THE INFLUENCE OF TIME AND PATH OF SUPPLY OF NITROGEN ON THE GROWTH RESPONSE OF WHEAT (TRITICUM AESTIVUM L.)

A thesis submitted

by

David Rolfe Lungley, B.Sc. (Hons.)

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Department of Agronomy
Waite Agricultural Research Institute
University of Adelaide
South Australia

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Summary

A stratagem of deferment of application of nitrogen fertilizer to wheat has been suggested as offering advantages in southern Australia (Russell 1969). The influence of time and path of supply of nitrogen on the growth response of wheat has been studied.

From an examination of the literature it is concluded that, provided the crop is nitrogen-deficient and nitrogen applied is recovered, wheat is capable of a grain yield response to application via the soil at any stage during vegetative development. The relative magnitudes of response to applications at different stages are influenced mainly by the relative recoveries of applied nitrogen. Application after the vegetative phase generally increases grain nitrogen concentration rather than grain yield. Where the two paths of supply have been separated, growth response to foliar-applied nitrogen has been inferior to that from equivalent application to the soil.

Three experiments are described. In the first, the recovery of nitrogen and growth response of wheat to a deferred application of ^{15}N-labelled urea, at three rates, made either to the foliage or to the soil, were measured in pot culture. Uptake of applied nitrogen by each path was rapid. Absolute recovery at maturity was similar and less than 50% in all treatments, except

at the lowest foliar rate where greater recovery was obtained. Growth response to foliar- was less than to soil-applied nitrogen. Lower response to the foliar path was associated with poorer translocation of urea-N away from the sprayed foliage, to a lesser extent at the lowest rate.

In the second experiment the response of four cultivars was compared to application of urea in the field at three different stages of development. Urea was sprayed at sowing time, or early or late in the tillering phase. Apparent recovery of N did not exceed 30%, but was higher for the first deferred than for the sowing time or late applications. It did not differ between cultivars. Grain yield response was mainly related to apparent recovery of N; however two cultivars showed superior responsiveness to deferred application of nitrogen.

In the third experiment the root growth response to time and path of nitrogen application was studied on one cultivar in flowing solution culture. Additional nitrogen was supplied to plants growing in nitrogen-deficient solution at stages equivalent to those of the second experiment, either as ammonium nitrate to the solution or by foliar application of urea. The growth of the first seminal and first nodal root per plant was studied. No growth response was obtained to foliar-applied urea. Seminal root members had a lower elongation rate and a higher branching density in high-N than in low-N solution; they responded to

addition of nitrogen in solution at each stage. In the nodal root, elongation rate and branching density tended to be higher in high-N than in low-N solution. Possible consequences for the water economy of nitrogen-fertilized wheat in the field are considered.

The implications of the study are discussed.

DECLARATION

This thesis contains no material that has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge and belief, it contains no material previously published or written by another person, except when due reference is made in the text.

D.R. Lungley

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1 Introduction

The use of nitrogen fertilizer together with responsive cultivars is the major means of increasing the grain yield of temperate cereals. Nitrogen is required by crops in the greatest amount after carbon, hydrogen and oxygen; where other factors are not limiting, the dry matter response to an increase in the supply is generally considerable (Viets 1965).

Nitrogen fertilizer is not used extensively in the southern Australian wheat belt, however, where response is limited by the water supply. A high daily rate of solar radiation in spring leads to high rates of transpiration, and only the requirements of relatively low yield crops can be met by the limited seasonal rainfall. The increased leaf area which usually results from addition of nitrogen increases the water requirement of the crop. Depletion of the soil water is hastened and the transpiration demand in spring is heightened. This tends to lead to water stress in the later stages of development, which checks growth and prevents a potential increase in grain yield from being realised. Thus, grain yield is often increased only marginally, and is occasionally depressed (Colwell 1963; Barley and Naidu 1964; Storrier 1965a, b; Fisher and Kohn 1966a, b; Russell 1967, 1968a). Seasonal rainfall is variable. A proportion of seasons can support relatively large amounts of leaf area and allow favourable responses in grain yield to be obtained. However the frequent occurrence of unfavourable seasons

discourages the regular use of nitrogen.

The cost of nitrogen fertilizer in Australia has decreased steadily over the past two decades, and the prospects for its use have improved (Russell 1957; Donald 1960). Russell (1967) reported the results of extensive field trials conducted in South Australia, in which applications of nitrogen at sowing were made to one cultivar of wheat. 52 experiments were carried out over a range of sites during six seasons. The average yield response to 50 kg N ha^{-1} over all experiments was 230 kg ha^{-1}. At the recent value of wheat grain ($ 4.50 q^{-1}) and cost of nitrogen (22c kg^{-1}), this corresponds to an average loss of 65c ha^{-1}. However, responses varied considerably between experiments. Responses that would have been profitable under the above grain:fertilizer price ratio were obtained in 22 experiments. The highest yield response recorded was 900 kg ha^{-1}.

Russell (1969) showed that yield response correlated well with four of the environmental variables measured: May-August rainfall, October mean maximum temperature, initial nitrate content of the 0-6" soil horizon, and the 15 atmosphere soil moisture content. He proposed that one stratagem of nitrogen fertilizer use in southern Australia might be based on the regular application of nitrogen at sowing to sites showing favourable environmental characteristics. The stratagem was illustrated with yield response models derived from the experimental data.

The selection of sites climatically favourable for the use

of nitrogen at sowing time must be based upon site meteorological records. Detailed statistical examination of South Australian rainfall records (Cornish 1950) has shown that there is no correlation in the rainfall from season to season, or from month to month within a season. Therefore there is only a probability that a given season will be favourable, and the possibility of an unprofitable response cannot be avoided. Russell (1969) proposed an alternative stratagem of nitrogen fertilizer use, which provides a partial solution to this problem. The decision whether or not to apply nitrogen is deferred until after the crop is established. If there is a high early season rainfall then the season is a potentially favourable one and an economic response to nitrogen is likely to be obtained. In addition, the nitrogen status of the crop after establishment provides a further measure of potential response.

It is theoretically a realistic proposition that seasons likely to be favourable for the use of nitrogen can be selected on the basis of the early season rainfall. Figure 1.1 shows the mean monthly precipitation and pan evaporation at Roseworthy, a locality climatically representative of the wheat belt of South Australia. In most years more than half of the seasonal rain (May-October) falls during the first three months of the season. In this period evapotranspiration is usually less than precipitation because of the relatively low level of daily solar radiation and the small crop leaf area. Transpiration during the later stages of growth, when water stress most commonly

occurs, is mostly from water accumulated in the soil in the earlier part of the season. Because there is no correlation between the early and late rainfall in the season, a relatively high early-season precipitation increases the probability of a relatively high total season precipitation. Thus, it has been found that grain yield (Cornish 1950) and grain yield response to nitrogen (Russell 1968a) show a positive correlation with winter rainfall in South Australia.

The extensive field trial data of Russell (1967) can be shown to support the proposition that seasons more favourable to the use of nitrogen may be selected on the basis of the early rainfall. The mean grain yield response to 50 kg N ha^{-1} obtained on the low-fertility sites (<5 ppm nitrate in the 0-100 cm soil zone) was 270 kg ha^{-1} (41 experiments). The mean response obtained on these sites in the seasons in which the May-July precipitation exceeded the long-term average (17 experiments) was 500 kg ha^{-1}. Under the recent grain:fertilizer price ratio this corresponds to an average profit of $10 ha^{-1}. In only one of the 17 experiments was there an unprofitable response.

The stratagem of deferment of application of nitrogen depends for its success upon the capacity of the crop to respond to delayed nitrogen application. Field experiments conducted in South Australia in the past, in which the response of wheat to nitrogen applied at sowing has been compared with application later in the season, have shown delayed applications generally to give inferior grain yield.

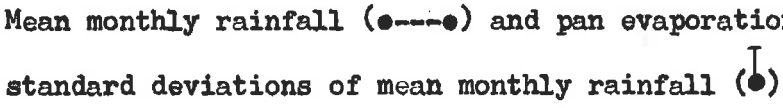
responses (Birks and Cole 1930; Richardson and Gurney 1935; Reuter 1967; Russell 1969). However, crop responses to applied nitrogen are known to be influenced by a number of environmental, climatic and cultural factors (Viets 1965). The data of Russell (1967, 1968a, b) demonstrate this clearly with respect to applications of nitrogen at sowing in South Australia. The results of pot experiments (Watson 1936; Thorne 1962), and field experiments conducted in Europe and North America have shown that similar grain yields are obtained from nitrogen application early and late in vegetative development in some circumstances (see section 2.2.2.e). Recently higher grain yields were obtained from deferred application than from sowing application of nitrogen to wheat in Western Australia (Mason, Rowley and Quayle 1972).

The earlier trials conducted in South Australia were limited to observation of the crop yield response and grain nitrogen content. Reasons for the inferiority of response to deferred application were not identified, and the extent to which the results apply to circumstances other than those of the experiments was not ascertained. The potential for response to deferred application of nitrogen has not been explored extensively in southern Australia.

The object of this thesis is to explore this potential. The growth response of wheat to time and path of nitrogen supply is examined. The grain nitrogen content response is included, because this is commonly linked with grain yield and is of potential economic

importance. The pertinent literature is reviewed in Chapter 2. Three experiments are described. They examine respectively: (A) the influence of the path of nitrogen supply to the plant on the uptake of applied nitrogen and the growth response; (B) the differential response of four contrasting cultivars to time of nitrogen application during the vegetative phase, at two plant densities; (C) the root growth response of one cultivar to path and time of nitrogen application during the vegetative phase, in solution culture. The reasons for the choice of each aspect examined are discussed in the introduction to each experiment. A general discussion and conclusions drawn from the study are presented in Chapter 6.

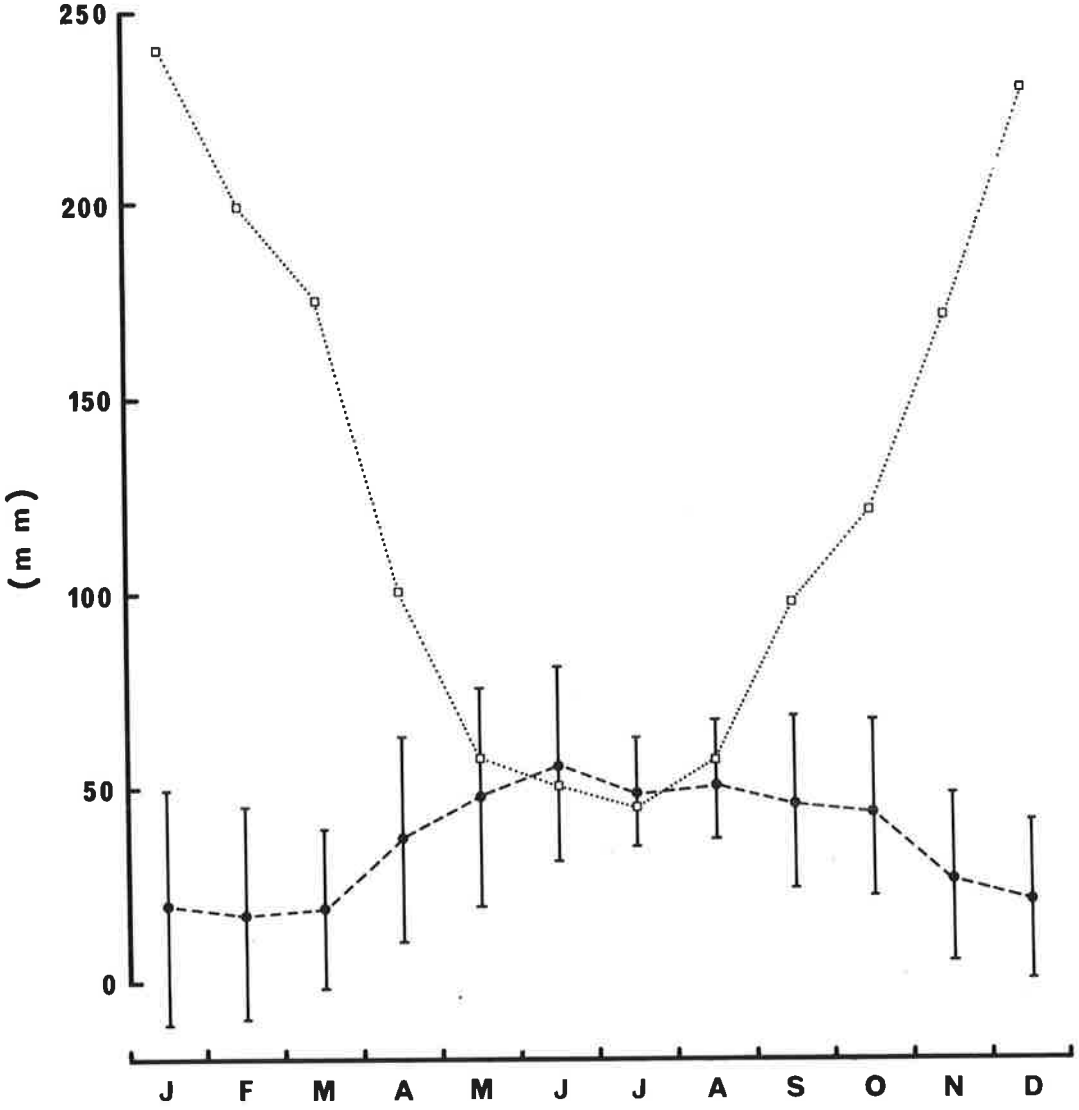
Figure 1.1



Mean monthly rainfall (●---●) and pan evaporation* (□···□), and standard deviations of mean monthly rainfall (I●), at Roseworthy, South Australia.

Data from Roseworthy Agricultural College records 1931-1968.

* Australian Tank.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2 Review of the literature

The literature relating to the influence of path and time of nitrogen application on the growth response of wheat is reviewed. The protein content of the grain at maturity is included as a relevant growth response. Literature relating to other plant species, particularly other temperate cereals, is referred to where appropriate.

The development of the wheat plant with respect to time is essential to the discussion. It has been described by Percival (1921), Hector (1936) and Peterson (1965). Wheat exhibits an annual habit of growth, normally passing through a complete cycle of development within one growing season. Germination of the seed is followed by a period of vegetative development, during which leaves are initiated and expand in turn on one or more shoots, or tillers. The reproductive phase succeeds the vegetative phase of development. It commences with the elongation of the shoot stems, following the expansion of the last-formed leaf on each shoot, termed the flag leaf. Not all tillers necessarily develop to the reproductive phase. Stem extension brings about emergence of the inflorescence, or ear, from the flag leaf sheath, which is followed by flowering of the inflorescence, otherwise termed anthesis. Grain development proceeds from flowering until maturity, following fertilization of the ovule. At maturity the grains are filled and partially dried, and the plant is senesced.

Two types of wheat are grown. Spring wheat is sown at the start of a wet, warm season, and develops uninterrupted from germination

to maturity. In cold temperate climates winter wheat is mainly grown. Winter wheat requires a period of vegetative development near 0°C, termed vernalisation, before reproductive growth may be induced. Winter wheat is sown in the autumn or winter preceding the main growing season, and undergoes limited vegetative growth before a period of winter dormancy. Vegetative growth recommences in spring, and continues until maturity.

2.1 Influence of the path of nitrogen supply on nitrogen uptake and growth response

2.1.1 Soil/root path

Nitrogen is normally acquired by non-leguminous plants during growth through absorption from the soil by the root system. Nitrogen fertilizer is normally applied to crops via the soil, whereby it supplements the supply of native soil nitrogen. The literature describing the effects of soil-applied nitrogen on the uptake of nitrogen and growth response of plants is large, but few experiments have compared the soil/root path with the foliar path of nitrogen application.

Roots are able to absorb inorganic ions from solutions that wet the root surface. The experimental evidence has been reviewed by Epstein (1972). The evidence indicates that ions move across the root cortex mainly by free diffusion within the cell walls. Some transfer occurs by diffusion within the cytoplasm. Ions are actively

(i.e. metabolically) accumulated into the cytoplasm of the endodermal cells, whence they move to the cells of the xylem by diffusion within the cytoplasm. Accumulation normally occurs against a concentration gradient and tends to be highly selective. The impermeable Casparian strip of the endodermis cell walls blocks free movement of ions into and out of the stele, so that once absorbed, nutrients are retained within the root for transport to the other parts of the plant. Roots normally absorb nitrogen as ammonium and nitrate ions, these being the principal forms of N in the soil solution (Russell 1961).

For absorption of soil-applied fertilizer to occur it is necessary for the fertilizer to be dissolved in the soil solution and transferred to the root surface. Limitations to the rate and extent of recovery of applied fertilizer by the plant are imposed by each of these prerequisite steps to absorption.

The fate of nitrogen fertilizer following application to soils has been reviewed by Allison (1966). Fertilizer may become unavailable for absorption by the root system in a number of ways: a proportion becomes biologically immobilised through incorporation into the soil microbial population; a proportion in the form of ammonium ions may be immobilised physically by adsorption on clay minerals, or chemically by reaction with certain organic compounds to form resistant complexes; a proportion may be lost from the soil altogether The chief mechanisms of nitrogen fertilizer loss from the soil are considered to be nitrate leaching and denitrification, and ammonia

volatilisation. Where precipitation sufficiently exceeds evaporation, nitrate may be lost by leaching out of the root zone, or less readily recovered after leaching to the lower regions of the root zone. Under anaerobic conditions nitrate may be lost to the atmosphere as gaseous nitrous oxide and nitrogen, as a result of the denitrifying activities of certain microorganisms. Anaerobic conditions probably exist at the moist centres of fine-textured soil crumbs. Under alkaline conditions urea and ammonium fertilizers may suffer volatilisation losses of ammonia. A number of other quantitatively less important mechanisms of gaseous nitrogen loss from nitrite, an intermediate in nitrification, are also known to occur.

The movement of inorganic ions through the soil to plant roots has been reviewed by Olsen and Kemper (1968), and discussed by Nye (1968a, 1968b) and Tinker (1968). The movement of nitrogen in soil has been reviewed by Gardner (1965). Net movement of ions to plant roots occurs in the soil solution, predominantly by (1) diffusion down the concentration gradient between the bulk soil solution and the root surface - the gradient arises because of the removal of ions at the root surface by the absorbing root - and (2) convection, or mass flow, in the soil water that moves to the plant roots in response to transpiration.

The availability of mineral nitrogen for uptake by the root system is dependent on the soil moisture content (Bartholomew 1971). The rate of movement of ions through the soil to the root surface

decreases with decreasing soil moisture content. As the soil moisture content decreases pores become increasingly filled with air, and the tortuosity of the path of ion movement through the soil increases. As the soil dries to "wilting point", the moisture content at which the roots are unable to withdraw sufficient water from the soil because of the suction at which the water is held, convective flow of ions ceases. The soil suction at which wilting point occurs has been most commonly determined as pF 4.2. Below this moisture content soil nutrients are essentially unavailable for absorption by roots.

The rate of uptake of a given ion species from unit volume of soil at a given moisture content is influenced by the ionic concentration, and the length of roots within the unit volume, or root density L_v (Barley 1970). The greater the root density the shorter the mean distance travelled by ions to the root surface, and the greater the extent of the absorbing surface. The influence of root density on the rate of ion uptake depends on the mobility of the ion species in the soil. The rate of depletion of nitrate, a mobile ion , is unlikely to be influenced by L_v except where values are small ($<1\ cm^{-2}$).

The apparent recovery of applied nitrogen by crops (the difference in total nitrogen uptake between treated and control plants, expressed as a percentage of the amount of nitrogen applied) has been determined from applications made via the soil in a large

number of experiments (Allison 1966). Distinguishing fertilizer from native soil nitrogen by the use of ^{15}N-labelled fertilizer, the absolute recovery of applied nitrogen (the uptake of ^{15}N by the plants expressed as a percentage of the amount of ^{15}N applied) has been determined in some instances. In pot exerpiments with temperate cereal plants in which the soil was maintained moist and no leaching occurred, absolute recoveries of fertilizer nitrogen were found to fall in the range 50-60% (MacVicar, Garman and Wall 1950; Turtschin et al. 1960; Jansson 1963; Paul and Myers 1971; Zamyatina 1971). 20-30% was found to be incorporated into the soil organic matter, and 10-20% to be lost as gas. In pot experiments where no leaching and little denitrification could occur, Broadbent and Nakashima (1968) showed that nitrate-N was more fully recovered than ammonium-N. Although readily hydrolysed, urea came intermediate between the two. Significant (25-40%) volatilisation losses of ammonia from ammonium fertilizers occurred even though the soil was kept moist, unless the fertilizer was applied in a band below the soil surface. In field experiments with temperate cereal crops absolute recoveries of 50-60% have been obtained (Bobritskaya and Moskalenko 1969; Westerman, Kurtz and Hauck 1972), but more commonly lower recoveries by the crop (20-40%) and higher losses from the soil have been found (Bartholomew, Nelson and Werkman 1950; Bobritskaya and Moskalenko 1969; Myers and Paul 1971; Hamid 1972). Where absolute recovery by a barley crop (Hordeum vulgare) was 40-50%, recovery in the grain was found to be

20-30% (Bobritskaya and Moskalenko 1969).

Plants' needs for and uses of nitrogen have been reviewed by Viets (1965). Nitrogen absorbed by the roots of a plant is rapidly utilised, following transport to different parts of the plant. It is used for protein synthesis, in enzymes, metabolic intermediates and chlorophyll, and in deoxyribonucleic acids. It is therefore needed in all cells of the plant, particularly at the growing points. Ammonium is available immediately for incorporation into amino acids, but nitrate must be first reduced to ammonium before it can be utilised. Nitrogen is a mobile element within the plant and is readily translocated from one site to another (Pate 1971). It may accumulate in the plant as free nitrate when the supply exceeds the need for protein synthesis, and may be remobilized from protein compounds in some parts for use elsewhere (Williams 1955).

Urea has been shown to be rapidly absorbed from nutrient solution by the roots of rice (Oryza sativa) and bean (Phaseolus vulgaris) seedlings (Hirose and Goto 1961; Hentshel 1970). Kirkby and Mengel (1970) found that the yield of sunflower plants (Helianthus annuus) supplied in nutrient solution with nitrogen as urea was greatly inferior to that of plants supplied with nitrogen as nitrate at an equivalent concentration. Analysis of the plant amino acids suggested that urea-fed plants suffered disturbed protein metabolism.

The principal response of nitrogen-deficient temperate

cereal plants to an increased nitrogen supply to the roots is an increase in the leaf area (Watson 1952). The rate of assimilation per unit leaf area (net assimilation rate) is increased (Ryle 1970), but the influence of this on crop growth rate rapidly becomes dominated by the influence of an increasing assimilating area. The nitrogen supply to crops is commonly sub-optimal because high-yielding crops have a need for nitrogen greater than the capacity of most soils to supply it from native sources (Viets 1965).

The growth responses of temperate cereals to nitrogen applied via the soil are reviewed in section 2.2.2, in relation to time of application of nitrogen.

2.1.1. Foliar path

Inorganic salt solutions of heavy metal ions originally were applied to plant foliage to control pathogens. It was found that such applications could remedy a nutrient deficiency. Reports of the foliar absorption of mineral nutrients were published as early as 1844 (Wittwer, Bukovac and Tukey 1963). The ability of plants to absorb other nutrients from solutions applied to the foliage, including nitrogen, was subsequently recognised, and is utilised in citrus and plantation crop culture (Wittwer, Bukovac and Tukey 1963). In the past twenty years interest has grown in the possibility of supplying nitrogen to temperate cereals in foliar sprays.

Foliar absorption of nitrogen, and other nutrients, was first reviewed by Boynton (1954), and the accumulating literature on foliar absorption has been reviewed since by Thorne (1955b), Wittwer and Teubner (1959), Wittwer, Bukovac and Tukey (1963), Jyung and Wittwer (1965), Franke (1967) and Wittwer and Bukovac (1969). The experimental evidence suggests that absorption into leaves of substances applied to the leaf surface is a 3-phase process: (i) penetration of the leaf cuticle and cellulose wall of the epidermis by diffusion, (ii) adsorption to the surface of the plasma membrane (plasmalemma) by some form of binding, (iii) absorption into the cytoplasm in a process requiring metabolically derived energy against a concentration gradient - i.e.active accumulation. Inorganic ions and small organic molecules have been shown to be absorbed by leaves. Passage of substances to the plasmalemma appears to occur mainly via specific sites in the cuticle opposite the numerous ectodesmata of the outer epidermal cell walls, the ectodesmata being the paths of penetration of the cell walls (Franke 1967, 1969). Rates of absorption are found to depend upon the substance concerned, the plant species, and a range of chemical, physical and biological factors. Except for the influence of the cuticle, foliar absorption would appear to be analagous to root absorption.

Cations are absorbed more rapidly than anions, because of a predominance of negative charge sites within the cutin. Urea penetrates extremely rapidly. Consequently nitrogen is most commonly applied

as a foliar dressing to plants in this form. Urea has the additional advantage of being the most concentrated form of nitrogen fertilizer.

Application of nitrogenous solutions to the foliage of plants usually causes some leaf discolouration, indicative of injury. Chesnin and Schafer (1953) showed that applications of almost saturated solutions of urea (80% w/v) could be made to wheat plants at flowering time without causing injury. Other species were more sensitive, especially to sprays of larger droplets. Insofar as a dilute solution becomes concentrated on the leaf surface as it dries the initial concentration employed is not likely to be of significance, except as it affects the quantity of solute received by unit area of leaf or per droplet. Hinsvark, Wittwer and Tukey (1953) found that susceptibility to leaf injury from urea spray of a range of plant species tested was positively correlated with the urease activity of the leaves, and hence the rate of hydrolysis of the urea. A correlation is known between the rate of absorption of foliar-applied nitrogen (as affected by additives such as sucrose) and sensitivity to leaf injury (Franke 1967).

Foliar nitrogen fertilization is most commonly practised with fruit and plantation crops, in situations where the soil is dry or otherwise unsuitable to give results from soil applications, or where accurately timed inputs have valuable benefits on the quality of the fruit (Wittwer, Bukovac and Tukey 1963). Consequently most studies of foliar absorption of nitrogen have been performed with citrus and

plantation crop species. Various techniques have been employed to study absorption, some more satisfactory than others. A simple, commonly used procedure involves washing unabsorbed residue from the leaf surface before chemical analysis of the leaf, or alternatively of the leaf washings. The procedure is the only practical one where a spray is applied to the whole plant, but is not wholly satisfactory. Although it is usually demonstrated that washings beyond the first few contain negligible amounts of nitrogen, it is possible that sprayed material may be held within the leaf but not absorbed into the leaf cells, either bound within the cuticle (see Yamada, Wittwer and Bukovac 1965; Franke 1969), or within stomatal cavities (a thin cuticle lines stomatal cavities - Franke 1969). A more unequivocal demonstration is possible if labelled solution is placed on only a part of the leaf or plant and the presence of labelled material is detected in the untreated parts, as demonstrated elegantly for a range of cations and anions, not including nitrogenous ions, by Bukovac and Wittwer (1957).

Using the method of leaf washing, Cook and Boynton (1952) found that urea applied as a 1% solution spray to McIntosh Apple leaves (Malus pumila) was apparently rapidly absorbed into the leaf, up to 50% disappearing from the leaf surface within two hours of application, and 85% within 48 hours. Less than 50% of the absorbed urea was still in soluble form after 48 hours, indicating reaction or translocation of the absorbed material, and there was evidence of

translocation of absorbed nitrogen out of the leaf. The rate of absorption was increased by inclusion of a surfactant in the spray. Freiberg and Payne (1957) measured greater than 50% absorption of urea within 24 hours, when sprayed as a 0.3% urea-Bordeaux mixture containing surfactant onto the surface of banana leaves (Musa sapientum). Analysis of an entire banana plant 12 days after application of ^{14}C-labelled urea-Bordeaux mixture showed that 22% of the total detected radioactivity was located in the youngest expanded leaf and in the unfurled leaf. Analysing the washed pine needles taken from pine seedlings (Pinus elliottii) that had been dipped in 0.3% N solutions containing a sticker-spreader, Eberhardt and Pritchett (1971) calculated that 70, 45 and 40 per cent of foliar applied N as urea, calcium nitrate and ammonium sulphate respectively were absorbed into the needles within 24 hours. In an experiment where total recovery of applied N was 90%, about 80% was found in the needles, 10% in the stem and 1% in the roots.

Cain (1956) and Impey and Jones (1960) obtained more rapid uptake of urea by leaves of coffee (Coffea arabica), cacao (Theobroma cacao), banana, and orange (Citrus sinensis), but their results may be high because the urea solution was applied over the leaf surface with a glass rod. Volk and McAuliffe (1954) demonstrated that damaging the epidermal hairs on tobacco leaves (Nicotiana tabacum) by gentle brushing with a camel's hair brush increased absorption of urea tenfold, the brushing being shown to rupture the

impermeable cutinized layer of the epidermal hairs, permitting the urea solution to come into direct contact with the epidermal cell wall. Freiberg and Payne (1957) obtained more than 50% absorption of urea by banana leaves within 25 minutes when it was spread on the surface with a glass rod, compared with 24 hours when it was sprayed on.

Cain (1956) found that urea absorbed by coffee, cacao and banana leaves was apparently readily translocated out of the absorbing leaf. Loss of N from the leaf commenced within a few hours of absorption, and reached values of 60-70% of N applied after one or two days. There was a suggestion that some urea was incorporated into amino acids, which increased in coffee and banana following absorption.

Analysing washed sugar beet plants (Beta vulgaris) at the end of a season during which the leaves had been sprayed daily for a period with 0.35% N solutions of ammonium sulphate, calcium nitrate or urea, containing surfactant, Thorne (1954) found that all three nitrogen sources increased the total nitrogen weight per plant by about 25%. Plants were grown in pots and the soil surface was protected from spray. In further experiments (Thorne 1955a) it was estimated that recovery of the foliar-applied nitrogen was between 50 and 100%.

Wittwer and Tukey (1953) measured absorption of ^{14}C-labelled urea applied to leaves as the evolution of radioactive

CO_2 following hydrolysis by the enzyme urease, assumed to occur within the leaf. Solution concentrations of 0.1 - 0.5% urea were employed. Experiments were carried out in the dark to avoid re-entry of CO_2 evolved in photosynthesis. The rate of hydrolysis varied among 6 species compared, but in all cases appeared to be complete within 12 hours of application.

Volk and McAuliffe (1954) sprayed ^{15}N-labelled 3.2% urea solution, without surfactant, onto single leaves of young, nitrogen-deficient tobacco plants. The washed sprayed and unsprayed parts were analysed after varying absorption periods. A third of the applied urea had been absorbed into the sprayed leaf after 24 hours. Absorption continued at a slow but steady rate from 6 to 24 hours, even though the urea solution had dried on the leaf surface after 10 minutes (Impey and Jones - 1960 - also noted that absorption of urea, applied to orange leaves, continued for many hours after the urea appeared to be quite dry). The concentration of urea had little effect on the total absorption. Within 24 hours of spraying almost half of the absorbed nitrogen had been translocated out of the sprayed leaf to other parts of the plant, the centres of accumulation being the meristem, the root, and leaves adjacent to the sprayed leaf. Absorption was the same through upper and lower leaf surfaces, but was greater through younger than through older leaves and was somewhat sensitive to the pH of the applied solution.

The experiments of Kuykendall and Wallace (1954), and

Webster, Varner and Gansa (1955), in which detached leaves were immersed in urea solutions are invalid measures of absorption (in the former case leaves were washed by rubbing and wiped dry before treatment, and in the latter leaves were wholly immersed permitting absorption through the petiole), but demonstrated the ability of leaves to metabolise foliar-absorbed urea. Kuykendall and Wallace showed cultivar, species and leaf-age differences in the activity of urease in plant leaves, and calculated that sufficient urease activity was present for urea hydrolysis never to be a limiting factor in the assimilation of foliar-applied urea. Webster, Varner and Gansa showed that carbon from ^{14}C-labelled urea absorbed into bean leaves was incorporated into free and protein-bound amino acids in a similar pattern to carbon from absorbed sodium bicarbonate, suggesting that urea may be metabolised by its hydrolysis to carbon dioxide and ammonia. Yatazawa, Takemoto and Yamamoto (1958) reported assimilation of ^{15}N into all N compounds in the leaves of spinach plants (Spinacia oleracea) sprayed 1-2 weeks previously with ^{15}N-labelled urea. Dilley and Walker (1961) demonstrated the assimilation of N and C into amino acids, amides and protein within 24 hours, from ^{15}N, ^{14}C-labelled urea solution absorbed through the petioles into detached apple and peach (Prunus persica) leaves. An observed failure of peach leaves to utilise foliar-applied urea in the field was concluded to be due not to an inability to metabolise urea within the leaf but to an inability to absorb urea.

Few unequivocal demonstrations of the absorption of nitrogen compounds into the leaves of temperate cereal plants have been made. Makarevich (1964) sprayed ^{15}N-labelled ammonium citrate solution of an unspecified concentration onto the leaves of young barley plants growing in nutrient solution. Analysis of leaf washings suggested that 50% of the nitrogen applied was absorbed within the first 30 minutes, a further 25% within 3 hours, and a further 14% within 48 hours. Ammonium entering the leaves during the first six hours was shown to be partially incorporated into amino acids in the aerial tissues, and partially translocated to the roots where it was incorporated into amino acids and amides. Pavlov (1960) applied 2.5% ^{15}N-labelled urea solution to leaves of maize plants and reported translocation of ^{15}N to the roots, unsprayed leaves and cobs within five days. Alkier, Racz and Soper (1972) obtained contrasting results. ^{15}N-labelled urea, ammonium nitrate and ammonium sulphate solutions of unspecified concentrations were sprayed onto 8-week old wheat plants in pots, the soil surface being protected from spray. Recovery of ^{15}N in the grain finally harvested was found to be less than 2% in each case.

Petinov and Pavlov (1960) reported that uptake and incorporation into the grain of ^{15}N from solutions of labelled ammonium nitrate and ammonium sulphate applied to wheat plants was much greater when applied to the ears than when applied to the leaves. Pavlov and Kolesnik (1966) showed that ^{15}N-labelled urea absorbed from dilute

leaf sprays by maize and oat (Avena sativa) plants was translocated to the grain and utilised for the synthesis of various proteins qualitatively in the same way as ^{15}N-urea absorbed through the roots.

A number of experiments have been reported in which foliar sprays of nitrogen have been applied to temperate cereals in the field. These suffer from the difficulties that (i) a proportion of the spray may fall on the soil, or drip off the leaves onto the soil, (ii) rainfall subsequent to application may wash the sprayed nutrient off the leaves onto the soil. Therefore it is not possible to be sure whether resulting nutrient uptake and growth responses arise from foliar or root absorption of the sprayed nutrient. Thorne and Watson (1955, 1956) obtained apparent recoveries of applied nitrogen of 40-70% following spray applications of ammonium nitrate and urea to wheat and sugar beet plants in the field. Applications were made during vegetative growth, and the shoot material was analysed at maturity after washing. However rain always fell after application, in many cases within a few hours of treatment, and it is not known what proportion of the fertilizer recovered was in fact absorbed by the foliage. Reeves (1954) obtained an increase in the grain nitrogen content of wheat sprayed 7 weeks before flowering with urea at the rate of 40 kg N ha^{-1}, the apparent recovery in the grain at harvest being 18% of the applied nitrogen. Finney et al. (1957), Seth, Herbert and Middleton (1960), and Sadaphal and Das (1966) obtained marked increases in grain nitrogen content of wheat following spray

applications of urea near the time of flowering. Finney et al.(1957) and Sadaphal and Das (1966) recorded greatest apparent recoveries of applied nitrogen in the grain of 43% and 35% respectively in those experiments where single sprays were applied.

Except for experiments where foliar sprays have been applied in the field, most studies of the foliar absorption of nitrogen by plants have not included observation of the consequent growth responses. In pot experiments in which the soil surface was protected from spray (Thorne 1954), multiple sprays of 0.35% N calcium nitrate and urea solutions to the foliage of sugar beet plants caused 22% and 14% increases in plant dry weight respectively. Equivalent ammonium sulphate sprays caused no increase, possibly because of leaf injury which accompanied this treatment. Foliar applied N increased the leaf area per plant of sugar beet by increasing leaf size rather than leaf number, and did not alter the distribution of dry matter between root and shoot (Thorne 1955a; Thorne and Watson 1956). Barley plants grown in pot culture showed a normal tillering response to nitrogen when applied as a number of foliar sprays after anthesis (Aspinall 1963).

Moderate increases in shoot and grain yield from so-called foliar applications of nitrogen in the field have been reported in several instances for wheat (Thorne and Watson 1955; Finney et al. 1957; Thorne 1957; Simkins 1959; Hanley, Ridgman and Beveridge 1966; Sadaphal and Das 1966; Arnold and Dilz 1967; Alkier, Racz

and Soper 1972) and maize (Foy, Montenegro and Barber 1953). No response was reported by Mathur, Bhatnagar and Singh (1969) in the field, and by Alkier, Racz and Soper (1972) in pot experiments. Alkier, Racz and Soper concluded that the responses obtained by them from foliar application of nitrogen in the field were probably due to nitrogen washed off the leaves and absorbed by the roots from the soil.

2.1.3 Comparison of foliar and soil/root paths

A number of experiments have been conducted in which foliar applications of nitrogen have been compared with equivalent applications made to the soil, or substitute root medium. Makarevich (1964) supplied ^{15}N-labelled ammonium citrate to barley plants growing in solution culture either wholly to the solution bathing the roots or wholly to the foliage. The plants were grown for one day prior to application on nitrogen-free solution. Samples were analysed, after washing, at intervals up to 48 hours after treatment. Foliar application supplied 15% less nitrogen than application to the nutrient solution on account of spray losses. Foliar absorption initially was much greater than root absorption, but after 48 hours twice as much ^{15}N had been absorbed into the plant from the solution as from the foliar spray. With both paths of supply more than 90% of the ^{15}N absorbed was incorporated into non-protein and protein nitrogen fractions 48 hours after treatment, though a build-up of ammonium

nitrogen occurred with the foliar-treated plants up to 6 hours after treatment. In pot experiments with wheat Simkins (1959) reported that foliar-applied nitrogen increased yield when 90 kg N ha^{-1} or more was used, but yields were lower than those obtained from equivalent nitrogen application to the soil. Alkier, Racz and Soper (1972) obtained no yield response to ^{15}N-labelled nitrogen as a foliar spray or broadcast on the soil when applied to wheat in pots 8 weeks after sowing. However, approximately 30% of applied ^{15}N was recovered in the grain in the case of broadcast application compared with less than 2% in the case of foliar spray.

