Root restriction and root-shoot relationships in tomato (*Lycopersicon esculentum* Mill.)

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We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.

—T.S.Eliot *Four Quartets*

Abstract

The potential for controlling plant growth and productivity by manipulating root growth and development has not been realised because of a lack of understanding of how root growth influences shoot growth. Until such responses are understood, matching container design and volume to desired plant output will continue to be based solely on anecdotal evidence. A series of experiments were conducted to explore the role of physical root restriction on the vegetative growth and development of tomato (*Lycopersicon esculentum* Mill. 'Moneymaker'). Concurrent with these experiments, a statistical model was developed for non-destructively estimating leaf area, cluster analysis was adapted to improve experimental precision, and an improved form of growth analysis developed. Additionally, a review of oxygen and major nutrient uptake rates by tomato established the operational parameters of a hydroponic system developed specifically for the study.

Rooted tomato cuttings were grown in 0.025 or 10 litre (control) containers in the hydroponic system. After 31 days in 0.025 litre containers, plants were de-restricted into either 0.05 or 10 litre containers, or retained in the 0.025 litre containers. Plants with physically restricted root systems had lower total plant biomass and total leaf area, were shorter in both height and total root length, and had fewer roots, leaves, and lateral shoots than unrestricted plants. Restriction reduced root number after 31 days, but reductions in root length and dry biomass did not occur until after 45 days. Leaf dry biomass was reduced in restricted plants after 45 days; reductions in stem height, leaf area, number and total dry biomass) were apparent after 67 days. Short periods (31 days) of root restriction had long term (67-99 days) effects on leaf growth. Leaf expansion was more sensitive than leaf biomass accumulation to root restriction. A strong linear relationship, independent of root restriction, was observed between the relative rates of root elongation and leaf expansion. Similar relationships with the relative

rates of increase in root number and dry biomass were due to their covariance with root elongation. These data are consistent with the hypothesis that root elongation is functionally linked to leaf expansion via the synthesis of hormones in actively growing root apices.

The influence of partial root restriction on leaf expansion was also examined. One or both halves of a split root system was enclosed in a 30 cm^3 polyethylene cell. Leaf expansion was reduced in plants with only a portion of their total root system physically restricted. Compensatory growth in the unrestricted portion of the root systems resulted in total root growth at final harvest being similar to plants with all their root system unrestricted. Analysis of the relative rate of leaf expansion (R_A) of individual leaves along the stem axis revealed two distinct phases in response to root restriction. In the first phase, apparent about 28 days after treatments were initiated (DAI) and observed in leaves that started expansion 3, 7, and 14 DAI, R_A was reduced in plants with one or both root sub-systems in a restriction cell. The second phase, detected 42 DAI and observed in leaves that started expanding 21 and 28 DAI, was characterised by a higher R_A in plants with a portion of their root system restricted compared to unrestricted plants. Proportionately more assimilate was partitioned to stems of plants with two restricted root sub-systems compared to plants with either a single or non-restricted root sub-system. No differences in leaf water potential or photosynthesis of leaves were observed among treatments.

Conclusions drawn from these data support the involvement of chemical signals in maintaining coordination between root and shoot growth in container-grown plants. These conclusions are discussed with reference to the literature, and a model is proposed to explain root-shoot coordination in terms of root-sourced cytokinin and shoot-sourced auxin. Avenues for future research to test hypotheses arising from this model are identified and discussed, as are possible horticultural ramifications.

Emphasis was placed in the study on improving analytical methodology of growth analysis of whole-plant studies. Experimental precision was increased in these experiments by using cluster analysis to allocate plants to blocks based on leaf area, with a developmental study showing that the mean coefficient of variation of groups formed from cluster analysis was between two and fives times smaller than that of groups formed from visual assessment. A statistical model for non-destructively estimating the leaf area of tomatoes was developed based on the length of the mib-rib of each compound leaf and its position on the stem. Although the model was accurate to within about 2.5% of actual leaf area, it was not stable in time. It was concluded that when non-destructive estimation of tomato leaf area is required, the prediction model must be developed while the main experiment is being conducted. A hybrid method of growth analysis, incorporating both functional and univariate statistical approaches, provided more flexibility and information than standard functional or classical analytical methods. The hybrid method yielded replicated estimates of growth analysis indices, providing opportunity for further evaluation of the derived data using multivariate analytical techniques including path, canonical correlation, and canonical discriminant analysis.

keywords: allometric relationships, assimilate partitioning, biometrics, Chanter function, cluster analysis, containerised plants, hydroponics, leaf expansion, local error control, plant growth analysis, relative growth rate, Richards function.

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List of abbreviations

$\bar{E}_A$	mean net assimilation rate ($g_w \cdot m^{-2} \cdot day^{-1}$)
$\bar{R}_W$	mean plant relative growth rate ($g \cdot g^{-1} \cdot day^{-1}$)
ε_{ij}	residual error (of RCB model)
ε_{ja}	among-treatment error
ε_{jw}	within-treatment error
λ_i	*i*th eigenvalue of canonical discriminant function
χ^2_{obs}	observed chi-square value
ψ_W	leaf water potential (MPa)
ACC	1-amino-cyclopropane-1-carboxylic acid
ANCOVA	analysis of covariance
ANOVA	analysis of variance
BA	benzyladenine
CCA	canonical correlation analysis
CDA	canonical discriminant analysis
CDF	canonical discriminant function
$CDF_{1(31)}$	first canonical discriminant function of data at 31 DAI
C_p	Mallows statistic
CR	continuously restricted
CRD	completely randomised design
CV	coefficient of variation
CV_{HT}	coefficient of variation for plant height
CV_{LA}	coefficient of variation for leaf area
DAI	days after initiation (of experiment)
DCR	double cell restricted root system
DFT	Deep Flow Technique (hydroponic system)
E_A	net assimilation rate ($g_w \cdot m^{-2} \cdot day^{-1}$)
g_L	leaf dry weight (g)
g_R	root dry weight (g)
g_{ST}	stem dry weight (g)
g_W	plant dry weight (g)
INDEX	relative leaf position on stem
k	allometric coefficient
LAR	leaf area ratio ($m^2 \cdot g_W^{-1}$)
LIP	leaf insertion position
LNAREA	$\log_e$ leaf area (cm^2)
LNMRIB	$\log_e$ leaf mid-rib (cm)
LPI	leaf plastochron index
LWR	leaf weight ratio

MSE	mean square of error
n	number of observations
NAA	naphthaleneacetic acid
NFT	Nutrient Film Technique
NSC	Non-Split Control
OLS	ordinary least squares
$P_{1(13)}$	first predictor canonical variable of data at 13 DAI
P_{ij}	path coefficient between variables i and j
$R_{1(13)}$	first response canonical variable of data at 13 DAI
R^2	coefficient of multiple determination
R^2_{adj}	adjusted coefficient of determination
R_A	relative leaf expansion rate ($m^2 \cdot m^{-2} \cdot day^{-1}$)
R_{av}	mean relative growth rate (over a given period).
RCB	randomised complete block design
RD	restricted-derestricted
RDD	restricted-derestricted-derestricted
$R_{I(RN)}$	relative rate of increase in root number
$R_{I(variable)}$	relative rate of increase of variable (e.g. leaf or root number)
r_{ij}	correlation coefficient between variables i and j
R_L	leaf relative growth rate ($g \cdot g^{-1} \cdot day^{-1}$)
RPF	root produced factor
R_R	root relative growth rate ($g \cdot g^{-1} \cdot day^{-1}$)
R_{RL}	relative root extension rate ($m \cdot m^{-1} \cdot day^{-1}$)
R_S	shoot relative growth rate ($g \cdot g^{-1} \cdot day^{-1}$)
RSM	root specific mass ($mg \cdot m^{-1}$)
R_{ST}	stem relative growth rate ($g \cdot g^{-1} \cdot day^{-1}$)
R_W	plant relative growth rate ($g \cdot g^{-1} \cdot day^{-1}$)
RWR	root weight ratio
SCR	single cell restricted root system
SE	standard error of mean
SEOD	standard error of difference between means
SLA	specific leaf area ($m^2 \cdot g_L^{-1}$)
SLW	specific leaf weight ($g_L \cdot m^{-2}$)
SOC	Split Only Control
SPF	shoot produced factor
SR	shoot:root ratio
SRL	specific root length ($m \cdot g_R^{-1}$)
SWR	stem weight ratio
UR	unrestricted (control)

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Chapter 1

Introduction

1.1 Introduction

A greater understanding of the root-shoot relationships of plants growing in containers is required. Crop production in containers is a dominant feature of the nursery, cut flower and greenhouse vegetable sectors of the horticultural industry in New Zealand. Over 97% of nurseries listed in the New Zealand Nursery Register (Anon, 1993) produce plants in containers, and the majority of greenhouse vegetables are raised in some form of container system. Crops traditionally grown in the open ground are increasingly being produced in containers. Bare-rooted vegetable seedling transplants are now rarely seen, having been replaced by higher quality transplants produced in 5-15 cm^3 cells. Forestry seedlings are increasingly being produced in containers, with the conservatism and longstanding antagonism of this sector to container production (van Dorsser, 1982) now giving way to greater acceptance of the improved quality, growth control and flexibility in production scheduling achievable with container produced stock (Tinus and McDonald, 1979).

Producing plants in containers rather than in the field or greenhouse soils increases the grower's financial risk. Container production necessitates a considerable financial investment in containers and soilless growing medium before production starts, whereas both real and opportunity costs associated with soil-grown crops tend to rise towards the end of the production cycle. Growers are, therefore, more demanding of crops produced in containers. They require rapid growth to minimise the time required to produce the plant or its saleable component and to maximise the number of crops that can be cycled through their production areas (e.g. greenhouses) annually. Producers of woody ornamental plants, for example, aim to maximise growth at all stages of the production process from liner (small container) through the progression of potting-on (transplanting) up to the final container size. Seedling growers seek to produce high quality plants with either good shelf life (bedding plants) or capable of withstanding transplant shock (forestry and

vegetable seedlings). Growers of houseplants often seek to control excessive growth (e.g. height) to maintain a compact, aesthetically pleasing growth habit.

Planned reduction of growth of containerised plants is currently achieved by withholding water or through the use of synthetic chemical growth retardants. Neither option, however, is sustainable. Withholding water from a container growing medium can substantially increase its salinity (Handreck and Black, 1991). In doing so, the chance of root damage is increased, as is consequent attack from root-rot pathogens drawn to the roots by stress-induced exudates or by gaining direct access through damaged tissue (Snapp and Shennan, 1994). Future use of chemical growth retardants (e.g. daminozide, chlormequat) to control the growth and appearance of ornamental plants is itself likely to be retarded. Increasing public antagonism towards such chemicals, increasingly stringent environmental laws regarding their use, and increasingly high costs for developing and maintaining their registration for a relatively small market sector (Davis and Curry, 1991) are likely to combine to at least narrow the range of use of such chemicals, if not completely eliminate their use.

Plants with highly controlled characteristics, developed through the recent advances in molecular biology and plant breeding, are unlikely to provide a solution. The horticultural industry is a small component of plant-based production industries, and in turn, those sectors of the industry that grow plants in containers (e.g. nursery, greenhouse vegetables, cut flowers) are small components of the horticultural industry. As a consequence, the market is too small and the range and diversity of crops grown too large for breeding and associated crop improvement programmes to offer realistic solutions in a realistic time frame.

Some alternative, more environmentally-friendly growth control strategies have been developed. For example, careful management of day and night

temperatures provides predictive control of internode length and plant height in several pot and bedding plants (Erwin et al., 1989; Karlsson et al., 1989; Moe et al., 1992; Mortensen and Moe, 1993). Unfortunately, responses across species and environments are often variable (Vogelezang et al., 1993), and by requiring control over the aerial environment, use of this strategy outside of greenhouses is effectively precluded.

Moreover, such control strategies do not provide control over partitioning of assimilate, and it is in this area that considerable opportunities exist for improving productivity in container-grown crops. There are many production scenarios in which maximum growth *and* manipulation of that growth within the plant is required. As examples, a grower aiming to improve the transplanting success of container grown vegetable or forestry seedlings might seek, prior to transplanting, to increase reserves in the seedlings' root systems to sustain subsequent rapid root growth and establishment in the field. Greenhouse vegetable growers might seek to reduce the sink strength of the root system to 'free up' assimilate for fruit growth, and thereby increase the harvest index.

If this degree of crop management is to be realised, a greater understanding of the root-shoot relationships of plants grown in containers is required. The container environment is, in many ways, unique. This makes extrapolating current knowledge of root-shoot relationships under field conditions to anticipate the performance of plants in a container environment unlikely to be of benefit. Although a container environment has a limited volume, properly managed it can be regarded as being non-limiting in nutrient and water supply. While not impossible in a field situation, such non-limiting conditions are considerably more difficult to achieve. Furthermore, as supply of water and nutrients can be directed to the entire root system in a container, there is less 'need' for the expansive growth of the root system in the field to 'seek' supply. The container environment places a physical impediment to growth and development of the entire root system. In

contrast, physical restriction to the root system in the field is of less consequence as the root system is 'free' to explore other less restrictive areas.

Container design will be the dominant tool by which root growth is managed to achieve the desired pattern of whole-plant growth. Unfortunately, most studies into the effects of container design on plant growth have approached the task empirically, without any prior reasoning of expected root-shoot response. One approach has been to empirically test the growth response (i.e. total shoot biomass) of different species in containers of different linear dimensions. Some of these studies have reported that for containers of the same diameter, shoot growth is always less in long containers (Falloon and Schurink, 1982; Hanson et al., 1987; Smith and Schwabe, 1980). Other studies, however, have shown that the natural form of the root system must be taken into account, with naturally shallow rooted species performing better in wide, shallow containers than naturally deep-rooted species (Biran and Eliassaf, 1980; Keever et al., 1985).

The other main target of container design studies has been the reduction of root circling (or binding). Whitcomb (1981, 1984), for example, developed containers with either vertical slits to air prune root tips as they circled the container, or with 'steps' on the internal wall of the container to inhibit root elongation and promote lateral root development. More recent studies have focused on using a copper-based coating on the inside surface of the container to chemically prune root apices and thereby avoid root circling (Arnold and Struve, 1993; Beeson and Newton, 1992). As with the studies of container dimension, the results of these studies have been inconclusive with respect to root response and shoot growth.

While this general lack of consistent response of plant growth in container design studies may have been due, in part, to the confounding action of changes in aeration and water availability that occur down the vertical axis of a container (Bunt, 1988; Milks et al., 1989), a major factor has been the

lack of understanding of the relationships between root and shoot growth in containers. As a consequence, many studies have not measured the important variables. In this regard, the study by Liptay and Edwards (1994) is typical. Working with tomato seedlings in small cells, these workers reported that only shoot growth (height, total shoot dry biomass weight) responded to changes in the shape of the cells. Unfortunately, the only measure of root performance taken by these workers was the dry biomass of the root system. Yet numerous studies have demonstrated a link between the number of root apices and shoot growth. Ooyama and Toyoshima (1965), for example, reported a strong, positive correlation between the number of roots and subsequent growth in height of pine seedlings. Reduced shoot growth of peach seedlings and fruiting tomato plants was associated with lower numbers of root apices (Richards, 1981; Richards and Rowe, 1977a). Bentz et al. (1985) demonstrated that stem cuttings of woody plants required a minimum number of roots, which was species-dependent, before measurable shoot growth was initiated. Importantly, the number of roots is depressed when plants are grown in containers that are too small (by volume) to accommodate the root system without physically impeding it (Carmi and Heuer, 1981; Hameed et al., 1987; Richards and Rowe, 1977a; Tschaplinski and Blake, 1985).

By not using the opportunity to control root growth, current methods of producing plants in containers are not realising the full potential that containerised production provides. Future crop production in containers will be advanced by the development of specially designed containers that will sustain actively growing plants and manipulate partitioning of their assimilate to fulfill the growers' (or their clients) specifications. Such potential will not be realised, however, until a better understanding is gained of how root growth in the physically restricting environment of a container influences the various components that make up shoot growth. This study aims to increase our understanding of this aspect of root-shoot relationships.

Chapter 2

Development of a hydroponic system for root restriction studies

2.1 Introduction

A root environment in which a physical barrier is the only factor limiting root growth is critical to a successful study of the effect of root restriction on plant growth. If other factors present in the environment limit root growth, the results may be confounded. Even if these factors are known, it may be impossible to disentangle their respective contributions to the observed plant response. In some instances, reported responses to root restriction may be experimental artefacts of the design of the restricting system. For example, consider the *reduction* in shoot:root ratio (SR) of tomato plants with restricted roots reported by Ruff et al. (1987). These workers grew plants in containers filled with a soil-based potting medium under frequent (4-6 times per day) overhead irrigation. Soil-based potting media have inherently poor drainage and low aeration characteristics (Bunt, 1988; Handreck and Black, 1991). Add to this the reliance of tomato shoot growth on oxygen diffusion in the root zone (Doyle and MacLean, 1958), the high frequency of overhead irrigation and small container volume, and it is likely, even probable, that restricted plants lacked sufficient oxygen for 'normal' growth. In reaching a similar conclusion, Peterson et al. (1991b) concluded that oxygen deficiency was a confounding element of hydroponic systems which rely on passive uptake of solution into the restricting container (e.g. Al-Sahaf, 1984; Carmi and Heuer, 1981; Carmi et al., 1983; Hameed et al., 1987; Tschaplinski and Blake, 1985). Increases in SR of 60-170% in restricted plants (Al-Sahaf, 1984; Carmi and Heuer, 1981; Hameed et al., 1987; Tschaplinski and Blake, 1985) are markedly higher than those where solution was actively moved into, or agitated within, the restriction container (e.g. Richards and Rowe, 1977a).

Nevertheless, hydroponic systems are ideally suited to root growth studies, provided they are correctly designed. The ease of access to root system and the absence of particles of solid media on the roots simplifies manipulation, recovery, and measurement of root systems. Properly managed, the root

environment in hydroponic systems is more uniform and controllable than that in alternative growing systems (e.g. peat or bark-based solid media) where oxygen and water characteristics vary within the root environment (Bunt, 1988; Spomer, 1975). Furthermore, confounding interactions among roots and the physical characteristics of solid media are absent, making hydroponic systems of particular value to studies of the influence of root restriction on whole plant relationships.

Despite their wide use (Carmi, 1986a; Carmi and Heuer, 1981; Carmi et al., 1983; Hameed et al., 1987; Peterson et al., 1991a; Richards and Rowe, 1977a; Tschaplinski and Blake, 1985), few guidelines are available for designing hydroponic systems for research purposes. Peterson and Krizek (1992) provide a detailed description for constructing a complex hydroponic system for studies of root restriction, but the physiological basis for several components of the system is inadequately explained. In contrast, the work of Cooper (1975) and his colleagues from the Glasshouse Crops Research Institute in the mid 1970s to mid 1980s provided considerable information of the physiological responses of plants in hydroponic systems. Unfortunately, their system, the Nutrient Film Technique (NFT), is unsuited to studies of root growth because roots of adjacent plants within a channel quickly intertwine, making it difficult to selectively impose treatments on, and harvest, individual root systems. Nevertheless, their work identified potentially confounding factors that must be considered in designing a hydroponic system suitable for root restriction studies.

Hydroponic systems are either static or recirculating (Jensen and Collins, 1985). Static systems do not require expensive pumps or the associated distribution network, and their relative ease of construction makes them a popular research choice over the recirculating alternative (Carmi, 1986a; Carmi and Heuer, 1981; Carmi et al., 1983; Hameed et al., 1987; Richards and Rowe, 1977a; Tschaplinski and Blake, 1985). Unfortunately, static

systems have several disadvantages. The pH and concentrations of nutrients and oxygen (O_2) in the solution constantly change with plant uptake. This requires frequent replacement of solutions (e.g. Hameed et al., 1987) to avoid large changes in the chemical composition of the root environment. Not only is this time-consuming, but the risk of physical damage and temperature fluctuations in the root zone is increased. Inadequate flux results in localised zones of depleted O_2, particularly in densely packed root systems, leading to reduced growth (Jackson, 1980). Recirculating systems (e.g. Peterson and Krizek, 1992) avoid several of these problems. The root environment can be maintained at set levels of nutrient concentration and pH by adjusting the main reservoirs. Attention to the flow rate of the solution minimises occurrence of localised zones of low concentrations of both O_2 and nutrients near absorbing roots (Clement et al., 1974; Hurd, 1978).

Flow rates appropriate to the system are important if correct conclusions are to be made from data (Edwards and Asher, 1974). Rates of 60–240 litres·hour^{-1} are quoted (Gislerød and Kempton, 1983; Hurd, 1978; Jackson et al., 1984; Jenner, 1980; Mahler, 1977), but these are for NFT systems in which a single channel may hold over 40 plants. Willumsen (1983) reported that flow rates between 4.4–5.8 litres·hour^{-1} per plant in seven litre containers were sufficient to avoid O_2 deficiency in the root zone. Peterson et al. (1991a) used flow rates of 0.72 and 3.6 litres·hour^{-1} in 0.025 and 1.5 litre containers respectively, but did not justify the rates or the differences among treatments.

Whereas flow rates of the solution are important in replenishing nutrients in the root zone, solution temperature markedly influences nutrient uptake. Uptake, particularly of phosphorus (P) and iron (Fe), in tomatoes is reduced at temperatures below 15°C (Moorby and Graves, 1980). Ganmore-Neumann and Kafkafi (1980) reported that potassium (K) and nitrate-nitrogen (NO_3^--N) accumulated in tomato roots at low solution temperatures (<16°C). They

suggested that poor translocation of these ions at low temperature contributed to slow growth under such conditions. The broad optimum root temperature for tomatoes is between 20-30°C (Gosselin and Trudel, 1982; Moorby and Graves, 1980; Morgan and O'Haire, 1978).

Recirculating systems, and some static systems (e.g. Carmi et al., 1983; Richards and Rowe, 1977a), have a common reservoir into which solution from all, or single treatments, combine. This practice raises an important, and often overlooked (cf. Wilcox, 1982) statistical issue; true treatment differences may not be detected in experiments using a common reservoir due to potential interaction among treatments (Jarrett and Chanter, 1981). Contaminants arising from specific treatments will circulate to all plants in the system, and due to the closed nature of the system, their concentration will steadily increase. Statistical problems also exist with systems in which all replicates of each treatment share a single reservoir (e.g. Peterson and Krizek, 1992). In such systems, random fluctuations in the behaviour of the reservoir systems confound treatment effects, consequently increasing the risk of incurring Type I errors.

Development of a hydroponic system suitable for root restriction studies requires that these potential constraints be accommodated in the design of the system. This experiment identifies and quantifies the critical levels of inputs necessary to minimise any confounding of root restriction experiments by artefacts of the hydroponic system.

2.1.1 pH

Solution pH influences the solubility, and therefore availability, of nutrients to plants. Left unchecked, considerable drift in pH occurs in hydroponic solutions supporting tomatoes (Winsor et al., 1979). As tomatoes are

intolerant of ammonium-nitrogen (NH_4^+-N) (Pill and Lambeth, 1977; Winsor and Massey, 1978), NO_3^--N is the preferred nitrogen source. Solutions containing NO_3^--N become alkaline due to differential uptake of NO_3^- ions and principal cations (Salisbury and Ross, 1978). The more rapid absorption of NO_3^- ions than cations increases pH as bicarbonate (HCO_3^-) and hydroxyl (OH^-) ions are transported out of the root to satisfy the charge difference.

The pH recommended for NFT systems is between 5.5 and 6.5 (Graves, 1983; Willumsen, 1980). At this pH, phosphates remain in the more soluble dihydrogen form (Steiner, 1966) and iron chelates remain associated and therefore less liable to precipitation.

2.1.2 Nutrient regime

Tomatoes in recirculating systems tolerate a wide range of nutrient concentrations. Negligible differences in yield have been observed with plants grown at nitrogen (N) concentrations ranging from 10–320 mg NO_3^--N/litre (Massey and Winsor, 1980a), 20–375 mg potassium (K)/litre (Winsor and Massey, 1978), and 5–200 mg phosphate (P)/litre (Massey and Winsor, 1980b). Although growth is not depressed at low concentrations, high concentrations are generally used to provide a reserve of nutrients within the system (Winsor et al., 1980). As a consequence, the tolerance to a range of nutrient concentrations has generated several nutrient formulations (Benton-Jones, 1982; Cooper, 1979; Winsor et al., 1979).

2.1.3 Nutrient and oxygen replacement

An advantage of hydroponic systems over solid media systems is that a uniform environment within the root zone can be maintained. To maximise

this advantage, flow rates must be high enough to ensure that minimal depletion of nutrients and O_2 occurs as the solution passes through each root system (Asher, 1981). The lack of response of tomatoes to low concentrations of major elements (Massey and Winsor, 1980a, 1980b; Massey and Winsor, 1978) is attributed to flow rates sufficient to replace nutrients absorbed from the immediate root zone.

Leaves of poorly aerated tomatoes wilt, develop an epinastic curvature, become chlorotic, and often abscise prematurely (Erickson, 1946; Jackson, 1980). Rates of leaf expansion and dry matter accumulation are reduced, stem growth is slowed and yield reduced (Jackson et al., 1984). Anoxic roots produce 1-amino-cyclopropane-1-carboxylic acid (ACC), a precursor of ethylene production (Jackson and Campbell, 1976; Jackson et al., 1978). Physiological consequences of this include swollen stem bases and adventitious roots developing on aerial portions of the stem. Such responses will develop with only part of the root system affected.

Environmental factors strongly influence the O_2 content of hydroponic solutions. Water saturated with air contains about 9.1 mg·litre^{-1} of dissolved O_2 at 20°C. Nutrient salts reduce this capacity by 2-3% (Hitchman, 1978). The O_2 content decreases as the temperature of the solution increases, a rise from 20-30°C depressing the O_2 content of air-saturated water to 7.5 mg·litre^{-1} (Hitchman, 1978). Increasing temperatures also increase the respiratory demand for O_2 by the roots. Thus, a combination of lowered reserves and greater consumption of dissolved O_2 increases the possibility that the O_2 supply may become limiting. Microorganisms in the solution exacerbate the problem by competing with roots for O_2. At temperatures between 20-23°C, O_2 consumption by microorganisms in nutrient solutions is about 0.3–0.4 mg·litre^{-1}·hour^{-1} (Jackson, 1980).

Solution flow rates: theory

Depletion of nutrients and O_2 is reduced to negligible proportions if the rate of flow of the solution is controlled (Asher et al., 1965; Trelease and Livingstone, 1922). Determining flow rates appropriate to nutrient and O_2 concentrations of a hydroponic system relies on estimates of the rate of uptake or consumption. The absolute rate of uptake of a nutrient ion or O_2 is the product of the flow rate and the difference in concentration between the incoming and outgoing solutions (Edwards and Asher, 1974):

$$U = F(C_i - C_o) \tag{2.1}$$

where U is the absolute uptake per plant ($mg \cdot hour^{-1}$),
F is the flow rate per plant ($litres \cdot hour^{-1}$),
and C_i, C_o are the inlet and outlet concentrations ($mg \cdot litre^{-1}$).

Although the outlet flow rate is reduced from that of the inlet by transpiration, the amount is small and may be neglected. Adams and Winsor (1979), for example, reported an average daily water uptake of 24 $ml \cdot hour^{-1}$ by indeterminate tomato plants with one flower truss. Such rates of uptake would only affect the relationship in equation (2.1) if very low flow rates were being considered.

The percent decrease in solution concentration, D, from uptake U is given by:

$$D = \frac{100(C_i - C_o)}{C_i} \tag{2.2}$$

Substituting for $(C_i - C_o)$ in equation (2.2) and solving for F, the lowest flow rate that will maintain the outlet concentration within D percent of the inlet concentration is given by:

$$F = \frac{100U}{(C_i \cdot D)} \tag{2.3}$$

Besides estimates of O_2 consumption in hydroponic solutions, the theoretical replacement rate needed to satisfy the O_2 demand requires estimates of the rate of O_2 consumption by microorganisms, the size of root system and the volume of solution for each plant.

Each plant in the hydroponic system has V_p litres of solution where:

$$V_p = \frac{V_s}{n} \tag{2.4}$$

where V_s is the total solution volume of the system (litres), and n is the number of plants in the system.

The rate of O_2 consumption by microorganisms (O_m) on a per plant basis (O_{mp}) is thus:

$$O_{mp} = O_m \cdot V_p \text{ (mg } O_2\text{/plant/hour)} \tag{2.5}$$

Oxygen consumption by roots (O_c) is derived either from the product of the root respiration rate, R_r (mg O_2/g root fresh weight/hour) and the root fresh weight per plant, W_r (g root fresh weight/plant), thus:

$$O_c = R_r \cdot W_r \text{ (mg } O_2\text{/plant/hour)} \tag{2.6}$$

or from direct measurements of total plant root consumption (e.g. Gasim and Hurd, 1980).

Total O_2 consumption on a per plant basis (O_{tc}) is given by:

$$O_{tc} = O_{mp} + O_c \text{ (mg } O_2\text{/plant/hour)} \tag{2.7}$$

The O_2 content of the solution, O_{sc}, expressed on a per plant basis, is the product of the measured O_2 concentration of the solution, O_{conc}, and V_p.

$$O_{sc} = O_{conc} \cdot V_p \quad (\text{mg } O_2/\text{litre/plant}) \tag{2.8}$$

From equation (2.2), the rate of consumption of O_2 in a flowing solution is the product of the flow rate (F) and the difference in concentration between the incoming (O_{sc}) and outgoing solutions (O_{sco}):

$$O_{tc} = F(O_{sc} - O_{sco}) \quad (\text{mg } O_2/\text{plant/hour}) \tag{2.9}$$

The percent decrease, D, in solution concentration resulting from consumption, O_{tc}, is given by:

$$D = \frac{100(O_{sc} - O_{sco})}{O_{sc}} \tag{2.10}$$

Substituting for (O_{sc} - O_{sco}) in equation (2.10) and solving for F:

$$F = \frac{100 \cdot O_{tc}}{(O_{sc} \cdot D)} \quad (\text{litres} \cdot \text{hour}^{-1}) \tag{2.11}$$

Substituting for O_{tc} from equation (2.8)

$$F = \frac{100(O_{mp} + O_c)}{(O_{sc} \cdot D)} \quad (\text{litres} \cdot \text{hour}^{-1}) \tag{2.12}$$

2.1.4 Statistical independence

Although static systems allow statistically independent treatment × replicate combinations (Jarrett and Chanter, 1981), this advantage is outweighed by the lack of control inherent in these systems. Additionally, it is possible that tomato plants themselves interact in a shared hydroponic system. In a somewhat equivocal study, Tucker (1977) reported a correlation between root death and the concentration of cytokinin-like substances exuded into the solution from the roots. In a subsequent study, however, while confirming the presence of cytokinin-like substances, Tucker (1981) found no evidence of their accumulation or consequent root death. Certainly, Tucker (1977) was unable to show whether roots were killed by the buildup in contaminants or whether they were released by aged and dying roots. Similarly, neither Hurd and Gay (1977) nor Sims (1977) observed detrimental effects on germination or early seedling growth using solution from systems in which root growth was poor and dead roots existed. Nevertheless, it is not possible, within any experiment, to be absolutely certain that no interaction among treatments is taking place.

Interaction among treatments is aided primarily by the closed nature of hydroponic systems. This ensures complete dispersal of the contaminants throughout the system and facilitates an increase in their concentration. Tucker's (1977) demonstration was made under conditions likely to maximise occurrence of interaction. Plants were grown in NFT channels, in a serial layout, and the total volume of solution per plant was about 0.6–0.8 litres, which is a common feature of NFT systems (Graves, 1983). Low volume systems accentuate small increases in absolute levels of contaminants to comparatively large increases in their concentration (Hurd, 1978).

Jarrett and Chanter (1981) suggested that peristaltic pumps, supporting several different supply lines, would avoid problems arising from interacting

treatments, while still providing a recirculating system. This is an expensive option, and does not directly address the problem of contaminant buildup.

2.2 Methods

2.2.1 Base system

The hydroponic system used in this study was based on the Deep Flow Technique (DFT) system of Willumsen (1983). Plants were grown singly in opaque, ten litre containers (Fig. 2.1). A vertical overflow tube, linked to a return pipe, maintained a constant level of solution in each container. After allowing for the positioning of the return pipes, each container had an effective solution volume of nine litres, and with two 100 litre reservoirs (arranged in series), the total solution volume per plant was about 11 litres.

Maintaining an active flow past the root systems is important in reducing the boundary layer around the roots, as the uptake of O_2 in deep flow systems is highly dependent upon the O_2 concentration of the immediate root environment (Zeroni et al., 1983). Consequently, the DFT system was modified to permit solution exchange within the container. The recirculating solution entered each container via a microtube (3.5 mm internal diameter) from the main delivery manifold, and returning solution entered the overflow tube at the base of the container. This was confirmed by placing dye crystals at the top of the solution and watching the movement of the dye streams. Nutrient solution with the same composition was fed into each container by arranging the containers in parallel with respect to solution flow. A loose fitting polystyrene lid, draped with a black polythene film (40 μ), covered each container. Negligible light entered the container.

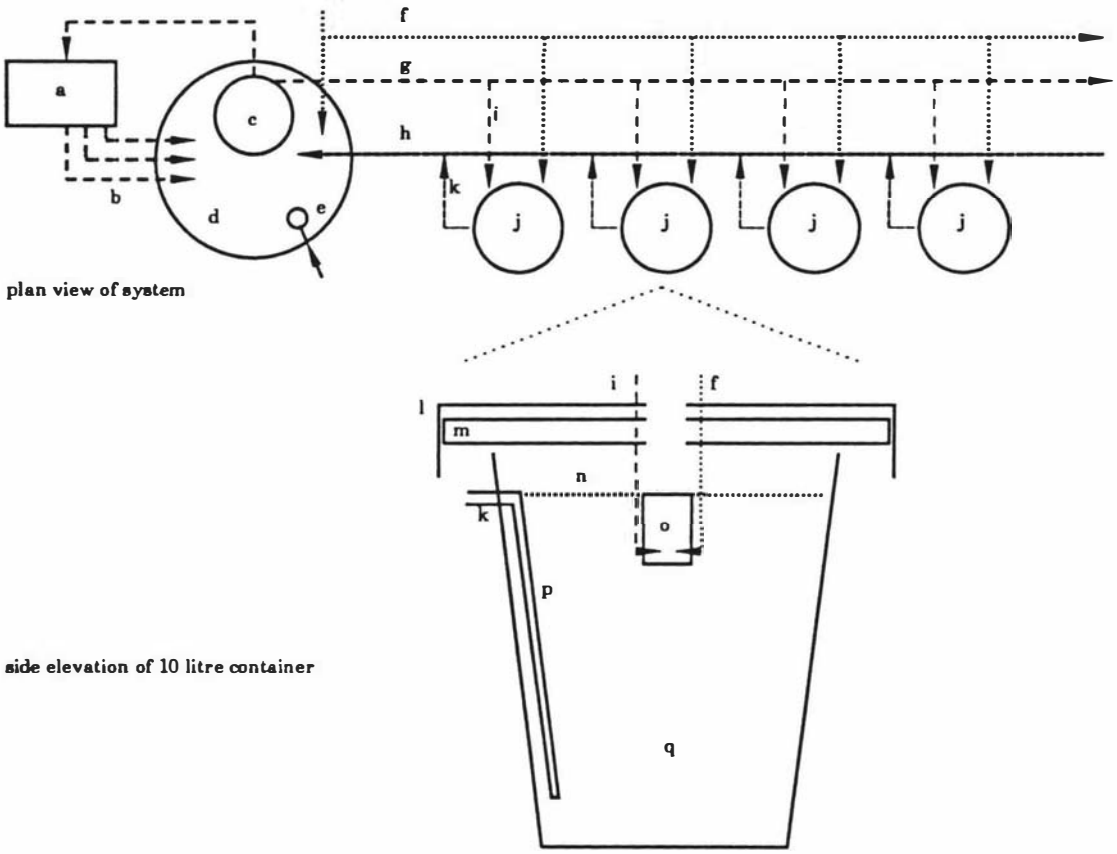

Fig. 2.1. Schematic view of hydroponic system.

A Terada submersible water pump (model SL100; 100 litre·min^{-1};[c]) in one of two 100 litre sumps [d] (each of which contained a float valve [e] connected to a fresh water supply) delivered nutrient solution to each 10 litre container [j,q] via a 3 mm feeder line [i] from the main 50 mm diameter polythene delivery pipe [g]. Solution from these containers flowed through an internal/external 12.5 mm diameter pipe [p/k] leading into a 50 mm diameter rigid PVC pipe [h], and back to the sumps. The solution was monitored for pH and EC levels [a] using a Dosetronic controller with necessary injections of acid or nutrient stock solution [b] automatically made to maintain the set points. Air lines [f; 2 mm diameter microtubes] were inserted into the sump, each 10 litre container, and into each restrictive container [o]. Each restrictive container was submerged below the level of solution [n] in the 10 litre container. A polystyrene lid [m] covered with a black polythene sheet [l], together with the opaque walls of the 10 litre container, inhibited light penetration to the root system.

2.2.2 Contaminant control

Only one of the two 100 litre reservoirs was needed for the system to be functional. However, given Tucker's (1977) demonstration of the type of plant × plant interaction possible, increasing the volume of solution of the system was the simplest way to reduce the effective concentration of any contaminants.

Possible buildup in concentration was further minimised by regularly replacing some of the solution. As total replacement after each cycle through the system was neither practical nor environmentally sound, a balance between excessive replacement rates and rates that minimise the potential buildup in concentration of contaminants was sought. A spreadsheet-based model, describing the effect of solution replacement on the buildup of contaminants in a hydroponic system, was used to help decide the percentage of the solution to be replaced daily. The fundamental equation of the model (2.13) assumed that both exudation rate of contaminants and volume of solution per plant were constant over time.

$$\Delta C = (C_t + C_{t-1}) - \left[\frac{(C_t + C_{t-1}) \times R_p}{100}\right] \qquad (2.13)$$

where ΔC is the daily change in concentration, C_t and C_{t-1} are the concentration of contaminants after t and t-1 days in solution, and R_p is the percentage of solution replaced in day t.

Using an estimate of the exudation rate of cytokinin-like contaminants (21 μg/g root dry weight/day; Tucker, 1981, Fig. 1), the volume of solution (11 litres/plant), and the dry weight of roots (g/plant) within the anticipated 100 day period of experiments in the system (2.3 to 5.2 g; pers. obs.), the model predicted the concentration of contaminants on a daily basis (Fig. 2.2).

Results from the model suggested that a daily replacement rate of 10% would avoid a buildup in the concentration of any contaminant.

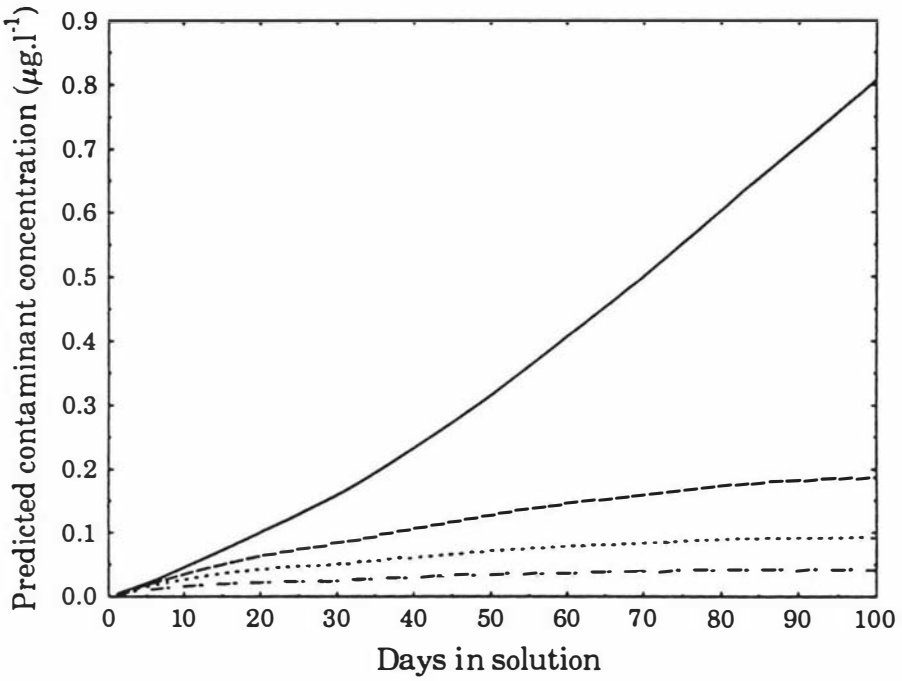

Fig. 2.2. Predicted influence of replacement regimes on concentration of contaminants in hydroponic system at a constant exudation rate of 21 μg/g root dry weight/day. *Key:* – 0% replacement, ---- 5% replacement, ····· 10% replacement, –·– 20% replacement.

Sensitivity analyses were conducted using various combinations of replacement rates (0, 5, 10 and 20%) and exudation rates (21, 21×10^1, 21×10^2, and 21×10^3 μg/g root dry weight/day). Even at the highest rate of exudation tested, the predicted concentration after 100 days, using a 10% daily replacement rate, was 92 $\mu g \cdot litre^{-1}$, considerably less than the 10–80 $mg \cdot litre^{-1}$ associated with root death (Tucker, 1977).

The replacement rate was obtained by allowing a delivery line from the pump to run to waste at 3.8 $litres \cdot hour^{-1}$. Float valves in the reservoirs

compensated for the loss by introducing fresh (uncontaminated) water to maintain a constant volume of solution in the system.

2.2.3 Solution composition

The nutrient solution used was based on Hoagland and Arnon (1938), modified by using chelated iron, and with the N:K ratio modified after Cooper (1975) and Sonneveld (1981) (Table 2.1).

Table 2.1 Major and minor elements in hydroponic solution.

Salt	Stock solution	Final solution	
	g·litre^{-1}	nutrient	ppm
Stock solution A.			
calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$)	196.64	Ca N	168 117
iron chelate $[CH_2 \cdot N(CH_2COO)_2]_2FeNa$	7.34	Fe N	5.6 1.4
Stock solution B.			
potassium dihydrogen phosphate (KH_2PO_4)	54.39	K P	78 62
potassium nitrate (KNO_3)	131.27	K N	254 91
magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	98.99	Mg S	49 64
boric acid (H_3BO_3)	0.37	B	0.32
manganous chloride ($MnCl_2 \cdot 4H_2O$)	1.58	Mn Cl	2.2 2.8
zinc sulphate ($ZnSO_4 \cdot H_2O$)	0.055	Zn S	0.1 0.1
copper sulphate ($CuSO_4 \cdot 5H_2O$)	0.05	Cu S	0.065 0.032
molybdic acid ($Na_2MoO_4 \cdot 2H_2O$)	0.004	Mo	0.1
potassium chloride (KCl)	0.63	K Cl	1.65 1.50

Stock solutions were made up in two 20 litre tanks (Stock solution A and Stock solution B), and diluted about 1 in 100 to the final solution (Table 2.1). Actual dilution was controlled automatically with a Dosetronic hydroponic controller (New Zealand Hydroponics Ltd, Tauranga) to maintain a solution conductivity of 2.5 (±0.1) $mS \cdot cm^{-1}$.

The same controller monitored and automatically controlled the pH of the solution to 6.0 (± 0.1). Nitric acid (HNO_3) was injected into the solution when pH rose above 6.0. On the few occasions that the solution pH constantly fell below the set level, the nitric acid was replaced by sodium hydroxide.

2.2.4 Solution flow rates

The difference in concentration of nutrients and O_2 between inlet and outlet was not allowed to exceed 5% in this study.

Nutrient calculations

Nitrogen and potassium are the dominant nutrients taken up by tomatoes (Gasim and Hurd, 1980; Willumsen, 1980). The minimum flow rate necessary to avoid nutrient depletion was estimated from published data of N and K uptake in tomato (Table 2.2), and the N and K concentrations (about 210 and 330 $mg \cdot litre^{-1}$ respectively, Table 2.1) of the system.

From the mean uptake rates of N and K (Table 2.2), the minimum flow rate per plant calculated to limit depletion to less than 5% of the inlet concentration was 0.4 $litre \cdot hour^{-1}$.

Flow rate was controlled with flow valves on the main delivery manifold, and clamps on each microtube entering the container (Fig. 2.2 [i]). The microtube was plugged whenever plants were harvested from the container.

Table 2.2 Nitrogen and potassium uptake data for tomato.

Uptake rate/plant ($mg \cdot hour^{-1}$)	Concentration ($mg \cdot litre^{-1}$)	Source of data
Nitrogen		
5.4	270	Attenburrow and Waller (1980)
3.4	≈200	Cooper and Charlesworth (1977)
1.4[x]	≈210	Gasim and Hurd (1980)
4.4[y]	≈210	Gasim and Hurd (1980)
8.4[z]	≈210	Gasim and Hurd (1980)
6.2	not specified	Khudhier and Newton (1983)
4.6	160	Massey and Winsor (1980a)
3.5	205	Schippers (1980)
2.9	185	Winsor and Massey (1978)
mean=4.5		
Potassium		
10.0	430	Attenburrow and Waller (1980)
5.3	330	Cooper and Charlesworth (1977)
10.0[y]	330	Gasim and Hurd (1980)
11.5	not specified	Khudhier and Newton (1983)
6.5	210	Schippers (1980)
8.3	100	Winsor and Massey (1978)
mean=8.6		

[x] 10 week old plants; [y] 20 week old plants; [z] 30 week old plants

Oxygen calculations

Oxygen consumption was calculated from data of Jackson (1980) and Gasim and Hurd (1980). In both instances, estimates of O_c were calculated from O_2 consumption data presented in units of mg O_2/hour/g root fresh weight and estimates of root fresh weight of tomatoes of different ages (Table 2.3).

Table 2.3 Oxygen consumption rates of tomato plants, calculated from data in the literature.

Description and conditions of plant	O_2 consumption per plant (O_c) (mg $O_2 \cdot hour^{-1}$)	Source of data
49 days old, 70 g root fresh weight, ~20°C solution temperature	24	Gasim and Hurd (1980)
70 days old, 190 g root fresh weight, ~20°C solution temperature	30	Gasim and Hurd (1980)
140 days old, 475 g root fresh weight, ~20°C solution temperature	60	Gasim and Hurd (1980)
~60 days old, 200 g root fresh weight, 20°C solution temperature	90	Jackson (1980)
~60 days old, 200 g root fresh weight, 25°C solution temperature	132	Jackson (1980)

Oxygen consumption rates calculated from Jackson (1980) are higher than those of similarly aged plants of Gasim and Hurd (1980). Whereas Gasim and Hurd (1980) directly measured O_2 consumption, Jackson (1980) estimated O_c with an assumed respiration rate. Consequently, Gasim and Hurd's (1980) data were used in designing the system (Table 2.4).

Table 2.4 Calculations of O_2 supply and solution flow rate for hydroponic system.

Variable	Minimum	Maximum
Volume of solution per plant (V_p)[w]	11	11
Rate of O_2 consumption by microorganisms (O_m)[x]	0.3	0.4
Rate of O_2 consumption by microorganisms per plant (O_{mp})	3.3	4.4
O_2 consumption by roots (O_c)[y]	60	90
Total O_2 consumption (O_t)	63.3	94.4
Measured O_2 concentration of solution (O_{conc})[z]	8.25	8.25
O_2 content per plant (O_{sc})	90.8	90.8
Percent decrease (D)	5	5
Required flow rate for <5% depletion (F)	7.0	20.8

[w] includes container and proportion of reservoir volume; [x] from Jackson (1980); [y] see Table 2.2; [z] measured directly at 20°C.

2.3 Discussion

Experimental systems must be developed not only with the objectives of the research in mind, but also recognising the constraints they may impose on 'normal' plant growth. Every effort must be made to identify and either remove or accommodate potentially confounding factors.

In developing this hydroponic system for root restriction studies, operating criteria for rates of solution replacement, flow, and nutrient and oxygen content have been quantified (Table 2.5). Importantly, several of these criteria identify minimum levels, below which operation of the system introduces factors that can confound treatment effects and obscure plant response.

Table 2.5 Summary of operating criteria of hydroponic system.

Component	Range of operating conditions	Actual operating condition
Solution management		
flow rate	minimum 14–21 litres·hour^{-1} for 5% depletion in O_2 minimum 0.4–0.5 litres·hour^{-1} for 5% depletion in N and K	30 litres·hour^{-1}
replacement rate	5–20%	10% (3.8 litres·hour^{-1})
temperature	min. 15–16°C	20-25°C
Solution composition		
N	10–320 mg·litre^{-1}	≈ 210 mg·litre^{-1}
P	20–375 mg·litre^{-1}	≈ 330 mg·litre^{-1}
K	5–200 mg·litre^{-1}	≈ 62 mg·litre^{-1}
EC	2.5–4.0 mS·cm^{-1}	2.5 ± 0.1 mS·cm^{-1}
pH	5.5–6.5	6.0 ± 0.1

This study highlights the relative importance of oxygen and nutrient concentrations. The flow rates of solution necessary to maintain a similar oxygen concentration throughout the root system are considerably higher than those required to maintain a similar nutrient environment (Table 2.5).

The hydroponic system developed here does not resolve the issue of statistical independence of experimental units (Jarrett and Chanter, 1981). It does, however, reduce this source of interaction to negligible physiological levels. The compromise reached between the possibility of statistical interaction and the need for a practical and uncomplicated system has taken the problem to one of being theoretically possible, but unlikely to occur. There is every reason to believe that results from experiments in this system are statistically and physiologically valid.

Chapter 3

Growth ontogeny under root restriction

3.1 Introduction

The physical restriction of roots of plants growing in containers induces a variety of responses, ranging from no response (McGowan and Devereux, 1989) to enhanced harvest index (Al-Sahaf, 1984; Carmi and Shalhevet, 1983; Richards, 1981; Ruff et al., 1987) through to senescence and death (Tschaplinski and Blake, 1985). Such divergence of response makes the management of container-grown plants an inexact science for plant growers, and frustrates the efforts of scientists seeking to explain the mechanistic basis of plant response to root restriction. Studies of the effects of root restriction on plant growth are difficult to interpret because the nature of the treatment changes through time. Unlike studies investigating growth responses to light and temperature, a root restriction treatment cannot be controlled at a given level. As roots grow throughout the container, the level of restriction they encounter increases, and therefore the intrinsic nature of the treatment changes. This factor is a major contributor to the diversity of both the reported effects of root restriction on plant growth and the mechanisms controlling them.

In their widely cited paper, Richards and Rowe (1977a) suggested, after observing a 39 per cent reduction in root dry weight and a 'consequent' comparable (34 per cent) reduction in top dry weight of container-grown peach (*Prunus persica* Batsch) seedlings, that the root regulated the growth of the top. Because application of benzyladenine partially restored shoot growth of the seedlings, Richards and Rowe (1977a) concluded that growth in small containers was restricted by a reduction in supply of cytokinins from root apices. Implicit in this view is the idea of an hormonal equilibrium (van Noordwijk and de Willigen, 1987) in which continuous activity of hormone-producing root meristems is required for normal shoot growth. Fluctuations in this production, possibly resulting from root restriction, limit plant growth independently of nutrient and water uptake.

Although internal regulation of root and shoot growth can be overruled by exogenous application of growth substances (Carmi and Heuer, 1981; Richards, 1980; Richards and Rowe, 1977a), the modification is usually short-lived, with the plants returning to their pretreatment balance between root and shoot growth even when exogenous hormone supply is maintained (Thuantavee, 1991). On the other hand, equilibrium between root and shoot growth is unlikely to be the simple maintenance of ratios of biomass (cf. Richards and Rowe, 1977a). Rather, any role that roots may have in regulating shoot growth through the supply of hormones is likely to be through maintenance of functional equilibria.

Functional equilibria describe precise balances between the respective outputs of the root and shoot systems: the product of root weight and uptake (nutrients and water) activity remains in constant proportion to the product of shoot weight and the photosynthetic activity of the shoot (Brouwer, 1963; Davidson, 1969a; Hunt, 1975; Thornley, 1975). Such equilibria relating root uptake functions with shoot and plant biomass production have been empirically described with data collected from single-harvest experiments involving a range of differently treated plants (Richards, 1977, 1980; Richards and Rowe, 1977b). Seeking to similarly describe relationships between the growth characteristics of root and shoots under conditions of root restriction have proven more difficult. Many studies have based their inferences on results from a single harvest, despite reports (e.g. Carmi et al., 1983) showing that the relative reductions in growth between restricted and unrestricted plants progressively increase with time. Assessment of single-harvest experiments invariably yields simple ratios of biomass and organ counts. Their use in interpreting results promotes, probably inadvertently, the idea of morphogenetic equilibria (i.e. "the more roots the better the shoot growth"; van Noordwijk and de Willigen, 1987), thus masking the possible involvement of a functional equilibrium between root and shoot growth characteristics. One possible solution to avoid this problem is to examine the dynamic nature of

plant response to root restriction treatments and seek, through appropriate growth analysis, those relationships between root and shoot growth remaining in proportion or balance over time, irrespective of the degree of root restriction encountered by the plant.

Two general approaches, the *classical* and the *functional*, dominate plant growth analysis (Causton and Venus, 1981; Hunt, 1982). The classical approach measures the efficiency and productivity of the plant based on calculations using data between adjacent harvests. In the functional approach, a single curve is fitted to the data from all harvests, effectively using the information content of the whole data set. Neither approach on its own, however, will provide the necessary information required to detect stable relationships between shoot and root growth under conditions of root restriction. Although the classical approach yields many estimates of indices that can be further analysed to detect treatment differences (e.g. analysis of variance (ANOVA)), local (short term) disturbances in growth at either adjacent harvest can increase the variance of mean values. The classical approach is also resource intensive, requiring large (many replicates) harvests taken frequently if short-term fluctuations in response are to be detected. The functional approach, on the other hand, is less demanding of resources and is more flexible in its requirements for replication and harvesting frequency. The data 'smoothing', however, that accompanies the curve fitting procedure can reduce the sensitivity of the approach to short-term changes in growth (Wickens and Cheeseman, 1988). As currently presented (e.g. Causton and Venus, 1981; Hunt, 1982; Hunt and Evans, 1980), many functional procedures inadequately accommodate structured experimental designs in non-constant environmental conditions (e.g. greenhouses). The "...economy of expression..." afforded by the functional approach (Hunt, 1982), in which large data sets are condensed into estimates of a few parameters, can be a disadvantage if further analysis of the parameters is desired. A hybrid

method, merging the advantages of the classical and functional approaches with the benefits of alternative approaches, is required.

A rapid improvement in the range of techniques available for analysing plant growth occurred during the late 1970s and early 1980s (reviewed by Causton and Venus, 1981; Hunt, 1982). Difficulties associated with pairing plants from adjacent harvests in calculating relative growth and net assimilation rates were overcome (Venus and Causton, 1979b), and in concert with concurrent developments in linear and nonlinear regression (Myers, 1990), the range of functions suitable for describing plant growth, and the rigour of their application, was improved (Causton and Venus, 1981; Elias and Causton, 1976; Nicholls and Calder, 1973). Allometric analysis, having its genesis in zoology (Huxley, 1924; 1932) and previously sporadic use in agronomy (e.g. Troughton, 1955), was increasingly used to describe the partitioning ratio between root and shoot growth in studies throughout the wider spectrum of plant science. More recently, however, plant growth analysis techniques have not kept pace with, or taken advantage of, advances in the computing and statistical tools now available to plant scientists. The few alternative approaches suggested (e.g. Poorter, 1989; Poorter and Lewis, 1986) have tended to be inwards-looking, seeking to merge existing approaches rather than incorporate new methods, or have been oriented towards crop growth analysis (Hardwick, 1984; Hunt et al., 1984; Warren Wilson et al., 1986). Use of both classical and functional approaches has tended to be self-terminating, with little subsequent use of their output for secondary analyses. In particular, use of multivariate analysis techniques has been conspicuously absent in recent studies involving growth analysis, yet these techniques have considerable potential for accessing and interpreting the interactional complexity of plant growth.

The main purpose of this study was to follow the ontogeny of relationships between root and shoot growth of tomato plants in response to root restric-

tion. Additionally, I sought to incorporate and compare the efficacy of different statistical methods suited to plant growth analysis to describe this ontogeny in a way that allowed insight into its complexity.

3.2 Materials and methods

3.2.1 Cultural methods.

Plant material

Seed of the indeterminate tomato *L. esculentum* Mill. 'Moneymaker' were germinated under mist in a glasshouse. Air temperature was maintained between a minimum of 16°C and a maximum of 25°C. Seedlings were transplanted to 5.0 × 5.0 × 8.0 cm tapered tubes (200 cm^3) when the cotyledons were fully expanded, and grown on in the glasshouse for six weeks. During this phase, the seedlings were grown in 3 peat: 2 pumice (by volume) medium amended with dolomite (3.0 $kg \cdot m^{-3}$) and PG Mix (14-7-15 NPK + trace elements, 3.0 $kg \cdot m^{-3}$, Windmill, Holland), a base starter fertiliser. Stem cuttings, 6-7 cm tall, were rooted in a 1 peat:9 pumice (by volume) propagation medium under closed mist. Four days later, the cuttings were transferred to an open mist system for hardening off. On average, plants were 16±0.5 cm tall, with a leaf area of 119±9 cm^2, 58±6 roots with a total length of 1.3±0.1 m, and a total plant dry weight of 0.9±0.08 g.

Stem cuttings, rather than seedlings, were used to limit the type of root being evaluated. Just as there is diversity in the components of a shoot system, a root system is a heterogenous collection of components with different internal structure and function (Waisel and Eshel, 1991; Zobel, 1992b). Using tomatoes as an example, Zobel (1986) identified four types of root: radicle, lateral, adventitious, and basal. By using stem cuttings, this potential

variation was limited to only adventitious and lateral roots. In addition, to distinguish between hypocotyl adventitious roots (Byrne and Aung, 1974) and true adventitious roots (Zobel, 1986), the cuttings were taken above the cotyledonary node. Studying the influence of adventitious root restriction was also consistent with the widespread production in containers of crops raised from cuttings.

Hydroponic system

The hydroponic system was based on the Deep Flow Technique (DFT) system of Willumsen (1983) as detailed in Chapter 2. In this system solution exchange is effected by vertical flow within the container. The containers were arranged in parallel with respect to solution flow, ensuring that the solution entering all containers had the same composition. The nutrient solution was a modified Hoagland solution (Table 2.1).

3.2.2 Environmental

Air temperature in the glasshouse was maintained at 15°C minimum at night, with ventilation triggered when temperature rose above 25°C. Daily mean temperature ranged from 18–23°C.

3.2.3 Experimental

The influence of a series of root restriction and de-restriction with container volumes of 0.025, 0.05, and 10 litres on plant growth was examined (Table 3.1). The 0.025 and 0.05 l containers were constructed from sections of PVC pipe, 2 cm and 4 cm internal diameter respectively. Both ends of the container were covered with black polythene film. A 3 mm irrigation fitting

was tapped into the base of the container for connection to the hydroponic delivery pipes. The smaller containers were fitted into polystyrene lids placed over the 10 l containers. As root temperature strongly influences shoot growth in tomatoes (Maletta and Janes, 1987), possibly through altered balances in the hormone content of xylem sap (Menhenett and Wareing, 1975), the small containers were immersed in the hydroponic solution flowing through the 10 l container to ensure that root temperatures in all treatments were similar. The polystyrene lids and 10 l containers were covered with black plastic film to exclude light from the root environment.

Treatments were initiated immediately after the plants were introduced into the hydroponic system. Roots present on cuttings at this time did not appear to continue growing. New roots appeared from the immersed stem after about 3 days; the root system developed from these latter roots. No visual signs of water stress were observed in the plants during this transition period.

Plants were supported vertically by crop training twine (Veg-Gro Supplies, Auckland) attached around the centre of the stem and connected to an overhead grid of supporting wires (2.1 m above the greenhouse floor). Plants were destructively harvested 13, 31, 45, 67, and 99 days later. After 31 days after initiation of the experiment (DAI), pre-assigned plants were de-restricted from 0.025 l containers into either 10 l containers (RD) or 0.05 l containers (RDD). After 67 DAI, plants in 0.05 l containers were de-restricted into 10 l containers (Table 3.1).

Table 3.1 Details of experiment

Treatment description	unrestricted, UR (10 l)
	continuously restricted, CR (0.025 l)
	restrict-derestrict, RD (0.025 l → 10 l)
	restrict-derestrict-derestrict, RDD (0.025 → 0.05 → 10 l)

3.2.3.1 Experimental design and data collection

A randomised complete block (RCB) design incorporating 6 blocks was used to provide local error control against known profiles of temperature and light within the glasshouse. From a pool of 168 rooted cuttings, 120 similarly sized plants (experimental units) were selected and randomly allocated to the blocks (i.e. 20 plants per block). Treatments were randomly allocated to positions within each block, and harvest dates then randomly allocated to plants within each treatment position. Thus, each block consisted of 4 restriction treatments, with each treatment consisting of 5 plants, one for each of the destructive harvests. At any one destructive harvest, 24 plants were removed from the system and assessed.

Blocks were harvested sequentially, with the shoot growth variables (component fresh weight, plant height, leaf area and leaf counts) completed for each plant within 30 minutes of removal from the hydroponic system. In contrast, the time required to record the length and number of roots of a single plant increased from 1.5 to 6.5 hours as the experiment progressed. Therefore, root systems were not measured until the shoot measurements for all plants were completed. After shoot removal, root systems were stored intact and submerged in water in 10 l plastic buckets in a cold (4-7°C) store. A compressed air line inserted into each root system avoided development of anaerobic conditions.

Total leaf area was measured with a Li-Cor LI-3100 leaf area meter (Lambda Instruments Co., Lincoln, NE, USA) and root length measured with a Comair root length scanner (Commonwealth Aircraft Co., Melbourne, Australia). Root number was measured by eye by counting the number of branches (Evans, 1976) of portions of the root system suspended in water in a petri dish laid against a dark background.

Root numbers of the entire root system of each plant were counted for the first two harvests (13 and 31 DAI). By 45 DAI, however, root systems were too large for this task to be practically accomplished and a sampling procedure was introduced. Roots were cut into about 2.5 cm lengths and laid onto a sampling tray (45 × 60 cm) in sufficient water to allow the roots to be distributed uniformly over the tray. Once achieved, the water was slowly removed through a drain at the centre of the tray. From a grid of 9 × 12 cm squares drawn on the base of the tray, 2.5 squares (10% of the area of the tray) were randomly selected, and the roots removed after cutting the outline of the grid through the root mass. The length of the complete root system was measured at each harvest.

Leaf water potential was measured early morning (730-830 h) and at solar noon (1200-1300 h) with a pressure chamber (model 3005, Soilmoisture Equipment Corporation, USA). A leaflet (≈60 cm^2) fully exposed to sunlight and next to the terminal leaflet was measured. The leaflet was enclosed in a plastic bag containing moistened filter paper, cut immediately below the petiole-stem junction, and within 10 seconds was transferred to the pressure chamber in a laboratory attached to the greenhouse. A humid environment was maintained in the pressure chamber by a lining of moistened capillary matting. Extrusion errors were avoided by ensuring the petiole length protruding from the chamber did not exceed 5 mm (Turner, 1981). The chamber was pressurised at about 0.2-0.25 $bar \cdot s^{-1}$ (Lakso, 1992) until the end point was approached after which the rate was reduced to 0.1-0.125 $bar \cdot s^{-1}$.

Leaf epinasty (downward growth) and appearance of adventitious roots on stems are symptoms of oxygen stress in tomato (Jackson and Campbell, 1976). Estimates of leaf epinasty were made at 31, 45, and 67 DAI by measuring the angle between the stem and the adaxial surface of the lower (oldest) six to eight petioles on the plant (Jackson and Campbell, 1976). Counts of adventitious roots were taken as required.

Dry weights of all components of plant biomass were measured after 48 h drying at 80°C in a forced air oven.

3.2.3.2 Pre-analysis adjustment of raw data

Adjustment for root senescence and turnover

Although root senescence and turnover is a normal characteristic of all crops (Klepper, 1991; Vogt and Bloomfield, 1991), root dieback in fruiting tomato plants grown in hydroponic systems can be severe. Little or no replacement of new roots occurs, leaves display symptoms of transient water stress and shoot growth is retarded (Hurd and Price, 1977). Although usually associated with the onset of fruit growth (Hurd and Gay, 1977), Tucker (1981) has suggested that root dieback is a consequence of a change in the auxin/cytokinin balance in the roots. As root restriction may influence cytokinin levels (Carmi and Heuer, 1981; Richards and Rowe, 1977a), and possibly their balance with auxin levels (Costa et al., 1992), restricted root systems might contain proportionately more dead root tissue than unrestricted systems. Without adjusting root data for these differences, direct comparisons among root characteristics (e.g. length, number and mass) and shoot growth functions of the treatments would not be valid.

At each harvest, two randomly selected subsamples (≈5%) were taken from roots of four plants in each treatment, and incubated in 0.5% tetrazolium solution for 2 hours at 30°C in complete darkness (Gordon and Rowe, 1982; Peacock, 1966). Live tissue (i.e. stained tissue) was physically separated from dead tissue and the length (Table 3.2) and number of roots in both groups measured, providing estimates of the proportion of dead tissue in the root systems. The amount of dead tissue in UR and de-restricted (RD, RDD) plants stabilised to about 11% of the total root system (Table 3.2). Root turn-over in CR plants was similar up to 67 DAI, after which considerable root death occurred. The number and length of roots reported were adjusted for the estimated proportion of dead tissue in the samples.

Table 3.2 Percentage of dead tissue in root system (by length).

Treatment	Harvest				
	13 DAI	31 DAI	45 DAI	67 DAI	99 DAI
UR	< 2%	6.5	10.5	11.1	11.0
CR	< 2%	5.4	13.5	12.5	69.3
RD	< 2%	5.5	9.8	10.0	10.9
RDD	< 2%	6.1	10.4	12.3	11.6

Adjustment for deterioration of roots in storage

Despite efforts to provide an environment conducive to storage, roots deteriorated during storage, making subsequent measurements of length and number suspect. As effective improvement of storage conditions was not possible, an experiment was conducted, concurrent with the main experiment, to detail the relationship between root deterioration and storage time, and thus allow appropriate adjustments to be made before analysing the data.

Twenty-four plants, originally surplus to the main experiment but grown on in the hydroponic system, were harvested after 34 days, their shoots removed and their intact root systems immediately submerged in aerated water in 10 l containers in the cold store. The experiment was conducted over twelve days with four root systems sampled every two days. An intact first order lateral and its associated sub-lateral branches was randomly selected and sub-sampled from each root system. Total length of each subsample was measured before drying at 80°C for 72 hours in a forced air oven.

Root deterioration was assessed by the root specific mass (RSM), the ratio of dry weight per unit length of root (equation 3.1).

$$\text{RSM} = \frac{\text{root dry weight (mg)}}{\text{root length (m)}} \qquad (3.1)$$

Weight loss through cell breakdown or root shrinkage, or both, will cause a change in RSM. Based on Logsdon and Reneau's (1988) report that the length of roots was unaltered following storage in ethanol and formaldehyde, I assumed that storage in water would have negligible, if any, affect on initial root length. Consequently, any reduction in RSM during storage would reflect weight loss due to cell breakdown.

Roots deteriorated rapidly during the first three days of storage, with a gradual reduction in the rate as the duration of storage increased (Fig. 3.1). Similar results have been recorded for stored wheat (*Triticum aestivum* L.) roots (van Noordwijk and Floris, 1979).

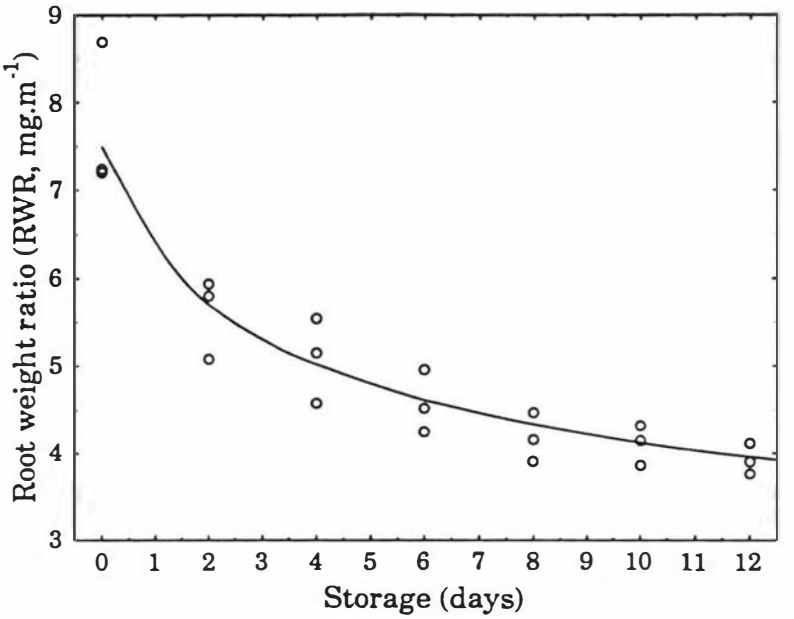

Fig. 3.1 Relationship between root specific mass (RSM) and storage. Fitted line from back-transformed values of $\log_e$ RSM = 2.02 - 0.249·$\log_e$ [days of storage + 1]. (r^2 = 0.92; lack of fit = NS.)

