|
| Species | Quadratic | Linear | Intercept | $R^2$ |
| Alfalfa | 0.00108 | -0.144 | 9.53 | 0.98 |
| Birdsft. trefoil | 0.00102 | -0.060 | 4.02 | 0.63 |
| Red clover | 0.00115 | -0.075 | 2.55 | 0.98 |
| Orchardgrass | 0.00326 | 0.041 | 10.84 | 0.87 |
| Bromegrass | -0.00311 | 0.355 | -3.73 | 0.75 |
| Switchgrass | -0.00227 | 0.065 | 0.81 | 0.53 |


PAL ACTIVITY (PER POT TOTAL UNITS)
10.0
8.0
6.0
4.0
2.0
0.0
Alfalfa
Birdsfoot trefoil
Red clover
PAL ACTIVITY (PER POT TOTAL UNITS)
40.0
30.0
20.0
10.0
0.0
Orchardgrass
Bromegrass
Switchgrass
LIGNIN DEPOSITION (g POT$^{-1}$ day$^{-1}$)
0.015
0.012
0.009
0.006
0.003
0.000
Alfalfa
Birdsfoot trefoil
Red clover
0
10
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)
LIGNIN DEPOSITION (g POT$^{-1}$ day$^{-1}$)
0.016
0.012
0.008
0.004
0.000
Orchardgrass
Bromegrass
Switchgrass
0
10
20
30
40
50
60
70
AGE (DAYS OF REGROWTH)

directed through the origin, by adding the data point 0, 0 to each plot, for a better representation of the data. These graphs show that, in some cases, trends in PAL total units as a function of age resemble trends in lignin deposition as a function of age. Close resemblance between PAL total units and lignin deposition was demonstrated in Figure 6 by alfalfa at days 14 through 70 and somewhat by smooth bromegrass and switchgrass at days 14 through 56.

## Conclusions

Changes in CW and lignin concentration were greater and decreased faster in legume stems compared to grass stems or orchardgrass sheaths at early days of regrowth. These trends were parallel to those of PAL activity and could be closely coupled within a species. Calculated concentrations of lignin produced by PAL overestimated changes in lignin concentration and indicated that PAL contributes to other metabolites in addition to lignin. Graphical representations indicated that PAL per pot resembled lignin deposition as a function of regrowth days. Close association between changes in lignin concentration and PAL support the idea that decreased enzyme activity may improve forage quality by decreasing lignin, but only with subsequent decreases of other phenylpropanoid metabolites.

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# GENERAL DISCUSSION

Sections I and II of this research contribute towards better understanding of forage physiology through developmental and physiological findings of forage CW growth. New information provided by this research enables scientists to better describe developmental differences between grass and legume CW growth and give molecular biologists preliminary information needed for improvement of forage quality through manipulation of phenylpropanoid metabolism.

In Section I, the order of component deposition was observed as hemicellulose and cellulose followed by lignin. This order was more apparent in grasses and timing of maximum component deposition appeared to be species-specific within grasses and legumes. Although species-specific timing of CW component deposition appeared to occur, 64 to 100% of the variability in CW component growth was attributed to changes in stem base weight. Thus, timing of maximum CW component deposition may be important only to the extent that it occurs coincident with maximum stem base weight deposition and is influenced by plant age. However, within the dry weight or CW of plant tissue, results demonstrated characteristic differences between grass and legume change in CW component concentration. Legumes could be distinguished from grasses

because legume CW and CW component concentration changes decreased faster than that of grasses as tissues matured. These differences imply that CWs of forage stem bases have a dynamic nature and expression of changes in CW and CW components on a dry weight basis provides evidence for species-specific developmental differences in CW structure.

With knowledge from rates of CW and CW component concentration changes, management of forage quality can be better keyed towards developmental aspects of stem tissue. Evidence from this study indicates that legume forage quality declines rapidly at early days of regrowth while grass forage quality decreases steadily throughout regrowth. From a management standpoint, it is evident that decreases in mature grass stem quality may be more detrimental to potential digestibility than that of legumes. Thus, timing harvests of grass forage may be more critical towards quality than that of legumes.

Section II provided additional evidence for faster concentration changes in legumes compared with grasses because PAL activity demonstrated a close parallel to the rapid decline in CW and lignin concentration changes as stems matured. Activity of PAL per pot (PAL total units) also resembled lignin deposition as a function of regrowth days. Paralleled PAL activity and change in lignin concentration as a function of regrowth days associates a physiological

parameter with CW development. From this close association, it is evident that manipulation of PAL may be used to change lignin concentration in forage tissues. However, altered PAL probably affects numerous other metabolic reactions as well as lignin biosynthesis.

Preliminary information from CW and CW component concentration changes in association with PAL shows promise for manipulation of forage quality. Future studies need focus on committed enzymes of lignin biosynthesis and their relation to CW deposition and forage quality. Once key enzymes are identified, more information will be needed to show how they are encoded, regulated, and synthesized and to provide additional information on their relation to PAL and other phenylpropanoid enzymes. Compiled information will enable molecularly altered forage quality. However, even with the institution of genetically altered cultivars to forage fields, molecular manipulation should not be viewed as a panacea to improved forage quality.

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