In field experiments uncertainty exists as to the actual path of entry into the plant of foliar-applied nitrogen (see section 2.1.2). Foy, Montenegro and Barber (1953) found that the yield response of maize to foliar application of nitrogen was the same as that to N applied as a side dressing, the soil being kept in a moist condition, but where leaf injury occurred at the high rate of foliar application yield response was reduced. Nitrogen uptake was not measured. Van Berg and Arnold (1963) reported that urea spraying was less effective at increasing yield of temperate cereals than addition of solid fertilizer. In comparing the effects of equivalent applications of ammonium sulphate, sodium nitrate or urea as solid applied to the soil, or as solution applied to the foliage of winter wheat early in the tillering phase in spring, Hanley, Ridgman and Beveridge (1966) found that solid applications consistently

resulted in greater growth and higher grain yields than sprays. In these experiments in which the plants were small at the time of treatment a large proportion of the "foliar" spray would have fallen on the soil. Alkier, Racz and Soper (1972) found no difference in the yield response and grain nitrogen content of wheat from applications of nitrogen during vegetative growth to the soil or the foliage. They concluded on the basis of results from a pot experiment (see section 2.1.2) that nitrogen received by the foliage in the field experiments was probably washed onto the soil by rain and absorbed by the roots.

Simkins (1959) reported that foliar applications of nitrogen to wheat were less effective in increasing grain nitrogen content than applications to the soil when made before the time of flowering, but the reverse was true when the applications were made after flowering. The same was reported by Soth, Herbert and Middleton (1960). The effect may be due to a drier surface soil later in the season, reducing the recovery of soil applied N later. However it may result from post-heading foliar applications of nitrogen falling partly on emerged ears, while pre-heading applications fall only on the plant foliage (see section 2.1.2).

2.2 Influence of the time of nitrogen application on nitrogen uptake and growth response

The literature relating to the effects of time of application of nitrogen on wheat falls into two distinct categories: (i) that concerned with autumn versus spring application to winter wheat in cold temperate climates, and (ii) that concerned with application at different stages in crop development to spring wheat, or to winter wheat after vernalisation. With winter wheat, nitrogen application in early spring generally produces a higher yield of dry matter and grain at maturity than an equivalent application at sowing in the preceding autumn (Bullen and Lessels 1957; Widdowson, Penny and Williams 1961; Van der Paauw 1962, 1972; Devine and Holmes 1964). The poorer yield from autumn application of nitrogen is associated with the amount of winter rainfall, and results from loss of fertilizer N by leaching during winter. Comparisons of autumn versus spring applications of nitrogen to winter wheat are not considered further, since they are not immediately relevant to the present study.

In the following account the nitrogen uptake and growth response from applications of soluble nitrogen fertilizers are considered. The essential features of the problem under consideration were clearly distinguished by Watson (1936). Watson concluded that the variable relation between time of application of nitrogen

fertilizer and the yield response of wheat in field experiments was due to the importance of meteorological factors in the results obtained. In particular Watson believed the amount and distribution of rainfall during the growing season to be important. Two effects of rainfall on the utilization of applied nitrogen were noted. Relatively high rainfall may leach the nitrogen out of the zone of soil from which it can be absorbed by the crop roots; while if rainfall is relatively low, water supply may become a limiting factor to growth. Thus the problem should be considered from two aspects: (i) the effect of meteorological factors in influencing the availability to the crop of the nitrogen applied; (ii) the ability of the crop to utilise in growth a given quantity of nitrogen fertilizer presented at different times to, and remaining within the sphere of activity of, its roots.

In the light of subsequent research other factors can be identified that may influence the response of wheat to an application of nitrogen, which should be added to those identified by Watson (see section 2.1.1): (i) the importance of moisture content of the soil in determining the availability of applied fertilizer for absorption, inorganic nitrogen being unavailable for absorption even though within the zone of root activity when the soil is dry (Gardner 1965; Clarke and Barley 1968; Bartholomew 1971); (ii) the importance of mechanisms of nitrogen loss from the root zone following application other than leaching (Allison 1966); (iii) the

modifying effects on growth response of other environmental factors such as light energy, temperature and supply of other essential nutrients (Milthorpe and Ivins 1965).

Clearly the growth response of crops to nitrogen application depends in the first instance on the amount of applied nitrogen that is recovered.

2.2.1 Recovery of applied nitrogen by the plant

Whenever sufficient nitrogen is not supplied by the soil, the recovery of applied nitrogen by temperate cereals or other nitrogen demanding crops has an overriding influence on the growth response obtained. This is because, in the absence of other limiting factors, the dry matter yield tends to be linearly proportional to N application up to relatively high levels of application (Viets 1965).

Nathan (1963) grew wheat plants in pots of Perlite supplied regularly with nutrient solution of either low or high nitrogen content. Pots were changed from the low-N to the high-N supply at 2, 4, 6, 8, 10 or 12 weeks after emergence. In this situation total nitrogen uptake at maturity was proportional to the duration of growth on the high-N supply. A similar experiment was conducted by Thenabadou (1972) with rice plants in sand culture. Pots were changed from low-N to high-N nutrient solution at fortnightly intervals from 32 to 130 days from sowing. At

maturity (175 days) plants that had been changed before anthesis (100 days) had utilised more N than those changed after anthesis. N utilisation decreased with delay in transfer to high-N nutrient solution after anthesis, but it did not differ significantly between plants transferred at various stages before anthesis.

In pot culture using soil, Watson (1936) obtained similar apparent recoveries of nitrogen from applications of sodium nitrate to wheat at seven different stages between germination and ear emergence. The same result was obtained for three rates of N application. The average apparent recovery, which did not differ significantly between the rates of application, was 51%. In a group of similar pot experiments conducted by Thorne (1962), nitrogen was applied either early in the tillering phase (early spring) or at ear emergence (late spring) to plants growing in soils to which varying amounts of ammonium nitrate had been added initially. In two experiments with winter wheat N uptake at maturity was the same for each time of application at each level of basal N. The average apparent recoveries were 83% and 73% respectively. In a third experiment with spring barley apparent recovery was unaffected by basal N level, but was slightly greater from early (58%) than from late (49%) application.

In these pot experiments it would appear as though all the nitrogen applied was rapidly taken up by the plants, excepting a constant proportion in each set of culture conditions that was

immobilised or lost from the soil. The favourable soil water regime and the high root densities that are rapidly developed in pots of limited dimensions would lend support to this conclusion.

Balba, Hassan and Mady (1972) reported the only known experiment in which absolute recovery of nitrogen applied at different stages of development to temperate cereals was measured, using ^{15}N-labelled fertilizer. Recoveries of N supplied as ammonium sulphate to pots at sowing, tillering (further definition not given), or ear emergence were 11, 27 and 12% respectively. The recoveries reflected the dry matter increases of the control plants in the periods following application, sowing-tillering, tillering-ear emergence and ear emergence-maturity (17, 56 and 27% of the total respectively). The low recoveries of applied nitrogen and the high soil moisture content employed (70% of field capacity) suggest the possibility of high denitrification losses of fertilizer from the soil. Under conditions of steady loss of N following application, recovery by the plant is likely to be correlated with the rate of absorption of N, or less immediately with the rate of growth.

In a review of English field experiments, Watson (1939) concluded that the ability of wheat to recover nitrogen applied early or late in spring was independent of the time of application of the fertilizer, up to ear emergence; and the variation between the times of application in individual years was determined by the

particular weather conditions of the season. Watson believed that the rainfall, particularly that falling in a short period following application, was likely to be the main factor responsible, as it was closely correlated with yield. The correlation was positive on a heavy clay soil and negative on a sandy loam. Rain following application of nitrogen fertilizer to the soil surface would wash the fertilizer in, making it more rapidly available for absorption; but with heavy rain on light soil it may pass through the soil profile, beyond the root zone. Davidson and Buchanan (1945) and McBeath and Toogood (1960) noted that the utilisation of nitrogen applied at various stages in the season in their field experiments was greatly dependent on precipitation following application.

The conclusion of Watson (1939) would appear to apply to the similarly variable results of many other field experiments on the time of application of nitrogen in spring to autumn- and spring-sown cereals in Britain (for example, Holmes and Tahir 1956; Holmes, Gill and Roger 1960; Beveridge, Jarvis and Ridgman 1965).

In North America Rankin (1946) obtained a greater apparent recovery from application of nitrogen to winter wheat early (70%) than from a similar application late (45%) in spring. The rate of nitrogen uptake by the crop was greatest from the late-supplied N, but this rate was not sustained for long enough for the final uptake to equal that from the earlier application. Apparent recovery from an equivalent amount supplied by regular small additions through winter and spring

was 85%.

In a field experiment on a coarse sand in Western Australia (Mason, Rowley and Quayle 1972), urea was applied to wheat 0, 2, 4 or 8 weeks after sowing, the last application being in the middle of the tillering phase of development. Apparent recoveries of nitrogen were respectively 21, 33, 38 and 47%. Sequential soil analyses showed considerable, rapid leaching of N into the lower part of the soil profile, which was more severe with the earlier applications. The apparent recoveries of applied N were attributed to the relative leaching conditions at each time of application, combined with the capacity for more rapid uptake the later the time of application. The rate of uptake of applied nitrogen by a crop is likely to be greater the later in vegetative development it is applied. This is because of the increase in crop growth rate and utilisation of nitrogen as vegetative development proceeds, and because of the increase in size of the root system. With a mobile ion such as nitrate, it has been shown that the rate of ion uptake by roots from unit volume of soil is unlikely to be limited by the root density, L_v, at values of L_v greater than 1 cm^{-2} (Barley 1970). Therefore, in the absence of leaching, the size of the root system is likely to influence the rate of uptake of applied nitrogen only until a root density greater than 1 cm^{-2} is developed throughout the surface soil. In the presence of leaching, the depth to which root density uniformly exceeds 1 cm^{-2} is likely to influence the rate of uptake. This normally increases as vegetative development

proceeds (Barley 1970).

Thus, the recovery of nitrogen applied to temperate cereals at different stages of development varies, probably depending on the availability and loss of fertilizer following application, and on the rate of uptake. Where the fraction applied that remains available for uptake is large, or is replenished, recovery is likely to be greater the earlier it is supplied because of the greater time available for uptake. If the fraction that remains available for uptake is completely taken up within a relatively short period following application, recovery may be the same from applications at different stages of development. This situation is likely with lower rates of application, or in pot culture where a high root density develops within a confined volume containing the applied fertilizer. Where severe losses of N occur following application, recovery is potentially greater from deferred than from sowing application of nitrogen because of a more rapid rate of uptake later in growth than immediately after germination.

2.2.2 Growth response

(a) Tillering

Aspinall (1961) examined the tillering behaviour of barley plants in pots in relation to nutrient supply. When the whole nutrient supply was added before sowing, a two-phase pattern of tillering was exhibited. An initial flush of tillers was

followed by a period of inactivity (weeks 8-18), with a smaller flush occurring 3-4 weeks after ear emergence. Plants supplied with part of their nutrient supply at various stages after sowing showed an immediate tillering response to added nutrients at any stage. In other experiments, Aspinall (1963) concluded that tillering during ear development is primarily controlled by competition between the grains and tiller buds for a limited nutrient supply.

Van Os (1966) also proposed that a state of competition for nitrogen exists within the cereal plant during vegetative development. He suggested that at the onset of grain-filling redistribution of nitrogen from the leaves begins to supply the needs of the grains, unless the external nitrogen supply is good, in which case there may be sufficient nitrogen available to support the leaves further or to promote tillering. Jewiss (1972) demonstrated that tillering in wheat and grasses is restricted during reproductive development, but that stem extension may be more important in controlling this restriction than changes at the stem apex.

In soil-culture pot experiments in which nitrogen was applied as a single addition of sodium nitrate to wheat on seven different occasions between sowing and ear emergence (Watson 1936), the maximum number of tillers produced declined the later nitrogen was applied after the middle of the tillering

phase. The latest application, made at 95% ear emergence, had no effect on tillering. Apparent recovery of applied N was similar for all times of application. The maximum number of tillers did not differ significantly between the first three times of application, made before the middle of the tillering phase. The middle of the tillering phase was defined as the stage at which the number of shoots per plant equalled half the eventual maximum. The number of ears matured per plant showed the same pattern as the maximum number of tillers.

Similar pot experiments were conducted by Thorne (1962), in which apparent recovery of N did not differ between treatments. In this case N applied at ear emergence stimulated tillering to the same degree as application early in the tillering phase, though the late-formed tillers were smaller than the early-formed ones.

In field experiments with winter wheat in Britain (Bremner 1969), N applied early in the tillering phase in spring increased the number of tillers and ears per plant. N applied later, but before ear emergence, had no effect on the maximum number of tillers, but increased their survival. The contribution to yield of tillers appearing after the date of the early application was small. Similar results were reported by Hunter and Hartley (1938), Thorne and Watson (1955) and Holmes and Tahir (1956) from field experiments in Britain, and by Van Dobben (1965) and

De Jong (1968) in reviewing the results of Dutch experiments.

(b) Leaf growth

The principal growth response of plants to applied nitrogen is increased leaf area (Watson 1952). In temperate cereals the tillering response to nitrogen increases the leaf area per plant, but increases may also occur in leaf size, leaf number per shoot and leaf longevity.

Khalil (1956) showed that the number of leaves produced by the main shoot of wheat may be influenced by nitrogen supply. Plants grown on nutrient solution of either high or low nitrogen content produced 9.2 and 6.5 leaves on the main shoot respectively at high light intensity. 6.0 and 5.8 leaves respectively were produced at low light intensity. Single (1964) showed that the number of leaves on the main stem of wheat was sensitive to nitrogen supply only during the period of leaf primordia production on the shoot apex - i.e. from germination until the stage of spikelet differentiation, or "double ridges".

The area of individual wheat leaves was shown to be increased by nitrogen application at sowing by Puckridge (1968). The magnitude of increase varied up to 300%, depending on the density of the plants. Other data on the stimulating effect of nitrogen application on leaf area has been expressed as total leaf area per plant or mean leaf area per shoot (Gregory 1937; Watson 1947; Watson, Thorne and French 1958, 1963).

Thorne and Watson (1955) examined the influence of nitrogen applied early in spring or at ear emergence on the leaf area of winter wheat in the field, in Britain. Early application of nitrogen increased leaf area per shoot as well as the number of shoots. The number of shoots remained greater than for the late N and control treatments until harvest. Late application of N had no effect on the number of shoots, but increased the leaf area per shoot and reduced the rate of leaf senescence after flowering. The net result was a similar leaf area duration after flowering from the two nitrogen treatments.

In pot experiments in which similar treatments were applied (Thorne 1962), maximum leaf area index was always greater from early than from late application of N. Late N application prolonged leaf area after flowering. In these experiments late application of N also produced a tillering response, and leaf area duration after flowering was greater than from early application of N. Delay in leaf senescence after flowering where nitrogen was applied to wheat late in vegetative development was also noted by Watson (1936).

An association between leaf senescence and the transfer of nitrogen from the leaves to the grain during grain growth was first shown by Knowles and Watkin (1931), and has been reviewed by Williams (1955). Applications of nitrogen to temperate cereals during the reproductive phase of growth raise the nitrogen content

of both straw and grain (see section 2.2.2f), and this undoubtedly accounts for the effects of late application of N on leaf area duration after flowering.

(c) Root growth

Little data exist on the root growth response of plants to nitrogen applied at different stages of growth. Brouwer, Jennekkens and Borggreve (1961) reported experiments in which maize plants were transferred at stages during vegetative development from Hoagland's nutrient solution lacking nitrogen to complete Hoagland's solution. Following transfer the rate of dry weight gain of the roots decreased or stopped until a lower root weight ratio (ratio of root weight to total plant weight) characteristic of the nitrogen-rich solution was reached. Thereafter shoot and root growth continued according to this ratio. The dynamic balance between root and shoot growth of cereals (growth in weight) in relation to nitrogen supply is discussed further by Brouwer (1965).

In soil-culture pot experiments Van Dobben (1962) reported that application of nitrogen at ear emergence to wheat offset the decline in root weight after flowering that occurred with unfertilized plants. Root weight increased marginally after flowering, but shoot growth was considerably stimulated by the late application.

The nitrate concentration of nutrient solution is known to have a pronounced influence on the growth in length of plant roots. The elongation rate of root axes is found to be increased (Bosemark 1954; Wiersum 1958; Brouwer, Jenneskens and Borggreve 1961; Brouwer and Loen 1962; Williams 1968) and the density of first-order laterals along the axes to be decreased (Wiersum 1958; Brouwer and Loen 1962) in solutions of low relative to high nitrate concentration[1].

Brouwer and Loen (1962), working with maize, showed that the elongation and branching of an axis was only influenced by the nitrate concentration of the ambient solution if the plant was nitrogen-deficient. If most roots, and therefore the shoot, were well supplied with nitrate, roots growing in solution lacking nitrate behaved as though they were well supplied with nitrate. Recently, Drew, Saker and Ashley (1973) observed the same behaviour with barley seminal axes growing in nitrate-deficient nutrient solution, when a zone of axis behind the apex, together with the associated laterals, was well supplied with

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(1) Factors additional to the concentration influence the supply of nutrients to roots in solution culture, such as the rate of solution replenishment and degree of stirring (Brewster and Tinker 1972). Therefore the relative terms "low" and "high" are convenient with reference to the solution concentration, where "low" implies deficiency and "high" implies sufficiency.

nitrate. However, they found that the elongation response of seminal axes growing entirely in nutrient solutions of contrasting nitrate concentrations altered with time. After elongating for the first few days after germination more rapidly in solution of low than of high nitrate concentration, subsequently elongation rate, as well as branching density, became inferior in the low nitrate concentration solution. The contrast in response with other reports in the literature may be accountable to the degree of nitrogen deficiency induced in the plants by the low-nitrate nutrient solution. This point is discussed in section 5.4.

The response of laterals to ambient nitrogen concentration when this has been applied uniformly over the root system has not been examined, except by Drew, Saker and Ashley (1973). Working with barley seminal roots, they observed the same elongation and branching behaviour of first- and second-order laterals as was ultimately exhibited by the axis; that is reduced elongation rate and branching density in low relative to high nitrate concentration solution.

May, Chapman and Aspinall (1965) applied nutrient solutions of varying overall concentration regularly to barley plants growing in pots of vermiculite. They showed that the elongation rate of the seminal root axes and first-order laterals was increased, while the density of first-order laterals along the axes was decreased, with decreasing overall nutrient concentration.

Brouwer, Jenneskens and Borggreve (1961) showed with oat, barley, rye (Secale cereale), maize and perennial ryegrass (Lolium perenne) that the elongation rate of the seminal root axes decreased following transfer from nutrient solution of low nitrate to solution of high nitrate concentration. Seedlings raised on Hoagland's nutrient solution (containing only nitrate-nitrogen) were grown in Hoagland's solution without nitrate for three days before transfer back to Hoagland's solution. Root growth was observed for 7 days following transfer.

Although an increased supply of nitrate to root systems tends to reduce the length of the root axes, except at excessive levels it increases root dry weight (Brenchley and Jackson 1921; Turner 1922; Meyer and Stork 1927; Ballard, Petrie and Cornish 1936; Goedewaagen 1942). The contrasting effects on weight and length are a result of the greater weight per unit length (i.e. thickness) of filaments in higher nitrogen concentration media.

An increased nitrate concentration around part of an otherwise nitrogen-deficient root system has been shown to produce a localised growth response. Drew, Saker and Ashley (1973), using barley seminal roots, showed that both the elongation rate and branching density of first- and second-order laterals were increased in a zone of the axis supplied with high-nitrate nutrient solution, relative to the remainder of the root which

was separately supplied with low-nitrate solution, and to the equivalent zone on roots supplied entirely with low-nitrate solution. Hackett (1972) obtained similar results for first-order laterals with wheat. Drew, Saker and Ashley showed that the local stimulation of lateral growth was accompanied by reduced lateral growth in the remainder of the root. Thus lateral growth in the enriched zone also exceeded that in the equivalent zone of roots supplied entirely with high-nitrate nutrient solution.

Localised enrichment of soil in pots (Nobbe 1862; Crist and Weaver 1924; Passioura and Wetselaar 1972) and in the field (Weaver 1926; Gliemeroth 1953) has been observed to bring about a proliferation of root length within the enriched zone of soil after an initial lag.

(d) Development of the inflorescence

The morphological development of the main shoot apex of wheat prior to its emergence from the flag leaf sheath as the immature ear has been described by several authors (e.g. Bonnett 1936; Barnard 1955; Williams 1966a). It has been reviewed by Williams (1966b). Attributes of the apex which are determined before ear emergence are the number of leaves and spikelets, and the number of florets differentiated per spikelet. After ear emergence the fertility of the florets (i.e. the number of

potential grains set) and the size of the individual grains are determined. Attributes of the inflorescence are sensitive to environmental conditions only before they have been determined.

Early development of the wheat shoot apex in relation to nitrogen supply has received little attention. Single (1964) showed that plants grown in nutrient solution containing 0, 0.14, 0.57 and 4.0 $\mu M\ l^{-1}$ N produced 6, 11, 13 and 16 spikelets on the main shoot apex respectively. The rate rather than the period of primordia production was increased. Spikelet number was increased on plants transferred from lower-N to high-N solution at the stage of spikelet differentiation ("double ridges"), but not on plants transferred later at the stage of differentiation of anther initials. The number of rachilla nodes (equivalent to the potential number of florets) similarly was increased with increasing nitrogen supply. The number was increased more in plants transferred to high-N solution at "double ridges" than in plants transferred at anther differentiation. The number of grains set was greatest on plants grown throughout on high-N solution, and equally greater on plants transferred at either stage than on those grown on lower-N solutions throughout.

Lucas (1971) also showed that spikelet number on the main shoot of wheat is sensitive to the nitrogen supply prior to determination of spikelet number, and that the length of the phase from germination to spikelet determination (shortly after "double

ridges") was relatively unaffected. Spikelet number was slightly increased on plants transferred from low-to high-N supply at the double-ridge stage.

Thenabadou (1972) found that the number of filled grains on rice plants grown in solution culture was severely depressed when plants were transferred from low-N to high-N solution at the time of flowering, relative to plants transferred a fortnight before or a fortnight after flowering.

Almost all pot and field experiments with temperate cereals report the attributes of the ear as mean values per plant, confounding the values for the different ears present. Two groups of ears are of interest - those present in a fairly advanced stage of development at the time of application, and those initiated in response to application which develop from the start at a relatively high plane of nitrogen nutrition. Thorne (1962) reported ear attributes of wheat plants in two pot experiments for the two categories respectively. In an experiment where only main shoots were present at ear emergence, when nitrogen was applied, N application did not alter the number of grains per main shoot but increased the mean weight per grain. The latter probably resulted from the delaying of leaf senescence after flowering that was caused by late application of N (see section 2.2.2.b). Tillers initiated in response to nitrogen produced fewer grains of a smaller mean weight than the main shoots. In

another experiment, tillers initiated in response to N application at ear emergence produced a smaller mean number of grains per ear than early-formed tillers where a basal nitrogen dressing had been applied before sowing, but a larger number where it had not. This reflected the relative sizes of the early-formed ears at each rate of basal N. Mean weight per grain was again considerably less for late-formed than for early-formed ears.

In detailed pot culture studies with wheat (Beveridge, Jarvis and Ridgman 1965), nitrogen increased the mean total number and fertile number of spikelets per ear, the mean number of grains per ear and the mean weight per grain, whether it was applied at sowing in winter or in the tillering phase in spring.

In field experiments, an increased mean number of grains per ear from application of N early in the tillering phase, and a decreased mean number from application after stem extension have been reported (Rankin 1946; De Jong 1968). The decrease from late application of nitrogen probably reflects the presence of late-formed, small ears. An increased mean weight per grain from late application of N has frequently been recorded (Holmes and Tahir 1956; Sadaphal and Das 1966; De Jong 1968; Bremner 1969). Rankin (1946) reported a slight decrease in mean weight per grain from N application late in the vegetative phase relative to early application. Bremner (1969) and Sadaphal and Das (1966) reported an increase in the mean number of grains per ear from N application late in the vegetative phase.

(e) Dry matter yield - shoot and grain

The recovery of nitrogen applied at different stages of development by the crop can vary (section 2.2.1). The growth response to application of nitrogen is considerably dependent on the proportion of nitrogen applied that is recovered by the crop. In a pot experiment with wheat where the apparent recovery of nitrogen applied at seven different stages of development between germination and ear emergence was constant (Watson 1936), shoot dry weight decreased steadily with lateness of application. Application at ear emergence produced no increase in shoot dry weight over unfertilized plants. Grain yield, however, was increased above the level of unfertilized plants to the same extent by nitrogen application at every stage, except for the last. This was a result of compensating effects on grain yield attributes from the application of nitrogen at different times (see sections 2.2.2.a, d).

Other pot experiments in which recovery of nitrogen applied at different times to wheat and barley was constant were conducted by Thorne (1962). In accord with Watson (1936), nitrogen applied at ear emergence increased shoot and grain yield less than nitrogen applied in the tillering phase.

The non-effectiveness of nitrogen applied as late as ear emergence in increasing shoot or grain yield relative to

applications during the vegetative phase was shown in carefully conducted field experiments in North America (Davidson and LeClerc 1917, 1918, 1923). A variety of nitrogen fertilizers were applied in solution to wheat and plots were subsequently watered to ensure fertilizer uptake.

In pot experiments in which the recovery of fertilizer applied at different stages of growth was not reported, varying results have been obtained. Gericke (1920, 1922a) obtained a higher grain yield of wheat from applications of sodium nitrate or ammonium sulphate during the tillering phase than from applications at or soon after sowing. The same was shown by Neidig and Snyder (1922) and Balba, Hassan and Mady (1972). In a pot experiment of Jonker (1961), quoted by Van Dobben (1965), nitrogen at low and high rates gave an increasing yield response with increasing lateness of application up to the beginning of stem extension, but thereafter gave a decreasing response. Straw yield decreased with lateness of application. Simkins (1959) and Alkier, Racz and Soper (1972) obtained a greater grain yield response of wheat from nitrogen applied at sowing than from nitrogen applied later in the vegetative phase. Spratt and Gasser (1970) obtained as great a yield of straw from an application of N made just prior to ear emergence as from an application at sowing, but a lower yield of grain.

Van Dobben (1965) discussed results of Dutch work in

pot and field experiments on the influence of time of application of nitrogen to temperate cereals on vegetative growth and grain yield. In general nitrogen applied early in growth (in spring) was found to increase shoot weight but late application near the time of ear emergence was not. Early N increased grain yield primarily by increasing the number of ears, while late N increased the size of existing ears. Which time of application gave the higher grain yield depended on the conditions. Under low fertility conditions plants receiving late N tended to be too poor to fully compensate by increases in ear size, whereas under higher fertility as high or higher grain yields were obtained from late as from early applications.

In reviewing the results of 270 regional field trials in Britain, Bullen and Lessels (1957) concluded that with both winter and spring wheats, at low rates of N application (up to 40 kg N ha^{-1}) there was no difference in grain yield response between early and late spring dressings, but at high rates (greater than 90 kg N ha^{-1}) there was a definite advantage of early over late applications. Widdowson, Penny and Williams (1961) reported that at a low level of application to winter wheat, gains in yield were greatest from a late spring application, but at a higher level an early spring application had a slight advantage.

Similar grain yield response from application of nitrogen early in spring as from later application up to the stage of

heading have been reported from many field experiments in northern Europe with winter wheat (Garner and Sanders 1936; Lewis, Proctor and Trevaines 1938; Watson 1939; Bacher 1941; Halliday 1948; Thorne and Watson 1955; Blackett 1957; Holmes and Tahir 1956; Beveridge, Jarvis and Ridgman 1965; Bremner 1969) and spring barley (Holmes, Gill and Roger 1960), and in North America with spring barley (Foote and Batchelder 1953). Straw yield, where reported, was increased more by early than by late application. A greater yield of grain from early than from late application of N with spring wheat and barley in Britain (Hunter and Hartley 1938; Widdowson and Cooke 1958), and with winter wheat in North America (Davidson 1922), have been reported.

Grain yield responses have been lower where N application has been delayed until ear emergence than where it has been applied earlier in vegetative growth (Rankin 1946; Davidson and Buchanan 1945; Long and Sherbakoff 1951; McBeath and Toogood 1960). Finney *et al.* (1957), Sadaphal and Das (1966), and Hucklesby *et al.* (1971) reported grain yield increases from N applied to winter wheat as late as ear emergence, but no comparisons with earlier application were made.

In field experiments in South Australia, application of nitrogen to wheat up to 12 weeks after sowing, during the vegetative phase, has generally given smaller grain yield responses than application at sowing (Birks and Cole 1930; Richardson and Gurney

1935; Reuter 1967; Russell 1969). In an experiment on a coarse sand in Western Australia, application of nitrogen at stages up to 8 weeks after sowing gave increasing grain yield with increasing delay in application (Mason, Rowley and Quayle 1972). The trend in grain yield response was probably a result of the parallel trend in apparent recovery of applied nitrogen (see section 2.2.1).

Applications of nitrogen to temperate cereals after ear emergence have not increased grain yield (Davidson and LeClerc 1917, 1918; Neidig and Snyder 1922; Hunter and Hartley 1938; Davidson and Buchanan 1945; Finney et al. 1957; Wood and Fox 1965; Sadaphal and Das 1966; Thenabadou 1972). Application of nitrogen at the time of flowering was found to depress grain yield by Finney et al. (1957), Simkins (1959) and Thenabadou (1972), probably through impaired grain set. In other experiments however this was not found (Hunter and Hartley 1938; Davidson and Buchanan 1945; Wood and Fox 1965).

Split applications of nitrogen to temperate cereals, where the first dressing is made at sowing and the second dressing is made at the stem extension or ear emergence stage, usually increase grain yield more than single applications either early or late (Coic 1954; Widdowson, Penny and Williams 1961; Arnold and Dilz 1967; De Jong 1968; Kodanev, Shibaev and Maslovskii 1968; Jain, Maurya and Singh 1971). The crop responds to each application in the same way as to equivalent single applications, though not to the same degree in the case of the second dressing.

(f) Grain protein content

The protein content of temperate cereal grain is usually estimated from the grain nitrogen content, and reported as the product of the grain nitrogen content and a factor. The factor is usually 5.7 in the case of wheat. In this case it is strictly referred to as crude protein content. The transformation into protein of nitrogen entering the wheat grain has been characterised in a number of studies (Woodman and Engledow 1924; Knowles and Watkin 1932; McCalla 1933; McCalla and Newton 1935).

An analysis of the processes determining grain nitrogen content is pertinent to an understanding of the influence of time of nitrogen application. Nitrogen enters the grain of wheat more or less steadily as it develops from anthesis to maturity (Brenchley and Hall 1908; Woodman and Engledow 1924; McCalla and Newton 1935). A proportion of the grain nitrogen is derived from N accumulated during vegetative growth in the leaves and stems, which is remobilised and transported to the developing grain after anthesis, accompanied by senescence of the leaves and stems (Williams 1955). Where the plant does not absorb nitrogen from external sources after anthesis all of the grain N is derived from the plant. Since nitrogen taken up late in development is not normally utilised in new growth (section 2.2.2.a), an additional supply of N is available for translocation to the grains where appreciable amounts of N are absorbed shortly before or after anthesis. The final nitrogen

content of the harvested grain is determined by the amount of grain produced relative to the amounts of nitrogen (i) remobilised from within the plant, and (ii) absorbed from external sources near the stage of anthesis (where this is not utilised in vegetative growth).

Where environmental factors, such as moisture stress, reduce grain yield below the potential level predisposed by vegetative growth, and nitrogen remobilisation and transport is not restricted, grain nitrogen content is increased (Neidig and Snyder 1922, 1924; Hutcheon and Rennie 1960; Dubetz 1961; Terman et al. 1969; Partridge and Shaykewich 1972). Where nitrogen uptake after anthesis is slight grain nitrogen content is likely to be low because the greater the vegetative growth before anthesis, which is the pool of N for the grain, the greater tends to be the grain yield. Thus, Hutcheon and Paul (1966) found in pot experiments in growth cabinets that the nitrogen content of Thatcher wheat could be effectively controlled in the range 1.9 - 3.9% by nitrogen supply and moisture stress. Where moisture supply was unlimiting high grain yields were obtained, which were only of a high nitrogen content if a fertilizer dressing, at sowing, was applied. Nitrogen contents higher than 2.8% were obtained only where a growth factor such as moisture stress prevented maximum yield from being realised.

Cereal plants continuously supplied with nitrogen, such as in nutrient solution culture, will absorb nitrogen at near maximum

rate almost up to maturity (McCalla 1933; Nathan 1963). Nitrogen uptake from unfertilized soil usually falls towards the end of vegetative growth because the soil soluble nitrogen fraction becomes depleted, and because the topsoil frequently dries out in the latter part of the season (Viets 1965). Nitrogen fertilizer applied at sowing promotes vegetative growth, and therefore will only raise grain nitrogen content appreciably if a proportion remains for absorption later in growth, such as where heavy applications are made (e.g. Russell, Smith and Pitman 1958).

In a pot experiment with wheat in which the apparent recovery of nitrogen was constant from applications at different stages between germination and ear emergence (Watson 1936), the nitrogen content of the grain was increased by the later applications of N. It was increased particularly by the latest application, made at 95% ear emergence. This occurred against a background of constant grain yield from every application of nitrogen except the latest, which produced a lower grain yield. Gericke (1920, 1922b) obtained similar results in earlier pot experiments with wheat, oat and rye using both sodium nitrate and ammonium sulphate sources of nitrogen.

Where a vegetative growth response was obtained from an application of N at ear emergence to spring wheat in pots, Spratt and Gasser (1970) found that the grain nitrogen content was only slightly raised relative to that obtained from an application at

sowing. In sand culture replenished regularly with nutrient solution (Thenabadou 1972), the grain nitrogen content of rice was raised to the same extent in plants transferred from low-N nutrient supply to high-N supply at any stage up to 2 weeks after anthesis. Simkins (1959) reported that in pot experiments where nitrogen was applied to wheat at different stages during growth, the nitrogen content of both grain and straw was increased with each additional increment of N applied. The nitrogen content of grain was increased from 1.9 to 3.8% by the use of 360 kg N ha^{-1} equivalent.

In field experiments in North America and northern Europe it has been shown repeatedly that application of nitrogen late in vegetative development to winter and spring wheat has increased grain nitrogen content more than early application (Davidson and LeClerc 1917, 1918, 1923; Davidson 1922; Davidson and Schollenberger 1926; Gericke 1927; Davidson and Buchanan 1945; Long and Sherbakoff 1951; Widdowson and Cooke 1958; Simkins 1959). The same has been shown with spring barley and oats (Hunter and Hartley 1938; McBeath and Toogood 1960). In these experiments the grain yield response was less to late-applied than to early-applied N. Davidson (1922) and McBeath and Toogood (1960) concluded that the effectiveness of N in increasing the nitrogen content of the grain increased as the effectiveness in increasing yield decreased. Nitrogen contents

were of the order of 2.1% and 2.6% from the early and late applications respectively.

In some experiments early and late applications of N have given the same grain yields and nitrogen contents (Thorne and Watson 1955; Holmes, Gill and Rodger 1960). In others early and late applications have produced the same grain yields, but a higher grain nitrogen content has resulted from the late application (Holmes and Tahir 1956; Widdowson, Penny and Williams 1961).

Where reported, late applications of nitrogen were shown to increase straw as well as grain nitrogen content at maturity (Davidson and LeClerc 1918; Simkins 1959).

The interaction between grain yield and grain nitrogen content with respect to time of nitrogen supply was clearly demonstrated by Wood and Fox (1965), in a field experiment with wheat conducted in Australia. In a treatment where natural rainfall was supplemented by irrigation, a large increase in grain yield and a small increase in grain nitrogen content were obtained from an application of nitrogen at sowing. A small increase in yield and a large increase in nitrogen content resulted from an equivalent application at flowering. Both high yield and high nitrogen content were obtained from an application at both times. A large mean weight per grain where both high nitrogen content and grain yield were obtained, spoiled

the otherwise apparent inverse correlation between mean grain size and grain nitrogen concentration, indicating an associative rather than a causative effect.

A lack of correlation between grain size and grain nitrogen content with respect to time of nitrogen application has been reported in other experiments where grain-filling was not restricted by limiting water supply late in development (Gericke 1922b; Davidson and LeClerc 1923; Hunter and Hartley 1938; Davidson and Buchanan 1945).

Applications of nitrogen to wheat as late as the milk stage of grain development have not increased grain nitrogen content (Davidson and LeClerc 1923; Davidson and Buchanan 1945), even when availability of the applied fertilizer was ensured by watering it in (Davidson and LeClerc 1917). Applying nitrogen to wheat in the field as foliar sprayed urea solution, Finney et al. (1957), shewed that the effectiveness of a single spray of urea in increasing grain protein content was greatest with an application made at flowering, and decreased steadily the later after and the earlier before flowering the application was made. Applications made 50 days before and 28 days after flowering did not increase protein content significantly above the level of untreated plants. The grain ripened 36 days after flowering. Grain yield was not influenced by applications after flowering. With multiple sprays the effects were the same but additive.

Fifteen consecutive sprays from 0 to 32 days after flowering produced the highest grain protein content, 21.0%. Simkins (1959) also reported that the most effective time of nitrogen application in increasing the grain nitrogen content of wheat was at flowering.

McCalla (1933) believed that wheat plants given a high nitrate supply during grain filling have a reduced rate of flow of assimilate into the grain. Plants grown in complete nutrient solution (containing only nitrate nitrogen) until maturity had a lower mean weight per grain than plants changed at ear emergence to nitrate free nutrient solution. Nitrogen absorbed by plants after ear emergence accumulated in vegetative parts in the form of non-protein compounds. McCalla concluded that the high nitrate content of the shoots continuing to absorb nitrate after ear emergence required carbohydrate to reduce it to non-protein nitrogen, resulting in less assimilate available for translocation to the grain.

Split applications of nitrogen to cereals, where a second dressing is made near the time of ear emergence, have much the same qualitative effects on grain nitrogen content as single late applications, though increases are less marked from the second dressing because of the influence of the earlier application (Widdowson and Cooke 1958; Arnold, Kersen and Van Berg 1964; Wood and Fox 1965; Arnold and Dilz 1967; Kodanev, Shibaev and Maslovskii 1968; Hucklesby et al. 1971).

Differences between wheat cultivars in the extent to

which nitrogen supplied at different stages of growth influenced grain nitrogen content were shown by Gericke (1927, 1933). Seth, Herbert and Middleton (1960) found no differences in the total N content of the vegetative parts of cultivars of characteristically high and low protein content prior to ear emergence. After ear emergence N content increased more rapidly in the ears of high protein cultivars. The increase in grain nitrogen content following foliar application of both nitrate- and urea-N after ear emergence was greater in the high-protein than in the low-protein cultivars.

With wheat, grain crude protein contents of the order of 12% are common, while contents of the order of 16% are considered high. Hutcheon and Paul (1966) found that the highest values that could be obtained for well-filled grain from sowing applications of nitrogen in their experiments was 16%. However, values of 21% (Finney et al. 1957) and 22% (Wood and Fox 1965) in the field, 21.5% (Simkins 1959) in pot soil culture, and 21.3% (McCalla 1933) in solution culture have been obtained where relatively large quantities of N have been supplied near the stage of flowering. A value of 29.6% was reported by Seth, Herbert and Middleton (1960) for a high-protein cultivar grown in pot culture supplied with nitrate as a soil dressing at flowering.