As this relationship was curvilinear, adjusting root dry weight for storage effects through analysis of covariance was not possible (Steel and Torrie, 1980). Regression analysis of $\log_e$ transformed storage data yielded the equation:

$$\log_e RSM_{as} = 2.020 - 0.249 \log_e T \quad (r^2 = 0.92, \text{ lack of fit:NS}) \qquad (3.2)$$

where RSM_{as} is the root weight ratio (g dry weight/m) measured after storage, T is days of storage + 1, and the rate constant (-0.249) measures the rate of weight loss per root length per day of storage ($g \cdot m^{-1} \cdot day^{-1}$).

The rate constant, with the coefficient terms, provide the adjustment for storage. Equation (3.2) has the form $y = a - bx$, where b is the rate constant, and a is the value of y when x equals zero. In this instance, a is the RSM before storage (RSM_{bs}). Rearranged, the equation yields:

$$\log_e RSM_{bs} = \log_e RSM_{as} + 0.249 \log_e T$$

or,

$$RSM_{bs} = RSM_{as} \cdot T^{0.249} \qquad (3.3)$$

where RSM_{bs} is an estimate of the initial (before storage) root dry weight per metre at harvest (i.e. RSM at t=0)

Through equation (3.3), raw data of root dry weight were adjusted to account for the duration the roots were stored before measurement.

3.2.4 Data analysis

Univariate analysis and time

Analyses of measured and derived variables were completed for each harvest. Time was not included as a variable in the statistical models used because it was possible that the changing nature of the treatments may have influenced different, or additional, physiological processes as the experiment progressed (Mead, 1990). Therefore, the nature of response would also change during the experiment. In this experiment, the nature of the treatment was expected to change during the experiment. For example, the root restriction 'treatment' encountered by CR plants 31 DAI was not the same root restriction treatment encountered by CR plants 67 DAI. Such change would have compromised the assumptions of heterogenous variance, additivity and normality, required for the analysis of variance of the more commonly used split-plot-in-time (with time used as the split plot factor) (Mead, 1990). In addition, the non-randomness of time would have created dependency in the error structure. Therefore, the ANOVA model (equation 3.4) was fitted to data from each month.

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij} \qquad (3.4)$$

where i=1, ..., 4 and j=1, ..., 6.

The appropriateness of the model, and absence of outliers, for each variable was checked using standard diagnostics of model residuals (Myers, 1990).

Significance testing

Standard statistical practice dictates that treatment differences are examined only if the overall F statistic is significant. The power of the F test to detect treatment differences, however, depends on the average of the squares of treatment deviations. As Mead (1990) argues, this ignores the possibility of detecting one substantial treatment effect that could be otherwise lost if all other treatment deviations are much smaller. Therefore, orthogonal contrasts were tested and reported irrespective of the overall F statistic being significant.

Unless otherwise stated, significance tests are presented at the 5% level of probability.

Missing data

A single missing value (root dry weight of treatment 1, block 4, 99 DAI) was estimated using analysis of covariance with dummy variables for the missing datum and non-missing data (Steel and Torrie, 1980).

Multivariate analyses

Multivariate statistical techniques enable simultaneous examination of observations on several different variables for several individuals. The many techniques available can be broadly classified as variable-directed, where primary concern is directed at the relationships among variables, and individual-directed, where primary concern is focused on the relationships among individuals (Chatfield and Collins, 1980). Variable-directed techniques

(e.g. canonical correlation analysis (CCA) and multiple regression analysis and its variants), reduce the dimensionality of the data set, exposing important underlying themes among variables, and highlighting redundant variables. Individual-directed techniques (e.g. canonical discriminant analysis (CDA)), provide the mechanism for discriminating among individuals or types on a quantifiable and repeatable basis.

Path analysis

Path analysis is a form of structured multiple linear regression analysis, investigating the relationships among standardised variables. The advantage of such an analysis is that the effect of one variable on another can be isolated from influences of other variables. By calculating the sign and significance of path coefficients, the direct effect of each variable on another is revealed following removal of the indirect effects exerted by other variables. Path coefficients significantly different from zero indicate that a change in a causal variable significantly affects the targeted response variable, with the greater the magnitude of the path coefficient, the greater its direct effect. Importantly, the terms *causal* and *effect* are used here in the statistical context of independent and dependent variables; a change in the direction of the path coefficients will yield coefficients of identical magnitude.

A major application of path analysis involves constructing and evaluating alternative structural models. Given a set of observed correlations among all the variables being considered, path analysis provides a tool to reproduce the correlations by a heuristic path diagram (Sokal and Rolfe, 1981). The technique relies heavily on path diagrams, which specify the proposed structure of the relationships among several variables. This structure is formed subjectively by the researcher as a plausible interpretation of these relationships, consistent with the observed data on all the variables involved. This characteristic distinguishes path analysis from multiple regression.

Whereas multiple regression is concerned solely with predicting an 'effect' from several 'causes', path analysis tests whether a proposed causal structure is compatible with the observed data.

Credited to Wright (1921), and popularised by Li (1975), path analysis has received considerable attention in social science and genetics research (e.g. Duncan, 1966; Heise, 1969; Kempthorne, 1957). Apart from use in evaluating yield components in agronomic crops (e.g. Dewey and Lu, 1959; McGiffen et al., 1994; Pandey and Torrie, 1973; Shasha's et al., 1973), path analysis is infrequently used in plant research (Hicklenton, 1990; Karlsson et al., 1988). The reasons for this are not clear. Certainly, the structural equations implied by a path diagram must be linear, and while linear relationships may often be unrealistic in some plant physiology contexts, they nevertheless exist in others. Moreover, transforming nonlinearly related variables may make their relationship linear or at least approximately so (Li, 1975). As it is not a fixed and routine method of handling data, path analysis is absent from statistical software packages, making it a less than readily available application for general data analysis.

In this study, path analysis provided the opportunity to examine several possible structural relationships between root and shoot growth. Moreover, given the considerable evidence existing that these relationships are often linear (Richards, 1981; Richards and Rowe, 1977a; Wilson, 1988), the linearity inherent in the analysis technique was considered to be biologically valid.

Correlation coefficients were calculated from the raw data using the SAS (Statistical Analysis System, Cary, N.C.; SAS Institute, 1989) procedure CORR. The path coefficients are equivalent to standardised partial regression coefficients. Raw data were standardised to zero mean and unity variance and multiple linear regressions conducted (using the SAS Institute (1989) procedures STANDARD and REG) as shown by the postulated path

diagrams. As estimates of regression coefficients are distorted if excessive collinearity exists among the independent variables in the model, appropriate measures of collinearity (e.g. condition indexes, variance decomposition and inflation factors; Myers, 1990) were calculated with each regression as a check.

Canonical correlation analysis

Latent relationships between the components of root and shoot growth were identified using canonical correlation analysis. The aim of canonical correlation analysis is to simplify the relationships between two groups of data. Pairs of linear functions (canonical variables) are defined which are linear and additive combinations of the original variables such that the correlation between the pairs of functions is as large as possible (Johnson and Wichern, 1988).

When variables in either group in a canonical correlation analysis are highly correlated (i.e. within-group correlation is high), interpreting the canonical variables solely on the magnitude and sign of the coefficients is prone to error. For example, if one variable of a group is highly correlated with one or more of the other variables in that group, then part of the effect of that variable will be accounted for by the coefficients of the other variables. Under such circumstances, interpretation based on these canonical coefficients is only approximate and potentially misleading (Reyment, 1972; Monmonier and Finn, 1973; Manly, 1986; Young, 1981) and should be supplemented by the canonical structure (i.e. the correlations between the canonical variables and the original variables).

More recently, however, this approach has been criticised as viewing a multivariate issue in a univariate way. Johnson and Wichern (1988) and Rencher (1992) favour assessing the contributions of the original variables

directly from the standardised coefficients since the correlations between the canonical variates and the original variables do not show how the original variables contribute jointly to the canonical analyses. This approach was adopted in this study, using guidelines from Hair et al. (1987) for determining which loadings were worth considering. In short, loadings greater than ±0.30 were considered important, loadings greater that ±0.40 considered more important, and if the loadings were ±0.50 or greater, they were considered very important. Thus, the larger the absolute size of the loading, the more important it is in interpreting the function.

Canonical discriminant analysis

By reducing the dimensionality of data sets, canonical discriminant analysis identifies and summarises important differences among treatments, while recognising the complex relationships among many characteristics (Cruz-Castillo et al., 1994). The aim of canonical discriminant analysis is to find linear functions of variables that separate two or more groups of individuals given measurements for these individuals on several variables. Heuristically, for the canonical discriminant functions (CDFs) $Z_1, Z_2, \ldots, Z_n$, Z_1 reflects group differences as much as possible; Z_2 captures as much as possible the group differences not displayed by Z_1; Z_3 reflects as much as possible the group differences not displayed by Z_1 and Z_2 and so on (Manly, 1986).

Canonical discriminant analysis proceeds under the assumptions of multivariate normal distributions and homogeneity of the within-group covariance matrix of all groups under examination. Failure of these assumptions influences the reliability of the significance tests (Manly, 1986). Curvilinear or nonlinear relationships between two variables will not be reflected in the results of the analysis unless suitable transformations are first performed on the original data for those variables (Matthew et al., 1994). The need for

such transformations was checked by plotting each variable pair by each harvest.

When correlation exists among some of the original variables, inferences based on the correlation between the CDFs and original variables (the canonical structure) is recommended (Afifi and Clark, 1990). But, as with canonical correlation analysis, this approach has been criticised (Rencher, 1992). In standardised form, the canonical coefficients of each CDF provide information about the joint contribution of the variables to that CDF—inferences from the canonical structure do not provide any information about the multivariate contribution of any variable.

3.2.5 Plant growth analysis

Relative growth rates and relative rates of increase

West et al. (1920) considered that the term 'relative growth rate' better described Blackman's (1919) efficiency index of dry weight production. Nevertheless, Blackman's (1919) explanation of this term, the efficiency of the plant as a producer of new material, is still widely accepted. When related to a specific organ (e.g. root) or group of organs (e.g. shoot), Warren Wilson (1972) suggested that the relative growth rate measured the sink activity of that organ group. As this also implies that the organ (sink) is entirely responsible for accumulating assimilate, Warren Wilson's (1972) view is not universally shared. Ho (1988), for example, pointed out that a considerable proportion of assimilate imported by a sink organ is used for respiration. Therefore, indices based upon biomass increase fail to assess the true ability of the sink organ to import assimilate, and only reflect the organ's *apparent* sink strength. Wareing and Patrick (1975) noted that Warren Wilson's (1972) definition of sink activity carried with it the implicit, and demonstrably

invalid, assumption that assimilate uptake was unaffected by neighbouring sinks.

These concepts of relative growth rates are not entirely relevant when comparing the relative change in the number or length of roots or length of stem over an interval. Yet some basis of standardisation is needed to compare differences in time as the size of a plant influences the increase in magnitude of its components. For this purpose, I have introduced the term *relative rate of increase* ($R_{I(variable)}$) to describe the relative increase of a variable (e.g. leaf or root number) over time, with respect to the initial size. While mathematically identical to whole plant relative growth rate, this term better describes the derived variable. This approach extends Hunt's (1982) attempt at distinguishing between whole and component 'relative growth rates' to assist in comparing the effects of treatments on the causal process contributing to the gross performance of the plant. Hunt (1982), however, supported use of the term *specific growth rate* to describe whole plant relative growth rates, and *relative growth rate* to describe that of the components. Specific growth rate has not gained widespread acceptance in scientific literature, possibly because the dubious association between organ efficiency and sink strength still exists. Consequently, in this thesis, the term relative growth rate is confined to whole plant dry biomass (R_W, $g \cdot g^{-1} \cdot day^{-1}$), shoot dry biomass (i.e. Σ[leaves, petioles, stem]; R_S, $g \cdot g^{-1} \cdot day^{-1}$), leaf dry biomass ($R_L$, $g \cdot g^{-1} \cdot day^{-1}$), stem dry biomass ($R_{ST}$, $g \cdot g^{-1} \cdot day^{-1}$) and root dry biomass ($R_R$, $g \cdot g^{-1} \cdot day^{-1}$). The standardised increase in leaf area is termed the *relative leaf expansion rate* (R_A, $m^2 \cdot m^{-2} \cdot day^{-1}$).

3.2.5.1 Classical approach

Relative growth rate

The relative growth rate, R_W, is a measure of the average efficiency of each unit of dry matter of the whole plant in producing new dry matter (Causton and Venus, 1981). Conventionally, R_W is calculated by dividing the difference in $\log_e$ transformed plant weight of a *j*th replicate pair of plants at two harvests by the time difference between those harvests. As such, it is an average ($\bar{R}_W$) of the instantaneous values of R_W within that period. In a blocked design, the *j*th pair are taken from the same block. If plants were allocated to the blocks on a size criterion, then such pairing is biologically valid (Causton and Venus, 1981). In this experiment, however, the block design was based on environmental differences within the greenhouse, with all blocks containing similarly sized plants. Thus, the actual pairing of plants was random and as such had no sound biological basis.

Therefore, the non-paired method of Venus and Causton (1979b) was used, where:

$$\bar{R}_W = \{1/(t_2-t_1)\} \cdot \{\mathscr{E}(\log_e W_2) - \mathscr{E}(\log_e (W_1)\} \quad (3.5)$$

$$\mathscr{V}(\bar{R}_W) = \{1/(t_2-t_1)^2\} \cdot \{\mathscr{V}(\log_e W_2) + \mathscr{V}(\log_e (W_1)\} \quad (3.6)$$

where $\mathscr{E}(\log_e W_i) = (1/n) \cdot \sum_{j=1}^{n} \log_e W_{ij}$,

$\mathscr{V}(\log_e W_i) = \{1/(n-1)\} \cdot \sum_{j=1}^{n} \{\log_e W_{ij} - \mathscr{V}(\log_e W_i)\}^2$;

n is the number of observations, *i* is the *i*th harvest,

and *j* is the *j*th pair of replicates.

Net assimilation rate

Net assimilation rate (E_A), the net increment of plant weight expressed per unit leaf area, measures the efficiency of the leaves in producing gains in dry matter (Causton and Venus, 1981). In deference to Evans' (1972) arguments, I retained the term net assimilation rate rather than unit leaf rate. The classical method for estimating E_A is by solving the equation (Causton and Venus, 1981):

$$\bar{E}_A = \frac{1}{(t_2 - t_1)} \int_{W_1}^{W_2} \frac{dW}{L_A} \qquad (3.7)$$

(where the subscripts 1 and 2 refer to the *t* harvest pairs).

to calculate the mean net assimilation rate, $\bar{E}_A$, between times t_1 and t_2. Expressed as a mean between two harvests, $\bar{E}_A$ serves as an approximate measure of the net photosynthetic rate during the period under examination, provided that mineral ion uptake is either neglected or allowed for (Causton and Venus, 1981). The integrative nature of this measure is arguably more meaningful in long-term experiments than estimates of instantaneous E_A (cf. Evans, 1976). The integral of equation (3.7) can be evaluated only if the relationship between W and L_A over the harvest period is known or assumed. The usual assumption is that of linearity (i.e. $W = a + bL_A$, where a and b are constants). Then, by the standard result of change of variable, equation (3.7) becomes:

$$\bar{E}_A = \frac{b}{(t_2 - t_1)} \int_{L_{A1}}^{L_{A2}} \frac{dL_A}{L_A} \qquad (3.8)$$

which is equivalent to:

$$\bar{E}_A = \frac{b(\log_e L_{A2} - \log_e L_{A1})}{(t_2 - t_1)} \tag{3.9}$$

(where L_{A1} and L_{A2} are the leaf areas at the first and second harvest).

For the respective harvests, $W_1 = a + bL_{A1}$ and $W_2 = a + bL_{A2}$. Rearranging these equations with respect to b yields:

$$b = \frac{(W_2 - W_1)}{(L_{A2} - L_{A1})} \tag{3.10}$$

Substituting for b in equation (3.9) gives:

$$\bar{E}_A = \frac{(W_2 - W_1)(\log_e L_{A2} - \log_e L_{A1})}{(L_{A2} - L_{A1})(t_2 - t_1)} \tag{3.11}$$

Whitehead and Myerscough (1962) generalised this equation for calculating $\bar{E}_A$ for relationships of the form $W = a + bL_A^n$ (of which linearity (n=1) is a special case) as:

$$\bar{E}_A = \frac{n(W_2 - W_1)(\log_e L_{A2} - \log_e L_{A1})}{(n - 1)(L_{A2} - L_{A1})(t_2 - t_1)} \tag{3.12}$$

The value of the index n is the ratio of the mean relative growth rates of the plant and leaf area (R_A):

$$n = \frac{(\log_e W_2 - \log_e W_1)}{(\log_e L_{A2} - \log_e L_{A1})} = \frac{\bar{R}_W}{\bar{R}_A} \tag{3.13}$$

Whale et al. (1985) noted that if the equation $W = a + b \cdot L_A^n$ is written in $\log_e$ form (i.e. $\log_e W = \log_e b + n \cdot \log_e L_A$), then n can be estimated as the regression coefficient of $\log_e W$ on $\log_e L_A$.

In classical growth analysis, equations (3.7)-(3.9) and (3.11)-(3.12) are solved for discrete pairs of data for the harvest interval ($t_2 - t_1$). As with $\bar{R}_W$, Venus and Causton (1979b) noted that unless such pairing was based on a size criterion, it lacked a sound biological basis. They proposed an alternative method for estimating the expected value of $\bar{E}_A$ that utilised standard theorems for a linear combination of variates for the equation (3.11):

$$\mathcal{E}\bar{E}_A = \frac{\mathcal{E}(W_2 - W_1)(\log_e \mathcal{E}(L_{A2}) - \log_e \mathcal{E}(L_{A1}))}{(\mathcal{E}L_{A2} - \mathcal{E}L_{A1})(t_2 - t_1)} \qquad (3.14)$$

where $\mathcal{E}(W_1) = 1/n\ (\Sigma\ W_{1j})$ etc and (n = no. observations, j ... n)

Whale et al. (1985) subsequently extended this approach for the equation form (3.12). As the four attributes W_1, W_2, L_{A1}, and L_{A2} are combined in the non-linear form of equation (3.11), only approximate estimates of $\mathcal{E}(E_A)$ and its variance, $\mathcal{V}(E_A)$, are obtainable. Venus and Causton (1979b) and Whale et al. (1985) provide such approximations, derived from formulae given by Kendall and Stuart (1977).

The assumption of linearity was tested by plotting $\log_e W$ against $\log_e L_A$ for each treatment. These plots indicated deviations from linearity which were confirmed subsequently by regression analysis with significant quadratic terms detected in the relationship for each treatment. Coombe (1960) and Evans (1972) tabulated the percentage differences between mean values of E_A calculated on the assumption that $W = a + bL_A^n$ and the corresponding value for the assumption that $W = a + bL_A^2$. Coombe (1960) noted that if the ratio of L_{A2}/L_{A1} is less than 2, the difference between estimates of E_A when n=1 or n=2 are negligible. As the L_{A2}/L_{A1} ratio ranged between 3 and 16 for all

combinations of treatment and harvest interval, the percentage differences between calculating $\bar{E}_A$ under the assumption of linearity (equation 3.11) and by using an estimate of n (equation 3.12) were evaluated. Figure 3.2 shows, for this experiment, that an error of approximately ±8% would be incurred if the wrong equation (3.11) was used. It also shows a clear association between high L_2/L_1 ratios, values of $n<1$, and under-estimation of $\bar{E}_A$. Consequently, equation (3.11) was discarded.

The non-paired method of Venus and Causton (1979b) was used in preference to the paired approach. The argument of Venus and Causton (1979b) of lack of biological validity for pairing was accepted, and their equation, modified for the index n (Whale et al., 1985), was adopted. Given the extensive mathematics required by this approach to estimate the variance of the estimate of $\bar{E}_A$, a spreadsheet was configured to expedite the calculations.

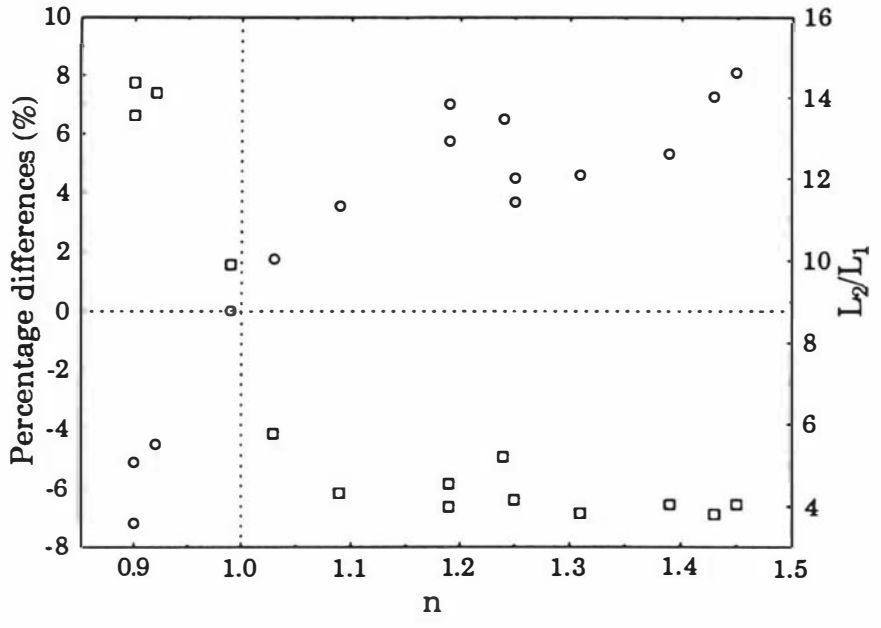

Fig. 3.2 Percentage difference in estimation of E_A between assumption that $W \propto L_A$ and $\log_e W \propto n \log_e L_A$, where n is the ratio of R_W:R_A, and L_2/L_1 is the ratio of leaf area at end and start of the period under review (see text for details). *Key:* □=percent error, ○=L_2/L_1 ratio.

Other ratio indices

The fundamental equation in growth analysis relates instantaneous plant relative growth rate as a linear function of E_A and the leaf area ratio (LAR), the ratio of leaf area (L_A) to total plant dry weight (W). The LAR reflects the ratio of photosynthesizing to respiring material in the plant (Hunt, 1978).

$$R_w = E_A \cdot LAR \tag{3.15}$$

$$R_w = E_A \cdot \frac{L_A}{W} \tag{3.16}$$

In turn, the LAR is the product of the leaf weight ratio (LWR), an index of the leafiness of the plant on a weight basis (equation 3.17), and the specific leaf area (SLA):

$$\begin{aligned} R_w &= E_A \cdot SLA \cdot LWR \\ &= E_A \cdot \frac{L_A}{W_L} \cdot \frac{W_L}{W} \end{aligned} \tag{3.17}$$

(where W_L = leaf dry weight)

The SLA is a morphological index of the expansion in space of the dry matter in leaves. Although commonly viewed as an indicator of leaf thickness, where a high ratio corresponds to a thin leaf (e.g. Causton and Venus, 1981; Friend and Helson, 1965; Hunt, 1982), changes in SLA may also result from sole or joint changes in leaf thickness, density, or composition (Dijkstra, 1990; van Arendonk and Poorter, 1994; Witkowski and Lamont, 1991). Dijkstra (1990) pointed out that although being a component of SLA, the index itself was not an appropriate measure of leaf thickness, because the ratio between dry weight and fresh weight of leaves may vary. Similarly, as leaf density may change independently of leaf thickness (Lewandowska and Jarvis, 1977; Purohit et al., 1988), SLA itself could only give a coarse estimate of density.

Dijkstra (1990) proposed that these problems of interpretation of SLA could be avoided, and a closer understanding gained of the underlying sources of change in the index, by considering its components:

$$\frac{1}{SLA} = \frac{FW_L}{L_A} \cdot \frac{W_L}{FW_L} \cdot \left(\frac{W_{L1}}{W_L} + \frac{W_{L2}}{W_L} + \cdots + \frac{W_{Ln}}{W_L} \right) \qquad (3.18)$$

where FW_L is the fresh weight of leaf; L_A is leaf area; W_L is the dry weight of leaf; $W_{L1, L2 \ldots Ln}$ is the weight of the different compounds 1, 2, ... n of the chemical composition of the leaf dry weight.

The ratio of fresh weight to leaf area approximates leaf thickness, and the ratio of dry weight to fresh weight reflects the density of the leaf tissue. The third term of the equation describes the chemical composition of the leaf dry weight. Of the three components, this term has the least interpretive value because as all components add to unity, a change in composition may accompany, but cannot cause, a change in SLA.

The variances of ratios such as LAR, SLA and LWR are frequently calculated in the same way as the variance of ratio scale measurements such as leaf area, plant dry weight and stem height. Such calculation is incorrect as it does not take into account the variability of numerator or the divisor. As a consequence, the standard errors of such ratios tend to be under-estimated (Oyejola and Mead, 1989). Moreover, statistical theory dictates that the ratio of two normally distributed variables is not itself normally distributed. As growth data tends to be log-normally distributed, the logarithm of each component is normally distributed, and thus by a standard result, the difference of two normally distributed variables is itself normally distributed (Causton and Venus, 1981). Oyejola and Mead (1989) provide empirical evidence that approximately normal distributions for ratios can be assumed if the variables involved are positively correlated and that the coefficient of variation (CV) of the denominator is less than 10%. These workers presented

a method for correcting the standard error of a ratio to account for the variability of the denominator term. When correlation is unlikely to exist between the numerator terms, the standard error of the difference of two ratios is calculated from the standard errors of the two separate ratios. When correlation exists, Oyejola and Mead (1989) recommend a conservative correction to the standard error using the larger of the two standard error to mean ratios (i.e. the less precise ratio). When the CV of the denominator term exceeds 25%, the distribution of the ratio is skewed, affecting inferences of significance involving t-tests. Thus, as the unadjusted standard errors are probably smaller than actual, a more conservative approach must be adopted when interpreting differences among treatments.

In the classical analysis component of this study, however, the CV of the denominator term sometimes exceeded 25%, making any interpretation of the data adjusted by Oyejola and Mead's (1989) method open to objective assessment. As both normal probability plots and the Shapiro-Wilk statistic (Shapiro and Wilk, 1965; calculated by the SAS (1989) procedure UNIVARIATE) indicated the data of each ratio could be treated as a random sample from a normal distribution, the ratios were treated as normally distributed variables.

3.2.5.2 Functional approach

Background and theory

Regardless of the experimental protocol taken, a reality of taking sequential observations of plant growth is that random errors will be incurred at each stage of the measurement process (Hunt, 1982). Any one of the subsamples harvested may not necessarily be representative of the whole population at the time of harvest. Both accidental and unavoidable differences between the

treatment of different individuals within each subsample will occur by manner of the physical layout of the plants in the experimental environment and harvesting techniques used. Seedling variation and variation within clonal material (Burdon and Harper, 1980; Skirvin et al., 1994) will result in individuals growing at slightly different rates. Thus, while observational data may parallel reality, it never mirrors it. Hunt (1982) argued that "... if attempts to assess the reality of growth result in a random scatter of observations about that reality, then a mathematical function fitted to those observations may be expected to regain much of the clarity with which the reality is perceived by the experimenter...". A fitted function, Hunt (1982) continued, reflected back towards the reality of which the observational data were imperfect estimates.

The correct choice of curve function to fit to the data is crucial to the success of the functional approach. This topic has received considerable attention and many papers published during the initial development and refinement of the functional approach focused on the choice of curve to fit (Hughes and Freeman, 1967; Nicholls and Calder, 1973). Towards the end of this period, Venus and Causton (1979b) compared the indices of growth analysis derived from fitted Richards functions with those from the family of polynomial exponential functions. Although no statistical differences between the two sets of indices were detected, they recommended the Richards function as the time trends of indices derived from it were biologically more meaningful than those from the polynomial exponential functions.

Incorporating structured experimental designs

An effort was made in this study to extend the work of Causton and Venus (1981) and Hunt (1982) to accommodate the structured experimental design of the randomised complete block in functional growth analysis. As previous analyses of variables in the data set had revealed significant block effects on

leaf area and the dry weights of leaves, stems, and roots, it was anticipated that similar block effects would influence the derived variables from the functions fitted to the data. In relating the functional approach to a structured experimental design, a simple approach is to add b-1 categorical variables to the model (where b is the number of blocks). If only the coefficients of the function are of interest, differences among blocks (i.e. equality of slope coefficients) can be examined using a t-test or partial F-test to test the significance of interactions between the time regressors and the categorical regressors (Myers, 1990). However, as growth analysis uses the first derivative of the function, other approaches are required.

Analytical procedures

The first approach, the 'control' (hereafter called the standard method), followed conventional functional protocols (Causton and Venus, 1981), but used block-adjusted data to account for environmental heterogeneity. Following this approach for the randomised complete block design (subsequently found to have been used by Whale et al., 1985), the model for the analysis of variance has the form:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij} \qquad (3.19)$$

where μ is the grand mean, α_i the main effect of the ith treatment, β_j the main effect of the jth block, and ϵ_{ij} is the residual error.

Adjusting the observation for the effect of the jth block is then straightforward:

$$\text{adjusted } Y_{ij} = Y_{ij} - (\beta_j - \mu) \qquad (3.20)$$

where β_j is estimated as the mean of the jth block.

The raw data were appropriately adjusted before $\log_e$ transformation and curve fitting through regression analysis. A single function was fitted to the

pooled (over blocks) adjusted data for each treatment. With several observations at each time node, this method also permitted a lack of fit analysis (Myers, 1990). A function was fitted (see next section), and its derivatives calculated from the resulting parameter estimates. The standard errors of the derivatives were calculated from the standard error of the predicted mean reported for each function.

The second approach, hereafter referred to as the hybrid method, sought to use a functional approach without losing block information, thereby maintaining *b* estimates of each derivative of interest for each treatment at each harvest. Not only did this approach account for variation in individual plant response to its environment (i.e. biotime; Bradford and Trewavas, 1994), but the derived data set could be used for subsequent analysis. In this method, a function was fitted to the $\log_e$ transformed raw data of each block × treatment combination (Mead, 1990). The derivatives of interest were calculated (see next section) and analysed by conventional analysis of variance (ANOVA) or its variants (e.g. analysis of covariance, ANCOVA), or in multivariate statistical techniques.

The nature of the computations involved for these two approaches create two important differences between them. First, the hybrid method provides a more robust estimate of residual variation than the standard method. The residual term of the standard method contains both within-time and between plot variation. In contrast, because the hybrid method is based on one value per plot, the residual term only contains between-time variation. Second, the response variables being regressed are not equal. The standard method regresses the quantity $\log_e(Y_{ij} - \beta_j)$ against time whereas the hybrid method regresses $\log_e(Y_{ij})$ against time. A linear change of scale between these response variables would not alter the rate coefficients derived from any function fitted to the two data sets. The logarithmic transformation, however, is non-linear and the change in scale coincident with this transformation

means that the difference between the response variables is not constant in time. The size of this difference in this study, however, was small as the block effects were very small relative to the values of the Y variables.

Curve fitting and calculating growth analysis indices

In choosing the function to fit to the $\log_e$ transformed data to calculate the relative growth rates of the whole plant, its root and shoot components, and its leaf area, preliminary scatterplots of the data revealed that within the limits of the information supplied by the experimental data (Hunt, 1982; Hurd, 1977), the second degree polynomial exponential function (hereafter termed the quadratic function):

$$\log_e Y = \alpha + \beta_1 t + \beta_2 t^2 \qquad (3.21)$$

was appropriate. When differentiated with respect to time, this function yields the relative growth rate of the dependent variable:

$$\frac{d\log_e Y}{dt} = \beta_1 + 2\beta_2 t \qquad (3.22)$$

No inherent physiological importance is given to the quadratic function by its use. Rather, its use is consistent with the primary aims of the functional approach to growth analysis: goodness of fit (Richards, 1969), and parsimony (Nicholls and Calder, 1973; Hunt, 1982). In some instances, the quadratic term was statistically insignificant. However, since the relative growth rate must decrease in time as an increasingly larger amount of the plant becomes purely structural and therefore incapable of providing further increases in dry weight, the term was included since the "biological expectation" (Hurd, 1977) of the term remained.

Statistically valid use of regression analysis techniques to fit curves requires three assumptions to be met (or at least closely met): that the independent variable (in this instance, time (t)) is measured without error; that the distributions of replicated dependent variables are normally distributed at each node of the independent variable; and that the variance of these distributions should be uniform and unchanged in magnitude with increasing levels of the independent variable. The first assumption was easily met since time is measured without error. With plant data, the latter two assumptions are largely met by analysing the logarithmic transformation of the dependent variable.

With quadratic functions fitted to logarithmic transformed data of total leaf area, total plant dry weight and leaf, shoot, and root dry weights, estimates of LAR, LWR, SLA, E_A and SR were also made. As it is usually assumed that plant weights and leaf areas are lognormally distributed (Causton and Venus, 1981; Flewelling and Pienaar, 1981), calculating these ratios followed the pattern described here for the LAR:

At time t (i.e. a particular harvest), and for $l_A = \log_e L_A$ and $w = \log_e W$, then (after Causton and Venus, 1981):

$$\mathcal{E}\{\log_e(L_A/W)\} = \mathcal{E}(l_A) - \mathcal{E}(w) \qquad (3.23)$$

$$\mathcal{V}\{\log_e(L_A/W)\} = \mathcal{V}(l_A) - \mathcal{V}(w) - 2 \cdot \mathcal{C}(l_A, w) \qquad (3.24)$$

where $\mathcal{E}$ denotes an expected value, $\mathcal{V}$ is a variance, and $\mathcal{C}$ is a covariance.

From the relationship between the lognormal and normal distribution (Flewelling and Pienaar, 1981), then after Causton and Venus (1981):

$$\mathcal{E}(L_A/W) = \exp[\mathcal{E}(\log_e(L_A/W)) + \tfrac{1}{2} \cdot \mathcal{V}(\log_e(L_A/W))] \qquad (3.25)$$

$$\mathcal{V}(L_A/W) = \exp\,[2\cdot\mathcal{E}(\log_e(L_A/W)) + \mathcal{V}(\log_e(L_A/W))] \times (\exp\,[\mathcal{V}(\log_e(L_A/W)]-1) \quad (3.26)$$

The net assimilation rate (E_A) was calculated after Causton and Venus (1981):

$$\mathcal{E}(E_A)=\mathcal{E}(R_W)/\mathcal{E}(L_A/W)+\mathcal{E}(R_W)\cdot\mathcal{V}(L_A/W)/\{\mathcal{E}(L_A/W)\}^3 - \mathcal{C}(R_W, L_A/W)/\{\mathcal{E}(L_A/W)\}^2 \quad (3.27)$$

$$\mathcal{V}(E_A)=\mathcal{V}(R_W)/\{\mathcal{E}(L_A/W)\}^2 + \{\mathcal{E}(R_W)\}^2\cdot\mathcal{V}(L_A/W)/\{(L_A/W)\}^4 - 2\cdot\mathcal{E}(R_W)\cdot\mathcal{C}(R_W, L_A/W)/\{\mathcal{E}(L_A/W)\}^3 \quad (3.28)$$

where $\mathcal{C}(R, L_A/W) = r(\mathcal{V}(R)\cdot(L_A/W)\}^{1/4}$

3.2.5.3 Allometric relationships

The study of the relative size of different parts of an organism and how the parts grow in relation to each other is called allometry. Huxley (1924, 1932) proposed a simple model for allometric growth in which the ratio of the relative growth rates of two components remains constant. Taking two organs or plant parts, denoted by W_1 and W_2, the model describes an allometric relationship by:

$$\frac{1}{W_2}\cdot\frac{dW_2}{dt} = \frac{k}{W_1}\cdot\frac{dW_1}{dt} \quad (3.29)$$

or

$$\frac{d\log_e W_2}{dt} = k\cdot\frac{d\log_e W_1}{dt} \quad (3.30)$$

where the parameter *k*, the allometric constant, is the ratio of the relative growth rates of components W_1 and W_2. Integrating both sides of equation (3.30) gives:

$$\log_e W_2 = \log_e a + k \log_e W_1 \tag{3.31}$$

or

$$W_2 = a W_1^k \tag{3.32}$$

This power function, commonly referred to as the allometric equation, expresses the size of one component as proportional to the size of another component raised to some power *k* (Reiss, 1989). Allometry has been widely used in plant science, particularly in the examination of root-shoot relationships (Hunt, 1978). In noting the constancy of *k* within a population of plants, often of different age and subjected to different manipulative treatments, several authors (Barnes, 1979; Chalmers and van den Ende, 1975; Richards, 1981; Troughton, 1968) concluded that a physiologically significant relationship exists between the relative growth rates of root and shoot. This relationship has important ramifications for crop husbandry. Factors which reduce the growth of the root system (expressed as R_R) will, indirectly through the allometric link, also proportionately limit shoot growth (expressed as R_S), and vice versa.

Despite many reports of an allometric relationship between root and shoot growth, some workers have expressed reservations. Some point out that with little evidence to suggest any substantial mechanistic basis to the relationship, the model is largely empirical (Richards, 1969; Thornley and Johnson, 1990; Wilson, 1988). Others have demonstrated that allometric relationships can be unstable, arising in a casual, inadvertant way. Dormer (1965), for example, showed that any two Gompertz curves with the same value for the rate constant will produce an approximately allometric relationship. Richards (1969) observed that an allometric relationship will appear to exist between two components whose growth rate is exponential, since the individual relative growth rate remain constant. Causton and Venus (1981) provided mathematical theorems demonstrating that linear

allometry may not physically exist, even though data plots suggest that it does. Thus, while all components (e.g. root, shoot) of a plant may be related allometrically, linear allometry cannot simultaneously exist among these components *and* between a component and the whole plant. Furthermore, for an allometric relationship between root and shoot growth to be *physically* possible, either the slope of the linear allometric relationship between sub-components of the shoot (i.e. leaf or stem) is unity, or no linear allometric relationships exist between any combinations of leaf, stem and root. Few, if any, authors have critically evaluated the apparent allometric relationships in their data against these criteria. Therefore, the assertion that root and shoot growth is allometrically bound in a relationship of physiological significance (Barnes, 1979; Chalmers and van den Ende, 1975; Richards, 1981 Troughton, 1968) may be founded on mathematical artefacts.

The correct use of allometry, like all mathematical tools, relies on several conditions that impinge on the validity of its interpretation being met. There is little doubt that an uncritical approach to allometry has been adopted in many previous reports of allometric relationships among plant components (Zar, 1968). Previous studies on root-shoot relationships have been inconsistent in the structure of the regression and allometric models tested. Some workers have viewed root growth as the dependent variable (Pearsall, 1927; Richards, 1981; Troughton, 1956), while others regard it as the independent variable (Chalmers and van den Ende, 1975; Drew and Ledig, 1980). Such inconsistency is surprising since the rhizosphere generally imposes greater limitations on growth than the atmosphere (Passioura, 1988a; Schulze et al., 1988; Sharp and Davies, 1989). Moreover, most studies of root function have been aimed at eventually improving shoot performance and productivity. It seems clear that root growth should be treated as the 'independent' variable.

The issue of dependent and independent variables, however, should not be over-emphasised. Although many authors refer to the power function $Y=aX^k$

as the allometric equation, the mathematically equivalent model $\log_e Y = \log_e a + k \cdot \log_e X$, is invariably fitted using the method of least squares, also called ordinary least squares (OLS) regression. However, the models are not statistically equivalent for OLS solutions, particularly with respect to the structure of the residuals (Zar, 1968). An assumption of OLS regression that one variate is measured without error is untenable as both variables are random (i.e. subject to random error). Under such circumstances the use of OLS regression is inappropriate, leading to inefficient estimation of the parameters, and inaccurate estimates of the variability structure of the data (Causton and Venus, 1981). In instances where two variates are both subject to independent random error, the general method of maximum likelihood must be used to calculate the parameter estimates.

In this study, the components of the maximum likelihood solution given by Causton and Venus (1981) were programmed into a Quattro Pro (ver.4, Borland) spreadsheet. Although some attempts have been made to provide a physiological role for the intercept of equation (3.31) (White and Gould, 1965; Gould, 1966), none are convincing. Thus, while the intercept value is reported for completeness, its standard error is not.

3.3 Results

3.3.1 Stress indicators

Leaf water potential

Despite very dense root systems developing in the restrictive containers, leaf water potential, measured 44 and 66 DAI, did not reveal any signs of water stress (Table 3.3).

Table 3.3 Leaf water potential of tomato plants (n=6).

Time	Treatment	Leaf water potential (ψ_w, MPa)	
		800 hr	1200-1300 hr
44 DAI	UR	-0.125	-0.160
	CR	-0.162	-0.217
	RD	-0.155	-0.197
	RDD	-0.147	-0.200
	SEOD[z]	0.027	0.024
66 DAI	UR	-0.102	-0.112
	CR	-0.093	-0.107
	RD	-0.113	-0.105
	RDD	-0.100	-0.093
	SEOD	0.041	0.041

[z] standard error of difference between means

Leaf epinasty

Up to 67 DAI, the mean petiole angle of plants in all treatments was similar and showed no sign of epinastic response (Table 3.4). Petiole angle of CR plants, however, decreased considerably between 67 and 99 DAI, indicating an epinastic response (Jackson and Campbell, 1979). This decrease was preceded by a substantial increase in the number of adventitious root primordia. Transient wilting was first observed 76 DAI in CR plants only. Together with the observed relative increase in root death in CR plants at the final harvest, it seems probable that the roots of CR plants came under water and oxygen stress some time between 67 and 99 DAI. Presumably, the high root density in the 0.025 l container restricted entry of nutrient solution into the root system. As similar symptoms were absent in other treatments, it was assumed that only CR plants had been subjected to this stress. As the growth responses under such conditions were probably due more to artefacts

of the restriction treatment, rather than restriction itself, data from the CR treatment collected 99 DAI were excluded from all analyses.

Table 3.4 Effect of root restriction treatments on epinastic curvature of petiole and adventitious root formation.

Treatment	Days after treatment (DAI)			
	31	45	67	99
Epinastic curvature[z] (°)				
UR	80.2	77.6	78.5	73.3
CR	78.4	76.5	75.5	55.3
RD	79.5	76.7	79.6	78.7
RDD	83.6	79.1	78.1	78.3
SEOD (n=6)	3.1	3.4	3.4	4.5
	NS	NS	NS	***
Adventitious root number				
UR	0	22.2	128.0	[y]
CR	0	35.8	267.8	
RD	0	35.2	111.5	
RDD	0	25.7	97.8	
SEOD (n=6)		9.5	44.7	
		NS	***	

NS, *, **, ***, ****Nonsignificant or significant F test at $P \leq 0.05$, 0.01, 0.001, or 0.0001 respectively
[z]Angle between the stem and adaxial surface of the petiole
[y]Adventitious roots present but not counted.

3.3.2 Growth ontogeny

Unrestricted (UR) and de-restricted (RD, RDD) plants grew exponentially during the first 45 days of the experiment and continued to increase, albeit at a slightly slower rate. Continuously restricted (CR) plants followed this

trend up to 67 DAI, after which a stress-induced decline in the rate of increase of all measured variables was observed.

Root growth

Root length was similar in all treatments for 31 days. After 45 days CR and RDD plants had shorter root systems than UR or RD plants. By 67 DAI, plants in 0.025 l containers (CR) had significantly shorter root systems than those in 0.05 l containers (RDD) and this pattern continued to 99 DAI.

Physically restricting root systems consistently reduced the number of roots (Fig. 3.3b, Tables 3.5a-c). The number of roots of all restricted plants was significantly smaller than unrestricted plants 31 DAI, while effects on other variables were inconsistent. By 45 DAI, UR plants had more roots than CR, RD or RDD plants, although by 67 DAI, UR and RD plants had similar numbers of roots. Continuously restricted (CR) plants had significantly fewer roots than UR plants from day 31 onwards. Plants released from restriction (RD and RDD) tended to have fewer roots than UR plants.

By 45 DAI, restricting roots in 0.025 l containers had significantly reduced root dry biomass increment. By 67 DAI, restricting roots in 0.05 l containers also significantly depressed accumulation of dry root biomass relative to plants growing in 10.0 l containers (i.e. UR and RD plants). Releasing roots from the 0.05 l containers stimulated accumulation of dry biomass by roots, so that by the final harvest the root dry weight of RDD and RD plants was similar.

There was considerable treatment and ontogenetic drift in the indices of root morphology (Fig. 3.4ab). By 31 DAI, roots of restricted plants (CR, RD, RDD) had a lower branching density and were either thicker or had a higher tissue density than unrestricted plants. By 45 DAI, however, the branching density

of UR and CR plants was similar while that of RD and RDD plants was significantly lower. The SRL of de-restricted plants was significantly lower than CR or UR plants. As CR, RD and RDD plants differed in root dry weight rather than root length at this harvest (Table 3.5b), de-restriction appears to have stimulated a proportionately greater increase in root biomass than elongation. De-restricted roots may have produced fewer sub-laterals than UR or CR plants, with the result that the average density of the root tissue increased. A large change in the tissue morphology of RDD roots occurred between 45 and 67 DAI, with roots being substantially thinner than at previous harvests. The branching density of RDD root systems also increased relative to RD plants. Together with CR plants, restricted plants had more roots per unit length than unrestricted (UR and RD) plants.

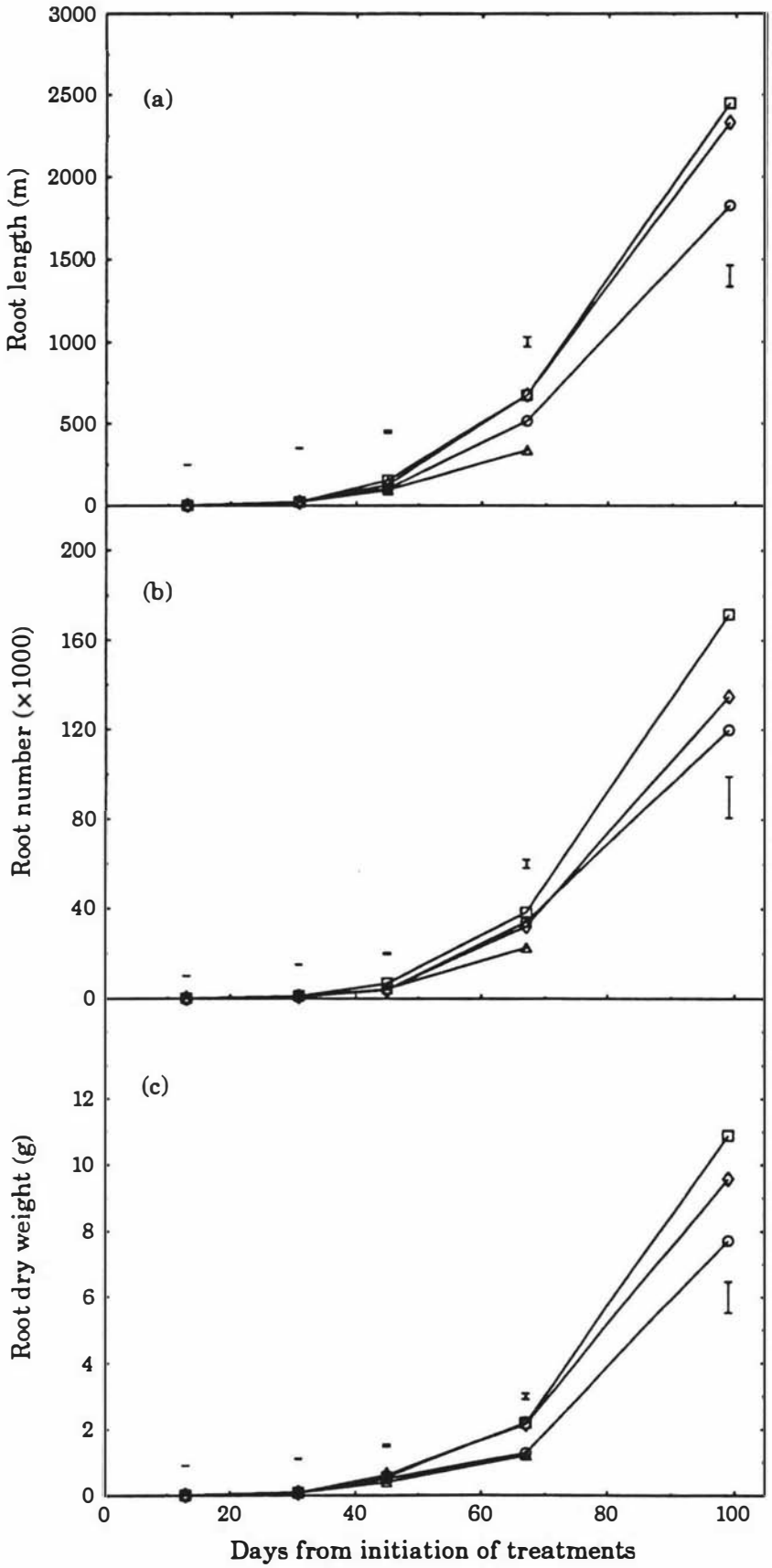

Fig. 3.3. Ontogeny of (a) root length (b) number and (c) dry weight of UR plants (□), RD plants (◇), RDD plants (○) and CR plants (△). Data points represent means, with n=6. Vertical bars are SEOD among treatments at a given harvest

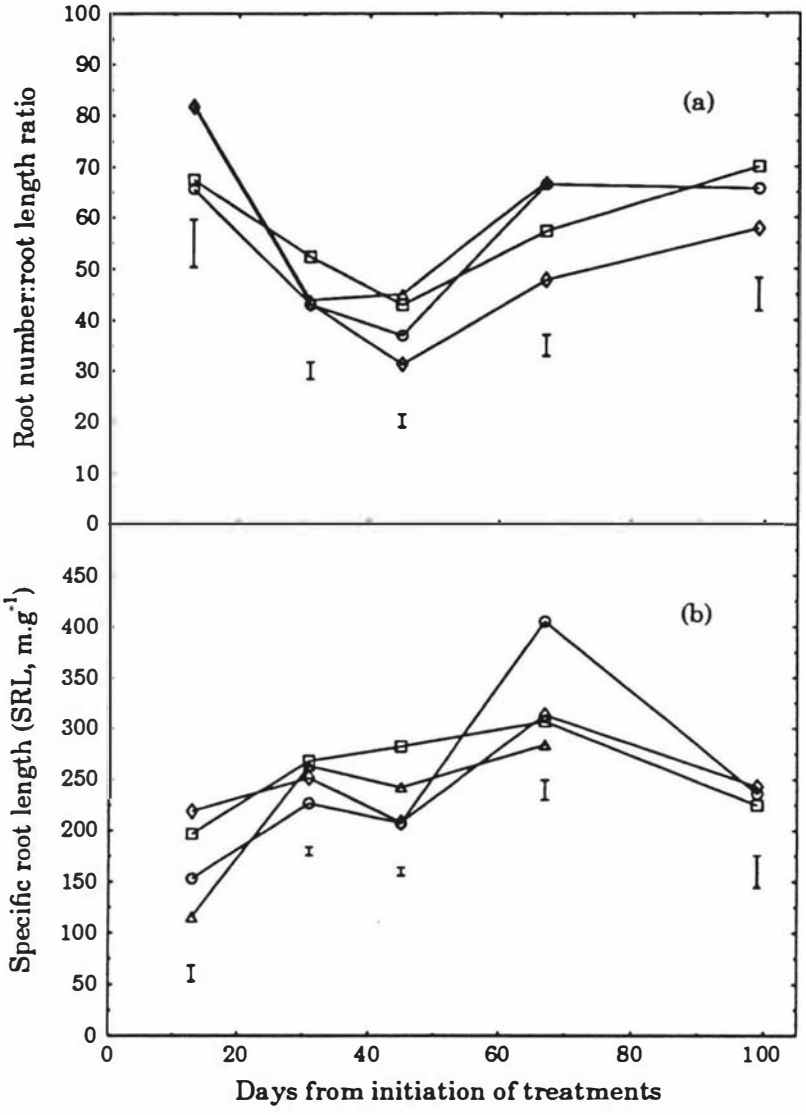

Fig. 3.4. Ontogeny of (a) ratio of root number:root length (m) and (b) specific root length (SRL; $m \cdot g_R^{-1}$) of UR plants (□), RD plants (◇), RDD plants (○) and CR plants (△). Data points represent means with n=6. Vertical bars are SEOD among treatments at a given harvest.

Table 3.5a Summary statistics from univariate analysis of plant response to root restriction at 13 and 31 DAI[z].

Treatment	Plant component								
	Root length (m)	Root number	Root dry weight (g)	Total leaf area (m^2)	Leaf number	Average leaf area (cm^2)	Leaf dry weight (g)	Stem height (m)	Stem dry weight (g)
13 DAI									
UR	1.97	133	0.01	0.005	9.0	5.3[NS]	0.08	0.011	0.03
CR	2.31	190	0.02	0.005	9.3	5.6	0.09	0.009	0.03
RD	2.19	179	0.01	0.005	8.5	5.4	0.09	0.009	0.03
RDD	3.06	201	0.02	0.007	9.7	6.9	0.12	0.011	0.04
SEOD (n=6)	0.433	25.6	0.003	0.0008	0.57	0.86	0.011	0.0047	0.004
	NS	NS	NS	NS	NS	NS	NS	*	*
contrasts									
RD vs RDD	NS	NS	NS	*	NS	NS	**	*	NS
CR vs [RD+RDD]	NS	NS	NS	NS	NS	NS	NS	NS	NS
UR vs [CR+RD+RDD]	NS	*	NS	NS	NS	NS	NS	*	NS
31 DAI									
UR	24.15	1264	0.09	0.079	14.3	56.5	1.13	0.026	0.36
CR	23.72	1042	0.09	0.083	14.0	59.6	1.22	0.029	0.42
RD	20.16	872	0.08	0.070	13.8	49.9	1.07	0.028	0.38
RDD	22.69	977	0.10	0.070	13.7	51.5	1.33	0.027	0.38
SEOD (n=6)	2.579	101.9	0.013	0.0140	0.37	10.41	0.185	0.0146	0.039
	NS	*	NS	NS	NS	NS	NS	NS	NS
contrasts									
RD vs RDD	NS	NS	NS	NS	NS	NS	NS	NS	NS
CR vs [RD+RDD]	NS	NS	NS	NS	NS	NS	NS	NS	NS
UR vs [CR+RD+RDD]	NS	**	NS	NS	NS	NS	NS	NS	NS

[z] Days after initiation of treatments

NS, *, **, ***, ****Nonsignificant or significant F test at $P \leq 0.05$, 0.01, 0.001, or 0.0001 respectively

Table 3.5b Summary statistics from univariate analysis of plant response to root restriction at 45 and 67 DAI[z].

Treatment	Plant component								
	Root length (m)	Root number	Root dry weight (g)	Total leaf area (m^2)	Leaf number	Average leaf area (cm^2)	Leaf dry weight (g)	Stem height (m)	Stem dry weight (g)
45 DAI									
UR	155.3	6668	0.55	0.274	32.2	85.2	4.39	0.097	2.88
CR	94.6	4262	0.39	0.285	29.7	96.5	4.39	0.093	2.81
RD	126.9	3967	0.61	0.344	36.0	95.7	4.97	0.109	3.10
RDD	103.7	3838	0.50	0.290	30.3	93.8	4.54	0.099	3.03
SEOD (n=6)	15.84	491	0.065	0.0417	3.70	6.90	0.563	0.0124	0.413
	••	••••	•	NS	NS	NS	NS	NS	NS
contrasts									
RD vs RDD	NS	NS	NS	NS	NS	NS	NS	NS	NS
CR vs [RD+RDD]	NS	NS	•	NS	NS	NS	NS	NS	NS
UR vs [CR+RD+RDD]	••	••••	—	NS	NS	NS	NS	NS	NS
67 DAI									
UR	672.1	38508	2.19	1.467	92.7	158.4	21.40	0.496	18.95
CR	338.1	22671	1.19	0.971	71.5	137.3	16.81	0.337	15.58
RD	673.6	32199	2.15	1.117	76.5	147.0	17.86	0.369	15.45
RDD	514.9	34259	1.27	1.056	66.5	162.6	16.99	0.342	13.86
SEOD (n=6)	59.98	3778	0.178	0.133	8.58	15.23	1.575	0.0502	1.964
	••••	••	••••	••	•	NS	•	•	NS
contrasts									
CR vs RDD	••	••	NS	NS	NS	NS	NS	NS	NS
UR vs RD	NS	NS	NS	•	NS	NS	•	•	NS
[UR+RD] vs [CR+RDD]	—	—	••••	—	•	NS	—	—	NS

[z] Days after initiation of treatments

NS, •, ••, •••, ••••Nonsignificant or significant F test at $P \leq 0.05$, 0.01, 0.001, or 0.0001 respectively

Table 3.5c Summary statistics from univariate analysis of plant response to root restriction at 99 DAI[z].

Treatment	Root length (m)	Root number	Root dry weight (g)	Total leaf area (m^2)	Leaf number	Average leaf area (m^2)	Leaf dry weight (g)	Stem height (m)	Stem dry weight (g)
99 DAI									
UR	2449.5	171685	10.89	4.21	359.2	117.28	127.33	2.022	80.12
CR	(465.3)	(44596)	(2.37)	(1.24)	(122.3)	(101.4)	(46.7)	(0.526)	(40.4)
RD	2331.2	134893	9.58	3.67	307.2	121.6	114.00	1.698	74.74
RDD	1824.7	119987	7.72	2.99	242.8	123.1	107.27	1.198	68.52
SEOD (n=6)	126.98	18379	0.953	0.221	22.60	7.55	8.116	0.2694	4.765
	••	•	•	•••	••••	NS	NS	•	NS
contrasts									
UR vs RD	NS	NS	NS	•	•	NS	NS	NS	NS
[UR+RD] vs RDD	•••	#	•	—	—	NS	NS	•	NS

[z] Days after initiation of treatments
NS, •, ••, •••, ••••Nonsignificant or significant F test at $P \leq 0.05$, 0.01, 0.001, or 0.0001 respectively
( ) Data not included in analysis

Shoot growth

By 13 DAI, plant height and the dry weights of leaves and stems differed significantly among treatments (Table 3.5a). These differences did not follow any discernible pattern with respect to the extent of restriction. Although statistically significant, in all instances the differences were negligible and considered unimportant. Differences among treatments in total leaf area were not detected until 67 DAI. The total leaf area of UR plants was significantly larger than CR or previously restricted (RD, RDD) plants. By 99 DAI, however, there was little difference among UR and RD and RDD plants (Fig. 3.5d, Table 3.5c).

Apart from an apparently random difference among treatments 13 DAI, total leaf dry weight was not affected by root restriction until at least 45 DAI (Table 3.5b). From 67 DAI, however, all plants with, or having had, a restricted root system had lower total leaf dry weights than plants with unrestricted root systems.

By 67 DAI, plants with, or having had, restricted roots were significantly shorter than unrestricted plants. Although root restriction decreased stem extension, dry matter accumulation in stems was unaffected. By 67 DAI, CR plants had similar stem dry weights to unrestricted (UR and RD) plants. On the other hand, plants in 0.05 l containers, while having partitioned a similar proportion of total assimilate to stem tissue as CR and RD plants, had significantly less dry biomass in stem tissue than UR plants (Table 3.5b).

Up to 45 DAI, the number of new internodes or leaves initiated, as characterised by the number of leaves (Fig. 3.5e), was unaffected by root treatment. Between 45 and 67 DAI, plants with roots either restricted (CR and RDD) or previously restricted (RD) initiated fewer leaves than unrestricted plants (Table 3.5b). A similar pattern of response was apparent for lateral shoots (data not presented).

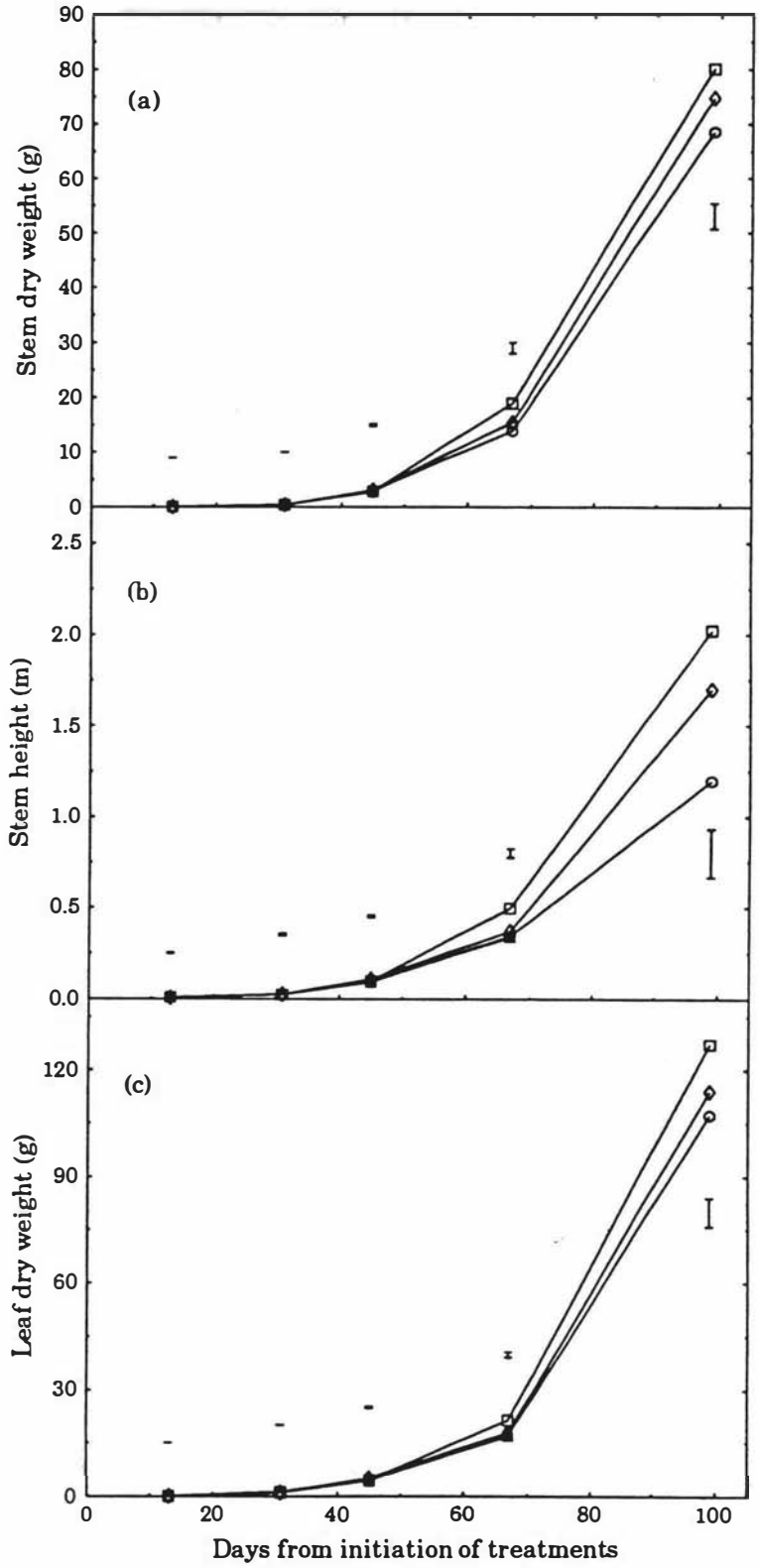

Fig. 3.5a-c. Ontogeny of (a) stem dry biomass, (b) stem height, and (c) leaf dry biomass of UR plants (□), RD plants (◇), RDD plants (○) and CR plants (△). Data points represent means, with n=6. Vertical bars are SEOD among treatments at a given harvest.

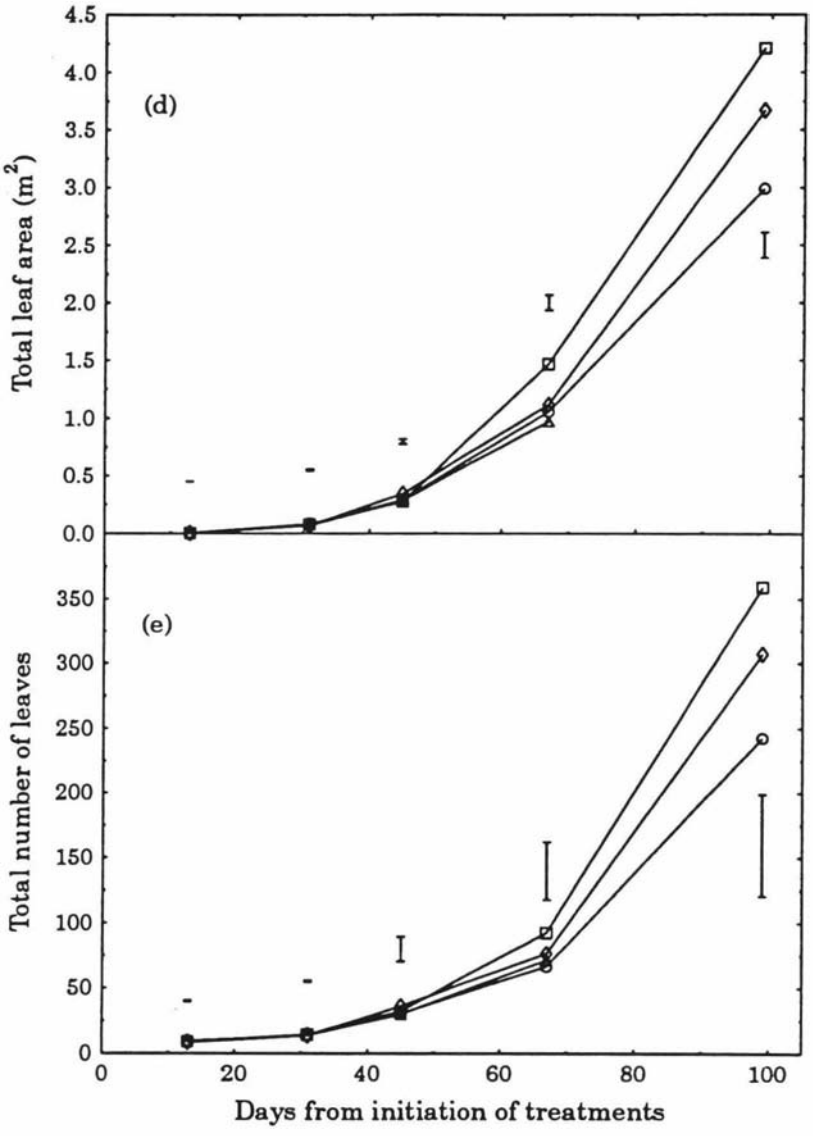

Fig. 3.5d-e. Ontogeny of (d) total leaf area and (e) leaf number of UR plants (□), RD plants (◇), RDD plants (○) and CR plants (△). Data points represent means, with n=6. Vertical bars are SEOD among treatments at a given harvest.

Among treatment comparisons of within-plant relationships

The canonical discriminant functions extracted from data collected 13 DAI did not reveal significant differences among groups (Fig. 3.6a). At 31 DAI, treatment groups were separated by a single CDF ($CDF_{1(31)}$), accounting for about 77% of total variance, which contrasted root mass against shoot mass (Table 3.6, Fig. 3.6b). Plants with unrestricted root systems had significantly higher scores than those with restricted root systems, reflecting the early depression of root growth (particularly root number) relative to shoot growth (particularly leaf dry biomass) in restricted plants.

Two discriminant functions ($CDF_{1(45)}$, $CDF_{2(45)}$) together accounted for about 99% of total variation of data collected 45 DAI. Treatment groups were maximally separated by $CDF_{1(45)}$, which contrasted root number with leaf dry weight. Stem dry weight was contrasted with root and stem length, although these contributions to the function were small. Plants with high scores for this function would be characterised by either a highly branched root system and low leaf dry weight, or vice versa. This function reflects different sites of growth of plants in the treatments. Unrestricted (UR) plants had significantly higher scores for $CDF_{1(45)}$ than all other treatment groups. While having a more multibranched root system than plants in the CR, RD and RDD treatments, UR plants had similar leaf dry biomass. Between the restricted or previously restricted groups, RD and RDD plants had significantly lower mean scores for $CDF_{1(45)}$ than CR plants. The RD and RDD plants tended to have fewer roots and higher leaf dry weights than plants under continuous root restriction. Taken with their low scores for $CDF_{1(45)}$, this suggests that following de-restriction 31 DAI, RD and RDD plants diverted energy to leaf growth rather than increasing root number.

Root dry weight dominated $CDF_{2(45)}$, with smaller contrasts with root length and shoot biomass (stem and leaf dry weight). Plants in continuous

restriction had significantly lower scores for this function than plants in the other treatments, while UR plants could not be distinguished from those in either the RD or RDD treatments. The significantly higher score of RD plants compared with RDD plants suggests that upon de-restriction, RD plants directed proportionately more assimilate to their root system and possibly increased root branching and stem elongation compared with RDD plants. The difference between RD and RDD plants also indicates that even partial de-restriction (i.e. into the 0.05 l container) was sufficient to influence growth.

By 67 DAI, treatment groups were distinguished by two CDFs accounting for similar proportions of total variation. Plants with low scores for the first function, $CDF_{1(67)}$, were characterised by relatively thick or dense roots (low SRL), dense ($g_{ST}.m^{-1}$) stems and 'heavy' leaf dry biomass. Plants in the CR and UR treatments has similarly low scores for this function, in both instances being significantly lower than the respective scores for RD and RDD plants. An increased stem density in UR plants is consistent with the large plants requiring structural strength in the stem to support the increasing foliage weight being carried. In the CR plants, an increase in the density of the stem is consistent with enhanced partitioning to the stem as the sink strength of the root system is weakened due its restriction, and thick roots are consistent with physical impedance (Goss and Russell, 1980). Thin roots, on the other hand, would be consistent with rapid root growth following removal (or partial removal) of physical restriction (i.e. RD and RDD plants) (Richards and Rowe, 1977a). The second function, $CDF_{2(67)}$, describes a pattern of growth in space and between organs, with unrestricted plants (UR and RD) characterised by being taller, and having longer root systems, larger leaf area and longer and heavier root systems than restricted plants (CR and RDD), which by comparison, had proportionately more assimilate in stem biomass.

The main discriminator between unrestricted and de-restricted groups at the final harvest (99 DAI) contrasts leaf dry weight with root length and stem dry weight. $CDF_{1(99)}$ is interpreted as a balance among these variables. The near-zero score of UR suggests the 'control' balance. Relative to this, RDD plants appear to have partitioned more assimilate to increasing leaf biomass than to stem biomass or root length. On the other hand, the partitioning pattern in RD plants appears to have been in the opposite direction. The remaining CDF ($CDF_{2(99)}$) contrasts stem growth (biomass) with leaf growth (area and biomass). From the scores, it appears that de-restricted plants (RD and RDD) had poorer developed canopies than stems compared to UR plants.

Table 3.6 Canonical discriminant functions and treatment means of growth variables at each harvest.

Growth variable	31 DAI	45 DAI		67 DAI		99 DAI	
	CDF_1	CDF_1	CDF_2	CDF_1	CDF_2	CDF_1	CDF_2
Leaf area	-0.53	0.32	-0.62	-0.35	0.92	-0.98	-5.13
Stem length	0.05	-0.72	1.85	-1.84	0.59	0.57	1.30
Leaf dry weight	-1.67	-2.25	-0.92	-1.77	-0.53	-4.61	-3.67
Stem dry weight	-0.90	0.98	-1.88	2.02	-1.22	2.77	6.31
Root dry weight	1.24	-0.43	4.08	-2.59	0.84	0.29	-0.21
Root number	1.50	3.97	1.18	0.63	0.17	-1.71	-1.22
Root length	0.65	-0.66	-2.17	3.18	0.67	4.54	1.65
λ_i	2.01	10.91	2.37	2.46	2.35	4.19	1.65
χ^2_{obs}	19.3^{*}	43.3^{****}	21.3^{**}	21.7^{**}	21.2^{**}	19.8^{*}	17.1^{NS}
df	9	9	7	9	7	8	6
percent total variance	77	81	18	45	43	65	34
ANOVA means							
UR	2.15	4.84	0.91	-1.74	1.43	0.17	-1.90
CR	-0.25	0.06	-2.17	-0.98	-2.17		
RD	-1.30	-3.13	1.51	0.86	1.00	2.19	1.06
RDD	-0.59	-1.77	-0.26	1.87	-0.27	-2.37	0.84
SEOD (n=6)	0.59	0.62	0.59	0.61	0.51	0.58	0.59
	****	****	****	****	****	****	***

NS, *, **, ***, ****Nonsignificant or significant χ^2 test (CDFs) or F test (ANOVA means) at $P \leq 0.05$, 0.01, 0.001, or 0.0001 respectively
λ_i:eigenvalue of ith canonical discriminant function

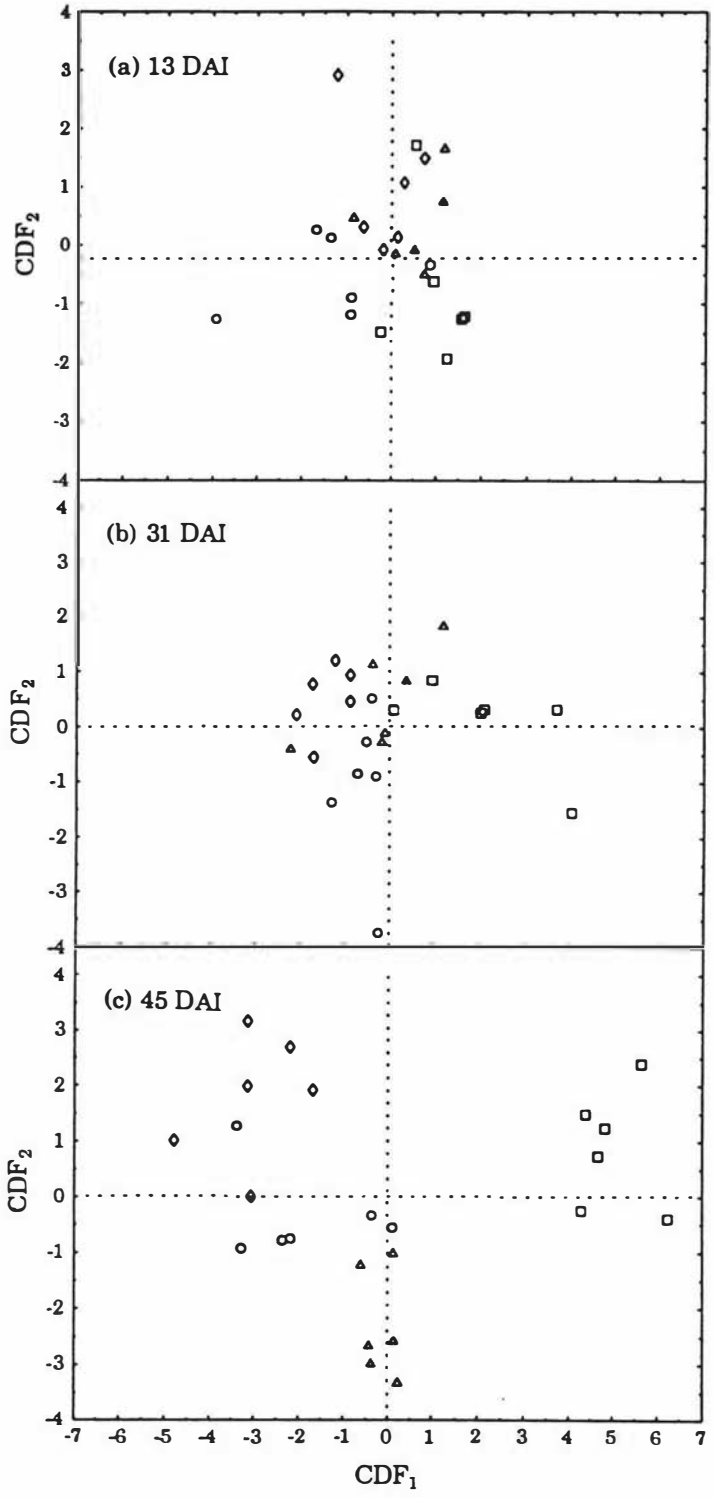

Fig. 3.6a-c. Plots of canonical discriminant functions from harvest data at 13, 31, and 45 DAI for UR plants (□), RD plants (◇), RDD plants (○) and CR plants (△).

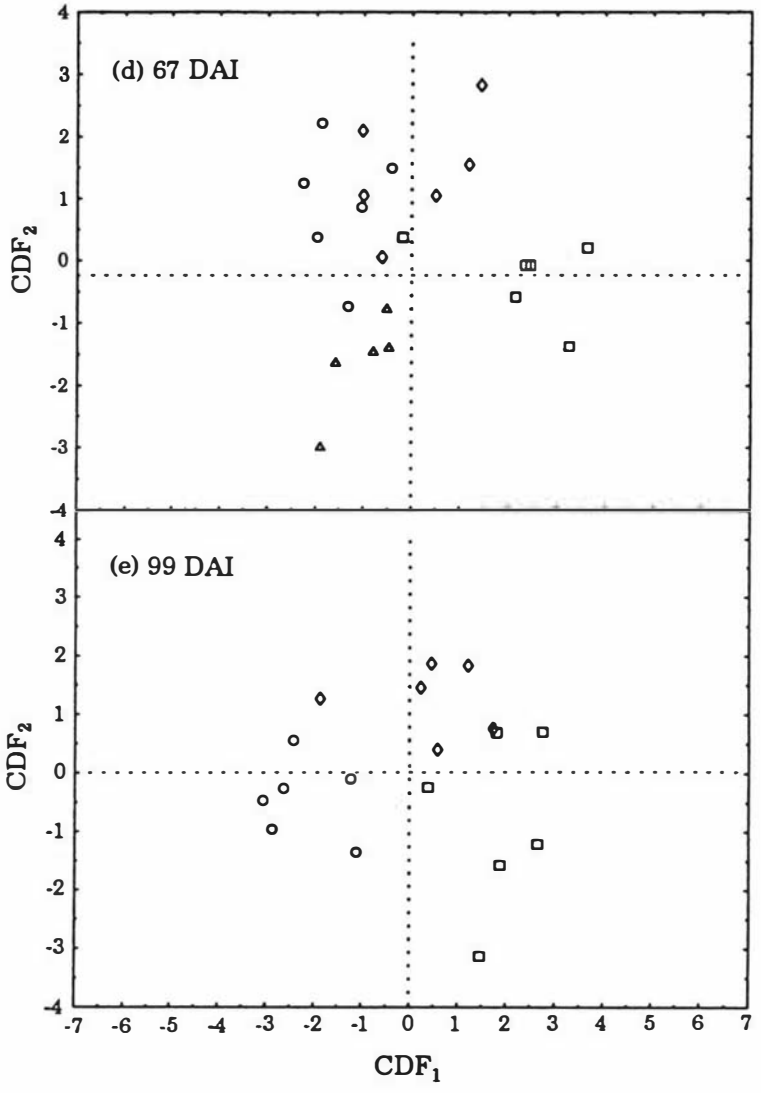

Fig. 3.6d-e. Plots of canonical discriminant functions from harvest data at 67 and 99 DAI for UR plants (□), RD plants (◇), RDD plants (○) and CR plants (△).

Relationships between the components of root and shoot growth

There was no consistent pattern in the percent reduction between CR and UR plants for root and shoot variables (Table 3.7).

Table 3.7 Percent (%) change in plant response between plants with restricted root systems (CR treatment only) and unrestricted root systems (UR treatment).

Harvest (DAI)	Plant variable							
	Root length	Root number	Root dry weight	Shoot dry weight	Leaf dry weight	Leaf area	Leaf number	Stem length
13	21	43	10	6	10	10	3	-12
31	0	-18	8	10	8	5	-2	8
45	-39	-36	0	-1	0	-5	-8	-4
67	-61	-41	-21	-18	-21	-34	-23	-32

Path analysis was used to gain more insight to the inter-relationships between root and shoot variables. The hypothesized model investigated (Fig. 3.7) explicitly gave an independent role to root size (dry biomass weight) in addition to root number and length. Although a mechanistic role for root size in the model is not immediately apparent, its inclusion in this analysis is warranted as root biomass increased independently of increases in root length (SRL; Fig. 3.4b).

Simple correlations alone would have led to the erroneous conclusion that the number, weight and length of roots were all strongly associated with total leaf area, leaf number, shoot dry weight *and* stem height. The path analysis revealed that the strong correlations between root number and dry weight and the shoot variables were invariably due to the covariance of these root variables with root length (Tables 3.8a-b). With the contribution of root length removed, the direct effects between both root number and weight on

plant height, leaf number and area and shoot dry weight were small and negative. The direct effect between root number and leaf area was significantly negative (Table 3.8b). Root dry weight had a positive direct effect on shoot dry weight.

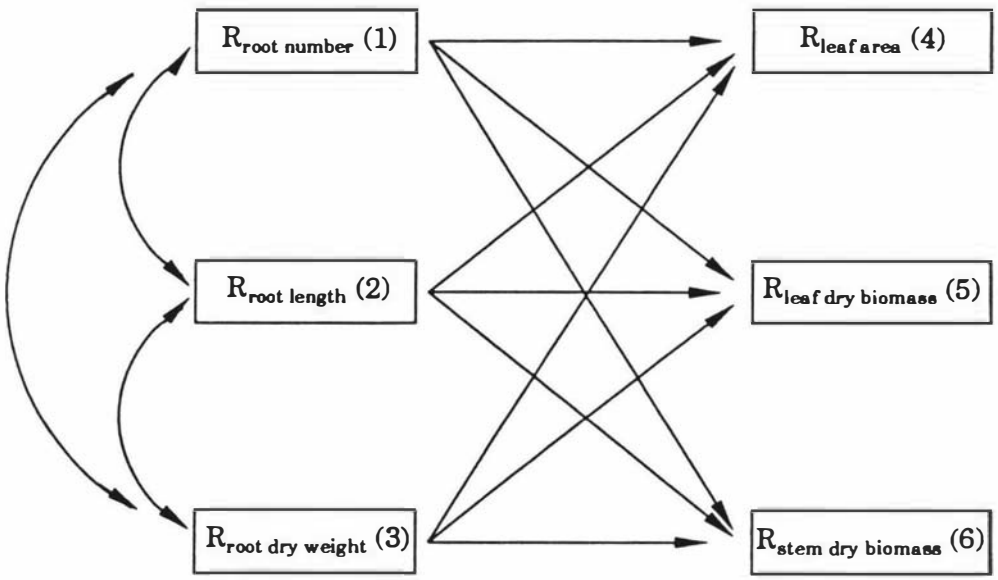

Fig. 3.7. Path analysis diagram of relationships between the components of root growth (i.e. root number, length and dry biomass) and shoot growth (i.e. leaf area and number, shoot dry biomass, and stem height).

Table 3.8a Path analysis of the effects of root growth components on shoot dry weight and plant height. Significance levels are presented for the direct effects and simple correlations only. (n=114.)

Pathway and compound pathways and designation of effect	correlation coefficient (r_{ij})	path coefficient (P_{ij})	influence $r_{ij} \cdot P_{ij}$
Root number vs. shoot dry weight (observed r=0.97****)			
Direct effect, P_{51}		-0.01	-0.01
Indirect effect via root length, $r_{56}P_{61}$	0.98	0.80	0.78
Indirect effect via root dry weight, $r_{57}P_{71}$	0.96	0.20	0.20
Root length vs. shoot dry weight (observed r=0.99****)			
Direct effect, P_{61}		0.80	0.80
Indirect effect via root number, $r_{65}P_{51}$	0.98	-0.01	-0.01
Indirect effect via root dry weight, $r_{67}P_{71}$	0.98	0.20	0.20
Root dry weight vs. shoot dry weight (observed r=0.97****)			
Direct effect, P_{71}		0.20	0.20**
Indirect effect via root number, $r_{75}P_{51}$	0.96	-0.01	-0.01
Indirect effect via root length, $r_{76}P_{61}$	0.98	0.80	0.78
Root number vs. plant height (observed r=0.95****)			
Direct effect, P_{52}		-0.11	-0.11
Indirect effect via root length, $r_{56}P_{62}$	0.98	1.13	1.11
Indirect effect via root dry weight, $r_{57}P_{72}$	0.96	-0.06	-0.05
Root length vs. plant height (observed r=0.97****)			
Direct effect, P_{62}		1.13	1.13****
Indirect effect via root number, $r_{65}P_{52}$	0.98	-0.11	-0.10
Indirect effect via root dry weight, $r_{67}P_{72}$	0.98	-0.06	-0.06
Root dry weight vs. plant height (observed r=0.95****)			
Direct effect, P_{72}		-0.06	-0.06
Indirect effect via root number, $r_{75}P_{52}$	0.96	-0.11	-0.10
Indirect effect via root length, $r_{76}P_{62}$	0.98	1.13	1.11

NS, *, **, ***, ****Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, or 0.0001 respectively

Table 3.8b Path analysis of the effects of root growth components on leaf area. Significance levels are presented for the direct effects and simple correlations only. (n=114.)

Pathway and compound pathways and designation of effect	correlation coefficient (r_{ij})	path coefficient (P_{ij})	influence $r_{ij} \cdot P_{ij}$
Root number vs. leaf area (observed r=0.95****)			
Direct effect, P_{53}		-0.19	-0.19*
Indirect effect via root length, $r_{56}P_{63}$	0.98	1.22	1.20
Indirect effect via root dry weight, $r_{57}P_{73}$	0.96	-0.06	-0.05
Root length vs. leaf area (observed r=0.99****)			
Direct effect, P_{63}		1.22	1.22
Indirect effect via root number, $r_{65}P_{53}$	0.98	-0.19	-0.18
Indirect effect via root dry weight, $r_{67}P_{73}$	0.98	-0.06	-0.05
Root dry weight vs. leaf area (observed r=0.96****)			
Direct effect, P_{73}		-0.06	-0.06
Indirect effect via root number, $r_{75}P_{53}$	0.96	-0.19	-0.18
Indirect effect via root length, $r_{76}P_{63}$	0.98	1.22	1.20
Root number vs. leaf number (observed r=0.97****)			
Direct effect, P_{54}		0.23	0.23*
Indirect effect via root number, $r_{65}P_{54}$	0.98	0.70	0.69
Indirect effect via root dry weight, $r_{57}P_{74}$	0.96	0.06	0.05
Root length vs. leaf number (observed r=0.98****)			
Direct effect, P_{64}		0.70	0.70****
Indirect effect via root number, $r_{65}P_{54}$	0.98	0.23	0.22
Indirect effect via root dry weight, $r_{67}P_{74}$	0.98	0.06	0.06
Root dry weight vs. leaf number(observed r=0.97****)			
Direct effect, P_{74}		0.06	0.06
Indirect effect via root number, $r_{75}P_{54}$	0.96	0.23	0.22
Indirect effect via root length, $r_{76}P_{64}$	0.98	0.70	0.69

NS, *, **, ***, ****Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, or 0.0001 respectively

3.3.3 Growth analysis

Methodology

The simple ratio method and the functional approaches estimated similar mean values for comparative indices at all harvests. No differences in $\bar{R}_W$, $\bar{E}_A$ (Fig. 3.8) or LAR among treatments (data not presented) were detected by classical growth analysis methods at any harvest assessed.

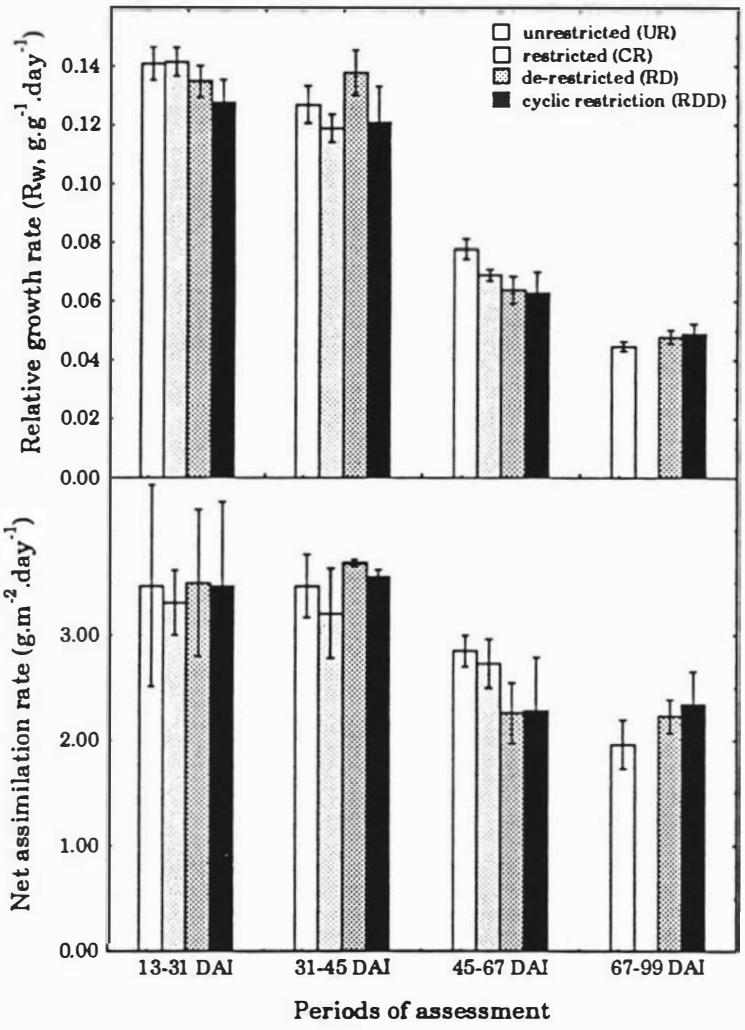

Fig. 3.8. Mean relative growth rates (a) and net assimilation rates (b) of plants. Data bars represent means with n=6. Vertical bars are ± SE.

Given the apparent uniformity of the plant material at the initiation of treatments and major differences in whole and component growth between UR and CR plants (Tables 3.5a-c) detected through the univariate analysis, differences in R_W and either or both E_A and LAR between these treatments at least must have occurred during the experiment. Either the duration between adjacent harvests was too long or the standard errors too large for relative changes in $\bar{R}_W$ to be detected.

Apart from E_A, the estimates of the indices obtained from the hybrid and standard method were almost identical. Differences were generally within ±1 unit of the last decimal place reported (Table 3.9). For E_A values, differences between estimates were within ±0.1 $m^2 \cdot m^{-2} \cdot day^{-1}$ of each other. Standard errors tended to be smaller for the hybrid method, although this varied among indices (Fig. 3.9).

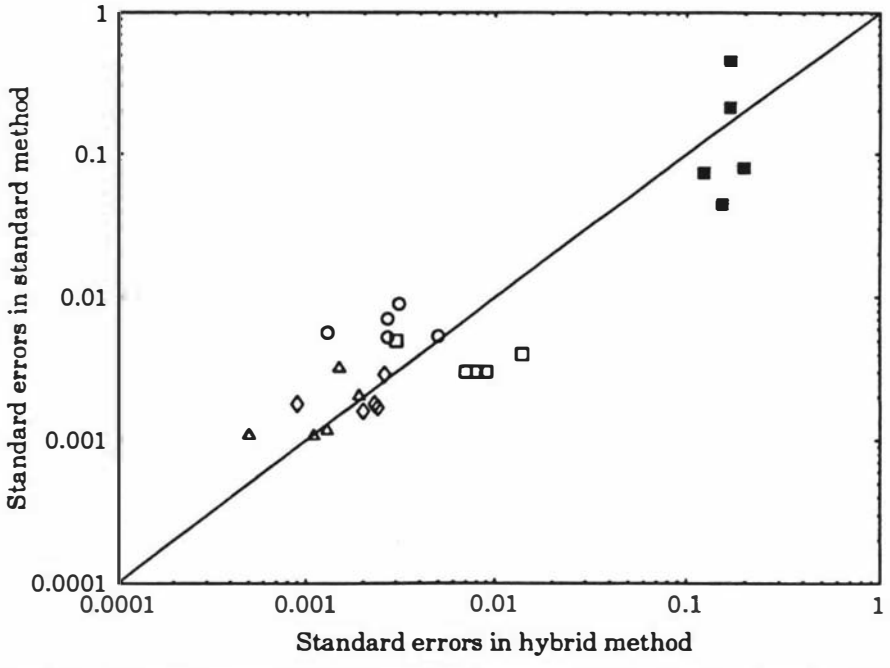

Fig. 3.9. Comparison of pooled standard errors of the standard and hybrid methods of analysis in calculating R_W (■), E_A (○), LAR (△), SLA (◇) and LWR (□).

As the hybrid method yielded near-identical means and standard errors of similar magnitude compared with the standard method, it was adopted for all subsequent analyses and all further data presented is from this analysis method. When, however, the two methods diverged considerably in their output, mention is made in the text.

Relative growth rate

The relative growth rate of plants in all treatments decreased during the experiment as the relative amount of tissue not directly contributing to overall growth increased (Fig 3.10). Small differences, inconsistently associated with root environment, were detected between treatments 13 and 31 DAI (Table 3.9). By 45 DAI, R_w of restricted plants (CR and RDD) was significantly lower than that of unrestricted (UR and RD) plants. Following release from the 0.025 l container 31 DAI, the R_w of RDD plants increased relative to unrestricted plants so that by 67 DAI the R_w of RDD plants was similar to that of unrestricted plants. By 67 DAI, the continued restriction in 0.025 l containers manifested in R_w of CR plants being markedly lower than all other treatments.

Table 3.9 Influence of root restriction on major growth analysis indices.

Treatment	Time (DAI)				
	13	31	45	67	99[z]
Plant relative growth rate (R_w; g g^{-1} day^{-1})					
UR	0.158	0.128	0.105	0.068	0.015
CR	0.167	0.127	0.096	0.048	
RD	0.154	0.125	0.102	0.067	0.015
RDD	0.141	0.116	0.096	0.065	0.019
SEOD (n=6)	0.0071	0.0038	0.0018	0.0038	0.0044
	•	•	•••	•••	NS
Net assimilation rate (E_a; g m^{-2} day^{-1})					
UR	3.95	3.55	3.21	2.51	0.70
CR	4.22	3.23	2.81	2.24	
RD	4.09	3.49	3.10	2.42	0.63
RDD	3.62	3.26	2.99	2.49	1.05
SEOD (n=6)	0.279	0.218	0.371	0.238	0.240
	NS	NS	NS	NS	NS
Leaf area ratio (LAR; m^2 · g$_w$$^{-1}$)					
UR	0.041	0.037	0.033	0.027	0.019
CR	0.040	0.040	0.035	0.022	
RD	0.039	0.036	0.033	0.027	0.018
RDD	0.039	0.035	0.032	0.026	0.016
SEOD (n=6)	0.0027	0.0021	0.0018	0.0016	0.0007
	NS	NS	NS	•	•
Specific leaf area (SLA; m^2 g$_L$$^{-1}$)					
UR	0.061	0.056	0.052	0.044	0.033
CR	0.059	0.059	0.052	0.036	
RD	0.058	0.055	0.051	0.044	0.032
RDD	0.055	0.052	0.048	0.041	0.028
SEOD (n=6)	0.0037	0.0034	0.0033	0.0028	0.0013
	NS	NS	NS	NS	•
Leaf weight ratio (LWR; g$_L$ g$_w$$^{-1}$)					
UR	0.68	0.66	0.65	0.62	0.58
CR	0.68	0.69	0.68	0.61	
RD	0.67	0.66	0.65	0.62	0.57
RDD	0.71	0.68	0.66	0.62	0.58
SEOD (n=6)	0.019	0.011	0.009	0.013	0.004
	NS	NS	NS	NS	•

NS, •, *, **, ***Nonsignificant or significant F test at $P \leq 0.10$, 0.05, 0.01, or 0.001, respectively.

Table 3.10 Influence of root restriction on partitioning patterns.

Treatment	Time (DAI)				
	13	31	45	67	99[z]
Stem weight ratio (SWR; $g_{ST}\,g_w^{-1}$)					
UR	0.22	0.26	0.29	0.34	0.38
CR	0.21	0.25	0.29	0.37	
RD	0.23	0.27	0.29	0.33	0.39
RDD	0.19	0.24	0.29	0.35	0.38
SEOD (n=6)	0.018	0.013	0.011	0.014	0.007
	NS	NS	NS	#	NS
Root weight ratio (RWR; $g_R\,g_w^{-1}$)					
UR	0.105	0.066	0.053	0.044	0.050
CR	0.116	0.060	0.041	0.028	
RD	0.094	0.065	0.054	0.046	0.049
RDD	0.109	0.062	0.046	0.036	0.042
SEOD (n=6)	0.0095	0.0027	0.0025	0.0024	0.0045
	NS	NS	•••	••••	NS
Shoot:root ratio (SR)					
UR	9.0	14.2	18.4	22.6	19.6
CR	7.8	15.6	23.5	36.2	
RD	10.1	14.3	17.5	20.9	20.6
RDD	8.4	15.3	21.3	27.6	23.3
SEOD (n=6)	0.91	0.69	1.16	2.73	2.67
	NS	NS	•••	•••	NS

NS, #, •, ••, •••, ••••Nonsignificant or significant F test at $P \leq 0.10$, 0.05, 0.01, 0.001, or 0.001, respectively.

Table 3.11 Influence of root restriction on relative growth rates of root and shoot components

Treatment	Time (DAI)				
	13	31	45	67	99[z]
Relative leaf growth rate (R_L; $g_L g_L^{-1}$ day^{-1})					
UR	0.157	0.126	0.103	0.066	0.012
CR	0.169	0.127	0.093	0.041	
RD	0.154	0.124	0.101	0.065	0.012
RDD	0.139	0.113	0.093	0.062	0.017
SEOD (n=6)	0.0068	0.0037	0.0020	0.0040	0.0045
	••	••	•••	••••	NS
Relative leaf expansion rate (R_A; $m^2 m^{-2}$ day^{-1}					
UR	0.153	0.122	0.097	0.058	0.002
CR	0.173	0.121	0.082	0.019	
RD	0.153	0.120	0.095	0.056	0.000
RDD	0.137	0.109	0.087	0.053	0.004
SEOD (n=6)	0.0061	0.0034	0.0023	0.004	0.0034
	••	••	••••	••••	NS
Relative stem growth rate (R_{ST}; $g_{ST} g_{ST}^{-1}$ day^{-1})					
UR	0.169	0.137	0.112	0.073	0.017
CR	0.178	0.138	0.107	0.059	
RD	0.163	0.133	0.109	0.072	0.018
RDD	0.158	0.129	0.106	0.071	0.019
SEOD (n=6)	0.0078	0.0044	0.0023	0.0038	0.0049
	NS	NS	#	••	NS
Relative root growth rate (R_R; $g_R g_R^{-1}$ day^{-1})					
UR	0.129	0.108	0.091	0.065	0.026
CR	0.127	0.096	0.073	0.036	
RD	0.133	0.109	0.091	0.063	0.022
RDD	0.105	0.089	0.078	0.060	0.034
SEOD (n=6)	0.0109	0.0059	0.0028	0.0055	0.0068
	NS	•	••••	•••	NS
Relative shoot growth rate ($g_s g_s^{-1}$ day^{-1})					
UR	0.161	0.129	0.106	0.068	0.014
CR	0.171	0.129	0.098	0.047	
RD	0.156	0.127	0.103	0.067	0.014
RDD	0.144	0.117	0.097	0.065	0.019
SEOD (n=6)	0.0068	0.0037	0.0018	0.0038	0.0045
	••	•	•••	••••	NS

NS, #, •, ••, •••, ••••Nonsignificant or significant F test at $P \leq 0.10$, 0.05, 0.01, 0.001 or 0.0001, respectively.

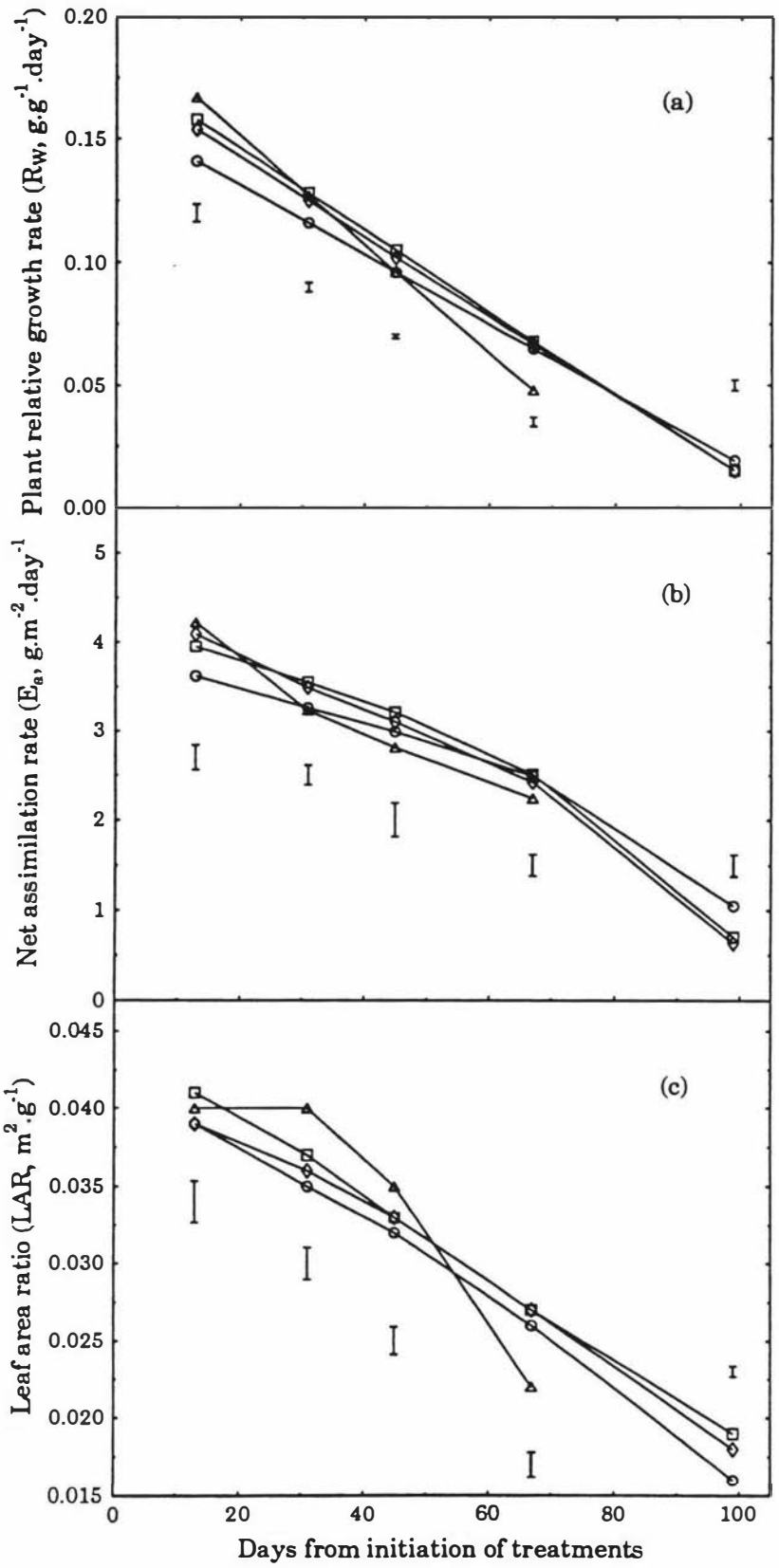

Fig. 3.10. Effect of root restriction on the relative growth rates, net assimilation rates and leaf area ratio of UR plants (□), RD plants (◇), RDD plants (○) and CR plants (△). Data points represent means with n=6. Vertical bars are SEOD among treatments at a given harvest.

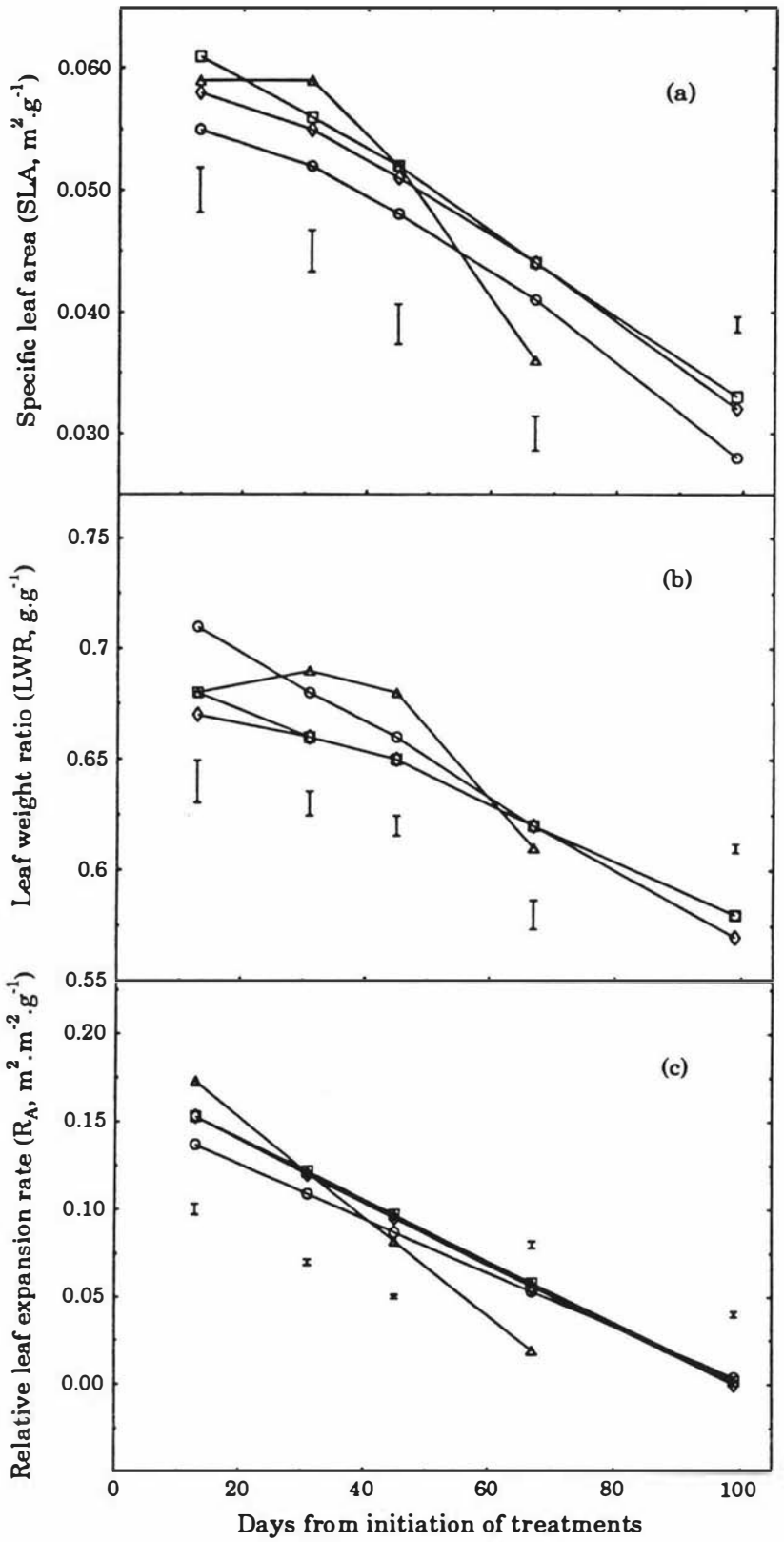

Fig. 3.11. Effect of root restriction on (a) the specific leaf area, (b) leaf weight ratio and (c) relative leaf expansion rate of UR plants (□), RD plants (◇), RDD plants (○) and CR plants (△). Data points represent means with n=6. Vertical bars are SEOD among treatments at a given harvest.

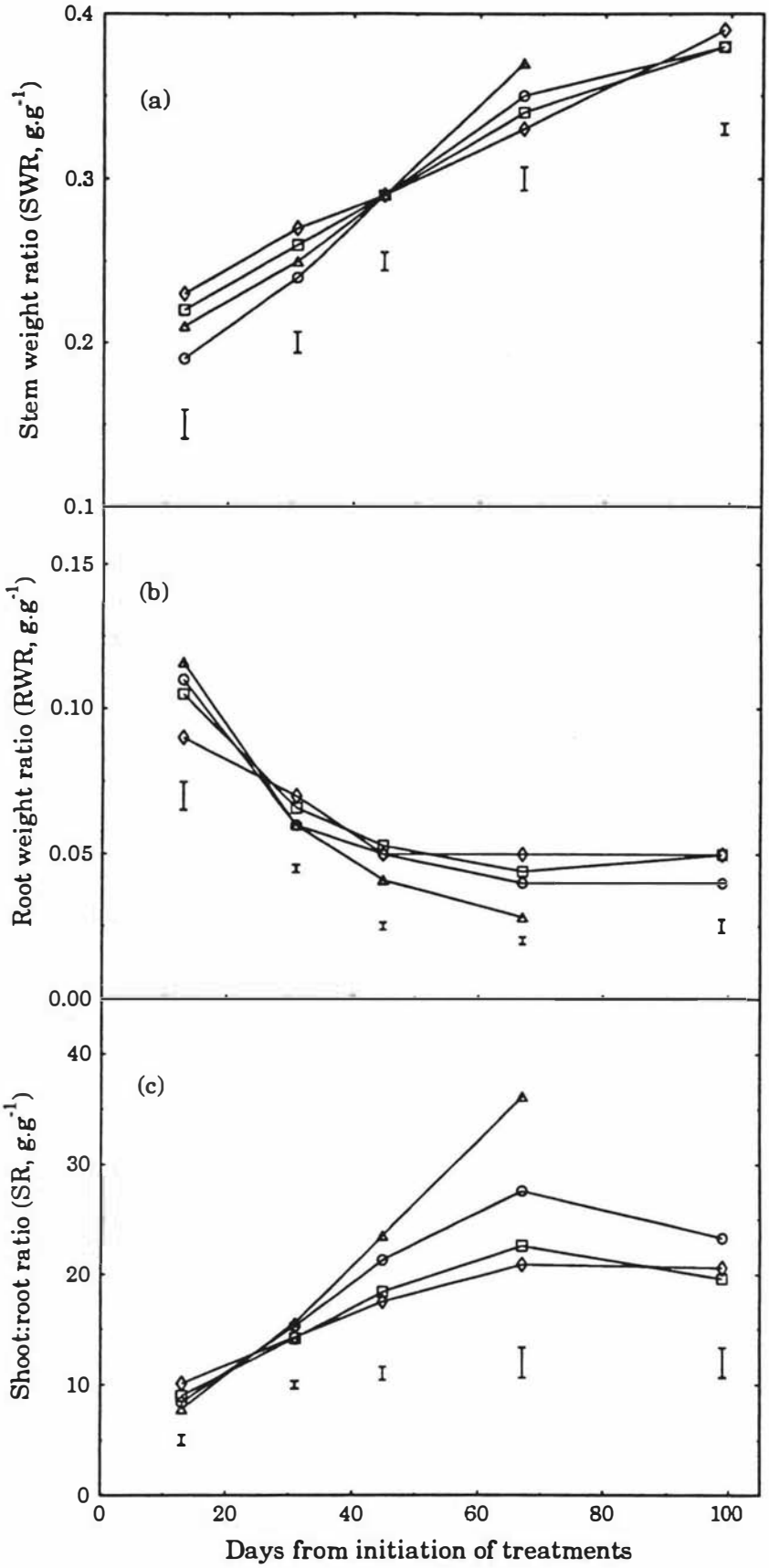

Fig. 3.12. Effect of root restriction on (a) stem weight ratio, (b) root weight ratio and (c) shoot:root ratio of UR plants (□), RD plants (◇), RDD plants (○) and CR plants (△). Data points represent means with n=6. Vertical bars are

Net assimilation rate

Restricting roots depressed E_A mid-way during the experiment, but the decline was not significantly different from unrestricted plants (Table 3.9, Fig. 3.8). The E_A of CR and RD plants was lower than unrestricted plants 31 DAI. Although the standard method recorded the differences as significant, the hybrid method was more conservative. By 45 DAI, however, both methods detected a significantly lower E_A in CR plants compared to less or unrestricted plants. No differences among any treatments were detected 12 days later.

Relative growth rates and indices of leaf growth

The duration of the observed differences among treatments in E_A seemed insufficient to cause the observed decrease in R_W. Given the relationship among the indices (equation 3.15), the difference in R_W between restricted and unrestricted plants must also have resulted from differences in leaf growth. Path analysis of the relationship between R_W and its components (Fig. 3.13, Table 3.12) revealed that E_A and LAR were equally 'important' in determining R_W. Although differences in LAR between CR and unrestricted (UR and RD) plants were not detected until 67 DAI (Table 3.9), the LAR of CR plants showed a rapid decline, relative to the other treatments, after 45 days of restriction (Fig. 3.10c). This was consistent with the observed decline in R_A of CR plants (Fig. 3.11c). Plants de-restricted into 0.05 l containers had a lower LAR than unrestricted plants throughout the experiment, although these differences were not significant until at least 67 DAI.

In the absence of differences among treatments in the pattern of assimilate partitioning to leaf dry biomass (Table 3.9, Fig. 3.11b), the observed influence of root restriction on LAR must have occurred through SLA. The relationship

between LAR and its components confirms this (Table 3.12, Fig. 3.13). From 31 DAI the SLA of CR plants decreased more rapidly than unrestricted plants (Fig. 3.11a). By 67 DAI, restricted plants (CR and RD) had made less leaf area per unit leaf biomass (lower SLA) than unrestricted plants (Table 3.9). Restriction apparently influenced the relative rate of leaf expansion more than the relative rate of increase in leaf dry biomass (Table 3.11). De-restricting plants from the 0.05 l container did not induce any detectable change in SLA.

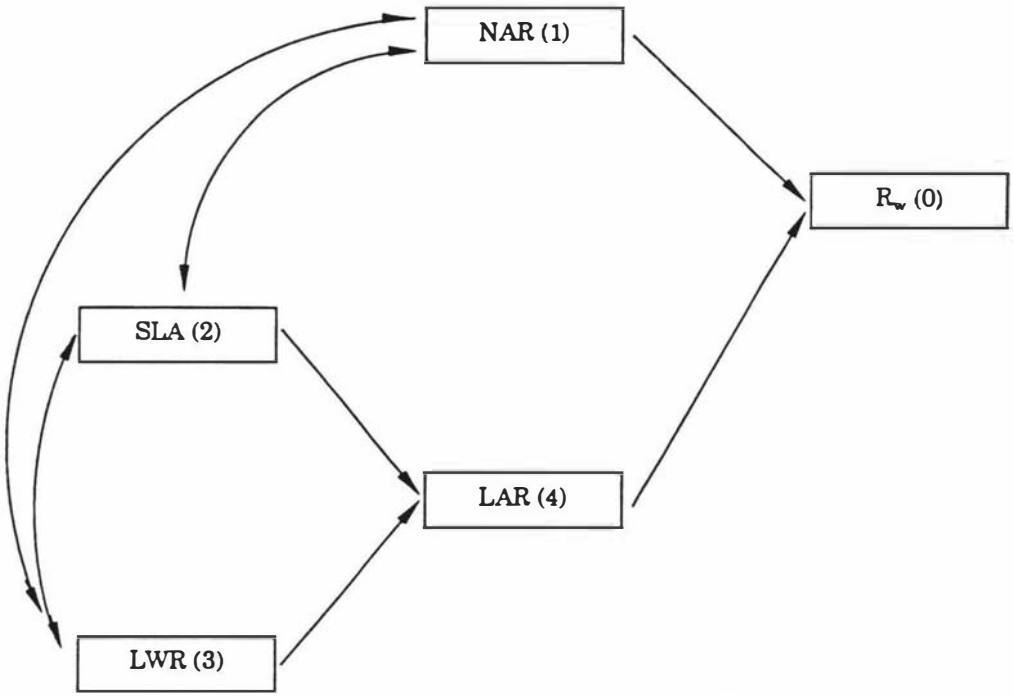

Fig. 3.13. Path diagram of structural relationship between the components (variables 1-4) of the whole plant relative growth rate (variable 0 [R_w]). Single-headed arrows represent the direct effects, measured by path coefficients (P_{ij}, Table 3.13), and the double-headed arrows depict simple correlations (r_{ij}, Table 3.13) between R_w and net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA), and leaf weight ratio (LWR).

Table 3.12 Path analysis[z] of the effects of growth components on the relative growth rate of tomatoes ($g \cdot g^{-1} \cdot day^{-1}$). Significance levels are presented for the direct effects and simple correlations only. (n of correlation matrix=19.)

Pathways and designation of effect	correlation coefficient (r_{ij})	path coefficient (P_{ij})	path coefficient (P_{ij})	influence $r_{ij} \cdot P_{ij}$
SLA vs. R_w				
via LAR, $P_{24}P_{40}$		0.77	0.56	0.43
via LWR, LAR, $r_{23}P_{34}P_{40}$	0.94	0.24	0.56	0.13
via NAR, $r_{21}P_{10}$	0.95	0.45		0.43
total correlation				0.98
LWR vs. R_w				
via LAR, $P_{34}P_{40}$		0.24	0.56	0.13
via SLA, LAR, $r_{32}P_{24}P_{40}$	0.94	0.77	0.56	0.41
via NAR, $r_{31}P_{10}$	0.91	0.45		0.41
total correlation				0.95
LAR vs. R_w				
direct, P_{40}			0.56	0.56****
via LWR, NAR, $P_{43}r_{31}P_{10}$	0.91	0.24	0.45	0.09
via SLA, NAR, $P_{42}r_{21}P_{10}$	0.94	0.77	0.45	0.33
total correlation				0.98
NAR vs R_w				
direct, P_{10}			0.45	0.45****
via SLA, LAR, $r_{12}P_{24}P_{40}$	0.94	0.77	0.56	0.41
via LWR, LAR, $r_{13}P_{34}P_{40}$	0.91	0.24	0.56	0.12
total correlation				0.98
SLA vs. LAR				
Direct effect, P_{10}		0.77		0.77
Indirect effect via LWR, $r_{12}P_{20}$	0.94	0.24		0.22
total correlation				0.99
LWR vs. LAR				
Direct effect, P_{20}		0.24		0.24
Indirect effect via E_A, $r_{21}P_{10}$	0.94	0.77		0.72
total correlation				0.96

NS, *, **, ***Nonsignificant or significant at $P \leq 0.10$, 0.05, 0.01, or 0.001, respectively.

[z] Path diagram in Figure 3.13

The changes in SLA among treatments were due to differences in leaf density. Leaf thickness, approximated by the ratio of leaf fresh weight to area (Table 3.13), declined during the experiment, but was not influenced by root restriction. Continuous root restriction, on the other hand, increased leaf density.

Table 3.13 Influence of root restriction on leaf 'thickness' and density components of specific leaf area.

	Time (DAI[z])				
Treatment	13	31	45	67	99
Leaf density ($10^2 \times g_{dw} \cdot g_{fw}^{-1}$)[y]					
UR	7.8	6.9	7.4	7.4	7.9
CR	8.1	7.1	7.8	9.4	(10.4)
RD	7.8	7.3	7.5	7.8	8.0
RDD	8.0	7.4	7.6	8.0	8.6
SEOD (n=6)	0.21	0.13	0.21	0.52	0.52
	NS	**	NS	*	NS
Leaf 'thickness' ($g_{fw} \cdot m^{-2}$)					
UR	229.6	229.2	167.1	168.6	386.4
CR	218.9	214.1	155.0	164.7	(371.9)
RD	245.1	217.2	154.9	167.2	390.3
RDD	232.2	249.2	158.4	166.9	433.3
SEOD (n=6)	11.06	26.1	8.15	7.07	34.35
	NS	NS	NS	NS	NS

[z] Days after initiation of treatments
[y] g_{fw}=leaf fresh weight (g); g_{dw}=leaf dry weight (g).
( ) Data not included in analysis.

Patterns of assimilate partitioning

Across all treatments, the relative amount of assimilate partitioned to roots declined from ≈10% to ≈5% over the course of the experiment (Fig 3.12b). By 31 DAI, proportionately less assimilate was partitioned to the restricted root systems (CR and RDD). Although this change in partitioning pattern was only statistically significant in the standard analysis, within two weeks the influence of restriction had become more pronounced and by 67 DAI, CR plants had allocated significantly less assimilate to their roots than RDD plants and unrestricted roots. During this period, plants in the 0.05 l containers had significantly lower RWR than unrestricted (UR and RD) plants, a pattern retained at 99 DAI despite a slight increase by all these treatments. Changes in partitioning patterns to the stem were less pronounced. By 67 DAI, plants in 0.025 l containers had partitioned comparatively more assimilate to stems than unrestricted plants, with the response of plants in the 0.05 l containers midway between these groups. These relative changes in assimilate partitioning among individual organs were amplified by a significant increase in the SR ratio in plants with restricted root systems between 31 and 45 DAI (Fig. 3.12c). De-restriction from the 0.05 l container resulted in an immediate decrease in the SR ratio as assimilate was re-directed to leaf tissue (Fig. 3.11b) at the expense of root and stem tissue (Figs. 3.12a,b).

Allometric relationships

For an allometric relationship between root and shoot growth to be *physically* possible, either the slope of the linear allometric relationship between leaf and stem is unity, or linear allometric relationships cannot exist between any combinations of leaf, stem and root. Paraphrasing Causton and Venus (1981), a tomato plant consists of two components, the root and shoot (Q_R and Q_S).

The shoot component, in turn, consists of two further sub-components of stem and leaf ($Q_{S(stem)}$ and $Q_{S(leaf)}$). If a linear allometric relationship exists between $Q_{S(stem)}$ and $Q_{S(leaf)}$, and between $Q_{S(leaf)}$ (or $Q_{S(stem)}$) and Q_R, then a linear allometric relationship can exist between Q_R and Q_S only if the linear allometric relationship between $Q_{S(stem)}$ and $Q_{S(leaf)}$ is unity. Evaluating the data according to this theorem indicated that a linear allometric relationship between shoot and root was physically impossible as allometric relationships with *k* values significantly different from one existed between the root, leaf and stem components (Table 3.14).

Following Causton and Venus (1981), curves were segmented visually into two or more linear phases (Fig. 3.14). The visual criterion was made on graphs of harvest means, not only because data trends are easier to see, but because harvest mean values were used to estimate the parameters.

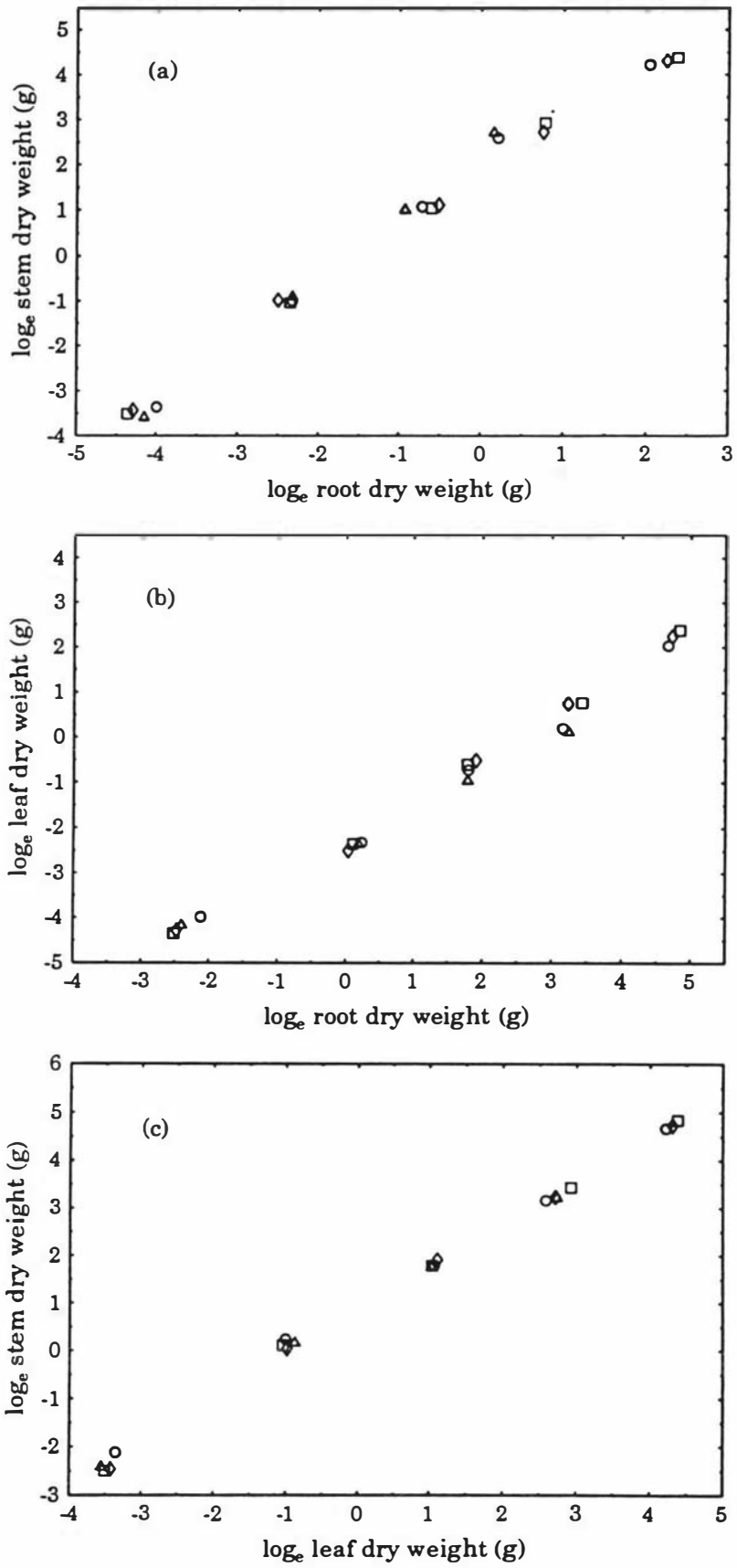

Fig. 3.14. Harvest means of log$_e$ transformed data between (a) stem and root (b) leaf and root and (c) leaf and stem weight of UR plants (□), RD plants (◇),

Regardless of treatment, comparatively more assimilate was proportioned to stem tissue than root or leaf (Table 3.14). Using Nelder's (1963) terminology, the demand of stem tissue for assimilate was greater than that of leaves and roots. Up to 67 DAI, leaves and stems of unrestricted plants (UR) exhibited higher demand ($k>1$) for assimilates than roots. During this time, the demand of stem tissue for assimilates was higher than that of leaves. A change occurred between 67 and 99 DAI, when assimilate demand by roots increased substantially ($k<1$). In both instances, this change was brought about by the relative growth rates of stem and leaf tissue declining faster than that of root tissue (-0.00237 and -0.00220 vs. -0.00168 $g \cdot g^{-1} \cdot day^{-1}$).

The response of RDD plants was consistent with these patterns. In the periods immediately preceding de-restriction (13-31 DAI, 45-67 DAI), comparatively more assimilate was partitioned to leaf and stem tissue than roots ($k>1$). Following de-restriction 31 DAI, the relative growth rates of leaves and roots was similar (i.e. $k=1$), while that of stem tissue was greater than leaves or roots.

Table 3.14 Maximum likelihood estimates of intercept (a) and slope (k) of linear allometric relationships between combinations of leaf, stem and root dry weight.

Model variables	Interval (DAI)	a	k^y	SE_k
Unrestricted (UR)				
Leaf vs. root[z]	13–67	2.57	1.14^{***}	0.014
	67–99	2.74	0.88^{**}	0.021
Stem vs. root	13–67	1.89	1.24^{***}	0.018
	67–99	2.22	0.91^{*}	0.035
Leaf vs. stem	13–99	0.84	0.92^{***}	0.020
Restricted (CR)				
Leaf vs. root	13–67	3.05	1.30^{***}	0.016
Stem vs. root	13–67	2.46	1.45^{***}	0.020
Leaf vs. stem	13–67	0.85	0.89^{***}	0.007
De-restricted (RD)				
Leaf vs. root	13–31	3.55	1.40^{***}	0.072
	31–99	2.48	0.99^{NS}	0.011
Stem vs. root	13–31	2.44	1.37^{***}	0.053
	31–99	1.79	1.13^{***}	0.015
Leaf vs. stem	13–31	1.05	1.03^{NS}	0.033
	31–99	0.90	0.88^{***}	0.008
Cyclic restriction (RDD)				
Leaf vs. root	13–31	3.53	1.42^{***}	0.044
	31–45	2.50	0.97^{NS}	0.107
	45–67	2.85	$1.46^{\#}$	0.216
	67–99	2.98	0.83^{**}	0.050
Stem vs. root	13–67	2.21	1.40^{***}	0.025
	67–99	2.40	0.89^{**}	0.034
Leaf vs. stem	13–31	1.24	1.00^{NS}	0.054
	31–45	0.99	0.75^{***}	0.064
	45–99	0.81	0.91^{NS}	0.098

[z] $\log_e$ leaf dry weight (g) = $\log_e a + k \cdot \log_e$ root dry weight (g)

[y] $H_O: k=1$ vs. $H_A: k \neq 1$

NS, #, *, **, ***Nonsignificant or significantly from 1 at $P \leq 0.10$, 0.05, 0.01, or 0.001, respectively.

Relationships between relative growth rates of components

The *k* values of the allometric analysis demonstrate that root restriction altered the distribution patterns of assimilate and resulted in different relative growth rates of the plant's components. The SLA of root restricted plants decreased more rapidly than that of unrestricted plants (Fig. 3.11a). Against a LWR that changed uniformly across all treatments (Fig. 3.11b), this decrease occurred because the restriction of root growth depressed the relative rate of leaf area expansion more than the relative rate of growth in leaf biomass (Table 3.11). The rate of root growth is composed of changes in the number and length of roots and biomass of the whole system. The relationships between the relative growth rates of growth of these components (i.e. relative rate of increase in dry biomass, R_R; relative rate of elongation, R_{RL}; relative rate of increase in number, R_{RN}) and the components of shoot growth (i.e. relative rate of increase in leaf (R_L) and stem (R_{ST}) biomass; relative rate of leaf area expansion (R_A)) was examined.

Canonical discriminant analysis of this data showed that, up to 67 DAI, the main source of variation separating the treatments was a difference in the relative growth rates of root components and shoot components (Table 3.15). $CDF_{1(13)}$ contrasts the relative rates of leaf growth, particularly leaf expansion, against the relative increase in root number and, to a lesser extent, root length. Similarly, $CDF_{1(31)}$ contrasts the relatives rates of leaf (biomass and expansion) growth with root (biomass, elongation and number) growth. At 45 DAI, the discriminant function maximally separating the treatments ($CFD_{45(1)}$) was dominated by the relative rate of root elongation. From the mean scores, it appears that the two levels of root restriction (0.025 and 0.05 l) reduced R_{RL} to different degrees, with the score for CR significantly lower than RDD, which in turn was significantly lower than that for the unrestricted treatments (UR and RD). After 67 days of treatment, plants in CR treatments has significantly lower scores than all other treatments,

suggesting substantially lower relative rates of root elongation and leaf expansion. No significant CDFs were detected at 99 DAI.

Table 3.15 Canonical discriminant function (CDF) and mean scores for relative growth rates of components of root and shoot growth.

Growth rate variable	13 DAI	31 DAI	45 DAI	67 DAI
	CDF_1	CDF_1	CDF_1	CDF_1
R_L	-0.56	-1.30	-0.93	-1.88
R_A	-2.31	-1.70	-0.20	1.34
R_{ST}	-0.06	-0.31	-0.88	-0.53
R_R	0.27	0.96	0.52	0.29
R_{RL}	0.64	1.66	2.95	5.78
$R_{1(RN)}$	1.79	1.07	-0.10	-1.11
λ_i	6.45	3.27	3.73	31.29
χ^2_{obs}	36.1	26.1	27.9	62.5
	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$	$P \leq 0.0001$
percent total variance	0.86	0.68	0.79	0.99
Treatment means				
UR	2.21	1.55	1.34	2.84
CR	-3.81	-2.78	-2.84	-8.82
RD	0.14	0.48	1.61	3.50
RDD	1.46	0.75	-0.11	2.49
SEOD (n=6)	0.577	0.577	0.577	0.532
	••••	••••	••••	••••

NS, *, **, ***, ****Nonsignificant or significant F test at $P \leq 0.05$, 0.01, 0.001, or 0.0001 respectively

Throughout the period 31-67 DAI, when the major changes in growth and development occurred, the rate of root elongation was consistently an important component in separating the treatment groups (Table 3.15). Canonical correlation analysis revealed strong relationships between R_{RL} and

R_A and R_{RL} and R_L during this period (Table 3.16) and separation of treatments through these relationships (Fig. 3.15). The relative rate of increase in root number was not an important component in any dominant function. A difficulty with this analysis, however, was the strong correlation between R_{RL}, R_R and the shoot components ($r>0.60$). As such correlations can confound interpretation of the canonical variates (Manly, 1986), the relationship between R_L and R_A was further investigated by path analysis. Because the observations of each block × treatment combination at each harvest were not independent, a path analysis was completed for each harvest of interest.

Table 3.16 Canonical correlation analysis of relative growth rates of root and shoot components between 31-67 DAI. All correlations are based on 24 observations.

Canonical vectors	Standardised canonical coefficient of significant canonical correlations at each harvest		
	31 DAI	45 DAI	67 DAI
Predictors (P)			
R_R	0.75	-0.17	0.33
R_{RN}	-0.19	-0.06	-0.27
R_{RL}	0.41	1.13	0.94
Responses (R)			
R_L	0.39	0.59	1.12
R_A	0.82	0.63	0.18
R_{ST}	-0.24	-0.24	-0.34
Canonical correlation	0.89^{****}	0.98^{****}	0.84^{*}
Rd_r^z	0.67	0.78	0.52

^{z} Standardised variance of responses explained by the canonical correlation of predictors
$^{NS, *, **, ***, ****}$ Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001 or 0.0001, respectively.

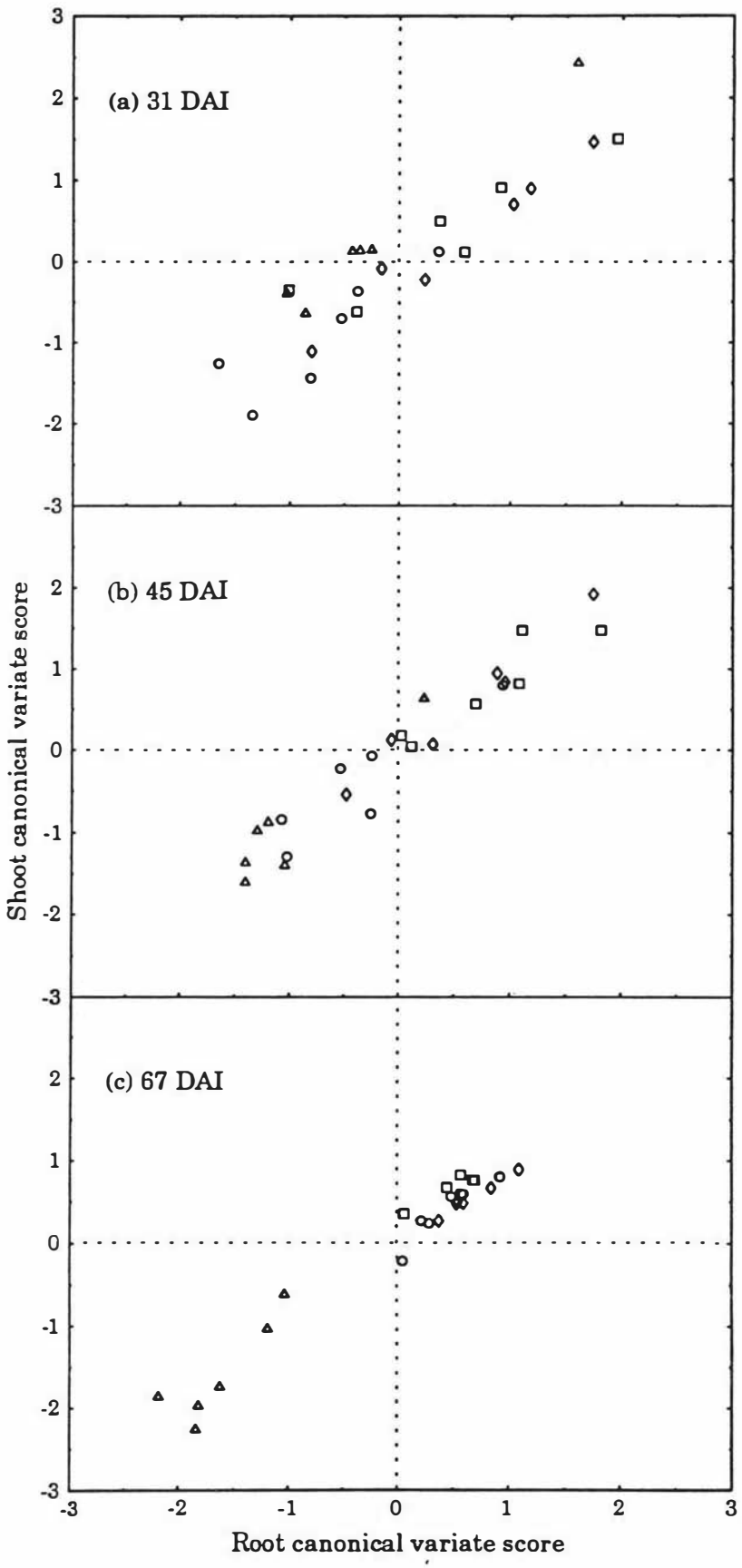

Fig. 3.15. Canonical correlation between relative growth rates of root components (dry biomass, extension and number) and shoot components (leaf and stem dry biomass, leaf expansion) of UR plants (□), RD plants (◇), RDD plants

The previous model (Table 3.8) was extended to reflect the relationship among relative rates of growth (root, leaf and stem dry biomass), expansion (leaf area), elongation (root length) and quantity (root number) at each harvest (Fig. 3.16). As the relative rates of increase in leaf number and plant height were constant throughout the experiment, these terms were excluded from the model.