Although the greatest increases in grain crude protein content are obtained from applications of nitrogen late in development, crude protein yield is more closely related to grain

yield than to grain nitrogen content. Crude protein yield is almost always greatest from applications of nitrogen early in development (Gericke 1920, 1922b; Widdowson and Cooke 1958; McBeath and Toogood 1960; Thenabadau 1972; cf. Blackett 1957). This is a reflection of the much greater capacity of cereals for increase in grain yield than in grain nitrogen content from applications of nitrogen. Thus Wood and Fox (1965) obtained grain yields of 2600, 1300 and 2800 kg ha^{-1} from application of nitrogen to wheat in the field at sowing, flowering, and both sowing and flowering, crude protein contents of 13.4, 16.4 and 15.3%, and crude protein yields of 270, 170 and 330 kg ha^{-1}. Crude protein yield was 100 kg ha^{-1} where no N was applied.

3 Experiment A - Growth response and recovery of nitrogen by wheat from urea application to the foliage or to the soil.

3.1 Introduction

When nitrogen is applied to wheat as a dressing at sowing the roots provide the only path of entry into the plant. In the case of deferred application of nitrogen the shoot provides an alternative pathway. Absorption of nitrogen from nitrogenous solutions applied to the leaves of plants has been demonstrated for a number of plant species (section 2.1.2).

Published evidence on the foliar absorption of nitrogen would suggest possible advantages of the foliar path over the soil/root path in a stratagem of deferment of application of nitrogen. Nitrogen in the form of urea has been shown to be rapidly absorbed by leaves, even after the solution has dried on the leaf surface, and losses have been found to be small (section 2.1.2). In contrast, soil applied nitrogen is dependent on a wet soil surface or following rain before it is available for uptake, and losses are often large (section 2.1.1). Therefore foliar application of urea may enable greater and more rapid recovery of nitrogen than application of nitrogen fertilizer to the soil. As well as being the most suitable form of nitrogen for foliar application, urea has the advantage of being the cheapest and most concentrated form

of nitrogen fertilizer available in Australia.

Although it has been shown for some species, the foliar absorption of urea by wheat has not been clearly demonstrated in the literature (section 2.1.2). The growth response of wheat to foliar absorbed nitrogen has not received much study. 'Foliar' applications of nitrogen to wheat in the field are liable to fall partly on the soil or be washed onto the soil by following rain, and plant responses measured from such applications may have been due to nitrogen absorbed by the roots. Recently a much lower recovery of ^{15}N in the grain of wheat grown in pots was recorded from an application of ^{15}N-labelled urea exclusively to the foliage during vegetative development, than from an equivalent application exclusively to the soil (Alkier, Racz and Soper 1972).

The experiment described below was designed to compare the rate of uptake and the recovery of nitrogen by wheat from application of urea exclusively to the foliage or exclusively to the soil, using ^{15}N-labelled urea. The growth responses are described also. The experiment was conducted in pot culture.

3.2 Experimental method

3.2.1 Design and treatments

The experiment had a factorial design, with three rates of urea-nitrogen application, each via two paths, and a control

or zero application. The treatments were randomised within each of three replicate blocks. The blocks were arranged in a row, contiguously. Four post-treatment harvests were allowed for. Therefore each replicate block contained 28 pots. After each harvest remaining pots within each block were congregated together, without change in their original randomisation, to maintain the adjacency of pots. The treatments were:-

Control	N_o (1)	No N applied
	$N_{1,F}$	Equivalent of 22 kg N ha^{-1} to the foliage
Foliar path	$N_{2,F}$	" 55 " " "
	$N_{3,F}$	" 110 " " "
	$N_{1,R}$	Equivalent of 22 kg N ha^{-1} to the soil
Soil/root path	$N_{2,R}$	" 55 " " "
	$N_{3,R}$	" 110 " " "

Nitrogen was applied 45 days after plant emergence, when the plants had just commenced stem extension. Harvests were carried

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(1) Nitrogen treatment symbols in the thesis are used according to the notation $N^{i}_{j,k}$, where

i = stage of plant development
j = rate of nitrogen application
k = path of nitrogen application

The superscripts and subscripts are omitted where unnecessary.

out 4, 10, 18 and 88 (maturity) days after nitrogen application.

3.2.2 Culture method

The experiment was conducted in an evaporatively cooled glasshouse from February - May 1969. During the first two months the glass was thinly covered with whitewash, which reduced solar radiation to about 40% incident radiation. In the glasshouse the mean maximum and minimum temperatures (^{o}C) for February, March, April and May respectively were 30/15, 30/13, 25/12 and 25/9. Relative humidity varied between 30 and 75% in every month.

A nitrogen-deficient topsoil (0 - 15 cm) was obtained from Roseworthy Agricultural College in January 1969. The soil was of a sandy loam texture. The profile from which it was excavated and the nitrate content are described in section 4.2.2. The soil was air-dried, passed through a 5 mm mesh sieve, and mixed before use.

Cylindrical enamel pots, 30 cm x 23 cm diameter, were used to contain layers of soil and 'Perlite'[(1)] as illustrated in Fig. 3.1. Superphosphate equivalent to 200 kg ha^{-1} P was mixed with the soil forming the 0 - 5 cm layer. The Perlite was used in the bottom of the pots as a reservoir to supply water to the soil above by upward capillary transfer. The Perlite was replenished periodically with water through a central polythene access tube. In this way watering of closed pots from below was achieved. Only

(1) An inert porous granular material, particle diameter 2mm.

Figure 3.1

Elevation (top) and plan diagram illustrating the assembly of pots used in Experiment A.

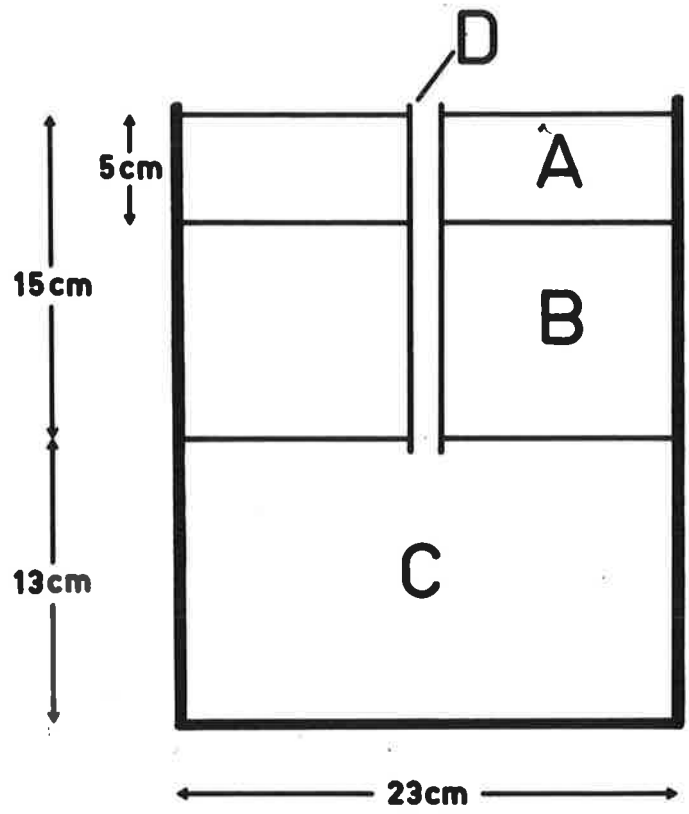
A = Superphosphate-enriched layer

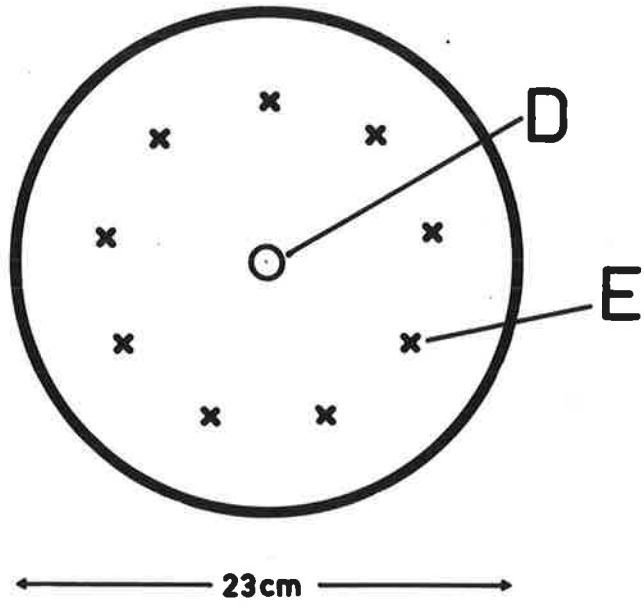
B = Nitrogen-deficient topsoil

C = Perlite

D = Polythene access tube for addition of water to Perlite

E = Position of plant


D
A
B
C
5cm
15cm
13cm
23cm


D
E
23cm

water equivalent to average weekly rainfall during the first four months of the growing season (May - August) at Roseworthy, South Australia, was added to the soil surface. The procedure was designed to simplify the watering of pots, and avoid washing of urea added to the soil surface to the bottom of the pot. Preliminary trials with different depths of Perlite and soil showed the arrangement adopted to be the most suitable. The quantity of water required to be added to the Perlite to bring the soil to a mean suction of 100 cm water (0.1 bar), after equilibrium had been established, was determined before the experiment. Water suction of the soil as the plants transpired was monitored in two extra pots of each treatment, by means of a vacuum-gauge tensiometer inserted in the soil layer. Pots were watered to the 0.1 bar weight whenever a tensiometer reading reached a suction of 0.7 bar. All pots were watered at each occasion, which occurred approximately weekly.

The cultivar "Heron" was used, a medium-maturity, medium tillering cultivar grown widely in South Australia. 27 seeds per pot, selected for uniformity of size and sound appearance, were sown on a circle of 7 cm radius (Fig. 3.1), on January 24th. Seedlings were thinned to leave 9 evenly spaced plants per pot after 10 days. The mean soil surface area per plant was 48 cm^2, which approximately equals the mean area per plant in field crops sown at 60 kg ha^{-1}. Plants commenced stem extension 42 days

after emergence, and flowering began 15 days later. Main shoot ears matured 133 days after emergence, when the final harvest was made. Ears of tillers initiated in response to the nitrogen treatments were still green at harvest. Mature and green ears were harvested and analysed separately.

3.2.3 Nitrogen application

Urea-nitrogen was applied to appropriate pots of replicate 1 on March 13th (45 days after plant emergence), of replicate 2 on March 14th, and of replicate 3 on March 15th. Rates are given in section 3.2.1. 3.0 g ^{15}N-enriched urea, 30.65 atom % excess, were mixed with 67.0 g 'Analar' urea ($<$0.4% biuret), and the mixture dissolved in deionised water to make 350 ml of 20% (w/v) solution. The total solution required was prepared on March 13th, and stored for use in replicates 2 and 3 in a stoppered bottle at room temperature.

The soil in pots to receive foliar application of urea was covered with absorbent paper. The soil surface was dry. The paper was cut into a central disc and an outer anulus to accommodate the plant stems. The foliage of the plants was enclosed in a clear plastic cylinder (75 cm x 23 cm diameter), and this was lined with absorbent paper. A known volume of 20% urea solution, containing B.A.S.F.(1) rapid wetting agent, was sprayed onto the

(1) Badische Anilin- und Soda-Fabrik Ag., Ludwigschafen am Rhein, W. Germany.

foliage from above through a Varian Techtron A4 variable atomiser at a pressure of 0.5 kg cm^{-2}, giving droplets of solution of the order of 0.1 mm diameter. One minute after spraying, when the mist had dispersed, the cylinder was removed. The absorbent paper was detached quickly and placed in a plastic cup, which was immediately sealed, and then weighed. The weight of the cup containing the dry paper was determined before spraying. The weight of solution received by the foliage was calculated from the volume sprayed and the weight retained by the paper. The value was an overestimate because some of the spray remained suspended in the air, evaporated during spraying, or was lost to surfaces contacted during paper detachment. It was judged to be within 10% of the true value.

The volume of solution required to spray the desired amount of urea onto the foliage for each foliar treatment was predetermined from dummy runs with deionised water. The volumes needed were 2.0, 5.0 and 10.0 ml for the N_1, N_2 and N_3 rates, applied in two sprayings of 1.0, 2.5 and 5.0 ml. The first sprayings were performed in the morning and the second in the afternoon of each day. Approximately half the volume of solution sprayed was intercepted by the absorbent paper, independent of the volume sprayed. Control pots were sprayed with 5.0 ml deionised water. The weights of nitrogen estimated to have been added to the plant shoots by the foliar application are shown in Table 3.1.

Table 3.1

Mean weight N (mg) added per plant by foliar application.

	Harvest							
	1		2		3		4	
Treatment	Per rep.	Mean	Per rep.	Mean	Per rep.	Mean	Per rep.	Mean
$N_{1,F}$	6.7		4.2		3.9		4.2	
	6.9		5.9		7.7		4.0	
	10.7	8.1	11.1	7.1	11.7	7.8	11.5	6.5
$N_{2,F}$	37.7		23.1		26.6		20.3	
	22.2		25.7		20.7		20.5	
	28.8	28.5	25.4	24.8	28.6	25.3	27.6	22.8
$N_{3,F}$	57.2		55.6		45.3		45.9	
	48.1		49.0		43.6		43.6	
	54.7	53.3	52.6	52.4	55.7	48.2	55.1	48.2

Note: foliar applications calculated as described in section 3.2.3.

Pots to be treated with urea-nitrogen applied to the soil received 250 ml of solution containing the desired quantity of ^{15}N-enriched urea for each rate of application (10.1, 25.2 and 50.4 mg N per plant respectively), applied to the soil surface. The pots receiving soil-applied nitrogen were watered to 250 g below the 0.1 bar weight before treatment. Foliar supplied and control pots were watered to the 0.1 bar weight as usual.

3.2.4 Harvesting and morphological measurements

Harvests of shoot and root were carried out at 4 (H1), 10 (H2), 18 (H3), and 88 (H4) days after application of nitrogen. At each occasion the three replicates were harvested on consecutive days, so that the interval between nitrogen treatment and harvest was constant (see section 3.2.3).

Shoots were severed at the crown of each plant. At the first three harvests plant and tiller number were counted per pot, and shoot dry weight was determined after drying at 80°C. Shoot material was not washed to remove surface residues of foliar-applied urea, because residues were not visible on the leaf surface and elution of free nitrate or urea from within the leaf was wished to be avoided. Analysis of foliar-sprayed shoot material after surface washing was believed to be an unsatisfactory measure of foliar absorption, for reasons discussed in section 2.1.2. 10 g crystalline urea placed in an oven at 80°C for 24 hours

showed no measurable loss in weight.

At the final harvest ears were divided into those derived from tillers present before nitrogen application - exclusively main culms - and those derived from tillers produced after the application of nitrogen. The latter were mostly green (see section 3.2.2). The number of ears per pot and the number of spikelets per ear for each class were determined. Grain weight and grain number were determined after hand-threshing.

Following excision of the shoots pots were soaked in water for one hour. Each was inverted over a 2 mm mesh steel sieve, and the soil slid from the pot. As root penetration of the Perlite was negligible the Perlite was washed away immediately. A spray of water was directed onto the soil/root mass, and the soil was washed through the sieve. Spraying was carried out so as to minimise loss of root material. The root mat finally left on the sieve was dried. Non-root organic material and a little fine sand remained after washing. The dry roots were picked clean with tweezers, and rewashed before drying at 80°C and weighing.

3.2.5 Total N determination

The following fractions derived from each pot were analysed: root and shoot fractions at harvests 1, 2 and 3; the roots, straw + chaff, and the grain at harvest 4. Samples

other than grain were ground to pass a 0.9 mm mesh sieve. The grain from "pre-N" and "post-N" tillers was ground separately to pass a 1.3 mm mesh sieve. Ground material was mixed thoroughly before duplicate samples were taken for digestion. 0.25 g samples were digested "cold" overnight in Kjeldahl flasks with 5 ml concentrated sulphuric acid containing 33 g salicylic acid per litre. After addition of a catalyst tablet containing 1.50 g K_2SO_4 and 0.0075 g Selenium the samples were digested fully by boiling for 5 hours. The digests were diluted to 50 or 100 ml and stored.

A Technicon Auto-Analyser was used to determine N content of the digests. The analysis is based on the spectro-photometric determination of colour intensity developed when an aliquot of sample solution is reacted under controlled conditions with sodium phenate followed by the addition of sodium hypochlorite. Aliquots of solution were drawn off with a syringe after samples had been allowed to stand and all suspended material had settled. The nitrogen concentrations of the samples were determined by comparison with a standard curve of readings obtained from prepared standard solutions. The method was checked by comparison with the steam distillation method of Bremner (1965). It gave satisfactory agreement providing the temperature of the reagents, samples, and mixing coils was kept constant during any one group of analyses.

3.2.6 ^{15}N determination

A 5-20 ml aliquot of digest was acidified with sulphuric acid and evaporated to a concentration of 0.5 mg N/ml for analysis. ^{15}N determination was made with an A.E.I. MS2 mass spectrometer according to the procedure given by Grasmanis and Barley (1969).

The amount of nitrogen in plant fractions considered to have been derived from the applied fertilizer was calculated from the ^{15}N and total N concentration of the plant tissue, and the ^{15}N : ^{14}N ratio in the applied fertilizer. The ^{15}N : ^{14}N ratio of the applied fertilizer was 1 : 77 (see section 3.2.3). The calculation assumes that the plants absorbed and utilised the isotopes ^{15}N and ^{14}N indiscriminately, and no isotopic exchange occurred between applied ^{15}N and soil or atmospheric ^{14}N.

3.3 Results

3.3.1 Tillering

Plants tillered weakly throughout the experiment, presumably as a result of the nitrogen deficiency of the soil (section 4.2.2). The deferred application of nitrogen did not stimulate production of new tillers, but influenced tiller survival. The mean number of tillers per plant was 1.9 at harvest one (four days after nitrogen application), and declined to 1.8 at harvest two and 1.4 at harvest three (ten and eighteen

days after nitrogen application), in all treatments. Standard errors of the means (s.e.) were 0.06, 0.07 and 0.06 for harvests 1 - 3 respectively. Survival of tillers to maturity was influenced by path and rate of nitrogen supply. Plants of treatments N_0, $N_{1,F}$, $N_{2,F}$, and $N_{1,R}$ matured only the main shoot. In treatments $N_{2,R}$ and $N_{3,F}$ 1.15 tillers per plant, and in treatment $N_{3,R}$ 2.10 tillers per plant, survived to maturity. The treatments for which a common mean has been given did not differ significantly from each other (P=0.05).

Tillers additional to the main culm were slow to senesce, and were still green at the final harvest. Their contribution to grain yield per plant was 2.7, 2.1 and 40.0% in treatments $N_{2,R}$, $N_{3,F}$ and $N_{3,R}$ respectively. Such ears would not normally be recovered by machine harvesting in the field, and they have not been included in the analysis of grain yield. Data for grain yield, the components of grain yield, and grain nitrogen content refer to grain produced by the main culms. Data for total shoot weight, shoot nitrogen content and uptake, and recovery of applied nitrogen by the whole plant include both the immature shoots and the shoots with mature ears.

3.3.2 Shoot and root dry weight

The "shoot" denotes all of the plant above and including the crown, except where indicated otherwise. Application of nitrogen

increased the dry weight of shoot and root at maturity (Fig. 3.2). Increases were proportional to the rate of application, but they were greater when made by the soil/root path than by the foliar path. There were no significant differences between treatments at the first three harvests. Mean shoot dry weights were 0.98, 1.15 and 1.51 g per plant, and mean root dry weights were 0.56, 0.51 and 0.48 g per plant, at harvests 1 - 3 respectively. Standard errors of the means were 0.051, 0.056 and 0.082 for shoot weights and 0.031, 0.033 and 0.026 for root weights respectively.

Root weight ratio (the ratio root weight/plant weight) declined from the first to the final harvest. The mean values per plant were 0.38, 0.32, 0.25 and 0.16 for harvests 1 - 4 respectively. Standard errors of the means were 0.015, 0.015, 0.014 and 0.007. There were no significant differences between treatments. The constancy of the root weight ratios suggests that the method of harvesting roots was reliable.

3.3.3 Grain weight, and the components of grain yield

Application of nitrogen increased grain yield (Fig. 3.2.b). As with shoot and root yield, the soil/root path was more effective than the foliar path. The increase was proportional to the rate of application up to the intermediate rate N_2. It must be noted that at the highest rate for the soil/root path plants produced a fertile tiller additional to the main culm, but grain from this additional

Figure 3.2

Influence of rate and path of urea application on dry weight per plant at maturity.

(a) Total

(b) Grain

(c) Straw plus chaff

(d) Root

■———■ = soil/root path

▲———▲ = foliar path

L.S.D.(I), P = 0.05

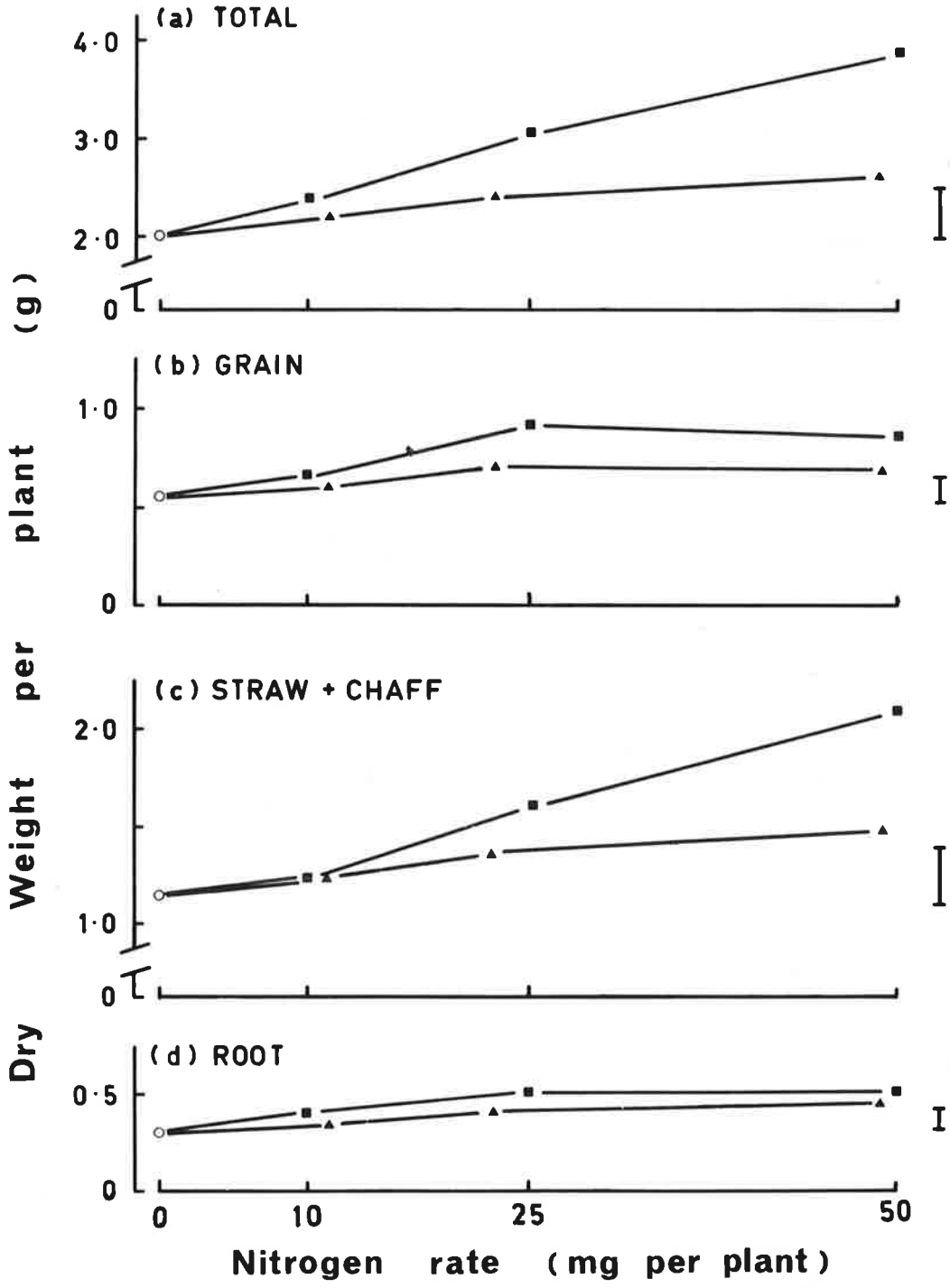

(a) TOTAL
4·0
3·0
2·0
0
(b) GRAIN
1·0
0
(c) STRAW + CHAFF
2·0
1·0
0
(d) ROOT
0·5
0
Dry Weight per plant (g)
0
10
25
50
Nitrogen rate (mg per plant)

Table 3.2

Influence of rate and path of urea application on the components of grain yield.

	Nitrogen path and rate						
	FOLIAR			SOIL/ROOT			L.S.D.
N_o	N_1	N_2	N_3	N_1	N_2	N_3	(P=0.05)
Total number of spikelets per ear							
11.8	11.4	12.0	11.7	11.9	12.1	12.5	n.s.
Number of fertile spikelets per ear							
8.2	8.7	9.5	9.6	8.9	10.0	10.7	1.31
Mean number of grains per fertile spikelet							
1.8	1.8	1.8	2.0	1.9	2.2	2.1	0.15
Mean weight per grain (mg)							
40	42	40	38	40	42	40	n.s.

tiller was not included in the measure of grain yield (see section 3.3.1).

The components of grain yield are shown in Table 3.2. Total number of spikelets per ear (main culm only) and mean weight per grain were not influenced by the deferred application of nitrogen. Spikelet fertility and mean number of grains per fertile spikelet tended to be increased more by nitrogen supplied via the soil/root than via the foliar path, and to be proportional to the rate of application up to the intermediate rate.

3.3.4 Grain nitrogen content

Application of nitrogen increased the nitrogen concentration in the grain (Table 3.3). The increase tended to be proportional to the rate of application, and was greater when supplied by the soil/root path. The increases in grain nitrogen were commensurate with the amounts of N derived from the fertilizer (Table 3.3 column 2, Fig. 3.4.i.c and ii.c).[1] The greater effectiveness of the N supplied via the roots was associated mainly with higher translocation of fertilizer-N to the grain, and to a lesser extent with an increase in the amount of fertilizer recovered (Table 3.3, and section 3.3.6).

---

(1) The amounts of N in plant fractions given as derived from the applied fertilizer and from native soil sources were calculated as described in section 3.2.6.

Table 3.3

Influence of rate and path of urea application on grain nitrogen content.

Nitrogen path and rate		Grain nitrogen concentration (%)	Per cent of total grain nitrogen derived from applied urea	^{15}N in the grain per cent of ^{15}N in the whole plant
	N_o	2.07		
	N_1	2.19	16.5	47.4
FOLIAR	N_2	2.19	20.6	31.6
	N_3	2.40	34.7	27.8
	N_1	2.09	20.8	72.4
SOIL/ ROOT	N_2	2.44	37.1	79.8
	N_3	2.77	50.6	62.2
L.S.D. (P=0.05)		0.19	3.6	7.0

3.3.5 Shoot and root nitrogen content

Shoot material was analysed for N content without washing, for reasons discussed in section 3.2.4. Consequently the nitrogen content of the shoot includes not only absorbed nitrogen, but also any fertilizer-nitrogen located internally but not absorbed, and any surface residue of fertilizer-nitrogen. Nitrogen concentration of the shoot increased following urea application, the increase being proportional to the rate of application, and being greater when supplied by the foliar than by the soil/root path (Fig. 3.3). Nitrogen concentration subsequently decreased, as a result of the relatively slow rate of uptake of fertilizer-N compared with growth after the first harvest. Table 3.4 indicates that there was no loss of foliar-applied nitrogen subsequent to the first harvest. By maturity in the soil/root treatment almost all additional nitrogen that had been accumulated in the foliage as a result of nitrogen application had been translocated out of the shoot, apparently to the grain. However, in the foliar treatment only part of the additional nitrogen located in the foliage had been translocated to the grain (Fig. 3.5.a).

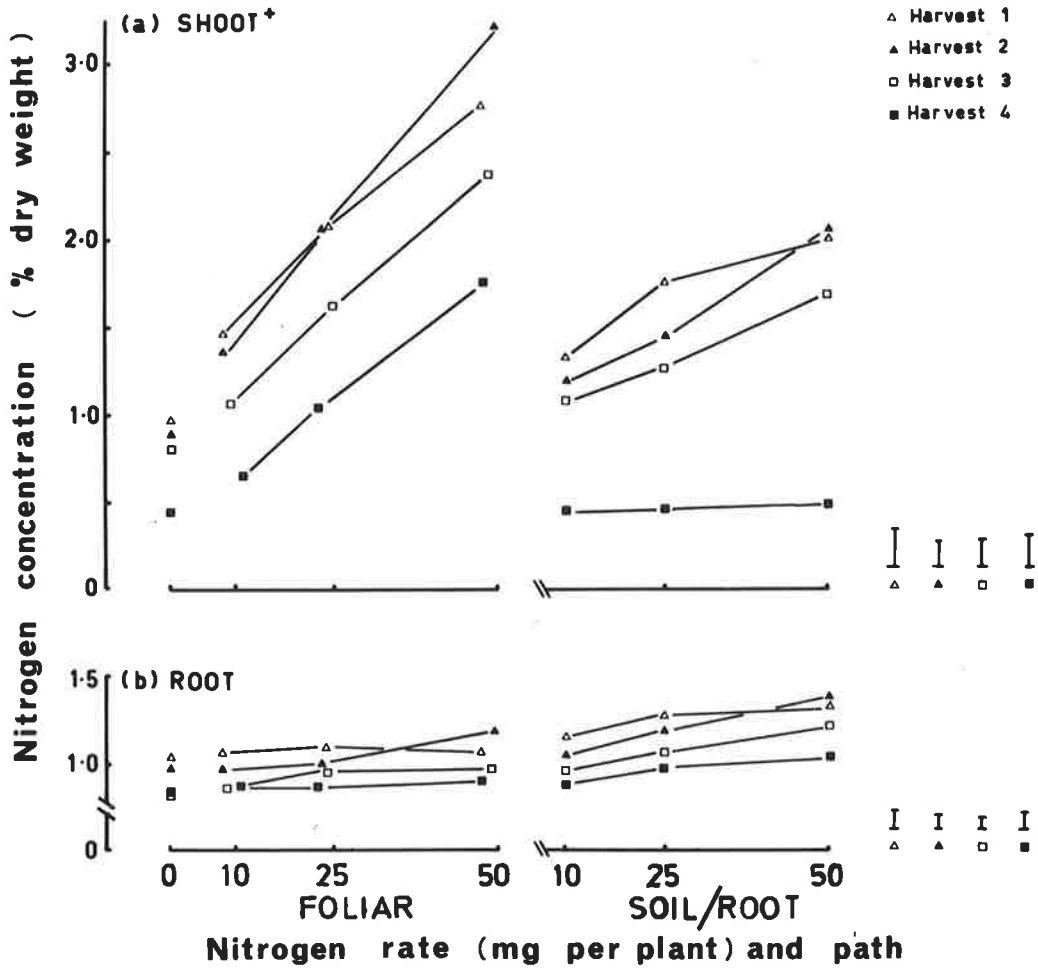
Figure 3.3

Influence of rate and path of urea application on shoot and root nitrogen concentration.

Harvests 1, 2, 3 and 4 were taken 4, 10, 18 and 88 (maturity) days after nitrogen application.

L.S.D.(I), P = 0.05

+ Excluding grain at harvest 4


(a) SHOOT+
(b) ROOT
Nitrogen concentration (% dry weight)
3·0
2·0
1·0
0
1·5
1·0
0
Harvest 1
Harvest 2
Harvest 3
Harvest 4
0
10
25
50
10
25
50
FOLIAR
SOIL/ROOT
Nitrogen rate (mg per plant) and path

3.3.6 Nitrogen uptake and absolute recovery of applied urea

Application of urea caused a sharp increase in nitrogen uptake within four days of treatment (harvest 1), whether supplied via the foliage or via the root system (Fig. 3.4.a). The increase was roughly proportional to the rate of application in each case. Initially the increased uptake was derived wholly from the applied urea (Fig. 3.4.b and c). After 18 days (harvest 3) an increase in the uptake of soil-derived nitrogen was evident where urea had been applied. The pattern of nitrogen uptake for the shoot and root fractions was similar to that for the whole plant (Fig. 3.5). Most of the nitrogen recovered from the fertilizer was retained in the shoot, whether supplied by the foliar or soil/root path. As noted in section 3.3.4, where urea had been supplied by the soil/root path, more of the additional nitrogen that accumulated in the shoot following urea application was translocated to the grain. Fertilizer-derived nitrogen supplied by the soil/root path was present in the shoot system, and that supplied by the foliar path was present in the root system, four days after treatment. However the translocation from root to shoot was much greater than that from shoot to root.

The absolute recoveries of applied nitrogen for each harvest are shown in Table 3.4. The results for the lowest rate of application by the foliar path ($N_{1,F}$) differ distinctly from those for the higher rates. A markedly higher recovery of

Figure 3.4

Influence of rate and path of urea application on nitrogen uptake by the whole plant.

(a) total N

(b) fertilizer-derived N^{+}

(c) soil-derived N^{+}

Harvests 1, 2, 3 and 4 were taken 4, 10, 18 and 88 (maturity) days after nitrogen application.

L.S.D.(I), P = 0.05

+ See section 3.2.6.

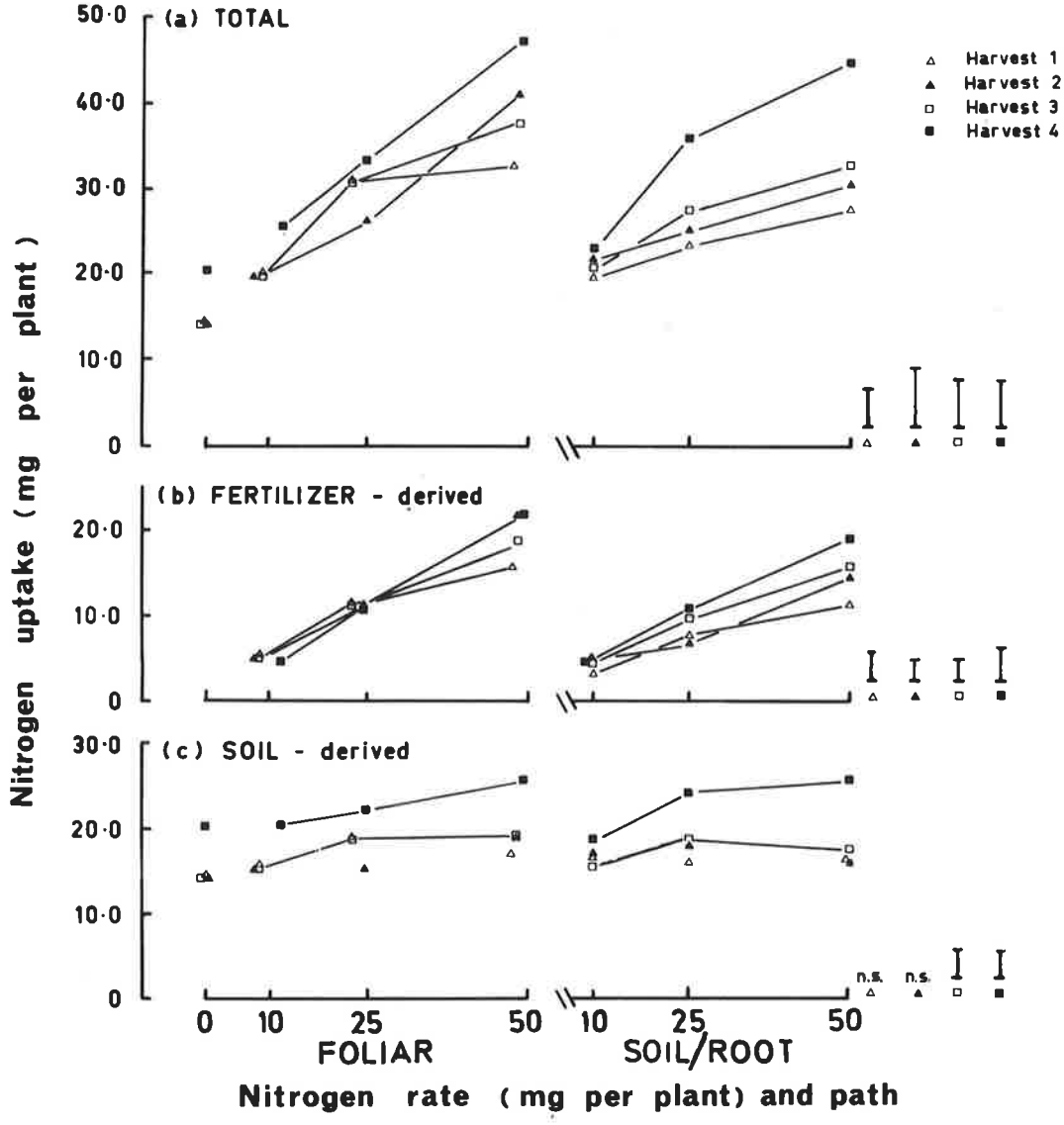

(a) TOTAL
(b) FERTILIZER - derived
(c) SOIL - derived
Harvest 1
Harvest 2
Harvest 3
Harvest 4
50·0
40·0
30·0
20·0
10·0
0
n.s.
n.s.
0
10
25
50
FOLIAR
SOIL/ROOT
Nitrogen uptake (mg per plant)
Nitrogen rate (mg per plant) and path

Figure 3.5

Influence of rate and path of urea application on nitrogen uptake by plant parts.