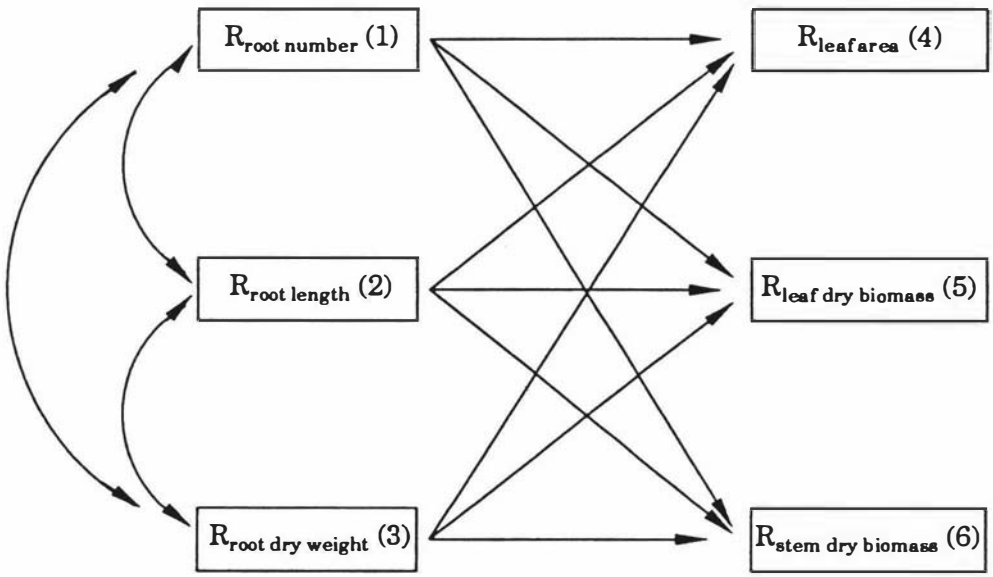

Fig. 3.16. Path diagram of structural relationships between the relative growth rates of the components of root growth (variables 1-3; number [R_{RN}], length [R_{RL}], and dry biomass [R_R]) with those of shoot growth (variables 4-6; leaf area expansion [R_{LA}], leaf dry biomass [R_L], and stem dry biomass [R_{ST}]). Single-headed arrows represent the direct effects, measured by path coefficients (P_{ij}, Table 3.19a-c), and the double-headed arrows depict simple correlations (r_{ij}, Table 3.19a-c).

Individually, R_R, R_{RN}, and R_{RL} were strongly correlated with R_{LA}, R_L and R_{ST} (Tables 3.19a-c), but the direct and indirect components of these correlations revealed considerable differences among the root growth indices. The relative rate of leaf expansion was positively associated with R_{RL}, with the strength

of this relationship increasing during the experiment. The observed correlations between R_{LA} and R_{RN} and R_R were due to the covariance of the latter two variables with R_{RL}. In contrast, R_L was positively associated with R_R, with the influence of R_{RL} and R_{RN} negligible. The relative growth rate of stem dry biomass was not strongly associated with any of the root variables until 67 DAI when R_R and possibly R_{RN} had direct effects. But, as with the univariate analysis of the point-in-time data, root length expressed as a rate, was the dominant feature of the analysis.

This analysis suggests, therefore, that the reduction in R_W arising from the root restriction treatments (Table 3.9) was strongly associated with a reduction in leaf expansion, which in turn, was strongly associated with reduced root elongation (Fig. 3.17).

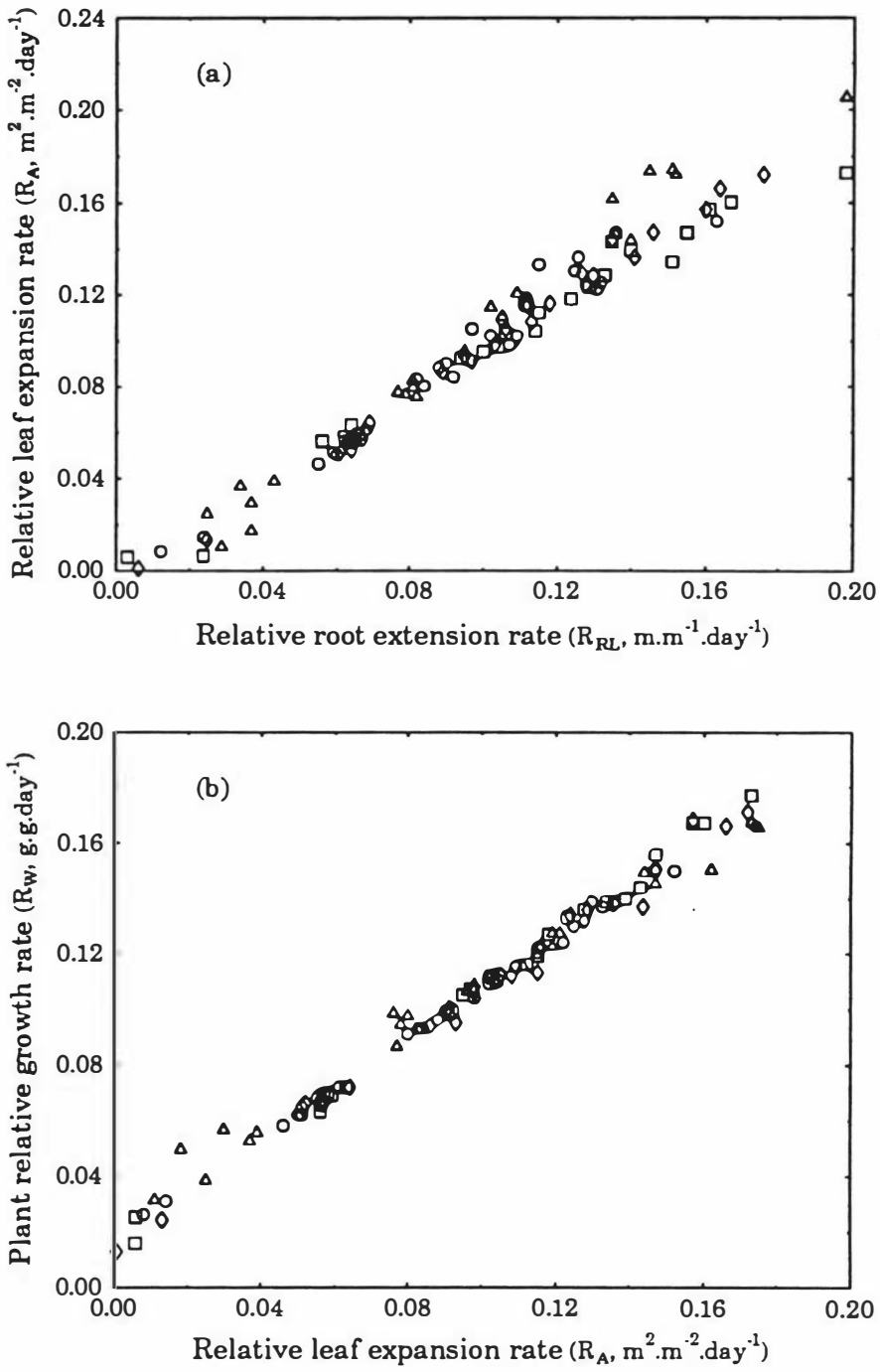

Fig. 3.17. Relationship between relative plant growth rates (R_W), relative leaf expansion rates (R_{LA}) and relative root elongation rates (R_{RL}) of UR (□), RD plants (◇), RDD plants (○) and CR plants (△).

Table 3.17a. Path analysis of the relationship between the relative rates of growth of root dry biomass (R_R), number (R_{RN}) and length (R_{RL}) on the relative leaf expansion rate (R_A; $m^2 \cdot m^{-2} \cdot day^{-1}$) of tomato plants. Significance levels are presented for the direct effects and simple correlations only. (n=24.)

Pathway and designation of effect	31 DAI			45 DAI			67 DAI		
	correlation coefficient (r_{ij})	path coefficient (P_{ij})	influence ($r_{ij} \cdot P_{ij}$)	correlation coefficient (r_{ij})	path coefficient (P_{ij})	influence ($r_{ij} \cdot P_{ij}$)	correlation coefficient (r_{ij})	path coefficient (P_{ij})	influence ($r_{ij} \cdot P_{ij}$)
R_{RN} vs. R_{LA}									
Direct effect, P_{14}		-0.14	-0.14^{NS}		0.07	0.07^{NS}		-0.06	-0.06^{NS}
Indirect effect via R_{RL}, $r_{12}P_{24}$	0.78	0.49	0.39	0.68	0.83	0.57	-0.04	1.05	-0.04
Indirect effect via R_R, $r_{13}P_{34}$	0.70	0.50	0.35	0.56	0.09	0.05	-0.21	-0.11	0.07
total correlation			0.60^{**}			0.69^{***}			-0.03^{NS}
R_{RL} vs. R_{LA}									
Direct effect, P_{24}		0.49	0.49^{NS}		0.83	0.83^{***}		1.05	1.05^{****}
Indirect effect via R_{RN}, $r_{21}P_{14}$	0.78	-0.14	-0.11	0.68	0.07	0.05	-0.04	-0.06	0.00
Indirect effect via R_R, $r_{23}P_{34}$	0.93	0.50	0.47	0.91	0.09	0.08	0.82	-0.11	-0.09
total correlation			0.86^{****}			0.96^{****}			0.96^{****}
R_R vs. R_{LA}									
Direct effect, P_{34}		0.50	0.50^{NS}		0.09	0.09^{NS}		-0.11	-0.11^{NS}
Indirect effect via R_{RN}, $r_{31}P_{14}$	0.70	-0.14	-0.09	0.56	0.07	0.04	-0.21	-0.06	0.01
Indirect effect via R_{RL}, $r_{32}P_{24}$	0.93	0.49	0.46	0.91	0.83	0.76	0.82	1.05	0.85
total correlation			0.87^{****}			0.88^{****}			0.75^{****}

^{NS}, *, **, ***, **** Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001 or 0.0001, respectively.

Table 3.17b Path analysis of the relationship between the relative rates of growth of root dry biomass (R_R), number (R_{RN}) and length (R_{RL}) on the relative growth rate of leaf dry biomass (R_L; $g \cdot g^{-1} \cdot day^{-1}$) of tomato plants. Significance levels are presented for the direct effects and simple correlations only. (n=24.)

Pathway and designation of effect	31 DAI			45 DAI			67 DAI		
	correlation coefficient (r_{ij})	path coefficient (P_{ij})	influence ($r_{ij} \cdot P_{ij}$)	correlation coefficient (r_{ij})	path coefficient (P_{ij})	influence ($r_{ij} \cdot P_{ij}$)	correlation coefficient (r_{ij})	path coefficient (P_{ij})	influence ($r_{ij} \cdot P_{ij}$)
R_{RN} vs. R_L									
Direct effect, P_{15}		-0.10	-0.10^{NS}		0.14	0.14^{NS}		0.03	0.03^{NS}
Indirect effect via R_{RL}, $r_{12}P_{25}$	0.78	0.23	0.18	0.68	0.19	0.13	-0.04	0.83	-0.03
Indirect effect via R_R, $r_{13}P_{35}$	0.70	0.72	0.50	0.56	0.66	0.37	-0.21	0.16	-0.03
total correlation			0.59^{**}			0.64^{***}			-0.03^{NS}
R_{RL} vs. R_L									
Direct effect, P_{25}		0.23	0.23^{NS}		0.19	0.19^{NS}		0.83	0.83^{****}
Indirect effect via R_{RN}, $r_{21}P_{15}$	0.78	-0.10	-0.08	0.68	0.14	0.10	-0.04	0.03	-0.00
Indirect effect via R_R, $r_{23}P_{35}$	0.93	0.72	0.67	0.91	0.66	0.60	0.80	0.16	0.13
total correlation			0.82^{****}			0.88^{****}			0.95^{****}
R_R vs. R_L									
Direct effect, P_{35}		0.72	0.72^{*}		0.66	0.66^{**}		0.16	0.16
Indirect effect via R_{RN}, $r_{31}P_{15}$	0.70	-0.10	-0.07	0.56	0.14	0.08	-0.12	0.03	-0.01
Indirect effect via R_{RL}, $r_{32}P_{25}$	0.93	0.23	0.22	0.91	0.19	0.17	0.80	0.83	0.67
total correlation			0.86^{****}			0.91^{****}			0.82^{****}

$^{NS, *, **, ***, ****}$ Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001 or 0.0001, respectively.

Table 3.17c. Path analysis of the relationship between the relative rates of growth of root dry biomass (R_R), number (R_{RN}) and length (R_{RL}) on the relative growth rate of stem dry biomass (R_{ST}; $g \cdot g^{-1} \cdot day^{-1}$) of tomato plants. Significance levels are presented for the direct effects and simple correlations only. (n=24.)

Pathway and designation of effect	31 DAI			45 DAI			67 DAI		
	correlation coefficient (r_{ij})	path coefficient (P_{ij})	influence ($r_{ij} \cdot P_{ij}$)	correlation coefficient (r_{ij})	path coefficient (P_{ij})	influence ($r_{ij} \cdot P_{ij}$)	correlation coefficient (r_{ij})	path coefficient (P_{ij})	influence ($r_{ij} \cdot P_{ij}$)
R_{RN} vs. R_{ST}									
Direct effect, P_{16}		0.09	0.09^{NS}		0.28	0.28^{NS}		0.18	0.18^{*}
Indirect effect via R_{RL}, $r_{12}P_{26}$	0.78	0.56	0.44	0.68	0.13	0.09	-0.04	0.14	-0.00
Indirect effect via R_R, $r_{13}P_{36}$	0.70	0.10	0.07	0.56	0.40	0.22	-0.21	0.79	-0.16
total correlation			0.60^{**}			0.59^{**}			0.01^{NS}
R_{RL} vs. R_{ST}									
Direct effect, P_{26}		0.56	0.56^{NS}		0.13	0.13^{NS}		0.14	0.14^{NS}
Indirect effect via R_{RN}, $r_{21}P_{16}$	0.78	0.09	0.07	0.68	0.28	0.19	-0.04	0.18	-0.01
Indirect effect via R_R, $r_{23}P_{36}$	0.93	0.10	0.10	0.91	0.40	0.36	0.80	0.79	0.64
total correlation			0.73^{****}			0.68^{***}			0.77^{****}
R_R vs. R_{ST}									
Direct effect, P_{36}		0.10	0.10^{NS}		0.40	0.40^{NS}		0.79	0.79^{***}
Indirect effect via R_{RN}, $r_{31}P_{16}$	0.70	0.09	0.06	0.56	0.28	0.16	-0.21	0.18	-0.04
Indirect effect via R_{RL}, $r_{32}P_{26}$	0.93	0.56	0.52	0.91	0.13	0.12	0.80	0.14	0.11
total correlation			0.69^{***}			0.67^{***}			0.86^{****}

$^{NS, *, **, ***, ****}$ Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001 or 0.0001, respectively.

3.4 Discussion

The data obtained in the present study provide additional information on the time course of the response of shoot growth to root restriction, new information on the response of root growth to physical restriction and provide support for a suggested mechanism through which physically inhibited root systems influence shoot growth.

General growth characteristics Tomato plants with physically restricted root systems had lower total plant biomass and total leaf area, were shorter in height and total root length, and had fewer roots, leaves, and lateral shoots than unrestricted plants. The extent of depression parallelled the duration and extent of restriction (i.e. CR > RDD > RD). Importantly, relatively short periods of root restriction were sufficient to have long term effects on leaf growth. The foliage of plants whose roots were restricted for 31 days before being released (RD) was as small and light 36 days later (67 DAI) as those plants whose roots were continually restricted (CR, RDD). By 99 DAI, RD plants were still shorter with fewer and smaller leaves than UR plants, although their leaf dry biomass was similar.

Components of root growth responded differently to root restriction treatments. Reduced root number was the first detected response to root restriction (Table 3.5). By 31 DAI, restricted (CR, RDD, RD) plants had produced fewer roots than unrestricted (UR) plants. Corresponding decreases in root dry weight and length were not detected until 45 DAI (Table 3.5, Table 3.6). The reverse response occurred upon de-restriction, with recovery in root length in RD plants after 67 DAI occurring before recovery in root number. Such recovery is consistent with Grime's (1979) description of tomato as adopting a competitive-ruderal strategy to environmental stress. According to Grime (1979), competitive-ruderals respond to stress with rapid morphogenetic responses to maximise vegetative growth (e.g. changes in SR,

leaf area, root surface area), or if capable of flowering, will rapidly redirect resources from vegetative growth to flowering.

The recovery in root elongation occurred without a concurrent increase in laterals, with the root systems of plants de-restricted 31 DAI considerably less branched than those of the unrestricted (UR) plants. Continuously restricted roots had a similar branching density to those of unrestricted (UR) plants throughout the experiment. By 67 DAI, however, restricted plants (CR and RDD) had more roots per unit length than unrestricted (UR and RD) plants. This is probably a consequence of the pattern of acropetal lateral root initiation in tomatoes being stable and independent of the rate of root elongation (Barlow and Adam, 1988). It is also possible that roots of unrestricted plants produced fewer sublaterals than those of restricted plants. As such sublaterals would be thinner than the 'parent' lateral, the net result to the root system would be denser root tissue (low SRL), as Table 3.6 reports.

The reported SRL values (Fig. 3.4) were similar to the 300-400 $m \cdot g_R^{-1}$ range reported in other studies with cv. 'Moneymaker' (de Willigen and van Noordwijk, 1987). Although it is difficult to uncouple the effect of ontogenetic drift from treatment responses, roots of continually restricted (CR) plants tended to have thicker roots (lower SRL) than unrestricted plants. With the increase in root branching density towards the end of the experiment (67 DAI), this suggests that restriction promoted development of thick, highly branched sublateral systems. Derestriction at 31 DAI (RD and RDD plants) and at 67 DAI (RDD plants) resulted in a major decrease in SRL. This probably reflects the reduced number of laterals produced (Fig. 3.4), rather than an increase in the density or thickness of roots. The large increase in SRL of RDD plants between 45 and 67 DAI reflected the development of thinner roots as the root branching density of these plants was low during this period (Fig. 3.4).

As to overall response, however, these variations in root biomass and number among treatments were considerably smaller than the variation in length. Although root biomass and number varied in relation to each other and to the restriction treatments, root length was consistently the major component of linear combinations of variables discriminating among the treatments (Table 3.6). It is possible that the apparent importance of root length reflects its close relationship to root surface area, and hence could be explained in terms of water absorptivity. This is unlikely as the water absorption capacity of tomato roots changes with transpirational demand and is independent of root length (Tan and Fulton, 1985; Tan et al., 1981). Moreover, Hameed et al. (1987) noted that the ratio between root length and leaf area was substantially larger in tomato plants with a restricted root system than those with unrestricted root systems, suggesting that restricted roots were more effective in water uptake per unit length. Richards et al. (1979) also reported evidence of changes in the absorptive activities of tomato root systems, in concert with changes to shoot activity, in maintaining a functional equilibrium between root and shoot that was independent of ontogenetic drift, root restriction, and a change from vegetative to reproductive growth.

Root-shoot relationships All three root components were strongly correlated with each other and with variables of shoot growth. Path analysis of these associations, however, revealed that the strong correlations between the shoot variables and both root length and dry weight could be explained by the covariance between the latter two variables and root length (Tables 3.8a-b). Thus, although a strong correlation was observed between root number and leaf area, consistent with Richards' (1981) observation, the direct effect of root number when assessed together with root length and dry weight was actually negative. The major canonical discriminant functions extracted from 31 and 45 DAI also revealed a negative relationship between total root number and leaf growth (Table 3.6). These results may reflect the greater increase in length relative to root number for the first 45 days of the experiment (Fig.

3.4), during which time leaf area was increasing. It is also possible that as root elongation and leaf expansion were impeded by restriction, the relative number of roots, presumably lateral and sublaterals, increased. The simple conclusion from the path analysis is that impeding root elongation was more detrimental to both leaf expansion and overall shoot growth than any decrease in root number.

The pattern and manner of distribution of assimilates within a plant has a major role in determining horticultural yield and the relationships between plant parts. Plots between the logarithms of shoot and root dry weights for each treatment suggested that a linear relationship existed between these components (data not presented). Critical evaluation of the relationship between stem, leaf, and root dry weights, however, revealed that allometry between shoot and root was not physically possible as the allometric coefficients between stem, leaf, and root growth were greater than unity. Thus, as the respective sizes of stem, leaf and root system did not increase at a fixed proportion to each other during the experimental period, then neither did the root and shoot system increase in size proportionally with each other. This suggests that a functional relationship between root and shoot based on relative sink strength was not a factor in the response of the plants to root restriction.

When roots were restricted, their mobilising ability was either decreased, or that of the stems and leaves increased. This was most apparent with CR plants, but appeared to also occur in the period prior to de-restriction of RD plants, and the first de-restriction of RDD plants. Following de-restriction, R_L and R_R were similar, suggesting that the relationship between leaf and root growth was supply-limited. Relatively more assimilate was proportioned to stem tissue than the roots over the same period (Table 3.14). This result is consistent with those from other studies on root restriction involving tomatoes (Al-Sahaf, 1984; Hameed et al., 1987; Peterson et al., 1991a) and

other crops (Carmi et al., 1983; Richards and Rowe, 1977a). These results contrast with those of Ruff et al. (1987) who reported that the relative amount of assimilate in roots of root-restricted plants of tomato was significantly greater than in leaves and stems. On the other hand, this result and the lower SR reported by these workers could also be interpreted as symptoms of water stress (Dale, 1988; Krizek et al., 1985). Root restriction may cause symptoms of water stress in plants even under conditions in which water is available in the root zone in non-limiting amounts. Under conditions of high transpiration for example, such a phenomenon may result from a physiological change that leads to a decreased ability of water absorption (Hameed et al., 1987). Alternatively, the difference in results may reflect cultivar differences. Krizek et al. (1985), for example, reported considerable differences in relative assimilate partitioning to roots among various cultivars of soybeans.

The magnitude and time trends of R_W, E_A and LAR of unrestricted plants (controls) were similar to those previously reported for tomato plants of comparative age in growing conditions of similar temperature and light (Hurd and Thornley, 1974; Paul et al., 1984; Thornley and Hurd, 1974) and hydroponic culture (Hameed et al., 1987). In contrast, the R_W and E_A values reported here are considerably lower than those reported by Peterson and Krizek (1992) and Nagel et al. (1994) for *L. esculentum* 'Moneymaker' grown in hydroponic systems in greenhouses and controlled environment rooms.

Restricting roots reduced the relative growth rate of the plant by influencing the assimilatory efficiency of the plant (E_A) and the extent and morphology of the leaves (LAR). Path analysis revealed that E_A and LAR were similarly important in influencing R_W (Table 3.12). The net assimilation rate is largely the balance between the rate of photosynthesis and that of respiration (of both photosynthetic and non-photosynthetic tissue) in the whole plant (Konings, 1990). Between 12-30% of assimilate produced daily is consumed

by root respiration (Lambers, 1985, 1987; Lambers et al., 1990; Veen, 1981). As respiration of restricted tomato roots can decline to less than 50% of unrestricted controls (mm^3 $O_2 \cdot g^{-1}$ root dry weight; Peterson et al., 1991b), a rise in E_A might therefore be expected in plants with restricted roots. As a slight decrease was detected in restricted (CR and RDD) plants (Table 3.9, Fig. 3.8), root restriction must have resulted in a decline in the rate of photosynthesis (assuming similar rates of shoot respiration among treatment groups). Reduced assimilate supply changes partitioning of assimilate so that root growth is inhibited to a greater extent than shoot growth (Brouwer, 1962; Mooney, 1972; Nagarajah and Schulze, 1983). The partitioning patterns were consistent with this response (Tables 3.10, 3.14), suggesting that photosynthesis may have been inhibited in root restricted plants.

In view of reports of increased starch concentrations in leaf tissue of root restricted plants of cotton (*Gossypium hirsutum* L.), cucumber (*Cucumis sativus*) and tobacco (*Nicotiana tanacum*) (Herold and McNeil, 1979; Robbins and Pharr, 1988; Thomas and Strain, 1991), it is possible that accumulated starch grains were responsible for the increase in leaf density (Table 3.13). The decrease in SLA between restricted and unrestricted plants is certainly consistent with previous reports of buildup of assimilates in leaves of restricted tomato plants (Al-Sahaf, 1984) and other crops (Carmi et al., 1983; Cresswell and Causton, 1988; Herold and McNeil, 1979; Robbins and Pharr, 1988; Thomas and Strain, 1991).

Starch accumulation is also consistent, albeit indirectly, with the slight reduction in E_A in root restricted (CR, RDD) plants (Fig. 3.8). Herold and McNeil (1979), Robbins and Pharr (1988) and Thomas and Strain (1991) all reported an inverse relationship between photosynthetic rate and level of accumulated starch in leaves. In tomato, decreases in SLA are associated with reduction in leaf photosynthetic rate as a result of negative feedback control resulting from low sink demand (Starck, 1983). Such 'sink regulation'

(Gifford and Evans, 1981; Goldschmidt and Huber, 1992; Herold, 1980; Neales and Incoll, 1968) has been described in tomato plants by Heuvelink and Buiskool (1995) under 'extreme' conditions of a single fruit per truss. In the current study, plants had no fruit and restricted root systems, conditions that could also be described as extreme. If translocation rates of assimilates to 'usual' sinks are reduced, as would occur if root metabolism (and hence root sink strength) was reduced by root restriction, and partitioning to alternative sinks (e.g. stem tissue: Table 3.11; Al-Sahaf, 1984; Friis-Nielsen, 1973a; Hameed et al., 1987; Hammond et al., 1984; Hewitt et al., 1982), sink-limited feedback inhibition of photosynthesis would result in lower rates of E_A.

Root restriction affected total leaf biomass and leaf area differently. Of the components defining LAR, SLA was dominant (Table 3.12). Similar proportions of total assimilate were apportioned to leaf biomass (LWR) no matter the level of restriction (Fig. 3.11). On the other hand, the ratio of leaf area to total plant weight (LAR) decreased with restriction (Fig. 3.10). Leaves were smaller in area, but weighed more per unit area (i.e. SLA declined; Fig. 3.11). Taken together, these indices suggest that leaf area expansion was more sensitive to root restriction than accumulation of biomass. These results show that the faster decline in R_W of CR plants compared with the other treatments was due more to the effect of continued restriction on leaf development in space (i.e. leaf expansion, reflected through SLA) than the amount of assimilate invested in leaf tissue (reflected by LWR). The strong association between the leaf expansion and whole plant growth, revealed through their respective relative growth rates (Fig. 3.17), is consistent with observations made with many species grown under a range of environments (Delaney and Dobrenz, 1974; El-Sharkawy et al., 1965; Hanson, 1971; Patterson et al., 1978; Potter and Jones, 1977; Watson, 1947a, 1947b). Potter and Jones (1977), for example, reported that for nine species they studied, growth responses due to temperature shifts were more sensitive to changes in relative leaf expansion rates than to net assimilation rates.

Adventitious root formation and petiole epinasty Appearance of both adventitious root primordia and petiole epinasty are common responses to waterlogging in tomatoes (e.g. Bradford and Dilley, 1978; Jackson and Campbell, 1976; Railton and Reid, 1973). The epinastic movement, due to more rapid expansion of cells on the adaxial side of the petiole compared to cells on the abaxial side (Bradford and Yang, 1981), is triggered by ethylene. Under waterlogged conditions, anaerobiosis of the root system stimulates 1-aminocyclopropane-1-carboxylate (ACC) synthase production in roots and shoots (Bradford and Yang, 1980). As conversion of ACC to ethylene in tomato roots is inhibited by low levels of O_2, ACC is apparently transported to the leaves where it is converted to ethylene (Wang and Arteca, 1992).

It is unlikely, however, that waterlogging initiated the epinastic response and development of adventitious roots (Table 3.4) given the attention placed on aerating the hydroponic solution (2.2.2.3). Two lines of evidence from the literature suggest the responses were due to the involvement of ethylene mediated by the physical stress encountered by the restricted roots. First, the rate of production of ethylene and its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) is positively correlated with the extent of root impedance. Kays et al. (1974) reported a sixfold increase in ethylene production by physically restricting roots of bean (*Vicia faba* L.). Rates of ethylene production increased proportionately with reduced axial growth of roots and shoots growing under different pressures, returning to normal rates after the impedance was removed (Sarquis et al., 1991). Whalen (1988) recorded a significant increase in ACC biosynthesis within 5 hours of physically impeding primary roots of maize (*Zea mays*). Sarquis et al. (1992) similarly reported significant accumulation of ACC within 1 hour of applying physical impedance to maize roots, although considerable conjugation also occurred, presumably reducing release of stress-induced ethylene. Second, application of exogenous ethylene reproduces the physiological responses to impedance (Dawkins et al., 1983; Whalen and Feldman, 1990) and inhibitors

of either ethylene production or action counteract them (Clarke and Moore, 1986; Sarquis et al., 1991; Zacarias and Reid, 1992). Third, ethylene retards extension of stems in tomatoes (Woodrow et al., 1988), suggesting that the reduced height and epinasty observed in CR plants may have had a common mediator. Finally, both the epinastic response and the appearance of adventitious roots may have resulted from an auxin-ethylene interaction. Zimmerman and Wilcoxon (1935) reported that 'gaseous emanations' following application of the endogenous auxin indoleacetic acid (IAA) to tomato shoots caused epinasty in adjacent marigolds (*Tagetes* spp.). They speculated (subsequently verified by Morgan and Hall, 1964) that ethylene had been released. Wample and Reid (1979) suggested that ethylene, produced in response to flooding, stimulated adventitious roots by causing auxin to accumulate in the shoot (i.e. ethylene-inhibited auxin transport). This is supported by Jusaitis's (1986) observation of stimulated production of adventitious roots in the presence of ACC and auxin.

Many of the observed plant responses were very similar to those reported in studies involving plant growth in high resistance soils. The rate of root elongation declines as the level of resistance increases (Barley, 1962; Goss, 1977; Goss and Russell, 1980). The rate of leaf expansion quickly declines (Ludlow et al., 1989; Masle, 1990), independently of leaf water potential, osmotic potential and turgor (Andrade et al., 1993), through reduced leaf number and average leaf area (Masle and Passioura, 1987). In the current study, as roots of CR plants became increasingly restricted, their rate of elongation and the ratio between dry root biomass weight per length of root declined, while the ratio between the number of roots and root length increased. The decline in R_A occurred early in the experiment (≈31 DAI) before morphological differences were detected in root growth. Restricted roots tended to be thicker and more branched than unrestricted roots (Fig. 3.4), a result consistent with those of other workers who have observed fewer total roots with increased radial growth and enhanced development of lateral

branches in physically impeded root systems (Barley, 1962; Goss, 1977; Goss and Russell, 1980; Lachno et al., 1982; Masle, 1992; Veen, 1982; Wilson et al., 1977).

There are, however, inconsistencies in response. High resistance soils affect shoot dry biomass more than root biomass, leading to a decrease in SR (Masle, 1992; Masle and Passioura, 1987). By contrast, the SR increased in the current study as root dry biomass was affected more than shoot dry weight as restriction increased. As the partitioning coefficient between leaf and stem shows (Table 3.14), restriction affected leaf dry biomass more than stem dry biomass. As Masle's studies (1990; 1992) were conducted with young wheat seedlings that lack appreciable stem tissue, the preferential partitioning to the root may have been a storage mechanism and not a reflection of root activity. On the other hand, there is no reason to assume that partitioning patterns in a monocotyledonous annual should be similar to those in a dicotyledonous herbaceous perennial (Schulze, 1983). Rates of photosynthesis were higher in plants in high resistance soils (Masle et al., 1990; Masle and Farquhar, 1988). Carmi et al. (1983) also reported enhanced rates of photosynthesis in leaves of root restricted beans although in other container-based studies, the photosynthetic rates of restricted plants have been either reduced (Robbins and Pharr, 1988; Thomas and Strain, 1991) or unaffected (Krizek et al., 1985). Masle's (1990; 1992) results imply a weak association between R_A and R_L as the reduction in R_A due to high resistance soils could be transitory whereas R_L of plants in high resistance soils was consistently lower than control plants. In contrast, the relationship of R_A and R_L in the current study was strong and consistent in time.

Mechanisms Multivariate analysis of the relationships among the relative growth rates of the components of root and shoot growth (Tables 3.15-3.17a-c) confirmed a close association between root elongation and leaf area expansion that was foreshadowed in the univariate analysis (Tables 3.5a-c, Fig. 3.3, 3.5).

In addition, the analysis showed that the strong relationships among root number and root dry weight and shoot growth variables were due to their covariance with root elongation. It seems logical, at least in a teleological sense, that a functional balance should exist in plants between the transpiration area of canopy (largely leaf area) and the absorptive area of the root system (approximated in this study by root length). It would similarly follow that the dynamic nature of such a balance would be reflected in the comparative rates of increase of these components.

The functional equilibrium model empirically interprets relationships between root and shoot growth from the view that their outputs (size × activity) are in precise balance (Brouwer, 1963; Davidson, 1969a). The plasticity of root function and its independence of root size predicted by this model has been demonstrated in root restricted (Al-Sahaf, 1984; Richards, 1981) and water stressed (Tan et al., 1981) tomato plants. Tan et al. (1981) have shown that water uptake per unit length can be at least doubled to maintain the root's functional balance with the shoot. Richards (1981) demonstrated that uptake of N was proportional to the growth increment, and independent of root size. Al-Sahaf (1984) reported that the efficiency of K, Ca and Mg uptake was greater in plants with restricted root systems compared to those with unrestricted root systems. As root length is a major determinant of water and nutrient acquisition (Molz, 1981; Nye and Tinker, 1977), it seems logical to infer a similar interpretation on the relationship between R_{RL} and R_A demonstrated in this experiment. Thus, whatever the level of root restriction being encountered, the plants maintained a functional equilibrium between uptake potential (root length) and transpiration surface (leaf area). As the gain in size (length) of the root system in restricted containers declined, the activity of the root (e.g. water uptake) would increase to maintain the equilibrium. The higher ratio of leaf area:root length of CR plants suggests that water uptake per unit length of root must have been higher in these roots than those of less or unrestricted plants. Such plasticity, however, is

a response mechanism to short term periods of stress, and thus either leaf size (expansion) or activity (photosynthesis) or both would be expected to decline. Such a decline in leaf expansion with declining root elongation (increasing root restriction) was clearly observed, and albeit indirectly through E_A, there is some evidence that photosynthesis rates also declined. As leaf area is a function of cell expansion (Dale and Milthorpe, 1983), an expected outcome of this model would be an increase in leaf density associated with decreased cell size (Casal et al., 1987; Myers et al., 1987). Such an increase would be accentuated if the restricted roots were unable to maintain an adequate supply of water to the leaves, thereby inducing leaf water stress (Hsaio, 1973; Rascio et al. 1990). An important observation, therefore, is that root restriction increased the density component of SLA (Table 3.13). Furthermore, if water stress was a feature of the response, then the observed reduction in leaf number would also be expected. Krizek et al. (1985), for example, reported a large reduction in the emergence rate of new leaves of soybean plants and their rate of growth when subjected to water stress in the exponential phase of development.

Several lines of argument suggest, however, that the relationship between root elongation and leaf expansion was not functionally based on water uptake. Although data on leaf turgor would be more definitive, differences in leaf water potential during the period of most rapid growth change between treatments were not detected (Table 3.3). In addition, other indirect pieces of evidence taken together suggest that water stress was not a major factor in this experiment. Average leaf area was similar in all treatments, yet a reduction in this parameter would be expected under conditions of water stress as the effect of stress progressively reduces expansion of leaves unfolding at higher stem positions (Clough and Milthorpe, 1975). Increased partitioning of assimilate to the root system and a large decrease in the ratio between leaf area and root length are commonly encountered responses to water stress (Brouwer, 1983; Hsaio, 1973; Krizek et al., 1985). In the current

experiment, however, plants with restricted root systems increasingly partitioned assimilate to stem biomass at the expense of root biomass (Table 3.14), and the leaf area:root length ratio of restricted plants was consistently higher than those with unrestricted root systems (Fig. 3.4c). Given the indirect measurement of leaf density used in this study (i.e. $g_{dw} \cdot g_{fw}$), it is also possible that the observed increase was due to a buildup of 'unused' assimilate in the leaves due to reduced sink demand elsewhere in the plant (Heuvelink and Buiskool, 1995; Rufty and Huber, 1983). Increases in starch concentration in leaves of root restricted plants have been previously observed (Herold and McNeil, 1979; Robbins and Pharr, 1988). Finally, with average leaf area similar, and total leaf area quite different among treatments (Tables 3.5a-c), the reduction in total leaf area appears to have been effected through fewer or slower initiation of leaves.

Cell initiation and division are both hormonally-directed activities, and as Richards and Rowe (1977a) speculated, better related to the synthesis of hormones from root apices than water uptake along the length of a root system. As synthesis of hormones is a normal function or activity of the root system (Itai and Birnbaum, 1991), a functional equilibrium might exist between the root-based synthesis of hormones and the shoot-based response to their effect. The growth responses, particularly the relationship between leaf expansion and root elongation, reported in the current study are consistent with such an equilibrium. Under such a model, export of hormones to the shoot is reduced or impeded as the root tip (the site of hormone production) is either damaged or alters activity as physical impedance to root elongation increases. Consequently, the function of the target organs in the shoot would similarly decline or be impeded. Partitioning to the root system would also be expected to decline as the mobilizing ability (Daie, 1985; Gifford and Evans, 1981) of the root system is weakened due to its reduced activity.

Expansion of this model is premature and is reserved to a more speculative chapter (7.0). Comment is confined here to features of the observed responses consistent with an hormonally-mediated system. As root apices are sites of hormone synthesis, such a model would predict that an association exists between root number and shoot growth or function. The immediate and critical issue to address is the apparent lack of association between root number and leaf growth. While the ontogeny of root number, length and dry weight all mirrored that of leaf area (Fig. 3.3, 3.5), in all the multivariate analyses when its association with leaf area was assessed jointly with root length and root dry biomass (Tables 3.8a-b, 3.15-3.17), root number was a minor contributor to the multivariate function being described. The contribution of root number relative to length decreased between harvests as the effects of the treatments on root and shoot growth likewise increased. The strong association between R_{RL} and R_A (Tables 3.16, 3.17; Fig. 3.17) was particularly interesting as it presents a stable and biologically meaningful relationship that incorporates, through the calculation of the respective relative growth rates, the ontogeny of the plant.

The expectation that, as the biosynthetic source of the putative hormone signal for the shoot system, root number should be closely linked with leaf area may be reconciled with these results with the hypothesis that as root elongation requires an intact, active root apex (Barlow and Rathfelder, 1985; Biddington and Dearman, 1982; 1984; Clowes and Stewart, 1967), the R_{RL} of a root system may be a better indicator of root activity (i.e. hormone synthesis) than R_{RN}. This assumes that root elongation and hormone biosynthesis are concurrent processes. Although the literature does not address this issue directly, there are reports that provide indirect support for this assumption. The quiescent centre of the root apex has been suggested as the site of hormone biosynthesis (Clowes, 1969; Short and Torrey, 1972), although more recent studies suggest that synthesis results from an interaction between the quiescent centre and the proximal meristem (Barlow,

1994; Feldman, 1979a, 1979b, 1980; Sossountzov et al., 1988; Zavala and Brandon, 1983). Removing carbohydrate sources from roots results in a cessation of meristematic activity in the apex (Van't Hof, 1968) and a reduction in the rate of root elongation (Barlow and Adam, 1988). Low temperatures inhibit root elongation (Bowen, 1991 and references therein). At the root apex, low temperatures result in a virtual cessation of meristem activity. Returning chilled roots to room temperature is followed sequentially by cell division in the quiescent centre, the formation of a new meristem at the root apex, the appearance of a new quiescent centre within this meristem, and resumption of root elongation (Clowes and Stewart, 1967; Barlow and Rathfelder, 1985). In high resistance soils, the decrease in root extension rate results from a reduced rate of cell elongation accompanied by a possible decrease in rate of cell production at the apex (Bennie, 1991). With respect to inter-organ relationships, root elongation is more sensitive to soil temperature than gain in root biomass (Cumbus and Nye, 1982) or increase in lateral numbers (Nambiar et al., 1979). Finally, root elongation × shoot relationships are not necessarily limited to relationships between the exogenous root functions of water and mineral absorption and transpiration by leaf area. Ješko (1981), for example, reported an apparent causal relationship between cytokinins produced by emerging (elongating) nodal roots of maize and a transient increase in photosynthetic activity of leaves close to the site of roots.

Several other observed responses are compatible with an hormonally-mediated control system. Root restriction increased branching density (Fig. 3.4). Although the pattern of lateral root initiation in tomato is apparently stable and resistant to experimental modification (Barlow and Adam, 1988), adventitious lateral initiation appears subject to the interplay between stimulatory and inhibitory influences that are largely hormonal in nature (Wightman and Thimann, 1980; Wightman et al., 1980). Studies with cytokinin mutants suggest that whereas auxin is necessary for primary root

growth, cytokinins counteract growth of primary roots and stimulate growth of root hairs in normal plants (Su and Howell, 1992). Benzyladenine (BA) stimulated the number of lateral root primordia on the cultured tomato roots (Finnie and van Staden, 1985) and the primary root of tomato seedlings (Aung, 1982). In the same experiment, interference of auxin transport with TIBA resulted in a significant reduction of root primordia on hypocotyls. Low concentrations of other cytokinins have stimulated lateral root primordia in peas (Street, 1969; Wightman et al., 1980) and lettuce (Biddington and Dearman, 1982; MacIssac et al., 1989). Cytokinins and auxins are both inhibitors of root growth (not initiation) (Stenlid, 1982), and together stimulate ethylene production, often synergistically, (Stenlid, 1982; Yu et al., 1981), which reduces root growth. The interaction between auxin and ethylene in the development of adventitious roots on the stem has been discussed. The possible involvement of auxin in this response is supported by the report by Costa et al. (1992) of enhanced IAA oxidase activity in the lower stem region of root-restricted peach rootstocks. In addition, a buildup of auxin in the lower stem of tomatoes may have attracted assimilate towards the stem (Gersani et al., 1980), thereby explaining the preferential partitioning to stem biomass (Fig. 3.12, Table 3.12). Finally, other studies have reported that reduced root elongation under mechanical impedance, as observed in this study (Fig. 3.3), was associated with altered levels of IAA in the root apex (Lachno et al., 1982).

Growth analysis methodology Growth analysis methods should not impose excessive requirements on experimental resources such as greenhouse space, plant material and technical input. It is often difficult to find a balance between collecting sufficient data to detect differences over time, and having sufficient space and time to accommodate experiments involving several treatments. Causton and Venus (1981), for example, suggested that with data with a coefficient of variation of about 20% and 10 replicates, the harvest frequency should be about 6 days. Such a frequency, however, may

be impractical. For example, in the experiment reported here, delays in completing harvests altered a feasible harvest frequency of 14 days, given the resources available, to one that ranged between 14 and 32 days. Such difficulties withstanding, like all tools, growth analysis techniques can only be expected to work within their operational boundaries. In this study, the duration between successive harvests was probably too long for differences in $\bar{R}_W$ calculated by the classical approach to be detected. As the functional approaches yielded equivocal results of the relative magnitude of E_A among treatments, it is not possible to decide whether the harvest periods were too long for meaningful estimates of $\bar{E}_A$. Nevertheless, calculation of $\bar{E}_A$ without cognisance of the relationship between leaf area and plant dry weight would have decreased the sensitivity of the analysis about 8% (Fig 3.3).

The hybrid method, an amalgam of functional and univariate approaches to growth analysis, performed well against the established functional approach of Causton and Venus (1981). Estimates of mean values of parameters were almost identical, and standard errors were similar (Fig. 3.9). The principle benefits of the hybrid approach were the replicated estimates of the important growth analysis indices (Maindonald, 1992), its flexibility in accounting for differences in biotime between individual plants (e.g. Bradford and Trewavas, 1994), and the flexibility that the derived data sets provided. The hybrid method provides many more observations (estimates) than are possible from the standard method. This provides the opportunity to use other relevant statistical procedures, such as path analysis, canonical correlation and canonical discriminant function analysis. Although not utilised in this experiment, the hybrid method would allow analysts to account for ontogenetic drift in interpreting growth analyses (Evans, 1972; Hunt, 1978) by using appropriate variables as covariates in analysis of covariance procedures or as concomitant variables in regression analyses.

The hybrid method does, however, require improvement. Causton and Venus's (1981) functional approach is inherently more robust than the hybrid method as the regression procedure at its base has more degrees of freedom to estimate the residual (or error) variance. For example, with the data sets used in this experiment, 27 error degrees of freedom were present in the per-treatment regressions in the standard method compared with 2 degrees of freedom in each of the treatment × block regressions of the modified method. This is important because the estimate of the residual variance plays a major role in calculating the estimated standard errors of the coefficients in the model for hypothesis testing. The residual variance is also used to estimate the standard errors of predicted values, values of which are required in the subsequent calculations of the rates and derived ratios. In addition, with repeated observations at each level of the independent variable (in this instance, DAI), the standard method provides an opportunity, lacking in the hybrid method, of objectively determining the validity of the model being fitted with a lack-of-fit test (Myers, 1990), rather than relying on the more subjective assessment of residual plots.

The low number of error degrees of freedom is the principle weakness of the modified method. If the raw data are highly variable, or if high order polynomial or other functions (e.g. Richards function) must be fitted to best model the data, there may be insufficient error degrees of freedom available to accurately fit the function and test the quality of its fit. In general, it is unlikely that more than 4 degrees of freedom will be required for fitting the function. A Richards function will 'consume' one degree of freedom for each of its four parameters. Fitting polynomials of three or more terms is considered unwise because overfitting leads to spurious changes in such derived indices as R_W, E_A and LAR (Causton, 1967; Elias and Causton, 1976; Hurd, 1977; Nicholls and Calder, 1973; Poorter, 1989; Venus and Causton, 1979a). Therefore, a minimum of six observations for each block × treatment combination are required to avoid over-parameterisation of the model. This

only provides a single degree of freedom for estimating the residual variance, which would concern most biometricans. Increasing either the number of harvests or the number of subsamples per block × treatment combination will lessen or avoid this problem.

The steady and uniform decline in the relative growth rates of the components of root and shoot growth (Table 3.11) are probably artefacts of the second order exponential polynomial fitted to the raw data. It is possible that the 'smoothing' action of the regression has masked momentary, but physiologically important, changes in the relative growth rates or their rate of decline. Fitting the data to a different function (e.g. a Richards function) may have overcome this difficulty (Venus and Causton, 1979a). Harvests were, however, too infrequent and too widely spaced for either of these issues to be resolved with this data set.

The hybrid method lacks the definition present in more recent hybrids of the classical and functional approaches. For example, Poorter (1989) sought to merge the strengths of the classical and functional approaches by fitting polynomials through stepwise regression to indices calculated using classical techniques. However, while having merit for data sets that do not lend themselves to easy curve fitting, the reliance of Poorter's (1989) technique on many frequent harvests to adequately calculate the indices is a major practical constraint. For example, Poorter (1989) used data from an experiment lasting 38 days that consisted of a single treatment with 24 harvests each of five plants, with either 1 or 2 days between each harvest. Following this protocol with four treatments running for 99 days would have required about 1200 plants, 6 greenhouses and 6 hydroponic systems.

The blocked design used in the present study was successfully accounted for in the standard method by using data adjusted for block effects. This adjustment, however, was difficult to program with SAS. Future use of this

approach should aim to increase the number of harvests or observations per harvest to gain sufficient 'spare' degrees of freedom to include additional terms in the model to account for block and block × time interactions. This would avoid the need to adjust the raw data for block effects before analysis.

Experimental system Despite selecting similarly sized plants for the experiment, early differences in growth between similarly treated plants were observed. By 13 DAI, the R_w of plants in CR treatments was significantly greater than those in RD treatments. At 31 DAI, the relative growth rates of the plant and its components in RD and RDD treatments were significantly lower than CR plants. It is unclear why such differences in growth response should exist between plants in these treatments (Table 3.5a) when the plants were in identically-sized (0.025 l) containers. The differences may have been artefacts of the polynomial fitted to the data in the functional methods. Analysis of $\bar{R}_w$ between 13-31 DAI calculated using the non-paired classical method did not detect treatment differences (data not presented). Other workers have also encountered problems with values at the extreme of fitted curves. Hurd and Thornley (1974), for example, ignored such values, only reporting those 'well within the limits of the curves'. The differences may have arisen either by chance, or resulted from poor grading and randomisation of plants during the set up of the experiment. Alternatively, the problem may have resulted from using non-homogenous plant material being distributed among the treatments, with the observed differences due to ontogenetic drift. Although unlikely given the double random allocation method used, it is possible that a disproportionate number of 'large sized' plants were allocated to CR treatments. Similarly sized plants were chosen for this experiment. Given the importance of leaf area and leaf area expansion rates to plant growth, it is possible that similarly *sized* plants are not equivalent to similarly *leafed* plants. Re-analysis of the data at 13 and 31 DAI using plant dry weight or leaf area as a covariate adjustment accounted for a significant portion of the variation and resulted in no

significant treatment effects being detected (data not presented). Clearly, greater care must be taken in selecting plants for use in studies, particularly when the variables being measured are sensitive to ontogenetic drift.

Rooted cuttings inserted into the hydroponic system developed a new set of roots before appreciable shoot growth occurred. Presumably the electrical conductivity of the hydroponic solution was too high for the roots present at planting and damage occurred. New roots, presumably conditioned to the osmotic potential of their environment, were then produced. While not explaining the observed differences between similarly treated plants 13 DAI (Table 3.5a), this disruption to growth, effectively a transplant shock, may have increased the variation in growth between the plants. As the rate of plant growth during early vegetative stages is often exponential (Fig. 3.3, 3.5; Moorby and Graves, 1980), it is critical that young plants establish quickly in the hydroponic system. Early growth of young tomato plants in hydroponic solutions is influenced by the manner in which they are raised (Cooper, 1978). A contributory factor is the different morphology and physiology of roots grown in solid substrates compared to those in hydroponic solutions (de Lint and Klapwijk, 1986). In solid substrates, roots are characteristically thin, with root hairs appearing behind the growing tip, whereas those developed in hydroponic solutions are thicker and more fleshy, with root hairs generally absent (de Lint and Klapwijk, 1986). Roots developed in hydroponic solution will continue to grow and develop when placed in a solid medium. But, as observed in this experiment, roots developed in solid substrates die when transferred into hydroponic solution. It is possible that the change in osmotic potential arising from the roots being shifted from an environment of low conductivity (i.e. tap water: 0.2 $mS \cdot cm^{-1}$) to the relative high conductivity (2.5 $mS \cdot cm^{-1}$) hydroponic solution may have damaged the root cells (Lee-Stadelmann and Stadelmann, 1989). It is likely that the same response was responsible for the problems encountered with root storage (Fig. 3.1). Future studies on growth responses to root restriction

must reduce such transplant shock, particularly if early growth responses to restriction are being studied.

Difficulties in assessing root restriction studies arise because the nature of the treatment changes in time as the degree of root restriction increases, making general inferences based on point-in-time comparisons difficult to sustain. As with all external environmental factors (Hunt and Nicholls, 1986), root restriction operates on a continuum that induces sub-optimal, optimal and supra-optimal responses from the plant. Physically restricting roots for 31 days in a 0.025 l container caused changes to root and shoot growth that were still apparent 68 days later. Unfortunately, until a method for quantifying the level of root restriction encountered by the plants is developed, the lack of an adequate benchmark against which these responses can be compared makes any comment solely qualitative.

Summary Richards and Rowe (1977a) based their premise of distinct regulatory and uptake functions of roots on similarities between the percent reduction in root number and stem height, shoot dry weight, leaf number and between the percent reduction in root length and total water uptake, nitrate uptake and leaf area. A similar analysis of my data did not reveal similar groupings. It also showed that the relationships between the percent reductions in the variables change over time (Table 3.7), and in doing so, revealed the fragility of Richard and Rowe's (1977a) analytical technique. Indeed, in the same way that calculating absolute growth rates to compare the performance of a 10 g and 1 g plant is flawed (see Hunt, 1978), comparing absolute values of components of root and shoot growth to explain how root and shoot growth might be connected is unlikely to be revealing. Changes in shoot growth arising from changes to root growth can be episodic (Bevington and Castle, 1985; Drew, 1982; Drew and Ledig, 1980; Williamson and Coston, 1989), or simply delayed. Restricting root systems reduced root numbers after 31 DAI, but differences in shoot dry weight between restricted and

unrestricted plants were not detected until 67 DAI (Table 3.5b). Moreover, such quantitative approaches imply that a 'morphogenetic equilibrium' exists (i.e. the more roots, the better shoot growth), a concept refuted (van Noordwijk and de Willigen, 1987) and largely replaced with the concept of a functional equilibrium (reviewed by Brouwer, 1983). One solution to this problem of interpretation was to assess root and shoot relationships over time, incorporating ontogenetic drift into the analysis, and seeking stable and biologically meaningful relationships. As the data presented in this study show (Fig. 3.3, 3.5), the relative reductions in growth between restricted and unrestricted plants progressively increased with time. Estimates of the relative rates of increases of the components of root and shoot growth provided insight into how physically restricted roots might influence shoot growth.

Three important physiological responses dominated the results of this analysis. First, a consistent, strong linear relationship was observed between the relative rates of root elongation and leaf expansion of the plants that was independent of root restriction. This was interpreted as indicating a physiologically meaningful relationship exists between the root and leaf growth. It was not possible, however, to disentangle the relative contribution of leaf count and individual leaf area to the reduction in total leaf area resulting from root restriction. Leaf area expansion is the product of the number of leaves and the area of each of those leaves. Although a reduction in leaf number resulting from root restriction was detected in the previous experiment, it was not possible to decide whether this reduction was due to reduced initiation or delayed emergence of initiated leaves. Similarly equivocal is the information about individual leaf expansion. Although average leaf area was similar in all treatments, expansion of individual leaves at similar positions on the stem may have differed between treatments according to the extent of root restriction being encountered. The similarity in average leaf area among all treatments at all harvests suggests that root

restriction did not influence the expansion of individual leaves. On its own, however, average leaf area does not provide sufficient definition of the response of individual leaves to root restriction. Leaves at different positions on the stem axis might respond differently to the treatments because the nature of the restriction treatments changed throughout the experiment. Similarly, greater movements in SLA, along with differences among treatments, would probably have been apparent had individual, or at least newly developing leaves (after each harvest), been analysed separately. For example, root restriction may have reduced the rate of expansion or the maximum final area, or both of individual leaves. Derestriction may have had the opposite effects. As every leaf or perhaps every leaflet of each leaf (Ho and Shaw, 1977) on an axis is unlikely to be equally affected by root restriction (Peterson et al., 1991a) because of the changing 'intensity' of restriction with time, further studies must analyse leaf growth on a leaf by leaf basis. Therefore, greater definition of the effect of root restriction on the components of total leaf area will only be gained if a closer assessment of individual leaf expansion was made. Besides requiring a method for measuring leaf expansion, such assessment also requires that the leaves being measured were at similar stages of growth at the time measurement commenced. Relative growth rates are extremely sensitive to ontogenetic drift (Figs. 3.10, 3.11), and small differences in biomass or leaf area between treatment groups can seriously confound between-group interpretation of relative growth rates derived from these variables. Solving this problem requires greater attention to obtaining uniformity in the initial plant material and the individual leaves being compared.

Second, comparatively short periods of qualitatively low levels of root restriction can have long term consequences for components of shoot growth. At the time growth responses were first detected in plants in small containers (31 DAI), the root system in these containers did not appear 'restricted'. Although roots filled the area of the container and were wrapping around its

interior surface, the root matrix was very porous. Roots approaching the interior surface of the container would presumably have been impeded, but there was ample space elsewhere in the matrix for expansion of the root system. This raises the possibility that only part of a root system needs to be 'restricted' for a response to be generated. Responses from localised parts of a root system causing physiological and developmental changes are well documented; the chemical signal ABA sent from drying *parts* of a root system to the shoot brings about stomatal closure in the absence of detectable shoot water deficit (Davies and Zhang, 1991 and references therein). As several of the observed responses to restriction were speculatively interpreted as being hormonally-mediated, it is possible that if chemical signals move between a restricted root system and the leaves, then restricting elongation of part of the root system should restrict expansion of leaf area.

It is also interesting that plants derestricted after 31 days had fewer leaves and smaller total leaf area, yet similar leaf dry biomass and average leaf areas compared to control (unrestricted) plants at the end of the experiment. This implies that the photosynthetic efficiency per unit area of these plants was higher than unrestricted plants, and raises the possibility that derestriction stimulated a compensatory growth response.

The third important result is the discrimination of response and effect achieved within the variable commonly called 'root growth'. There was little evidence to suggest that the reduction in root numbers *per se* caused by root restriction had any direct influence on shoot biomass or its major determinant, leaf area. The direct influence of R_R, a measure of the relative increase in the size of the root sink, in its strong correlation between R_A was small, suggesting that activity of the root system had a more important role in leaf growth. It was postulated that this activity was connected with hormone synthesis in actively growing roots, and as such, the relative rate of elongation of a root system was a better indicator of synthetic activity than

the relative rate of increase in root number. By implication, the relationship between the relative rates of root elongation and leaf expansion can be described as a functional, rather than morphogenetic, equilibrium.

In the following chapters I report on further investigation into this relationship and on efforts to address some of the constraints identified in the current experiment. In Chapter 4, an improved method for propagating the tomato cuttings destined for hydroponic systems is introduced and an empirical model for non-destructively estimating the expansion of individual leaves of tomato developed. In Chapter 5, a multivariate technique is adapted to better characterise and group plant material in order to reduce within-block variation. These tools are used in Chapter 6 to further investigate and characterise the relationship between root restriction and leaf expansion, with particular emphasis on partial root restriction and the response of individual leaves on the stem axis. Finally, in Chapter 7, I summarise the results of my research programme and outline future research directions.

Chapter 4

Non-destructive estimation of leaf area

4.1 Introduction

The development and extent of leaf area is a fundamental aspect of analysis and interpretation of plant growth (Causton and Venus, 1981; Hunt, 1982). A common method for describing the ontogeny of leaf area involves destructively harvesting whole plants throughout the experimental period and analysing the leaf area data using classical or functional growth analysis techniques (Hunt, 1982). This approach, however, is not always suited to studies following the development of individual leaves. It assumes that sampled leaves on sequentially harvested plants follow identical physiological time traces. Thus, the physiological age of leaf l on plant j harvested at time t must have been identical to that of leaf l on plant i harvested at time t-1. Physiological age is important as both cell division and expansion contribute to development throughout lamina expansion (Steer, 1971; Sunderland, 1960).

Leaf area is measured with leaf scanners or estimated using empirical models, with the choice of method usually depending on whether leaves are destructively or non-destructively harvested. Many leaf area scanners require detached (i.e. destructively harvested) leaves for measuring. Use of those scanners capable of *in situ* measurements (i.e. non-destructive) is limited by the width of the leaf the scanner can process. Empirical models based on leaf length and width are commonly used to non-destructively estimate leaf area (Marshall, 1968). The area of simple leaves of apple (*Malus domestica* Borkh), peach (*Prunus domestica* L.), pear (*Pyrus communis* L), bean (*Phaseolus vulgaris*), sweetcorn (*Zea mays*) and rice (*Oryza* spp.) can be rapidly and accurately estimated using polynomials based on length and width measurements (Ackley et al., 1958; Bhan and Pande, 1966; McKee, 1964). Estimating area of compound leaves, however, is made difficult by the different shapes of leaflets. Nevertheless, Akoroda (1993), Hughes and Proctor (1981), Lim and Narayanan (1972) and Wiersma and Bailey (1975) reported accurate predictive models of total compound leaf area using the

terminal leaflets of fluted pumpkin (*Telfairia occidentalis*), ginseng (*Panax quinquefolius* L.), rubber (*Hevea brasiliensis*) and soybean (*Glycine max* L.) respectively. Ross (1946) and Salter (1958) non-destructively estimated tomato leaf area using either length and width of leaflets or mid-rib length of the compound leaf.

Empirical models arise from applying mathematical or statistical formulae to observational data. Although this process is unconstrained by the need for any knowledge of scientific principles or mechanism, a basic understanding of the system being modelled is generally necessary when deciding what to observe and measure. Although empirical models can be deterministic, whenever the data is statistically analysed a probability distribution is associated with the model and its output. Many reported empirical models for predicting leaf area inadequately address this issue. Estimates of the variation associated with the model are often ignored (e.g. Hoffman, 1971; NeSmith, 1991; Whitworth et al., 1992). Moreover, models designed for prediction purposes are often evaluated on current performance, rather than predictive ability (e.g. Hughes and Proctor, 1981; Sepaskhah, 1977).

The robustness of models for environments or cultivars different from those under which the models were developed has received limited attention. Neither cultivar nor plant age influenced coefficients of a model estimating leaf area of cotton (Ashley et al., 1963). McKee (1964) reported that a derived relationship for corn leaf area was stable under field and greenhouse environments with seven varieties. On the other hand, Nautiyal et al. (1990) recommended correction factors for predicting leaf area of different cultivars of apples. This model was, however, stable over location. Sepúlveda and Kliewer (1983) reported site and cultivar differences in linear equations for estimating leaf area of grapes. Robbins and Pharr (1987) observed that the relationship between length, width and area leaves of several cucumber cultivars changed with cultural system. Hoffman (1971) reported that

relative humidity and root medium salinity had no effect on a log-linear relationship between leaf length and area for onion (*Allium cepa* L. hyb). Hoffman's data, however, shows clear evidence of heteroscedasticity, and slope and intercept coefficients show consistent change with increasing salinity. Hoffman (1971) does not present estimates of residual variation, perhaps because residual variation was too great to detect real differences. Salter (1958) applied his model (relating compound leaf mid-rib length to leaf area) to an experimental system in which plants of tomato were subjected to different water regimes, subsequently reporting that the model was robust.

Few authors report the robustness over time of their models. The study by McKee (1964) is cited (Hoffman, 1971; Hughes and Proctor, 1981) as evidence that predictive leaf area models are robust over time, but McKee's description, "... *two quite different seasons* ...", provides insufficient detail to warrant such confidence. Nautiyal et al. (1990) developed their models over six years, and although they tested for robustness over location, they did not report on robustness over time. The apparent lack of concern by model developers about the influence of time is surprising since there is good evidence that leaf morphology is significantly influenced by prevailing temperature and light conditions. Warrington and Norton (1991) demonstrated in several crops, including corn and cucumbers, that specific leaf weight (SLW) was linearly related to daily quantum integral. Van Volkenburgh and Davies (1977) reported that SLW of cotton and soybean was negatively correlated with day temperature. It seems reasonable to expect that seasonal changes in daily light integrals and temperature could alter leaf morphology to such an extent that empirical models developed from data collected during the winter, for example, would prove unreliable when used to estimate leaf area of crops grown during summer.

This chapter reports the development of an empirical model for non-destructively estimating leaf area of tomato. It builds on the studies of Ross

(1946) and Salter (1958), and discusses factors which should be taken into account when developing such models.

4.2 Materials and methods

4.2.1 Cultural

Seed of the indeterminate tomato *L. esculentum* Mill. 'Moneymaker' were germinated in trays (28 cm long × 40 cm wide × 6 cm deep) containing peat and pumice (3:2 v/v) with bottom heat under mist in a heated glasshouse. When their cotyledons were fully expanded, seedlings were transplanted to 200 cm^3 tubes containing a peat:pumice (3:2 v/v) growing medium, and grown on in the glasshouse. Plants were fed daily with Peters 20+8.6+16.6 (NPK, Grace-Sierra Horticultural Products, USA) at 100 ppm N.

After six weeks, shoots were excised immediately above the cotyledon node and rooted in an aerated water bath under mist. The water bath was constructed from an opaque plastic container (30 × 40 × 8 cm) covered with an inverted 273 plug tray. Cuttings were inserted through the cells into the water, immersing the lower 2 cm of stem. The cuttings 'hung' from their cotyledons resting on the top of the plug cells. As the extent of rooting is influenced by the O_2 content of the solution (Soffer and Burger, 1988) an aquarium pump supplied air continuously to the water bath via three microtubes (2 mm internal diameter). The O_2 content of the solution, measured at 20°C with a YSI Dissolved Oxygen Monitor (model 57), was 8.55 $mg \cdot litre^{-1}$. Excess water, accumulating from the mist system, drained to waste from the water bath. Cuttings rooted after about five days and were weaned from the system by gradually reducing the mist frequency from six seconds/minute (daylight hours) to zero over the ensuing four days.

Rooted cuttings were placed in individual 10 litre containers in a deep flow hydroponic system (Section 2.0). The electrical conductivity of the solution was increased daily in 0.5 $mS \cdot cm^{-1}$ increments from 0.5 to the final operating level of 2.5 $mS \cdot cm^{-1}$. The solution was replaced at monthly intervals.

Lateral shoots and flower trusses were removed as soon as practicable to restrict the assimilate sinks to the primary shoot system and roots.

4.2.2 Environmental

Glasshouse air temperature was maintained at 15°C minimum at night, with ventilation triggered when air temperature rose above 25°C during the day. Daily mean temperature ranged from 18°C during the spring and autumn months to 26°C during summer.

4.2.3 Experimental

Experiment 1 Conducted in October 1989, the objective of this experiment was to estimate total compound leaf area from length and width measurements of leaflets. Measurements were taken from the terminal and two adjacent leaflets on leaves at nodes 4-6, 9-11 and 13-15 (numbered acropetally from the cotyledonary node). Width measurements were taken at positions 25, 50 and 75% along the leaflet blade. Leaflet length was exclusive of the petiole. Twenty five plants were used in the experiment, with 27 leaflets measured on each plant.

Experiment 2 Total compound leaf area was estimated from the length of the mid-rib of the leaf (i.e. the combined length of the rachis and the terminal leaflet). This experiment was repeated four times: January and

March, 1990, April 1991, and April 1992. The number of plants used varied; 40 plants were harvested in January 1990, 34 in March 1990, 15 in April 1991, and 25 in April 1992. Depending on the size of the plant, between 6 and 13 leaves were harvested from each plant.

4.2.4 Development of predictive models

Models were developed in four phases (Neter et al., 1990):

(i) data collection and preparation
(ii) reduction of the number of independent variables
(iii) model refinement
(iv) model validation and selection

4.2.4.1 Data collection and preparation

Collection

All length measurements were made to the closest millimetre using a flexible tape. The smallest mid-rib length was about 30 mm; shorter leaves could not be measured without physically damaging the expanding lamina. The area of each compound leaf was measured with a Li-Cor LI-3000 leaf area meter (Lambda Instruments Corporation, USA). Leaflets were removed from the petioles before area measurement and then dried for 72 hours at 80°C in a forced air drying oven before weighing.

Tomato leaf size changes with position on the stem axis. The area of fully expanded leaves at nodes immediately above the cotyledon node are smaller than fully expanded leaves at higher insertions, despite being both physiologically and chronologically older. Thus, leaves become progressively larger then progressively smaller as insertion position increases. Positional and

developmental indices were used in the model building process to account for this trend.

Positional indices

Two positional indices were used: the leaf insertion position (LIP), measured acropetally from the cotyledon node; and INDEX, calculated as leaf insertion position divided by the number of leaves on the stem (i.e. LIP_n/LIP_{max}). This index standardised leaf position between plants of different age or height.