(i) fertilizer-derived N^+

(ii) soil-derived N^+

(a) shoot‡

(b) root

(c) grain

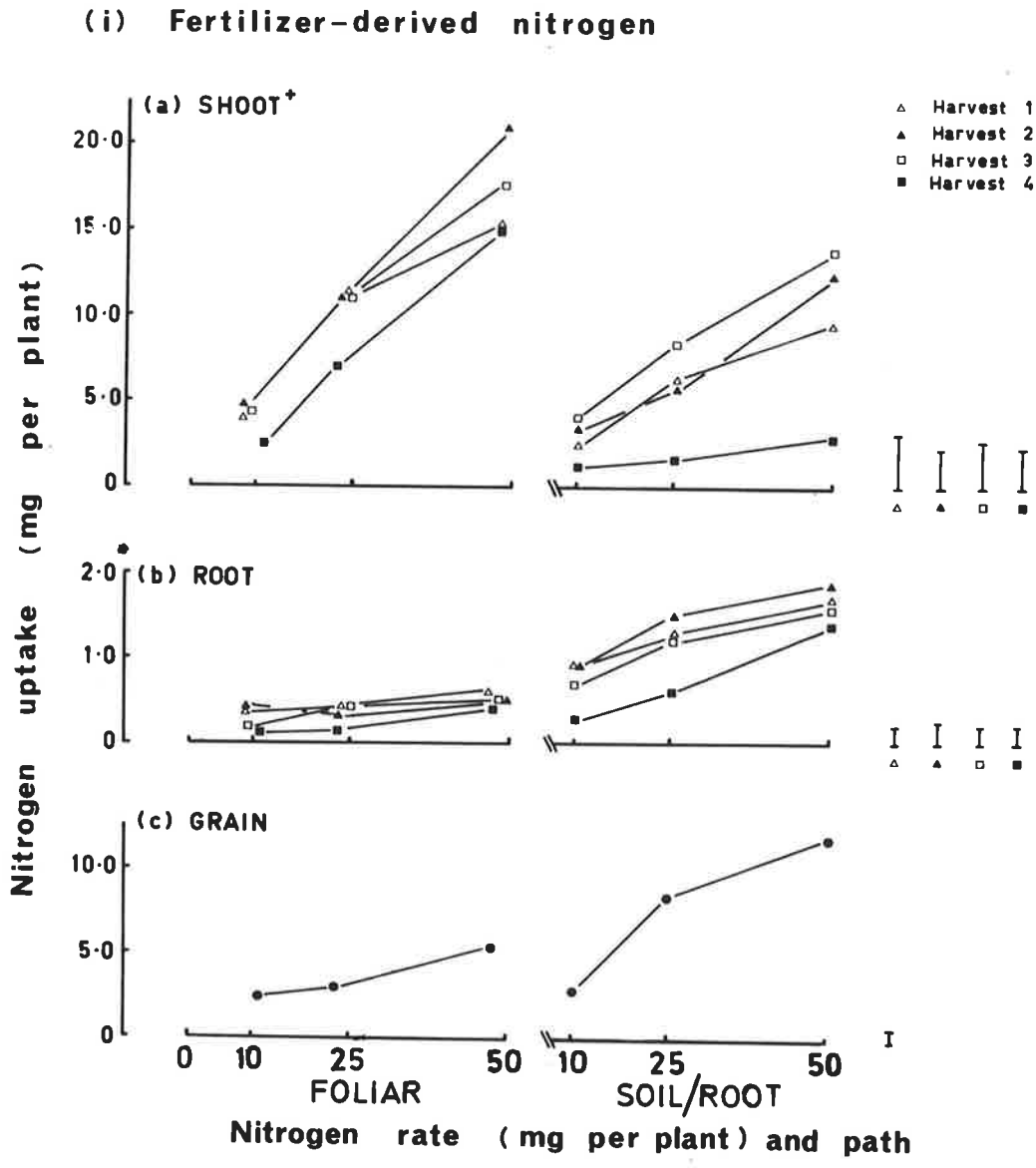
Harvests 1, 2, 3 and 4 were taken 4, 10, 18 and 88 (maturity) days after nitrogen application.

L.S.D.(I), P = 0.05

* Note scale

\+ See section 3.2.6

‡ Excluding grain at harvest 4


(i) Fertilizer-derived nitrogen
(a) SHOOT+
(b) ROOT
(c) GRAIN
Harvest 1
Harvest 2
Harvest 3
Harvest 4
Nitrogen uptake (mg per plant)
20·0
15·0
10·0
5·0
0
2·0
1·0
0
10·0
5·0
0
0 10 25 50
FOLIAR
10 25 50
SOIL/ROOT
Nitrogen rate (mg per plant) and path

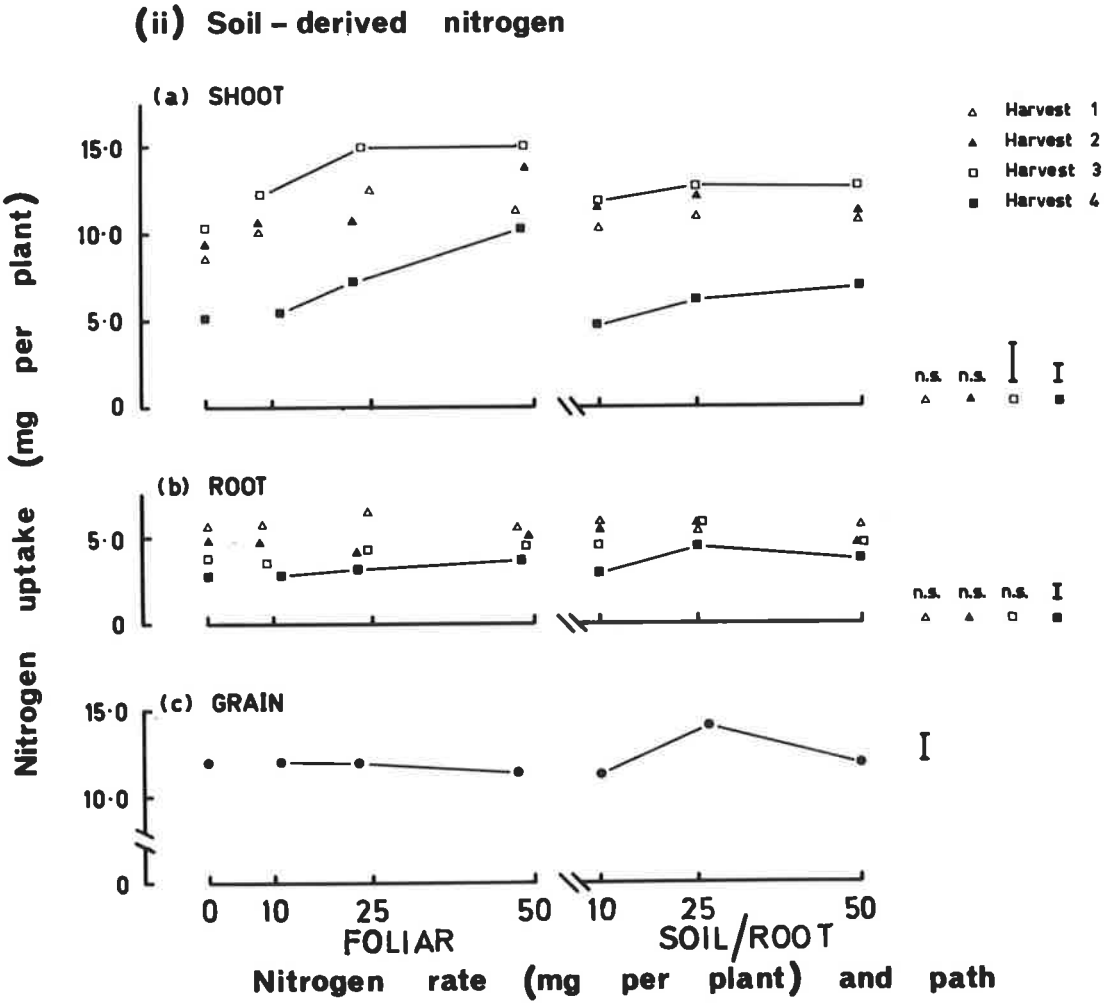

(ii) Soil – derived nitrogen
(a) SHOOT
(b) ROOT
(c) GRAIN
Harvest 1
Harvest 2
Harvest 3
Harvest 4
n.s.
15·0
10·0
5·0
0
Nitrogen uptake (mg per plant)
0 10 25 50 10 25 50
FOLIAR
SOIL/ROOT
Nitrogen rate (mg per plant) and path

Table 3.4

Influence of rate and path of urea application on the recovery of urea-N.

(a) Recovery in the whole plant (%)

Harvest		Nitrogen path and rate: FOLIAR N_1	FOLIAR N_2	FOLIAR N_3	SOIL/ROOT N_1	SOIL/ROOT N_2	SOIL/ROOT N_3	L.S.D. (P=0,05)
1	(4)*	58	37	29	32	27	23	9.8
2	(10)	86	46	42	43	28	25	18.9
3	(18)	70	46	40	41	36	28	n.s.
4	(88 - maturity)	94	46	46	41	44	37	18.5

(b) Recovery in the grain (%)

43	14	12	28	33	24	n.s.

* Days after application of urea.

fertilizer was obtained. It can be seen from Table 3.1 that in this treatment the amount of urea received by the foliage was below the intended amount for the first two replicates, and above the intended amount for the third replicate. The mean applications per plant over all harvests were 4.7, 6.1 and 11.2 mg for replicates 1, 2 and 3 (standard errors of the means were 0.65, 0.80 and 0.22 respectively). The mean recoveries of fertilizer over all harvests for the three replicates were 101, 83 and 44% (standard errors of the means were 11.0, 16.1 and 4.2 respectively).

Except for treatment $N_{1,F}$, recoveries did not exceed 50%. With either path of supply the bulk of the applied nitrogen ultimately recovered was taken up within the first few days after application. Initial recovery was generally less by the soil/root path, but gradual further recovery until maturity brought about similar final recoveries for the two paths of supply.

3.4 Discussion

The percentage recovery of nitrogen applied by the soil/root path in this experiment was at a level commonly found in experiments of nitrogen fertilizer application via the soil to temperate cereals (Allison 1966). The fraction of urea-nitrogen not recovered by the plants may have been lost by ammonia volatilisation to the atmosphere following hydrolysis by microbial urease, or by immobilisation, or by denitrification

Allison 1966), as discussed in section 4.4 with respect to losses of urea-nitrogen from the same soil in the field.

The rapid recovery of large amounts of fertilizer-nitrogen by roots is of interest. Rapid uptake was facilitated by the watering in of the fertilizer and presence of a favourable soil moisture content for uptake.

The percentage recovery of nitrogen applied to the foliage was lower than has generally been found in experiments of a similar nature (section 2.1.2). Since the harvested shoot material was not washed before chemical analysis, it appears that a substantial proportion of the nitrogen applied to the shoot was lost shortly after application. The fact that near 100% recoveries of foliar-applied N were obtained in some instances (the first two replicates of treatment $N_{1,F}$) indicates that losses occurred before harvest rather than as a result of any treatment between harvesting and chemical analysis. No rain fell on the plants after application because they were in the glasshouse. Morning dew, which was observed, would probably have been only sufficient to wash urea into the leaf axils. The most likely mechanism of such large losses of nitrogen in a short period would seem to be as ammonia following hydrolysis of urea by the enzyme urease. Urease may have come from within the leaf, or it may have been of microbial origin on the leaf surface. The first step in the assimilation of foliar-absorbed urea by plants is believed to be hydrolysis by

urease (Wittwer and Bukovac 1969). An examination of the leaf urease activity of four citrus species indicated that sufficient activity was present for urea hydrolysis not t o be a limiting factor in the assimilation of foliar-applied urea by these species (Kuykendall and Wallace 1954). No comparable investigation of the leaf urease activity of cereals is known, nor is it known whether the enzyme could act exogenously. The ubiquity of microorganisms and their known urease activity in soils (Gasser 1964) suggests the alternative possibility of microbial hydrolysis on the leaf surface.

Loss of urea from the shoot following application raises doubt about the validity of estimates of urea absorption from the nitrogen content of leaf washings after application (section 2.1.2). However it must be noted that losses were not detected at the lowest rate of application (replicate 1 of treatment $N_{1,F}$), which corresponds most closely with the low rates of application used in the experiments where foliar absorption has been estimated in this way.

The relatively small growth responses and large increases in grain nitrogen content obtained from the application of nitrogen to the soil are consistent with the general results of other experiments in which nitrogen has been applied to temperate cereals at the beginning of the reproductive phase of development (sections 2.2.2.e and f).

Although the recovery of fertilizer-N by the two paths of supply was comparable at each rate of application, the growth responses induced were larger where application was made to the soil. Similar results have been reported by Simkins (1959) and Alkier, Racz and Soper (1972), who conducted pot experiments with wheat in which the two paths of supply were examined separately, and also by Van Berg and Arnold (1963) and Hanley, Ridgman and Beveridge (1966) from field experiments comparing foliar sprays with application of solid forms of nitrogen fertilizer to the soil. However the significance of these observations has been overshadowed by a relatively extensive literature advocating foliar spraying as a method of applying nitrogen fertilizer to crops (section 2.1.2). This literature may be misleading, for the following reasons: (1) many demonstrations of the absorption of foliar-applied nitrogen have not extended to observation of the growth responses induced; (2) when the soil surface is not protected from spray and plants are subject to rainfall following application, so-called "foliar" applications of fertilizer result in part of the nitrogen being added to the soil. It should be noted also that increases in cereal grain nitrogen content from foliar sprays applied late in development may be greater than growth responses from sprays applied earlier in development, because of a greater capacity of ears than of leaves to utilise nitrogen applied in a spray (Petinov and Pavlov 1960).

Poor growth response to foliar-applied N was associated with poor transfer of the nitrogen received by the foliage to the remainder of the plant. This was evident both from the low ^{15}N contents of the roots and grain, and from the comparatively large quantities of ^{15}N retained in the straw at maturity (Table 3.3, Figs. 3.3, 3.5). The sites of shoot growth response of temperate cereal plants are the undeveloped tiller buds, the developing ears, and the unexpanded leaves on existing tillers, and it is likely that poor transfer of applied nitrogen out of the sprayed leaves was directly responsible for the poor growth responses. The growth attributes that responded to applied nitrogen all responded less to foliar-applied than to soil-applied N, and the response to foliar-applied N was lower to a similar degree for each attribute.

Incomplete transfer may have been due either to incomplete absorption of urea into the leaf cells or to poor assimilation and translocation of absorbed urea. Assimilation of foliar-absorbed urea into amino-acids and proteins has been shown to occur for a range of plant species (Webster, Varner and Gansa 1955; Yatazawa, Takemoto and Yamamoto 1958; Dilley and Walker 1961), including barley (Makarevich 1964) and oat and maize (Pavlov and Kolesnik 1966). The observations of Kuykendall and Wallace (1954) on the extent of urease activity in plants have been referred to on p.90. Dilley and Walker (1961) demonstrated assimilation of labelled

urea supplied through the petiole of detached leaves of apple and peach, and they concluded that the failure of peach leaves in the field to utilise foliar-applied urea was due to an inability to absorb rather than an inability to metabolise urea. Urea-nitrogen supplied by the soil/root path, which clearly was assimilated and translocated within the plant, is more likely to have been absorbed as nitrate or ammonium than as urea, following transformation in the soil.

Retention of urea unabsorbed on the leaf surface might seem the simplest explanation of the poor translocation from sprayed leaves. However this should be questioned. Crystals visible on the leaf surface soon after application were no longer visible after a few days. Previously it has been found that absorption continues after apparent drying of the solution on leaves (Volk and McAuliffe 1954; Impey and Jones 1960). The hygroscopic nature of urea would cause a thin film of solution to be present over the crystal surfaces, and this is invoked to explain the considerable absorption of urea from solutions which dry within minutes of application (Wittwer and Bukovac 1969). Alternatively, disappearance of unabsorbed urea from the leaf surface may have been due to volatilisation following hydrolysis.

Absorption of foliar-applied urea into plant leaves has been shown unequivocally in only a few experiments (see section 2.1.2). Methods in which unabsorbed urea residue is washed from the

leaf surface before analysis, do not exclude the possibility that the urea recovered in analysis is held in the cuticle, stomata, and inter-cellular spaces, rather than within the leaf cells. More definite evidence of absorption may be obtained by measurement of the translocation of absorbed nitrogen out of the treated area. In experiments on temperate cereals, in which translocation of foliar-applied nitrogen out of the receptor leaf was shown (Pavlov 1960; Petinov and Pavlov 1960; Makarevich 1964; Pavlov and Kolesnik 1966), the authors either did not use nitrogen in the form of urea, or they did not indicate the proportion of the nitrogen applied that was translocated away from the leaves.

Experiments which have shown the translocation of applied urea out of the receptor leaf of a plant have employed dilute solutions (1 - 3%, w/v) (Volk and McAuliffe 1954; Cain 1956; Freiberg and Payne 1957; Pavlov 1960; Eberhardt and Pritchett 1971). The concentration of the solution applied is not in itself important, since the concentration increases after application as the solution dries; but it does affect the quantity of urea applied per unit area of leaf. In the present experiment recovery of urea-nitrogen and translocation out of the sprayed leaves were greater at the low than at the higher rates of application. It seems possible that leaf cells might have been damaged at the higher rates of application by the production of excessive amounts of ammonia following hydrolysis of absorbed urea, and that this

could have resulted in retention of nitrogen in the damaged leaf cells. A correlation between leaf urease activity and susceptibility to leaf damage from urea spray is known (Hinsvark, Wittwer and Tukey 1953). Visible damage of the sprayed leaves was however confined to the leaf tips, as is commonly found when high concentrations of urea solution are applied to wheat (Chesnin and Schafer 1953).

The translocation of foliar-applied urea-^{15}N to the maturing grain, although low relative to root-absorbed ^{15}N (Table 3.3), was considerably greater than that reported recently in a similar experiment by Alkier, Racz and Soper (1972), in which less than 2% of urea-^{15}N applied was recovered in the harvested grain.

4 Experiment B - The response of four cultivars of wheat in the field to deferment of nitrogen fertilizer application.

4.1 Introduction

The number of ears produced per plant is an important component of grain yield of a wheat crop. At a given plant density it is determined by the tillering behaviour of the plant. Wheat has been shown to be capable of a tillering response to an application of nitrogen at any stage during vegetative development, though the capacity tends to reduce with increasing lateness of application (section 2.2.2.a).

Cultivars are known to differ in their tillering capacity, and in their tillering response to nitrogen applied at sowing time (Barley and Naidu 1964). In previous field experiments conducted in South Australia in which the response of wheat to nitrogen applied at different stages during vegetative development has been examined (Birks and Cole 1930; Richardson and Gurney 1935; Reuter 1967; Russell 1969), a single cultivar was used in each case. The present experiment was designed to compare the response of different cultivars. Cultivars of contrasting tillering propensity were chosen.

Two sowing rates were compared. The tillering behaviour of wheat is markedly influenced by the plant density (Puckridge and Donald 1967). The tillering response at relatively low

plant density is of interest with respect to a stratagem of deferment of nitrogen application, because a low density crop (a) has a greater tillering capacity per plant for a given supply of nitrogen, (b) has a lower leaf area index, and hence a smaller water requirement, for much of the season. The sowing rates used were normal and one-fifth the normal sowing rate of wheat in South Australia. These rates were found to give similar grain yields in a study in South Australia of the tillering and grain yield of wheat at a wide range of plant densities (Puckridge and Donald 1967).

4.2 Experimental method

4.2.1 Design and treatments

The experiment had a split-split-plot design with three replicates. The treatments were assigned as follows:-

Main plots — Sowing rate: S_1 70 kg ha^{-1} germinable seed

S_2 14 " " "

Sub-plots Cultivar: M Mexico 120 – High tillering capacity, early maturity, semi-dwarf Mexican

G Gabo – Low tillering capacity, early maturity, tall

H Halberd – Intermediate tillering capacity, intermediate maturity, tall

P Pinnacle – High tillering capacity, late maturity, tall

Sub-sub-plots
Nitrogen: N_o no nitrogen applied

N^1 55 kg ha^{-1} urea-N applied one day before crop emergence (12 days after sowing)

N^2 55 kg ha^{-1} urea-N applied at the stage of spikelet differentiation of the main shoot apex (53 - 67 days after sowing, according to cultivar).

N^3 55 kg ha^{-1} urea-N applied at the stage of floret differentiation on the terminal spikelet of the main shoot apex (86 - 105 days after sowing, according to cultivar)

Developmental stages of the main shoot apex were defined according to Barnard (1955).

4.2.2 Site, and crop establishment

The experiment was conducted on a sandy red-brown earth (Dr. 2.33 - Northcote 1965), at Roseworthy Agricultural College. The site had grown cereals for two preceding years. Analysis of

soil samples taken from the site showed a NO_3-N content of <0.5 p.p.m. Quadruplet samples were analysed according to the procedure of Clarke and Jennings (1965), from a composite of 9 soil cores of the 0-30 cm depth interval, taken on April 15th 1969.

The site was ploughed and harrowed, and plots were sown with a 9-hoe drill on June 6th, 1969. Sowing rates were adjusted for seed size and viability. Superphosphate was drilled with the seed at a rate of 50 kg P ha^{-1}. A urea spray was supplied as a basal nitrogen dressing of 5 kg N ha^{-1}, three days after sowing. Plots were hand-weeded during early growth, treated with herbicide spray(1) on July 13th and August 12th, and with an insecticide spray (2) on August 12th. The arrangement and dimensions of plots is shown in Fig. 4.1.

Seedlings emerged on June 19th. Establishment was even over the site, but depressed for the cultivar "Gabo" for no apparent reason. The number of plants established per sq. metre is shown below:

		Cultivar			
		Mexico 120	Gabo	Halberd	Pinnacle
Sowing rate	S_1	160	115	175	160
	S_2	35	25	33	35

(1) "Buctral M.A." - Manufactured by May and Baker Ltd., Paramount Road, West Footscray, Victoria.

(2) "Imidan 15" - Manufactured by I.C.I. Australia Ltd., 24 Sutton Terrace, Marleston, South Australia.

Figure 4.1

The arrangement and dimensions of plots in Experiment B.

Key as given in section 4.2.1.

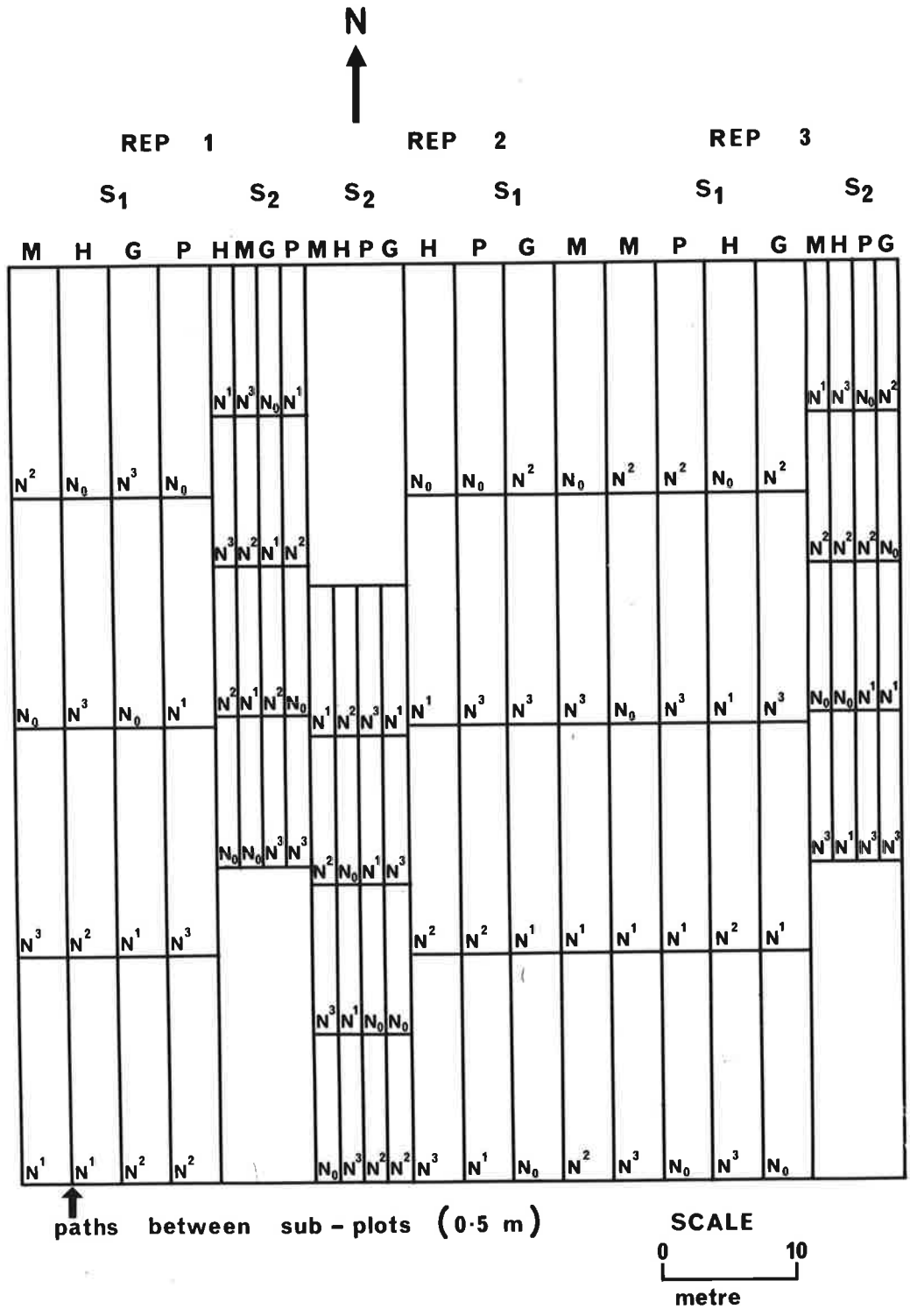

N
REP 1
REP 2
REP 3
S1
S2
S2
S1
S1
S2
M H G P H M G P M H P G H P G M M P H G M H P G
paths between sub – plots (0·5 m)
SCALE
0
10
metre

Table 4.1

(a) Monthly rainfall and pan evaporation* for the 1969 growing season compared with long-term average values (1931 - 1968), and (b) monthly mean minimum and mean maximum temperatures for the 1969 growing season, at Roseworthy, South Australia.‡

(a)	Monthly rainfall (mm)		Monthly pan evaporation (mm)*	
	Mean	1969	Mean	1969
May	47	89	57	58
June	55	30	50	41
July	48	66	44	42
August	50	33	56	65
September	44	47	97	74
October	43	1	120	143
November	25	18	170	190
Total	312	284	594	613

(b)	Mean minimum temperature (°C)	Mean maximum temperature (°C)
	1969	1969
May	7.5	18.0
June	6.5	15.0
July	6.5	15.5
August	6.5	17.0
September	5.5	16.0
October	9.5	23.5
November	11.0	24.5

* Australian Tank

‡ Data from Roseworthy Agricultural College records.

Table 4.2

Daily rainfall (R - mm) and mean daily pan evaporation (E - mm)* June - October 1969 at Roseworthy, South Australia‡, and dates on which nitrogen treatments were applied in Experiment B. The cultivar(s) to which each nitrogen treatment refers is indicated.

*Australian Tank.

‡Data from Roseworthy Agricultural College records.

Date	June R	July R	August R	September R	October R
1	4.6				
2				0.8	
3			6.4	0.5	
4			0.3		
5	1.5	4.3	(N^2-HM)*	0.3	
6	0.3	3.0		3.3	
7			4.3	11.2	
8			1.3	0.8	
9	15.7		0.5	(N^3-G)	
10	0.3				
11		7.6		1.0	
12		14.0	(N^2-P)	1.0	
13		0.8		7.4	
14			2.5	4.8(N^3-H)	
15				0.3	
16					
17					
18	1.5(N^1-MGHP)				
19				(N^3-P)	
20				0.8	
21		22.6		8.1	
22		4.1		4.3	
23	0.8	2.5	0.8	2.3	
24	1.0	2.0			
25	2.5				
26	0.8				
27			4.3		
28		0.5			1.0
29		4.8(N^2-G)	0.3		
30			12.2		
31			(N^3-M)		
E	1.3	1.4	2.1	2.5	4.6

*N^2 to "Mexico 120" was applied late. Spikelet differentiation for this cultivar occurred on July 24th.

Long-term average climatic data at the site for the growing season, and data for the 1969 growing season, are shown in Tables 4.1 and 4.2. The data were obtained approximately 1 km from the experimental site. The season was slightly drier than average.

4.2.3 Nitrogen application

10% (w/v) urea solution (I.C.I. commercial fertilizer, 46% N, <0.3% Biuret content) was sprayed onto plots with a boom-spray with attachments to limit spread, 25% of the required amount being sprayed in each of four traverses. The boom-spray was mounted on a garden tractor, which travelled on paths between plots. Three McPherson Flat Spray 'Tee-Jet' 6501 nozzles, 51 cm apart, delivered 0.5 litres each per minute at a line pressure of 2.8 kg cm^{-2}. Treatments were applied at given developmental stages of each cultivar. The development of cultivars was followed by apical dissection of sample plants. Apices were dissected under a light microscope, and developmental stages assessed using photographs from Barnard (1955). The calendar dates on which applications were made to each cultivar are given in Table 4.2.

4.2.4 Harvesting and measurements

Plots were sampled at four stages during the season:

(H1) early in the tillering phase, (H2) in the middle of the tillering phase, (H3) ear emergence, (H4) maturity. Harvest dates (day/month) for each cultivar were as follows:- 1/8 (H1-MGHP), 25/8 (H2-MGHP), 30/9 (H3-M), 15/10 (H3-G), 20/10 (H3-H), 30/10 (H3-P), 26/11 (H4-M), 3/12 (H4-GH), 8/12 (H4-P). At the first three harvests plants were pulled up from five strips of row selected at random, and stored at 2°C for up to three days while measurements were made. At maturity ten strips per plot were taken. Strips were 1.6 m at S_1 and 0.4 m at S_2, containing approximately 10 plants in each case. At least two rows or four plants were allowed as borders.

The number of tillers, dry weight (80°C), and total nitrogen content (as described in section 3.2.5) were determined on the samples of total shoots from the first three harvests. At maturity dry weight and nitrogen content of the straw + chaff and of the grain were determined separately. The components of grain yield were determined - number of ears per sq. metre, mean number of grains per ear, mean weight per grain. The proportion of the grains harvested that were incompletely filled was measured as the percentage that passed through a 1.5 mm mesh sieve.

Counts of the number of emerged ears in the strips of row designated to be harvested at maturity were made at several occasions near ear emergence. The date when 50% of ears finally harvested at maturity had emerged was calculated. Similar counts of the number of heads fully senesced (no green colouration visible)

in the strips were made near maturity, from which the date when 50% of the ears finally harvested had matured was determined. An estimate of grain-filling duration was derived from these dates, to detect any effects from the treatments of hastened maturation.

4.3 Results

4.3.1 Tillering

The pattern of tillering during the season for each nitrogen treatment is shown in Fig. 4.2.a. Urea-nitrogen stimulated tillering at each stage of development at which it was applied. The greatest stimulus apparently derived from the N^2 treatment - the first deferred application. This treatment produced the highest number of ears per sq. metre (210).

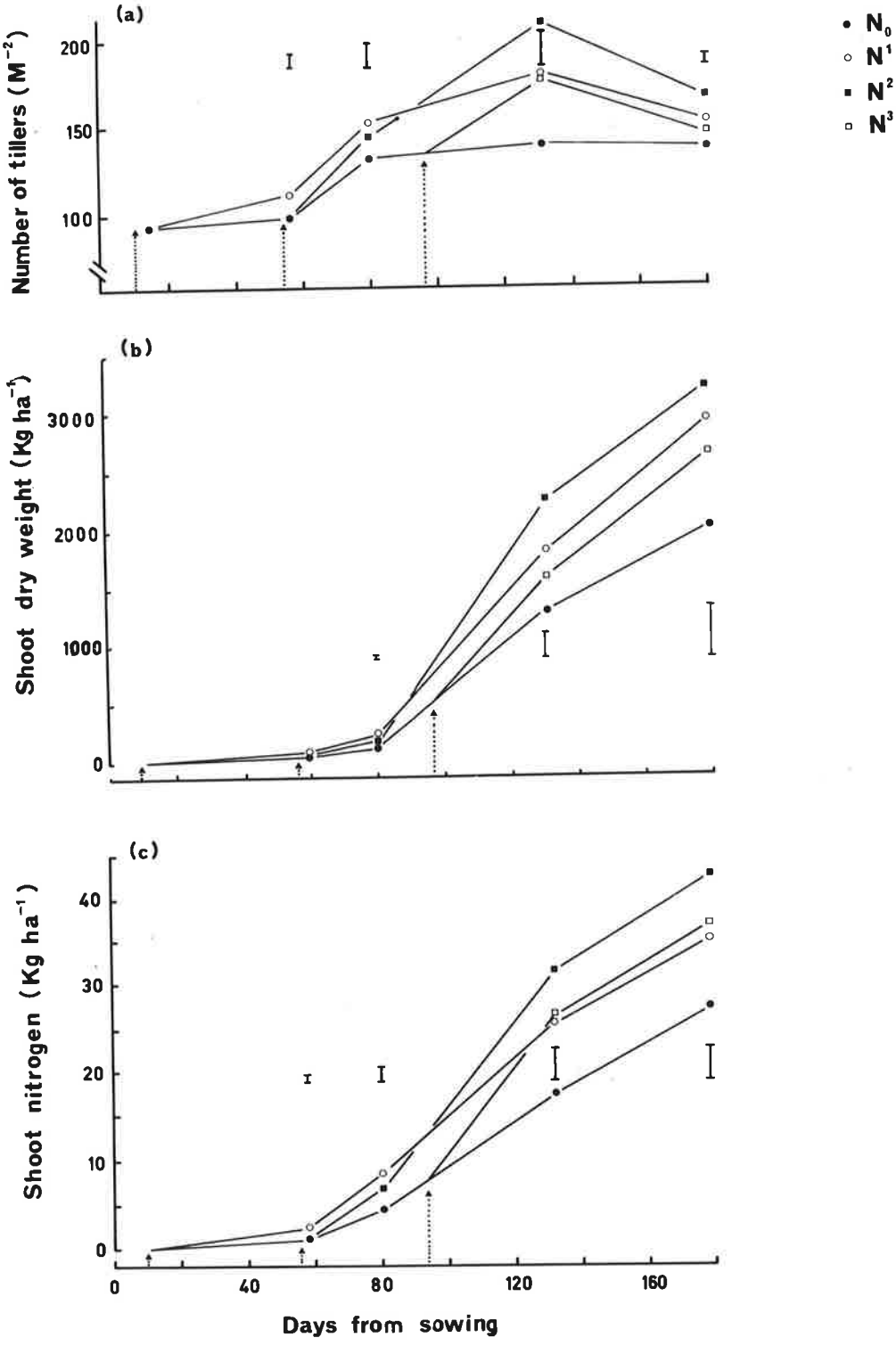
The mean main effects of Sowing rate and Cultivar on tillering, and certain minor interactions between them, are tabulated in Appendix 2, Table A1. Although individual plants tillered more, and tiller survival was greater at low than at normal sowing rate, the density of tillers and ears per sq. metre remained less at the low sowing rate. 'Gabo' showed a relatively low and 'Mexico 120' a relatively high tiller production. These differences were reflected in the number of ears per sq. metre at maturity (Table 4.3). There were no interactions between Nitrogen treatment and either Cultivar or Sowing rate.

Figure 4.2

Main effects of time of nitrogen application on (a) tillering, (b) total shoot dry weight, and (c) shoot nitrogen uptake, through the season.

Arrows (↑) mark the mean dates of nitrogen application for treatments N^1, N^2 and N^3 respectively (the calendar dates of nitrogen application for cultivars within each nitrogen treatment were not uniform - see section 4.2.3).

L.S.D.(I), P = 0.05


(a)
Number of tillers (M^{-2})
200
150
100
(b)
Shoot dry weight ($Kg\ ha^{-1}$)
3000
2000
1000
0
(c)
Shoot nitrogen ($Kg\ ha^{-1}$)
40
30
20
10
0
0
40
80
120
160
Days from sowing
N_0
N^1
N^2
N^3

4.3.2 Shoot dry weight

As with tillering, urea-nitrogen accelerated the increase in shoot dry weight at each stage of development at which it was applied (Fig. 4.2.b), and the greatest increase occurred in the N^2 treatment. Shoot dry weight per sq. metre was less at low than at normal sowing rate during development, but by maturity no significant difference between sowing rates was detectable (Table A2 - Appendix 2). Interactions between Sowing rate and Cultivar, and between Sowing rate and Nitrogen treatment at the first two harvests are shown in Table A3, Appendix 2. A Cultivar x Nitrogen interaction was evident at maturity (Fig. 4.3.a). A significantly greater response to deferred application of nitrogen was shown by 'Halberd' and 'Mexico 120' compared to 'Gabo' and 'Pinnacle'. 'Halberd' in particular showed a greater response.

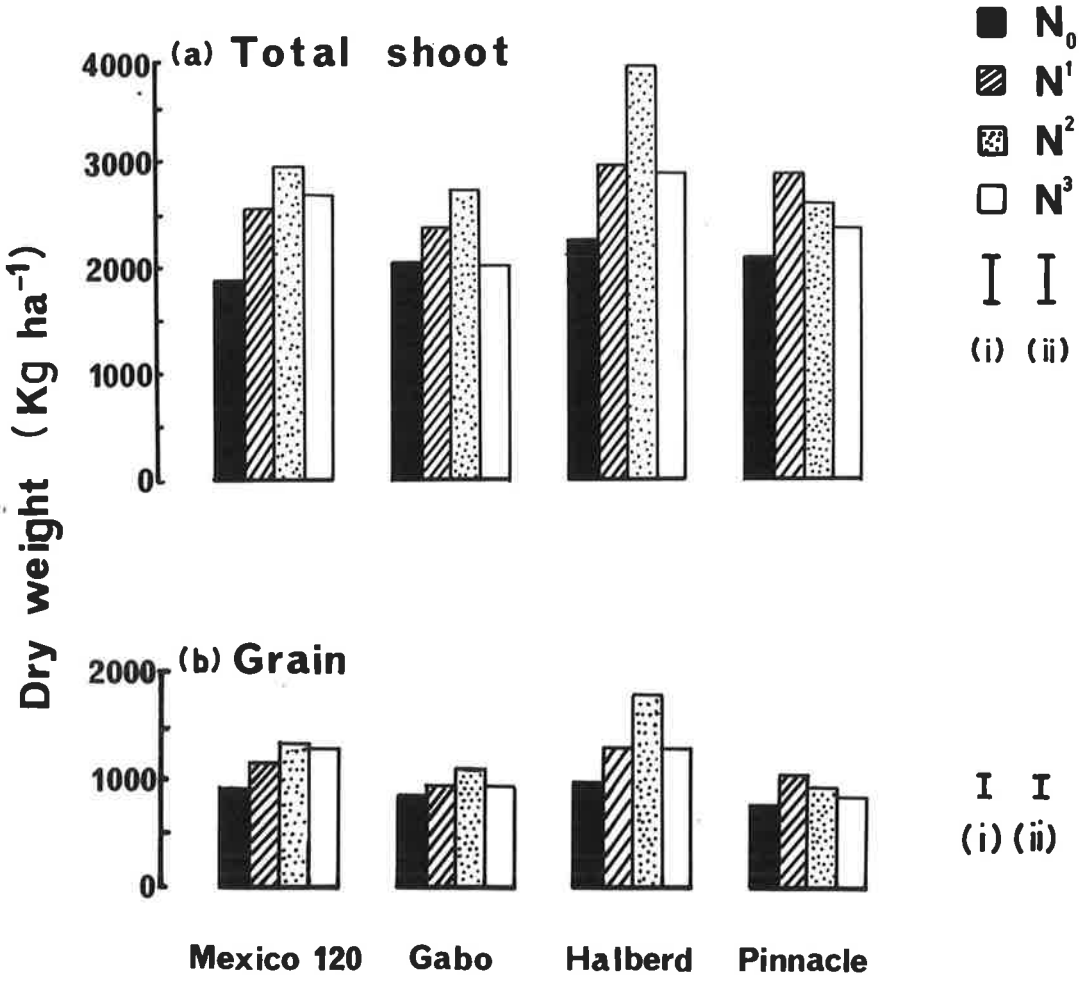
4.3.3 Nitrogen uptake

Urea increased the rate of nitrogen uptake at each stage of development at which it was applied (Fig. 4.2.c), and the greatest increase occurred in the N^2 treatment. No significant differences between sowing rates or cultivars occurred at any harvest. Interactions at harvests 1 and 2 between Sowing rate and Nitrogen are shown in Table A4, Appendix 2.

Figure 4.3

Cultivar x Nitrogen effects on dry weight of (a) total shoot and (b) grain, at maturity.

L.S.D's (P = 0.05) apply to (i) cultivar comparisons within one nitrogen treatment, and (ii) nitrogen treatment comparisons within one cultivar.


(a) Total shoot
4000
3000
2000
1000
0
Dry weight (Kg ha^{-1})
(b) Grain
2000
1000
0
N_0
N^1
N^2
N^3
(i) (ii)
I I
(i) (ii)
Mexico 120
Gabo
Halberd
Pinnacle

4.3.4 Development

The number of days to 50% ear emergence was 128, 132, 135 and 143 (L.S.D.* = 1.2) for 'Mexico 120', 'Gabo', 'Halberd' and 'Pinnacle' respectively. Plants reached 50% ear emergence significantly* earlier at normal than at low sowing rate (133 cf 136 days), and with the two earlier times of nitrogen application (136, 133, 134 and 136 days for treatments N_0, N^1, N^2 and N^3, L.S.D.* = 1.1). Small but statistically significant differences occurred between cultivars in the numbers of days between 50% ear emergence and 50% ear senescence (32, 32, 35 and 33 for 'Mexico 120', 'Gabo', 'Halberd' and 'Pinnacle', L.S.D.* = 1.1) and nitrogen treatments (38, 34, 33 and 33 for treatments N_0, N^1, N^2 and N^3, L.S.D.* = 1.0). There were no interactions.

4.3.5 Components of grain yield

The main effects of treatments on the components of grain yield are shown in Table 4.3, and the statistically significant interactions that occurred in Table 4.4. A smaller number of ears per sq. metre at low than at normal sowing rate was compensated by a larger ear size (as indicated by mean number of grains per ear). 'Halberd' showed a greater capacity to compensate at low sowing rate than did the other cultivars

---

* P = 0.05

Table 4.3

Main effects of Sowing rate, Cultivar and Nitrogen treatment on components of grain yield.

		Number of ears M^{-2}		Mean number of grains per ear		Mean weight per grain (mg)	
Sowing rate:	S_1	100	a*	31.6	a	32.9	n.s.
	S_2	191	b	20.0	b	31.3	
Cultivar:	M	182	c	33.6	c	30.5	a
	G	127	d	25.8	d	32.5	b
	H	145	e	28.8	e	33.9	c
	P	127	d	24.9	cd	31.5	d
Nitrogen:	N_o	133	f	24.2	f	32.4	
	N^1	146	g	26.5	gh	31.9	n.s.
	N^2	158	h	26.9	g	32.0	
	N^3	143	g	25.6	h	32.1	

* Values followed by a common letter are not significantly different from other values within the main treatment group (P = 0.05)

Table 4.4

Statistically significant (P = 0.05) interactions on components of grain yield.

(a) Mean number of grains per ear

		Cultivar			
		M	G	H	P
Nitrogen treatment:	N_o	20.7	25.4	26.0	24.5
	N^1	22.7	25.6	30.0	26.4
	N^2	25.7	26.9	30.3	24.4
	N^3	24.0	25.1	29.0	24.6

L.S.D. (P = 0.05) for comparisons between cultivars within a nitrogen treatment = 1.4

" " for comparisons between nitrogen treatments within a cultivar = 1.2

		M	G	H	P
Sowing rate:	S_1	30.4	31.0	35.0	29.8
	S_2	16.8	20.5	22.0	19.7

L.S.D. (P = 0.05) for comparisons between cultivars within a sowing rate = 0.8

" " for comparisons between sowing rates within a cultivar = 1.7

Table 4.4, continued

(b) Mean weight per grain (mg)

		Cultivar			
		M	G	H	P
Nitrogen treatment:	N_o	31.0	32.9	33.6	32.0
	N^1	31.3	32.5	33.4	30.5
	N^2	28.9	32.2	34.8	32.2
	N^3	30.7	32.6	34.0	30.7

L.S.D. (P = 0.05) for comparisons between cultivars within a nitrogen treatment = 1.5

" " for comparisons between nitrogen treatments within a cultivar = 1.4

(Table 4.4.a). 'Mexico 120' matured a relatively large number of ears per sq. metre, but these held relatively few and small grains. Compared with the other cultivars, 'Halberd' matured ears containing the highest mean number of grains per ear, and having the highest mean weight per grain. Next to 'Mexico 120', 'Halberd' matured the highest number of ears per sq. metre.

Urea application increased the number of ears matured per sq. metre, the greatest increase resulting from the first deferred application (N^2). This occurred for all cultivars at both sowing rates. Urea increased the mean number of grains per ear, but this response differed between cultivars. 'Gabo' and 'Pinnacle' showed relatively little response to nitrogen applied at any stage, in contrast to 'Mexico 120' and 'Halberd' (Table 4.4.a). There was no main effect of nitrogen on mean weight per grain. With the exception of 'Halberd' this component tended to be inversely related to mean number of grains per ear (Table 4.4.b).

4.3.6 Grain yield

Grain yields reflected low straw + chaff weights at maturity and were low throughout. There was no significant difference ($P = 0.05$) between sowing rates, but an interesting interaction occurred between Cultivar and Nitrogen treatment (Fig. 4.3.b). With three cultivars the first deferred application

(N^2) gave a greater grain yield response than either the time of sowing (N^1) or the second deferred application (N^3). However, 'Pinnacle' showed the greatest response to nitrogen applied at sowing. 'Halberd', and to a lesser extent 'Mexico 120', showed a greater grain yield response to deferred application of urea than did 'Gabo' or 'Pinnacle'.

The percentage of grain yield that passed through a 1.5 mm mesh sieve was lower for 'Halberd' (10%) and higher for 'Mexico 120' (45%) than for 'Gabo' and 'Pinnacle' (20%). Except in the case of 'Mexico 120', which showed an increase in the percentage with the first deferred application, there was no effect of nitrogen treatment for any of the cultivars. There was also no effect of Sowing rate. The mean weights per grain that passed through the sieve were 15, 23, 21 and 21 mg for 'Mexico 120', 'Gabo', 'Halberd' and 'Pinnacle'.

4.3.7 Grain nitrogen content

Grain nitrogen concentrations were low for all treatments (Table 4.5), the general mean being equivalent to 11.0% crude protein content. There was no effect of Sowing rate. 'Halberd' showed a slightly lower value than the other cultivars. Small increases resulted from the second deferred application of nitrogen (N^3). There were no interactions.

Table 4.5

Main effects of Sowing rate, Cultivar and Nitrogen treatment on grain nitrogen concentration.

		N (% of dry matter)	
Sowing rate:	S_1	1.95	n.s.
	S_2	1.91	
Cultivar:	M	1.96	a*
	G	1.95	a
	H	1.82	b
	P	2.00	a
Nitrogen:	N_o	1.88	c
	N^1	1.91	c
	N^2	1.93	c
	N^3	2.00	d

* Values followed by a common letter are not significantly different from other values within the main treatment group ($P = 0.05$)

Table 4.6

Main effect of time of nitrogen application on apparent recovery (%) of applied nitrogen at 4 stages of growth.

	Harvest‡			
Nitrogen treatment	1	2	3	4
N^1	2.4	7.4	14.0	16.1
N^2	(0.2)	3.5	26.7	24.5
N^3	(0.3)	(0.1)	15.6	18.1
L.S.D. (P = 0.05)	1.1	2.6	5.2	6.3

‡ See section 4.2.4

4.3.8 Apparent recovery of applied nitrogen

There were no significant effects of either Sowing rate or Cultivar at any harvest on apparent recovery of applied nitrogen. The effects due to Nitrogen treatments are shown in Table 4.6. Recovery of N was low, never exceeding 27% of that applied. Recovery increased up to harvest 3, but did not increase significantly thereafter. A significantly higher recovery was obtained from N^2 than from N^1 or N^3. No interactions occurred.

4.4 Discussion

Soil and plant variability resulted in only the most prominent effects of the treatments imposed being discernible. The limitations of low replication were offset to some extent in the case of sub- and sub-sub-plot treatments by the use of three factors in a split-split-plot design.

The apparent recoveries of applied nitrogen in this experiment were low by general standards (Allison 1966). In view of the results of Experiments A and C, incomplete plant cover of the soil at the times when fertilizer was applied, and the incidence of rain following spraying (Table 4.2), it seems probable that fertilizer was recovered by the crop mainly from the soil. Low recoveries may have been caused by losses of urea from the soil following application, and partial immobilisation of

that retained. The near neutral pH of the soil together with its relatively low buffering capacity (Northcote 1965) would predispose it to local alkalinity, and lead to loss of ammonia following hydrolysis of urea (Gasser 1964). A high C:N ratio of the soil would be expected from the site history of two successive cereal crops, which were preceded by a pasture of low legume content. This would have favoured immobilisation of applied nitrogen (Allison 1966). Losses of nitrogen by denitrification at rates up to 1.7 g N $week^{-1}$ m^{-3} of soil from ammonium, and up to 3.3 g N $week^{-1}$ m^{-3} from nitrate, have been demonstrated in a similar red-brown earth soil at field capacity in the presence of plants (Stefanson 1972). These rates correspond to 5 and 10 kg N $week^{-1}$ ha^{-1} respectively, for a 30 cm depth of soil. Loss of urea may also have occurred from the crop foliage, as in Experiments A and C. Precipitation did not exceed pan evaporation appreciably following any nitrogen application (Table 4.2). Therefore leaching of nitrate below or ahead of the root zone following hydrolysis of urea and nitrification does not seem likely to have been a major cause of nitrogen unavailability (cf Mason, Rowley and Quayle 1972).

A greater apparent recovery of nitrogen was obtained from the first deferred than from the sowing time or second deferred applications. After germination in the pre-tillering phase the absorbing capacity of the crop would have been small because of the limited root development and the small dry weight

of the plants. Given a continuous loss of fertilizer from the soil, low recovery from sowing time application may be expected. If the rate of nitrogen loss or immobilisation were no greater at a later stage, a greater recovery of nitrogen would be expected because of the more extensive root development and the larger crop. Similar reasoning has been applied by Mason, Rowley and Quayle (1972) to explain results of an experiment conducted in Western Australia, in which leaching was shown to be the principal cause of loss of nitrogen following application. It has also been applied by Gunasena and Harris (1968) in discussing equivalent results with a potato crop (Solanum tuberosum).

Lower recovery of nitrogen from the later deferred application may have been due to a greater proportion falling on the crop foliage, and either remaining unabsorbed or being lost by volatilisation from the leaves. Appreciable rain fell shortly before or after each N^3 application (Table 4.2), and it seems less likely that lower recovery was due to urea remaining unavailable for uptake on a dry soil surface.

It is of interest that no significant differences between cultivars in the apparent recovery of each deferred application of nitrogen were induced by differences in the calendar date of application.

Grain yields in this experiment were low by regional standards (Russell 1967), as a result of the severe nitrogen

deficiency of the site and the low recoveries of applied nitrogen. No significant difference in grain yield was detectable between the normal and 1/5th normal sowing rates, the small number of plants per unit area in the latter case being compensated by a greater number and size of ears per plant. This is in accord with the results of Puckridge and Donald (1967), but not of Walter (1971), who obtained a significantly lower grain yield from a density of 20 than from a density of 90 plants per sq. metre (cf. section 4.2.2).

Cultivars yielded similarly where no fertilizer was applied, and where it was applied near sowing. The greater grain yield from the first deferred application of nitrogen than from the sowing time application, except for 'Pinnacle', was associated with the greater apparent recovery of fertilizer from the deferred than from the sowing time application. The greater responses of 'Halberd' and 'Mexico 120' than of 'Gabo' and 'Pinnacle' to deferred application reflected different abilities to respond to a given supply of nitrogen rather than differences in the nitrogen supply, since cultivars did not differ significantly in their apparent recoveries of applied nitrogen.

All cultivars tillered in response to nitrogen application, but 'Halberd' and 'Mexico 120' achieved an increase in mean ear size with deferred application, whereas the other cultivars only maintained ear size. It is interesting that the cultivars of

similar responsiveness were not similar in development. 'Mexico 120' and 'Gabo' matured relatively early and 'Halberd' and 'Pinnacle' relatively late (section 4.3.4). 'Mexico 120' and 'Halberd' were both strong-tillering cultivars, but 'Gabo' and 'Pinnacle' contrasted markedly in tillering rate (Table A1, Appendix 2).

Added nitrogen produced a growth response rather than an increase in nitrogen content of the grain. This was in conformity with most experiments in which nitrogen has been applied before the end of the vegetative phase of development (sections 2.2.2.e and f). Grain nitrogen contents were low, the level of 1.82% for 'Halberd' corresponding to a crude protein content of 10.4%. The slightly increased grain nitrogen content from the second deferred application corresponded to an increase of crude protein content from 10.7 to 11.4%. The values reflect the nitrogen-deficiency of the site (Russell 1963) and the low recoveries of applied nitrogen.

Observations on ear emergence and maturation, and on the amounts of incompletely filled grain produced, showed no evidence of hastened ear senescence or grain hardening due to nitrogen-induced water stress during the late stages of development, as has been reported elsewhere in southern Australia (Colwell 1963; Barley and Naidu 1964; Storrier 1965a, b; Fischer and Kohn 1966b). The absence of such effects was probably due to the moderate growing season rainfall (266 mm, May - October) and relatively small leaf

area of the N-deficient crop.

The grain yield response to applied nitrogen differed from that found in previous experiments in South Australia, in which comparisons have been made between sowing time and deferred applications of nitrogen (Birks and Cole 1930; Richardson and Gurney 1935; Reuter 1967; Russell 1969). In previous experiments yield responses were inferior when application was deferred. The results correspond, however, with those of Mason, Rowley and Quayle (1972) obtained in Western Australia. A number of arguments may be advanced to account for the differences in result in different instances.

The crop response to application of nitrogen is dependent in the first instance on the percentage recovery of applied N. This is very sensitive to environmental conditions; losses from the system following application, and availability for absorption by the roots, are both affected by rainfall for example. Both losses and availability may differ with application at different times of the season. Where losses do not differ, a greater recovery of applied nitrogen may be found with deferred application, because of the larger root system present. On the other hand, positional availability of the fertilizer may be less with late applications, because of the dependence of rainfall to wash the fertilizer into the soil. The possibility that "foliar" applications of nitrogen may in fact be supplied largely via the soil has already been mentioned. The

recovery of such applications would not be independent of rainfall following application.

In this experiment the comparison of sowing time and deferred applications of nitrogen was not wholly realistic, since, although the method of sowing time application was comparable with that of later applications, it was possibly not the most effective method for applying N. For example, drilling an equivalent rate of ammonium sulphate with the seed may have given a greater recovery of N by the crop, as losses of nitrogen are generally reduced when N is applied as ammonium (Allison 1966).

The response by a crop to nitrogen application is influenced by its state of nitrogen deficiency. Inasmuch as (i) the means by which the plant may respond to nitrogen are less the later in development the nitrogen is applied, and (ii) early nitrogen application predisposes a crop to increased uptake later in development, it is likely that the efficacy of deferred relative to early application decreases with increasing nitrogen-status of the site.

The inclusion in the experiment of a range of cultivars, which exhibited differing capacities for response to N applied at each growth stage, influenced the conclusions drawn. Earlier experiments conducted in South Australia contained only one cultivar, unnamed in the case of Birks and Cole (1930) and Richardson and Gurney (1935), Gabo in the case of Reuter (1967) and Russell (1969).

The use of commercially established genotypes which have been selected for their performance under traditional methods of husbandry favours the success of these methods when compared with innovative ones. While genotypes selected for performance under innovative methods are not available, a greater chance of success with an innovative method will be provided by the use of a range of genotypes.

5 Experiment C - The root growth response of wheat, cv. Halberd, to deferment of nitrogen application, and to path of application, in solution culture.

5.1 Introduction

As stated in chapter 1, application of nitrogen to wheat in southern Australia often leads to only marginal increase, and occasionally to depression, in grain yield. A potentially increased yield at anthesis, through an increased leaf area and number of ears, is frequently not realised at maturity because of post-anthesis water stress. The likelihood and severity of water stress in the post-anthesis period are generally increased by the use of nitrogen, because of the stimulation of leaf area, and hence transpiration, and the consequent earlier depletion of soil moisture.

Water stress to the crop occurs when the rate at which water can be absorbed from the soil lags behind the rate of transpiration. Provided it is not too high, the length of roots per unit volume of soil, L_v (cm^{-2}), has a major influence on the rate of flow of water from a volume element of soil to the shoot. With increasing L_v the resistance to flow of water is reduced (i) from the soil to the set of roots present in a volume element (Gardner 1960, 1964), (ii) within the root system (Passioura 1972). In the first case the mean length of the flow path in the soil is

affected, and in the latter the number of vertical conducting channels. The capillary conductivity of the soil, which decreases sharply as soils dry below field capacity, also governs the soil's resistance to flow (Gardner 1964). Walter (1971) studied the water economy of a wheat crop in relation to its root development, at Roseworthy, South Australia (see also Walter and Barley, in press). It was found that, although the upper part of the soil profile dried as the root system developed, 'available' water (i.e. at a suction <pF 4.2) was present below 90 cm depth at the end of the season, where the crop had been sown at normal sowing rate. Poor utilisation of the deeper water was attributed to the low root densities measured at depth, and to the small rate of upward flow of water within the soil. Root density was shown to decline sharply with depth, ranging from 13 (cm^{-2}) at 5 cm, through 5 at 50 cm, 1 at 100 cm, to 0.1 below 125 cm, at maturity. Occurrence of 'available' water at depth in South Australian soils at the end of a season during which water stress was observed has also been reported by Schultz (1971).

Root growth is sensitive to the nitrogen concentration of the root medium (section 2.2.2.c). The distribution of root weight beneath a barley crop in the field was shown by Welbank and Williams (1962) to be altered by application of nitrogen at sowing time. An increase in the proportion occurring in the upper soil horizons was observed. Root distribution in relation to deferment

of nitrogen application has not been investigated, but an influence less apparently disadvantageous to a South Australian wheat crop might be expected. The elongation rate of wheat seminal root axes has been shown to be increased in nutrient solution of low relative to high nitrate concentration (section 2.2.2.c). A low nitrogen status of the soil during early growth resulting from deferment of nitrogen application might result in deeper penetration of seminal roots than when nitrogen was applied at sowing.

The object of the present experiment was to examine the influence of deferment of nitrogen application on the root development of wheat. The influence of path of application was also examined.

The root density in any layer of the soil profile is the result of the combined effects of the rate and duration of elongation of the individual filaments, and their density of branching (Barley 1970; Lungley 1973). The integrated effects of deferred application of nitrogen on root distribution could have been measured in the field. A more basic study was undertaken in solution culture in order to separate the responses of elongation and branching and of different classes of root member, and to facilitate the observation of response to alteration of the nitrogen supply during development.

Definition of the attributes which govern root length development in cereal plants, and the possibility of evaluating

quantitatively the effects of local or temporal alterations in them on the spatial distribution of root length, led to the formulation of a numerical simulation model of the growth of a cereal root system (Lungley 1973). The model served as a valuable aid to the design and interpretation of the experiment. The model is given in Appendix 1.

5.2 Experimental method

5.2.1 Design and treatments

Six nitrogen treatments were replicated three times in randomised blocks. Single plants of wheat, cv. Halberd, were grown in flowing solution culture, in tanks designed to allow periodic observation of root growth. The plants were grown in N-deficient nutrient solution and, at two different stages of growth, either changed to solution well supplied with N, or supplied with nitrogen to the foliage in the form of a urea spray. The stages of growth corresponded with the stages of deferred application of nitrogen in Experiment B. In control treatments plants were grown continuously in N-deficient solution, and in solution well supplied with N. Plants were grown for 94 days from germination.

The treatments were as follows:

N_1 — N_1 solution throughout

N_R^1 — N_2 solution throughout

N_F^2 — N_1 solution throughout, but with foliar applications of urea from the stage of spikelet differentiation of the main shoot apex

N_R^2 — N_1 solution until the stage of spikelet differentiation of the main shoot apex, N_2 solution thereafter

N_F^3 — N_1 solution throughout, but with foliar applications of urea from the stage of floret differentiation on the terminal spikelet of the main shoot apex

N_R^3 — N_1 solution until the stage of floret differentiation on the terminal spikelet of the main shoot apex, N_2 solution thereafter

N_1 solution (low-N) was such as to grow nitrogen-deficient plants, while N_2 solution (high-N) was such as to grow nitrogen-sufficient plants. Deficiency is defined, following Greenwood (1966), as the condition where an increase in the supply of the deficient element causes an increase in the rate of growth, in contrast to sufficiency, and is quantifiable as to degree in the manner described by the same author.

Growth stages were defined as in Experiment B.

5.2.2 Culture apparatus

(a) General construction

The apparatus was designed to allow the growth of roots in solutions of nearly constant composition, comparable as far as possible with soil solution concentrations. The solution temperature was controlled at a level commensurate with that of field soil. The roots were grown in glass-walled tanks, through which they could be observed, and measurements of the lengths of root members made, after removal of an insulation cover. Use of a flowing culture technique permitted a large volume of dilute, temperature-controlled solution to be cycled continuously through culture tanks. A large volume of solution per plant buffered the concentration against rapid depletion.

The apparatus is shown in Plate 5.1. A diagram illustrating the construction of one half is given in Fig. 5.1. The other half was identical, except that the main and constant-head reservoirs contained solution at the second concentration of N. For each nutrient solution, a large fibre-glass reservoir (260 l capacity) containing solution was immersed in a water bath, the temperature of which was thermostatically controlled so that the temperature of the nutrient solution was 13°C ($\pm$ 2°C). The water in the jacket was stirred continuously. A "Webster"[1] water pump drove nutrient

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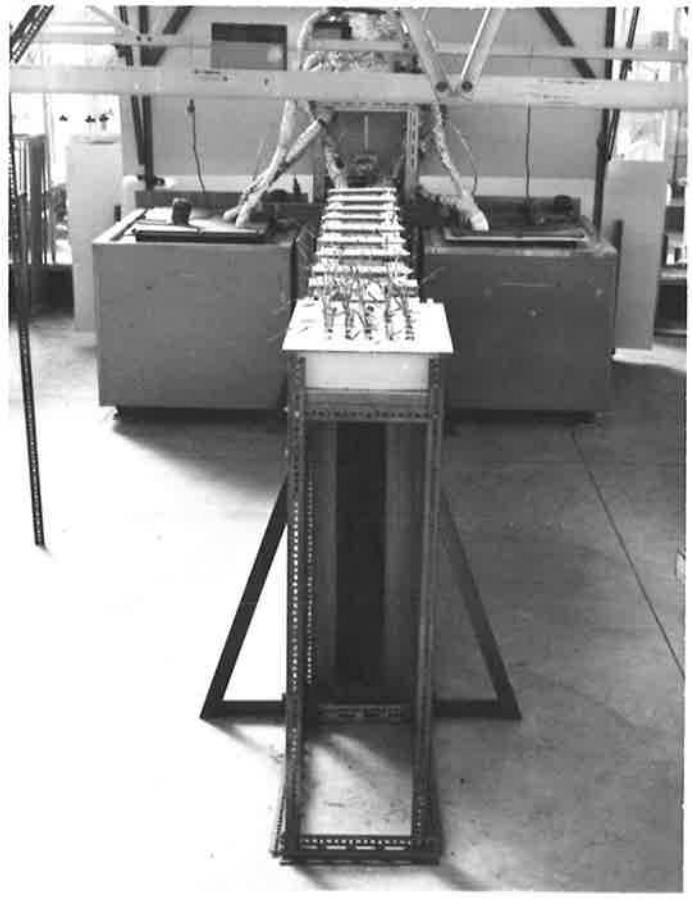
[1] Manufactured by Webster Mfg. Co. Ltd., Adelaide, South Australia.

Plate 5.1

General views of Experiment C, showing (i) wooden frame containing 18 insulated root observation culture tanks and associated plumbing, together with a container (foreground) growing extra plants for shoot apex observation; (ii) the two main solution reservoirs immersed in temperature-controlled water baths, and the elevated constant-head reservoirs (background).



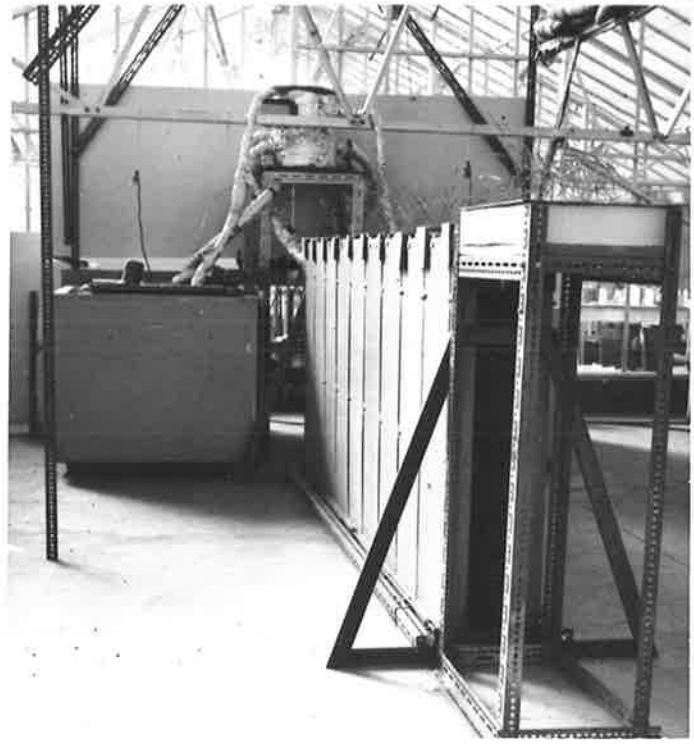


Figure 5.1

Diagram of the main components of one half of the flowing solution culture apparatus used in Experiment C, showing the system of flow.

A Main reservoir of nutrient solution (fibre-glass) - 260 l capacity

B Water pump (stainless steel, Polystyrene)

C Elevated constant-head reservoir (Polythene) - 20 l capacity

D Main arterial pipe (Polyvinylchloride (PVC) hose)

E Main return pipe (PVC hose)

F Master-tap (rigid PVC)

G Sloping shelf

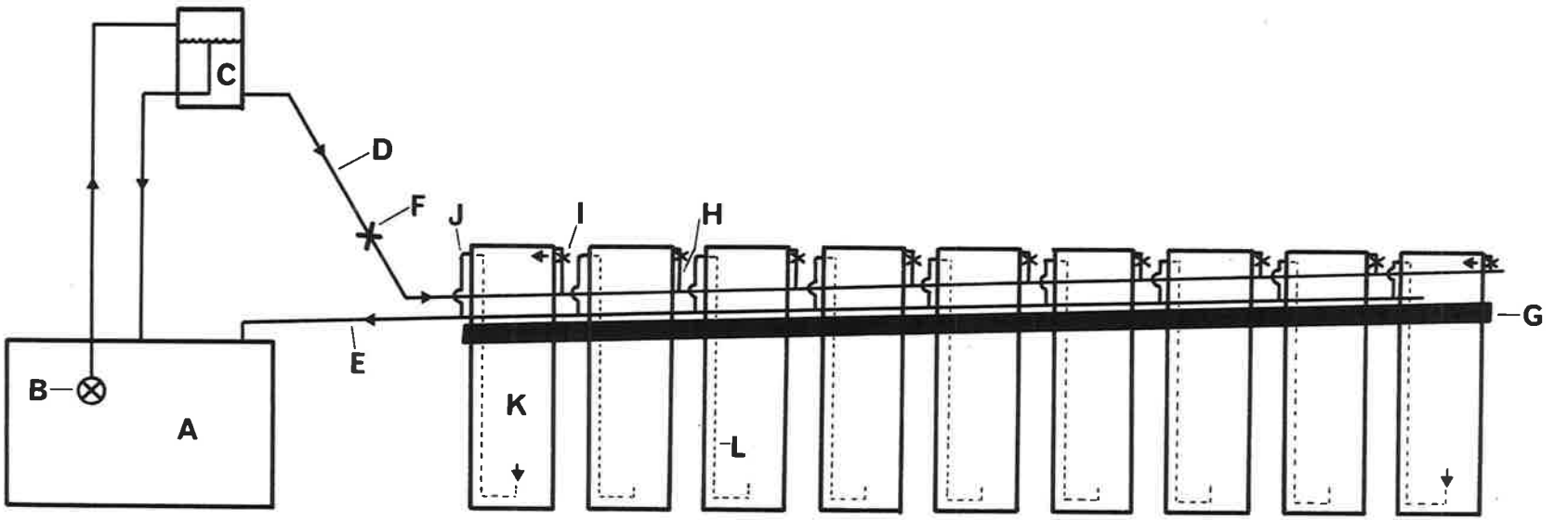
H Inflow pipe to root observation culture tank (Polythene)

I Flow-regulating tap (Polythene)

J Outflow pipe from root observation culture tank (Polythene)

K Root observation culture tank (stainless steel, plate glass) - 9 l capacity

L Internal L-shaped tube connecting the solution exit point at the base of the culture tank with the outflow point at the top (glass)


C
D
F
J
I
H
G
E
B
A
K
L

solution to an elevated constant-head reservoir. Solution flowed under gravity from the constant-head reservoir to supplied root observation culture tanks via a main arterial pipe. The arterial pipes supplying each nutrient solution ran the length of a wooden frame, which supported eighteen culture tanks arranged in two rows. The pipes rested on a central sloping shelf. Inflow pipes led at intervals from each arterial pipe to the tops of the culture tanks. Adjustment of a tap in each inflow pipe regulated the flow into each tank. Tanks were supplied with each nutrient solution according to the randomised allocation of treatments. The outflow was taken from the base of each tank, via an exit at the top (see Fig. 5.2). Solution flowed out of the tank under a small gravitational head into one of two main return pipes. Each return pipe drained under gravity into the appropriate main reservoir, completing the circuit.

The solution in each main reservoir was continually mixed by an attachment to the "Webster" pump. A nylon fine-mesh sock-filter was placed over the exit pipe in each constant-head reservoir to trap macroscopic detritus. Small amounts of what appeared to be dust, sloughed root material, and bacterial growth were trapped. The apparatus was light-proofed and insulated with soft-board, wood, wool and aluminium foil.

Some problems were experienced initially with toxic substances derived from the apparatus. A subsidiary experiment

indicated that flexible PVC tubing released substances into nutrient solution with which it was in contact, which were toxic to the growth of wheat plants. The substances were apparently lead- and zinc- based plasticisers. The materials finally used in the apparatus are indicated in the keys to Figs. 5.1 and 5.2.

(b) Root observation culture tanks

The tanks were designed to accommodate a well-developed root system. The dimensions, 30 cm x 2.5 cm x 120 cm internal, were chosen so as to spread the root system laterally and expose the network, the smaller lateral dimension being the minimum necessary to contain the crown of the plant without constriction. A diagram illustrating the construction of a tank is given in Fig. 5.2. Roots were viewed after removal of an opaque insulation panel. A view of two tanks with root systems exposed is shown in Plate 5.2.

Robust culture tanks were constructed by an aquarium-maker from plate-glass and stainless steel, cemented with an inert soft-setting silicone glue "Silastic 732 RTV"(1). Attachments were cemented with "Araldite"(2) or fitted by means of plastic-to-plastic self-sealing connections. Inflowing solution was

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(1) Manufactured by Dow Corning Corporation, Midland, Michigan 48640, U.S.A.

(2) Manufactured by CIBA-GEIGY (U.K.) Ltd., Duxford, Cambridge, U.K.

Figure 5.2

Diagram illustrating the construction of a root observation culture tank.

A 6 mm plate-glass panels

B Stainless steel side and base panels

C Stainless steel gauze for supporting one wheat plant (2 mm mesh)

D Perspex plates containing inflow and outflow apertures

E Inflow pipe, from main arterial pipe (Polythene)

F Flow-regulating tap (Polythene)

G Outflow pipe, to main return pipe (Polythene)

H 'Nick' in pipe to facilitate gravitational flow

I "Distributor" tube (Polythene)

J Distributing capillary (stainless steel)

K Internal L-shaped tube connecting the solution exist point at the bottom of the tank with the outflow point at the top (glass)

L Internal L-shaped tube connecting capillaries at the bottom of the tank with the compressed air supply at the top (glass)

M 0.11 mm diameter capillaries bubbling filtered compressed air

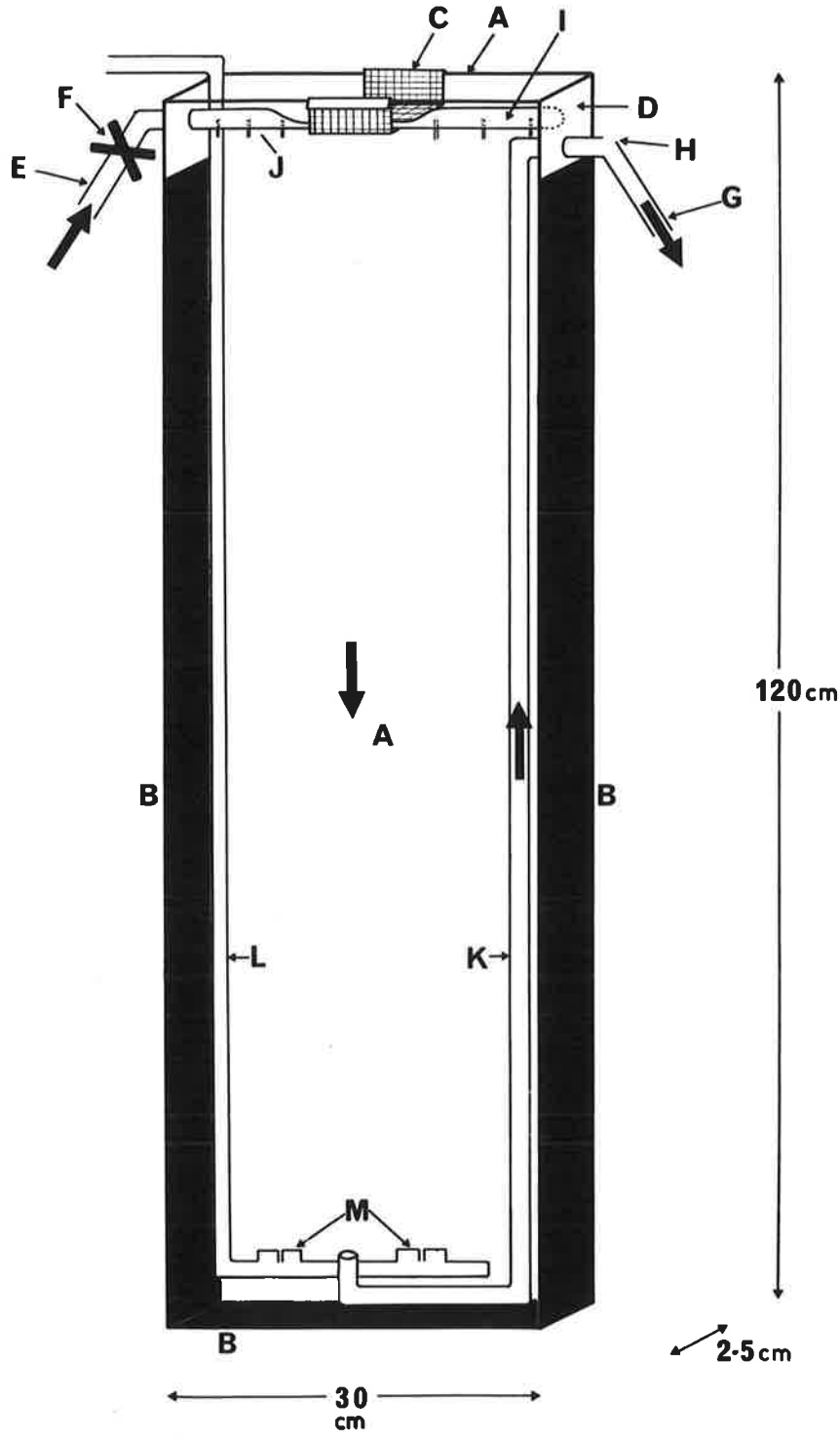

C
A
I
F
D
E
J
H
G
A
B
B
120 cm
L
K
M
B
2·5 cm
30
cm

Plate 5.2

View of the upper halves of two root observation culture tanks, containing 94-day old plants, with root systems exposed.

N_R^1 N_1

Day 94

distributed across the top of the tank as shown in Fig. 5.2. Solution flowed from the bottom of the tank via an internal tube to an outflow point at the top of the tank. Outflow occurred under the small gravitational head arising from continual inflow.

A flow of fine bubbles of compressed air was continuously supplied at the bottom of each tank from two capillaries, for the purpose of mixing the solution. The compressed air was passed at 1.5 kg cm^{-2} through a water droplet trap and a charcoal filter before connection to a manifold, from which the supply to each tank was taken. The rate of bubbling was controlled with a needle valve at the manifold connection.

5.2.3 Culture solution

The ionic composition of the culture solutions is shown in Table 5.1. The two solutions were identical in all respects except concentration of nitrogen, nitrogen being supplied as ammonium nitrate. The pH of the solution when prepared was 5.5. 0.009 m.equivs./1 of Fe-EDTA were added to each batch of solution, but symptoms of iron deficiency were only eliminated when a 2 metre length of 12-gauge soft iron wire was immersed in the main reservoir. Addition of boron was not necessary, probably because of traces supplied to the solution from impurities and contaminants in the apparatus, such as glass and steel. The concentration of nitrogen at N_1 was reduced after 30 days as no differences had

Table 5.1

Ionic composition of the nutrient solutions N_1 and N_2 employed in Experiment C.