Developmental indices

Plant height (HT) was used as a coarse index of whole plant development and the leaf plastochron index (LPI) as a precise measure.

The LPI is an established index of plant development and has been successfully used with tomato (Andrews and Chalmers, 1989; Coleman and Greyson, 1976a). The duration between initiation of successive leaf primordia on the main stem apex defines a plastochron. A nondestructive estimate of a plastochron is the time required for successive leaves to reach a specified length (termed the reference length). Erickson and Michelini (1957) extended the plastochron concept to the plastochron index (PI) with a method that estimates the number of developed plastochrons plus the fractional component of the plastochron currently undergoing development (equation. 4.1).

$$PI = i + \frac{(\log_e L_i - \log_e L_{ref})}{(\log_e L_i - \log_e L_{(i+1)})} \qquad (4.1)$$

and,

$$LPI_a = PI - j \qquad (4.2)$$

where i is the serial number of that leaf (counting from the cotyledon node) that first exceeds the reference length (L_{ref}; 30 mm), $\log_e L_i$ is the $\log_e$ length (mm) of leaf i, $\log L_{(i+1)}$ is the $\log_e$ length (mm) of leaf $i+1$, $\log_e L_{ref}$ is the $\log_e$ reference length, and j is the serial number for any given leaf j on the shoot apex.

Valid derivation of PI assumes that early leaf growth occurs at an exponential rate, early growth of successive leaves on a single plant occurs at the same relative rate, and successive plastochrons are the same length for a particular plant (Lamoreaux et al., 1978). This was confirmed for all the sets of data examined (data not shown). A reference length of 30 mm was used to calculate LPI.

Environmental indices

Light and temperature data were not available for all data sets (months). The demonstration that SLW is functionally related to daily quantum integral and day temperature (van Volkenburgh and Davies, 1977; Warrington and Norton, 1991) suggested that this index might better reflect light and temperature effects than simultaneous measurements. Specific leaf area (SLA), the reciprocal of SLW, was used as an environmental index since the focus of the study was to develop a leaf *area* model. Two randomly selected leaves were destructively harvested from guard plants every 2-3 weeks during each repeat of the experiment. The average SLA during respective repeats was used in the model.

Data preparation

Gross data errors and extreme outliers were identified through scatterplots of model variables against leaf area. Data entry mistakes were corrected, and in seven instances, a data case was removed from the main data set.

Data partitioning

Each data set was randomly divided into a model-building sample and a validation sample (Snee, 1977). In the absence of firm guidelines for respective sizes of each sample, a 2:1 split was used. Both sets satisfied the liberal rule that sample number n should be greater than $2p + 20$, where p is the number of regressor variables in the model (Myers, 1990).

A preliminary analysis (Table 4.1) revealed that the relationship between compound leaf area and leaflet length and width was not significantly improved if either the degree of development of the leaflet (assessed by the position of the leaf on the stem), or the position of the leaflet in the compound leaf was included in the model. As a consequence, the data set of experiment 1 was randomly divided into a model building set and a validation set without reference to the age or position of the leaflet.

Table 4.1 Partial sums of squares and means squares for preliminary model for estimating compound leaf area from linear measures of leaflets.

Model term	df*	Partial sums of squares	Mean square	F value	$P > F$
Leaflet length	1	2780.2	2780.2	218.9	0.0001
Leaflet width at 30%	1	376.0	376.0	29.6	0.0001
Leaflet width at halfway	1	241.6	241.6	19.0	0.0001
Leaflet width at 60%	1	61.0	61.0	4.8	0.0308
Age of leaflet	2	62.2	31.1	2.5	0.0912
Position of leaflet in leaf	7	127.3	18.2	1.4	0.2017
Mean square error	97	1232.2	12.7		

* degrees of freedom

Reduction of the number of independent variables

An all-possible-regressions selection procedure (PROC REG; SAS Institute, 1988) identified subsets of regressors worthy of further, more detailed examination. An intercept term was included in all models.

Subsets of each data set were identified using four criteria: the coefficient of multiple determination, R^2; the adjusted coefficient of multiple determination, R^2_{adj}; the mean square for error, MSE_p; and Mallows statistic, C_p.

The coefficient of multiple determination (R^2) is defined as the fraction of the total sums of squares (SSTO) accounted for by a regression model of p parameters[1] (SSR_p):

$$R^2 = \frac{SSR_p}{SSTO} \tag{4.3}$$

This criterion is used subjectively; the intent is to find the point where the gain in R^2 after adding a further regressor is very small.

Subjective use of R^2 is necessary as incorporating additional regressors into the model will, by definition, increase its value, a point which is frequently overlooked (e.g. Gamiely et al., 1991; Hughes and Proctor, 1981; NeSmith, 1992; Robbins and Pharr, 1987; Sepúlveda and Kliewer, 1983; Whitworth et al., 1992). The coefficient of multiple determination varies inversely with the error sums of squares for a given regression (SSE_p), as the SSTO is constant for all possible regressions from a given data set. As a consequence, R^2 is maximised when all p - 1 regressors are added to the model. The adjusted coefficient of multiple determination, R^2_{adj}, takes into account the number of parameters in the regression model:

$$\begin{aligned} R^2_{adj} &= 1 - \left(\frac{n - 1}{n - p}\right) \frac{SSE}{SSTO} \\ &= 1 - \frac{\frac{MSE}{SSTO}}{n - 1} \end{aligned} \tag{4.4}$$

The adjusted coefficient of multiple determination (R^2_{adj}) increases only if MSE decreases, since SSTO/(n-1) is fixed for the given data set; hence, R^2_{adj} and MSE_p are equivalent criteria. The favoured model is that for which

[1] p - 1 predictor variables plus the intercept term

MSE_p is at the minimum, or so close to the minimum that adding more regressors is not worthwhile.

Under-specification of the model (i.e. when important regressors are absent from the model) results in biased coefficients and prediction while large variances in both coefficients and prediction arise from over-specification. Mallows' C_p statistic provides a compromise between bias and variance:

$$C_p = \frac{SSE_p}{MSE_{p_{max}} - (n - 2p)} \tag{4.5}$$

Models with a value of C_p close to p are favoured as they do not contain estimated bias. In other words, all error in the predicted y estimates is variance, and the model is not under-specified.

4.2.4.2 Model refinement and selection

Decision rules

The basic decision rule for accepting or rejecting a fitted regression equation is whether or not the regression F ratio (F_{model}) exceeds the critical F factor ($F_{crit.}$), the 1-α percentile of the central F with p and $n - p - 1$ degrees of freedom (where p is the number of parameters in the model, excluding the intercept, and n is the number of observations). When F_{model} is statistically significant, it implies that a significantly large amount of the variation in the data about the mean has been accounted for by the regression equation. While some authors record F_{model} for candidate models (e.g. Robbins and Pharr, 1987), it is usually assumed that $F_{crit.}$ has been exceeded.

Accepting the decision rule has limited value; acceptance simply means that the fitted equation is a better predictor of the response than an equation

containing only the mean. As Draper and Smith (1981, p.93) noted: "Unless the range of values predicted by the fitted equation is considerably greater than the size of the random error, prediction will be of no value even though a 'significant' *F*-value has been obtained, since the equation will be 'fitted to the errors' only". Box and Wetz (1973) proposed that statistically significant and worthwhile prediction equations could be distinguished from statistically significant equations of limited practical value by the γ_m criterion. This criterion quantifies the ratio of changes in predicted response over the variation of the estimators of these changes. Algebraically, the usual test for significance of regression tests the hypotheses:

$$H_0{:}\gamma = 0 \text{ versus } H_1{:}\gamma > 0$$

by comparing the F_{model} to $F_{crit.}$. The γ_m criterion tests:

$$H_0{:}\gamma = \gamma_0 \ (\gamma_0 \geq 0) \text{ versus } H_1{:}\gamma > \gamma_0$$

The value of F_{model} is compared to $F'_{crit.}$ where $F'_{crit.} = (1 + \gamma^2)F_{\alpha,b,n-m-1}$, m is the number of parameters in the model, not including the constant term, and $b = m(1 + \gamma^2)^2/(1 + 2\gamma^2)$ (Suich and Derringer, 1977).

In all tests, γ_m was taken as 4 (Draper and Smith, 1981). In other words, I required assurance that the standard deviations of the estimators of leaf area were at least four times smaller than the range of changes in leaf area in the regression zone.

Residual analysis

Models selected during the screening process underwent detailed residual analysis, using the PROC REG procedure of SAS (SAS Institute, 1988). Residual plots illustrated the degree of variance heterogeneity and highlighted systematic trends usually indicative of an incorrectly specified model. Studentised residuals and HAT matrix diagonals pointed to outliers and influential data points. Benchmarks for the average HAT and highly

influential observations were calculated as $p \cdot n^{-1}$ and $2p \cdot n^{-1}$ respectively (Belsley et al., 1980). Distributional normality of residuals was assessed using box and normal probability plots of studentised residuals.

Stability

The stability in time of models common between months was tested by examining the homogeneity of slopes and intercepts of the models with time (months) added to the model as qualitative (dummy) variables. The significance of the coefficients of the interaction terms between the time and positional, developmental or environmental indices within the model were tested using partial F tests of the general linear model approach (Neter et al., 1990).

Multicollinearity

Multicollinearity is a problem of models involving regressors that are dependent on each other and thus incorporate redundant information (Myers, 1990). The potential for multicollinearity was assessed from the correlation matrix of regressors (the eigenstructure $\mathbf{X'X}$); highly correlated regressors indicate a potential multicollinearity problem.

The seriousness and number of dependencies was determined by the condition number and condition index of $\mathbf{X'X}$. The condition number of the eigenstructure is given by ϕ, the ratio of the largest to the smallest eigenvalue.

$$\phi = \frac{\lambda_{max}}{\lambda_{min}} \qquad (4.6)$$

If the condition number is less than 100, there is no serious problem with multicollinearity. Condition numbers between 100 and 1000 imply moderate

to strong multicollinearity. Severe multicollinearity is indicated when the condition number exceeds 1000 (Montgomery and Peck, 1992; Myers, 1990).

The condition index of the ith eigenvalue of the eigenstructure, ϕ_i, is the ratio of the largest to the ith eigenvalue.

$$\phi_i = \frac{\lambda_{max}}{\lambda_i} \tag{4.7}$$

The number of large ϕ_i (i.e. ≥ 1000) indicates the number of multicollinearities in the data set.

Variance decomposition proportions of $\mathbf{X'X}$ indicated the extent of involvement of regressors in each dependency, i.e. they reflect the proportion of the variance of the coefficient of the ith regressor that can be attributed to the collinearity characterised by the jth eigenvalue (Myers, 1990).

Variance inflation factors (VIF) reflected the extent of damage of the dependency to individual regressor coefficients. For each regressor term, VIF is given as:

$$\text{VIF} = \frac{1}{1 - R_i^2} \tag{4.8}$$

where R^2_i is the coefficient of determination of the regression of the ith regressor variable against the other regressor variables.

The VIFs represent the inflation that each regression coefficient experiences due to associations among the regressor variables. Generally accepted guidelines for interpretation are that regression coefficients with VIFs exceeding 10 are poorly estimated (Myers, 1990).

The extent of multicollinearity was minimised by removing offending regressors from the model wherever justified, and by centering the raw

regressor data (i.e. $(x_{ij} - x_j)$, where x_{ij} is the ith observation of the jth regressor). Removing regressors from the model was not always desirable as performance of the model was detrimentally affected. Although it is conventional practice to retain non-significant main factors if they contribute to significant interaction terms, such factors were removed if they contributed to a multicollinearity problem.

4.2.4.3 Model validation

Ordinary residuals do not generally indicate how well a regression model will predict, as the predicted value of an observation is dependent on the observation. Thus, ordinary residuals measure quality of fit, not quality of future prediction (Myers, 1990). The quality of future prediction of candidate models was assessed by (i) cross validation, (ii) calibrating the predictive capability of the model, and (iii) using the PRESS (prediction sum of squares) criterion.

Several procedures exist for cross validation (Myers, 1990; Neter et al., 1990; Snee, 1977). In this study, leaf area of observations in a validation sample was predicted by the candidate models. The coefficients of determination (r^2) between actual and predicted leaf area and the candidate model (R_p^2) were compared. A decrease between these values of more than 10% (i.e. $R_p^2 - r^2 \geq 0.1$) signalled a poor model.

Preliminary choice of a regression model is based, at least in large part, on how well it fits the data at hand. Because different models may have been chosen for different random outcomes in the data set, the MSE of the selected model will tend to underestimate the inherent variability of future predictions made from it. One method of calibrating the actual predictive capability of the selected model is to use it to predict each case in the new data set and

then to calculate the mean of the squared prediction errors (i.e. the MSPR). The MSPR is given as:

$$MSPR = \frac{\sum_{i=1}^{n^*} (Y_i - \hat{Y}_i)^2}{n^*} \tag{4.9}$$

where: Y_i is the value of the response variable in the ith validation observation, $\hat{Y}_i$ is the predicted value for the ith validation observation based on the model-building sample, and n^* is the number of cases in the validation sample.

If the MSPR is similar to the MSE of the regression fit to the model-building sample, the MSE is not seriously biased and reflects the predictive ability of the model (Neter et al., 1990). However, if considerably larger than the MSE, the MSPR provides a better indication of how well the candidate model will predict.

The PRESS criterion is the sum of n PRESS residuals associated with a given model, where a press residual at the ith observations (d_i) is the difference between the actual Y value (Y_i) and the predicted Y ($\hat{Y}_i$) when the regression model is fitted without the ith observation:

$$d_i = Y_i - \hat{Y}_{i(i)} \tag{4.10}$$

$$\begin{aligned} PRESS_p &= \sum_{i=1}^{n} d_i^2 \\ &= \sum_{i=1}^{n} (Y_i - \hat{Y}_{i(i)}) \end{aligned} \tag{4.11}$$

Models with small PRESS values are considered favourably.

PRESS residuals were used to generate the statistic R^2_{PRESS}, whose similar format to R_p^2 aids interpretation of the PRESS criterion, where:

$$R^2_{PRESS} = 1 - \frac{PRESS}{\sum_{i=1}^{n} (y_i - \bar{y})^2} \qquad (4.12)$$

R^2_{PRESS} values are interpreted as the percent of variability in predicting new observations 'explained' by the model.

The 'best' model of each month selected by these procedures was re-calculated using the PROC REG of SAS (SAS Institute, 1988) with the full data set since accuracy of coefficients and MSE is a positive function of n. The standard error of individual predicted values was determined, and from these the mean standard error of leaves with mid-rib lengths of 10, 20, 30, 40 and 50 cm (± 0.5 cm) calculated.

4.3 Results

4.3.1 Predicting compound leaf area from leaflet area (experiment 1)

Preliminary analysis indicated that neither leaflet age nor position in the compound leaf were important factors in the prediction model (Table 4.1). Consequently, data were pooled on these variables for subsequent analyses. Mallows C_p statistic was optimised only when leaflet length and all three width measures were in the model. A second-order model accounted for about 99% of total variation and exceeded the γ_m criterion (Table 4.2). Using models with fewer terms, but less optimal values of Mallows C_p statistic, would have resulted in predicted values with inflated confidence intervals relative to this second order model (Fig. 4.1; model A vs. models B-H).

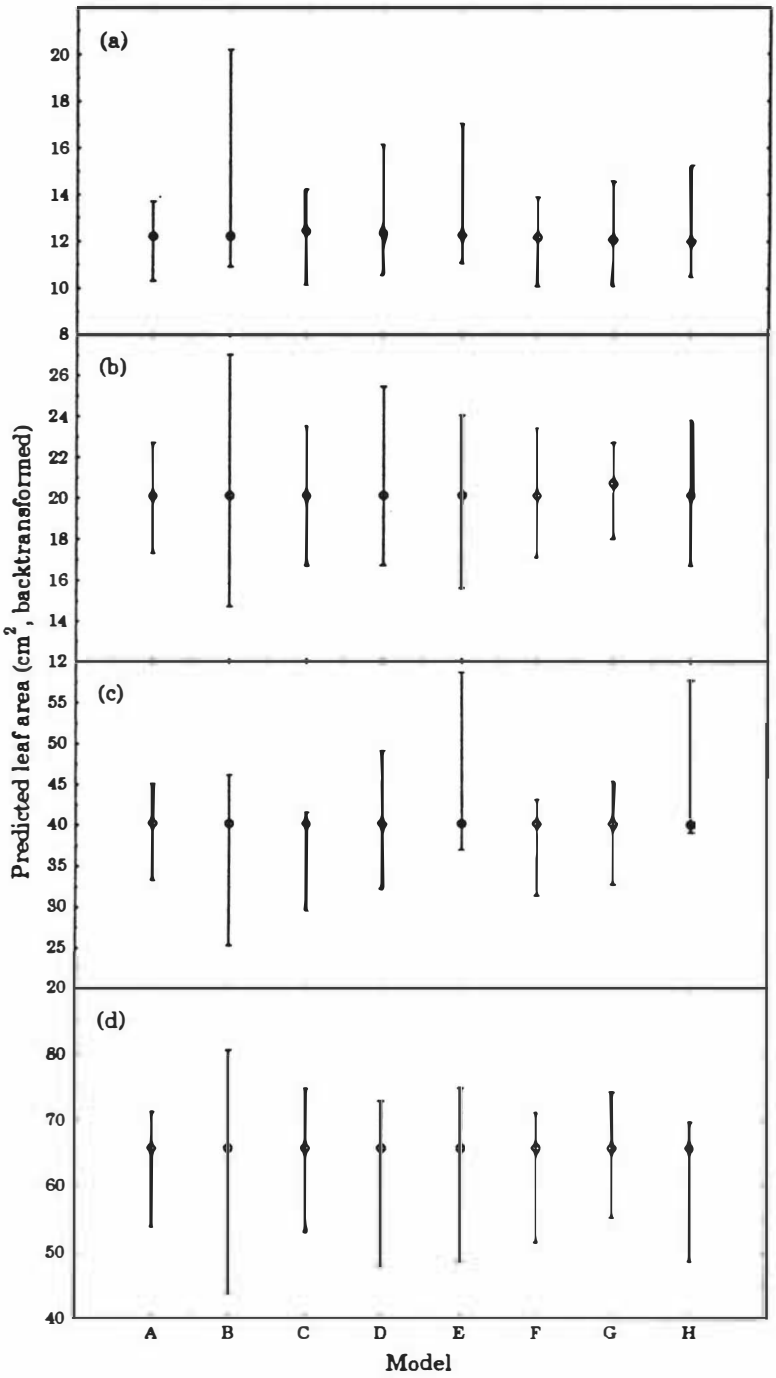

Fig. 4.1. Upper and lower 95% confidence levels of an individual prediction of the leaf area of randomly selected leaves of (a) 5.5 cm, (b) 7.3 cm length, (c) 10.5 cm, and (d) 14.0 cm length. Data are backtransformed (e^x) values from the models:

A: $\log_e$ area = intercept + X_{length} + X_{width1} + X_{width2} + X_{width3} - $X_{length \times width1}$ - $X_{length \times width3}$ + $X_{width1 \times width3}$

B: $\log_e$ area = intercept + X_{length}

C: $\log_e$ area = intercept + X_{length} + X_{width1} - $X_{length \times width1}$

D: $\log_e$ area = intercept + X_{length} + X_{width2} - $X_{length \times width2}$

E: $\log_e$ area = intercept + X_{length} + X_{width3} - $X_{length \times width3}$

F: $\log_e$ area = intercept + X_{length} + X_{width1} + X_{width2} - $X_{length \times width1}$ - $X_{length \times width2}$ + $X_{width1 \times width2}$

G: $\log_e$ area = intercept + X_{length} + X_{width1} + X_{width3} - $X_{length \times width1}$ - $X_{length \times width3}$ + $X_{width1 \times width3}$

H: $\log_e$ area = intercept + X_{length} + X_{width2} + X_{width3} - $X_{length \times width2}$ - $X_{length \times width3}$ + $X_{width2 \times width3}$

A natural logarithm transformation of leaflet area remedied variance heteroscedasticity and centering the regressors avoided moderate multicollinearity (Table 4.2). Minimal shrinkage between the respective coefficients of determination of the model and validation set, and between the MSE and MSPR values showed that area of compound leaves in the validation set of data was closely predicted by the model. The practical value of the model was limited, however, as four measurements from each of the three leaflets on the compound leaf were required for its accurate prediction. Further development was not considered worthwhile.

Table 4.2 Regression and validation diagnostics of candidate model predicting compound leaf area from leaflet area.

Model term	Raw model				Regressors centered			
	coefficient	SE	VIF	ϕ	coefficient	SE	VIF	ϕ
intercept	0.605	0.093	0.0	1.0	3.635	0.011	0.0	1.0
length	0.286	0.018	59.8	5.9	0.116	0.005	4.2	1.1
width1	0.196	0.042	49.8	13.2	0.081	0.013	4.5	2.5
width2	0.039	0.012	3.8	15.9	0.039	0.012	3.8	3.3
width3	-0.045	0.066	63.7	21.5	0.049	0.016	3.6	4.2
length × width1	-0.025	0.003	177.2	55.9	-0.025	0.003	4.7	4.6
length × width3	-0.014	0.005	165.5	112.3	-0.014	0.005	4.0	5.8
width1 × width3	0.048	0.011	135.2	125.1	0.048	0.011	4.2	6.7

Model fit statistics		*Model validation statistics*	
R_{adj}^2	0.986	r^2	0.989
MSE	0.0045	MSPR	0.0043
F_{model}	750.6		
$F_{crit.}$	1.53		
$F'_{crit.}$	26.0		

4.3.2 Predicting compound leaf area (experiment 2)

Preliminary model selection

The relationship between mid-rib length and leaf area is curvilinear (Fig. 4.2). Separate analyses of regression using the model *compound leaf area = a + b(mid rib length)*^{2} (Lyon, 1948; Ross, 1946; Salter, 1958) were conducted for each month. This model accounted for at least 87% of total variance (Table 4.3) with intercept and slope coefficients of similar magnitude to those reported by Ross (1946) and Salter (1958), although larger than those of Lyon (1948).

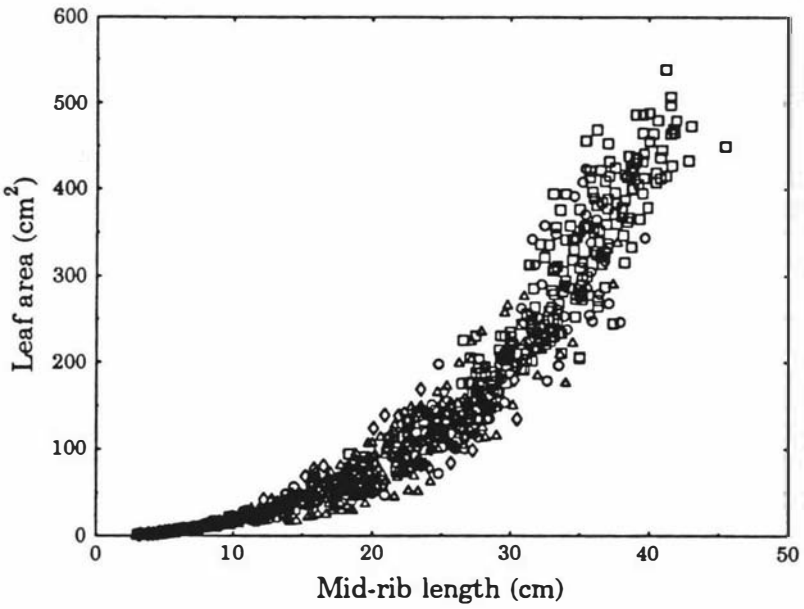

Fig. 4.2 Relationship between mid-rib length and area of *L. esculentum* 'Moneymaker'.

Both intercept and slope, however, differed between months, revealing changes in the relationship between mid-rib length and leaf area in time (Table 4.3). Furthermore, residual plots revealed heteroscedasticity with the variance of predicted leaf areas increasing as mid-rib length increased.

Although logarithmic transformation of the dependent variable can correct this problem (Draper and Smith, 1981), its use with models from all months failed to reduce the heteroscedasticity problem. In addition, Mallows C_p statistic indicated the model was under-specified and biased (i.e. important terms were absent from the model, and the sign and magnitude of the coefficients of terms in the model were badly estimated). Consequently, this model was abandoned and a search for better performing alternative models made.

Table 4.3 Performance of Ross's (1946) and Salter's (1958) model for predicting tomato leaf area from the model *leaf area* (cm^2) = a + *mid-rib length*^{2}.

	Month			
Regressor	January 1990	March 1990	April 1991	April 1992
intercept	-16.17	-5.48	3.33	-9.95
(SE)	(2.196)	(1.757)	(3.454)	(7.431)
mid-rib length^2	0.269	0.207	0.197	0.241
(SE)	(0.003)	(0.004)	(0.007)	(0.009)
R^2	0.95	0.87	0.88	0.89
MSE	1014.12	478.54	449.81	1554.02

Other models

The range of leaf areas examined differed slightly between months; the widest ranges examined were in January 1990 and April 1992 (Table 4.4). Values of LPI, INDEX, and LIP were similar between months.

Results from correlation analysis and scatterplots of various linearising transformations of leaf area and mid-rib length revealed a strong linear

relationship between $\log_e$ area (LNAREA) and $\log_e$ mid-rib length (LNMRIB) in each month (Fig. 4.3).

Table 4.4 Range of values of data examined.

Month	n	Area (cm^2)		Mid-rib length (cm)		SLA ($m^2 \cdot g_L^{-1}$)	
		range	mean	range	mean	range	mean
January 1990	397	1-539	186	3.0-45.5	24.6	233-341	277
March 1990	270	1-339	70	2.9-37.7	17.4	313-425	359
April 1991	90	1-221	83	3.1-31.3	17.8	383-1020	518
April 1992	115	2-624	87	3.4-49.7	24.0	260-348	289

	LPI		INDEX		LIP	
	range	mean	range	mean	range	mean
January 1990	0-12.0	5.7	0.25-1.00	0.59	4-16	9.1
March 1990	0-11.1	4.5	0.27-1.00	0.63	4-15	8.4
April 1991	0-10.6	4.9	0.28-1.00	0.61	4-14	8.6
April 1992	0-12.6	6.5	0.24-1.00	0.59	4-17	9.9

Residual plots from the model *$\log_e$ compound leaf area* = $a_i + b_i$*($\log_e$ mid-rib length)*, (where i=month) exhibited an asymmetric pattern of variance, with the area of leaves with mid-ribs longer than 22-27 cm (depending on month) being over-estimated (Fig. 4.4(a-d)). Subsequent linear transformations of the terms in the model failed to stabilise this heterocedasiticity. Furthermore, slight departures from linearity in normal probability plots indicated that the residuals were not normally distributed.

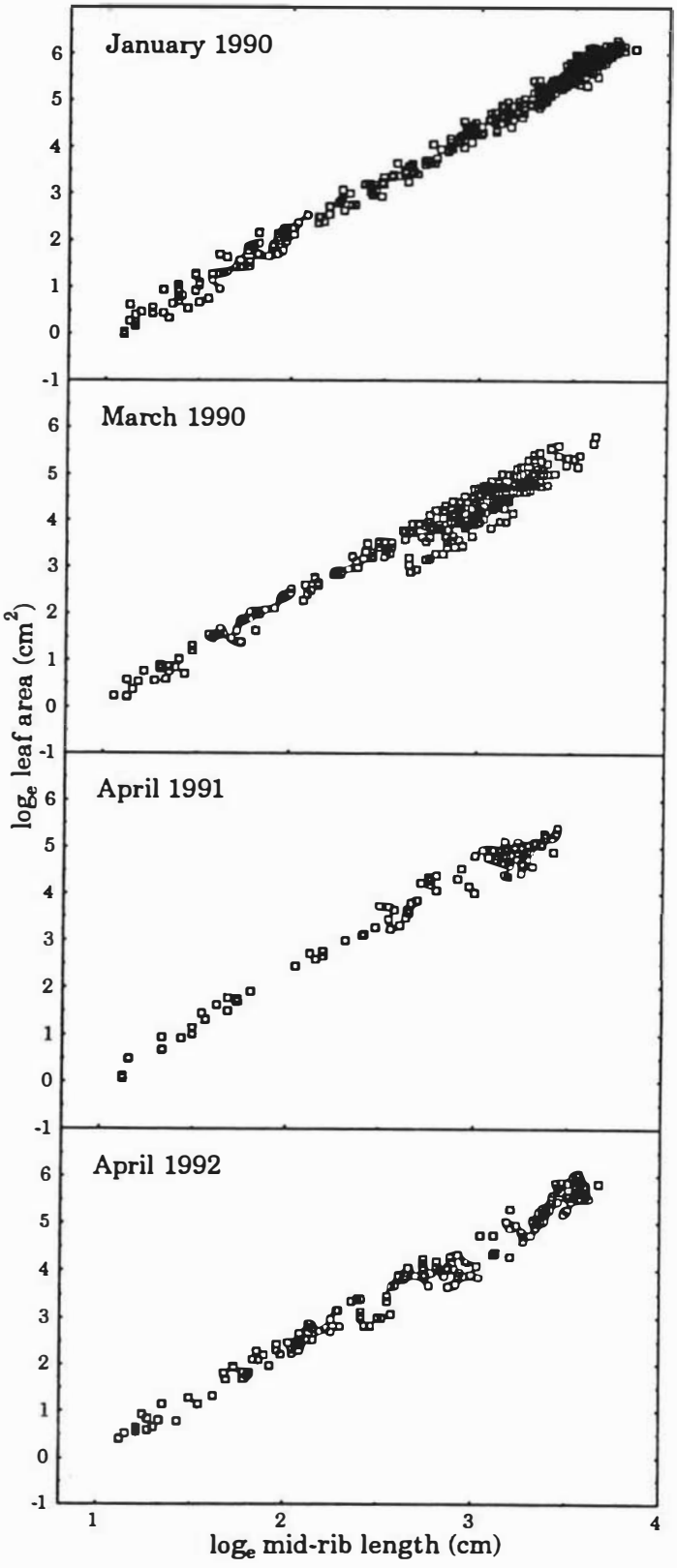

Fig. 4.3 Relationship between log$_e$ mid-rib length and log$_e$ leaf area of *L. esculentum* 'Moneymaker' at each month.

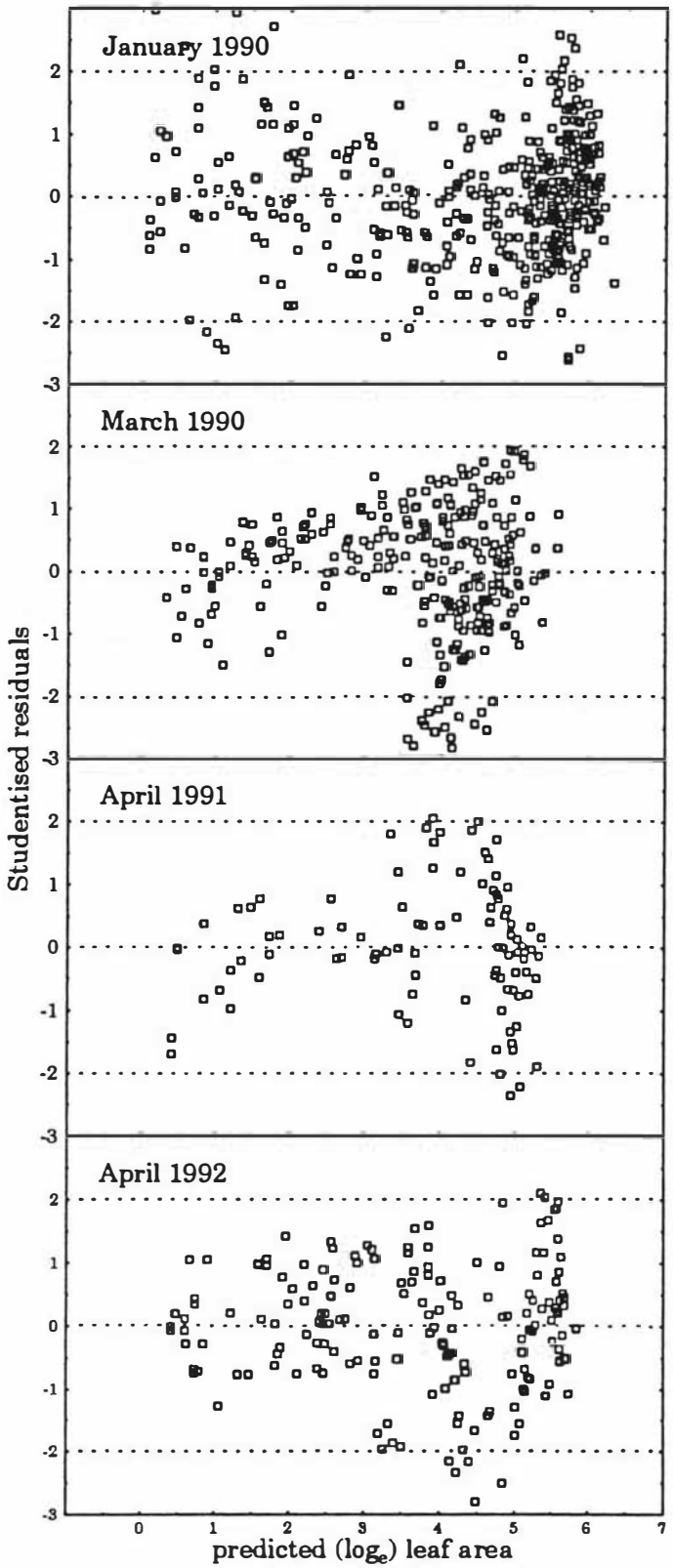

Fig. 4.4 Plots of studentised residuals from regression of log$_e$ mid-rib length and log$_e$ leaf area for each month.

Preliminary regression analyses of models containing INDEX, LIP, LPI terms in addition to LNMRIB revealed that no simple model was consistently superior across all months (data not shown). Significant interaction terms between month and other regressors in the model indicated that the relationship between the regressor terms and the $\log_e$ transformed leaf area term varied through time.

Although LNMRIB accounted for most of the variation, distribution of residuals and overall model performance was improved by adding positional and developmental indices to the model. The number and form of terms (e.g. main factor or interaction term) required to satisfy selection criteria varied between months. Criteria for R^2_{adj}, C_p, and MSE_p were met with a single term model for the January 1990 data set, two terms in March 1990 and April 1991, and three terms for April 1992 (Table 4.5). With the exception of January 1990, the analysis confirmed that models comprising of only LNMRIB were under-specified.

Extreme multicollinearity was revealed in some models fitted to raw data, with VIF and ϕ exceeding their benchmark values. This was not surprising given the high correlation between INDEX, LIP, LPI and LNMRIB (Table 4.6, Fig. 4.5). As there was no need to study the efficiency with which the intercept (in the natural variables) was being estimated, or the role of the intercept in the multicollinearity problem, the natural data were centered. This reduced, and in some models, eliminated the problem.

Table 4.5 'Best' main factor models for each month from all-possible-regressions selection procedure.

Regressors	R^2_{adj}	C_p	MSE
January 1990			
X_{LNMRIB}	0.9922	1.86	0.02186
$X_{LNMRIB}X_{LIP}$	0.9922	1.73	0.02177
$X_{LNMRIB}X_{SLA}$	0.9923	1.99	0.02179
$X_{LNMRIB}X_{INDEX}$	0.9923	2.25	0.02181
$X_{LNMRIB}X_{LPI}$	0.9922	2.86	0.02186
$X_{LNMRIB}X_{SLA}X_{LIP}$	0.9923	1.80	0.02169
$X_{LNMRIB}X_{SLA}X_{INDEX}$	0.9922	2.50	0.02175
$X_{LNMRIB}X_{SLA}X_{LPI}$	0.9923	3.07	0.02179
March 1990			
$X_{LNMRIB}X_{INDEX}$	0.9849	1.71	0.02626
$X_{LNMRIB}X_{INDEX}X_{LPI}$	0.9853	2.59	0.02559
$X_{LNMRIB}X_{INDEX}X_{LIP}$	0.9851	3.99	0.02581
$X_{LNMRIB}X_{INDEX}X_{SLA}X_{LPI}$	0.9852	4.02	0.02566
$X_{LNMRIB}X_{LPI}X_{SLA}X_{INDEX}$	0.9852	4.29	0.02570
April 1991			
$X_{LNMRIB}X_{LIP}$	0.9822	-0.05	0.03584
$X_{LNMRIB}X_{INDEX}$	0.9815	1.80	0.03739
$X_{LNMRIB}X_{LPI}X_{INDEX}$	0.9819	1.66	0.03638
$X_{LNMRIB}X_{LIP}X_{INDEX}$	0.9819	1.75	0.03646
$X_{LNMRIB}X_{LIP}X_{SLA}$	0.9819	1.89	0.03658
$X_{LNMRIB}X_{LIP}X_{LPI}$	0.9819	1.95	0.03663
$X_{LNMRIB}X_{LPI}X_{HT}X_{INDEX}$	0.9818	3.05	0.03668
$X_{LNMRIB}X_{LIP}X_{LPI}X_{INDEX}$	0.9818	3.13	0.03675
April 1992			
$X_{LNMRIB}X_{LIP}X_{SLA}$	0.9849	3.65	0.03386
$X_{LNMRIB}X_{LIP}X_{SLA}X_{LPI}$	0.9851	4.08	0.03353
$X_{LNMRIB}X_{LIP}X_{SLA}X_{INDEX}$	0.9849	4.61	0.03382

Table 4.6 Correlation coefficients between leaf area and predictor variables.

	LNMRIB	LIP	INDEX	LPI	SLA	HT
January 1990						
LNAREA	0.99	-0.81	-0.82	0.82	0.03	0.09
LNMRIB		-0.81	-0.83	0.83	0.05	0.09
LIP			0.98	-0.97	0.004	0.06
INDEX				-0.98	0.01	-0.03
LPI					0.009	0.08
SLA						0.21
March 1990						
LNAREA	0.98	-0.56	-0.64	0.67	-0.01	0.26
LNMRIB		-0.68	-0.76	0.78	-0.001	0.23
LIP			0.95	-0.88	-0.03	0.23
INDEX				-0.97	-0.02	-0.02
LPI					0.01	0.19
SLA						-0.02
April 1991						
LNAREA	0.99	-0.81	-0.83	0.82	-0.01	0.07
LNMRIB		-0.86	-0.87	0.87	-0.02	0.07
LIP			0.98	-0.95	-0.02	0.09
INDEX				-0.99	-0.01	-0.05
LPI					0.03	0.13
SLA						-0.002
April 1992						
LNAREA	0.98	-0.70	-0.70	-0.71	0.06	0.03
LNMRIB		-0.77	-0.77	-0.78	0.05	0.03
LIP			0.99	-0.98	-0.19	0.06
INDEX				-0.99	-0.11	-0.03
LPI					0.05	0.09
SLA						-0.47

LNAREA= $\log_e$ leaf area; LNMRIB=$\log_e$ mid-rib length; LIP=leaf position on stem; INDEX=relative leaf position on stem; LPI=leaf plastochron index; SLA=specific leaf area; HT=plant height.

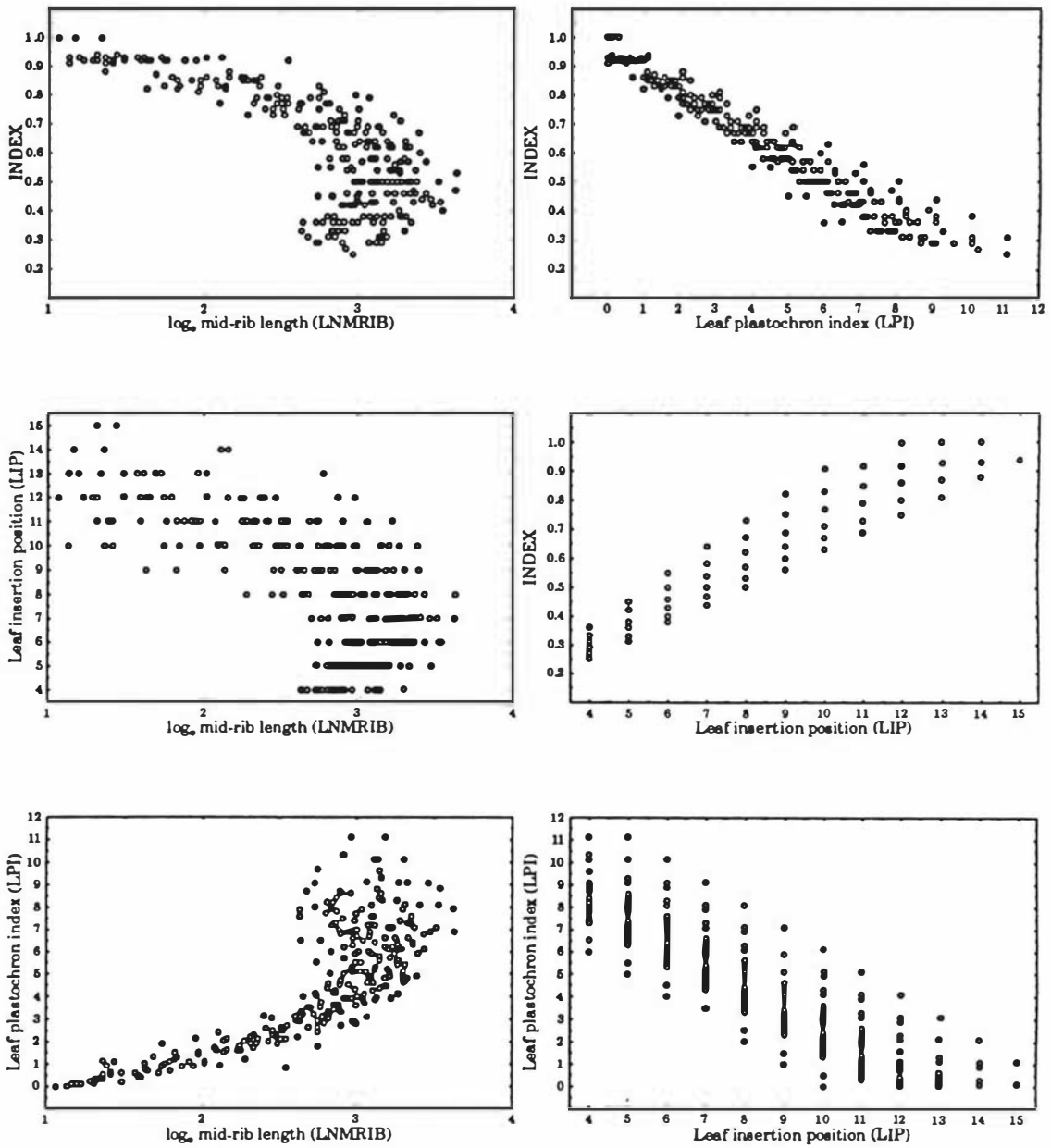

Fig. 4.5 Inter-relationships between regressors in March 1990 data set, but typical of all data sets.

Model refinement

In refining the models, emphasis was placed on minimising MSE. This was reduced by half in some models after second-order interaction terms were added (Table 4.7). Identifying and, when warranted, deleting influential

outliers also improved the models. For example, influence diagnostics of the model $Y_{LNAREA} = X_{LNMRIB} + X_{INDEX} + X_{SLA}$ (January 1990 data set) identified eight observations, all from a single plant, responsible for the SLA term. This cluster of observations suggests that either the plant was a rogue, or that the plant was poorly measured (i.e. human error). Both alternatives are reasonable grounds for deleting data. As a consequence, SLA was eliminated from the model as both a main factor and interaction term.

January 1990 Although all refined models performed well, conditions for fit and validation statistics were maximised in the model $Y_{LNAREA} = X_{LNMRIB} + X_{INDEX} - X_{LNMRIB \times INDEX}$.

March 1990 The model $Y_{LNAREA} = X_{LNMRIB} + X_{LIP} + X_{LIP \times INDEX}$ was the best performing model. The INDEX term was only significant in combination with LIP, and as its presence as a main factor caused a slight multicollinearity problem, it was removed from the model. While the MSPR statistic of the simpler model $Y_{LNAREA} = X_{LNMRIB} + X_{INDEX}$ suggested the MSE was inflated, this model had the largest PRESS residuals and lowest R^2_{adj} of the candidate models.

April 1991 Models with three or more terms were superior to two factor models, with PRESS residuals and MSE markedly smaller than the two factor models. In all higher term models the MSPR values were over 30% larger than the MSE estimates, indicating that the MSE estimates were biased and that predictive precision of the models was poorer than the MSE indicated. Of the higher term models, $Y_{LNAREA} = X_{LNMRIB} + X_{LPI} + X_{INDEX} + X_{LPI \times INDEX}$ and $Y_{LNAREA} = X_{LNMRIB} + X_{LIP} + X_{INDEX} + X_{LIP \times INDEX}$ exhibited superior residual and fit statistics. Moderate multicollinearity was detected in both models, with VIF_{max} associated with the INDEX term. In the model $Y_{LNAREA} = X_{LNMRIB} + X_{LPI} + X_{INDEX} + X_{LPI \times INDEX}$ variance decomposition proportions showed the problem due to INDEX and LIP as main factor terms. In the

model $Y_{LNAREA} = X_{LNMRIB} + X_{LIP} + X_{INDEX} + X_{LIP \times INDEX}$ variance decomposition proportions showed the problem 'shared' between all terms. While there is little to distinguish between the two models, the model $Y_{LNAREA} = X_{LNMRIB} + X_{LIP} + X_{INDEX} + X_{LIP \times INDEX}$ was favoured by the smaller disparity between MSE and MSPR and its smaller MSPR.

April 1992 The relationship between LIP and INDEX was stronger in this data set than others, with the consequence that all models incorporating these regressors showed moderate multicollinearity. Differences in the SLA of leaves in the April 1992 data set were sufficiently large to be a useful determinant of leaf area. The considerable effort required to obtain estimates of SLA, however, makes models not requiring this regressor more attractive. The model $Y_{LNAREA} = X_{LNMRIB} + X_{LIP} - X_{INDEX} - X_{LIP \times INDEX}$ had slightly poorer regression fit diagnostics than other models, but its estimate of future residual was considerably smaller than that of other models, and for this reason is the favoured model. Given its moderate level of multicollinearity, however, prediction of leaf area outside of the data region would be inaccurate. If extrapolation was likely, the model $Y_{LNAREA} = X_{LNMRIB} + X_{LPI} + X_{LFPI \times LPI}$, with minimal multicollinearity problems and comparable performance characteristics, would be favoured.

As the $F_{crit.}$ at $\gamma_m=4$ was exceeded for all models in all months, it had no discriminatory value. The maximum value of γ_m (γ_{max}) before $F_{crit.} > F_{model}$ was iteratively calculated and revealed that changes in leaf area were 24-185 times as large as the variation of the estimators of these changes (Table 4.7). The criterion γ_{max} provided similar discriminatory power to R^2. Within each data set, and for models with the same number of terms, the model with the highest γ_{max} coincided with the model with the largest R^2.

Table 4.7 Summary of regression fit and validation statistics for refined candidate models for predicting $\log_e$ leaf area.

Month	Model	γ_{max}	R^2	R^2_{adj}	r^2	R^2_{PRESS}	PRESS	MSE	MSPR	VIF_{max}	ϕ
Jan. 1990	$4.53 + 2.28X_{LNMRIB}$	185	0.995	0.995	0.995	0.994	3.319	0.014	0.015	1.0	1
	$4.47 + 2.38X_{LNMRIB} + 0.01X_{LIP} - 0.02X_{LNMRIB*LIP}$	110	0.995	0.995	0.995	0.995	3.240	0.013	0.015	6.6	25
	$4.47 + 2.44X_{LNMRIB} - 0.02X_{LPI} + 0.03X_{LNMRIB*LPI}$	112	0.995	0.995	0.995	0.995	3.009	0.013	0.014	10.0	39
	$4.47 + 2.45X_{LNMRIB} + 0.31X_{INDEX} - 0.47X_{LNMRIB*INDEX}$	113	0.996	0.995	0.995	0.995	2.952	0.012	0.014	9.6	38
March 1990	$3.72 + 2.45X_{LNMRIB} + 1.54X_{INDEX}$	68	0.987	0.986	0.990	0.986	3.780	0.023	0.015	2.4	7
	$3.88 + 2.17X_{LNMRIB} + 0.07X_{LIP} - 0.28X_{LIP*INDEX}$	63	0.990	0.989	0.992	0.989	2.894	0.018	0.015	2.9	9
	$3.80 + 2.33X_{LNMRIB} + 1.11X_{INDEX} + 0.042X_{LNMRIB*LPI} + 0.26X_{LPI*INDEX}$	52	0.989	0.989	0.987	0.988	3.272	0.019	0.020	5.2	26
April 1991	$3.73 + 2.45X_{LNMRIB} + 0.08X_{LIP}$	34	0.989	0.988	0.991	0.987	1.249	0.026	0.029	3.3	11
	$3.75 + 2.48X_{LNMRIB} + 1.19X_{INDEX}$	30	0.984	0.983	0.990	0.982	1.742	0.034	0.032	3.8	13
	$3.78 + 2.42X_{LNMRIB} - 0.07X_{LPI}$	28	0.982	0.981	0.988	0.979	1.985	0.039	0.031	3.6	12
	$3.91 + 1.94X_{LNMRIB} + 0.15X_{LPI} + 1.63X_{INDEX} + 0.37X_{LPI*INDEX}$	39	0.996	0.996	0.992	0.995	0.434	0.009	0.028	44.9	204
	$3.98 + 1.87X_{LNMRIB} + 0.13X_{LIP} - 2.34X_{INDEX} - 0.38X_{LIP*INDEX}$	32	0.994	0.994	0.992	0.993	0.579	0.012	0.022	37.8	193
	$3.92 + 2.00X_{LNMRIB} + 0.03X_{HT} + 0.33X_{LPI*INDEX} - 0.09X_{HT*INDEX}$	30	0.993	0.992	0.986	0.991	0.831	0.017	0.046	1.4	3
	$3.95 + 1.99X_{LNMRIB} + 0.11X_{LIP} + 0.13X_{LPI} - 0.24X_{LNMRIB*LPI} - 3.45X_{LNMRIB*INDEX} - 0.36X_{LFIP*INDEX}$	24	0.993	0.992	0.991	0.991	0.880	0.018	0.027	76.9	610
April 1992	$4.74 + 1.82X_{LNMRIB} + 0.15X_{LIP} + 0.003X_{SLA} - 2.83X_{INDEX} - 0.43X_{LIP*INDEX}$		0.995	0.994	0.992	0.993	0.899	0.013	0.025	101.3	529
	$4.76 + 1.75X_{LNMRIB} + 0.14X_{LIP} + 0.005X_{SLA} + 0.17X_{LPI} + 0.03X_{LIP*LPI}$		0.995	0.995	0.992	0.994	0.824	0.012	0.029	77.1	396
	$4.72 + 1.79X_{LNMRIB} + 0.08X_{LIP} - 1.71X_{INDEX} - 0.44X_{LIP*INDEX}$		0.994	0.994	0.993	0.993	0.969	0.015	0.018	67.8	334
	$4.75 + 1.72X_{LNMRIB} + 0.04X_{LPI} + 0.03X_{LIP*LPI}$		0.994	0.993	0.990	0.993	1.010	0.015	0.024	20.6	80

γ_{max}: Box and Wetz *F* test criterion; PRESS: prediction sums of squares; MSE: mean square error; MSPR: mean squared prediction residuals; VIF_{max}: maximum variance inflation factor; ϕ: condition number

Model performance within and between months was not improved by including either HT or SLA, despite large differences in these variables between months (Table 4.8). Changes in SLA appeared to parallel changes in leaf morphology. Sequential sums of squares showed that SLA accounted for a significant portion of total variance when added as the first term in the models. However, its contribution in the presence of other terms, adjudged by partial sums of squares, was always non-significant (data not shown), indicating the other terms in the model accounted for this variance.

Table 4.8 Plant height and SLA between months.

	Height (cm)		SLA ($cm^2 \cdot g_L^{-1}$)	
Month	mean	SE	mean	SE
January 1990	48.5	0.35	277.3	1.31
March 1990	34.1	0.33	359.2	1.83
April 1991	46.2	0.39	402.0	1.30
April 1992	36.7	1.06	296.1	2.36

Model stability

Although no single model structure was common to all months, some structures were common between pairs of months (Table 4.9). Coefficients of the model $Y_{LNAREA} = X_{LNMRIB} + X_{LIP} + X_{INDEX} + X_{INDEX \times LIP}$ did not significantly change between April 1991 and April 1992. Similarly, coefficients of the model $Y_{LNAREA} = X_{LNMRIB} + X_{INDEX}$ were not significantly different between the March 1990 and April 1991 data sets. The common models accounted for similar proportions of total variation, and the MSE of the common model was similar to the model from the contributing data sets. In both instances, however, the predictive ability of the common model was suspect. Despite little shrinkage between r^2 and R^2, the expected residual mean squares

(MSPR) were considerably larger than that of the common model (MSE). The estimation bias of MSE this represents would be reflected in an increase in the width of the confidence limits of prediction and decreased prediction accuracy.

The relationship between mid-rib length and leaf area along the stem axis changed between months. In January 1990, the interaction between $\log_e$ mid-rib and positional and developmental indices was significant, indicating that the relationship between mid-rib length and leaf area varied along the stem axis. This inconsistency, however, was largely absent in plants grown in other months.

Both INDEX and LPI indices varied in their respective contributions between models. In particular, INDEX often contributed to multicollinearity in the model (data not shown), reflecting the robust relationship between it and LNMRIB, LIP and LPI (e.g. Fig. 4.5a,b).

Final selection

In the absence of a single model that was consistent in terms and stable in time (between months), a model was selected for each month (Table 4.10). Coefficients for LNMRIB and LIP were consistent in sign and magnitude between models. The sign of the coefficient of the INDEX term varied between models, making a positive adjustment in the January 1990 model, and negative adjustments in the April 1991 and 1992 models. The negative signs in April 1991 and 1992 were due to the inclusion of LIP. The change in sign is probably a consequence of multicollinearity.

Table 4.9 Relative performance of common model structures between months.

Model	Data set		Combined model
$Y_{LNAREA} = a + X_{LNMRIB} + X_{LIP} - X_{INDEX} - X_{LIP \times INDEX}$	April 91	April 92	
intercept	3.980	4.716	4.526
LNMRIB	1.868	1.791	1.869
LIP	0.135	0.075	0.118
INDEX	-2.342	-1.709	-2.039
LIP×INDEX	-0.384	-0.441	-0.387
R^2	0.9942	0.9940	0.9939
R^2_{adj}	0.9936	0.9936	0.9936
r^2	0.9923	0.9925	0.9901
MSE_e	0.0123	0.0147	0.0142
MSPR	0.0222	0.0178	0.2211
$MSPR^z_{April\ 1991}$			0.0496
$MSPR_{April\ 1992}$			0.3142
$Y_{LNAREA} = a + X_{LNMRIB} + X_{INDEX}$	March 1990	April 1991	
intercept	3.722	3.752	3.825
LNMRIB	2.452	2.478	2.452
INDEX	1.542	1.196	1.448
R^2	0.9865	0.9839	0.9872
R^2_{adj}	0.9864	0.9832	0.9871
r^2	0.9899	0.9900	0.9842
MSE_e	0.0227	0.0344	0.0223
MSPR	0.0148	0.0323	0.0748
$MSPR_{March\ 1990}$			0.0827
$MSPR_{April\ 1991}$			0.0472

[z]Validation data set for indicated month only

Table 4.10 Coefficients and prediction intervals of selected models

	Month			
Parameter[z]	January 1990	March 1990	April 1991	April 1992
intercept	4.456	3.856	4.034	3.814
(SE)	(0.0106)	(0.0129)	(0.0362)	(0.0216)
LNMRIB	2.460	2.174	1.841	2.022
(SE)	(0.0254)	(0.2264)	(0.0937)	(0.0604)
LIP		0.077	0.124	0.075
(SE)		(0.0043)	(0.0239)	(0.0129)
INDEX	0.303		-2.352	-0.848
(SE)	(0.0576)		(0.4578)	(0.3328)
LNMRIB×LIP				-0.026
(SE)				(0.0092)
LNMRIB×INDEX	-0.497			-0.398
(SE)	(0.0726)			(0.0458)
LIP×INDEX		-0.253	-0.415	
(SE)		(0.0201)	(0.0572)	
n	379	264	83	160
MSE	0.0126	0.0159	0.0158	0.0183
$SE_{1P(10)}$[y]	0.1127	0.1265	0.1269	0.1363
$SE_{1P(20)}$	0.1127	0.1265	0.1301	0.1371
$SE_{1P(30)}$	0.1130	0.1265	0.1276	0.1369
$SE_{1P(40)}$	0.1129	na[x]	0.1286	0.1374
$SE_{1P(50)}$	na	na	na	0.1381

[z]Centered regressors

[y]mean standard error of individual predicted values of leaves with mid-rib length of (x)±0.5 cm.

[x]No leaves within the range were present in the data set.

4.4 Discussion

A conventional statistical approach, standard in its sequence and procedures (Draper and Smith, 1981; Myers, 1990; Neter et al., 1990), was used to develop the models reported here. Many of the regression diagnostics and measures of model adequacy used were developed between 1970-1980. Allen (1971a, 1971b, 1974) proposed the MSPR and PRESS statistics; Cook (1977) proposed the D statistic to identify influential points; Snee (1977) introduced data-splitting as an effective method of evaluating model performance; Hoaglin and Welsch (1978) discussed the role of the HAT matrix in identifying influential observations; and Belsley et al. (1980) are credited for statistics that assess multicollinearity (e.g. ϕ) and the DFBETAS, DFFITS, and COVRATIO statistics to identify those observations influencing regression coefficients. The techniques are now well established in statistical literature and software (e.g. Minitab, 1991; SAS Institute, 1988), yet their utility has largely been overlooked in recent literature presenting empirical models for predicting leaf area.

The position of the leaf on the stem was an important factor in predicting leaf area. Both positional factors accounted for this change, with LIP being slightly more stable in the sign of its coefficient than INDEX. Significant interactions between positional indices and LNMRIB in some months but not in others (Table 4.7) signalled a variable relationship between mid-rib length and leaf area along the stem axis. It is unclear whether this change is due to position or stage of development. The strong relationship between INDEX and LPI (Table 4.6) makes it difficult to distinguish whether INDEX was important for expressing position or for indirectly expressing the stage of leaf development.

Variation in the relationship between mid-rib length and leaf area is possibly due to non-uniform expansion either between leaflets within the compound leaf, or within leaflets. Ho and Shaw (1979) reported leaflets on the same compound leaf mature at different times. The latter report is supported by

the observation that tomato leaflets expand in at least six layers of cells (Maksymowych, 1973). Moreover, varying rates of cell division within the different layers (Coleman and Greyson, 1976b) results in different sizes and shapes of cells and air spaces in the mature leaf (Picken et al., 1986). This developmental response was not reflected in models incorporating developmental indices. On the other hand, models incorporating LPI were consistently out-performed by those incorporating positional indices.

While closely paralleled by INDEX (Table 4.6, Fig. 4.5), LIP was an important main factor in many models, apparently contributing information not provided by INDEX. Within a sample of plants, INDEX values define the relative stem position of the leaves, whereas LIP values reflect absolute position. Leaves at absolute nodal positions have predefined source-sink functions (Picken et al., 1986) which may influence the rate at which leaflets reach full expansion in a manner independent of the development stage (LPI) or relative position (INDEX) of the compound leaf.

Due to changes between months in the predictive value of positional and developmental regressors (Table 4.7), no single model accurately predicted leaf area. Thus, although LNMRIB accounted for most of the variance in each month's data set, the relative importance of regressors for leaf position and development stage differed between months. Model structures common in some months (e.g. $Y_{LNAREA} = X_{LNMRIB} + X_{INDEX}$ in March 1990 and April 1991) were not robust over time, with the magnitude of all partial regression coefficients changing between months (Table 4.8). These changes were not due to multicollinearity, as models with negligible multicollinearity also displayed this condition.

Incorporating SLA into models did not improve model performance between months, despite there being significant differences in this index between months (Table 4.9). Presumably the mid-rib length and positional and development indices were sufficiently robust to accommodate changes in leaf 'thickness' or 'density'. This also indicates that changes in the partial

coefficients of these regressors between months were not due to morphological changes associated with SLA. Although seasonal changes in average plant SLA of greenhouse grown tomatoes are of sufficient magnitude to be detected (Heuvelink, 1995), the sample SLA values made in the current study may have been too imprecise. Steer (1971), for example, reported that large changes in SLA in sunflower and maize leaves occurred with stem position and stage of lamina development. The mean SLA calculated from leaves randomly selected from any position on the stem may have provided such a coarse measure to be of no value in the model.

Photoperiod influences leaf length in some tomato cultivars (Aung and Austin, 1971). Under long days, leaves at node positions less than about 6 were shorter than similarly positioned leaves under short days; at positions greater than about 6 the response was reversed. The SD treatments of Aung and Austin (1971) were 8 h, and the LD treatments 11-13 h. Although the response of 'Moneymaker' to photoperiod is unknown, because day length exceeded 11 hours in all months in which data was collected, it is unlikely that the differences between months were due to photoperiod.

Potential collinearity between the regressors was signalled by strong correlation between LNMRIB and the developmental and positional indices (Table 4.6), and later confirmed by VIF and eigensystem condition diagnostics. Deleting ill-conditioned variables was not a satisfactory solution as the resulting models were under-specified. Use of variables uncorrelated with LNMRIB (e.g. SLA and HT) was also unsuccessful as these variables were weak regressors. Centering raw data before regression analysis reduced, and in some models eliminated, multicollinearity. The assumption required by this procedure, that prediction of zero levels of the response variable (i.e. $\log_e$ leaf area) was not important (Myers, 1990), was justified.

The best performing models from the March 1990, April 1991 and 1992 data sets exhibited moderate levels of multicollinearity (Table 4.10). Potential problems associated with multicollinearity must be assessed in context with

the intended use of the model. Models accounting for a high proportion of total variation (i.e. high R^2) and having a small residual mean square can be fitted despite multicollinearity between the fitted regressors. Prediction outside the data region using such models, however, is usually associated with unacceptably large prediction confidence intervals. Valid use of the models developed in this study outside the month in which data was collected is precluded by their instability in time, and is thus restricted to data sets collected concurrently from plants of the same cultivar under the same cultural conditions. Under these restrictions, physiological age, mid-rib lengths, and stem position would be within the data region on which the model was based, and the presence of multicollinearity would not decrease the accuracy of prediction.

Estimating compound leaf area as the sum of predicted leaflet area is statistically rigorous but impractical. Three measures of leaflet width, and one of length, were required for satisfactory performance of the model (Table 4.1). Ross (1946) estimated leaflet area from the product of their length and maximum width. My results (Table 4.4) suggest this model structure is under-specified and increasingly inaccurate as either leaflet length or width increases.

Insufficient attention is given to validating and checking the robustness of models in time. None of the models in the surveyed literature were apparently validated. While use of PRESS residuals (Allen, 1971a, 1971b, 1974) has been made easier through recent advances in computing power, there seems no reason to ignore the value of split data sets (Snee, 1977) to develop and test proposed models. Stability of models in time is closely linked with the overall concept of prediction. Prediction describes the future; it follows that predictive models should be tested in time as part of a formal validation procedure.

Indeed, the essential nature of prediction is often overlooked. Authors present statistics that reflect current, rather than future, performance of the

model. In this regard, authors routinely misrepresent R^2 values, interpreting them as measures of the predictive ability of the model. The R^2 criterion is not, however, conceptually prediction orientated (i.e. prediction performance based), but rather a measure of the capacity of the model to fit the *present* data. Moreover, a large R^2 does not imply the fitted model is a useful one. The fitted model may not be useful if most predictions require extrapolation outside the region of observations. Again, a large R^2 can often be accompanied by a MSE too large for inferences to be useful when high precision is required (Neter et al., 1990). As demonstrated in my study, the R^2 criterion can be a poor discriminator of models, particularly when all models have high R^2 values. For these reasons, R^2 is not recommended as the sole criteria for choosing the best prediction model from a set of candidate models (Myers, 1990).

Confidence intervals and standard errors of prediction are rarely presented in reports of predictive models. Many authors present estimates of MSE, but the magnitude of standard errors of prediction calculable for this statistic depends on the position of the observation in the data space, with observations close to the mean having smaller standard errors of prediction than those distant. Authors who do present MSE estimates do not provide sufficient information concerning the data space from which the model was developed to allow estimates of confidence intervals to be calculated. Indeed, recently published models have not presented estimates of MSE at all (Hoffman, 1971; NeSmith, 1991; Whitworth et al., 1992), or have overlooked their interpretation. NeSmith (1992) concluded that summer squash leaf area was best estimated by a squared function of width since only one measurement need be collected. The proposed model, however, exhibited increasing variability with increasing width, and the MSE of an alternate model using the product of leaf length and width was 50% smaller than that of the chosen model. NeSmith (1992) was apparently prepared to sacrifice considerable precision for modest gains in efficiency of measurement.

The potential utility of models that are stable in time is questioned by this study. The performance of models whose structure was common between pairs of months was poor (Table 4.8). Thus, while stability in time is a basic premise in developing a predictive model, the whole issue of stability becomes irrelevant if the stable model does not predict accurately. Model developers must distinguish between structural and performance stability. Consistency of regressors between different data sets reflects structural stability; only model validation can confirm performance stability.

Evaluating a model is not a wholly objective exercise. The researcher's opinion of the utility, simplicity, and appropriateness to objectives of the model also contributes to its final worth (Thornley and Johnson, 1990). As different researchers attach different weights to these items, it is not surprising that models in the same problem area are ranked differently by different people. Nevertheless, although the level of precision gained with one model might be sacrificed for gains in utility in another (e.g. NeSmith, 1992), complete information on the precision and predictive ability of both models is still required if an informed decision is to be made.

Developing statistical models to predict leaf area from length and width measurements requires more effort than assessment of R^2. Because poorly specified models are often masked by high R^2 values, analysis of residuals is required to detect such models. Validating the model with independent data is necessary to assess its predictive ability. In tomato, the relationship between area and leaf or leaflet length and width changes within the plant, and in time. Neither the physiological stage of development of the plant, nor SLA nor photoperiod are influential factors in these changing relationships. When non-destructive estimation of tomato leaf area is required, the prediction model must be developed while the main experiment is being conducted.

Chapter 5

Reduction of experimental error using cluster analysis

5.1 Introduction

Biometrics texts recommend blocked designs for overcoming differences within the immediate environment of an experiment (e.g. Cochran and Cox, 1957; Zar, 1984). Less attention is given to the use of blocked designs to manage initial (before-treatment) differences in the morphology of the experimental units. Such differences can inflate within-group variation, particularly if the variables being measured are subject to ontogenetic drift. This problem confounds analysis of many indices used in growth analysis, e.g. relative growth rates and shoot:root ratios (Hunt, 1978). Additionally, accommodating uniform groups of plants of different size or shape in a structured experimental design increases the range of experimental units over which conclusions can be made.

An underlying premise of experimental design is the valid imposition of restrictions to the otherwise random allocation of treatments to experimental units. These restrictions partition the total variance into comparisons of interest to the researcher, and provide local error control. Local error control uses a form of restricted randomisation commonly termed blocking to reduce experimental error. Experimental units are blocked into groups in which they are homogeneous in one or more of the variables characteristic of the units (Gill, 1978). By allocating blocks along existing clines (e.g. a gradient in soil fertility, temperature, light, or size of experimental units), these additional sources of variation are accounted for in the analysis.

Although experimental error is reduced using homogeneous experimental units, the results of the experiment are only valid for the range encompassed by those units. A wider range of experimental units makes conclusions valid over a broader range (Neter et al., 1990; Pearce, 1979), but increased variation among experimental units reduces the precision of the experiment.

Blocking resolves this dilemma, accounting for the increased variation without a concomitant reduction in precision.

Blocking reduces among-treatment error, leaving the residual mainly as within-treatment variation. As variation within blocks is reduced through blocking, block differences represent variation across clines. For example, in the unblocked completely randomised design (CRD), the residual is the sum of among-treatment and within-treatment error. By contrast, in the blocked randomised complete block (RCB) design, the block effect estimates among-treatment error and the residual estimates within-treatment error. Thus, residual variance in the RCB model is partitioned into variation across contours (among treatments), and variation along clines (within treatments), with the result that the residual error of a RCB design is less than that of a CRD design. Insignificant block effects in an RCB analysis suggest the presence of a homogeneous environment or experimental units, or use of an inappropriate blocking practice. In the latter, blocks lie along the cline (across contours), and the block effect is therefore composed of both among- and within-treatment components. Under such circumstances the residual error of the incorrect RCB will approach that of the CRD.