	Element	m.equiv./l	p.p.m.	Salts
Basal:	P	0.011	0.34	KH_2PO_4
	K	0.21	3.3	KH_2PO_4, K_2SO_4
	Ca	0.43	8.7	$CaCl_2$
	Mg	0.31	3.7	$MgSO_4$
	Cl	0.43	15.4	$CaCl_2$
	S	0.51	8.2	$MgSO_4$, K_2SO_4
	Fe	-	-	Iron wire + Fe-EDTA
	(B)	-	-	-
	Mn	0.18×10^{-3}	5.0×10^{-3}	$MnSO_4$
	Zn	0.06×10^{-3}	2.0×10^{-3}	$ZnSO_4$
	Cu	0.016×10^{-3}	0.5×10^{-3}	$CuSO_4$
	Mo	0.021×10^{-3}	1.0×10^{-3}	$(NH_4)_2MoO_4$
N_1 solution: 0-30 days	N	0.036	0.50	NH_4NO_3
30- "	N	0.018	0.25	"
N_2 solution	N	0.72	10.0	"

appeared in shoot or root growth between the solutions at the initial level of N_1. Fresh solution was prepared by the addition of appropriate amounts of stock solutions to the main reservoirs, after the system had been filled with deionised water. Where depleted solution was being renewed, the main and constant-head reservoirs were isolated from the culture tanks by means of the arterial pipe master-tap, and the main reservoir emptied and refilled with deionised water. The prepared solution was allowed to mix before the master-tap was re-opened. Nitrogen stock solution was added to each main reservoir at a rate calculated to produce the required initial concentration throughout the system after mixing, allowing for the volume and nitrogen concentration of the solution in the isolated culture tanks. This allowance was not made for the other ions, for which it was considered unimportant.

The number of culture tanks connected to each reservoir altered as nitrogen treatments were imposed (section 5.2.5). The volume of solution per plant altered accordingly from 27 to 40 l in the N_1 system, and 103 to 40 l in the N_2 system. Removal of nitrogen from the N_1 solution by the root systems rapidly reduced the concentration to undetectable levels ($<$0.1 p.p.m) after each renewal of solution.

The concentration of nitrogen in the two solutions was monitored by regular analysis of samples drawn from the main reservoirs. Total N was determined by steam distillation with

Figure 5.3

Environmental data, Experiment C.

(a) Estimated total daily Photosynthetically Active Radiation on plants (see section 5.2.4).

(b) Measured nitrogen concentrations of N_1 (•) and N_2 (o) nutrient solutions vs. time.

•, o = assayed concentrations of solution samples (values for N_1 solution on day 19 = 0.4, day 38 = 0.1, days 52, 65, 70, 75, 80, 87, 94 = 0 ppm)

↑ = replacement of N_2 solution in the main reservoir

↓ = replacement of N_1 solution in the main reservoir

(c) Measured pH values of N_1 (•) and N_2 (o) nutrient solutions vs. time.

▲, △ = replacement of N_1 and N_2 solutions respectively (initial pH = 5.5)

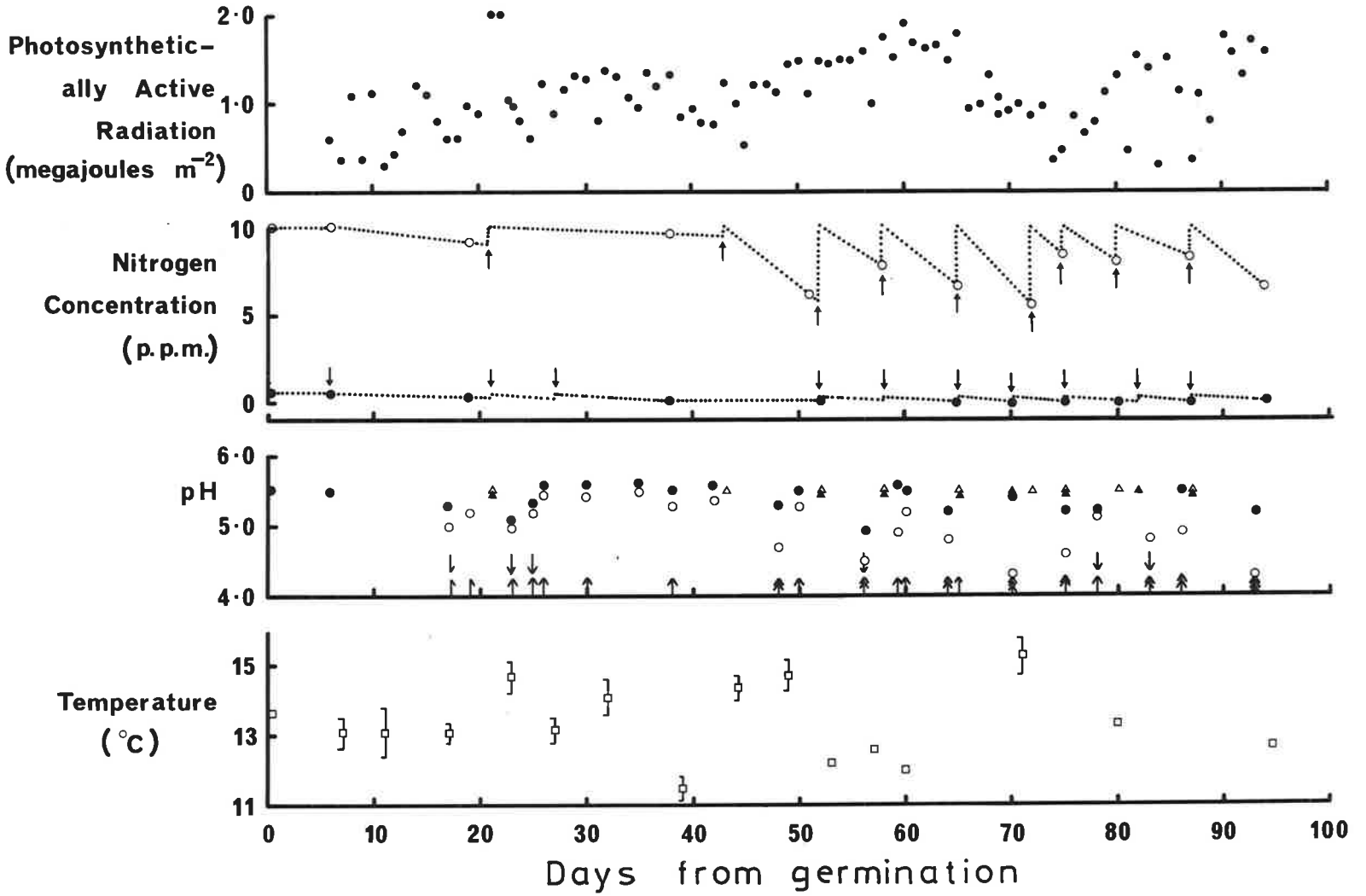
↑, ↑, ↑↑, ↑↑ = addition of 5, 10, 20, 30 ml respectively of 1N NaOH to N_2 solution, after pH measurement

↓, ↓ = addition of 5, 10 ml respectively of 1N NaOH to N_1 solution, after pH measurement

(d) Measured temperatures in root observation culture tanks vs. time.

⊡ = mean value over all tanks ( ] = range of individual values)

□ = value for one sample tank


Photosynthetic-
ally Active
Radiation
(megajoules m⁻²)
2·0
1·0
0
Nitrogen
Concentration
(p.p.m.)
10
5
0
pH
6·0
5·0
4·0
Temperature
(°C)
15
13
11
0
10
20
30
40
50
60
70
80
90
100
Days from germination

MgO and Devarda alloy and titration of liberated ammonia, as described by Bremner (1965), except that the titrant used was 0.05N $KH(IO_3)_2$. The method estimates concentrations down to 0.1 $\pm$ 0.1 p.p.m. N. Solutions were renewed at intervals determined by the rate of depletion of nitrogen at N_2, and the degree of nitrogen stress evident in the shoots at N_1. Figure 5.3.b shows the changes in nitrogen concentration with time experienced by each set of plants. Depletion of nitrogen in the N_2 solution did not exceed 50%.

Solution pH tended to fall, and was kept within the range 4.3 - 6.0 by regular monitoring and addition of 1N NaOH, at the main reservoir. The time courses of solution pH are shown in Fig. 5.3.c.

The temperature of solution in the main reservoirs was monitored by continuous-recording mercury-in-steel thermometers. Temperature in each culture tank was measured intermittently with a mercury-in-glass thermometer. The measurements of solution temperature in the culture tanks are shown in Fig. 5.3.d.

The solution in each root observation culture tank was 'stirred' continuously by two fine streams of compressed air bubbles arising from the bottom of the tank, in order to improve solution homogeneity within the tank. Uniform colouration of solution in a tank following addition of a drop of dye at the top was observed within 10 minutes where the solution was mixed by bubbling, compared with 1 hour where it was not. The bubbles

caused no disturbance of the root system, except momentary displacements of smaller filaments.

The nutrient solution was aerated by the compressed air arising within each culture tank, and by solution turbulence at several points along the flow cycle.

Flow rates of solution into each culture tank were adjusted regularly by removal of the "distributor" at the inflow point and insertion of a rate-meter. The rate of inflow was 75 ml min^{-1}, corresponding to a vertical flow velocity of 0.017 cm sec^{-1}, and to theoretical replacement of solution in a tank every 2 hours.

5.2.4 Culture method

On June 12th 1971, seeds weighing between 0.035 and 0.045g, the modal grain size interval, were placed groove-down on a stainless steel grid in contact with either the N_1 or N_2 solution, in a 500 ml glass germination dish. The dishes were placed in a dark cabinet at a constant temperature of 13°C. After two days the first seminal root was just visible on most seeds, and this was taken as day zero of the experiment (D0). On day 6, a seedling having 2.5 cm of coleoptile and 3 seminal roots was placed on the stainless steel gauze support of each culture tank, and surrounded, except for the tip of the coleoptile, with non-absorbent cotton-wool.

The experiment was conducted in an evaporatively cooled glasshouse. The plants were shaded during the experiment in an attempt to limit tillering at N_2 to 4-5 tillers per plant. However it proved impossible to do this without inducing etiolation, and a higher tiller number was allowed - see Fig. 5.5.a. Layers of coarse-mesh hessian were stretched over the apparatus on a frame 2 m above the culture tanks. As the season progressed and radiation increased, this was supplemented with hessian hung at the sides. Figure 5.3.a shows the estimated daily photosynthetically active radiation (P.A.R.) incident on the plants during the course of the experiment. Values were estimated from the daily short-wave radiation recorded by a Kipp solarimeter at the Waite Institute Meteorological Station, assuming that 45% of total radiation was photosynthetically active, and multiplying this external P.A.R. by a transmission factor. The transmission factor was determined from measurement of the radiation outside the glasshouse and that inside beneath the shade, with an EEL photometer.

The mean daily maximum and minimum air temperatures within the glasshouse during the months of June, July, August and September were 18/10, 18/11, 20/11 and 20/11°C respectively. Relative humidity in each month varied between 30 and 90%.

5.2.5 Application of nitrogen

Development of the main shoot apex was monitored by

regular sampling and dissection of plants (section 4.2.3) grown in an extra container connected to the end of the apparatus. The N^2 and N^3 nitrogen treatments were imposed on days 46 and 75 respectively.

Nitrogen was applied by the foliar path in the same manner as described in section 3.2.3, except that 10% urea solution was used, and the surfactant included was 'Agral 60'(1), at a rate of 1 ml/10 l. Approximately 37 mg urea-N were applied per plant, in two sprayings. This was equivalent to an application of 80 kg N ha^{-1} to a field crop plant sown at normal density. An additional foliar application of urea-N at the same rate was made to plants of treatment N_F^2 on day 62, and of treatment N_F^3 on day 84, in order to make the additional foliar supplies of N comparable with the additional solution supplies.

Additional nitrogen was supplied by the root path by disconnecting the appropriate tanks from supply of N_1 solution and reconnecting them to supply of N_2 solution. The connections were altered at the main arterial pipe and tank inflow pipe junctions, and at the main return pipe and tank outflow pipe junctions (see section 5.2.2 and Fig. 5.1). The procedure caused the numbers of culture tanks connected to each level of N supply to change during the course of the experiment. The allocation of tanks to each

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(1) Manufactured by I.C.I. Australia Ltd., 24 Sutton Terrace, Marleston, South Australia.

N level at different times is shown in the table below:

	Number of culture tanks connected to N_1 system	Number of culture tanks connected to N_2 system
Days 0 - 46	15	3
" 46 - 75	12	6
" 75 - 94	9	9

5.2.6 Root measurements

(a) Growth attributes considered

The terminology used to describe root morphology, and the growth attributes of a cereal root system that govern increase in root length and the spatial distribution of root length, have been defined in the simulation model of root growth, given in Appendix 1. They are illustrated in Fig. 5.4. The following growth attributes of the root system were considered in the experiment:

1. The rate of appearance of root axes at the crown of the plant
2. The rates of elongation of axes, first-order and second-order laterals (cm/member/day)
3. The inter-branch distances of first-order and second-order laterals (cm)
4. The lengths of apical unbranched zone of the axes and first-order laterals (cm)

Inter-branch distances were measured and expressed more conveniently in practice as the reciprocal, branch density (cm^{-1}). Seminal and nodal roots were distinguished.

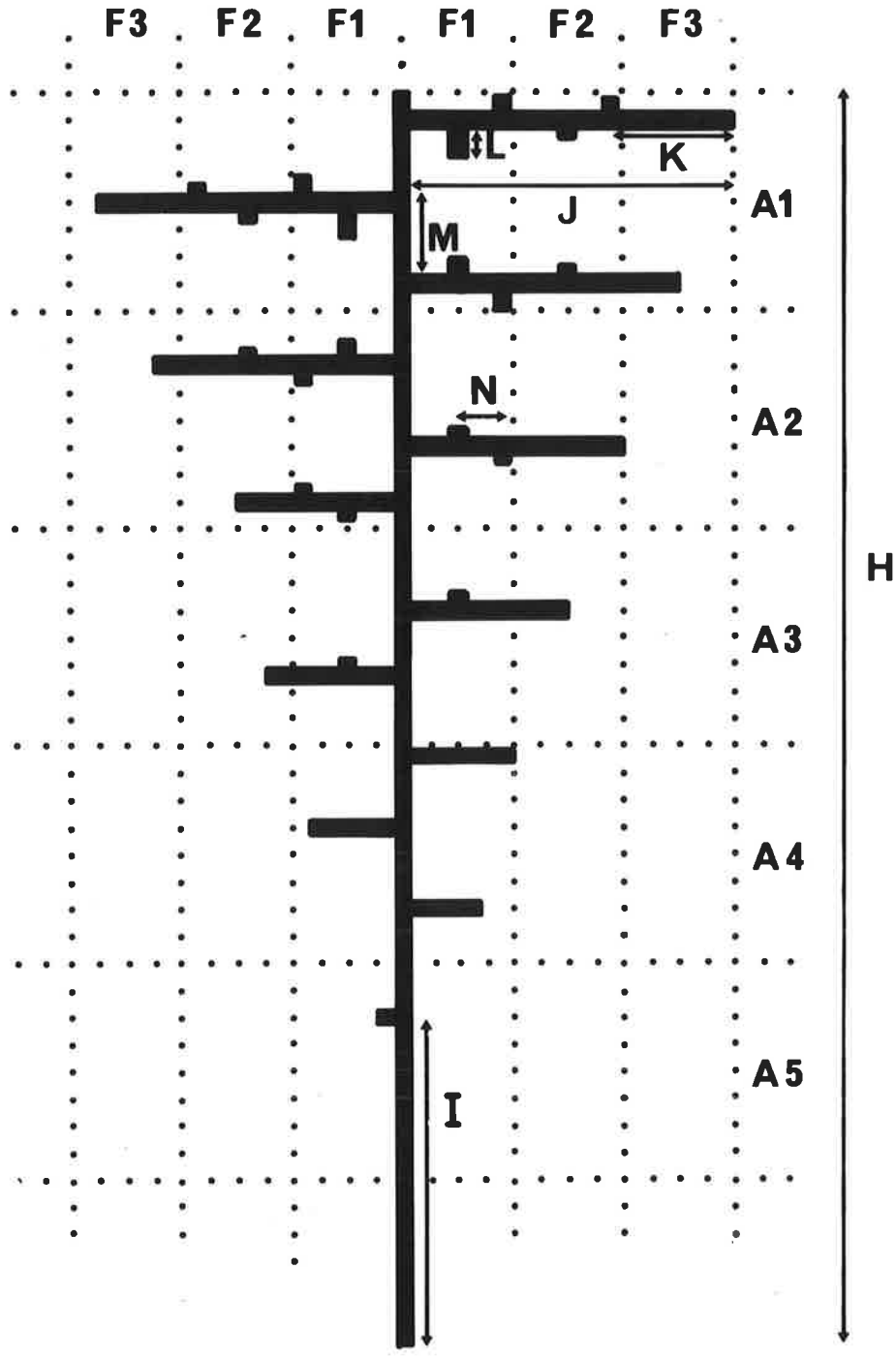
(b) Sampling procedure and morphological analysis

A cereal plant rapidly develops a root system that is composed of too many filaments to allow a comprehensive measurement of their attributes, and a sampling procedure is needed. Two roots were selected for detailed analysis - the first seminal root (Seminal 1) and the first nodal root (Nodal 1) produced by each plant. Within each root, sample measurements were made of the lengths of root members, branching densities, and lengths of apical unbranched zones. Sampling was carried out over the whole root. A morphological analysis of the root was obtained by deriving mean values of each attribute for successive segments of the axis ("axis segments"), and successive segments of the first-order laterals arising within each axis segment ("first-order lateral segments"). The structural analysis of a root employed is illustrated in Fig. 5.4. Axis segments were of 10 cm length, and numbered consecutively from the base of the root. First-order lateral segments were 5 cm length, and numbered within each axis segment from the bases of the first-order laterals. As a root developed the number of axis and first-order lateral segments present increased.

Figure 5.4

Diagrammatic representation of a root, showing the morphological attributes measured in Experiment C, and the structural analysis employed to facilitate mensuration.

H	Length of the axis
I	Length of apical unbranched zone of the axis
J	Length of the basal first-order lateral
K	Length of apical unbranched zone of the basal first-order lateral
L	Length of the basal second-order lateral on the basal first-order lateral
M	Inter-branch distance between two first-order laterals
N	Inter-branch distance between two second-order laterals
A1,A2,A3	"Axis segments" (10 cm)
F1,F2,F3	"First-order lateral segments" (5 cm)
.....	Segment boundaries


F3
F2
F1
F1
F2
F3
L
K
J
M
A1
N
A2
H
A3
A4
I
A5

The sampling procedure was based on measured variability within certain axis and first-order lateral segments. Data are shown in Table 5.2.

Morphological attributes were measured on several occasions 'in situ' on the growing root system, and also on the roots harvested after 94 days growth. For the 'in situ' measurements, arbitrary selection of root members was necessitated (section 5.2.6.c). For the harvested roots sampling was systematic (section 5.2.6.e).

(c) Measurement of attributes on growing roots

At frequent intervals measurements were made on the two roots selected for examination, visible through the glass panels of the culture tanks. The lengths of axis, first-order and second-order laterals were traced on clear polythene sheet. The lengths of apical unbranched zone of the axis and first-order laterals were also marked. A tracing was prepared by removing the front insulation panel from a tank, fitting a polythene sheet fixed to a wooden frame against the exposed glass panel, and tracing the outline of as many filaments as possible with a felt pen. Selection of laterals for tracing at random, or recording the progressive length of specific laterals was not possible, because as the root systems became complex many laterals became obscured from view over part of their length. Different coloured

Table 5.2

Variability of morphology within certain axis segments of the first seminal root and the first nodal root of two plants, grown in N_1 and N_2 solutions, 94 days after germination.

m = mean value per segment

n = number of first-order laterals contributing to the mean

se(%) = (standard error of mean/mean) x 100%

A = axis segment number

F = first-order lateral segment number

f.o.l. = "first-order lateral"

s.o.l. = "second-order lateral"

a.u.z. = "apical unbranched zone"

Table 5.2, continued

(i) N_1 solution

		A1			A5			A10		
Seminal 1		m	n	se(%)	m	n	se(%)	m	n	se(%)
f.o.l. length (cm)		17.1	25	5	12.8	27	5	11.7	27	5
f.o.l. a.u.z. (")		3.1		7	2.0		6	2.2		8
s.o.l. number	F1	14.4	25	5	5.7	27	5	7.0	27	6
	F2	12.5	25	8	10.3	24	10	6.6	23	7
	F3	8.8	17	11	9.3	11	12	12.7	7	10
	F4	8.0	7	20	5.0	1	-			
s.o.l. length (cm)	F1	3.3		4	2.2		5	1.3		6
	F2	3.5		6	1.7		10	1.5		8
	F3	3.3		11	1.9		7	1.3		14
	F4	3.1		10	1.2		-			
Nodal 1										
f.o.l. length (cm)		5.0	18	9	7.2	34	4			
f.o.l. a.u.z. (")		2.1		15	2.9		4			
s.o.l. number	F1	5.4	17	11	6.6	34	7			
	F2	1.0	1	-	5.0	1	-			
s.o.l. length (cm)	F1	1.7		13	1.2		6			
	F2	1.0		-	1.2		-			

Table 5.2, continued

(ii) N_2 solution

		A1			A5			A10		
Seminal 1		m	n	se(%)	m	n	se(%)	m	n	se(%)
f.o.l. length (cm)		17.1	23	8	7.6	27	5	3.0	39	5
f.o.l. a.u.z. (")		0.8		10	0.7		7	1.6		7
s.o.l. number	F1	12.3	23	5	10.4	27	5	2.3	35	9
	F2	19.4	21	5	16.0	17	7			
	F3	19.8	16	5						
	F4	19.1	11	5						
s.o.l. length (cm)	F1	0.9		5	0.5		9	0.2		10
	F2	2.0		10	0.4		9			
	F3	2.0		16						
	F4	1.3		18						
Nodal 1										
f.o.l. length (cm)		5.9	42	11	5.3	37	5			
f.o.l. a.u.z. (")		0.6		7	0.8		11			
s.o.l. number	F1	11.3	42	4	4.9	28	6			
	F2	15.9	18	5	12.6	5	30			
	F3	24.5	2	2						
	F4	22.5	2	5						
s.o.l. length (cm)	F1	0.8		14	0.3		6			
	F2	0.7		28	0.4		9			
	F3	1.6		34						
	F4	1.1		4						

pens were used for each order of root member, and a separate tracing was made of each root on each occasion. The roots selected for examination in each tank were identifiable on each occasion from the previous tracing of the axis. Filaments were viewed through jeweller's binocular magnifiers (x 3).

The lengths and apical unbranched lengths of the traced filaments of a given order on each segment of the root were measured, after marking in all segment boundaries, by running an opisometer wheel over the appropriate lines. The accumulated length shown on the dial was divided by the number of filaments measured, recorded on a counter, to obtain the mean.

Errors arose in tracing from parallax, the 2-dimensional representation, and the sampling procedure. An assessment of the errors was made by comparing tracings made one day before harvest with measurements made for the same segments on the harvested roots. The tracings were found to overestimate mean length per member by 1% for the axis, by 25% for first-order laterals (s.e.= 6, number of comparisons = 26), and by 50% for second-order laterals (s.e.= 13, number of comparisons = 34). There was no evidence of difference in overestimate for different lengths of the laterals of given order.

The extension of each axis from the crown of the plant into the solution followed a linear downward course initially (not usually vertical). The axes later became curved due to bending proximal to the apex in the zone of branch emergence.

The apex itself remained vertical or nearly so.

The number of root axes emerged from the crown of the plant was recorded at each date of tracing.

(d) Harvesting procedure

Plants were harvested on day 94 when the shoots were in the phase of stem extension. Before harvesting the two roots measured during growth were tagged near their base with coloured wool, and all tillers were numbered in order of number of leaves emerged. Tillers were severed at their base and placed in polythene bags for further measurements (section 5.2.7). Each crown and its associated root system was removed and placed in a tray containing preserving fluid. The preserving fluid was "Craf II" (Sass 1958). The root systems were kept, with one change of preserving fluid, for the four months required to complete displaying and photography of the roots.

(e) Mensuration of harvested roots

The procedure for mensuration of a preserved root system involved (i) separation of the members, (ii) display of the roots for black-and-white photography, (iii) measurement of morphological attributes on projections of the photographic negatives. The root systems were dried (80°C) and weighed after photography. The

photographs were measured later.

Individual roots were severed at the crown, and separated from the root mass without difficulty under water. The tensile strength and brittleness of roots grown in the two nutrient solutions differed markedly. While filaments grown in N_1 solution were slender and remarkably tough, those from N_2 solution, particularly those of the seminal roots, were easily broken, despite being much thicker.

Separated roots, other than the two selected for detailed study, were spread on black cloth under water in a tray and photographed, with a Carl Zeiss Jena "Praktika IV" camera on 35 mm Plus-X Pan ASA 125 black-and-white film. Labels indicating the number and tiller of origin of each root, the number of the culture tank, and a 10 cm scale were included in each photograph. Photographs were taken at a slight angle to avoid camera reflection, from a tripod, at a height of 1 m, in an alcove lined with black cloth. The roots were illuminated from each side by a bank of fluorescent lights, and exposure (1 second, F16 aperture) was constant for all photographs.

The first seminal and first nodal root of each plant were displayed in detail, and photographed in the same way. The space needed to display the numerous filaments on these roots necessitated severing the axis into sections, each being photographed separately. Each section was displayed by combing with a blunt

steel needle. Using random numbers, one in every three consecutive first-order laterals was displayed; the other two were discarded. Each second-order lateral on the chosen first-order member was displayed. The roots were spread on a fine-weave black cloth stretched over a cloth-wrapped pane of glass, which rested in a shallow tray (60 x 40 x 1 cm). Water lay in the tray around the cloth but did not cover it, since the filaments adhered conveniently to the wet cloth. Trays were photographed after slowly covering the displayed network with a thin film of water. The display and photography of one root system required 4 man-days.

The photographic negatives were projected via a mirror onto the undersurface of a sheet of high-quality tracing paper, resting on a glass panel. Projection was adjusted to produce an actual scale image, with the aid of the 10 cm scale included in each photograph. Axis and first-order lateral segment boundaries were drawn on the image, and measurements were made with an opisometer and counter, as described in section 5.2.6.c. The numbers of branches per segment were measured from the photographs. Examples of the photographs are shown in Plates 5.3 - 5.7. These particular root segments were displayed fully for measurements of morphological variability within a segment (Table 5.2).

Plates 5.3 - 5.7

Photographs of segments of wheat roots harvested after 94 days growth in flowing solution culture, from which morphological attributes were measured. Each photograph depicts either a 10 cm axis segment of the first seminal root, with its associated laterals (R1), or the complete first nodal root (R2). The axes were severed into sections to enable display of the laterals.

B = base of the axis

Plates 5.3 and 5.4 (Tank 6) - N_1 treatment (replicate 1)

A1 = 0 - 10 cm axis segment

A5 = 40 - 50 cm axis segment

A10 = 90 - 100 cm axis segment

Plates 5.5 and 5.6 (Tank 4) - N_R^1 treatment (replicate 1)

A1 = 0 - 10 cm axis segment

A5 = 40 - 50 cm axis segment

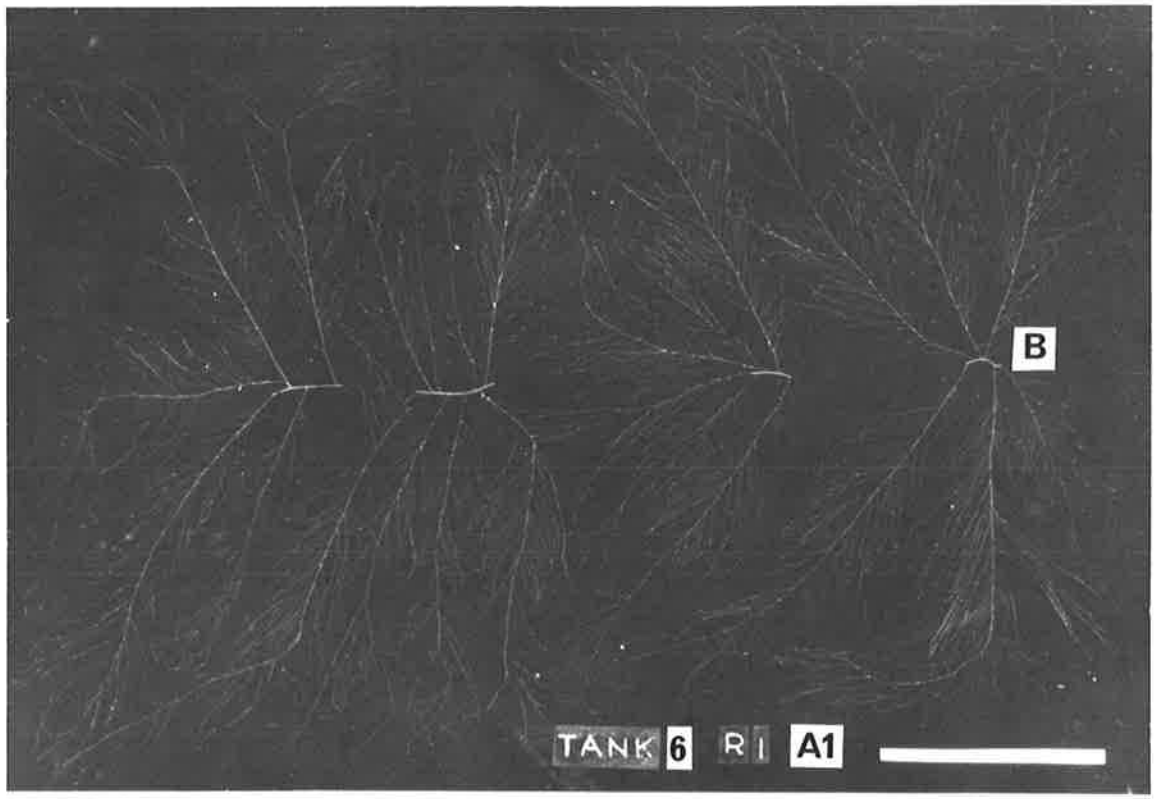
A10 = 90 - 100 cm axis segment

Plate 5.7 (Tank 3) - N_R^2 treatment (replicate 1)

A1 = 0 - 10 cm axis segment

Plate 5. 3

(a)



(b)

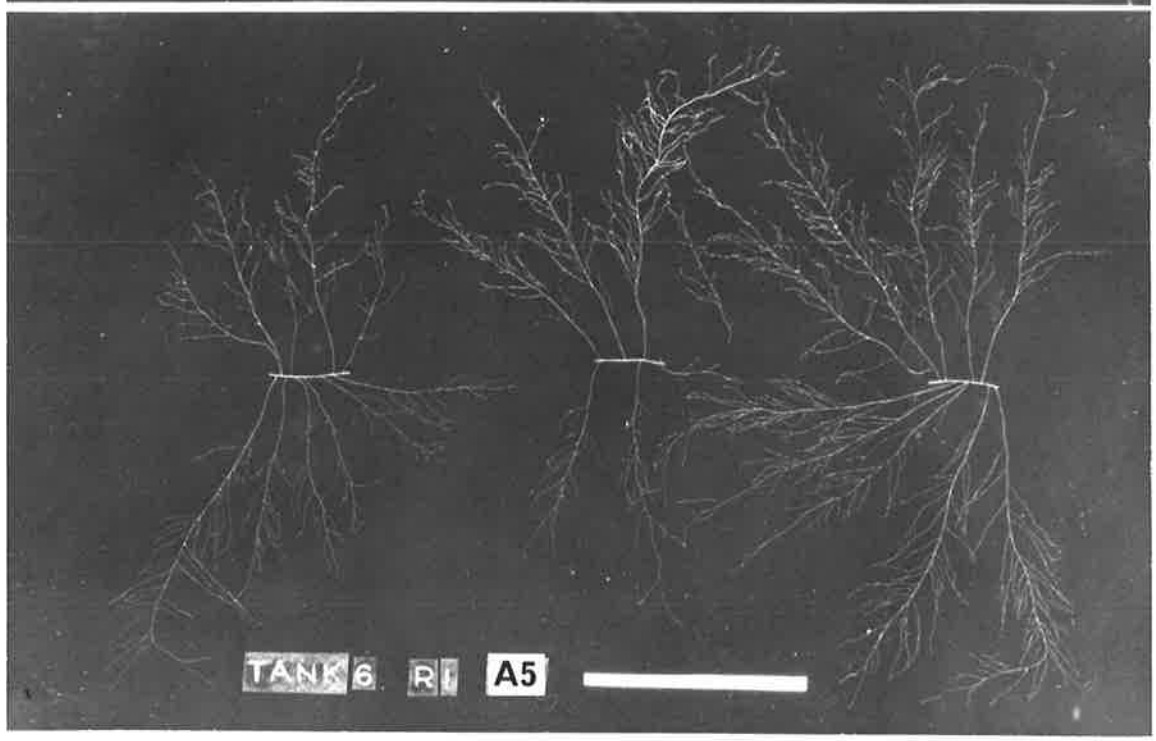
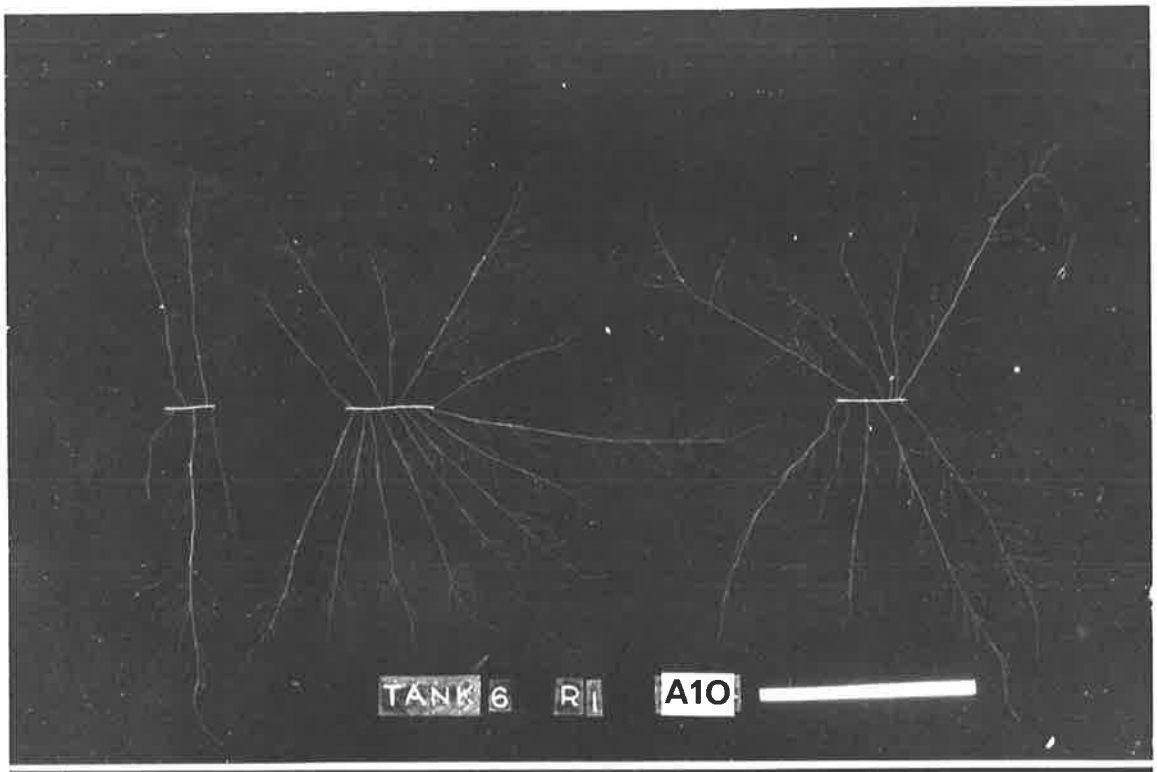


Plate 5.4

(a)



(b)

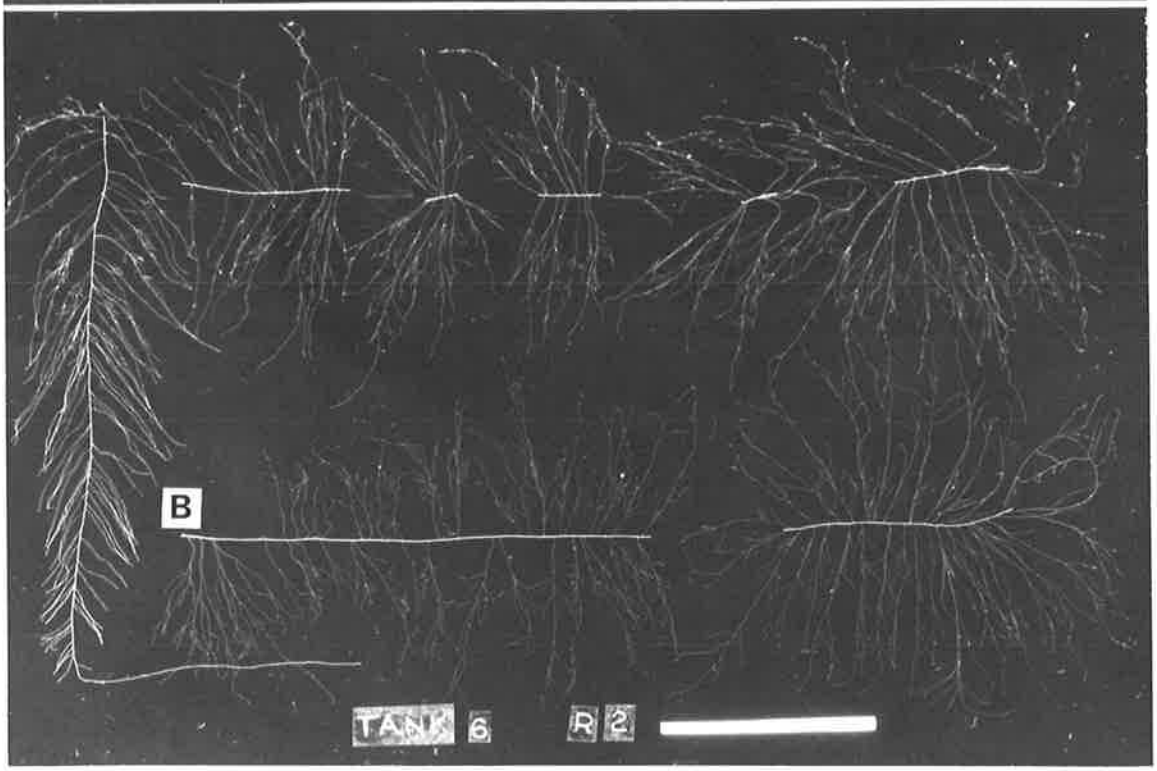
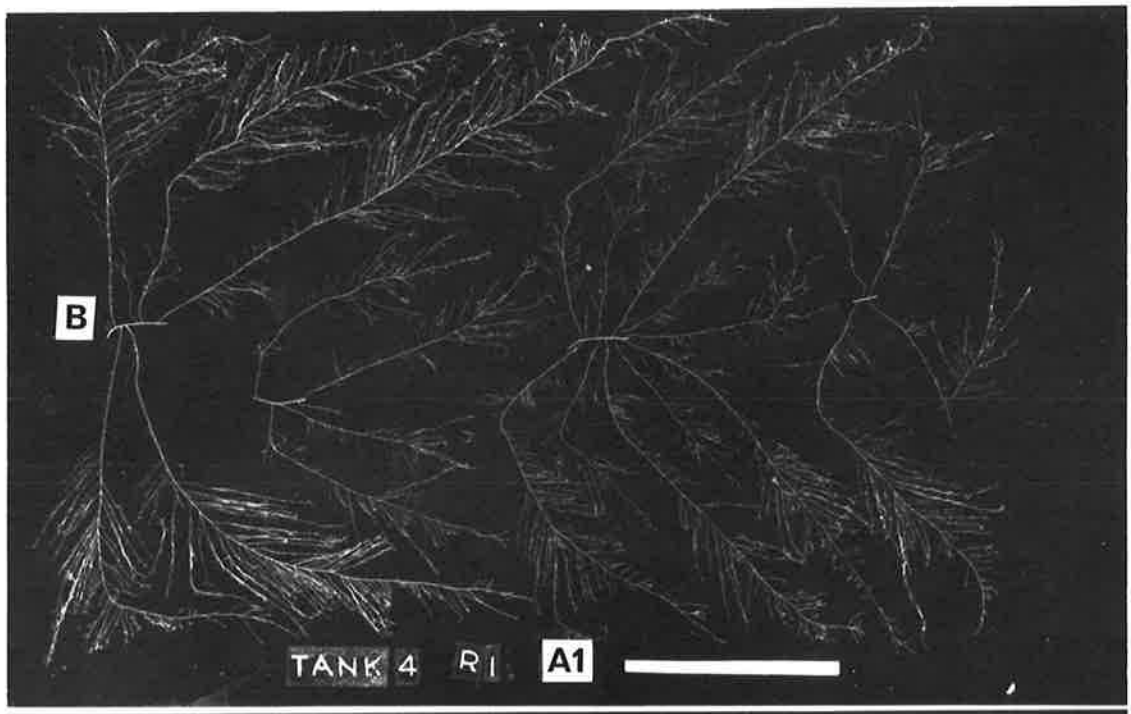


Plate 5.5

(a)



(b)

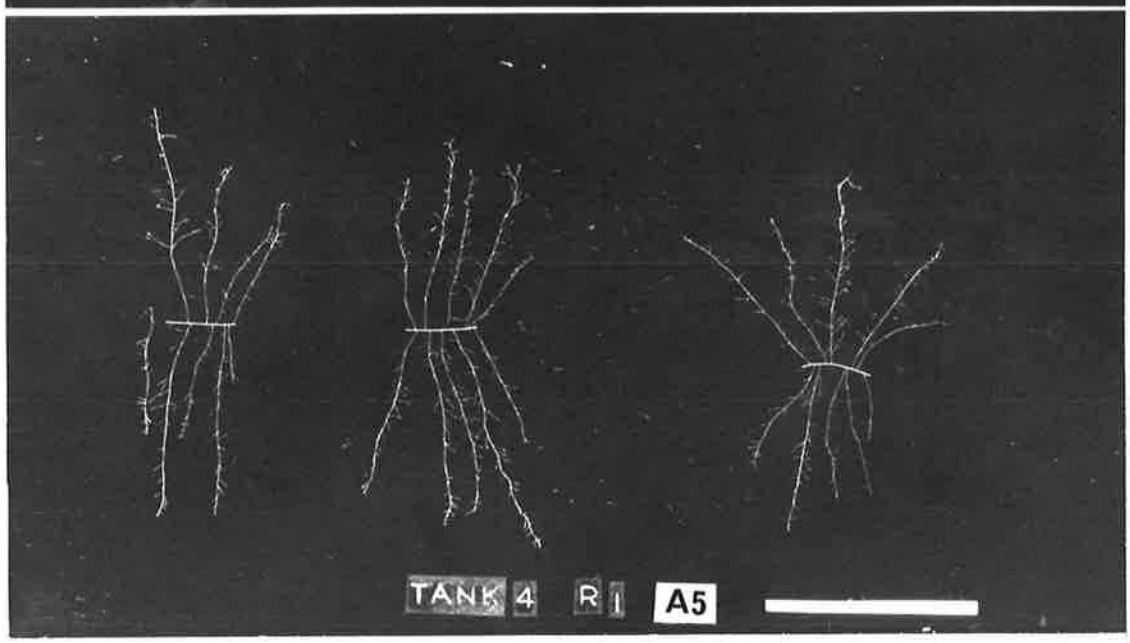
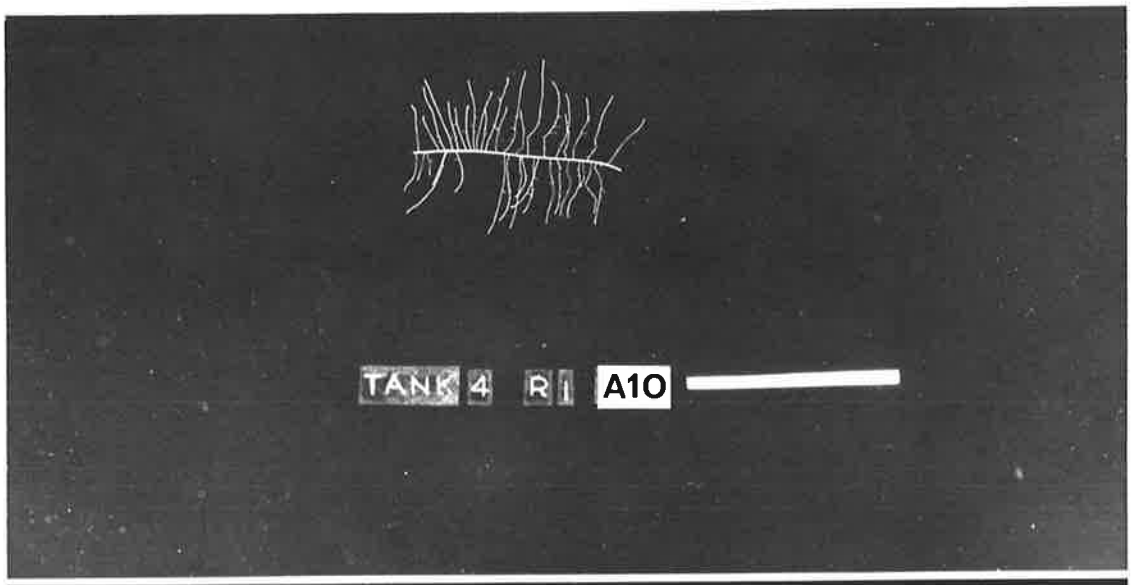


Plate 5.6

(a)



(b)

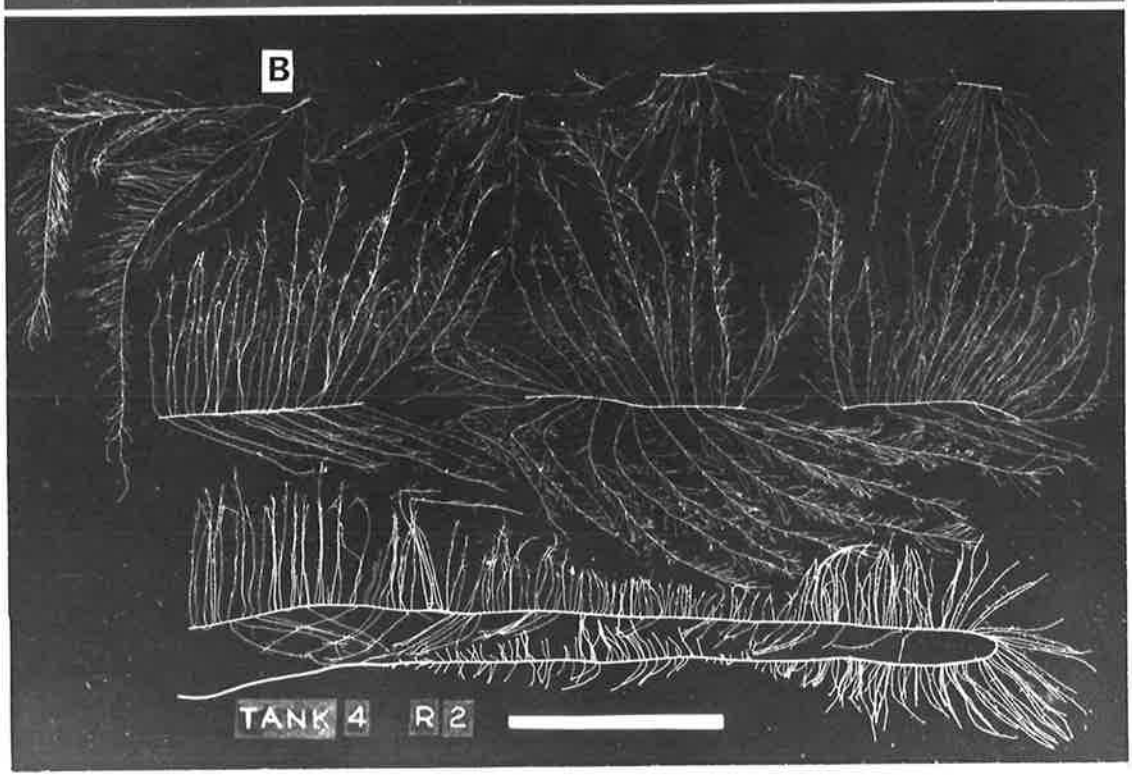
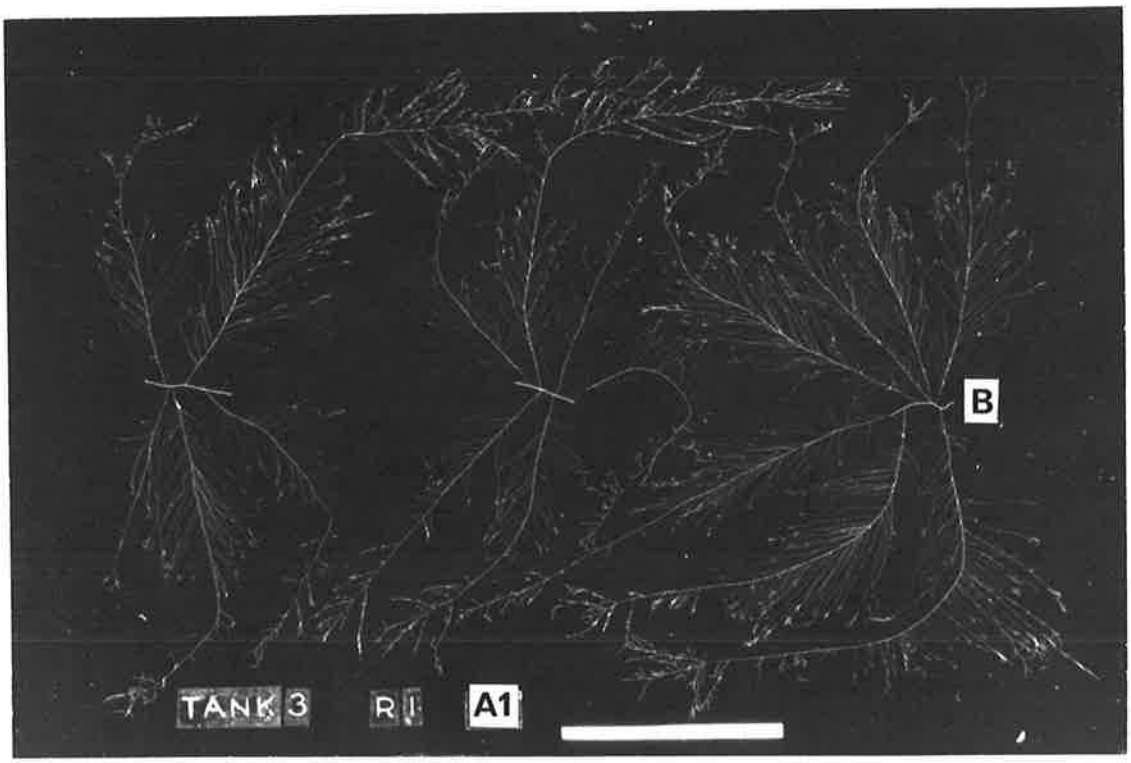


Plate 5.7



5.2.7 Shoot measurements

The number of tillers per plant was recorded during the course of the experiment at each occasion of root tracing. More frequently, the length L (from the tip to the point of extrusion from the subtending leaf sheath, or to the blade base) and the width W (at the widest point) of the elongating leaves on each tiller were recorded, up to the fourth tiller per plant. The area of each leaf, A, on each occasion was estimated by the method of Lal and Subba Rao (1951):

$$\text{Log } A = \log L + \log W - \log F$$

where F is a correction factor for the particular leaf shape concerned, derived from a sub-sample of leaves. The leaf area per tiller at each occasion was computed by summation of the areas of expanded and expanding leaves.

The shoot apex of tillers that had commenced stem extension was dissected before oven-drying, and the number of spikelets present and the stage of development were recorded.

5.3 Results

5.3.1 Dry weight and nitrogen uptake

Additional nitrogen increased shoot and root dry weight per plant at harvest when supplied via the root, but not when

supplied via the foliage (Table 5.3). The increase in growth when the additional nitrogen was supplied via the root was greater the longer the time during which the additional nitrogen had been supplied. At harvest plants had a root weight ratio characteristic of the nitrogen concentration of the solution in which they were growing, except where transfer from low-N to high-N solution had been too recent for adjustment to be completed (N_R^3). As with absolute growth, the distribution of growth between root and shoot was little affected by foliar-supplied N.

Shoot nitrogen concentration at harvest (Table 5.3) reflected the nitrogen status of the plant, and, where N was supplied to the root, the recency of application. The shoots were not washed before chemical analysis, and it is not known what proportion of the shoot nitrogen, if any, consisted of urea on the leaf surface. The excess of shoot nitrogen at harvest in treatment N_F^2 over treatment N_1 was only 5% of the amount of urea-N estimated to have been applied to the foliage in treatment N_F^2. This suggests a loss of 95% of the foliar-applied urea from the shoot surface following application. The corresponding excess for treatment N_F^3 was 25%, suggesting a loss of urea-N from the shoot surface of 75%. The shoot nitrogen weights per plant in treatments N_1 and N_R^1 were 55% and 15% respectively of the total nitrogen supplied per plant during the experiment in solutions N_1 and N_2. The total nitrogen supplied per plant of these treatments was

Table 5.3

Influence of time and path of nitrogen addition on shoot and root dry weight per plant at harvest, dry weight distribution, and shoot nitrogen content.

	Path and time of nitrogen addition						
		ROOT			FOLIAR		
	N_1	N^1	N^2	N^3	N^2	N^3	L.S.D. (P=0.05)
Shoot dry weight (g)	2.4	25.0	17.0	3.1	2.2	2.0	1.3
se (%)	11	8	3	15	8	5	
Root dry weight (g)	1.2	3.2	2.2	0.8	0.9	1.0	0.2
se (%)	5	4	9	18	16	12	
Root weight ratio (%)*	0.33	0.11	0.11	0.21	0.30	0.32	0.025
se (%)	4	5	6	5	8	8	
Shoot nitrogen concentration (%)	2.2	3.8	4.0	4.8	2.6	3.7	0.4

* Root weight ratio = dry weight of root/dry weight of plant

calculated from the nitrogen additions to each solution, allowing for the total numbers of plants in each solution during different periods of the experiment.

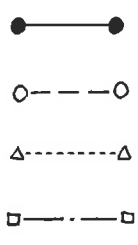
5.3.2 Tillering, leaf growth, and apical development of the shoot

Tillering of the plants in low-N solution was not curtailed until after day 40, following renewal of the low-N solution at half the original N concentration from day 30 (see section 5.2.3). After day 40, supply of additional nitrogen to the foliage had no effect on tillering. In contrast, supply of additional nitrogen to the roots caused a large increase in tillering (Fig. 5.5). The number of tillers present at harvest was related to the duration of growth on high-N solution. The plants tillered rapidly at the higher level of N supply.

Supply of additional nitrogen to the roots increased leaf area of the plants by increasing both tiller number and leaf area per tiller. The leaf area development of the first tiller per plant (main culm) is shown in Fig. 5.6. After day 40, the response to increased nitrogen supply was rapid. Tillers achieved increase in leaf area after supply of additional nitrogen through an increase in the rate of appearance of leaves, as well as in the areas of unexpanded leaves after full expansion. Thus at harvest the ninth leaf on the main culm was fully expanded in treatment N_R^1, half expanded in treatment N_R^2, and was not present

Figure 5.5

Influence of time of supply of additional nitrogen to the roots on the number of (a) tillers and (b) roots per plant, during the growing period.



●——● = treatment N_1

○— — —○ = " N_R^1

△·····△ = " N_R^2

□—·—□ = " N_R^3

L.S.D.(I), P = 0.05

↑ = dates of application of N^2 and N^3 treatments

↓ = date of appearance of first nodal root on plants

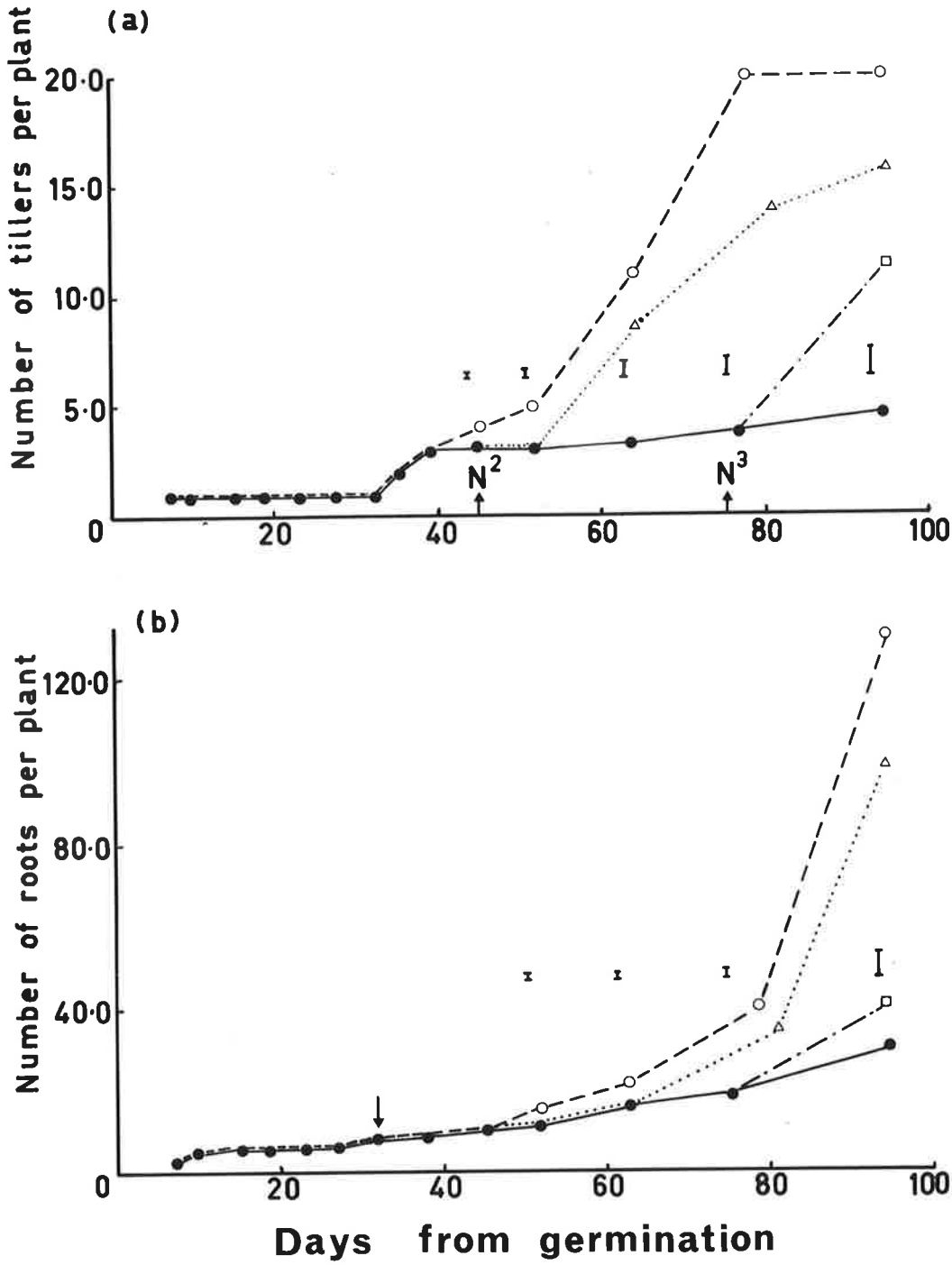
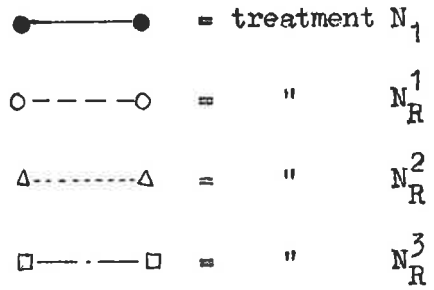
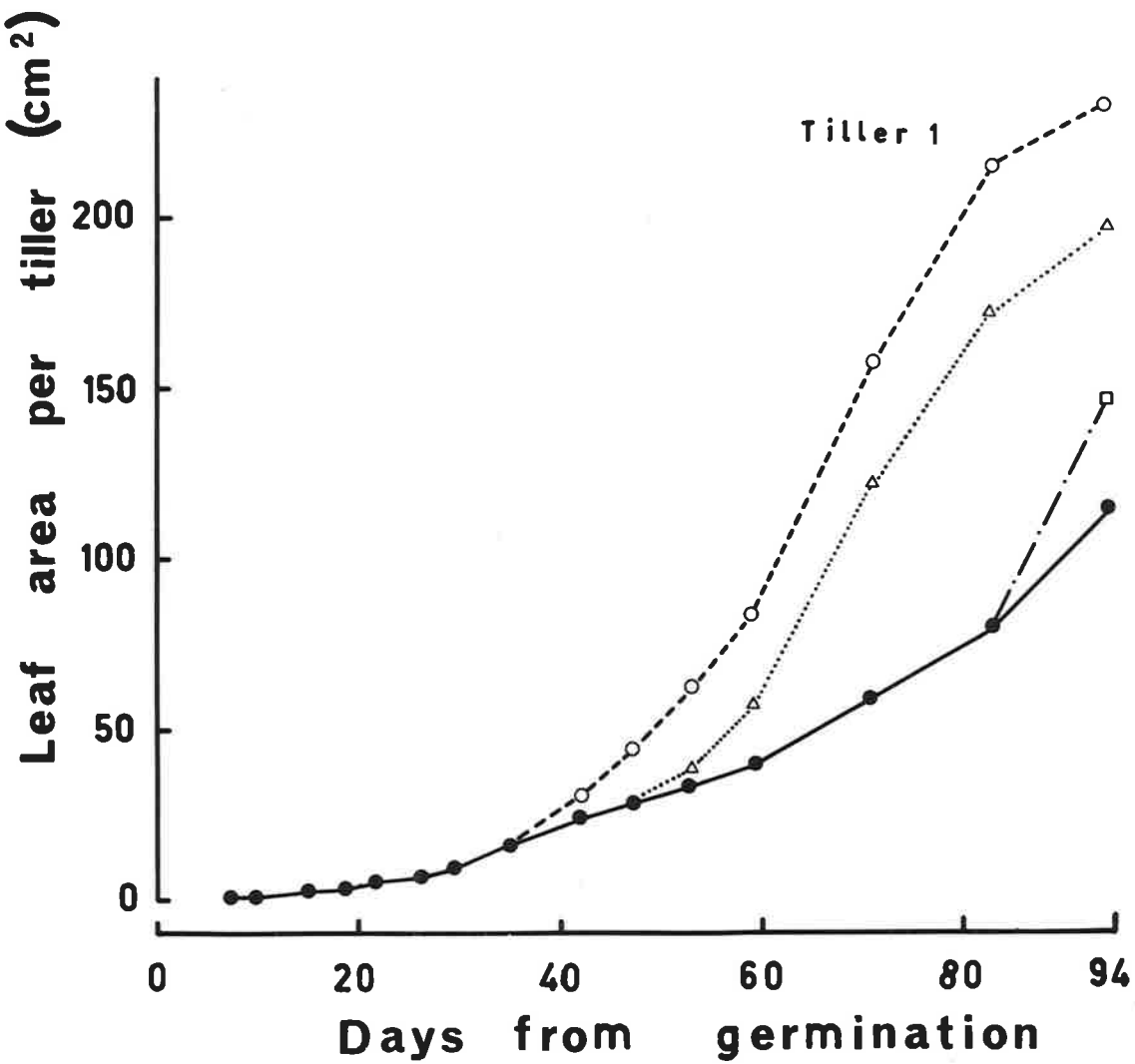

(a)
Number of tillers per plant
20·0
15·0
10·0
5·0
0
20
40
60
80
100
N2
N3
(b)
Number of roots per plant
120·0
80·0
40·0
0
20
40
60
80
100
Days from germination

Figure 5.6

Influence of time of supply of additional nitrogen to the roots on leaf area development of the main culm.



The standard error associated with each point was always less than the height of the symbol plotted.


Leaf area per tiller (cm²)
Tiller 1
200
150
100
50
0
0
20
40
60
80
94
Days from germination

at all in treatment N_1.

The apices of tillers that had commenced stem extension were dissected at harvest. The number of spikelets per ear was constant for all the tillers examined of each treatment (the first 12 tillers per plant for treatments N_R^1 and N_R^2, the first three tillers per plant for the other treatments), but differed between treatments. The mean number of spikelets per ear was 16.7 for treatments N_1, N_F^2, N_F^3 and N_R^3, 20.0 for treatment N_R^2 and 21.7 for treatment N_R^1 (L.S.D. = 0.51).*

5.3.3 Root emergence, and number of roots per tiller, and lengths of root axes at harvest

Additional nitrogen had no effect on the number of seminal roots per plant (Table 5.4). Additional nitrogen supplied via the foliage had no effect on nodal root emergence or the number of nodal roots per tiller at harvest (Table 5.4). Additional supply via the root system increased the number of nodal roots per plant after day 45, the response to an improved nitrogen supply commencing later than the associated tillering response (compare Figs. 5.5.a and 5.5.b). Additional supply of N to the roots increased both the number of tillers producing nodal roots, and also the number of nodal roots associated with at least each of

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* P = 0.05

Table 5.4

Influence of time and path of nitrogen addition on the number of seminal roots per plant, the number of nodal roots associated with each of the first five tillers per plant, and the mean number of nodal roots per tiller, at harvest.

	Path and time of nitrogen addition						
		ROOT			FOLIAR		
	N_1	N^1	N^2	N^3	N^2	N^3	L.S.D. (P = 0.05)
Number of seminal roots:	6.0	6.0	5.3	6.0	5.3	5.3	n.s.
Number of nodal roots attached* to tiller number:							
1	13.0	19.5	19.0	14.3	14.3	13.7	4.0
2	4.3	10.5	10.7	4.7	5.7	4.0	3.8
3	5.0	9.5	9.7	8.3	4.0	4.7	1.5
4	0.7	11.0	8.3	3.0	0.0	0.0	2.8
5	0.0	7.5	7.3	2.0	-	0.0	4.0
Mean number of nodal roots per tiller:							
tillers 1-5	5.2	11.6	11.0	6.5	5.4	4.9	0.9
all tillers	5.2	6.2	5.8	3.2	5.4	4.9	0.8

* Tillers were numbered in order of number of leaves emerged.

Table 5.5

Influence of time and path of nitrogen addition on the lengths of the seminal and nodal root axes at harvest.

	Path and time of nitrogen addition						
		ROOT			FOLIAR		
	N_1	N^1	N^2	N^3	N^2	N^3	L.S.D. (P=0.05)
Axis length (cm) of given seminal root numbers:* 1	265	121	139	214	256	261	12
2	196	106	125	192	186	188	15
3	154	103	101	155	161	151	18
4	108	79	76	105	100	102	22
5	96	70	72	73	92	89	n.s.
6	43	57	(50)+	33	(46)	(39)	n.s.
Number of nodal roots of given axis length:							
0-10 cm	5	72	54	19	5	6	6.3
10-20 "	6	22	15	7	6	6	3.4
20-30 "	6	14	11	4	5	4	1.8
30-40 "	3	5	5	2	4	3	n.s.
40-50 "	1	3	2			1	n.s.
50-60 "	1	2	5	1	1	1	1.9
60-70 "		3	2	1	1		
70-80 "	1	1	1			1	
80-100 "		1			1		
Length (cm) of nodal root 1	78	92	78	66	97	59	n.s.

* Seminal roots 2 - 6 are numbered in order of decreasing axis length. Seminal root 1 and nodal root 1 were selected for detailed morphological analysis.

\+ Sixth seminal root not present in all replicates (Table 5.4). Values are the mean lengths of those axes present.

the first five tillers (Table 5.4).

The lengths of the seminal and nodal axes at harvest are shown in Table 5.5. The seminal and longest nodal roots not analysed in detail showed a qualitatively similar response to nitrogen treatment to those that were selected for analysis in detail (see section 5.3.5.b). The seminal axes showed characteristic differences in length, probably reflecting their respective origins in the seed, though this was not distinguished for root numbers 2 - 6. The majority of the nodal roots per plant were relatively short at harvest, especially where nitrogen increased the number produced.

5.3.4 Branching density of roots

The root morphological data are presented in the form of mean values per root segment for the three replicates (see section 5.2.6.b). Bartlett's test indicated homogeneity of variance between treatments, and therefore the pooled (mean) standard error of the treatment means is presented for each element of data (see Snedecor and Cochran 1967). As a convenient approximation, where two treatment means differ by more than twice the pooled standard error, they are regarded as being significantly different. The morphology of the two roots examined on each plant - the first seminal and the first nodal root - did not differ significantly from treatment N_1 where additional nitrogen was supplied by the

foliar path (treatments N_F^2, N_F^3). For simplicity, these latter treatments are not reported further, and the following account refers to the response to N supplied via the roots only.

The branching density of first-order laterals, determined at harvest, is shown in Fig. 5.7. Branching density varied considerably along root axes, even in treatment N_R^1 where the level of nitrogen supply was not altered during growth. An indication of periodicity in branching was obtained along the seminal axes. For each time of supply, branching density along the seminal root increased in the axis segments distal to that in which the apex had been located at the time of supply; that is, in the segments which branched subsequent to the supply of additional N. The nodal root differed from the seminal in showing this effect only where additional N had been supplied early in growth. The mean numbers of first-order laterals per cm of axis for treatments N_1, N_R^1, N_R^2, N_R^3 were 2.71, 3.43, 3.14, 3.02 (L.S.D. = 0.18)* for the seminal root, and 2.89, 4.52, 3.55, 2.61 (L.S.D. = 0.31)* for the nodal root.

Similar effects of increasing the nitrogen supply on branching density of second-order laterals were obtained as on branching density of first-order laterals (Figs. 5.8 and 5.9). Branching density tended to be less in the basal 5 cm of

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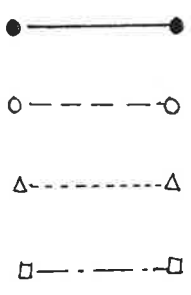
* P = 0.05

Figure 5.7

Influence of time of supply of additional nitrogen on the branching density of first-order laterals along (a) the first seminal, and (b) the first nodal root.



= treatment N_1

= " N_R^1

= " N_R^2

= " N_R^3

= twice the pooled standard error of the treatment means. Bartlett's test indicated homogeneity of variance over all treatments (P = 0.05)

= Position of most distal first-order lateral in treatment N_R^2 when plants were supplied with high-N solution

= Position of most distal first-order lateral in treatment N_R^3 when plants were supplied with high-N solution

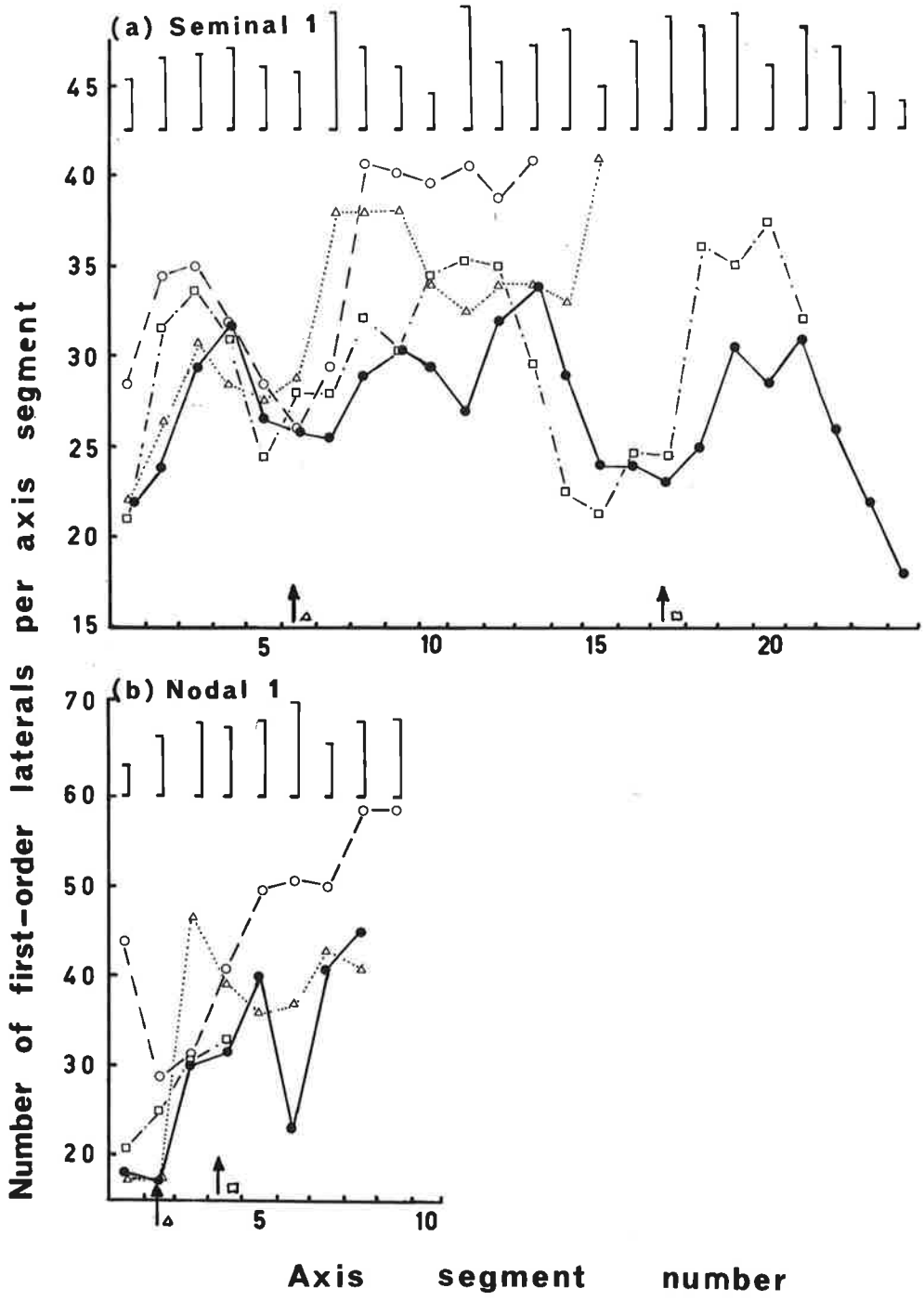

(a) Seminal 1
(b) Nodal 1
Number of first-order laterals per axis segment
Axis segment number
45
40
35
30
25
20
15
5
10
15
20
70
60
50
40
30
20
5
10

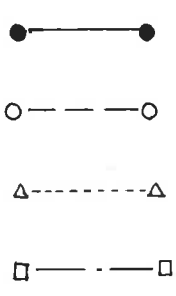
Figure 5.8

Influence of time of supply of additional nitrogen on the branching density of second-order laterals along the first seminal root:

(a) 0-5 cm first-order lateral segment (F1)

(b) 5-10 cm " " " (F2)

(c) 10-15 cm " " " (F3)



= treatment N_1

= " N_R^1

= " N_R^2

= " N_R^3

= twice the pooled standard error of the treatment means. Bartlett's test indicated homogeneity of variance over all treatments (P = 0.05)

= position of most distal first-order lateral in treatment N_R^2 when plants were supplied with high-N solution

= position of most distal first-order lateral in treatment N_R^3 when plants were supplied with high-N solution

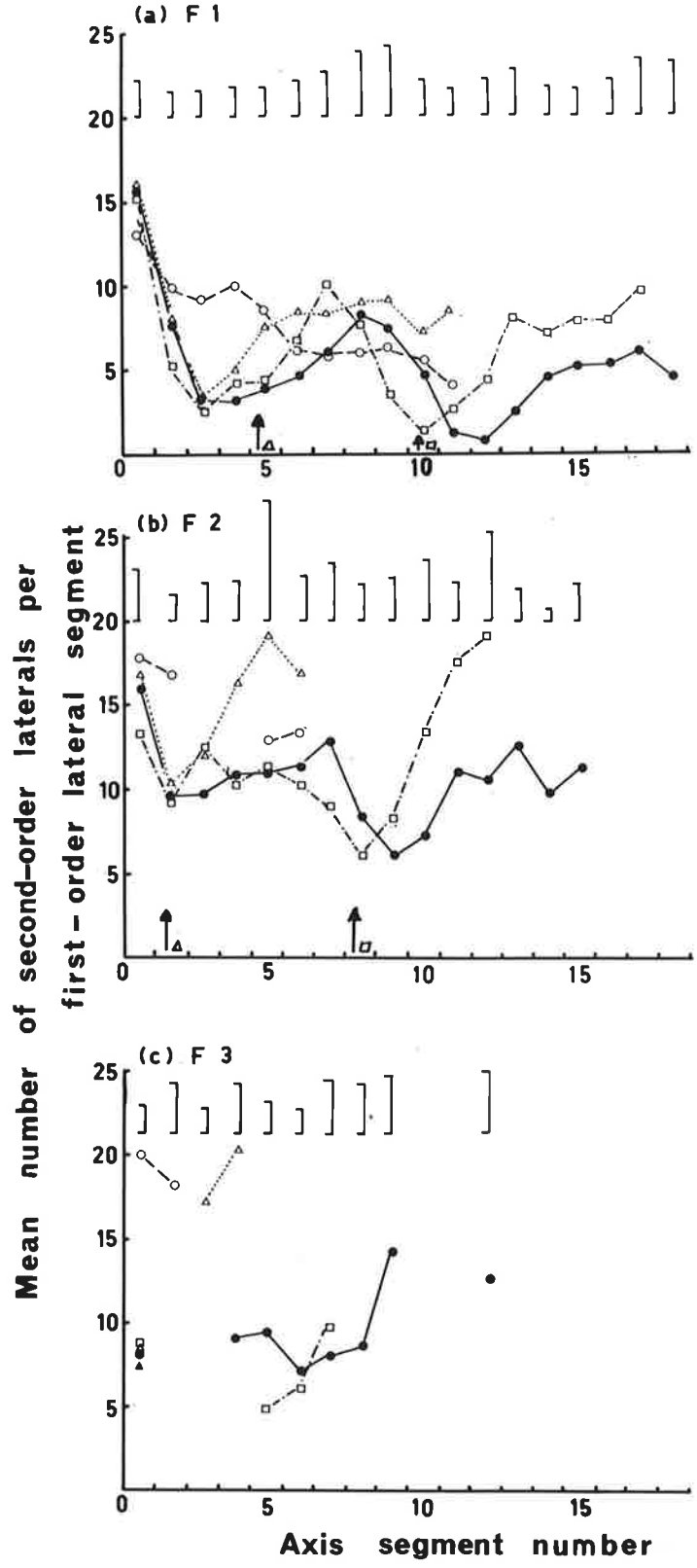
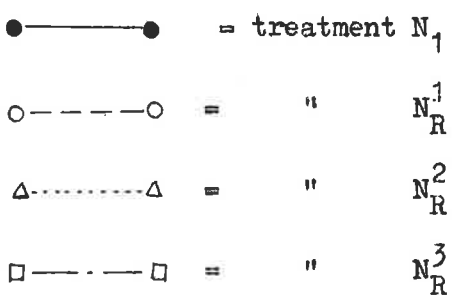