Control of experimental error in plant growth studies

It is often difficult in plant growth studies to obtain a uniform population of plants (experimental units). Morphological characteristics of plants of the same chronological age may vary due to inherent variability. In such cases similar plants are blocked into groups by visual size (Lieth et al., 1991; Proebsting et al., 1989), height (Biasi et al., 1989; Early and Martin, 1988; Erwin and Heins, 1990), trunk circumference (Erf and Proctor, 1989; Maust and Williamson, 1994), leaf and lateral shoot number (Erwin and Heins, 1990; Erwin et al., 1991), or fruit ripeness (An and Paull, 1990), shape or size (Cabrera and Saltveit, 1990) before randomly allocating treatments to the plants within each block.

Common to these examples of blocking strategy is a single source of variation. Simple classification becomes more difficult, however, when the sources of potential variation among units increase. Although additional sources of potential variation can be fitted to the model as covariates (Mead, 1990; Neter et al., 1990), this approach requires covariates to be linearly related to the response variable. This restriction may not always be met by these sources of potential variation.

The variable used as the blocking criterion is often chosen more for its ease of assessment than direct value to the study. Such blocking strategies implicitly assume a correlation between this variable and other variables more relevant to the study's objectives. For example, blocking visually by size or plant height assumes a strong correlation between these variables and such variables as total leaf area, leaf number, or stage of development. For example, Biasi et al. (1989) blocked peach seedlings by height, but assessed treatment effects through leaf area, leaf number, and fresh and dry weights. These assumptions are rarely acknowledged or tested, raising doubt about the degree of local error control actually achieved by the blocking strategy.

Cluster analysis

Cluster analysis is a multivariate statistical technique. Its basic aim is to find natural groupings within a set of individuals. Individuals are allocated to mutually exclusive groups; individuals within a group are similar to each other while individuals in different groups are dissimilar. Clear groups that emerge are named and their properties summarized, enabling efficient organising and retrieval of the information available. In other words, simplification with minimal loss of information is sought.

Cluster analysis procedures fall into three groups: hierarchical, partitioning, and clumping methods (Pimentel, 1979). All groups impose a certain structure on the data. A hierarchical approach summarises how groups in a

partition are related at different levels. Hierarchical techniques are either agglomerative, which proceed by a series of successive fusions of n observations into groups, or divisive, which successively partition the set of n observations into finer partitions. Both types of hierarchical technique find the most efficient juncture at each stage of the progressive subdivision or synthesis of the population. Results of both subdivisions are presented in a dendrogram, a two-dimensional diagram illustrating the fusions or partitions made at each successive level.

Partitioning methods partition the set of n observations into a specified number of disjoint groups, g, so that each observation belongs to one and only one group. The value of g is specified by the researcher and analyses are usually repeated using different values. Groups produced by partitioning methods have, by definition, no members in common with one another. On occasion this is unnecessarily restrictive; in clumping methods the groups are allowed to overlap.

An assumption made in these methods is that all the relevant relationships within the set of n observations can be summarized by a matrix containing 'proximity' or distance values between each pair of observations. Thus each pair of observations will have an associated numerical value, p_{ij}, which depicts the extent to which the ith and jth observations resemble each other. Similarity and dissimilarity are terms also used in this context. Thus, in performing cluster analysis, the similarity (or dissimilarity) of every pair of individuals is measured, with the raw data matrix $\mathbf{X}$ (n observations $\times$ p attributes) replaced by a matrix of proximity coefficients.

This step is important and substantial information is lost if done incorrectly. The information within the set of variables describing the observations is subsumed into the set of proximity coefficients. It is important that these coefficients accurately reflect the relevant features of the data. Thus, atten-

tion must be given to scaling the data, so that the (dis)similarity matrix represents the relationships within the set of observations. This is particularly important when variables are measured in non-commensurable units, or are strongly correlated with each other. Both conditions are characteristics common to biological data.

Cluster analysis and local error control

Pimentel (1979) defined cluster analysis as allocating individuals to mutually exclusive and exhaustive groups, such that individuals within a group are similar to each other while individuals in different groups are dissimilar. Clearly, for local error control, these groups could be regarded as blocks. Moreover, the multivariate nature of cluster analysis would allow several potential sources of variation to be considered concurrently.

To my knowledge, cluster analysis has not been used to improve the local error control afforded by blocked experimental designs. This chapter presents results from experiments comparing cluster analysis with visual grading in allocating tomato plants to blocks based on total leaf area and plant height. In focusing on these variables, I assumed that (i) the area of leaves available for photosynthesis was an important contributor to growth, and (ii) total leaf area was closely related to the stage of plant development.

5.2 Materials and Methods

5.2.1 Plant culture

In both experiments, seedlings of tomato 'Moneymaker' were transplanted into 200 cm^3 plastic pots at first true leaf stage (10 days after sowing). Six weeks later, shoots were excised below the cotyledonary node and rooted under mist in an aerated water bath (Chapter 4). When adventitious root

length was approximately 2 cm, plants were individually numbered and transferred to a deep flow hydroponic system (Chapter 2).

5.2.2 Experiment 1

Plants were randomly divided into two groups of 50. Total leaf area of each plant was determined as the sum of the individual leaf areas, the latter estimated from linear, positional and developmental characteristics (Chapter 4) according to the formula:

$$\begin{aligned} LNAREA &= 1.841(LNMRIB) + 0.124(LIP) - 2.352(INDEX) \\ &\quad - 0.415(LIP \cdot INDEX) \end{aligned} \qquad (5.1)$$

where LNAREA = $\log_e$ leaf area (cm^2), LNMRIB = $\log_e$ leaf mid-rib (cm), LIP = leaf insertion position, and INDEX = relative leaf position on stem.

Two independent observers were each asked to arrange, from the pool of 50 plants, six sub-groups each containing five similarly 'sized' plants based on a visual assessment of 'leafiness' and height. Plants in the second group were allocated to six groups containing five plants by cluster analysis of their height and estimated leaf area, individually and together. Whenever a clustered group contained more than five plants, the first five plants listed in that group were chosen.

5.2.3 Experiment 2

Fifty-one plants were prepared in the manner described. Height, leaf mid-rib lengths and leaf position on the stem were recorded and total leaf area of each plant calculated from equation (5.1). Two independent observers each visually graded the plants, on the basis of leafiness and height, into sub-

groups containing 2, 4, 6, 8, and then 10 plants each. Plants were recombined after all the sub-groups of a given size (i.e. *n*) had been formed. As in experiment 1, whenever a clustered group contained more than *n* plants, the first *n* listed plants were chosen. Variation in leaf area and height of these sub-groups was compared to those classified by cluster analysis.

Group size was based on a review of the number of experimental units per block of blocked designs reported in the Journal of the American Society for Horticultural Science. During the period 1989-1991 (vol. 114-116 inclusive) over 60% of reported experiments using blocked designs had fewer than ten experimental units per block (Table 5.1).

Table 5.1. Number of experimental units per block of blocked designs reported in the Journal of the American Society for Horticultural Science 1989-1991 (vol. 114-116) inclusive.

Number of experimental units per block	2	3	4	5	6	7	8	9	10	11+
percentage of total (n=101)	13	6	18	9	8	0	6	1	2	37

5.2.4 Data collection and analysis

Cluster analysis

Excessive claims cannot be made about the objectivity of cluster analysis. Sensitivity to data structures differs among clustering methods, and subjective decisions must be made before constructing a dissimilarity matrix. Thus, results of a single clustering method cannot be accepted uncritically. It is usual practice to compare groups 'uncovered' by one method with those 'uncovered' by different methods before making final decisions (Orlóci, 1978). In this study, a hierarchical clustering approach was adopted using Ward's

method to corroborate the group compositions formed by the group-average method (Milligan, 1980).

The agglomerative group-average method (also known as the average linkage or unweighted pair group methods) uses the average distance from individuals in one cluster to individuals in another cluster as the clustering criterion. This approach tends to combine clusters with small variances. Ward's method (also known as the sum of squares method) is based on the proposition that the loss of information from grouping observations into clusters can be measured by the total sum of squared deviations of every point from the mean of the cluster to which it belongs. At each step in the analysis, union of every possible pair of clusters is considered and the two clusters whose fusion results in the smallest increase in the error sum of squares are combined.

As the scale of measurement influences the dissimilarity measure, height (cm) and leaf area (cm^2) data were standardised to equally weight both variables in the analysis. The SAS procedures CLUSTER and TREE hierarchically clustered the individual plants and listed the members of the major clusters of the data set.

Assessment criteria and analysis

Uniformity within each block was assessed by the within-block coefficient of variation (CV):

$$CV = \frac{SD_i}{\bar{Y}_i} \cdot 100\% \qquad (5.2)$$

where i is the ith block, SD_i is the standard deviation, and $\bar{Y}_i$ is the mean response for block i.

As $\bar{Y}$ and SD have identical units, CV is unitless. Thus, CVs may be compared even if they are calculated from measurements obtained in different units.

The coefficient of variation is conventionally used as an index of the reliability of the experiment, indicating the degree of precision with which the treatments were compared (Gomez and Gomez, 1984). Without published benchmarks against which to assess within-block CV, 15% was adopted as the upper desirable limit. This was based on my experience of whole experiment CV in plant research, and that of Gomez and Gomez (1984) and Colwell (1994). Gomez and Gomez (1984) reported acceptable ranges of CV for yields of rice of 6-8% for variety trials, 10-12% for fertiliser trials, and 13-15% for insecticide and herbicide trials. Colwell (1994) considered 10-15% an average range of CV for fertiliser experiments.

In Experiment 1, differences between the within-block CV of each clustered or visually graded group were analysed by t-tests. In the second experiment, ANOVA sums of squares were partitioned into *a priori* orthogonal contrasts. Orthogonal orthogonal contrasts are calculable when n_i, the number of individuals within each treatment group, is different (Hicks, 1982). Despite this, I adopted a simpler approach by assuming that I had the best available estimates of the respective treatment population means and consequently calculated the contrasts as if n_i were equal in each group.

5.3 Results

There was consistent agreement in both experiments between the group-average method and Ward's method in the composition of clustered groups. Of the two methods, only results arising from the group-average method are presented.

5.3.1 Blocking by leafiness (Experiment 1).

Cluster analysis of estimates of total plant leaf area was superior to visual grading in reducing within-block CV. The mean CV of clustered blocks was about four times smaller than visually graded blocks (Table 5.2). Mean leaf area, averaged over all blocks, was similar between visually graded blocks and those established by cluster analysis. Cluster analysis increased the range of leaf area between the blocks, with the mean leaf area of visually graded blocks ranging from about 200 to 500 cm^2, and from 150 to 600 cm^2 for clustered blocks.

Table 5.2. Mean plant leaf area and coefficient of variation of blocks determined by cluster analysis and visual differentiation.

Blocking method	Block number	Total leaf area (cm^2)	
		mean	CV[z]
Cluster analysis	1	602.6	2.9
	2	364.1	1.5
	3	332.2	4.1
	4	274.9	4.9
	5	207.1	6.8
	6	150.1	10.8
	mean	321.8	5.2
Visual	1	484.8	26.3
	2	465.4	23.3
	3	396.7	19.4
	4	355.7	13.0
	5	255.1	30.2
	6	218.0	10.3
	mean	362.6	20.4
t-tests (cluster analysis vs visual)		NS	••

NS, *, **, ***Nonsignificant or significant t test at $P<0.05$, 0.01, or 0.001 respectively.
[z] Coefficient of variation

5.3.2 Blocking by leafiness and height (Experiment 2)

Preliminary analysis of data showed that the block discrimination of the independent observers was similar (data not shown), and therefore their data were pooled. Blocks established after cluster analysis of either height or leaf area, or both, were less variable in those variables than visually graded blocks (Table 5.3, Fig. 5.1). The CV of variables not involved in the clustering procedure was, on the other hand, higher than those of the visually graded blocks.

Table 5.3. Mean coefficient of variation of leaf area (CV_{LA}) and height (CV_{HT}) within determined blocks.

		Coefficient of variation (CV)	
Source	Level	Leaf area CV_{LA}	Plant height CV_{HT}
Blocking technique (T)	visual	16.4	7.5
	$cluster_{LA+HT}$	8.9	3.6
	$cluster_{LA}$	3.6	12.6
	$cluster_{HT}$	18.8	2.1
		***	***
Block size (S)	2	10.7	5.5
	4	14.2	6.9
	6	14.9	7.2
	8	16.8	8.4
	10	17.2	9.3
		***	**
Interactions			
T x S		NS	NS
Contrasts			
Clustering vs. visual discrimination		***	***
Uni- vs. bivariate clustering		**	NS
S_{linear}		***	***
$S_{quadratic}$		NS	NS

NS, *, **, *** Nonsignificant or significant F test at $P<0.05$, 0.01, or 0.001 respectively

The mean CV of both variables increased linearly as the number of plants (experimental units) per block increased, irrespective of blocking technique (Table 5.3, Fig. 5.1). Relative performance of the clustering techniques depended on which variables were clustered. Within-block variation of height was similar irrespective of whether the blocks were clustered by height alone or in combination with leaf area (Table 5.3). On the other hand, within-block variation of leaf area was higher when the blocks were clustered by both leaf area and height than by leaf area alone.

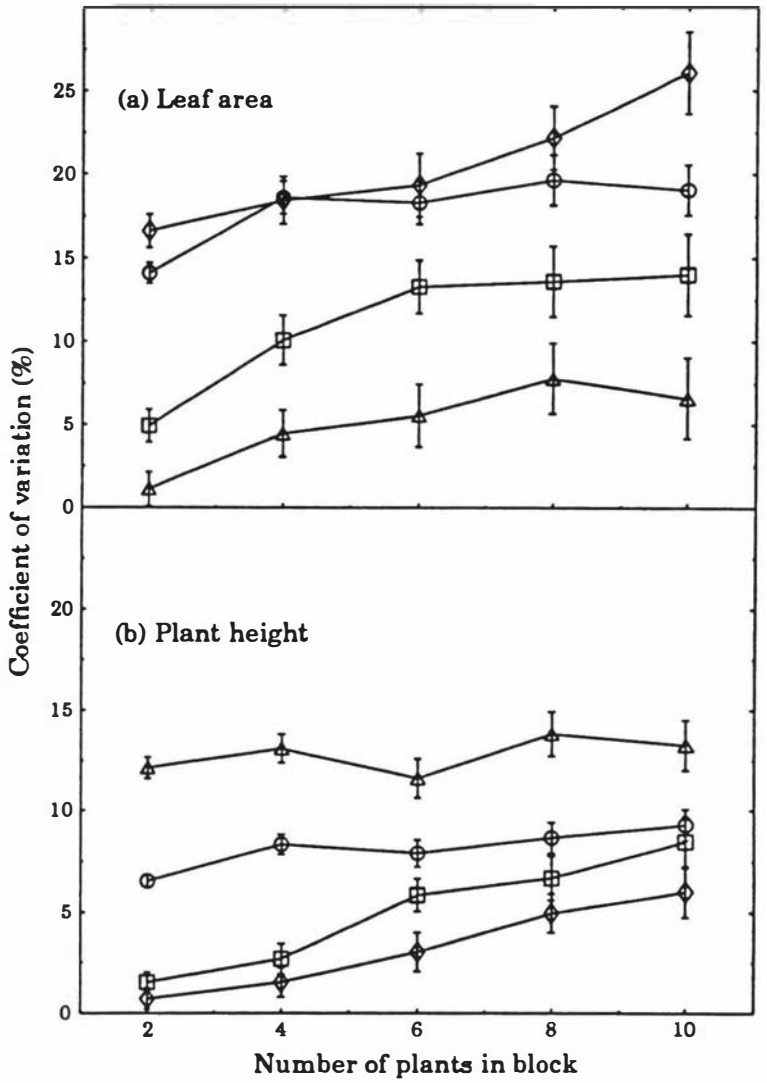

Fig. 5.1. Effect of blocking technique on within-block CV of (a) leaf area and (b) plant height in blocks containing different numbers of plants. *Key*: visual (○), leaf area-specific cluster analysis (△), stem height-specific cluster analysis (◇), and bivariate cluster analysis (□). Data presented as means ± SE; n ranges from 3 to 46 (see Table 5.4).

The assumption that plants visually graded by height into blocks will also have uniform within-block leaf area was not supported by the data. The CV for height in all visually graded blocks was significantly smaller than leaf area, irrespective of the number of plants in the block (Table 5.3, Fig 5.1). While this may simply reflect a greater ease of visually discriminating differences in height compared to leaf area, variation about the curvilinear relationship between height and total leaf area (Fig. 5.2) may also have contributed to the poor relationship between leaf area and height CVs. For example, the leaf area of plants between 13 and 15 cm ranged between about 60 to 170 cm^2 (Fig. 5.2). Indeed, when the precision of blocking-by-height was further increased using cluster analysis on height alone, the uniformity of leaf area between plants within each block decreased further (Table 5.3, Figs. 5.1, 5.3).

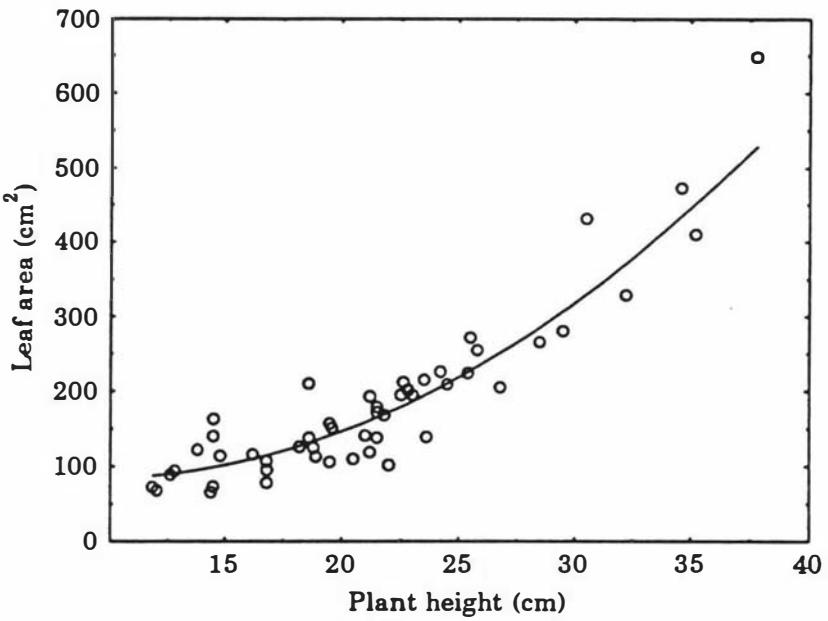

Fig. 5.2 Relationship between plant height and total leaf area for cutting-grown tomato plants. Second degree polynomial regression (SE coefficient in brackets) is: Leaf area = 199.34(57.43) - 17.29(5.08) × height + 0.72(0.11) × height², R^2=0.89, P<0.0001.

The slow increase in leaf area relative to height in those plants below 25 cm had a large effect on the height CVs of groups blocked through cluster analysis of leaf area. For example, plants with a leaf area of about 100 cm^2 ranged in height from 13 to 22 cm (Fig. 5.2). Thus, while the cluster analysis yielded blocks with low leaf area CVs, the CVs of height were relatively large (Table 5.3, Fig. 5.3).

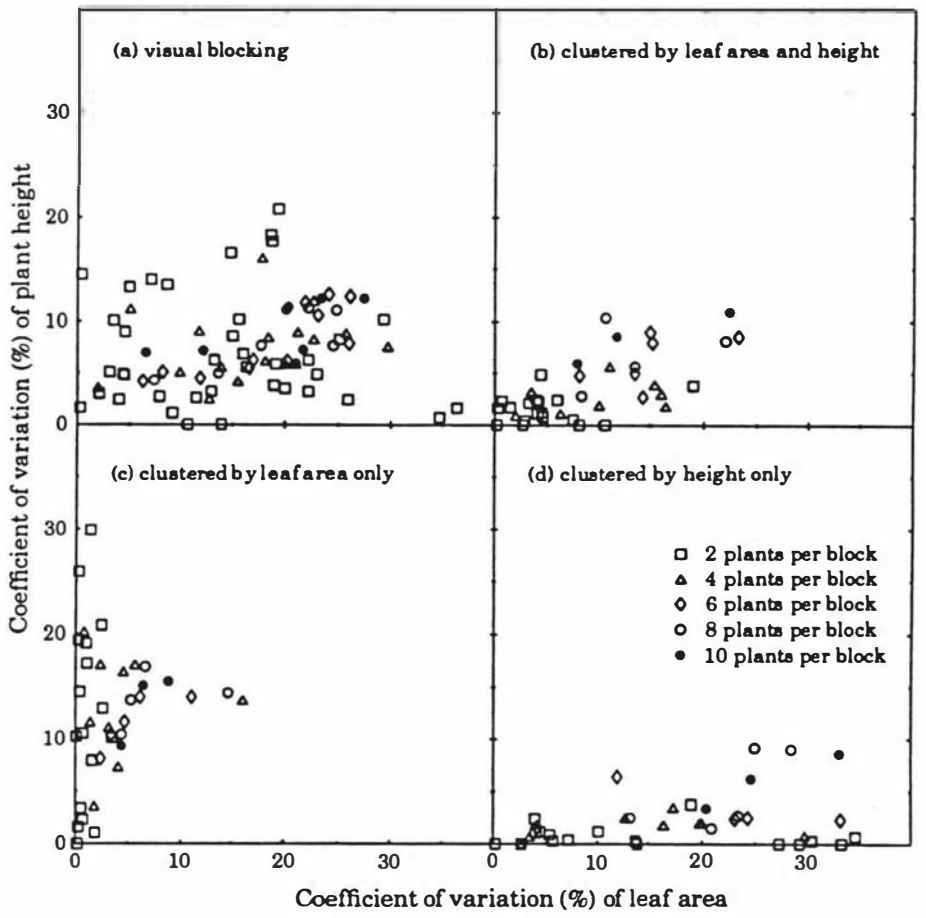

Fig. 5.3 Relationship between coefficients of variation of leaf area and height within blocks discriminated visually or through cluster analysis.

The correlation between height and leaf area CV across all visually graded blocks was 0.23, and while significantly greater than zero (P<0.03) was,

nevertheless, very low. Clustering by height alone also yielded blocks in which total leaf area was highly variable (Table 5.3, Figs. 5.1, 5.3).

Reduced block CV through cluster analysis of measured variables did not occur at the expense of fewer blocks. When two or ten plants per block were required, cluster analysis tended to be more conservative; with six plants per block bivariate cluster analysis discriminated one block more than visual discrimination (Table 5.4).

Table 5.4. Number of blocks of given size (*n*) 'extracted' from the pool of plants by visual or clustering methods.

Number of plants within block (*n*)	Number of blocks				
	visual #1[z]	visual #2[z]	cluster$_b$[y]	cluster$_{LA}$	cluster$_{SH}$
2	18	18	18	17	18
4	9	10	8	9	9
6	6	6	7	5	5
8	4	4	4	4	5
10	4	4	3	3	3

[z] data of two independent observers
[y] cluster$_b$: bivariate cluster analysis; cluster$_{LA}$: cluster analysis using leaf area only; cluster$_{SH}$: cluster analysis using stem length only

5.4 Discussion

Allocating plants to blocks by cluster analysis of measured characteristics consistently resulted in smaller within-block CV than blocks formed by visual grading. Over both experiments, the mean CV of clustered blocks was between two and five times less than visually discriminated blocks. Blocks clustered on a single variable had smaller CVs than identical sized blocks formed by bivariate clustering.

Although cluster analysis was more effective than visual grading in reducing block CV, regardless of the number of plants required per block (Table 5.3), the relative effectiveness of the technique decreased as the number of plants required per block increased (Fig. 5.1). Clustering specifically for leaf area was significantly more effective than visual grading for all block sizes evaluated. Specific or bivariate clustering for leaf area was more effective in reducing block heterogeneity than visual grading when the block consisted of four or fewer plants. In the wider scope of reported experimental unit sizes (Table 5.1), a cumulative 36% of reported units had four or fewer individuals.

The improvement (i.e. reduction) in CV achieved by group through cluster analysis of the variables of interest did not reduce the number of blocks that could be 'extracted' from the pool of plants (Table 5.4). This indicates that the reduced CV was due to better blocking of the plants available, rather than an increased number of plants deemed as being unsuitable. Given the similar number of blocks between the methods, it appears that grading visually or via cluster analysis were similarly effective in rejecting unsuitable plants.

Within-block CV was a good discriminatory measure of the different blocking strategies. By standardising within-block variation against the mean size of the variable, allowance is made for the tendency in biological studies for variation to increase with plant size. The CVs reported, however, should not be taken as absolute values suitable for extrapolation to other studies. Although the 15% benchmark proved appropriate for leaf area, it was probably too conservative for plant height. Simpson et al. (1960) concluded that CVs less than 4% in zoomorphological studies indicated that the sample size was insufficient to show variability. Applying this benchmark to the present study gives ambiguous results. The CV of blocks of up to six plants clustered by height was less than 4%, yet the CV of blocks of identical size grouped visually exceeded the benchmark. Determining appropriate benchmark CVs for blocking strategies requires further study.

Mean CVs of plant height and leaf area in visually graded blocks suggest that visual grading is influenced more by plant height than leafiness (Table 5.3). Taken together, height and leafiness reflect plant size. Therefore, blocking by plant size may yield blocks of plants more similar in height than leafiness. The effectiveness of the local error control realised under such conditions would depend on the variables being measured. For example, Proebsting et al. (1989) blocked peach seedlings by size, but measured evapotranspiration and leaf water relations. These variables are physiologically related more to leaf area than height; local error control may have been improved by blocking on some estimate of leaf area.

There was no support for the often implied assertion that blocks of plants of similar height or 'size' will have similar leaf area. Despite a relatively strong relationship between the height and leaf area of the tomato plants used in this study (Fig. 5.2), both visual grading and cluster analysis by height yielded blocks of plants with leaf area CV over the 15% lower limit (Table 5.3, Fig. 5.1). This suggests that blocking tomato plants visually by height to obtain homogeneous leaf area within blocks will not be successful. As leaf area is considerably more important than height or size to subsequent expressions of growth (Brown, 1984), the use of the latter characteristics (e.g. Biasi et al., 1989; Early and Martin, 1988; Erwin and Heins, 1990; Lieth et al., 1991; Proebsting et al., 1989), as suitable criteria for blocking requires review.

Unfortunately, leaf area is difficult to assess non-destructively. In this study, leaf area was estimated using a known mathematical relationship between leaf mid-rib length and leaf area. As such relationships are usually not immediately available for the crop under investigation, researchers may resist the added burden of developing such relationships before block membership can be determined. Development of simpler methods to non-destructively estimate leaf area, for example video imagery (Lindsey and Bassuk, 1992),

may provide more accurate and more easily obtainable data to effectively block on leaf area. Alternatively, non-destructive estimates of canopy volume (Ludwig et al., 1975) or leaf area index (e.g. Devitt et al., 1994) may be more consistent indicators of leaf area than height and size, and should be investigated as possible blocking criteria.

The differences in within-block CV among the methods reflect, to some extent, the difference between qualitative (visual blocking) and quantitative (cluster analysis of measured characteristics) assessment. Following this reasoning, it is possible that had the two observers had access to the plant height and leaf area data, then by ranking the plants by either characteristic, they would have discriminated blocks with lower CVs than they achieved visually. Further processing of the data through cluster analysis must add value to the blocking procedure if it is to become an accepted procedure. Cluster analysis achieves this requirement in two ways. First, it retains objectivity. Ranking data carries the problem of deciding which individual will be the first member of the group—in other words, where should the group boundaries be drawn on the list. This objective decision must be made with each group formed, and as a consequence, different people may draw the line at different places. Cluster analysis avoids this problem by producing a unique classification from a given sample of individuals (Orlóci, 1978). Second, cluster analysis facilitates easy blocking of individuals when more than one characteristic contributes to among-treatment error. Although grouping individuals is possible from a single ranked measurement, it becomes very difficult when the individuals are to be grouped based on the rankings of two or more characteristics (Manly, 1986).

A design that assesses treatment effects across a range of some specific attribute (e.g. height, age, or leaf area) offers considerably more scope for general interpretation or application of the results. For example, if a uniform grade of short plants is used within the experimental units, the results of the

experiment are strictly only applicable to short plants. On the other hand, assigning plants of different height to particular blocks to reduce within-block variation broadens the population range to which the conclusions of the experiment are applicable. This widens the inferential base, and helps prevent misleading generalisation of results. These results show that, in addition to reducing within-block CV, blocking plants by cluster analysis of measured leaf area effectively broadens the range of leaf area over which the treatments are evaluated.

Chapter 6

Leaf expansion under partial root restriction

6.1 Introduction

The often balanced reduction in root and shoot biomass or relative growth rates that accompanies root restriction is interpreted by several authors as evidence of a coordinated and dynamically controlled relationship between roots and shoots (Krizek et al., 1985; LaRoche, 1980; Menzel et al., 1994; Reiger and Marra, 1994; Richards and Rowe, 1977a; Robbins and Pharr, 1988). Several mechanisms have been advanced to explain the nature of this relationship. Hameed et al. (1987) concluded that reduced growth of tomato plants in small containers grown in a hydroponic solution resulted from drought stress induced by increased hydraulic resistance in the restricted root system. Tschaplinski and Blake (1985), working with alder (*Alnus glutinosa* Gaertn.), and Proebsting et al. (1989) with peach, also concluded that the mechanism involved changes in the plant water balance. Proebsting et al. (1989) found evidence that these changes were mediated through changes in stomatal conductance, rather than leaf water potential or osmotic potential. However, water stress is not always responsible for reduced growth in small containers. Carmi and Heuer (1981) reported higher leaf water potential in restricted compared to unrestricted bean seedlings. Krizek et al. (1985) concluded that growth impairment caused by water stress and restricted root volumes involves different physiological mechanisms. Water stress reduced leaf water potentials and stomatal conductance, induced preferential partitioning to the root, inhibited leaf initiation and decreased the rate of photosynthesis. Such responses were absent in restricted plants which showed a balanced overall decrease in growth throughout the plant (i.e. the SR was similar to unrestricted plants). On finding no inferential evidence of water stress in the reduced expansion rates of cotton leaves, Mutsaers (1983) postulated that limiting mineral nutrition was the principal cause. Reiger and Marra (1994) reached a similar conclusion with young peach trees under severe root restriction. In contrast, neither Carmi and Heuer (1981), Carmi and Shalhevet (1983), Menzel et al. (1994), Peterson et al. (1984) nor Robbins

and Pharr (1988) found any relationship between measured nutrient levels and reduced shoot and root growth of root-restricted plants of bean, cotton, passionfruit (*Passiflora* sp.), wheat or cucumber. On finding that exogenous applications of cytokinins (Richards and Rowe, 1977a) and gibberellins (Carmi and Heuer, 1981) partially restored shoot growth in restricted plants, a possible role for root-synthesised hormones in controlling shoot growth, possibly via photosynthesis (Carmi and Koller, 1978; Carmi et al., 1983; Herold and McNeil, 1979), has been postulated.

The involvement of cytokinins in this control system has received popular support. Apparently initiated by the work of Richards and Rowe (1977a) and Carmi and Heuer (1981) with the synthetic cytokinin benzyladenine, this support has yet to be validated by any demonstration of a correlative relationship among restricted roots, endogenous levels of any cytokinin[1] and shoot growth. There is no doubt that cytokinins are synthesised in roots (Goodwin, 1978; Koda and Okazawa, 1978; Letham, 1978; Vaadia and Itai, 1968), probably at the root apex (Feldman, 1975). The concentration of cytokinins in xylem sap responds to changes in environment, plant condition and ontogeny. Export is reduced in plants under water or salinity stress (Bano et al., 1993; Itai et al., 1968; Torrey, 1976; Vaadia and Itai, 1968) while increased concentrations have been measured following disbudding, floral initiation and removal of flower buds (Beever and Woolhouse, 1973; Colbert and Beever, 1981; Menary and van Staden, 1976) and decapitation of the shoot (Bangerth, 1994). Considerable qualitative and quantitative variation in cytokinin fluxes in xylem sap through time have been linked to their postulated regulation of reproductive growth (Heindl et al., 1982). Exogenous application of cytokinins to shoot tissue has increased chlorophyll production and reduced its degradation, stimulated chloroplast development, mobilised nutrients to the site of application, regulated leaf expansion, and promoted

[1]Carnes et al. (1975) identified eight different endogenous cytokinins in root exudate of tomato.

the production and increased the activity of ribulose bisphosphate carboxylase, an essential photosynthetic enzyme (Beltrano et al., 1994; Brock and Cleland, 1990; Feierabend, 1969; Kaminek, 1992; Leopold and Kawase, 1964; Lerbs et al., 1984; Ulvskov et al., 1992). Missing, however, is any *in situ* evidence linking root restriction to shoot growth through the mediation of cytokinins.

Positive, negative, accumulative, and debit chemical signals have been implicated linking the function of root and shoot under conditions in the rhizosphere which limit growth (Cannell and Jackson, 1982; Jones, 1990; Jackson, 1993). A positive signal may be produced by the root when a stress induces or increases supply of some physiologically active substance (PAS) to the shoot; reduced supply of a PAS normally transmitted to the shoot results in a negative signal. Accumulative signals may occur when a substance normally transmitted to the root accumulates in the shoot, while debit signals are formed when the root system becomes a more active sink for such a substance.

Negative signals have not received widespread support in the literature. For example, Jones (1990) considered that a negative signal generated by a drying soil did not contain sufficient information, since the magnitude of the signal would vary with root volume, whether or not some roots were stressed. Jackson (1990) reached a similar conclusion after observing that only a few unstressed roots were sufficient to maintain growth when the larger part of the root system was waterlogged. He concluded that a system consisting of a negative signal could not provide sufficient sensitivity to maintain shoot growth and avoid leaf senescence. Although Kramer (1988) and Boyer (1989) cautioned against a perceived abandonment of the concept of water potential and consequent transmission of hydraulic signals in response to falling shoot water status, it is generally accepted that the root is the primary sensor of changes in the water status of rhizosphere (Passioura, 1988a; Schulze et al.,

1988; Sharp and Davies, 1989). Accordingly, the root is directly implicated in the altered propagation of a biochemical signal to the shoot system. Accordingly, neither accumulative nor debit signals have been regarded as likely candidates for the operational signal and have largely been ignored.

In contrast, Davies and Zhang's (1991) review of root signals and water stress revealed that the hypothesis that positive signals convey chemical information from roots to stomata in response to drying soil is widely accepted. Gowing et al. (1990a) provided the earliest evidence that leaf growth was reduced by an inhibitor synthesised in the root, when they showed apple leaf growth recovered to control levels after the water-stressed half of the root system was excised. A characteristic of a positive signal is that its 'signalling', and the consequences of that signalling, should increase as the level of stress it is reporting increases (e.g. Fig. 4, Cornish and Zeevaart, 1985), up to some point after which damage caused by the stress perturbs propagation or transport of the signal. It is now clear that ABA is the positive signal produced by plant roots in response to drying soil. As soil dries, ABA is synthesised in roots and transported to leaves where it causes stomata to close (Cornish and Zeevaart, 1985; Hartung and Radin, 1989; Neales et al., 1989; Schurr and Gollan, 1990; Zhang and Davies, 1987, 1989, 1990a, 1990b, 1991).

The nature of the communication system linking root and shoot growth has not been characterised in previous studies of root restriction. These studies were pre-occupied with restricting the whole root system, an approach which has impeded understanding of the relationship between roots and shoots. Root systems are rarely completely restricted. With the exception of bonsai, plants grown in containers are usually repotted into larger containers, and in most cases (e.g. bedding plants, shrubs and trees) finally transplanted into the soil. More commonly, when growth is slowed or impeded in one part of the root, it is enhanced elsewhere to compensate (Drew and Saker, 1975;

Klepper, 1990). In addition, it is difficult to apply and maintain root restriction at levels that can be quantified. Although root restriction is commonly quantified by the volume available for root growth, the actual degree of restriction encountered will range from slight to extreme as roots progressively occupy the available space.

The objectives of this study were to use split root system experiments to investigate further the influence of root restriction on leaf area expansion in tomatoes and the nature of the signal involved.

6.2 Materials and Methods

6.2.1 Cultural

Seed of the indeterminate tomato variety *L. esculentum* Mill. 'Moneymaker' were germinated under mist in a heated glasshouse. Seedlings were transplanted to 5.0 × 5.0 × 8.0 cm tubes (200 cm^3) when the cotyledons were fully expanded, and grown on in the glasshouse for six weeks. Plants were excised immediately above the cotyledon node and rooted in an aerated water bath under mist (Chapter 4.2.1). After five days, rooted cuttings were weaned from the mist system by gradually reducing the mist frequency to zero over the next four days.

Rooted cuttings were placed in individual 10 l containers in the deep flow hydroponic system previously described (Chapter 3). The electrical conductivity of the solution was increased daily in 0.5 $mS \cdot cm^{-1}$ increments from 0.5 to the final operating level of 2.5 $mS \cdot cm^{-1}$.

Lateral shoots and flower trusses were removed as soon as possible to restrict the assimilate sink to the primary shoot system and roots.

6.2.2 Environmental

Air temperature in the glasshouse was maintained at 15°C minimum at night, with ventilation triggered when temperature rose above 25°C. Daily mean temperature ranged from 18–23°C. Light levels ranged between 500 to 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation (PAR) at solar noon under the frequently cloudy conditions encountered during the experiment.

6.2.3 Experimental

Experimental design

A randomised complete block design with 4 blocks and 2-3 single plant sub-samples per block was used in the study. The block design provided local error protection for known temperature and light profiles within the glasshouse. Cluster analysis of total estimated leaf area at the start of the experiment, following the methods outlined in Chapter 5.0, provided an objective basis for allocating plants of similar leaf area to a given block. The average total leaf area of cuttings when treatments were initiated was 234 ± 14 cm^2.

Treatments

The influence on growth characteristics of root restriction to none, one, or two sub-systems of the root system was evaluated (Table 6.1). Root systems were divided into two sub-systems of visually similar size by gently teasing the roots apart in the hydroponic solution, and a 2 cm longitudinal cut made up the stem from the cutting base. Each sub-system to be restricted was placed in a 10.5 × 4 cm (30 cm^3) polyethylene bag 'cell' drawn up the split stem to enclose the sub-system. The top of the cell was left open to allow solution

escape. Two air lines and two nutrient supply lines were provided for each container. Each restricted root sub-system had an air line and a nutrient supply line inserted into the cell. Both lines discharged at the base of the cell and overflowed through the open top.

Two unrestricted control treatments were established: a split stem and an intact (non-split) stem. The latter was included as a reference against possible growth responses induced by the stem splitting. New roots that developed from the stem above this point were permitted to develop freely. It was not possible to direct them into the bags and because ethylene is released by wounded roots (Saltveit and Locy, 1982), removing the roots may have introduced an unnecessary source of variation into the experiment.

Table 6.1 Treatment description and codes

Treatment code	Treatment description
DCR	Split stem with both root sub-systems placed in individual restriction cells (Double Cell Restriction)
SCR	Split stem with a single root sub-system placed in a restriction cell (Single Cell Restriction)
SOC	Split stem with no root restriction (Split Only Control)
NSC	Intact stem with no root restriction (Non-Split Control)

6.2.4 Data collection

Estimation of leaf area

Leaf expansion was closely monitored on a total plant and individual leaf basis. It was anticipated that any possible treatment effects would not

influence all leaves equally, and thus, treatment responses would be a function of the stage of development of each leaf. Consequently, individual leaf area was estimated non-destructively from the leaf's linear, positional and developmental characteristics (Chapter 3) according to the formula:

$$\begin{aligned} LNAREA = {} & 3.814 + 2.022 \cdot LNMRIB + 0.075 \cdot LFIP - 0.848 \cdot INDEX \\ & - 0.026 \cdot (LNMRIB \cdot LFIP) - 0.398 \cdot (LNMRIB \cdot INDEX) \end{aligned} \quad (6.1)$$

where LNAREA = $\log_e$ leaf area (cm^2), LNMRIB = $\log_e$ leaf mid-rib (cm), LFIP = leaf insertion position, INDEX = relative leaf position on stem

Seven days after treatments were initiated (DAI; 14 days after transfer to the hydroponic system), leaves of similar size were identified by cluster analysis (Chapter 5.0) of estimates of leaf area. It was assumed that treatment effects would not have been present at this stage of the experiment. Furthermore, at this early stage leaves of similar area would also be at a similar developmental stage. Analysis of total plant leaf area at day 7 did not detect treatment differences (Fig. 6.6), supporting this assumption. Total estimated leaf area was calculated as the sum of individual leaf areas.

Leaf area ontogeny

Concise quantification of the ontogeny of leaf growth is aided by its determinant nature. Under conditions where the measured attribute reaches and maintains a maximum level, a class of asymptotic functions provides a biologically meaningful insight to the nature of the observed growth pattern. For example, these functions estimate maximum values for the y axis parameter, points of inflexion, and mean relative and absolute growth rates.

Causton and Venus (1981) and Hunt (1982) both provide excellent reviews of the applications of the major asymptotic functions used in biological studies. Individual leaf growth has been described using the monomolecular, the logistic and the Gompertz functions (e.g. Amer and Williams, 1957; Constable

and Rawson, 1980; Hackett and Rawson, 1974). The Richards function (equation 6.2) has received by far the most support (see Hunt, 1982) due primarily to its flexibility in estimating the point of inflexion. Both the logistic and Gompertz functions have fixed points of inflexion whereas the Richards function does not place this restriction on the model.

The Richards function is defined by the differential equation:

$$\frac{dL}{dt} = \frac{\kappa L}{\nu A^{\nu}}(A^{\nu} - L^{\nu}) \qquad (6.2)$$

where L is the leaf size at time *t*, A is a constant expressing the estimated maximum leaf area, κ is a growth rate constant, and ν controls the presence of the point of inflexion.

This integrates to:

$$L = A(1 \pm e^{(\beta - \kappa t)^{\nu-1}}) \qquad (6.3)$$

where β positions the curve on the time axis

or in its logarithmic form, where the only parameter affected by the transformation is A which becomes $\log_e A$:

$$\log_e L = \log_e A - (\frac{1}{\nu})\log_e(1 \pm e^{(\beta - \kappa t)}) \qquad (6.4)$$

More recently, France and Thornley (1984) and Thornley and Johnson (1990) recommended the Chanter function in preference to the Richards function. The Chanter function is a hybrid of the logistic and Gompertz functions providing a flexible point of inflexion. It is defined by the differential equation:

$$\frac{dL}{dt} = \mu L \left(1 - \frac{L}{B}\right) e^{-Dt} \qquad (6.5)$$

which integrates to:

$$L = \frac{L_0 B}{L_0 + (B - L_0)\, e^{\{-[\mu(1 - e^{-Dt})D^{-1}]\}}} \qquad (6.6)$$

where L is leaf area, μ is the initial relative growth rate, L_0 is the leaf area at t=0, B is substrate level, and the product Dt is interpreted as differentiation, development, or senescence.

This function is intuitively attractive, predicting first that the relative growth rate depends linearly on substrate level ($1 - LB^{-1}$), as in the logistic function, and secondly, e^{-Dt} (where D is a constant), on the passage of time. The latter may be interpreted as differentiation, development or senescence, and is parallelled in the Gompertz function. There is, however, no reported use of the Chanter function, making it difficult to assess its claimed superiority over the widely supported Richards function. Consequently, both the Chanter and the Richards function were chosen to describe the ontogeny of individual leaf area expansion.

The SAS procedure NLIN (SAS Institute, 1989) was used to fit the data to the logarithmic form of the Richards function (equation 6.4) and the standard form of the Chanter equation (equation 6.6).

Leaf sampling

In choosing leaves for curve fitting and evaluation, it was assumed that leaves of a similar area would also be at a similar developmental stage. Identifying leaves of similar size or developmental age by stem position

proved impossible. This was possibly due to, or at least accentuated by, some leaf abscission from cuttings while under propagation. Analysis confirmed the lack of correlation between leaf area and stem position with large coefficient of variation (CV) of estimated area of leaves at day 7 at acropetal stem positions 10-15 (Table 6.2).

This problem was overcome by identifying leaves of similar size at the start of the experimental period. Cluster analysis using the group average method (through the SAS procedure CLUSTER) grouped leaves into three major size categories and 16 smaller categories. The major size categories coincided with leaves having just commenced (L_3) or undergoing (L_7, L_{14}) expansion growth, where the subscripts denote the number of days after initiation of the treatments (DAI). Smaller groups were rejected as they did not have a full complement of treatments in any single group. The CV of the three major groups was substantially smaller than groups based on stem position (Table 6.2). Using Lewontin's (1966) procedure for testing the difference between CVs, the CV of pooled clustered groups was significantly ($P<0.001$) smaller than the CV of the pooled stem position groups.

Each group contained leaves from different stem positions (Table 6.3). There was, however, clear bias to older leaves (lower stem position) as leaf area of the cluster increased.

Table 6.2 Individual leaf area and within-group variation by stem position and clustered group

	Leaf position on stem[z]							Clustered groups				
	9	10	11	12	13	14	15	L_3	L_7	L_{14}	L_{21}	L_{28}
mean leaf area (cm^2)	147.0	85.4	41.1	18.3	6.9	2.7	1.2	58.7	24.2	3.1	3.3	1.2
Coefficient of variation	56.6	66.6	81.6	117.2	129.7	125.2	63.3	13.6	36.7	53.2	25.6	47.3

[z] acropetal from cotyledon node

Table 6.3 Percentage (%) of leaves in clustered groups from different stem positions.

	Leaf position on stem[z]									
Clustered group	9	10	11	12	13	14	15	16	17	18
L_3	22	41	37	0	0	0	0	0	0	0
L_7	0	21	39	40	0	0	0	0	.0	0
L_{14}	0	0	0	35	29	36	0	0	0	0
L_{21}	0	0	0	0	0	30	37	27	6	0
L_{28}	0	0	0	0	0	0	0	8	50	42

[z] acropetal from cotyledon node

Relative rate of leaf area expansion

A functional approach (Hunt, 1982) to calculating relative leaf expansion rates (R_A) was adopted. Components and derivatives from the Chanter and Richards functions estimated R_A at the start of leaf growth, point of inflexion, and overall average. More focused information of the comparative ontogeny of R_A among treatments was gained by calculating the R_A at each harvest. For leaves developing at, or within 14 DAI, the first derivative of the Richards function estimated R_A at seven day intervals from the start of expansion:

$$\text{RLER} = \frac{1}{L}\cdot\frac{dL}{dt} = \frac{\kappa e^{\beta - \kappa t}}{\nu(1 \pm e^{\beta - \kappa t})} \qquad (6.7)$$

Leaves developing later than 14 DAI had not developed sufficiently by the completion of the experiment for a Richards function to be fitted to the data. A second order polynomial of the form:

$$\log_e L = a + bt + ct^2 \qquad (6.8)$$

where L is leaf area (cm^2) at t days (DAI)

was fitted to the individual leaf data with coefficients of determination (R^2) consistently exceeding 0.98 (data not shown). The R_A at given t was calculated by the first derivative of the polynomial:

$$R_A = b + 2t \qquad (6.9)$$

A characteristic of this function is that differences between t_x and t_{x-1} are equal.

Duration of leaf expansion

The duration of leaf expansion from 5 to 95% of maximum estimated area (A) was calculated for each leaf based on the Richards function coefficients fitted for that leaf. For a given leaf area L_i, rearranging the Richards function provides an estimate of the time t at which that area was reached as:

$$t = [\log_e((e^{\beta(A-\log_e L_i)} - 1) - \kappa)] - \nu^{-1} \quad (6.10)$$

Rate of leaf appearance

A mid-rib length of 3 cm, the minimum length practically and safely measurable, was used as the reference length for leaf appearance rates. The number of days required for each leaf to reach the reference length was either measured directly (during the weekly measurements), or estimated by solving the exponential function between mid-rib length and time that existed during the first 3 weeks of development (data not presented). The rate of leaf appearance was estimated from the slope of the curve relating leaf number to time.

Actual leaf area measurement

At the completion of the experiment, leaf area was measured with a Li-Cor LI-3000 leaf area meter (Lambda Instruments Corporation, USA). Leaflets were removed from the petioles prior to measurement.

Water potential measurements

Leaf water potential was measured at solar noon using a pressure chamber (model 3005, Soilmoisture Equipment Corporation, USA). A leaflet fully

exposed to sunlight and next to the terminal leaflet was measured. Leaflet size was about 60 cm^2. The leaflet was enclosed in a plastic bag containing moistened filter paper, cut immediately below the petiole-stem junction, and transferred to the pressure chamber, in a laboratory attached to the green-house. Time from excision to measurement did not exceed 10 seconds. A humid environment was maintained in the pressure chamber by a lining of moistened capillary matting. Extrusion errors were avoided by ensuring the petiole length protruding from the chamber did not exceed 5 mm (Turner, 1981). The chamber was pressurised at about 0.2-0.25 $bar \cdot s^{-1}$ (Lakso, 1992) until the end point was approached after which the rate was reduced to 0.1-0.125 $bar \cdot s^{-1}$.

Photosynthesis measurements

Photosynthetic functions of the leaves was measured with a Li-Cor LI-6200 portable photosynthesis system (Lambda Instruments Corporation, USA). Primary parameters used in the operation of the unit were 1020 mbar atmospheric pressure, 1149 cm^3 system volume, 0.1775 $s \cdot cm^{-1}$ boundary layer resistance, 11.40 cm^2 leaf area, and maximum desiccant flow rate of 1392-1460 $\mu mol \cdot s^{-1}$. A stomatal ratio of 0.5 was used, reflecting the ratio of stomata on the adaxial:abaxial surface of the tomato leaf (Rudich and Luchinsky, 1986). Measurements routinely commenced one hour prior to solar noon and were completed two hours later.

Biomass measurements

Plants were harvested by block over two days. Harvested material was dried for 72 hours in metal tins in a forced air oven operating at 80°C. Dried material was sequentially removed from the oven to standardise the equilibration period to room conditions to about 30 minutes.

6.2.5 Data analysis

Analysis of variance

The analysis of variance (ANOVA) model for the growth curve coefficient analysis followed a split-plot design with a factorial arrangement of four levels of root restriction and three levels of leaf size. The root restriction treatments were the whole plots and leaf sizes the split (sub) plots:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \rho_k + (\alpha\beta)_{ij} + (\alpha\rho)_{ik} + \varepsilon_{ijk} \qquad (6.11)$$

where μ = overall mean, α_i = root restriction effects, β_j = block effects, ρ_k = leaf size effects, $\alpha\beta_{ij}$ = whole plot error term for root restriction treatments, $\alpha\rho_{ik}$ = interaction term for root restriction and leaf size, and ε_{ijk} = residual error term.

The model for the final, destructive harvest data and derived parameters followed the model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} \qquad (6.12)$$

where μ = overall mean, α_i = root restriction effects, β_j = block effects, and ε_{ijk} = residual error term.

Photosynthesis measurements were made under intermittent cloudy conditions. Changes in photosynthetically active radiation (PAR) during and between measurement periods were accounted for by using PAR as a concomitant variable in analysis of covariance (Saunders, 1991). This approach assumed that the photosynthesis rate x PAR relationship was linear in the range encountered. The covariate adjustment reduced the estimate of error variance estimate and standardised photosynthesis measurements with respect to the different light levels encountered. Reported mean values are adjusted for the PAR covariate.

Two orthogonal sets of contrasts were constructed *a priori* and tested within each ANOVA. Although contrasts are conventionally limited to a single orthogonal set within any analysis, the questions asked of the data did not fit this constraint. In the ANOVA analyses, attention focused on the influence of restriction on the variables being examined. The primary set of contrasts tested some restriction (SCR and DCR) against no restriction (SOC and NSC), SCR versus DCR, and SOC versus NSC. Interpreting a significant contrast between restricted and unrestricted plants became irrelevant if the contrast between the unrestricted controls (SOC vs NSC) was also significant. Consequently, a second set of orthogonal contrasts that focused on the split stems was required. Linear and quadratic contrasts for leaf types were calculated by the algorithm of Khanizadeh and Fanous (1992), using the average leaf area of the respective groups at 14 DAI.

In all ANOVA analyses, the generalised linear models procedure (GLM) of SAS (SAS Institute, 1989) was used. A consequence of using cluster analysis to distinguish groups of leaves at similar developmental stage is that there is no guarantee that each treatment will be represented in each block. Leaves of plants of each treatment were represented in each group in similar, but not identical numbers. In some instances, plants of a particular treatment within a particular block were not represented. Therefore, the presented means are least square means (LSMEANS), an option of PROC GLM that accounts for missing data.

Standard errors of differences between means of variables expressed as ratios (e.g. SLA, LWR, and SR ratio) were adjusted for the variability of the divisor following the method of Oyejola and Mead (1989).

Principal component analysis

Principal components reduced the dimensionality of the original data set of vegetative growth components into a set of new variables (components) retaining most of the variation present in the original variables. In achieving this, a perspicuous description of the vegetative growth components was expected. The SAS procedure PRINCOMP (SAS Institute, 1989) calculated principal components of the standardised (mean=0, variance=1) vegetative data. Following Jolliffe (1972), all principal components with a variance (eigenvalue) less than 0.7 were rejected. Use of ANOVA of the important principal components provided discrimination of the relative influence of root restriction on the new composite variables.

Repeated measures analyses

Analysis of measures taken from the same experimental units over time usually requires some form of repeated measures analysis, because a major assumption of any variance analysis or curve-fitting technique is that sample observations at each harvest, t_i, are independent of one another (Mead, 1990). Comparison of the weekly increment of individual leaf area, through either estimation from the growth curve of the leaf, or directly from mid-rib measurements, would lead to problems of dependence. This was avoided by one of two approaches, depending on the data source. Parameter estimates and derivatives of the Richards and Chanter function of each monitored leaf were analysed by conventional ANOVA (Mead, 1990). The second approach followed the contrast method of Mead (1990). Analysis of R_A and variables derived from these data were performed for each separate time variable and for derived variables representing averages or changes over time.

6.3 Results

6.3.1 Root growth

Restricted root systems appeared healthy. Some roots were translucent, but as they were present in all parts of the root systems (i.e. restricted and unrestricted), probably reflect normal turnover of root tissue. Although densely packed, the moist interior of the root mass and the white roots of roots in the cells indicated that nutrients and oxygen were available. New root growth on all split plants, first noticed about 21 DAI, developed from the stem immediately above the split. By the final harvest, slightly less than half of the root dry biomass of the DCR plants was from restricted portions of the root system (Table 6.4). The dry biomass content of restricted root sub-systems was similar irrespective of the number of restriction cells present. Double cell restriction reduced final root growth, while final root growth of SCR plants was similar to unrestricted plants.

Table 6.4 Dry weight of restricted and unrestricted portions of the root systems at final harvest (49 DAI).

Treatment	Root dry weight[a] (g ± SE)		
	restricted		unrestricted
	cell #1	cell #2	
NSC			7.52 ± 0.589
SOC			8.93 ± 0.507
SCR	1.57 ± 0.143		7.31 ± 0.733
DCR	1.48 ± 0.127	1.46 ± 0.152	3.22 ± 0.262

[a] Each value is the mean of 7-9 observations

It is important to note that in all restriction treatments, an unrestricted root sub-system eventually became established. In the DCR treatment, this sub-system consisted of adventitious roots which started to develop 21 DAI and eventually represented just over half of the total root system (1.48 + 1.46

versus 3.22, Table 6.4). Thus, responses of leaves can be classified into those present before unrestricted adventitious roots developed (i.e. prior to day 21) and those leaves that developed later.

6.3.2 Leaf growth

Validation of leaf area model

Regression analysis of estimated total leaf area against actual total leaf area at the final harvest showed reasonable agreement (Fig. 6.1). T-tests determined that the y intercept was not significantly different from 0 and the x coefficient not significantly different from 1 (SE 1136.7 and 0.1089 respectively, df(error)=35). The mean percentage difference between actual and estimated total leaf area for each plant was 2.6% (data not shown). This equates to about 270 cm^2 over an average final total plant leaf area of 10385 cm^2 (Table 6.18).

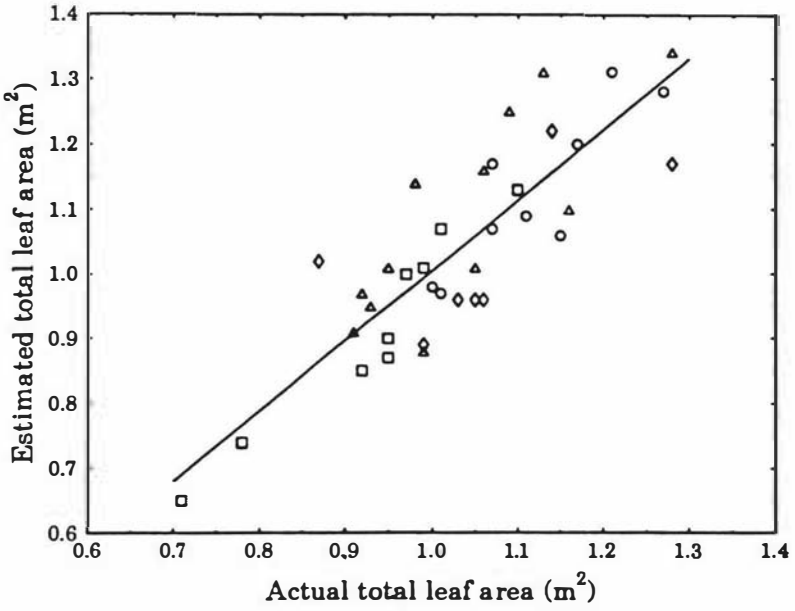

Fig. 6.1. Relationship between estimated total leaf area and actual area measured at final harvest for individual plants. The relationship is described by Y=0.074 + 1.078X, r^2=0.74. *Key:* □:DCR, △:SCR, ○:SOC, ◇:NSC.

6.3.3 Leaf expansion

6.3.3.1 Individual leaf ontogeny

Monitored leaf groups

The ontogeny of five groups of leaves was monitored during the experiment (Table 6.2). Leaves developing at, and subsequent to, 35 DAI were not monitored as there were insufficient harvests to fit a meaningful mathematical function for estimating R_A.

The leaf groups provided a range of developmental stages within which the relative effects of root restriction could be assessed. By the conclusion of the experiment, leaves in groups L_3 and L_7 had reached the final plateau phase of their sigmoidal growth curve (Figs. 6.2a,b - 6.3a,b). Based on these growth curves and percentage final expansion (Table 6.5), leaves that commenced expansion 14 DAI (L_{14}) approached maximum size, while leaves in L_{21} and L_{28} groups were approaching, or had just passed, the stage of maximum leaf expansion rate respectively.

Table 6.5 Characteristics of monitored groups of individual leaves.

Group	Commencement of expansion (DAI[w])	Initial area (cm^2 ± SE)	Area of control[y] plants at 49 DAI	% of estimated full expansion[z] at 49 DAI
L_3	3.5	4.2 ± 0.32[x]	878.7 ± 40.6	
L_7	7	1.1 ± 0.17	1023.1 ± 30.1	100
L_{14}	14	2.5 ± 0.29	841.1 ± 12.3	82
L_{21}	21	2.1 ± 0.19	597.6 ± 36.6	58
L_{28}	28	1.2 ± 0.09	327.2 ± 19.6	32

[w] days after treatment application
[x] at 7 DAI
[y] Split only
[z] Relative to maximum leaf area of L_7 controls

Leaves developing before day 21

Significant differences were detected between the two unrestricted controls in the rate of leaf area increase to A (i.e. κ), estimated maximum leaf area (Table 6.7), the mean R_A and the time taken to reach the point of inflexion (Table 6.8). These data indicated that the intact stem control (NSC) was not a valid control against which effects of restriction could be assessed. Accordingly, further comparison between restricted and unrestricted plants excludes this treatment. Similarly, the non-split control was not included in analyses of leaf groups at higher stem insertions (i.e. L_{21}, L_{28}).

Analysis of the Chanter estimates of maximum leaf area (A_{max}) of L_3, L_7 and L_{14} leaves indicated that root restriction reduced maximum leaf area (Table 6.6). A similar, albeit non-significant, pattern was observed in the Richards estimates of maximum leaf area (A, Table 6.7). Expressing the extent of reduction as a percentage of the estimated maximum leaf area of control (SOC) plants, L_7 leaves on restricted (DCR or SCR) plants were reduced by an average 15%, with L_3 and L_{14} leaves by about 8%. Reduced leaf area in restricted plants was detected about 20 DAI as L_3 leaves reached the point of inflexion, although reduction was more pronounced 5 days later as L_7 leaves reached the same stage of growth (Table 6.9, Fig. 6.4). The major differences in leaf expansion between restricted and unrestricted treatments apparently occurred after the point of inflexion (Figs. 6.2, 6.3), with a comparatively rapid fall in expansion rate occurring in restricted plants. As the duration of expansion was similar between all treatments (Table 6.8), the reduction of leaf area in restricted plants was apparently due to an overall lower mean absolute rate of leaf expansion (Table 6.9).

Leaf development within groups and stages of growth was similar, irrespective of treatment, confirming the assumption made in the preparatory grouping by cluster analysis. Initial relative growth rate of leaves (μ) of all treatments and sizes was similar (Table 6.6). The initial leaf area (L_0) at

which measurement was initiated, was also similar within any one category, although a statistically significant difference between plants in double cell restriction and those in single cell restriction (Table 6.6) was detected. This difference (1.3 cm^2) is, however, within the error bounds of the leaf area model. Analysis of variance of area at $t = 0$ within each clustered group did not detect differences among treatments, suggesting that the L_0 differences were by chance (i.e. Type II error). Indeed, analysis of the Chanter coefficients μ, D, B, and A_{max} with L_0 as a covariate confirmed that differences in L_0 among treatments was inconsequential to these components of leaf growth (i.e. the covariate term (L_0) was nonsignificant).

Table 6.6. Analysis of parameter estimates of the Chanter function for leaf expansion over time. Values presented are least square means (±SE).

Treatment			Chanter function coefficient and parameter estimates				
source	level	n	μ	D[x]	L_0	B	A_{max}
root restriction (R)	NSC	9	0.326 (0.0329)	0.014	3.71 (0.817)	924.7 (39.25)	922.1 (38.97)
	SOC	10	0.319 (0.0289)	0.017	3.43 (0.817)	931.4 (33.99)	929.9 (33.75)
	SCR	11	0.359 (0.0252)	0.019	3.25 (0.624)	870.4 (29.98)	867.5 (29.77)
	DCR	12	0.314 (0.0233)	0.011	4.97 (0.578)	808.2 (27.76)	807.82 (27.56)
			NS		NS	#	#
leaf size (LS)	L_{14}	15	0.312 (0.0174)	0.008	3.09 (0.380)	867.1 (17.02)	867.4 (16.46)
	L_7	12	0.348 (0.0222)	0.021	2.76 (0.485)	948.7 (21.47)	945.08 (20.77)
	L_3	15	0.329 (0.0188)	0.017	5.67 (0.414)	835.3 (17.33)	833.08 (16.76)
			NS		***	**	**
interaction	R × LS		NS		NS	NS	NS
contrasts							
root restriction							
[NSC+SOC] vs [SCR+DCR]			NS		NS	*	*
DCR vs SCR			NS		#	NS	NS
NSC vs SOC			NS		NS	NS	NS
[SCR+DCR] vs SOC			NS		NS	*	*
DCR vs SCR			NS		#	NS	NS
Stem split vs no split			NS		NS	NS	NS
leaf size							
linear			NS		****	#	*
quadratic			NS		*	**	**

[NS,#,*,**,***,****] Non significant or significant F test at P<0.1, 0.05, 0.01, 0.001 or 0.0001 respectively

[x]Means only given (see text for explanation)

Table 6.7. Analysis of parameter estimates of Richards function for leaf expansion over time. Values presented are least square means (±SE).

Treatment			Richards function coefficient estimate		
source	level	n	A	κ	ν
root restriction (R)	NSC	9	1092.9 (48.95)	0.126 (0.0084)	0.196 (0.0463)
	SOC	10	988.1 (42.39)	0.149 (0.0072)	0.271 (0.0401)
	SCR	11	941.6 (37.39)	0.144 (0.0064)	0.226 (0.0354)
	DCR	12	869.5 (34.6)	0.160 (0.0059)	0.307 (0.0328)
			*	*	NS
leaf size (LS)	L_{14}	15	1007.9 (24.33)	0.139 (0.0059)	0.278 (0.0346)
	L_7	12	1050.5 (30.71)	0.132 (0.0074)	0.200 (0.0436)
	L_3	15	860.7 (24.78)	0.162 (0.0059)	0.272 (0.0352)
			****	**	NS
interaction	R × LS		NS	NS	NS
contrasts					
root restriction					
[NSC+SOC] vs [SCR+DCR]			**	#	NS
DCR vs SCR			NS	NS	NS
NSC vs SOC			NS	#	NS
[SCR+DCR] vs SOC			NS	NS	NS
DCR vs SCR			NS	NS	NS
Stem split vs no split			**	*	NS
leaf size					
linear			****	**	NS
quadratic			**	#	NS

NS,#,*,**,***,**** Non significant or significant F test at P<0.01, 0.05, 0.01, 0.001 or 0.0001 respectively

Table 6.8. Analysis of derivatives of Richards function coefficients for leaf expansion over time. Values presented are least square means (±SE).

Treatment			Richards function coefficient derivatives			
source	level	n	point of inflexion (days)[x]	% final leaf area at poi[y]	leaf area at point of inflexion	duration of expansion (days)[z]
root restriction (R)	NSC	9	20.9 (0.45)	40.0 (0.73)	434.5 (19.79)	29.4 (1.77)
	SOC	10	18.9 (0.39)	41.2 (0.63)	407.8 (17.14)	26.2 (1.45)
	SCR	11	18.6 (0.34)	40.5 (0.55)	385.5 (15.12)	25.9 (1.3)
	DCR	12	18.0 (0.32)	41.8 (0.51)	361.4 (13.99)	26.2 (1.02)
			**	NS	#	NS
leaf size (LS)	L_{14}	15	20.6 (0.38)	41.3 (0.51)	419.5 (10.06)	26.8 (1.25)
	L_7	12	20.9 (0.48)	40.0 (0.65)	418.4 (12.69)	31.6 (1.90)
	L_3	15	15.7 (0.39)	41.3 (0.52)	353.9 (10.24)	22.2 (1.42)
			****	NS	***	**
interaction	R × LS		NS	NS	NS	NS
contrasts						
root restriction						
[NSC+SOC] vs [SCR+DCR]			***	NS	**	NS
DCR vs SCR			NS	#	NS	NS
NSC vs SOC			**	NS	NS	NS
[SCR+DCR] vs SOC			NS	NS	NS	NS
DCR vs SCR			NS	NS	NS	NS
Split stem vs no split			***	NS	*	NS
leaf size						
linear			****	NS	****	**
quadratic			***	NS	NS	**

[x] standardised to 0 DAI
[y] point of inflexion
[z] duration from 5 to 95% A
NS,#,*,**,***,**** Non significant or significant F test at P<0.10, 0.05, 0.01, 0.001 or 0.0001 respectively

Table 6.9. Analysis of parameter estimates of Richards function for relative and absolute rates of leaf expansion over time. Values presented are least square means (±SE).

Treatment			Richards function rate coefficient derivatives		
source	level	n	R_A ($cm^2 \cdot cm^{-2} \cdot day^{-1}$) at point of inflexion	mean R_A ($cm^2 \cdot cm^{-2} \cdot day^{-1}$)	mean absolute rate of expansion ($cm^2 \cdot day^{-1}$)
root restriction (R)	NSC	9	0.353 (0.0421)	0.103 (0.0026)	59.9 (3.49)
	SOC	10	0.292 (0.0343)	0.117 (0.0022)	64.4 (3.03)
	SCR	11	0.317 (0.0315)	0.117 (0.0019)	60.9 (2.67)
	DCR	12	0.323 (0.0243)	0.119 (0.0018)	57.9 (2.47)
			NS	**	NS
leaf size (LS)	L_{14}	15	0.288 (0.0207)	0.109 (0.0018)	61.4 (1.73)
	L_7	12	0.365 (0.0314)	0.109 (0.0022)	60.9 (2.19)
	L_3	15	0.311 (0.0234)	0.125 (0.0018)	60.0 (1.77)
			NS	****	NS
interaction	R × LS		NS	NS	NS
contrasts					
root restriction					
[NSC+SOC] vs [SCR+DCR]			NS	**	NS
DCR vs SCR			NS	NS	NS
NSC vs SOC			NS	**	NS
[SCR+DCR] vs SOC			NS	NS	NS
DCR vs SCR			NS	NS	NS
Split stem vs no split			NS	***	NS
leaf size					
linear			NS	****	NS
quadratic			NS	*	NS

NS,#,*,**,***,**** Non significant or significant F test at $P<0.10$, 0.05, 0.01, 0.001 or 0.0001 respectively

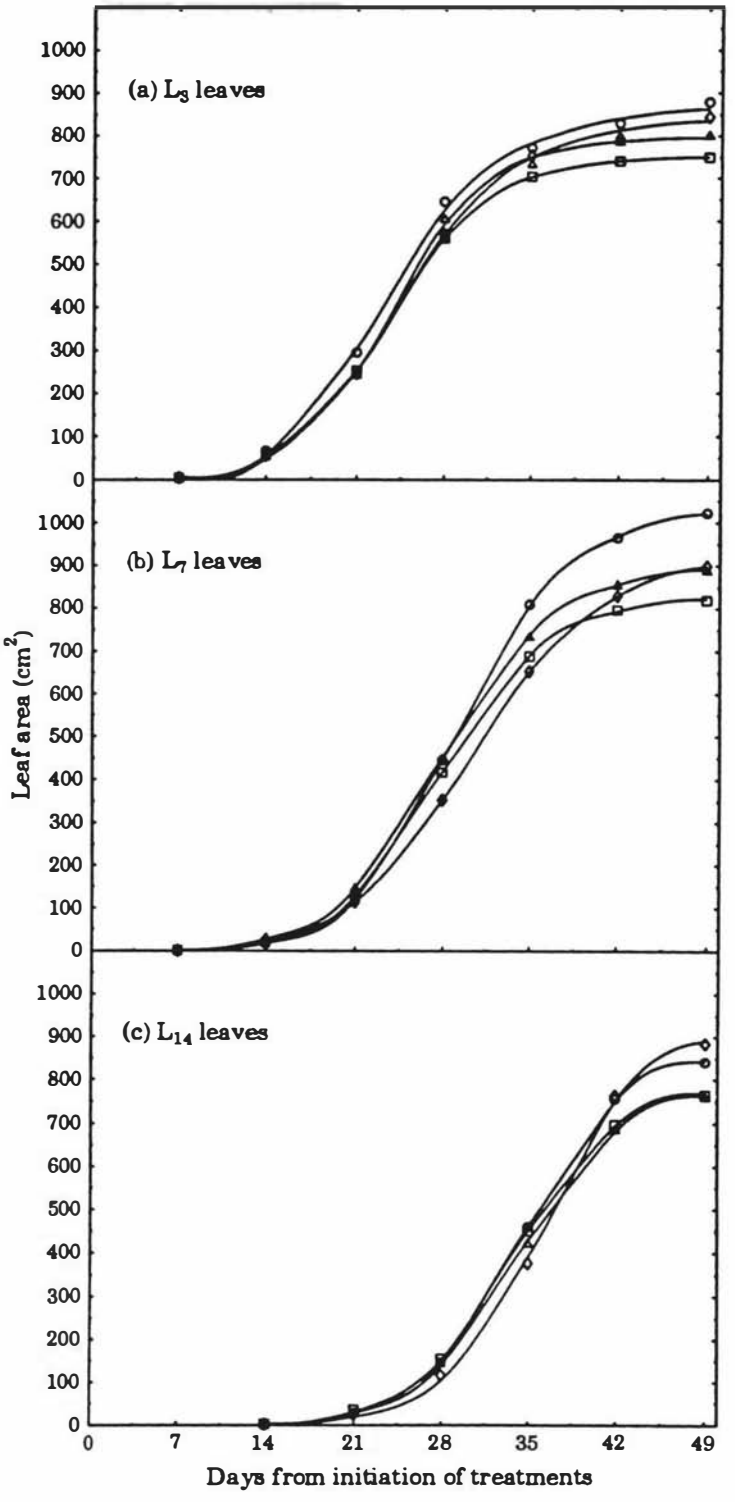

Fig. 6.2. Chanters functions describing growth of leaves commencing expansion about 3, 7, and 14 days after initiation of treatment. Each value is the mean of 9-12 plants. *Key:* □:DCR, △:SCR, ○:SOC, ◇:NSC.

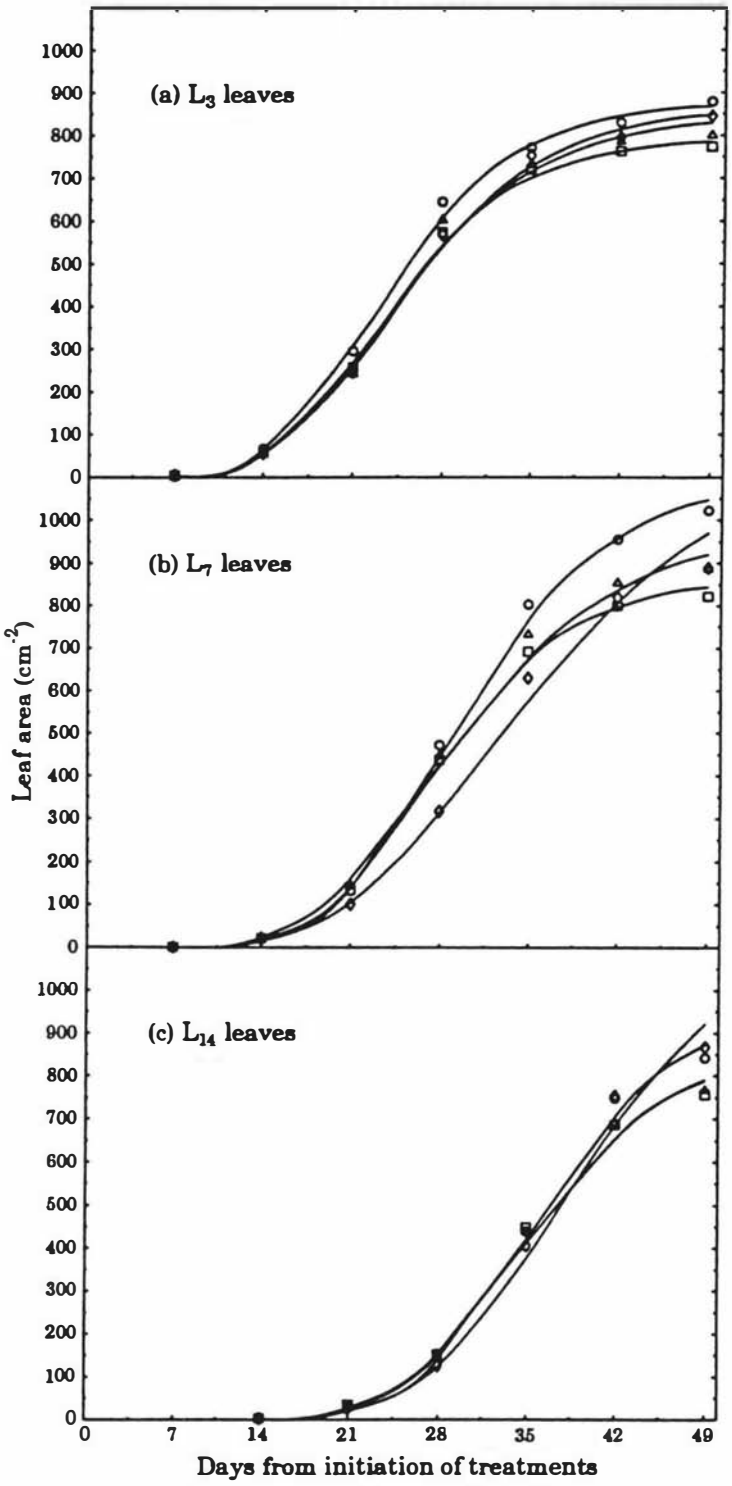

Fig. 6.3. Richards functions describing growth of leaves commencing expansion about 3, 7, and 14 days after initiation of treatment. Each value is the mean of 9-12 plants. *Key:* □:DCR, △:SCR, ○:SOC, ◇:NSC.

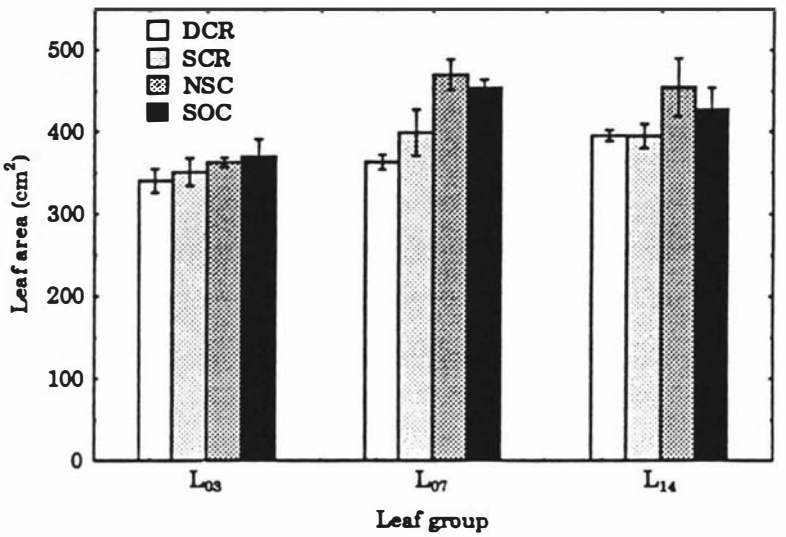

Fig. 6.4. Leaf area at the point of inflexion of L_3, L_7, and L_{14} leaves. Bars are ± SE

Inserting roots into the restriction cells

Early leaf ontogeny was not influenced by the root restriction treatments. Initial relative growth rates and days to the point of inflexion (Tables 6.6, 6.8, 6.9) were similar among all treatments in those leaves that began expanding soon after initiation of treatment (i.e. L_3, L_7 and L_{14}). These data, coupled with the absence of effect on photosynthesis and leaf water potential (see later results), indicated that either no physiologically significant damage to the roots occurred when inserted into the restriction cells, or that they had fully recovered from damage by the time these lower leaves commenced expansion. Ethylene is released by wounded roots (Saltveit and Locy, 1982) but epinastic leaf curvature, a characteristic response of tomato to ethylene (Jackson and Campbell, 1976), was not observed. Subsequent growth responses can therefore be assumed to be due to root restriction, rather than artefacts of treatment application.

Estimates of the D parameter of the Chanter function were extremely variable. This precluded meaningful statistical analysis and only means are presented (Table 6.6). By definition, D must lie in the range $0 \leq D \leq \mu[\log_e(A_{max} \cdot L_0^{-1})]$. As D approaches 0 the Chanter function reduces to the logistic equation; as D approaches the upper limit it reduces to the Gompertz equation (Thornley and Johnson, 1990). Values for $\log_e(A_{max} \cdot L_0^{-1})$ differed only among leaf sizes, ranging from 0.053 and 0.058 for the L_{14} and L_7 categories respectively to 0.068 for the L_3 category. Compared to these, the values for D were small, suggesting that curves tended to be logistic in character.

Leaves developing after day 21

The increment in leaf area of L_{21} leaves tended to be higher in SOC plants, although differences among all treatments were negligible (Fig 6.5). Leaves of both SCR and DCR plants that commenced expansion 28 DAI were significantly smaller than SOC leaves after seven days, but the total leaf area of both restriction treatments was similar to that of control treatments 49 DAI (Fig. 6.6).

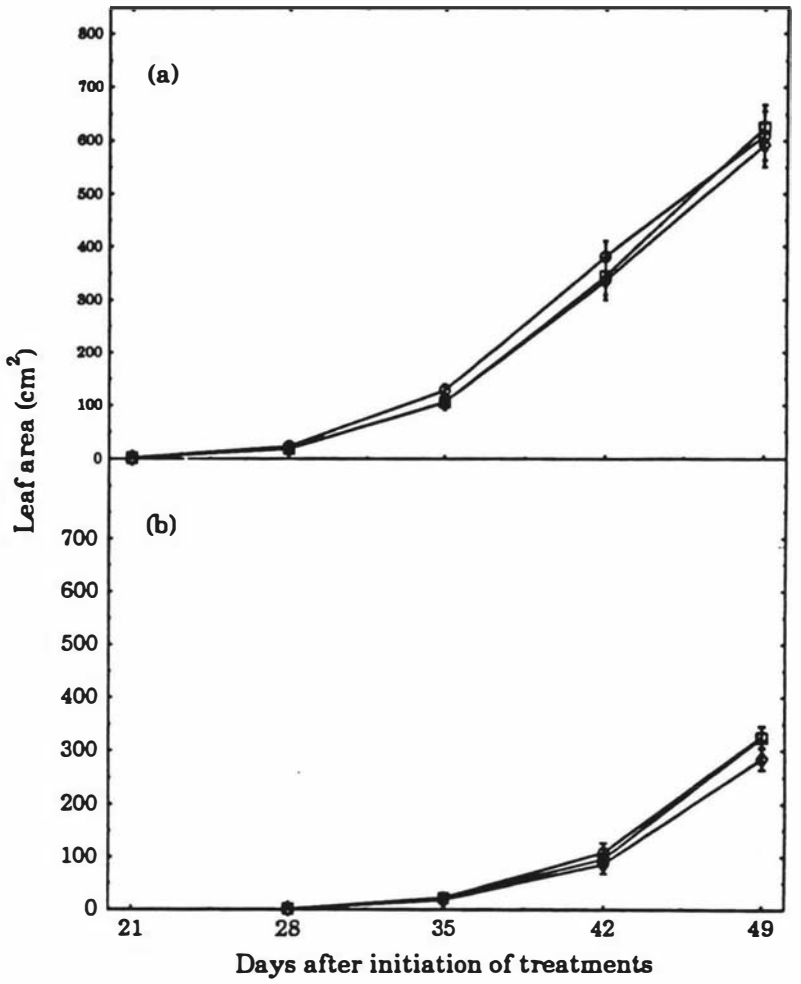

Fig. 6.5. Expansion ontogeny of leaves commencing development (a) 21 days or (b) 28 days after treatment application. Each point is the mean ± SE from 10-12 plants. *key:* □=DCR, ○=SCR, ◇=SOC.

Rate of leaf appearance

The rate of leaf appearance in each treatment was constant throughout the experiment (Table 6.10). Moreover, there were no significant rate differences among treatments. The rate coefficient calculated from the pooled data set equates to slightly over 2 leaves being produced per week.

Table 6.10. Individual and pooled regression coefficients relating appearance of leaves of 3 cm mid-rib length against time (days from transfer into hydroponic system).

Treatment	*x*-axis intercept (SE)	rate coefficient (SE)	r^2
NSC	9.25 (0.355)	0.29 (0.013)	0.92
SOC	9.59 (0.297)	0.30 (0.011)	0.93
SCR	9.80 (0.248)	0.31 (0.010)	0.94
DCR	8.85 (0.258)	0.31 (0.009)	0.95
pooled regression line	9.42 (0.149)	0.30 (0.006)	0.92

Relative leaf expansion rate

The relative leaf expansion rate of leaves of all groups, irrespective of treatment, gradually decreased over time with ontogenetic drift and the determinate nature of individual leaf growth.

The initial R_A of leaves developing soon after treatments were applied (i.e. L_3, L_7, and L_{14}) was similar for all treatments. Estimates of initial R_A, calculated from the first differential of the Richards function (equation 6.3), were higher than those estimated by the Chanter function but, as for the Chanter function estimates, no consistent treatment differences were detected. Relative leaf expansion rate at the point of inflexion was similar to that reported by Freijsen and Veen (1989) for tomato. Neither maximum R_A (i.e. R_A at the point of inflexion) nor mean R_A were influenced by restriction treatment (Table 6.9). Presence of a split stem, however, increased the mean R_A over plants with non-split stems.

Root restriction had no effect on point estimates of R_A of leaves in the L_3 group (Table 6.11). The presence of a split stem influenced R_A of leaves in the L_7 group more than whether the root system was restricted or not (Table 6.12). The R_A of leaves that had started expanding 14 DAI was lower in restricted (SCR and DCR) than unrestricted (SOC) plants between 28 and 35 DAI. In contrast, leaves of restricted plants that started expanding 21 and 28 DAI exhibited higher R_A than unrestricted plants from 42 and 49 DAI respectively (Tables 6.13, 6.14). Restriction slowed the rate of decrease in L_{28} leaves, but not in L_{21} leaves.

Table 6.11. Relative leaf expansion rates (R_A) of leaves commencing expansion growth 3.5 DAI at and between different times.

variable	Level of root restriction (R)				SD	contrasts			contrasts	
	NSC	SOC	SCR	DCR		[NSC+SOC] vs [SCR+DCR]	SCR vs DCR	SOC vs NSC	[SCR+DCR] vs SOC	Split vs no split
n	5	4	7	6						
R_{A7}	0.415	0.451	0.438	0.448	0.0685	NS	NS	NS	NS	NS
R_{A14}	0.295	0.297	0.305	0.304	0.0214	NS	NS	NS	NS	NS
R_{A21}	0.158	0.149	0.154	0.153	0.0110	NS	NS	NS	NS	NS
R_{A28}	0.065	0.057	0.057	0.058	0.0072	NS	NS	NS	NS	*
R_{A35}	0.024	0.019	0.018	0.019	0.0059	NS	NS	NS	NS	NS
R_{A42}	0.009	0.007	0.006	0.006	0.0036	NS	NS	NS	NS	NS
R_{A49}	0.003	0.002	0.002	0.002	0.0019	NS	NS	NS	NS	NS
$R_{A14} - R_{A7}$	-0.119	-0.154	-0.133	-0.144	0.0617	NS	NS	NS	NS	NS
$R_{A21} - R_{A14}$	-0.137	-0.147	-0.151	-0.152	0.0215	NS	NS	NS	NS	NS
$R_{A28} - R_{A21}$	-0.093	-0.093	-0.097	-0.095	0.0115	NS	NS	NS	NS	NS
$R_{A35} - R_{A28}$	-0.041	-0.038	-0.038	-0.039	0.0038	NS	NS	NS	NS	NS
$R_{A42} - R_{A35}$	-0.015	-0.013	-0.012	-0.013	0.0026	NS	NS	NS	NS	NS
$R_{A49} - R_{A42}$	-0.005	-0.004	-0.004	-0.004	0.0018	NS	NS	NS	NS	NS

NS,*,**,***,**** Non significant or significant F test at $P<0.05$, 0.01, 0.001 or 0.0001 respectively
df(error)=18

Table 6.12. Relative leaf expansion rates (R_A) of leaves commencing expansion growth 7 days after treatment application (DAI) at, and between, different days.

variable$_{DAI}$	Level of root restriction (R)				SD	contrasts			contrasts	
	NSC	SOC	SCR	DCR		[NSC+SOC] vs [SCR+DCR]	SCR vs DCR	SOC vs NSC	[SCR+DCR] vs SOC	Split vs no split
n	3	4	5	7						
R_{A7}	0.466	0.443	0.483	0.429	0.1025	NS	NS	NS	NS	NS
R_{A14}	0.355	0.354	0.368	0.335	0.0383	NS	NS	NS	NS	NS
R_{A21}	0.209	0.224	0.194	0.219	0.0283	NS	NS	NS	NS	NS
R_{A28}	0.118	0.114	0.091	0.104	0.0204	#	NS	NS	NS	NS
R_{A35}	0.064	0.048	0.039	0.037	0.0094	•••	NS	••	NS	**
R_{A42}	0.034	0.018	0.017	0.012	0.0054	•••	NS	••	NS	***
R_{A49}	0.018	0.007	0.006	0.004	0.0033	•••	NS	•••	NS	•••
$R_{A14} - R_{A7}$	-0.148	-0.097	-0.138	-0.094	0.0846	NS	NS	NS	NS	NS
$R_{A21} - R_{A14}$	-0.145	-0.129	-0.174	-0.116	0.0452	NS	•	NS	NS	NS
$R_{A28} - R_{A21}$	-0.092	-0.110	-0.103	-0.115	0.0117	NS	NS	NS	NS	•
$R_{A35} - R_{A28}$	-0.054	-0.066	-0.051	-0.067	0.0137	NS	#	NS	NS	NS
$R_{A42} - R_{A35}$	-0.029	-0.029	-0.022	-0.025	0.0056	#	NS	NS	NS	NS
$R_{A49} - R_{A42}$	-0.016	-0.011	-0.009	-0.008	0.0024	•••	NS	•	#	•••

NS,#,•,••,•••,•••• Non significant or significant F test at $P<0.10$, 0.05, 0.01, 0.001 or 0.0001 respectively
df(error)=15

Table 6.13. Relative leaf expansion rates (R_A) of leaves commencing expansion growth 14 DAI at and between different times.

variable	Level of root restriction (R)				SD	contrasts			contrasts	
	NSC	SOC	SCR	DCR		[NSC+SOC] vs [SCR+DCR]	SCR vs DCR	SOC vs NSC	[SCR+DCR] vs SOC	Split vs no split
n	10	6	7	11						
R_{A14}	0.397	0.425	0.415	0.401	0.0578	NS	NS	NS	NS	NS
R_{A21}	0.313	0.341	0.314	0.305	0.0370	NS	NS	NS	NS	NS
R_{A28}	0.213	0.226	0.191	0.191	0.0366	*	NS	NS	*	NS
R_{A35}	0.122	0.119	0.091	0.095	0.0304	*	NS	NS	#	NS
R_{A42}	0.059	0.052	0.037	0.042	0.0191	*	NS	NS	NS	*
R_{A49}	0.026	0.020	0.014	0.018	0.0099	#	NS	NS	NS	*
$R_{A21} - R_{A14}$	-0.084	-0.084	-0.098	-0.096	0.0406	NS	NS	NS	NS	NS
$R_{A28} - R_{A21}$	-0.099	-0.114	-0.123	-0.114	0.0263	NS	NS	NS	NS	NS
$R_{A35} - R_{A28}$	-0.091	-0.107	-0.099	-0.095	0.0159	NS	NS	#	NS	NS
$R_{A42} - R_{A35}$	-0.063	-0.068	-0.054	-0.053	0.0142	*	NS	NS	*	NS
$R_{A49} - R_{A42}$	-0.034	-0.032	-0.023	-0.024	0.0100	**	NS	NS	#	#

NS,#,*,**,***,**** Non significant or significant F test at $P<0.10$, 0.05, 0.01, 0.001 or 0.0001 respectively
df(error)=30

Table 6.14. Relative leaf expansion rates (R_A) of leaves commencing expansion growth 21 DAI at and between different times.

variable	Level of root restriction (R)			SD	contrasts	
	SOC	SCR	DCR		SOC vs [SCR+DCR]	SCR vs DCR
n	10	12	10			
R_{A21}	0.383	0.384	0.389	0.0368	NS	NS
R_{A28}	0.293	0.299	0.304	0.0274	NS	NS
R_{A35}	0.203	0.214	0.218	0.0206	NS	NS
R_{A42}	0.113	0.129	0.132	0.0190	•	NS
R_{A49}	0.023	0.044	0.046	0.0238	•	NS
$R_{Ax} - R_{A(x-1)}$	-0.090	-0.085	-0.086	0.0116	NS	NS

NS,•,••,•••,•••• Non significant or significant F test at $P<0.05$, 0.01, 0.001 or 0.0001 respectively
df(error)=29

Table 6.15. Relative leaf expansion rates (R_A) of leaves commencing expansion growth 28 DAI at and between different times.

variable	Level of root restriction (R)			SD	contrasts	
	SOC	SCR	DCR		SOC vs [SCR+DCR]	SCR vs DCR
n	8	8	10			
R_{A28}	0.468	0.378	0.436	0.0699	NS	NS
R_{A35}	0.378	0.306	0.323	0.0361	NS	NS
R_{A42}	0.195	0.233	0.209	0.0338	NS	NS
R_{A49}	0.059	0.161	0.096	0.0665	•	NS
$R_{Ax} - R_{A(x-1)}$	-0.136	-0.072	-0.113	0.0415	•	•

NS,•,••,•••,•••• Non significant or significant F test at $P<0.05$, 0.01, 0.001 or 0.0001 respectively
df(error)=23

6.3.2.2 Total leaf area

Double cell restricted plants had developed smaller leaf canopies than SCR plants by the conclusion of the experiment (Fig. 6.6a; Table 6.18). Analysis of estimated total leaf area at 49 DAI revealed a similar pattern, although the statistical difference between the two groups was less distinct ($P<0.057$). These results contradict those obtained for individual leaf growth (Tables 6.6, 6.7), where maximum area of individual leaves of plants under DCR and SCR was not significantly different. Closer examination of these data, however, shows that individual leaf area of DCR plants was consistently smaller than SCR. Presumably, the accumulated leaf area totals magnified the small differences between individual leaf areas.

Total leaf area was re-examined after apportioning total leaf area on any plant to three groups; (1) leaves present at treatment application (Fig. 6.6b), (2) leaves commencing expansion 0-20 DAI (includes L_3, L_7 and L_{14} leaf groups; Fig. 6.6c), and (3) leaves commencing expansion 21 DAI (includes L_{21} and L_{28} leaves; Fig. 6.6d). Ontogeny of total estimated leaf area of the 0-20 DAI group is consistent with the results of the individual leaf analysis (6.3.2.1), with unrestricted plants developing larger leaf area than restricted plants (Fig. 6.6c). No differences among treatments were observed in those leaves that started expanding 21 DAI (Fig. 6.6d).

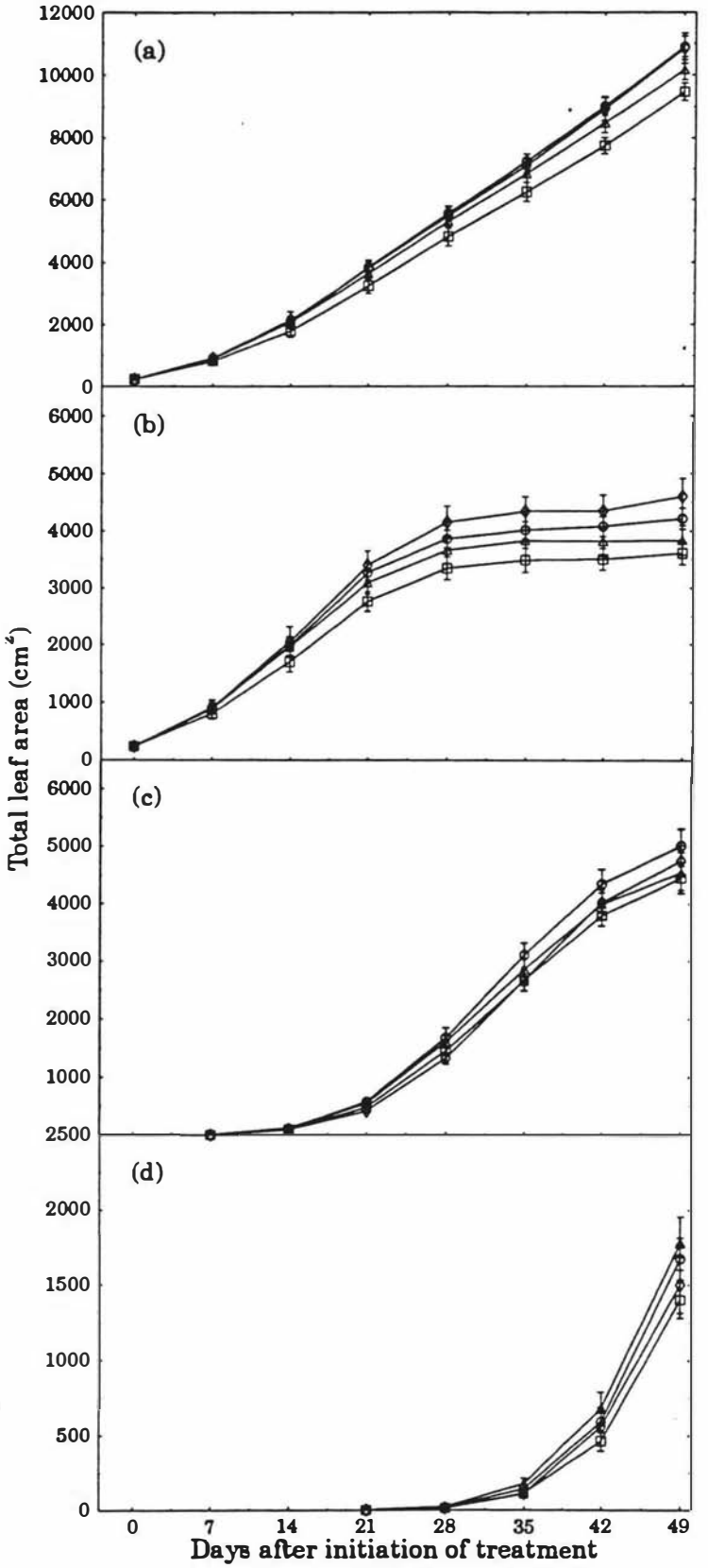

Fig. 6.6. Expansion ontogeny of (a) total plant leaf area, (b) leaves present at treatment initiation, leaves commencing expansion (c) 0-20 DAI, and (d) 21+ DAI. Each point is the mean ± SE from 10-12 plants. *Key:* □:DCR, △:SCR, ○:SOC, ◇:NSC.

6.3.4 Leaf function

Photosynthesis

No consistent differences in photosynthetic rates between restricted and unrestricted plants were detected throughout the experimental period (Table 6.16).

Leaf water potential

Midday leaf water potential measurements showed no indication of substantial water stress in any treatment throughout the duration of the experiment (Table 6.16). Small differences between restricted and split control plants were detected 21 DAI and between restriction treatments 49 DAI.

Table 6.16 Photosynthesis rates and leaf water potential during experimental period.

Variable	Level of root restriction (R)				*SEOD	contrasts			contrasts	
time (DAI)	NSC	SOC	SCR	DCR		Some R vs no R	SCR vs DCR	SOC vs NSC	Some R vs SOC	Split vs no split
photosynthesis (μmol· m^{-2}· s^{-1})										
13	14.71	14.10	13.97	14.97	0.76	NS	NS	NS	NS	NS
20	12.62	13.26	12.68	12.60	1.39	NS	NS	NS	NS	NS
27	13.13	12.69	11.98	11.72	2.55	NS	NS	NS	NS	NS
34	13.44	13.55	15.36	15.03	1.79	NS	NS	NS	NS	NS
41	11.19	12.75	13.02	12.26	1.97	NS	NS	NS	NS	NS
48	11.18	12.00	12.93	11.68	1.63	NS	NS	NS	NS	NS
leaf water potential (MPa)										
21	-0.323	-0.245	-0.355	-0.300	0.038	NS	NS	NS	*	NS
27	-0.335	-0.280	-0.280	-0.350	0.048	NS	NS	NS	NS	NS
35	-0.345	-0.350	-0.350	-0.393	0.045	NS	NS	NS	NS	NS
42	-0.395	-0.420	-0.383	-0.405	0.047	NS	NS	NS	NS	NS
49	-0.370	-0.390	-0.410	-0.300	0.052	NS	*	NS	NS	NS

NS,*,**,***,**** Non significant or significant F test at P<0.05, 0.01, 0.001 or 0.0001 respectively
* n_i=4, df(error)=8 for photosynthesis data and 9 for leaf water potential data

6.3.5 Whole plant growth

Vegetative growth components

Similar patterns of response to restriction were detected in the dry weights of leaf, stem, petiole, and root, and total leaf area and leaf number at final harvest. Principal component analysis of these parameters yielded a single component, accounting for over 90% of total variation, which reflected overall size (Table 6.17).

Table 6.17 Principal component of standardised vegetative growth parameters under levels of root restriction at final harvest.

Principal component	Eigenvalue	Cumulative % variance explained	Leaf area	Leaf dry wgt	Petiole dry wgt	Stem dry wgt	Root dry wgt
PC_1	4.58	91.6	0.428	0.458	0.461	0.446	0.443

In general, SCR and SOC plants accumulated more dry matter than DCR or NSC plants (Table 6.18). Double cell root restriction decreased overall size compared to unrestricted plants. Plants with a single cell of restricted roots (SCR), on the other hand, were similar in overall size to unrestricted plants. Control plants with intact stems (NSC) had similar, although consistently lower, dry biomass weights of leaf, root and petioles compared to SOC plants, while the number of leaves and stem dry biomass were significantly lower. This pattern was amplified in the principal components analysis with NSC. Unrestricted plants with split stems (SOC) were larger (PC_1) than unrestricted plants with intact stems.

Table 6.18 Influence of root restriction treatments on plant dry biomass at final harvest.

treatment		total leaf number	total leaf area[‡] (cm^2)	leaf dry wgt (g)	petiole dry wgt (g)	stem dry wgt (g)	root dry wgt (g)	overall size (PC_1)
source	level							
root restriction (R)	NSC	23.6	10219.	32.0	15.9	17.3	7.5	-0.578
	SOC	24.9	11230.	38.6	18.1	20.3	8.9	1.386
	SCR	24.6	10763.	37.2	17.4	20.2	8.9	0.971
	DCR	23.9	9399.9	28.3	13.8	17.2	6.2	-1.779
SEOD		0.52	437.12	3.36	1.37	1.13	0.95	0.969
		•	•	•	•	••	•	•
contrasts								
[NSC+SOC] v [SCR+DCR]		NS	NS	NS	NS	NS	NS	NS
DCR vs SCR		NS	•	•	•	•	•	••
SOC vs NSC		•	NS	NS	NS	•	NS	NS
[SCR+DCR] vs SOC		NS	NS	NS	NS	NS	NS	NS
DCR vs SCR		NS	•	•	•	•	•	••
Split vs no split stem		•	NS	NS	NS	•	NS	NS

NS,*,**,***,**** Non significant or significant *F* test at $P<0.05$, 0.01, 0.001 or 0.0001 respectively
df(error)=9
‡ actual leaf area

Assimilate partitioning

Double cell restricted plants partitioned considerably more assimilate to stem biomass and less to root biomass than any of the other treatments (Table 6.19).

The proportion partitioned to leaf biomass in DCR plants was less than in SOC plants, but similar ($P<0.06$) to that in SCR plants. Double cell restriction reduced root growth more than shoot growth (31 vs 23%), with the consequence that the shoot:root ratio increased. This increase, however, was not large, and as the data in Fig. 6.7 suggest, the reduction in root and shoot growth caused by restriction appears to have been balanced.

Table 6.19 Partitioning and growth analysis indices[a] at final harvest.

Treatment		LWR	PWR	SWR	RWR	SLA	LAR	SR
source	level	($g \cdot g^{-1}$)	($g \cdot g^{-1}$)	($g \cdot g^{-1}$)	($g \cdot g^{-1}$)	($m^2 \cdot g^{-1}$)	($m^2 \cdot g^{-1}$)	($g \cdot g^{-1}$)
Root restriction (R)	NSC	0.439	0.218	0.239	0.103	0.031	0.014	8.7
	SOC	0.448	0.211	0.237	0.104	0.034	0.013	8.7
	SCR	0.444	0.209	0.241	0.105	0.035	0.013	8.6
	DCR	0.432	0.211	0.264	0.094	0.030	0.014	9.7
SEOD		0.0058	0.0030	0.0082	0.0041	0.0020	0.0001	0.42
		NS	•	•	NS	NS	•	NS

[a]LWR: leaf weight ratio; PWR: petiole weight ratio; SWR: stem weight ratio; RWR: root weight ratio; SLA: specific leaf area; LAR: leaf area ratio; SR: shoot:root ratio
[NS,•] Non significant or significant F test at $P<0.05$ respectively
df(error)=9

Double cell restricted plants tended to be more 'leafy' (higher LAR) with thicker or more dense leaves (lower SLA) than SCR plants, although the differences, like those with SOC, were not significant.

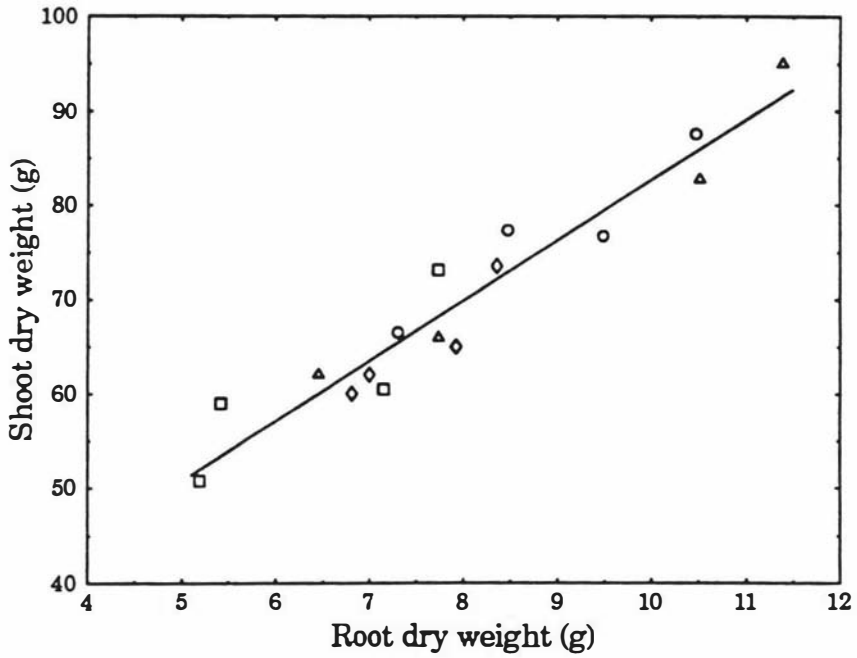

Fig. 6.7. Relationship between shoot dry weight and root dry weight. Each value is the block mean of 2-3 plants. $Y=18.7 + 6.39X$, $R^2=0.92$. *Key:* □:DCR, △:SCR, ○:SOC, ◇:NSC.

6.4 Discussion

A critical observation from this experiment was the reduction of leaf expansion in plants with only a portion of their root system physically restricted. This is, to my knowledge, the first demonstration that localised physical restriction of a root system can result in reduced shoot growth. The effect is even more noteworthy as compensatory root growth occurred in plants with partially restricted root systems, resulting in total root growth similar to unrestricted plants. A reasonable presumption of this interpretation is that because the SCR treatment had similar root dry biomass 49 DAI compared with the SOC control (Fig. 6.2), the respective root masses were also similar during the period that leaf expansion was reduced.

Leaf growth Effects of root restriction on the growth of individual leaves were first detected 23 DAI with reductions in leaf area at the point of inflexion of the expansion curve in leaves that started expanding 3, 7 and 14 DAI (Table 6.8). There was a significant quadratic component to the estimates of maximum leaf area (Table 6.7) with the maximum leaf area of L_3 leaves larger than that of L_{14} leaves. There was no evidence, however, that this decline was faster in restricted compared to restricted plants. Moreover, the experiment did not continue long enough for maximum leaf areas of L_{21} or L_{28} leaf groups to be estimated.

Analysis of R_A revealed two distinct growth phases in response to root restriction. In the first phase, apparent after about 28 DAI, R_A was reduced in restricted plants, presumably as the level of restriction encountered by the root sub-systems within the restriction cells increased. The R_A of leaves in both L_7 and L_{14} groups of restricted plants gradually declined from 28 DAI (Tables 6.11, 6.12). The R_A of unrestricted plants declined to that of restricted plants 42 and 49 DAI as leaf expansion approached its maximum. The second phase, detected 42 DAI and observed in leaves that started

expanding 21 and 28 DAI, was characterised by an increase in R_A in restricted plants compared to unrestricted plants (Tables 6.13, 6.14).

Root restriction affected leaf expansion at all stages of development. The nature of this response, however, changed in time. Reduced leaf area and R_A were first detected in L_3, L_7, and L_{14} groups during periods of decreasing, maximum, and increasing expansion rates respectively (i.e. after, during, and preceding the point of inflexion, Figs. 6.2, 6.3). It is a little puzzling that expansion of L_7 leaves was, on a percent basis of final size, reduced more than L_{14}. This may be an artefact of the curve fitting procedures; few data were available near the asymptote, and it is possible that the estimates of maximum leaf area do not accurately represent the actual final areas gained by the leaves in any of the treatments. Alternatively, the effect of root restriction may have been magnified in L_7 leaves because in reaching the point of inflexion, they were at their maximum rate of expansion, whereas L_{14} leaves had not yet reached this stage of development (Fig. 6.3; Table 6.9). Similarly, enhanced R_A of L_{21} leaves, but not L_{28} leaves, at 42 DAI probably reflects the differences in stages of development (Tables 6.14, 6.15). From estimates of maximum leaf area and time from emergence to the point of inflexion (Table 6.7, 6.8), at 42 DAI L_{21} leaves were about 40% fully expanded and nearing the maximum rate of expansion; L_{28} leaves, on the other hand, did not reach a similar stage of development until about 49 DAI (Fig. 6.5). An interesting parallel with this response is found in recent work by Ainley et al. (1993). Using transgenic tobacco plants in which endogenous cytokinin biosynthesis could be conditionally stimulated by heat shock, these workers reported that expansion was only increased in leaves that were rapidly growing at the time enhanced cytokinin levels were induced.

Responsiveness of leaf expansion to root restriction may depend on the stage of functional development. Ho and Shaw (1977) showed that net import of carbohydrate into a tomato leaf ceased when it was between 12-50% fully

expanded. In a later study, these authors (1979) observed that rates of accumulation of minerals, water and organic carbon were greatest when the leaf was 22-24 days old. In both instances, these stages of growth encompass the period around the point of inflexion (Table 6.8) during which the changes in expansion rates were detected. It is unlikely, however, that the changes in leaf expansion rates were due to changes in assimilate supply. Although a restriction-induced reduction in the sink strength of roots might provide more assimilate for shoot growth, the usual response to a reduction in sink strength is either a reduction in shoot activity or preferential partitioning of assimilate to the root system as, teleologically, the plant adjusts to maintain a functional equilibrium between the root and the shoot (Brouwer, 1963; Davidson, 1969a; Richards and Rowe, 1977a, 1977b). Neither of these responses were observed (Tables 6.18, 6.19). On the other hand, if maximum rates of accumulation of growth substances also coincide with the period of maximum leaf expansion, changes in growth would be most visible at the time of maximum expansion rate (i.e. the point of inflexion). Although exogenously applied cytokinins and gibberellins stimulate cell division in young leaves (Humphries and Wheeler, 1960) and induce rapid leaf cell enlargement (Brock and Cleland, 1989, 1990; Nielsen and Ulvskov, 1992; Powell and Griffith, 1960), it is only recently that a correlation between the rate of leaf expansion and the distribution of endogenous cytokinins in leaves has been demonstrated (Ulvskov et al., 1992).

The size of leaves is determined by the size and number of cells and inter-cellular spaces (Dale, 1976; Dale and Milthorpe, 1983; Paul, 1984a). Milthorpe and Newton (1963) observed both reduced leaf cell number and final cell size in leaves of cucumbers growing in '5 inch' versus '7 inch' containers. While some reduction in cell size was noted, Cresswell and Causton (1988) reported that reduced cell number largely accounted for the smaller leaf area of Brussels sprouts (*Brassica oleracea* var. *gemmifera* DC) seedlings grown in 90 cm^3 containers compared to those grown in larger volume (210, 550 and

1600 cm^3) containers. The small leaf size of root restricted bonsai trees was associated with reduced leaf cell number, not size (Körner et al., 1989). In instances where leaf cell division has been limited, for example by temperature (Paul, 1984a), initial rates of R_A (pre-point of inflexion) are similar, with the major contributor to variation in final leaf size between temperature regimes being the R_A following the point of inflexion (Fig. 1. in Paul, 1984a). In contrast, when leaf cell expansion is reduced, for example through reduced leaf water potential and turgor pressure (Takami et al., 1981), initial rates of R_A are reduced. Although the main features of leaf shape and structure are established at unfolding and rapid expansion of the lamina, over 70% of leaf cells arise from divisions occurring after this time and up to 40% of full expansion (Dale, 1988; Milthorpe and Newton, 1963; Nelson and Larson, 1984; Sunderland, 1960). It is noteworthy, therefore, that in the current study 40% of full leaf expansion coincided with the point of inflexion. Root restriction in SCR and DCR had, by this stage, already resulted in reduced leaf area (Table 6.8), despite similar leaf ontogeny (as inferred by the shape of the curve via the parameter v (Table 6.7), and duration of growth (Table 6.8)), and relative rates of leaf expansion (Table 6.9) among treatments. In addition, the major differences in leaf expansion between restricted and unrestricted treatments occurred after the point of inflexion (Figs. 6.2, 6.3). These data suggest that leaf expansion was reduced by root restriction through an effect on cell division.

Root growth In all previous studies in which leaf area was reduced by root restriction, the entire root system was restricted. A critical feature of the current experiment was that all 'restricted' plants were allowed to develop unrestricted root systems. Thus, while at the start of the experiment DCR plants had 100% of their roots in a restricting cell, by the end of the experiment over 50% of the root system (by dry biomass) of DCR plants was unrestricted, outside of these cells. Similarly, while initially 50% of the root system of SCR plants was restricted, by 49 DAI less than 20% of the total

root system was restricted (Table 6.4). Teleologically speaking, restricted plants could avoid the restrictive environment and clearly by 49 DAI they had done that.

Assimilate partitioning Plants respond to short term changes in the environment by altering the specific activity of their roots and shoots (Davidson, 1969a; Brouwer, 1963). If the environmental change persists, however, the long term response is a change in the SR ratio to one suitable for that environment (Chalmers and van den Ende, 1975). The balanced reduction in SR ratio (Table 6.19) indicates that the adjustment in root and shoot growth of restricted plants was towards an environment in which unrestricted root growth was the 'norm' (i.e. as reflected in the SR ratio of SOC and NSC plants). In other words, the SR ratio reflected the environment of the unrestricted part of their root systems, not the restricted part.

In contrast, the partitioning pattern of DCR plants appeared to respond to the restricted part of their root system. These plants partitioned more assimilate ($P < 0.005$) to the stem at the expense of root and leaf growth than SCR or unrestricted plants (Table 6.19). This pattern of within-shoot partitioning of DCR plants is consistent with those in experiments with whole-root restriction in which increased partitioning to the stem occurs at the expense of root and leaf growth (Carmi and Shalhevet, 1983; Peterson et al., 1991a; Richards and Rowe, 1977a). Increased partitioning to the stem of tomatoes is also observed when fruit trusses, flowers or leaves are removed. The common conclusion is that the stem tissue acts as an alternative sink in tomato (Al-Sahaf, 1984; Friis-Nielsen, 1973a; Hameed et al., 1987; Hammond et al., 1984; Hewitt et al., 1982; Peterson et al., 1991a). When fruiting trusses were removed from (unrestricted) tomato plants, the stem operated as a substitute sink for the excess assimilate produced by the leaves (Hammond et al., 1984). Al-Sahaf (1984) concluded that the stem was a more competitive accumulator of assimilates in restricted compared to unrestricted

plants because the former lacked active alternative sinks in roots and leaves. It is interesting, therefore, that despite have an unrestricted root sub-system, DCR plants still preferentially partitioned assimilate to the stem. This suggests that either the stem is a stronger sink than has been previously regarded, or that the partitioning patterns are not a simple function of relative sink strength.

The accumulation of assimilates in the stems could also have resulted from either reduced phloem unloading to normally strong sinks such as roots (Geiger, 1975), or through increased use within the stem due to enhanced activity of the cambial meristem arising from altered hormonal status (Chalmers, 1985). In my study, however, preferential partitioning of assimilate to the stem occurred in DCR plants despite the presence of a sink (i.e. the unrestricted root sub-system). One further explanation is that reduced activity in leaf and root sinks may have resulted in accumulating photosynthate (Robbins and Pharr, 1988; Warren Wilson, 1972) being stored in the stem. Whatever the mechanism, the absence of partitioning changes in SCR plants (Table 6.19) suggests that stem storage is a short term measure. Stored assimilates may have been consumed to support new leaf growth as the unrestricted root sections became free of the postulated correlative inhibition of the restricted root systems. Early growth of SCR leaves above L_{21} tended to be more rapid than DCR leaves (Fig. 6.5), possibly reflecting earlier recovery of SCR plants from root restriction. Richards and Rowe (1977a) reported a similar pattern of assimilate movement after peach roots were released from restriction, with partitioning from the butt in favour of leaf growth. Since both sets of data represent discrete points in time in a dynamic process, however, more detailed analysis of changes in partitioning patterns is required, with particular emphasis on the ontogeny of partitioning between restricted and unrestricted root sub-systems.

Clearly, the partitioning response to restriction is more complex than proposed (Al-Sahaf, 1984). Although the sink activity of the unrestricted root sub-system was likely to have been correlatively inhibited (Gersani and Sachs, 1992) by the restricted root sub-system, the substantial growth of the unrestricted root system in SCR plants indicates that such correlative control can be broken, and thus cannot be regarded as a factor influencing the perceived sink strength of the root system. Physical blockage of assimilate transport through the phloem arising from callose deposition in sieve tubes is also an unlikely explanation, as the high auxin/low cytokinin level expected in the stems of root restricted plants (Richards and Rowe, 1977a; Carmi and Heuer, 1981) would favour callose dissolution (Aloni et al., 1990, 1991). A possibility worthy of further investigation is that the observed increased SWR was a temporal artefact, having occurred when most of the root system of DCR plants was still in the restriction cells. Thus, the final harvest simply occurred before the assimilate stored in the stem had been redistributed to support the growth of either the unrestricted root system or leaf growth.

The pattern of assimilate partitioning is also relevant to discussion of root restriction in a container and root impedance in soils of high strength. As might be expected, many of the responses reported here to root restriction are similar to those of plants whose roots are physically impeded in soils of high strength. Root elongation and leaf expansion rates are reduced in both systems. There is good evidence that hormones are involved in the response to root restriction and root impedance. For the latter, Masle and Passioura (1987) suggested that the response of shoot growth to soil strength is controlled by hormones produced in the roots. Passioura and Gardner (1990) separated the confounding interaction of soil moisture content from soil strength and reported that the signal sent in response to soil strength and which lead to a reduction in R_A was independent of soil water content. Masle (1992) traced the time course of growth and stomatal response to root growth in compacted soil and concluded that the effect of soil resistance on cell

expansion and division involved either a different signal or a different perception of a common signal from that involved in regulating stomatal guard cells. Soil compaction had no significant effect on pre-dawn or midday leaf water potential, osmotic potential or leaf turgor of sunflower plants, yet R_A decreased linearly with increasing soil strength (Andrade et al., 1993). In contrast, Tardieu et al. (1991) found no evidence to support the effect of mechanical stress on roots in control of stomata in field-grown maize. These workers concluded that observed reductions in conductance of plants in compacted soils were due to reduced water uptake by the root system.

The major difference, however, between both phenomena is the partitioning patterns between root and shoot. In all but two studies of root restriction (Ruff et al., 1987; Ternesi et al., 1994), the SR either remains unchanged or increases. In studies of soil compaction, the SR decreases. Dawkins et al. (1983) working with peas (*Pisum sativum*), Atwell (1990a) with wheat (*Triticum aestivum*), and Andrade et al. (1993) with sunflowers all reported a decrease in SR of plants growing in compacted soils. In also observing preferential partitioning of assimilate to roots of wheat and barley growing in high strength soils, Masle et al. (1990) postulated that root-produced signal retarded shoot growth, providing more assimilate to become available for root growth. These workers speculated that the signal was similar to ABA in its effects on assimilate partitioning between shoot and root and stomatal conductance. Subsequent studies with ABA-deficient mutants of *L. esculentum* 'Moneymaker' discounted this possibility, with leaf area expansion and transpiration being reduced in high resistance soils at levels similar to the wild type controls (Masle, 1990). It would be interesting to grow this mutant in a container of restricting volume and compare the partitioning pattern with that observed by Masle et al. (1990).

Compensatory growth The spatiotemporal influence of root restriction on leaf area expansion rates was a critical observation in this experiment. Expansion

was reduced in leaves that started expanding within the first three weeks of the experiment, but in leaves that started expanding after this time (21 DAI), the R_A was higher in restricted plants than in unrestricted plants (Tables 6.14, 6.15). The experiment was terminated before these leaves were fully expanded, so it is not possible to determine whether this early difference in R_A would have resulted in larger leaves in plants with a restricted root system, or in smaller leaves which were fully expanded before those of unrestricted plants. Unfortunately, the ontogeny of root growth was not similarly followed. From observations, however, the period of reduced R_A occurred as the root system become restricted in the cells, while the period of increased R_A was coincident with the initiation and growth of new roots outside of the restricted sub-system. The increased R_A may have resulted from compensatory growth associated with the release from the stress conditions 'imposed' by the restricted root systems by the development of the unrestricted root sub-system some time after 21 DAI. Other studies have reported compensatory growth following release from stress, although almost all have been associated with water stress. BassiriRad and Caldwell (1992) recorded a three-fold increase (compared to well watered plants) in the relative rate of extension of roots of previously water stressed plants of the perennial shrub *Artemisa tridentata* following re-watering. Restoring irrigation to all quadrants of peach roots resulted in growth rates equal to or greater than fully irrigated trees (Proebsting et al., 1989). Acevedo et al. (1971) observed that reduced growth of maize leaves following a very mild and short period of water stress was fully compensated by a transitory phase of enhanced growth of the same leaves.

Compensatory growth is not, however, a widely accepted phenomenon. Measurements purporting to show such growth have often been confounded with other physiological 'by-products' of the stress. Hopkinson (1968) reported that relative leaf expansion rates of tobacco increased substantially after water stress was removed, to the extent that stressed or root damaged

plants had final total leaf areas that exceeded unstressed, undamaged plants. Flower initiation in stressed plants, however, was delayed and leaf number consequently increased in stressed plants, making it difficult to distinguish between the recovery effect and the displacement of development in time. Hsiao and Acevedo (1974) re-interpreted reports of compensatory growth in tomato lasting several days after stress was released (Gates, 1955a, 1955b) as showing that growth of control leaves had slowed upon maturation, rather than growth had increased in stressed leaves at the same developmental stage. Moreover, both Gates (1955a, 1955b) and Hopkinson (1968) restricted water to the extent that wilting was the criterion for the stressed treatment. According to Hsiao et al. (1976), the metabolic processes responsible for the 'stored growth' of Acevedo et al. (1971) would also be inhibited at such levels of water stress. In all these studies, compensatory growth following recovery from water stress was most noticeable on lower (older) leaves. Upper (younger) leaves either failed to respond (Hopkinson, 1968) or did not fully reestablish leaf areas similar to unstressed plants (Gates, 1955a, 1955b). On the other hand, Takami et al. (1981) reported enhanced leaf expansion of *H. annuus* leaves emerging after recovery from water stress.

In my study, compensatory growth was only detected in leaves that commenced development after the restriction treatments were started (i.e. 21 DAI). These growth responses are not easily discounted by the above arguments. Compensatory growth was not the resumption of a postponed growth event (Acevedo et al., 1971) because the duration of expansion of 'reduced' leaves was not increased (Table 6.8). Moreover, it occurred in leaves other than those in which expansion growth was reduced by the restriction treatments. The increased expansion rate of leaves of restricted plants was not due to slowing growth of control leaves as they approached maturity (cf. Hsiao and Acevedo, 1974); unrestricted leaves had only reached 40% full expansion (based on final leaf area of lower leaves). Finally, compensatory growth cannot be attributed to ontogenetic drift because the monitored leaves

can be assumed to have been at the same developmental stage due to the clustering procedure used to objectively sample the population (Table 6.5).

Under conditions of low nitrogen nutrition, levels of cytokinin nucleotides in young leaves and stem tissue increase relative to control plants despite reduced export of cytokinins from the roots (Wagner and Beck, 1993), suggesting that *de novo* synthesis *in situ* may contribute to the cytokinin level in these tissues (Chen et al., 1985). Perhaps such synthesis, stimulated by reduced levels of root-synthesised cytokinin in plants under root restriction, induces relatively stronger mobilising sinks or stimulates higher rates of cell division, expansion, or both, in these tissues than unrestricted plants.

Leaf number The rate of leaf appearance was similar to those reported under winter light levels (Coleman and Greyson, 1976b), day/night temperatures (Paul, 1984b), and over a range of leaf insertions (Klapwijk, 1981) similar to those encountered during this experiment. There was no evidence of delayed emergence of leaves arising from root restriction (Table 6.10). This result neither supports nor refutes that of the earlier experiment (Chapter 3) as reduction in leaf number was not detected in that experiment until after 67 days of restriction.

Leaf function Root restriction affected individual and total plant leaf area without precipitating the gross changes in leaf function and whole plant development characteristic of other studies into root restriction (e.g. Tschaplinski and Blake, 1985; Hameed et al., 1987). Neither photosynthesis nor leaf water potential was influenced markedly by root restriction. Measured rates of photosynthesis were similar to those published for well watered tomato plants (Saunders, 1991; Zerbi et al., 1990). Altered patterns of partitioning, for example, to the stem and leaves (Table 6.19), without any decrease in dry matter accumulation (Table 6.18) suggest that photosynthesis was not a major response centre during the initial responses of roots to

physical restriction. Small differences in leaf water potential were observed between restricted and split control plants 21 DAI and among restriction treatments at 49 DAI. In both cases the difference was small, and in context of the range of values recorded, of negligible physiological importance (Saunders, 1991).

On the other hand, the protocol for selecting leaves for measurement did not recognise the different growth responses occurring in different leaves on the same plant. Given the specific response of individual leaves to root restriction, it is possible that the measurements of leaf function taken did not accurately reflect the condition of leaves in which expansion rate was undergoing inhibition (i.e. L_3, L_7 and L_{14}) or stimulation (i.e. L_{21}, L_{28}) by root restriction. The measurement protocol for all measurements involved sampling a young leaf in the upper canopy. Consequently, the leaf function measurements can only be interpreted as indicating that no gross perturbations, sufficiently large to influence whole plant function, arose from the root restriction treatments. The leaf function data may not reflect the rate of photosynthesis or the leaf water status of leaves in which expansion was reduced. Future studies must account for localised effects of root restriction on leaf growth at different insertions into the main stem when measuring leaf function.

Source-sink relationships It is unlikely that reduced carbohydrate supply was a factor in leaf area reduction. Restriction did not noticeably reduce photosynthetic activity. The lack of effect on photosynthesis does not support the hypothesis that inhibited root growth leads to an increase of carbohydrate in the leaves with consequent feedback inhibition of leaf photosynthesis (Masle and Passioura, 1987; Neales and Incoll, 1968).

Water and other limiting exogenous resources Water relations measurements were not sufficiently rigorous to exclude the possibility that leaf expansion

was reduced by insufficient turgor resulting from decreased osmotic pressure of the cell (see Dale, 1988; Hsiao, 1973). Nevertheless, the data does not support the hypothesis that growth reduction induced by root restriction in these experiments is mediated predominately through a gross water stress response (Hameed et al., 1987; Tschaplinski and Blake, 1987). Although Hameed et al. (1987) did not report leaf water potential measurements of their root-restricted tomatoes, their observations of wilting and increased diffusive resistance of leaves on restricted plants suggests a water potential approaching the threshold Ψ_P range for stomatal closure in tomato of -0.8 to -1.0 MPa (Brix, 1962; Duniway, 1971). In my study, midday leaf water potential measurements did not approach the threshold Ψ_P range and did not indicate water stress occurred in any treatment throughout the duration of the experiment (Table 6.16).

Several lines of analysis of these and other data provide indirect evidence that water stress was not responsible for the observed growth reductions. As root growth is less sensitive to water stress than shoot growth, SR ratios are smaller in water stressed plants compared to unstressed plants (Bradford and Hsiao, 1982; El Nadi et al., 1969; Gales, 1979; Hsiao and Jing, 1987; Klepper, 1991; Robertson et al., 1990; Sharp and Davies, 1979). The same pattern was observed in tomatoes (Tan et al., 1981). In my study, however, DCR affected root growth more than shoot growth, resulting in a marginal increase in SR ratio (Table 6.19).

Root restriction need not affect plant water relations. Localised drying in the root zone is compensated by increased water uptake in other parts of the root system. In this way, the total flux of water and resulting water relations of the plant remain unaffected (Gallardo et al., 1994; Lawlor, 1973; Saab and Sharp, 1989; Tan and Buttery, 1982). In tomato, Tan et al. (1981) applied water to 50% of the root system without reducing transpiration, photosynthesis, stomatal conductance, or leaf area. Accordingly, little or no

reduction in leaf area would be expected in tomato plants with less than half of their root systems unrestricted. The significant reduction in leaf area (Table 6.18) supports the argument that the change was independent of the bulk water potential of the leaves, and thus indicative of a non-hydraulic signal between restricted roots and expanding leaves.

Water stress also inhibits leaf initiation (Gowing et al., 1990a; Krizek et al., 1985; Marc and Palmer, 1976; Ticha et al., 1985) or delays emergence (Takami et al., 1981). Root restriction did not reduce either leaf number (Table 6.18) or the rate of leaf emergence (Table 6.10), confirming the general trend of other root restriction studies reporting leaf initiation data. For example, Krizek et al. (1985) found no appreciable effect of root restriction on the number of new internodes and leaves initiated or on the rate of leaf initiation in soybean. Takami et al. (1981) reported that water stress increased the duration of leaf expansion in sunflowers. In the present study, the duration of expansion was similar, irrespective of the presence or extent of root restriction (Table 6.8).

Reduced growth resulting from root restriction has also been correlated with reduced availability of either nutrients or oxygen (Mutsaers, 1983; Peterson et al., 1991b). Given the experimental system and the plant material used in this experiment, however, there are no reasons to conclude such resources were limiting. Water, oxygen and nutrients were supplied in non-limiting amounts by an automatic, highly aerated, nutrient dispensing system. Furthermore, as unrestricted root systems were present in all treatments, and as tomatoes can fully maintain leaf function and root integrity under conditions of stress in the root environment (Tan et al., 1981; Taylor and Fenn, 1985), it is highly unlikely that growth responses observed were due to a lack of some exogenous essential factor.

Moreover, in studies supporting the hypothesis that exogenous resources limit growth following root restriction, morphological responses were greater and occurred more rapidly than those recorded in my study. For example, Ruff et al. (1987) reported that total leaf area in tomatoes restricted in a 450 cm^3 container filled with solid medium was 32% smaller after two weeks, compared to plants in 13,500 cm^3 containers. After three weeks in a 25 cm^3 container attached to a hydroponic system, Peterson et al. (1991a) reported a 50% reduction in total leaf area compared to unrestricted plants. Hameed et al. (1987) observed a 60% reduction in total leaf area of tomato seedlings after two weeks in 75 cm^3 containers in a hydroponic system. In my study however, total leaf area of DCR plants at 14 DAI was only 8% smaller than SOC plants 14 DAI, and 10% smaller 28 DAI. At the point of inflexion (≈28 DAI), the area of L_7 leaves, which showed the greatest response to restriction, was 20% smaller in DCR than SOC plants (Fig. 6.5). Furthermore, root restriction in my study resulted in a balanced growth reduction between roots and shoot (Fig. 6.12), which is characteristic of some (Krizek et al., 1985; LaRoche, 1980; Richards and Rowe, 1977a; Richards et al., 1979; Robbins and Pharr, 1988), but not other reports (Al-Sahaf, 1984; Carmi and Heuer, 1981; Hameed et al., 1987; Peterson et al., 1991a; Tschaplinski and Blake, 1985). In contrast to those reports of balanced reductions in root and shoot biomass, this response occurred regardless of the proportion of the total root system being restricted.

In nature, the entire root system is seldom restricted. As stress factors develop in the rhizosphere, plants adapt through compensatory growth and increased efficiency of water and nutrient uptake in other parts of the root system. In my study, shoot growth was reduced despite having unrestricted root sub-systems and the capacity to improve the efficiency of nutrient and water uptake (Tan et al., 1981; Taylor and Fenn, 1985). These data point to the conclusion that root restriction reduces shoot growth via an endogenous regulation system, but that many experiments designed to examine this

phenomenon have created artefacts that appear to implicate exogenous factors.

Root-shoot communication Expansion of specific leaves of restricted plants was reduced despite an unrestricted root sub-system being available with which to 'escape' restriction, and with leaf function (photosynthetic rates and leaf water potential) and normal leaf ontogeny apparently maintained. This response is consistent with the putative role of chemical signals exported from roots to stomata in response to drying soil (section 6.1; Davies and Zhang, 1991, and references therein). Moreover, the concurrent balanced reduction in root growth (Fig. 6.7) suggests that the signal(s) are an important component of the communication mechanism interrelating root and shoot growth.

It is possible that the signal depends on the build up of an inhibitor in the transpiration stream (i.e. a positive signal). Healthy roots were present in the restricted cells; it can be assumed they were metabolically active, and that tissue integrity was maintained (Tan et al., 1981, Taylor and Fenn, 1985). If such a positive, inhibitory signal operates under conditions of increasing root restriction, a steady reduction in leaf area expansion and possibly leaf function along the entire stem axis should be expected. Moreover, if the inhibitor was a compounding factor, then reductions in growth would also compound. Accordingly, a progressive decrease in individual leaf area would be expected. The decreasing rate of leaf expansion in 'restricted' leaves developing from 0-21 DAI is consistent with a positive signal (Fig. 6.6).

On the other hand, the higher R_A of leaves expanding after 21 DAI (Tables 6.14, 6.15) in restricted plants compared with unrestricted plants, together with the absence of marked changes in photosynthetic rates and leaf water potential, are inconsistent with a positive (inhibitor) signal model. In

addition, individual leaf growth from subsequent leaf groups did not exhibit progressive decreases in leaf area. This latter response parallels that reported by Mutsaers (1983, Fig. 4), in which the relative reduction in final area between successive leaves was similar, despite increasing root restriction. These results should be compared to those of Clough and Milthorpe (1975) with tobacco plants, in which the role of a positive inhibitor is well supported. In their study, leaf area at higher stem positions was progressively reduced as water stress increased; the area of leaves at positions 6-12 was 39 to 95% smaller than leaves at the same positions on fully irrigated plants.

Reduced leaf expansion during periods of root restriction could be due to reduced supply and transport of a chemical signal to the shoot system, i.e. a negative signal. Reduced expansion of leaves in groups L_3, L_7, and L_{14}, followed by recovery and stimulated expansion in L_{21} and L_{28} leaves, are consistent with a system in which a root produced factor (RPF) necessary for growth is available in limiting and then stimulating amounts. Insufficient data exist to identify whether such a process was occurring, but it is useful to consider the possibilities. Despite having unrestricted sub-systems, roots of SCR and DCR plants may have continued to grow in the restriction cells until a common level of restriction was reached. The similar dry root biomass in the restrictive cells of these plants (Table 6.4) supports this suggestion. During this phase (phase 1), synthesis or transport of the postulated RPF to the shoot system may have been reduced or inhibited as the rate of root elongation was progressively reduced by the increasingly physically restrictive environment. As a result, expansion of leaves was retarded (Tables 6.7, 6.7, 6.8). Once the common level of root restriction was reached, growth was presumably diverted to the unrestricted sub-systems because of their more favourable local environment (Gersani and Sachs, 1992), as indicated by the comparative differences between the dry biomass data of the restricted and unrestricted systems (Table 6.4). Enhanced growth (e.g. increased rates of

root elongation) of the unrestricted sub-system would have lead to the resumption of the supply of the RPF to the shoot system (phase 2), and as a consequence, the uninhibited growth of leaves expanding at this time. The kinetics of this proposed sequence were not, unfortunately, studied. However, the observed reduction in area of L_3 and L_7 leaves after 13-19 days growth (Table 6.7, Figs. 6.2, 6.3) and the absence of reduced leaf area in L_{21} leaves (Fig. 6.5c) suggests that phase 2 started about 33 DAI (21+13).

The spatially and temporally distinct responses of leaf expansion do not lend total support to a communication model in which both types of signals are involved, with leaf expansion being a function of their relative balance. The reduced expansion of leaves that commenced development between 0-21 DAI (Table 6.7, Figs. 6.2, 6.3) is consistent with a high inhibitor:promoter ratio. Such a ratio would have arisen as root restriction induced synthesis and transport of a growth inhibitor (positive signal) to the shoot system, while reducing the 'normal' output of a growth promoter (negative signal). However, the enhanced expansion of leaves developing after this period (Tables 6.14, 6.15; Fig. 6.5d), coincident with the development of a new, unrestricted root sub-system, and by implication, resumed export of growth promoter, is not consistent with a dual signal model. Resumption of export of a growth promoter would likely only decrease the inhibitor:promoter balance, particularly since the restricted root sub-system would still be exporting the inhibitor.

An interaction between the RPF and the assimilate accumulating in the shoot may have stimulated leaf growth. When severely restricted plants of tobacco were transferred to larger pots (de-restricted), previously reduced photosynthetic rates were rapidly restored to 'normal', with rates of CO_2 uptake and RuBP carboxylase activity frequently exceeding those of the non-restricted plants (Herold and McNeil, 1979). These authors proposed that the accumulated starch was mobilised, providing an immediate source of carbo-

hydrate building blocks. This resulted in anabolic activities to occur at rates that were not possible in unrestricted plants. My study did not continue long enough to determine the duration of enhanced expansion. It is possible that a sustained growth advantage was obtained, as found for stem splitting. Alternatively, L_{21} and L_{28} leaves may have reached full expansion sooner, because of enhanced rates of expansion. As compensatory growth cycles reported for root and/or shoot pruned plants diminish as the SR balance is regained (Abod and Webster, 1989; Buttrose and Mullins, 1968; Carmi and Heuer, 1981; Gilliam et al., 1986), the latter outcome seems more likely.

Negative signals have not received wide support in the literature, but the arguments provided are equivocal. Initially proposed to explain root-shoot responses to waterlogging (Cannell and Jackson, 1982), they were subsequently applied to explain responses to soil drying (Davies and Zhang, 1991). In reviewing root-shoot communication models under anaerobic root environments, Jackson (1990) concluded that negative signals lacked sensitivity to respond to oxygen stress, especially when only part of the root system was inundated. However, in both papers cited by Jackson (Drew et al., 1979; Jackson and Campbell, 1976), the time between inundation of part of the root system and assessment was sufficient for compensatory growth to have occurred in the unstressed part of the root system. Davies and Zhang (1991) also overemphasise the case against negative signals. They argue that any reduction in levels of a putative substance normally moving from root to shoot would be too small for the shoot to 'read' with sufficient precision. Yet in all the studies cited by these authors in which leaf conductance or growth was reduced, more than 30% of the root system was dehydrated. Jones (1990) considered that a negative signal would not have the required information content by itself; the magnitude of the signal would vary with root volume, whether or not some roots were stressed. Jones (1990) concluded that such a signal would need to be combined with other information, such as total root volume. Indeed, if the synthesis or transport of the signal is linked to the

rate of root extension (as suggested in Chapter 3), or is under correlative control within the root system, or both, then the magnitude of the signal would reflect the prevailing edaphic conditions throughout the entire root system. Extending Gersani and Sach's (1992) suggestion that an hormonally-driven mechanism was responsible for correlative relationships within a root system, if conditions for root extension are non-limiting throughout the entire root environment, synthesis and transport would be unimpeded. When conditions in part of the environment impeded root extension, the extent of synthesis and transport would be an integrative function of the conditions in the more favourable part of the environment and the influence of the other roots of the plant. Finally, given the considerable elasticity in root and shoot function (e.g. Ovaska et al., 1992; Tan and Buttery, 1982; Tan et al., 1981), the speed and precision of the response expected for stomatal control are probably greater than those expected for maintaining a balance between root and shoot growth that is appropriate to the prevailing environment.

Stem wounding Splitting the stem slightly reduced the expansion of those leaves present (Fig. 6.6b). On the other hand, split-stem plants commenced growth earlier than intact stem plants (Figs. 6.2, 6.3), and the rate at which leaf expansion increased to its maximum (κ), was higher in split than intact plants (Table 6.6). Splitting the stem enhanced final total leaf area and overall size (Table 6.18). It is unclear whether this growth was stimulated by incision of the stem, or arose through delayed growth following recovery from the incision. Richards and Rowe (1977a) suggested a functional relationship existed between shoot growth and growth substances produced by root apices. In propagating apple (*Malus pumila*) rootstocks from hardwood cuttings, Howard (1981) and Howard and Harrison-Murray (1982) promoted root initials by splitting the base of the cutting. Milligan and Dale (1988) noted that leaf expansion following excision of root tips coincided with appearance of new roots (apices). It is possible that splitting the tomato cuttings increased the number of root initials. This may have stimulated

production of growth substances (e.g. cytokinins; Forsyth and van Staden, 1981), providing an additional stimulus to growth. Certainly, the approximate time (21 DAI) when the development of new root initials in the SCR and DCR plants was first observed coincides with the time those leaves whose expansion rates were later observed to be higher in restricted plants first started expanding. Plant propagation and nursery production may benefit from further, more critical evaluation of this hypothesis.

Growth analysis methodology Thornley and Johnson (1990) preferred the Chanter function to the Richards function because the parameter ν was non-physiological and, in their experience, the Richards function was very sensitive to variation in ν. Although no sensitivity analysis was conducted, parameters derived from ν (Table 6.7) had CVs within acceptable bounds (Simpson et al., 1960). Furthermore, the highly variable estimates of the D parameter severely weakened the explanatory power of the Chanter function. Without other reports using Chanter function, it is unclear whether this is a characteristic of the function or this data set. Overall, the Richards function provided more consistent and revealing information than the Chanter function.

Summary In conclusion, this study has shown that responses to root restriction are specific in relation to time and leaf position on the stem (space). Restricting all or part of a plant's root system initially reduces rates of expansion of individual leaves. Expansion rates of leaves developing later are not affected by the presence of a restricted root sub-system. In some instances leaf expansion rates of 'restricted' plants were higher than unrestricted control plants. The spatiotemporal nature of these results is best explained by a root-shoot communication model which includes a negative, root produced signal acting in combination with a factor produced by the shoot. Further research is required to determine the role of correlative inhibition between restricted and non-restricted sub-systems of roots in the

propagation of the signal and growth recovery. Supply of exogenous factors such as water, nutrients, and oxygen was not limiting in this experiment. Indeed, experiments in which these factors were implicated are likely to have been confounded by artefacts. In this regard, experimental systems using split-root systems for root restriction studies appear to avoid such problems, and offer an improved technique for studying effects of root restriction on whole plant growth.

Chapter 7

General discussion

7.1 General discussion

The dynamism of plant response to root restriction and the plasticity of 'root restriction' as a treatment were highlighted throughout this study. Reduced root number preceded reductions in root length or dry biomass and shoot growth of plants in cells restricting root growth (Chapter 3). Although total leaf area was reduced in all root restricted plants (Chapter 3, 6), the extent of this reduction varied between leaves on the stem (Chapter 6). Such temporal responses to root restriction exposed the fragility of conclusions made from root restriction experiments involving only a single harvest. The multiple harvest approach taken in the current study overcame the single harvest problem, as did assessment (using multivariate techniques) of changes in within-plant relationships over time. Atkinson et al. (1989) argued that it was difficult to make realistic interpretations of the importance of instantaneously determined parameters in influencing control of growth, as it is a process that is integrated over time. The estimates of instantaneous parameters obtained using the hybrid functional approach to growth analysis developed in my study avoid this criticism because they arise from a fitted function that describes the progression of growth of an individual plant through time. Thus, growth responses to root restriction that might feed back through the growth process and influence subsequent response (Morrison, 1991) are accommodated by the fitted function.

The question of the usefulness of instantaneous measurements of plant function can be extended further. The possibility cannot be discounted that root restriction caused reduced leaf expansion via water stress or that reduced photosynthetic rates resulted in lower total plant biomass. Measurements of photosynthetic rates and leaf water potential discounted major perturbations in these processes (cf. Hameed et al., 1987; Tschaplinski and Blake, 1985), but were not sufficiently sensitive to detect mild, persistent reductions in either process resulting from root restriction. This criticism

accepted, it nevertheless remains difficult to decide on the appropriate level of measurement in studies at the whole plant or crop level in the organisational hierarchy of plant science research (Thornley, 1980). As Evans (1976) foreshadowed, integrating observations of photosynthesis, water status or transpiration made over periods of minutes to a plant or crop whose growth is measured in weeks, months or years has yet to be adequately resolved. The issue is further complicated by considerable variation during the day in stomatal responsiveness to water stress (Tardieu and Davies, 1992). Moreover, several authors (e.g. Evans, 1976; Hunt, 1978; Passioura, 1979; Thornley, 1980; Trewavas, 1986; Wallace and Zobel, 1994) have argued that an overly reductionist approach to plant science may actually remove the integrative picture of plant growth from sight. Thus, just as equipment capable of measuring the photosynthetic rate or water flux in real time have their place in the armoury of plant scientists, investigations into whole plant response to restriction will continue to find analysis of integrative plant growth more interpretable.

Allometric relationships Applying Causton and Venus's (1981) theorems revealed that an allometric relationship between root and shoot was not physically possible (Chapter 3). Richards (1969) considered that there was no *a priori* reason to suppose that during development the relative growth rates of different parts or dimensions should remain proportional to one another. The relative growth rate of an organ measures the efficiency by which that organ can produce new material. Strictly speaking, it is only of use during the early stages of plant growth when all tissue is contributing. From a physiological standpoint, the relative growth rate of an organ which consists largely of tissue that is mature and unable to grow is meaningless (cf. de Wit and Penning de Vries, 1983). Non-respiring tissues accounts for a higher proportion of total biomass of woody plants than of annuals and herbaceous perennials (Schulze, 1983). Early attempts were made to bypass this problem by defining a relative growth rate for only meristematic tissue

(Brouwer and de Wit, 1968), but knowledge of morphogenetic processes is too fragmentary to make this approach useful. When comparing the relative growth rates of different organs, a required, but infrequently acknowledged, assumption is that the proportion of growing tissue to total tissue is the same in both shoot and roots. There have been no apparent attempts to test this assumption. Indeed, it is difficult to accept that the proportion of actively growing tissue in shoots and roots is similar. Using tomato as an example, its fibrous root system could be expected to have a high proportion of actively growing tissue. On the other hand, the proportion of actively growing tissue in its shoot system is likely to be lower given the higher proportion of structural tissue.

Moreover, relative growth rate is, at best, a measure of *apparent* sink strength. For example, as a large proportion (40-60%) of assimilate imported by a root sink is used for respiration (Farrar, 1985; Lambers, 1988; Lambers et al., 1991) and a significant proportion (~5-10%) lost as root exudates and through root turnover (Atwell, 1990b; Keith et al., 1986; Lambers, 1988;), discussion of sink strength in terms of net accumulation rate of dry biomass fails to assess the true ability of the sink organ to receive assimilate (Ho, 1988). In other words, the *actual* sink strength of the root is probably considerably higher than that implied by the relative growth rate. Consistent with this, Ho (1988) concluded that Warren Wilson's (1972) definition of sink strength as the product of sink size and sink activity was flawed. Ho (1988) reasoned that sink size was better defined as the physical constraint and sink activity the physiological constraint upon a sink organ's assimilate transport. Indeed, Watson and Casper (1984) find sufficient evidence in the literature to question the validity of dry biomass weight as a measure of allocation, preferring instead the use of fixed carbon to reflect patterns of biomass allocation in response to environment.

This issue aside, many authors regard the allometric coefficient *k* as a useful summary variable. Hunt and Nicholls (1986) concluded that *k* was sufficiently stable to represent the whole of the vegetative phase of growth without serious loss of information, while remaining responsive to genetic and environmental influences. Causton and Venus (1981) extended Warren Wilson's (1972) notion of relative growth rates as measures of sink activity, suggesting that *k* summarised the ratio of sink activities of the two components. In the current study, however, interpreting *k* required previous interpretation of the univariate analyses of the variables being summarised through the allometric approach. An allometric coefficient significantly greater than unity is usually interpreted as a reflecting a higher demand for assimilate by the dependent variable compared to the independent variable (Causton and Venus, 1981; Nelder, 1963). Thus, under intense root restriction (i.e. CR), plants appeared to partition relatively more assimilate to leaves and stems than the roots, a result similar to that reported by Richards et al. (1979). Yet as both the univariate and CDF analyses revealed (Tables 3.5a, 3.6), root growth of CR plants was significantly lower than other treatments by 31 DAI, whereas stem dry biomass weight and leaf dry biomass weight was not significantly lower than other treatments until 67 DAI. The correct conclusion, therefore, was that the allometric coefficient reflected a lowered demand for assimilate by roots.

The conclusions drawn from the study of the allometric relationships between roots and the components of shoot growth also revealed changes in the partitioning behaviour of the plants. For example, stems and leaves tend to receive proportionately more assimilate than roots up to 67 DAI, after which roots were favoured. This observation is consistent with the high stem density-high leaf biomass-low root density CDF extracted from the 67 DAI data (Table 3.6). On the other hand, the observed reversal of partitioning (Table 3.14) was an inevitable consequence of dissimilar rates of decline in the relative growth rates, with the rates of decrease of R_{ST} and R_L faster than

R_R (Table 3.11). Changes in allometric relationships, often occurring abruptly, have been previously described, but have been associated with morphological switches such as the onset of flowering or fruiting (Richards, 1981; Troughton, 1956, 1960), phenomena avoided in the current study by removing flower clusters. It is possible that the fitted linear segments (Fig. 3.14) are poor estimates of a curvilinear allometric relationship (Barnes, 1979), reflecting a decrease in the proportion of actively growing tissue in shoot tissue to that in roots (Causton and Venus, 1981), or dissimilar rates of maintenance respiration (Barnes, 1979). Both Hansen and Jensen (1977) and Veen (1981) reported higher respiration rates in roots than shoots. Alternatively, it is possible that bias may have been introduced into that analysis through the subjective segmentation of the data. Others might be tempted to fit a single line over more harvests, arguing that the deviations are minor. Certainly, regression is used to 'smooth out' variations in response (Myers, 1990).

On the other hand, if we accept that leaf and stem growth are closely linked with root growth, then smoothing out variations will mask consequences of this close link and deny identity of an 'odd occurrence' (Popper, 1972). Comparing the data in Table 7.1 with those in Table 3.14 shows that for components i and j, the allometric coefficient k describes the ratio of their *mean* relative growth rates between times t and $t+1$, but provides no information of short term movements in the relative growth rates within this duration. As Poorter (1989) has shown, such changes do occur. This has important consequences when interpreting treatment responses. First, the major changes in partitioning patterns indicated in plots of the logarithms of the variables of interest may not be signalling real changes in partitioning or changes in relative sink strengths. The plots of stem, leaf dry biomass weight, and root dry biomass weight (Figs. 3.3, 3.5, 3.6) suggest that a major change in partitioning patterns between UR and CR plants occurred between 45 and 67 DAI. However, dissimilar rates of decrease in the relative growth rates

of stem, leaf and root dry biomass resulted in R_R exceeding that of R_L and R_{ST} between 67 and 99 DAI (Fig. 3.11, 3.12). As a consequence, the SR ratio declined (Fig. 3.12, Table 3.10), and the value of *k* fell below unity (Table 3.14). Second, as a consequence of these unequal rates of change in RGR, the value of *k* is not constant, as conventional application of allometry assumes. Third, because *k* is a mean value, short term variations in partitioning patterns between root and shoot are likely to be masked if the period covered by the data is too long. For example, despite observing stimulated vegetative growth upon releasing tomato plants from root restriction, Richards (1981) reported similar values of *k* in restricted plants and those released from restriction. In this example, the experiment lasted 140 days. In calculating *k* over this duration, Richards (1981) was effectively calculating the ratio between the mean shoot relative growth rate over 140 days and the mean root relative growth rate over the same period, i.e.:

$$k = \frac{\dfrac{\log_e W_{S(t=140)} - \log_e W_{S(t=0)}}{(140-0)}}{\dfrac{\log_e W_{R(t=140)} - \log_e W_{R(t=0)}}{(140-0)}}$$

Thus, it was not possible for the allometric analysis to reflect short term divergent movements in the relative growth rates of shoot or root.

On a broader level, the classical growth analysis in the current study did not detect differences in R_W among treatments. Nevertheless, R_W must have differed at some stage because similarly sized plants at the beginning of the experiment were significantly different in size at its conclusion. This lack of definition has been highlighted by other workers (e.g. Poorter, 1989; Poorter and Lewis, 1986). The lack of definition provided by the classical analysis in the current study was probably due to the 14 and 18 day gaps between harvests. Although allometric analysis does not arrive at its estimate of *k* via

Table 7.1 Estimate of the allometric coefficient, k, from the ratio of relative growth rates (R) of components i and j ($i \neq j$), using the non-paired method of Causton and Venus (1981; equation 3.5) with block-adjusted data (cf. Table 3.15).

Model variables (i/j)	Interval (DAI)	R_i	SE_i	R_j	SE_j	R_i/R_j (= k)
Unrestricted (UR)						
Leaf/root	13–67	0.110	0.0018	0.095	0.0024	1.16
	67–99	0.044	0.0015	0.049	0.0023	0.89
Stem/root	13–67	0.119	0.0018	0.095	0.0024	1.25
	67–99	0.045	0.0023	0.049	0.0022	0.92
Leaf/stem	13–99	0.085	0.0012	0.092	0.0010	0.93
Restricted (CR)						
Leaf/root	13–67	0.104	0.0014	0.080	0.0022	1.30
Stem/root	13–67	0.117	0.0014	0.080	0.0022	1.46
Leaf/stem	13–67	0.104	0.0014	0.117	0.0014	0.88
De-restricted (RD)						
Leaf/root	13–31	0.140	0.0061	0.099	0.0062	1.40
	31–99	0.069	0.0015	0.069	0.0016	1.00
Stem/root	13–31	0.136	0.0059	0.099	0.0062	1.37
	31–99	0.078	0.0008	0.069	0.0016	1.13
Leaf/stem	13–31	0.140	0.0061	0.136	0.0059	1.03
	31–99	0.069	0.0015	0.078	0.0008	0.88
Cyclic restriction (RDD)						
Leaf/root	13–31	0.131	0.0085	0.092	0.0082	1.42
	31–45	0.111	0.0126	0.115	0.0112	0.97
	45–67	0.062	0.0067	0.042	0.0067	1.47
	67–99	0.047	0.0031	0.057	0.0033	0.82
Stem/root	13–67	0.110	0.0026	0.078	0.0025	1.41
	67–99	0.051	0.0042	0.057	0.0025	0.89
Leaf/stem	13–31	0.131	0.0085	0.131	0.0065	1.00
	31–45	0.111	0.0126	0.148	0.0116	0.75
	45–99	0.053	0.0023	0.058	0.0025	0.91

the same route, it is nevertheless estimating a value based on durations far in excess of 14 and 18 days.

Moreover, as the ratio of two lines, each constructed from points at their extremities, is a straight line, the marked absence of reports of no allometric relationship between plant components is more readily understood. Other workers have recognised the coarseness of definition offered by allometric analysis, but have viewed it much more positively. Hunt and Nicholls (1986) argued *k* was a more useful measure of partitioning over a period of study as it was not simultaneously subject to genetic, ontogenetic and environmental control in the same way that instantaneous ratios between root and shoot size were subject. The difficulty in this view, and that of others who have reported a constancy of *k* over seasons and vegetative and generative growth, is that the degree of constancy becomes a function of the period being reviewed. Viewed over several short consecutive periods, *k* may be expected to show major deviations according to the treatments imposed (e.g. Table 3.14), whereas viewing the same data as a single period, *k* will 'appear' stable. Given these factors, the general utility of allometric analysis must therefore be questionable. Indeed, and in contrast to Thuantavee's (1991) conclusion, detecting short term changes in partitioning patterns between different organs is arguably one of the few useful applications of linear allometry, if only because it is arithmetically more efficient than calculating the respective relative growth rates.

Integrating the effects of root restriction into a model The emphasis placed in Chapter 6 on the possible nature and origin of the root signal responsible, directly or indirectly, for the reduction in leaf expansion in root restricted plants does not assume a single hormonal control system. In most, if not all, hormonal messenger systems, multiple rather than single hormonal control systems appear to operate (Leopold and Noodén, 1984), a point apparently forgotten in recent reviews of root-shoot communication (Jackson, 1990, 1993).

Root elongation is controlled by auxin (Björkman and Cleland, 1991; Jackson and Barlow, 1981; MacIssac et al., 1989; Wightman et al., 1980; Zeaden and McLeod, 1984) synthesised in the shoot (Rowntree and Morris, 1979). Hence, if R_{RL} influences R_A then we should consider a model with a root produced factor (RPF) and a shoot produced factor (SPF).

If we assume that auxin (or a derivative) is the SPF and cytokinin (or a derivative) is the RPF (given the strong qualitative support for this group of hormones), how consistent are the observed responses with an hormone control system? It is significant that leaf expansion was not increased by root restriction (Tables 3.5a-c; Table 6.8). Individual leaves are both sources and sinks (Ho and Shaw, 1977; Nielsen and Veierskov, 1990; Russell and Morris, 1983), yet a reduction in sink strength of roots is not mirrored by a comparative increase in leaf expansion, despite the close proximity of the source. This suggests that root restriction disturbed another factor(s) crucial to leaf expansion.

An important result in Chapter 6 was the observation of increased partitioning to the stem in DCR plants. Such partitioning is a characteristic of root restricted plants, and is regarded as evidence of a secondary sink operating in the absence of a functioning root sink. In DCR plants, however, a functioning and unrestricted root sink was present (i.e. those roots formed outside the restriction cells). Metabolism of accumulating amounts of a shoot produced factor (SPF) in stems may account for the preferential partitioning towards the stems of DCR plants. This would infer an asymmetric distribution or activity of a SPF along the stem axis. Auxin displays such characteristics under both stress and non-stress conditions. Everat-Bourbouloux and Bonnemain (1980) reported that labelled auxin (^{3}H-IAA) applied to the apical bud of broadbean plants (*Vicia faba* L.) accumulated in the cotyledonary node zone, encompassing the lower half of the first internode and the upper half of the hypocotyl. Changes in hormonal activity in this transitional zone

between the vascular systems of the root and shoot (Esau, 1965) have also been recorded in apple trees following root and shoot pruning (Grochowska et al., 1984; Grochowska and Karaszewska, 1990). Grochowska et al. (1994) applied TIBA to the transition zone of apple seedlings, inhibiting basipetal transport of ^{14}C-1-IAA. The resulting supra-optimal concentration of auxin above the TIBA 'girdle' induced ethylene which, in turn, resulted in leaf epinasty. Jaramillo et al. (1992) presented anatomical evidence from physically impeded roots showing that the structural arrangement of xylem vessels was disrupted at the junction of primary root and hypocotyl. The potential conductivity of this zone was estimated to be considerably less than in zones with normal vessel structure. This observation is consistent with the conclusion of Hameed et al. (1987) that increased hydraulic conductivity in roots of restricted plants was a major reason for growth reduction in root restricted plants. After reviewing the effect of stem girdling experiments, Chalmers (1985) concluded that stimulation of cambial activity above girdles was regulated by auxin turnover. Significantly, working with root-restricted peach rootstocks, Costa et al. (1992) reported enhanced IAA oxidase activity in the lower stem region, a region to which assimilates are preferentially partitioned (Richards and Rowe, 1977a). Such an increase in IAA metabolism in root restricted plants in which cytokinin export to the shoot has been reduced is consistent with reports that cytokinins may protect auxins from degradation. Noor-Saleh and Hemberg (1980) detected increased amounts of IAA in bean plants previously treated with kinetin. A similar response was observed in subsequent studies using roots of bean, maize and oats (*Avena* spp.) irrigated with a kinetin solution (Noor-Saleh, 1981).

There is also circumstantial evidence that auxin is involved in the control loop. Wareing et al. (1968) suggested that decreased competition between leaves for root-produced cytokinins was responsible for observed increases in photosynthetic rates of leaves remaining after partial defoliation treatments. Beever and Woolhouse (1973) reported that decapitation and continual

removal of axillary buds promoted export of cytokinins from the roots. Similar increases following removal of axillary buds (sources of IAA) have been observed in tomato and tobacco (Colbert and Beever, 1981). These reports, and those detailing increases in leaf area, SLA, protein content, Rubisco (Callow and Woolhouse, 1973; Carmi and Koller, 1979) and chloroplast ribosomal RNA synthesis (Callow and Woolhouse, 1973) following bud removal or decapitation, are consistent with demonstrated roles of cytokinin. Interestingly, root restriction reduces all these phenomena.

The postulated model presumes a dominant role for cytokinin in leaf expansion. The stimulated expansion of rapidly growing leaves in transgenic tobacco plants exhibiting enhanced biosynthesis of endogenous cytokinins (Ainley et al., 1993) clearly points to the involvement of cytokinins in leaf expansion. The actual nature of their role is, however, still unclear. Exogenously applied cytokinins stimulate cell division in young leaves (Humphries and Wheeler, 1960), induce rapid leaf cell enlargement (Brock and Cleland, 1989, 1990; Leopold and Kawase, 1964; Nielsen and Ulvskov, 1992; Powell and Griffith, 1960), and can partially compensate the retardative effects of root restriction on leaf expansion (Carmi and Heuer, 1981; Richards and Rowe, 1977a). Largely based on studies involving exogenous application of cytokinins (e.g. Crosby et al., 1981; Fletcher et al., 1970; Gersani et al., 1980; McDavid et al., 1974; Nakata and Leopold, 1967; Richards, 1980; Richards and Rowe, 1977a; Shindy and Weaver, 1970; Shindy et al., 1973; Weaver et al., 1969; Wittwer and Dedolph, 1963; Zack and Loy, 1984), the putative role of cytokinin in leaf growth was its ability to enhance sink activity by diverting and retaining assimilates to treated tissue. More recent studies, however, have cast doubt on this role, with the demonstration that while leaf expansion in pepper leaf disks is stimulated by BA, it occurs without significant changes in sucrose or leucine uptake, the concentration of fructose-2,6-bisphosphate (a promoter of glycolytic metabolism; Black et al., 1987), activities of acid invertase or phosphofructokinase, or partitioning of

photosynthates (Ulvskov et al., 1992; Nielsen and Ulvskov, 1992). It is also important to note that the use of BA or kinetin, often at unphysiologically high concentrations (e.g. Carmi and Heuer, 1981; Richards and Rowe, 1977a), in many of these studies limits their value in elucidating the role of cytokinins in leaf expansion because the metabolism of BA and kinetin is different to that of zeatin-type cytokinins (the dominant group of endogenous cytokinins; Letham and Palni, 1983) (Fox et al., 1973; Letham, 1994). Threshold ratios or direct interaction with other hormones may be required for response. Gibberellins, for example, promote leaf expansion (Brock and Cleland, 1990; Steffens et al., 1985; Ulvskov et al., 1992), and applying both gibberellin and cytokinin to root-restricted bean plants restores the growth of leaves and stems (Carmi and Heuer, 1981).

While the functional link between cytokinins and leaf expansion currently remains obscured, recent developments in three different areas give some hope of progress. First, a correlation between the rate of leaf expansion and the spatial distribution of endogenous cytokinins in leaves has been demonstrated (Ulvskov et al., 1992). The distribution of zeatin and zeatin riboside in rapidly expanding leaves of pepper was correlated with the rate of leaf expansion which was high in the basal leaf tissue and low near the root apex. Second, developments in understanding of the biophysical components of cell growth indicate that cytokinin (albeit BA) promotes leaf cell expansion by increasing cell wall extensibility (i.e. the relaxation ('loosening') of the cell wall prerequisite to its irreversible expansion; Cosgrove, 1993) (Brock and Cleland, 1989, 1990; Cleland, 1986; Tomos and Pritchard, 1994). Taylor et al. (1993) reported that the reduced leaf area and expansion rates of shrub willow (*Salix viminalis*) grown under sub-optimal supplies of nitrate were due to a reduction in cell wall extensibility. This result links the mechanism of leaf expansion to the third area of recent development, the relationship between nitrogen supply, cytokinin levels and assimilate partitioning. Kuiper (1988) and Kuiper et al. (1988, 1989) attributed the rapidity of growth

response (e.g. R_w, SR) of plantain (*Plantago major* ssp *pleiosperma*) to changes in nutrient regimes as being mediated by cytokinins. Samuelson et al. (1992), working with barley (*Hordeum vulgare*) reached a similar conclusion, although suggested that cytokinins responded to short-term rather than long-term changes in nitrogen supply. The concentration of trans-zeatin and trans-zeatin ribosides in roots and the daily fluxes of these hormones from roots to shoots in stinging nettle (*Urtica dioica* L.) were correlated with SR (Beck and Wagner, 1994; Fetene et al., 1993; Wagner and Beck, 1993) and similarly responded to nitrogen supply. These results suggest that reduced N concentrations result in reduced cytokinin synthesis and delivery to the shoots. Taken together, the advances in these three areas reaffirm a role for cytokinin in leaf expansion. More study is required, however, before the relative contributions to cell division and cell expansion of this role can be assessed. For example, the zones of rapid expansion in sweet pepper leaves in which high concentrations of cytokinins were found (Ulvskov et al, 1992) were likely to have contained both dividing and expanding cells.

The relationship described between the relative rate of root elongation and the relative rate of leaf expansion (Chapter 3) is a simple empirical model. Although some workers regard all empirical models of negligible value (Wilson, 1988), others are less dismissive, recognising that the deductions from empirical models become the hypotheses for further study and ultimately progress (Pease and Bull, 1992). Moreover, the line between empirical and mechanistic models is often blurred. For example, Thornley (1976) observed that most modelling exercises contain elements of both empiricism and mechanism, and McNaughton et al. (1985) acknowledged that empirical models often have, at their centre, a notional state of plant development that progresses continuously in time. The importance and need to validate statistical models was emphasised in Chapter 4. In keeping with the 'working hypothesis' status of a scientific theory, validation is assumed to mean a failed attempt at falsification (Popper, 1959, 1963). Indeed, as

Johnson et al. (1991) reflect, all models in science are probably false in a rigorous sense, yet their value in defining principles, providing clarity in analysis and aiding interpretation is enormous. The specific mechanism by which root restriction might regulate shoot growth in tomato cannot be determined from the data presented in the current study. Thus, the following model explaining the mechanism through which root restriction influences shoot growth seeks only to integrate ideas and to act as a guide to future experimental work.

This model is based on a control loop first considered by Thimann in 1937, and subsequently refined by Sachs (1972). Under steady-state conditions, auxins synthesised in the shoot are transported towards the roots where, in low concentrations, they stimulate root growth. In turn, cytokinins synthesised in the root system are exported to the shoot where they promote growth. When roots become physically restricted or their rate of elongation is reduced (Chapter 3), or both, auxin concentration within the root tissue increases (Lachno et al., 1982; Saugy and Rivier, 1988), possibly as a result of reduced metabolic activity, and root elongation declines further. As auxin can stimulate cytokinin degradation in a range of plant tissues (Palmer et al., 1984; Palni et al., 1988), it is possible that the increased concentration of auxin in the root apex may increase degradation of cytokinins produced in the quiescent centre (Clowes, 1969; Feldman, 1980; Short and Torrey, 1972). Alternatively, or in conjunction, synthesis of cytokinins may be reduced through a decline in the metabolic activity of the impeded root apex. Either path would result in decreased export of cytokinins from the root system, as found by Bangerth (1994), and as a consequence, their concentration in xylem and phloem tissue (Komor et al., 1993) would decline. Without their 'protection' (Bourquin and Pilet, 1990; Noor-Saleh, 1981; Yip and Yang, 1986), auxin in the transition zone between the vascular systems of the root and shoot is metabolised (Costa et al., 1992). This turnover of auxin and the resultant stimulation of cambial activity (Chalmers, 1985) would increase the

sink strength of the stem (Wyse and Saftner, 1982). With both a root sink weakened by reduced growth (elongation) and a leaf sink weakened through reduced expansion (Chapter 3) due to a deficiency of cytokinins needed for expansion (cf. Ulvskov et al., 1992; Zhang et al., 1995), the basal section of the stem becomes a dominant sink, and assimilate is preferentially partitioned to it (Chapters 3 and 6; Carmi and Shalhevet, 1983; Peterson et al., 1991a; Richards and Rowe, 1977a). The high concentrations of auxin either in the root tissue or at the transition zone would stimulate the synthesis of ethylene (Dubucq et al., 1978). The observed development of adventitious roots and epinastic leaves (Chapters 3 and 6) are consistent with reported responses to both hormones at elevated concentrations (Davis et al., 1988 and references therein; Woodrow et al., 1988). Alternatively, the ethylene might arise within the root system due to the physical impedance (Kays et al., 1974; Sarquis et al., 1991; Sarquis et al., 1992; Whalen, 1988).

Although this model nominates auxin as the SPF, it is also possible that root-synthesized cytokinin might cycle back, via the phloem (Komor et al., 1993), to the roots and regulate cytokinin biosynthesis. Thuantavee (1991) stimulated growth of tomato plants by applying a low concentration (10^{-8}M) of BA to the root system, and inhibited growth with a higher concentration (10^{-6}M). She postulated that cytokinin was part of the feedback loop responsible for manipulating *de novo* synthesis of cytokinin in the root system. Alternatively, if loading of cytokinin into the xylem stream is slower than its rate of synthesis, synthesis could be feedback-inhibited by the increasing concentrations in the root tissue.

Lockard and Schneider (1981) proposed a model similar to that developed here to explain the dwarfing mechanism of scion-rootstock combinations. If this model was valid and either cytokinin biosynthesis or export, or both, in roots was positively controlled by a shoot-produced factor, then assuming that vigour is a function of hormonal output, interactions in growth response

between scion and rootstock would be expected. Weak scions on vigorous rootstocks should produce vigorous trees, while weak scions on weak rootstocks would produce dwarf trees. On the other hand, vigorous scions would be expected to 'cancel out' vigorous rootstocks, yet have little effect on weak rootstocks. It is interesting that grafting studies involving a wide range of species have revealed that few such interactions occur, with both scion and rootstock contributing to the growth of the shoot (de Vries and Dubois, 1989; Lehman et al., 1990; Tubbs, 1977, 1980; Zijlstra and Nijs, 1987; Zijlstra et al., 1994). Moreover, the results from these grafting studies contrast uncomfortably with recent demonstrations of a possible regulatory role for auxin on cytokinin concentrations in shoot tissue. Bangerth (1994), for example, reported that applying naphthaleneacetic acid (NAA) to the decapitated shoots of bean seedlings almost completely eliminated the rapid increase in cytokinin concentration in the xylem exudate that followed decapitation. Zhang et al. (1995) also provide evidence of auxin-induced down-regulation of cytokinin levels through their demonstration that applying auxin to transgenic tobacco tissue expressing an *ipt* gene (encoding enzymes for cytokinin biosynthesis) promoted conversion of zeatin-type cytokinins to adenine derivatives.

Contrary to the general interpretation of others (e.g. Jackson, 1990, 1993; Wilson, 1988) of root-shoot communication models involving cytokinins, there is no implicit notion in the model postulated here that the potential supply of cytokinin to the shoot system is proportional to the number of active root apices. The important difference in this model is that it focuses on elongating and, by implication, biosynthetically active root apices. A decrease in root elongation, caused by physical restriction of part of the root system, would only be expected to result in decreased cytokinin delivery to the shoot system, and consequently reduced rates of leaf expansion, if elongation of the whole root system was impeded (Chapter 3). Provided roots in other parts of the root system were physically free to grow and were not correlatively inhibited

by the restricted sub-system, reduced activity of root apices in one part of the root system would result in increased activity of those in unrestricted portions, and initiation of new apices, possibly by the SPF, elsewhere in the root system (Chapter 6).

Jackson (1993) was critical of studies concluding links between root function (mineral acquisition and cytokinin synthesis) and shoot growth. He argued that models of root to shoot communication based on either of these factors were weakened by reports of large reductions in root size, achieved by volume restriction or pruning, apparently sustained with little change in shoot growth, and others in which increases in cytokinin levels and shoot growth occurred after root apices were removed. Taking Popper's (1959, 1963) view of validating an hypothesis, it is a useful exercise to re-evaluate Jackson's (1993) falsification of the hypothesis that root-synthesised cytokinins are significant components of root-shoot communication in view of the conclusions of the study reported here. Jackson (1993) cited Tschaplinski and Blake's (1985) study, in which they reported a reduction in the dry biomass of alder seedlings from 1.73 g to 0.70 g following extended (96 days) restriction in a small container without any accompanying decline in leaf area as evidence of the apparent insensitivity of shoot growth to a loss in the putative output of growth-promoting cytokinin. Buttrose and Mullins' (1968) study in which roots of grape vine (*Vitis vinifera*) were pruned to about 75% of the volume of unpruned control plants every week for 8 weeks without any statistically significant effects on shoot growth, and Carmi and van Staden's (1983) study in which large decreases in cytokinin concentrations in leaves and petioles of decapitated bean seedlings that occurred 8 days following removal of two-thirds of the root system were not accompanied by statistically significant changes in leaf area were similarly presented as evidence against the putative role of root-sourced cytokinin in shoot growth. In all these studies, the concerns raised by Jackson (1993) do not acknowledge the possibility of a spatiotemporal response to stress-induced changes in root growth and

function. It is likely that use of total plant leaf area in Buttrose and Mullins' (1968) and Tschaplinski and Blake's (1985) study masked treatment-induced changes in younger, expanding leaves. Although Jackson (1993) noted that in Carmi and van Staden's (1983) study reductions in total leaf area had not occurred within 8 days of partially excising roots, a significant decline in leaf area had occurred by the time of the next harvest (16 days). As reported in the present study, decline in total leaf area is not detected until some time after reduction in root growth occurs (Tables 3.5a-c), yet reduction in individual leaf growth is likely to be observed much sooner, particularly if the leaf is rapidly expanding at the time of onset of root restriction (e.g. Table 6.7).

Additionally, Jackson (1993) appears to assume that a reduction in its dry biomass signals a reduction in root length and number, and that a root system is an homogenous 'tissue'. As noted previously, root systems are heterogenous in size and function (Waisel and Eshel, 1991; Zobel, 1992b). The relationship between root number and dry biomass is not constant (Tables 3.5a-c; Richards and Rowe, 1977a) and neither should this be expected. Both physical and chemical stress, for example, can cause major alterations to the distribution of root size and type. Physically impeded roots have a greater proportion of lateral and sub-lateral roots compared to unrestricted roots (Chapter 3; Barley, 1962; Goss, 1977; Goss and Russell, 1980; Lachno et al., 1982; Ran et al., 1992; Veen, 1982; Wilson et al., 1977). Similar changes in distribution of root size occur when root apices are chemically pruned by copper coatings on the inside of containers. Such perturbation results in large changes in root dry biomass without concurrent changes in shoot growth (Gilman and Beeson, 1995).

Both ABA and gibberellins are absent from the postulated model. As root-sourced ABA plays an important role in shoot growth and physiology and gibberellins influence leaf growth, the reasons for their exclusions deserve

expansion. In addition to influencing stomatal aperture and plant water relations (Correia et al., 1995; Walton et al., 1976; Zhang and Davies, 1990a), ABA can increase sink strength when applied locally (Brenner, 1987; Karmoker and van Steveninck, 1979; Patrick, 1990) or when it accumulates at a certain site (Saab et al., 1990). Abscisic acid is synthesized in tomato roots, and only a mild water stress is required to stimulate biosynthesis in hydroponically grown plants (Cornish and Zeevaart, 1985; Parry et al., 1992). The role for ABA in reducing leaf expansion is, however, very weak. Zhang and Davies (1990b) observed a negative log-linear relationship between leaf extension in maize and sunflower leaves and ABA concentrations in xylem sap. However, the low light conditions under which the study was conducted, coupled with the significant reduction in stomatal conductance that occurred before leaf expansion started to decline, suggests that the results were confounded by carbon limitation (Munns and Sharp, 1993). Dodd and Davies (1994) also observed that the growth response of leaves to ABA was temperature-dependent, making it difficult to assign a unique relationship between leaf growth inhibition and ABA concentration. Furthermore, the concentration range within which ABA-induced leaf growth inhibition occurs is considerably higher than ABA concentrations in xylem sap (Munns, 1990; van Volkenburgh and Davies, 1983). Young expanding leaves on unstressed plants of the castor oil plant (*Ricinus communis*) contain higher levels of ABA than mature leaves (Zeevart, 1977), suggesting that, at least in these circumstances, it is not inhibitory to leaf growth. Abscisic acid is apparently not needed for expansion in tomato leaves; the *flacca* mutant, which does not produce ABA, develops normal leaves.

In addition, leaf expansion of ABA-deficient mutants of *L. esculentum* 'Moneymaker' grown in soils of high strength was reduced (Masle, 1990), suggesting a lack of involvement of ABA in inhibiting leaf expansion. Tardieu et al. (1991) reported enhanced ABA concentrations in the xylem sap of maize plants from field plots growing on compacted soil, but later (Tardieu et al.,

1992) concluded that the increase probably resulted from root dehydration due to a limited water supply to the roots in the compacted soil. A similar conclusion is reached from a subsequent study by Hartung et al. (1994). These workers recorded a 10-fold increase in ABA concentration in the xylem sap of young maize seedlings in fully compacted soils. This increase paralleled a decrease in leaf elongation and coincided with a reduction in leaf water potential and turgor. All these responses were, however, transitory and after 8-10 days no longer apparent, despite the continued presence of compacted soil. In a second experiment in which maize seedlings were placed in pots containing a compacted layer, neither ABA concentration in the xylem nor leaf elongation rates were affected, provided the plants were well watered and supplied with nutrients. It is also significant that although ABA accumulates in root tissue under water stress (Cornish and Zeevaart, 1985; Parry et al., 1992), physical root impedance only marginally increases ABA concentrations in root tissue (Lachno et al., 1982; Masle and Passioura, 1987; Moss et al., 1988; Tardieu et al., 1991, 1992).

A further argument against the role for ABA in plant responses to compacted soil is that high xylem concentrations of ABA generally promotes root growth while inhibiting shoot growth (Creelman et al., 1990; Saab et al., 1990, 1992). Yet studies with the ABA-deficient tomato mutants found no support for the hypothesis that ABA has a direct effect on the sink strength of plant organs, concluding instead that the changed biomass allocations result from altered water relations in the plants (Nagel et al., 1994). In lupin (*Lupinus cosentinii* L.), maize, sunflower, Scots pine (*Pinus sylvestris*), Sitka spruce (*Pinus sitchensis* (Bong.) Carr.), a threshold leaf water potential of about -1.0 MPa appears necessary before ABA concentrations increase in xylem (Gallardo et al., 1994; Hartung et al., 1990, 1994; Jackson et al., 1995; Zhang and Davies, 1990b). Specific data on the threshold leaf water potential for tomato is lacking. However, given that the threshold stress range for stomatal closure in tomato is -0.8 to -1.0 MPa (Brix, 1962; Duniway, 1971), it seems reasonable

to assume that a similar threshold level of stress before ABA concentrations increase in xylem sap exists in tomato. It is noteworthy then that measurements of leaf water potential throughout the study did not approach this threshold value (Table 6.15).

The study by Ternesi et al. (1994) is particularly relevant to the current study as it reports a seven-fold increase in ABA concentration in the xylem sap of root-restricted, hydroponically-grown seedlings of sunflower. This increase in ABA concentration was associated with a significant reduction in total leaf area expansion and ratio of shoot to root fresh weight. Similar ABA concentrations in hydroponically-grown sunflower seedlings have also been associated with a slight reduction in the rate of leaf expansion (Zhang and Davies, 1990b). Despite these associations, other results presented in this paper provide a basis for caution in concluding that the observed increase in xylem ABA concentration and reduced shoot growth is linked. Of particular concern was the observation that the daily transpiration rate of restricted plants was similar to control plants (2.5 $ml \cdot m^{-2}$ vs. 2.0 $ml \cdot m^{-2}$). This was unexpected because although transpiration rate is only an indirect measure of stomatal conductance, similar concentrations of ABA in xylem sap (≈50-100 nM) have significantly reduced the stomatal conductance of both soil- and hydroponically-grown sunflower seedlings to between 50-65% of unstressed control plants (e.g. Masia et al., 1994; Neales et al., 1989; Zhang and Davies, 1989). If ABA was responsible for reduced leaf expansion, it must have mediated this influence directly to leaf cells (e.g. by influencing cell wall extensibility). As developed above, however, the evidence for ABA influencing cell growth is weak. Indeed, the high ABA concentrations in the xylem sap may arisen from an accumulation of the hormone due to reduced export in the phloem to active sinks such as root apices, shoot apices and underdeveloped leaves (cf. Hocking et al., 1972; Shindy et al., 1973). As root restriction reduces the activity of all these sinks (Chapter 3, 6), the observed

increase in ABA in the restricted plants may be a symptom, rather than an agent, of root restriction.

Roots are a site of synthesis (Carr and Reid, 1968; Jones and Phillips, 1966; Sitton et al., 1967) or interconversion (Crozier and Reid, 1971) of gibberellins. As a group, gibberellins are involved in several responses frequently touted as evidence of cytokinin involvement. Exogenously applied gibberellins enhance RuDP carboxylase activity and increase chlorophyll content of leaves (Briant, 1974; Treharne and Stoddart, 1968) and increase the rates of cyclic and non-cyclic photophosphorylation (Yakushina and Pushkina, 1975). Wheeler (1960) reported a correlation between gibberellin content and leaf expansion in bean. Stem length and leaf area were increased when low doses of gibberellic acid (GA_3) were applied to the apex of intact tomato plants subjected to low root temperatures (Menhenett and Wareing, 1975). Exogenous gibberellins promote leaf expansion in bean (Brock and Cleland, 1990) and apple leaves (Steffens et al., 1985), but in contrast, Thuantavee (1991) reported increases in only stem length and dry biomass accumulation in tomato plants with foliage or roots treated with GA_3. Exogenous application of GA_3 to the *procera* mutant of tomato changes its leaf shape (Jones, 1987) and leaf growth is reduced in gibberellin-responsive dwarf mutants of lettuce (*Lactuca sativa* L.; Waycott and Taiz, 1991; Waycott et al., 1991), sweet pea (*Lathyrus odoratus* L; Ross et al., 1993), tomato (Koornneef et al., 1990), and turnip (*Brassica rapa*; Zanewich et al., 1990). It is possible that gibberellins act in concert with cytokinins in leaf growth; applying both gibberellin and cytokinin to root-restricted bean plants was necessary to restore the growth of leaves and stems (Carmi and Heuer, 1981). These reports withstanding, that shoot tissues are also vigorous producers of gibberellins (Jones and Phillips, 1966; MacMillan, 1987) to the extent that they may be self-sufficient in their production makes the notion of root-sourced gibberellins playing a major role in root-shoot communication considerably less tenable than that postulated for other hormones. For example, Ross et al. (1993) noted that the

precursors of GA_1, the gibberellin suspected of regulating leaf growth in sweet pea (Ross et al., 1990), were synthesised in young leaflets and internodes.

Considerations for future enquiry The literature dealing with the effects of root restriction on growth and development can be divided into two broad groups based upon the probable cause of the effects. One group concludes growth is reduced directly when 'normal' levels of either water, oxygen, or nutrients are reduced (Hameed et al., 1987; Mutsaers, 1983; Peterson et al., 1991b; Tschaplinski and Blake, 1985). The other group concludes that the activity of root-produced factors, responsible for the maintenance of balanced root and shoot growth, is perturbed by root restriction (Carmi and Heuer, 1981; Richards and Rowe, 1977a). As root restriction encompasses a wide spectrum of responses, the conclusions of both groups are probably correct. Under conditions where nutrients, oxygen, and water uptake and transport are not limiting, restricting root growth results in a balanced reduction in root and shoot growth. Shoot:root ratios are similar, irrespective of the level of restriction (Fig 6.12; Krizek et al., 1985; Richards and Rowe, 1977a; Robbins and Pharr, 1988). Later, however, as increasing root restriction results in the availability of water and oxygen to the root system becoming limiting, root sink strength would decline. Root growth would be detrimentally affected more than shoot growth and, as a consequence, the SR would increase. For example, availability of oxygen is likely to be limiting when plants are grown in passive hydroponic systems (e.g. Al-Sahaf, 1984; Carmi and Heuer, 1981; Carmi et al., 1983; Hameed et al., 1987; Tschaplinski and Blake, 1985). Finally, as the rhizosphere environment fully limits root growth, ensuing root death would result in a further increase in SR ratio (e.g. Tschaplinski and Blake, 1985).

Common to all these studies, and the current one, is the absence of a suitable method for quantifying the extent of root restriction. Unlike nutrition or plant growth regulator experiments, plants subjected to root restriction are

not treated with exact, statistically continuous levels of the treatment factor. Immediately after 'treatment' roots of plants in 'restricted' treatments share a similar immediate environment as those in 'unrestricted' treatments. As the experiment progresses, roots in restricted containers encounter ever changing levels of restriction. Not only does this make comparison of experiments difficult, but analysis of within-experiment treatments is also made difficult. Trend or regression analysis, using restriction volume is unreliable, as results depend greatly upon when the harvest is taken. Plants in containers of different volumes will encounter the same level of restriction, but at different ontogenetic stages of development. For example, Cresswell and Causton (1988), in reporting curvilinear relationships between container volume and growth responses of Brussels sprouts, observed that had the experiment been continued until root growth in the largest container had been limiting, a linear response might have been obtained. Additionally, the range of container volumes used is such that the largest volume often assumes a level of (statistical) influence in any regression analysis that far exceeds its importance to any physiological response. Al-Sahaf (1984), for example, regressed growth variables against container volumes of 0.1, 0.25, 0.6, 1.0 and 4.5 l. Given the asymmetric nature of this range, it seems reasonable to speculate that HAT diagonals or R-student values (Myers, 1990) associated with observations at the 4.5 l node would have signalled that these observations exerted undue influence to the coefficients of the fitted regression line.

What is required is a measure of the impact pressure of roots, or some index that reflects the extent of restriction. Standard techniques for measuring the mechanical impedance of soil to root growth (Bengough and Mullins, 1990) are not useful. Simple measures examined in this study such as root length per volume container, the resistance of the root ball against a penetrometer, or the porosity of the root matrix were all too coarse and variable (data not presented). Veen and Boone (1981) related the elongation

rate of corn roots to the pore volume of compacted soil. On the other hand, Henry (1993) found no evidence of a relationship between root growth and the percent of pore space occupied by grape (*Vitis vinifera* L.) roots. Bengough and MacKenzie (1994) reported a method for simultaneously measuring root force and elongation of a single root, but as the root is grown in a nutrient and water-free environment, the physiological validity of data from this method must be questioned. Without a baseline, the different responses to restriction will remain independent observations, and the progression of response to restriction will remain obscured. Without an understanding of the progression of response, management of root restriction in horticultural production will be impossible. It will remain solely as an experimental strategy for elucidating root-shoot relationships, and never develop to a rigorous horticultural practice for non-chemically controlling plant growth.

Although the bulk of the circumstantial evidence in Chapter 6 points to the involvement of a negative signal such as cytokinins in the response of tomato plants to root restriction, further study is required. As with all hormonal responses, it is not possible to view single hormones in isolation (Trevawas, 1986). For example, cell division and morphogenesis are very sensitive to the relative concentrations of cytokinin and auxin in tissue (Fosket, 1977; Greshoff, 1978; Maldiney et al., 1986; Morris et al., 1982; Skoog and Miller, 1957); cytokinin and auxin jointly regulate plant genes (Dominov et al., 1992; Mohnen et al., 1985; Shinshi et al., 1987); enhancement of nitrate reductase mRNA transcription by cytokinin is antagonised by ABA (Chen et al., 1992; Lu et al., 1992); ABA inhibits GA_3 induction of α-amylase synthesis and the level of its mRNA in aleurone layers (Chrispeels and Varner, 1966; Muthukrishnan et al., 1983); plant senescence involves interaction among auxins, cytokinins, ethylene, and ABA (Nooden et al., 1990; Thimann et al., 1982). Even the well documented response of plants to drying soil conditions through increased concentrations of ABA (Davies and Zhang, 1991) may also be due to a change in the balance between ABA and cytokinins, given their observed

decline in concentration in xylem sap with soil drying (Bano et al., 1993; Itai and Vaadia, 1965; Itai et al., 1968) coupled with their role in maintaining stomatal conductance (Incoll and Jewer, 1987).

The possibility that response to root restriction is also due to a balance between positive and negative signals therefore deserves investigation. In observing an increase in leaf expansion after the part of a root system in a drying environment was excised from the plant, Gowing et al. (1990a) reasoned that a positive inhibitor (speculatively ABA), produced by the roots but influencing shoot growth, had been removed. As this group's observation is also consistent with a positive signal model involving oligosaccharides (Campbell et al., 1995a, 1995b) or with a negative signal model from a root sub-system in drying soil, it would be interesting to examine whether root restricted plants responded similarly if a restricted portion of the root system was removed. Furthermore, if the response was a balance between positive and negative signals, then increasing the delivery rate of the negative signal in a plant with a restricted portion of the root system should overcome any reduction in shoot growth. Thuantavee (1991), for example, demonstrated that 10^{-8}M BA applied to tomato roots can supplement the endogenous concentrations of cytokinins in the plant. After enhancing import of zeatin riboside into shoots of stinging nettle (*Urtica dioica* L.) by immersing less than 10% of the root system (minus root apices) in solutions of various concentrations, Fetene and Beck (1993) observed substantial increases in both fixation of ^{14}C in expanding leaves and export of assimilates to the shoot apex. If cytokinins are the negative signal, then similarly supplementing endogenous levels should overcome any decline in leaf expansion in root-restricted plants.

Although this approach would progress understanding of the relative importance of hormones in response to root restriction, it is, nevertheless, difficult to interpret the effect of exogenously supplied hormones on internal

hormone ratios and concentrations (Feldman, 1984; Horgan, 1990; McGaw et al., 1984). Alternative approaches, such as the use of transgenic plants in which hormone ratios have been modified *in vivo* (Ainley et al., 1993; Guiltinan and Deikman, 1994; Hamill, 1993; Romano et al., 1991, 1993; Zhang et al., 1995) may help resolve many of the outstanding questions. If high concentrations of auxin or ethylene or low concentrations of cytokinins, induced by stress, are in some way responsible for inhibition of root or shoot growth, then the effect of root restriction on mutants with reduced biosynthesis or altered sensitivity to these hormones (Blonstein et al., 1991; Cao et al., 1993; King, 1988; Schiefelbein and Benfey, 1991; Zobel, 1986, 1991) would prove interesting. As the rate of leaf expansion of ABA-deficient mutants of *L. esculentum* 'Moneymaker' was reduced when grown in soils of high strength, Masle (1990) largely discounted ABA of having a major influence in plant response to the physical impedance of root growth. Zacarias and Reid (1992) concluded that ethylene was involved in the physiological response of plants to physical impedance after observing the failure of roots of an ethylene-resistant mutant of *Arabidopsis thaliana* to penetrate compressed media. Root growth of the *diageotropica* mutant of tomato (Zobel, 1974) normalises after treatment of the shoot with low concentrations of ethylene. Production of this mutant and its wild type under conditions of root restriction might provide an insight into the role of ethylene in the root restriction response. The *bushy root (brt)* mutant of tomato is characterised phenotypically by large numbers of roots developing from the basal portion of the hypocotyl and tap root and a small weak shoot, and physiologically by extensive starch deposition in the basal portion of the shoot and root (Zobel, 1972). If a normal shoot is grafted onto the mutant's roots, the shoot assumes the characteristics of a mutant shoot. Given the preferential partitioning of assimilate to the stem in restricted plants (Tables 3.10, 6.19), together with the possibility that response to root restriction is mediated through changes in the transition zone between root and shoot tissue (cf. Costa et al., 1992; Grochowska et al., 1994; Hameed et al., 1987; Jaramillo et

al., 1992), it would be useful to examine the anatomy of the transition zone in the mutant and its auxin:cytokinin ratio in stem and root tissue.

Direct comparative analysis is required of cytokinin delivery rates from roots to shoots of restricted and unrestricted plants. Measurement of cytokinin concentration is, by itself, not sufficient evidence of changes of the passage of the hormone into the xylem sap from roots. As Jackson and his coworkers (Else et al., 1994; Jackson, 1993) have warned, many reports of stress-induced changes in hormone concentrations in xylem sap have not taken into account the confounding effect of reductions in sap flow rate on concentration. It is important that analysis of delivery rates of cytokinins within root and stem tissue either side of the transition zone is undertaken. If cytokinin delivery rates in shoot tissue are reduced in root restricted plants, it must be determined whether this arises from inhibited transport of the cytokinins through the transition zone or reduced synthesis in the root apices. If only export is inhibited during restriction, continuing synthesis would lead, at least in the short term, to an increase in cytokinin concentration in root tissue. This increase may contribute to the reduced growth of restricted roots (Chapter 3, Chapter 6), because high concentrations of cytokinins inhibit root growth (Stenlid, 1982). If de-restriction, either by removal of a restricting container (Chapter 3) or development of an unrestricted portion of the root system (Chapter 6), removes a blockage within the transition zone, the increased delivery rates of cytokinin within the restricted root systems might 'flood' the shoot, inducing higher rates of leaf expansion. It would be interesting to determine whether, in restricted plants, the increase in R_A of leaves expanding at the same time as unrestricted roots were developing (Chapter 6) was mediated by higher-than-normal delivery rates of cytokinins to the leaves. Certainly, the study by Ainley et al. (1993) of the effects of pulsed enhancement of cytokinin levels on leaf growth indicates that such a response is possible, given that in their study, expansion rates were accelerated in actively growing leaves only.

A corollary of this hypothesis is that a reduction in supply of cytokinin from a physically restricted root system would result in reduced leaf expansion. In my study, however, analysis of the expansion parameters of the Richards functions fitted to individual leaves developing 3, 7 and 14 days after restriction treatments were initiated (Fig. 6.3; Table 6.5, 6.6) indicated that the effect of restriction was through reduced cell numbers, a conclusion consistent with results from studies in which cell number and size in leaves of root restricted plants were counted (Cresswell and Causton, 1988; Milthorpe and Newton, 1963). Although cell division and expansion are not mutually exclusive processes in explaining the effect of root restriction on leaf growth, it is clear that the relative importance of the two processes on leaf growth can not be resolved with the data available. Future studies need to consider both processes when quantifying the effect of root restriction on leaf growth.

Root restriction often (Chapter 3; Al-Sahaf, 1984; Hameed et al., 1987; Peterson et al., 1991a) but not always (Chapter 6; Robbins and Pharr, 1987) results in an increase in SR. Increasing the level of zeatin riboside in shoot tissue of stinging nettle increased the SR ratio (Fetene and Beck, 1993). As zeatin and zeatin riboside are also dominant cytokinins in tomato (Davey and van Staden, 1976), it would be interesting to relate their concentrations in roots as they progressively become restricted, to changes in the partitioning between roots and shoots. Such a study might use the dwarf root mutant of tomato (Zobel, 1975). The roots of this mutant are dwarfed under all conditions of culture. In contrast, high nutrient concentrations in hydroponic systems result in normal shoot development. If the concentration is reduced below 25% of those necessary for normal shoot growth, the shoot takes on a dwarf-like appearance (Zobel, 1986). Assuming this response is mediated by a nitrogen-cytokinin synthesis interaction, and that root restriction reduces cytokinin synthesis, physically restricting roots of this mutant in a high nutrient environment should cause the shoot to be dwarfed.

The relationship between the temporal distribution of cytokinin concentrations in leaves, particularly with respect to their daily modulation by temperature and light (photoperiod and irradiance), and the influence of these factors on leaf expansion requires further elucidation. Cytokinin concentrations in leaves and leaf expansion are both modulated by temperature and light (Dale, 1988 and references therein; Hansen et al., 1988).

More substantive analysis of the role of ABA in the root restriction response is required. Early emphasis should be given to determining the effect of root restriction on stomatal conductance. Stomatal response to root-sourced ABA is independent of leaf water potential or leaf turgor (Bates and Hall, 1981; Dwyer and Stewart, 1984; Gollan et al., 1986; Tardieu et al., 1992), and without more detailed attention to these variables, a role of ABA analogous to its role in plant response to drying soil cannot be ruled out. While photosynthesis, which is normally limited by stomatal conductance, was not manifestly affected by restriction (Table 6.16), the lack of data on stomatal conductance in this study is, in hindsight, a major constraint to full interpretation of the data collected. This stated, it is also important to note that future studies must measure leaf function throughout the stem axis, as the effect of root restriction has both a temporal and spatial element (Chapter 6). Measuring leaf function of the 'most recently expanded leaf' alone will not provide sufficient definition of the effect of restriction on leaf function throughout the plant.

Future attention should be directed to possible interaction of ABA with cytokinins. Cytokinins inhibit ABA biosynthesis (Cowan and Railton, 1987; Norman et al., 1983) and promote its conjugation (Even-Chen and Itai, 1975). Abscisic acid may change cytokinin transport and metabolism (Back et al., 1972; Sondheimer and Tzou, 1971). The antagonistic relationship (Evans, 1984; Thimann, 1992) that appears to operate between ABA and cytokinins is also reflected in the decline of cytokinin concentrations and increase in

ABA concentrations in water stressed plants. Parenthetically, this antagonistic relationship may explain why, despite reported reductions in cytokinin concentrations in xylem sap of water-stressed plants (Hubick et al., 1986; Itai and Vaadia, 1965; Rosa da Costa et al., 1987), the relationship between these and stomatal conductance has either been simply implied (Bano et al., 1993) or found to be uncorrelated (Fußeder et al., 1992).

The split root technique provides a flexible system for studying plant response to root restriction. Further development of this technique must focus on why growth is stimulated by splitting the stem (Figs. 6.2, 6.3; Table 6.17). Although plant propagators may be satisfied to vertically split the stems of their cuttings to stimulate growth, the split may be introducing a chemical factor into the plant system which confounds any subsequent physiological analysis of plant response.

An important area for more study using the split-root system is the extent of correlative inhibition within a root system under conditions of partial restriction. This is important because the correlative phenomenon in roots appears to involve the inhibition of initiation of root apices (Gersani and Sachs, 1992). Given the postulated importance of actively growing root apices to leaf expansion (Chapter 3), the possibility that restricted roots might inhibit formation of new roots in unrestricted portions of the root system deserves investigation. This issue also has horticultural relevance. The extent of correlative inhibition existing between a restricted root sub-system and unrestricted sub-systems will have a major impact on container design. For example, if a root (or root sub-system) that is impeded by a container wall, correlatively inhibits compensatory growth in other unrestricted parts of the root system, then container designers should aim to minimise the internal surface area of containers for those crops in which growth is to be maximised.

Future studies should consider test plants other than tomato. Leaf expansion of tomato is difficult to model non-destructively, requiring concurrent studies to develop a valid predictive model (Chapter 4). Orthostichy in tomato (Gifford and Evans, 1981; Russell and Morris, 1983) will make studies of assimilate or hormonal flows difficult. Furthermore, as internal and external phloem, usually responsible for transporting assimilate in opposite directions, develops at different rates in tomato, the pattern of translocation is subject to ontogenetic drift, depending on the developmental stage of the particular leaf and that of the plant. This could add unnecessary complication to any study of the postulated auxin-cytokinin control loop. In all these respects, sweet pepper and sunflowers offer a simpler system. Moreover, as both plants have been used in studies involving root stress and leaf expansion, there is depth in the literature to support further studies in these areas.

An unresolved difficulty in this study is that discussion of root growth has assumed that the root system is an homogenous mass of tissue. Comparing the relative growth rates of leaf expansion with root extension is probably an oversimplification. Treatment of roots as a single class in experiments is similar to pooling data from leaf, petiole and stem to determine the function of the shoot (Zobel, 1986). As with a shoot system, a root system is made up of constituent parts, each of which probably reacts differently to various environmental and physiological factors, and consequently affects the behaviour of the entire plant differently (Eissenstat, 1992; Waisel and Eshel, 1991). For example, the height and total dry biomass of pea (*Pisum sativum*), rape (*Brassica napus*), linseed (*Linum usitatissimum*), safflower (*Carthamus tinctorius*), sunflower and wheat grown under various levels of soil impedance was more strongly correlated with reductions in seminal root lengths than total root length (Whiteley and Dexter, 1982). Reducing the classes of roots on the plants to adventitious and lateral roots (Chapter 3) may have reduced variation in the observed correlations between root and shoot growth (Chapter 3), but future studies should attempt to discriminate the response

of different root classes to restriction, and relate these responses to those of the components of shoot growth.

Because of our lack of understanding of the respective functions of the different components of a root system, it is not yet possible to determine whether the cytokinin delivery from a root system containing a large number of roots each elongating a small amount is equivalent to that of a root system with a small number of roots, each of which elongates considerably. The relative rate of elongation of both root systems would be similar: whether their sum activity is also similar is unknown and requires study.

While important from a physiological standpoint, this issue also has important practical considerations. For example, should growers produce plants in containers designed to maximise root number or in those designed to maximise root elongation? Some studies on the effect of container size and shape on plant growth have reported that for containers of the same diameter, shoot growth is always less in long containers (Hanson et al., 1987; Smith and Schwabe, 1980). This suggests that restriction of tap root growth in shorter containers may enhance lateral root development, thereby increasing shoot growth. On the other hand, other studies have pointed to genetic factors being important to growth responses to container design. For example, both Biran and Eliassaf (1980) and Keever et al. (1985) concluded that naturally shallow rooted species should be grown in shallow, wide containers, and deep-rooted species in deep containers. Unfortunately, as studies in this area tend to use a solid soilless medium, such as peat or bark, it is difficult to avoid confounding the effect of container depth with changes in aeration and water and nutrient availability down the profile of the containers. Use of a deep flow hydroponic system (Chapter 2) would avoid the confounding actions of these factors, and should provide more conclusive evidence of the type of root system whose development should be encouraged.

High strength soils tend to depress biomass gain in shoot tissue more than in roots (Andrade et al., 1993; Atwell, 1990a; Dawkins et al., 1983; Masle, 1992; Masle and Passioura, 1987). In contrast, sink strength in roots declines with the extent of restriction within a container, and root growth is reduced more than shoot growth. It is possible that root restriction induces changes in the transition zone between the vascular systems of the root and shoot systems, thereby impeding transport of both assimilate and water. Certainly, the cambium in dicotyledons can change its orientation according to relations among organs (Gersani and Sachs, 1984; Kirschner et al., 1971), and under root restriction, differentiation of vascular tissue might be disrupted. Anatomical studies are required to investigate this possibility. Such studies might reveal whether the assimilate buildup in stems (Tables 3.10, 6.19) (presumably at the base of the stem) was due to disrupted transport, or the absence of alternative sinks (e.g. fruit; Heuvelink and Buiskool, 1995; Ho, 1992). If the latter is found true, and several reports (Al-Sahaf, 1984; Carmi and Shalhevet, 1983; Richards, 1981, 1986; Ruff et al. 1987) suggests this might be so, it may prove possible to manage root restriction to improve harvest index, particularly if root restriction can be applied without a concurrent depression in photosynthesis or development of water stress (Table 3.3, 3.9, 6.15).

Liberty Hyde Bailey (1907) wrote that research was "... not so much a matter of the subject, as of the intention, the point of view, and the method of work". I conclude this thesis by reviewing my doctoral programme from the perspective of the nature of research.

Progress in research (Thornley, 1980), as in learning (Kolb et al., 1979), follows a cyclic path. Current theories provide a starting point, and through a sequence of observation, hypothesis generation and testing, we arrive at a

new starting point with a more articulate and precise understanding of the problem or phenomenon being researched. The study of the ontogeny of tomato plants under varying levels of root restriction (Chapter 3) evolved, in the manner of Kuhn (1963, 1970), to focus on the relative dynamics of root elongation and whole-plant leaf area expansion, and from this, to study expansion of individual leaves of plants to which root restriction was spatially applied (Chapter 6). The demonstration of an increase in leaf expansion in the presence of a restricted root sub-system (Chapter 6) provided an unexpected leap (Popper, 1959) and adds significantly to our understanding of the type of communication between root and shoot under physically restrictive root conditions.

My progress through the cycles that this work reflects was tempered by a continuing conflict between substance and process. This conflict occurs when deciding between working directly to get the right answer to a problem (the substance), and working on the right way of getting the answer (the process) (Badaracco and Ellsworth, 1989). This conflict occurred in deciding between simply using a hydroponic system, and the concern about nutrient and oxygen uptake rates and the need to match the flow rate of the system with these (Chapter 2); between simply calculating the mean net assimilation rate for a particular group of plants, and determining the best method for calculating the net assimilation rate (Chapter 3); between assuming positional and temporal stability in a model that estimates leaf area, and testing those assumptions (Chapter 4); and between visually blocking plants by 'size' and blocking by highly discriminatory quantitative techniques (Chapter 5). The currency of this conflict is time. And in the finite time of my academic research programme, time spent on process was time lost to substance, to finding answers to the original problem.

Now, at the end of the study, I find myself at a beginning, with a clear understanding of where to target future research to characterise the mechanistic

basis of plant response to root restriction in containers. The strong circumstantial evidence of a negative signal that involves cytokinins and influences shoot growth in response to physical impedance of the root system must be validated with direct measurements of cytokinin delivery from root to stem to leaf. The probable roles of other hormones as 'co-respondents' to physical root restriction cannot be ignored, and in this regard, ABA (as a partner in the leaf growth response), and auxin (as a partner in the root growth and partitioning response) are likely participants. The response to root restriction of mutant or transgenic plants, deficient in and sensitive to one or more of these hormones, will progress this work. In particular, growth analysis of the ontogeny of root and leaf expansion in both mutant and wild type plants under partial root restriction (i.e. in a split root system) will provide needed information of the relative kinetics of response. Although tomato would be a satisfactory test plant, both sweet pepper and sunflower provide simpler leaf forms on which to cluster for local error control and to model expansion. In all these studies, a managed deep flow hydroponic system would provide the necessary rigour in maintaining a root environment that was only limiting in space.

> What we call the beginning is often the end
> And to make an end is to make a beginning.
> The end is where we start from.
>
> —T.S.Eliot *Four Quartets*

Chapter 8

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