(a) F 1
(b) F 2
(c) F 3
Mean number of second-order laterals per first-order lateral segment
Axis segment number
0
5
10
15
20
25

Figure 5.9

Influence of time of supply of additional nitrogen on the branching density of second-order laterals along the first nodal root :

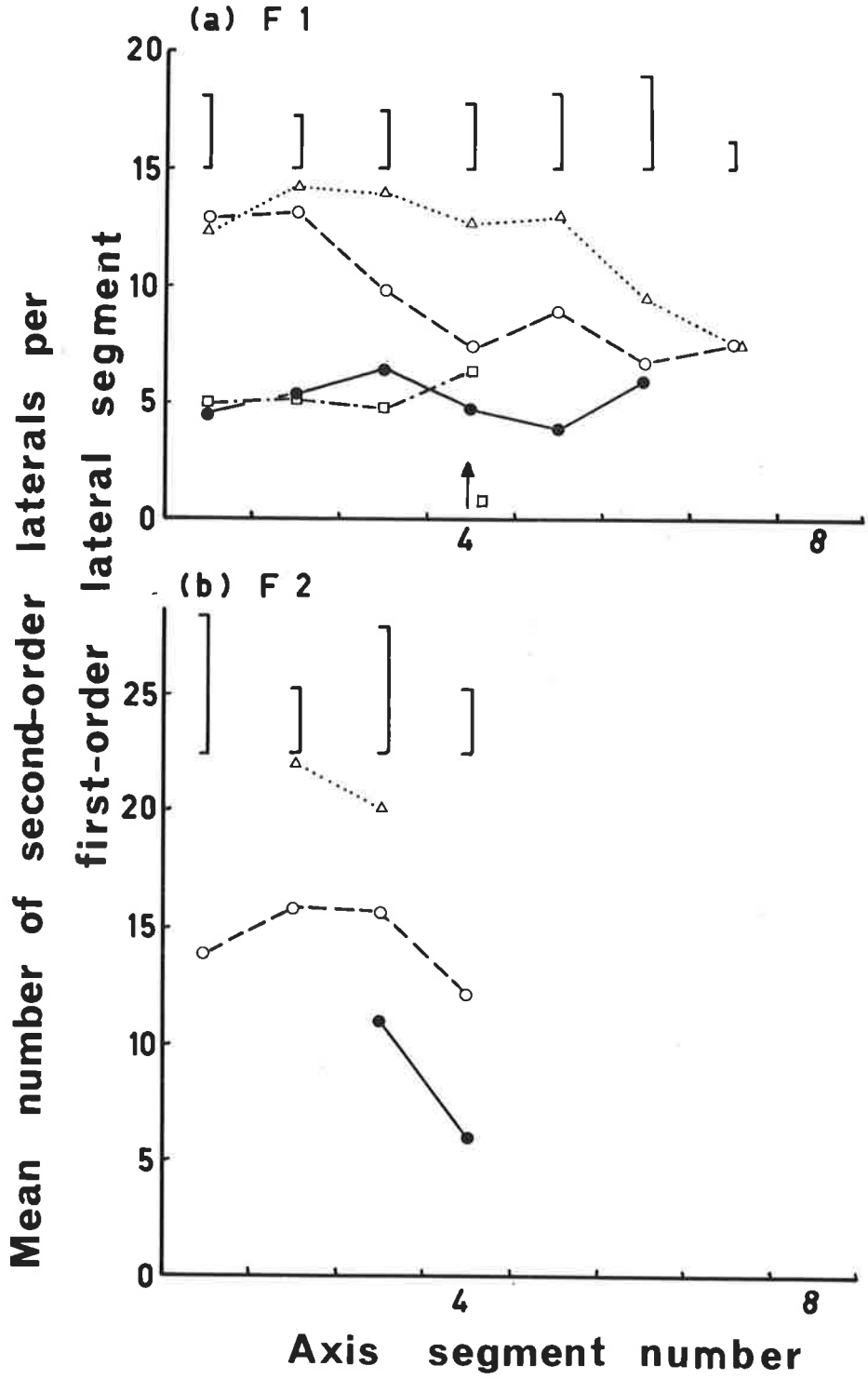
(a) 0-5 cm first-order lateral segment (F1)

(b) 5-10 cm " " " (F2)



= twice the pooled standard error of the treatment means. Bartlett's test indicated homogeneity of variance over all treatments (P = 0.05)

= position of most distal first-order lateral in treatment N_R^3 when plants were supplied with high-N solution


(a) F 1
20
15
10
5
0
4
8
(b) F 2
25
20
15
10
5
0
4
8
Mean number of second-order laterals per first-order lateral segment
Axis segment number

first-order laterals than in the more distal segments. The mean numbers of second-order laterals per cm of first-order lateral averaged over the whole root for treatments N_1, N_R^1, N_R^2, N_R^3 were 1.63, 2.09, 2.29, 1.81 (L.S.D. = 0.40)* for the seminal root, and 1.06, 1.86, 2.42, 1.05 (L.S.D. = 0.47)* for the nodal root.

5.3.5 Elongation of root members

The lengths of the curved and partly concealed root members could not be determined accurately during growth. Errors were larger for laterals than for axes (section 5.2.6.c). The lengths at harvest were determined precisely. The two sets of data are presented separately. As with lateral branching density, foliar application of nitrogen had no significant effect on the elongation of the root members, either during growth or at harvest, and the following account refers to the treatments that supplied N via the roots.

(a) Measurements during growth

After 40 days germination, when nitrogen deficiency became established in treatment N_1, the seminal axes elongated more rapidly in low-N than in high-N solution (Fig. 5.10.a). The mean rates of elongation in low-N and high-N solution after

---

* P = 0.05

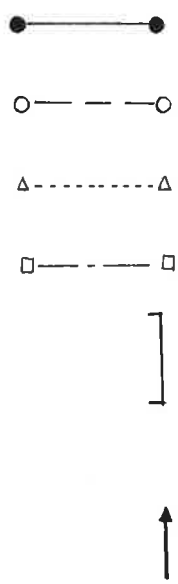
Figure 5.10

Influence of time of supply of additional nitrogen on elongation of the root axis:

(a) first seminal root

(b) first nodal root

Where treatments did not differ significantly from treatment N_1, points for treatment N_1 are plotted only.



= treatment N_1

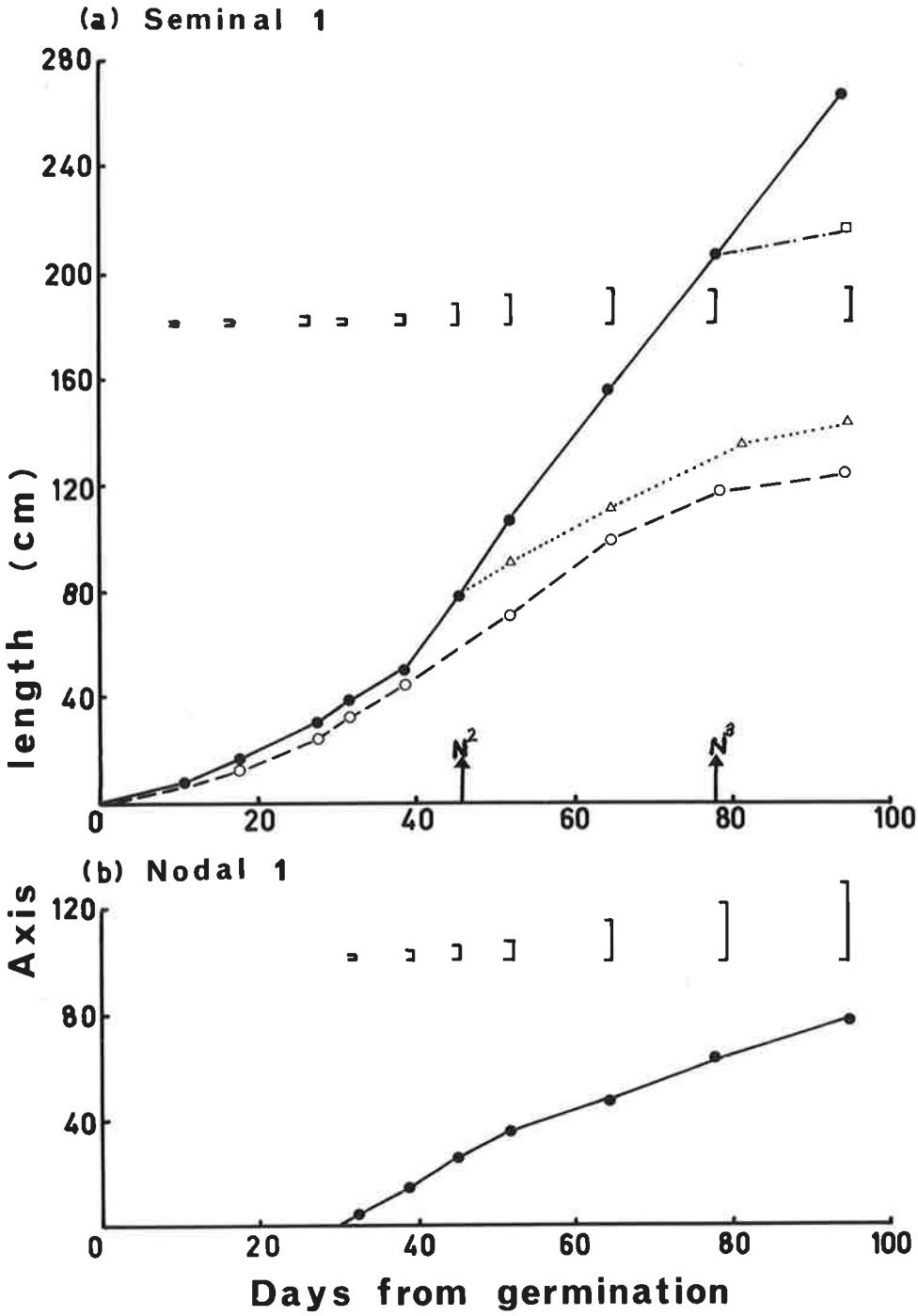
= " N_R^1

= " N_R^2

= " N_R^3

= twice the pooled standard error of the treatment means. Bartlett's test indicated homogeneity of variance over all treatments ($P = 0.05$)

= dates of application of treatments N^2 and N^3


(a) Seminal 1
280
240
200
160
120
80
40
length (cm)
0
20
40
60
80
100
N2
N3
(b) Nodal 1
120
80
40
Axis
0
20
40
60
80
100
Days from germination

day 40 were 4.0 and 1.0 cm day^{-1}. Elongation rate of the seminal axes was reduced without any detectable lag when plants were transferred from low-N to high-N solution at any stage. In contrast, the nodal axes showed a marked variability between replicates, and no significant difference was detected between N treatments (Fig. 5.10.a).

The mean lengths of the first-order laterals within each axis segment, estimated by observation through the glass walls of the tanks at a number of times during growth, were plotted against time for each N treatment and each root. Because of a discrepancy between estimates of length made during growth and direct measurements made at harvest (section 5.2.6.c), the lengths were uniformly multiplied by the factor 0.8. The measurements of first-order lateral lengths obtained through the glass wall were shown to overestimate the true lengths, as determined on the harvested roots, by about 25%, independent of filament lengths so far as could be determined. Graphs of the adjusted values for selected axis segments for treatments N_1, N_R^1 and N_R^2 are shown in Figs. 5.11 (seminal root) and 5.12 (nodal root). As treatment N_R^3 was imposed too late to reveal any subsequent change, data are not shown for this treatment. The time when the first-order laterals at the mid-point of each axis segment commenced to elongate, t_i, was interpolated from separate graphs showing the progression of branching along each axis. The graphs of the progression of first-order branching along each

axis were prepared from the graphs of axis elongation (Fig. 5.10) and the measurements of length of apical unbranched zone of each axis during elongation (section 5.3.6). The graphs were therefore similar, but offset in time, to those of axis elongation. Similar graphs were prepared of the progression of second-order branching along first-order laterals of each axis segment, in order to determine t_i for each first-order lateral segment for plots of second-order lateral elongation during growth (see on). Corrected first-order lateral lengths and lengths of apical unbranched zone were used (i.e. multiplied by 0.8). These graphs were similar, but offset in time, to those of first-order lateral elongation (Figs. 5.12 and 5.13). t_i could be determined with precision for the axis segments because of the precision of axis length measures during growth; it could be determined less accurately for first-order lateral segments because of the lower accuracy of first-order lateral length measures during growth (see section 5.2.6.c). The interpolated time, t_i, has been used as the base-point of each lateral elongation curve.

The only justified interpretation of the data for the period terminating at 80 days is that the points represent a linear trend in time. On this assumption, linear regression analysis was performed on the adjusted replicated values for each axis segment. The analysis was restricted to the more basal segments, because insufficient points were available for the later-formed,

Figure 5.11

Influence of time of supply of additional nitrogen on the elongation of first-order laterals of selected axis segments of the first seminal root:

(a) treatment N_1

(b) " N_R^1

(c) " N_R^2

↑ = time of application of treatment N^2.

Points plotted are generally means of three replicates (there were several missing values). Linear regression lines are shown for each axis segment where the regression was significant. For treatment N_R^2, the regression line is fitted to points occurring on or after day 45, when high-N solution was supplied. Regression coefficients and associated tests of significance are given in Table 5.6. Points in parentheses refer to data obtained at harvest by direct measurement. They are shown for comparison, but were not included in the regressions.

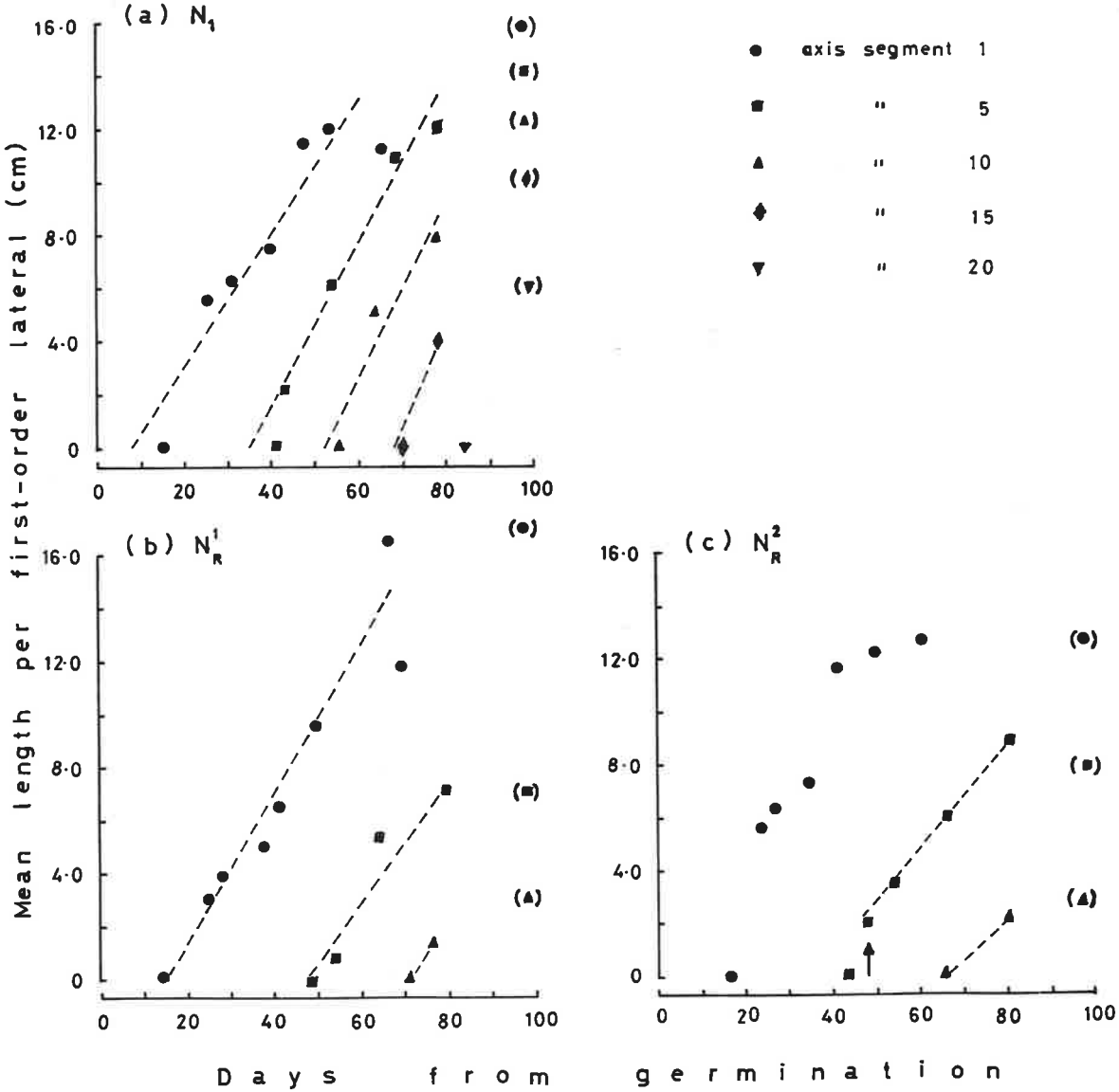

(a) N_1
(b) N_R^1
(c) N_R^2
Mean length per first-order lateral (cm)
Days from germination
16·0
12·0
8·0
4·0
0
0
20
40
60
80
100
(●)
(■)
(▲)
(♦)
(▼)
● axis segment 1
■ " 5
▲ " 10
♦ " 15
▼ " 20

Figure 5.12

Influence of the time of supply of additional nitrogen on the elongation of first-order laterals of selected axis segments of the first nodal root:

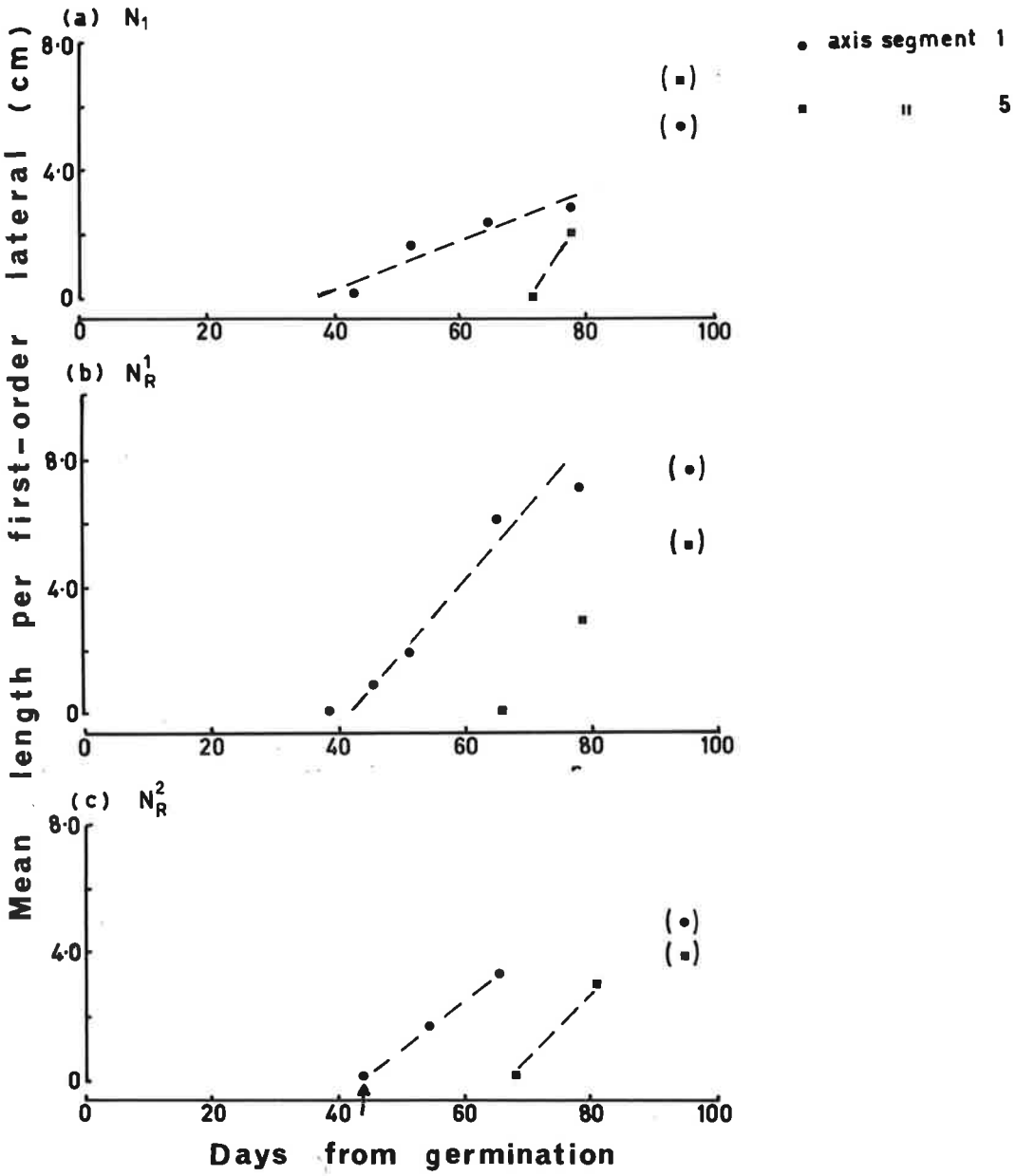
(a) treatment N_1

(b) " N_R^1

(c) " N_R^2

↑ = time of application of treatment N^2.

Points plotted are generally means for three replicates (there were several missing values). Linear regression lines are shown for each axis segment where the regression was significant. Regression coefficients and associated tests of significance are given in Table 5.6. Points in parentheses refer to data obtained at harvest by direct measurement. They are shown for comparison, but were not included in the regressions.


(a) N_1
(b) N_R^1
(c) N_R^2
Mean length per first-order lateral (cm)
8·0
4·0
0
0
20
40
60
80
100
(•)
(▪)
• axis segment 1
▪ " 5
Days from germination

apical segments. For treatment N_R^2, the regression was restricted to points occurring from day 45 onwards, after the change to the high level of N-supply. Linear regression curves are shown for selected axis segments, where significant, in Figs. 5.11 and 5.12. Pertinent statistics from the regression analyses are shown in Table 5.6. The mean lengths of the first-order laterals determined at harvest are shown in the figures for reference, but these were not used in the regressions.

In the seminal root the rates of elongation of first-order laterals did not differ significantly between the N_1 and N_R^1 treatments in the basal segments. In the more apical segments, however, the first-order laterals elongated more rapidly in low-N than in high-N solution. This difference was probably due to the delay in establishment of nitrogen-deficiency until after the appearance of the more basal first-order laterals. The response of the first-order laterals in general was similar to that of the axes. The later appearance of corresponding axis segments in high-N than in low-N solution was due to the effect of N on the elongation rate of the axis. Within treatments N_1 and N_R^1 the 10 regression lines were compared to test the hypothesis that the lines formed a parallel set. In N_R^1 the lines were not of significantly differing slopes ($P = 0.05$), but in N_1 significant divergence was found. There was no significant difference between treatments N_R^1 and N_R^2 in the effect of nitrogen concentration of

Table 5.6

Results of the linear regression analysis of corrected mean length per first-order lateral vs. time, for treatments N_1, N_R^1 and N_R^2 (see Figs. 5.12 and 5.13, and section 5.3.5.a): (i) the first seminal root (axis segments 1-10); (ii) the first nodal root (axis segments 1-5). Values shown for each axis segment and treatment are the regression coefficient β (cm day^{-1}), the statistical significance of β ($^{*}P = 0.05$, $^{**}P = 0.01$) and the standard error (s.e.), the intercept on the time axis Y_o (day), and the number of points regressed (N). Regression coefficients of an axis segment which are not significantly different (P=0.05) are followed by a common letter.

(i) Seminal 1

Axis segment number	Nitrogen treatment	β (cm day^{-1})		s.e.	Y_o (day)	N
1	N_1	0.24 **	a	0.04	8.0	19
	N_R^1	0.26 **	a	0.02	15.6	22
	N_R^2	0.04		0.06	-	5
2	N_1	0.16 **	a	0.03	13.1	16
	N_R^1	0.18 **	a	0.03	24.2	16
	N_R^2	0.06		0.07	-	6
3	N_1	0.28 **	ab	0.08	23.3	16
	N_R^1	0.25 **	a	0.05	36.0	14
	N_R^2	0.45 *	b	0.13	-	8
4	N_1	0.31 **	a	0.04	30.8	15
	N_R^1	0.14 **	b	0.03	41.0	12
	N_R^2	0.06		0.12	-	8

Table 5.6, continued

Axis segment number	Nitrogen treatment	β (cm day^{-1})		s.e.	Y (day)	N
5	N_1	0.32 **	a	0.05	36.8	14
	N_R^1	0.22 **	ab	0.04	44.7	11
	N_R^2	0.15 *	b	0.07	-	9
6	N_1	0.37 **	a	0.03	40.0	10
	N_R^1	0.15 *	b	0.07	36.1	10
	N_R^2	0.15 *	b	0.08	-	8
7	N_1	0.44 **	a	0.05	44.8	10
	N_R^1	0.19 *	b	0.07	46.0	9
	N_R^2	0.15 *	b	0.06	42.1	8
8	N_1	0.46 **	a	0.07	48.0	10
	N_R^1	0.18 *	b	0.08	56.2	7
	N_R^2	0.12 *	b	0.05	46.0	7
9	N_1	0.35 *	a	0.08	45.2	8
	N_R^1	0.16 *	b	0.07	63.4	6
	N_R^2	0.04		0.03	41.5	4
10	N_1	0.31 *	a	0.05	50.6	9
	N_R^1	0.22 *	a	0.06	70.9	6
	N_R^2	0.22 *	a	0.08	62.0	5

Table 5.6, continued

(ii) Nodal 1

Axis segment number	Nitrogen treatment	β (cm day^{-1})		s.e.	Y (day)	N
1	N_1	0.07 **	a	0.03	34.0	10
	N_R^1	0.23 **	b	0.03	41.5	11
	N_R^2	0.15 **	c	0.03	41.0	9
2	N_1	0.12 **	a	0.02	38.3	9
	N_R^1	0.43 **	b	0.08	44.5	8
	N_R^2	0.35 **	b	0.03	50.2	10
3	N_1	0.15 *	a	0.03	38.0	8
	N_R^1	0.25 *	a	0.07	45.9	9
	N_R^2	0.20 *	a	0.06	48.2	9
4	N_1	0.24 **	a	0.02	54.8	7
	N_R^1	0.18 *	a	0.08	50.3	5
	N_R^2	0.18 *	a	0.09	46.3	6
5	N_1	0.25 **	a	0.05	69.8	6
	N_R^1	0.14		0.09	64.4	5
	N_R^2	0.18 *	a	0.08	66.6	6

the nutrient solution on the elongation rate of the more apical laterals. The first-order laterals on the nodal root differed from those on the seminal root. Where differences occurred, as in segments 1 and 2, the laterals elongated less rapidly at low-N than at high-N supply; however on more apical segments no significant differences were found.

The mean lengths of the second-order laterals within each first-order lateral segment were plotted against time in a similar way as described previously for the first-order laterals within each axis segment. A correction factor of 0.67 was used for the lengths before day 80 (see section 5.2.6.c). Data for the seminal root are given in Fig. 5.13. The number of points available was too small and the accuracy of the data was too low to justify regression analysis, and no statistical evaluation has been attempted. However it appears that on the seminal roots second-order laterals tended to elongate less rapidly in the high-N than in low-N solution, as did the first-order laterals. Elongation rates appeared to be higher in the 5-10 cm than in the 0-5 cm first-order lateral segment. In treatment N_1 elongation rate appeared to be higher for laterals initiated later in development than for laterals initiated earlier, which again was probably due to the late establishment of nitrogen-deficiency. The later appearance of second-order laterals in corresponding segments of the root in high-N than in low-N solution results from the

Figure 5.13

Influence of time of supply of additional nitrogen on the elongation of second-order laterals of selected axis segments of the first seminal root:

(i) 0-5 cm first-order lateral segment (F1)

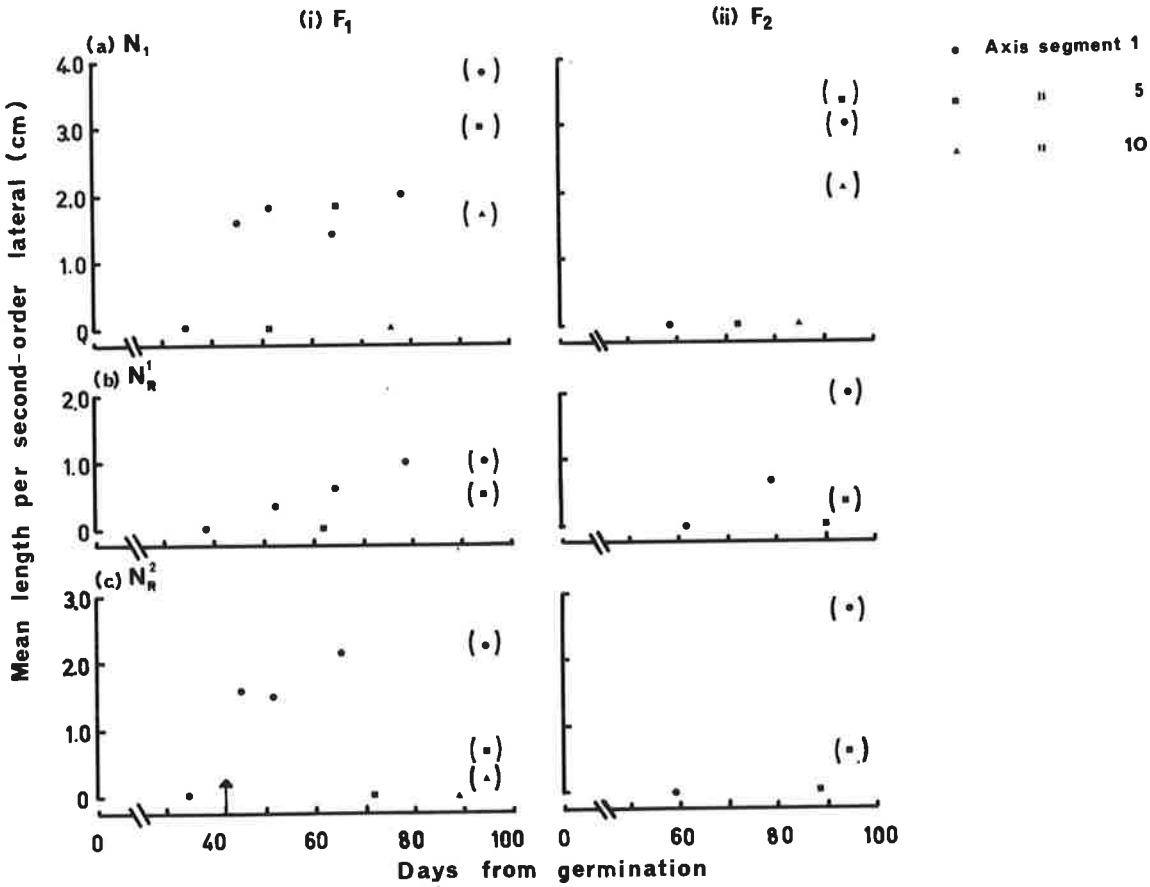
(ii) 5-10 cm " " " (F2)

(a) treatment N_1

(b) " N_R^1

(c) " N_R^2

↑ = time of application of treatment N^2


(i) F1
(ii) F2
(a) N1
(b) N'R
(c) N2R
Mean length per second-order lateral (cm)
Days from germination
Axis segment 1
5
10

lower rates of axis and first-order lateral elongation on the seminal root in the high-N solution. Little data was obtained during growth for the relatively few second-order laterals of the first nodal root (see following section).

(b) Lengths of root members at harvest

The lengths of the axis at harvest, 94 days from germination, are shown in Fig. 5.10 for the two roots examined, and also in Table 5.5. The effects of nitrogen treatments on the elongation of the axes have been noted in the preceding section.

The mean lengths at harvest of the first-order laterals within each axis segment, and of the second-order laterals within each first-order lateral segment, are shown in Figs. 5.14 and 5.15. In corresponding segments of the seminal root, first-order and second-order laterals were generally significantly longer where they had grown in low-N solution than where they had grown for all or part of the time in high-N solution. Laterals in corresponding positions along the axis were generally shorter on the plants that had been supplied with high-N solution for longer periods. Laterals in the basal segments of the axes commenced elongation early in growth when nitrogen-deficiency had not established, and these showed smaller differences between N treatments than the more distal segments. The response of first-order laterals on the nodal root to nitrogen treatment differed from the response of

Figure 5.14

Influence of time of supply of additional nitrogen on mean length per first-order lateral for successive segments of the axis, at harvest:

(a) first seminal root

(b) first nodal root

●——————● = treatment N_1

○— — —○ = " N_R^1

△·--------△ = " N_R^2

□—— · ——□ = " N_R^3

⌜————⌝ = twice the pooled standard error of the treatment means. Bartlett's test indicated homogeneity of variance over all treatments (P = 0.05)

▷——→ = position of most distal first-order lateral in treatment N_R^2 when plants were supplied with high-N solution

□——→ = position of most distal first-order lateral in treatment N_R^3 when plants were supplied with high-N solution

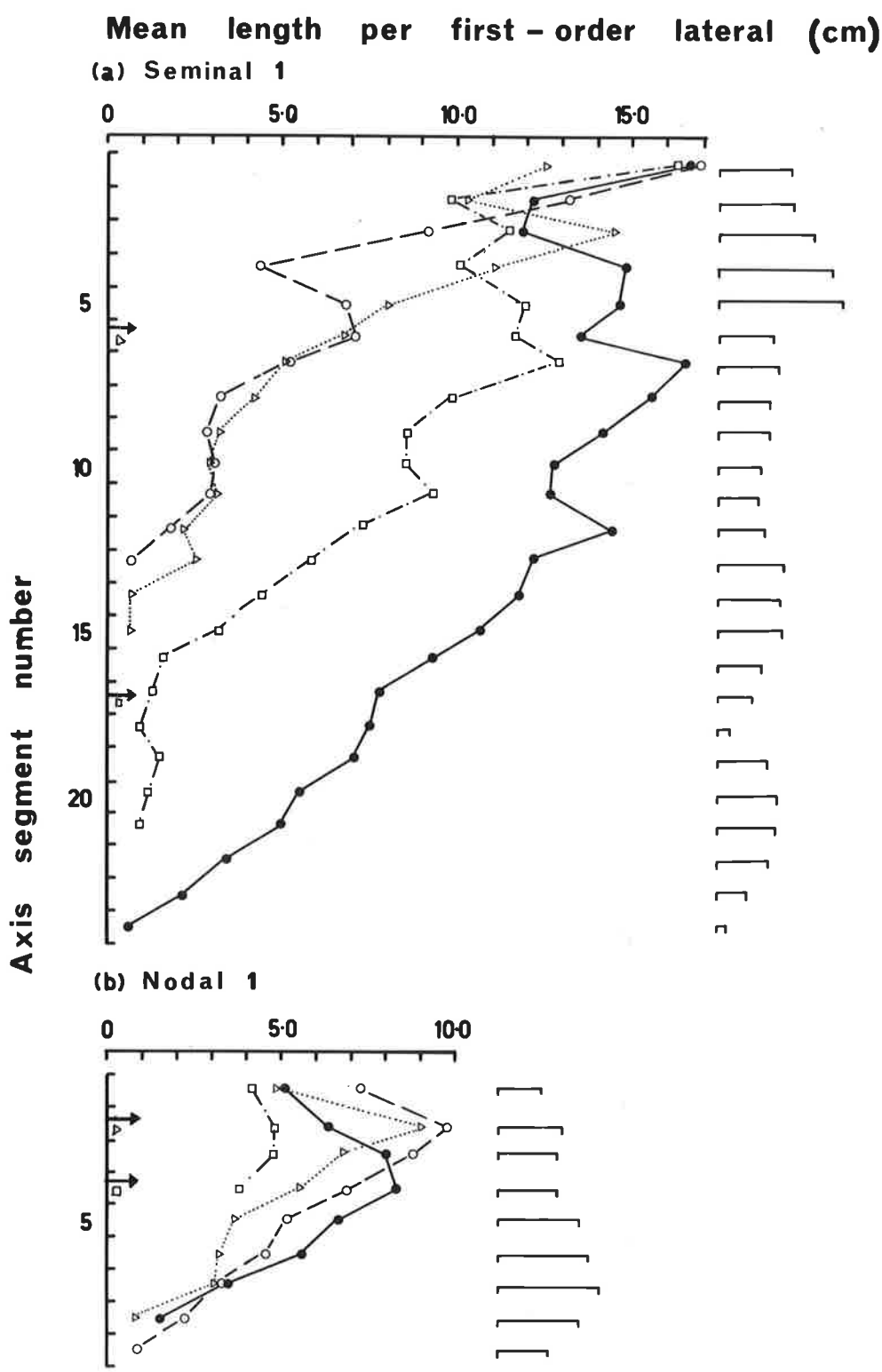

Mean length per first – order lateral (cm)
(a) Seminal 1
0
5·0
10·0
15·0
5
10
15
20
Axis segment number
(b) Nodal 1
0
5·0
10·0
5

Figure 5.15

Influence of time of supply of additional nitrogen on mean length per second-order lateral for successive first-order lateral and axis segments at harvest:

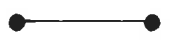
(a) first seminal root

(b) first nodal root

(i) 0-5 cm first-order lateral segment (F1)

(ii) 5-10 cm " " " (F2)

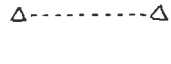
(iii) 10-15 cm " " " (F3)



= treatment N_1

= " N_R^1

= " N_R^2

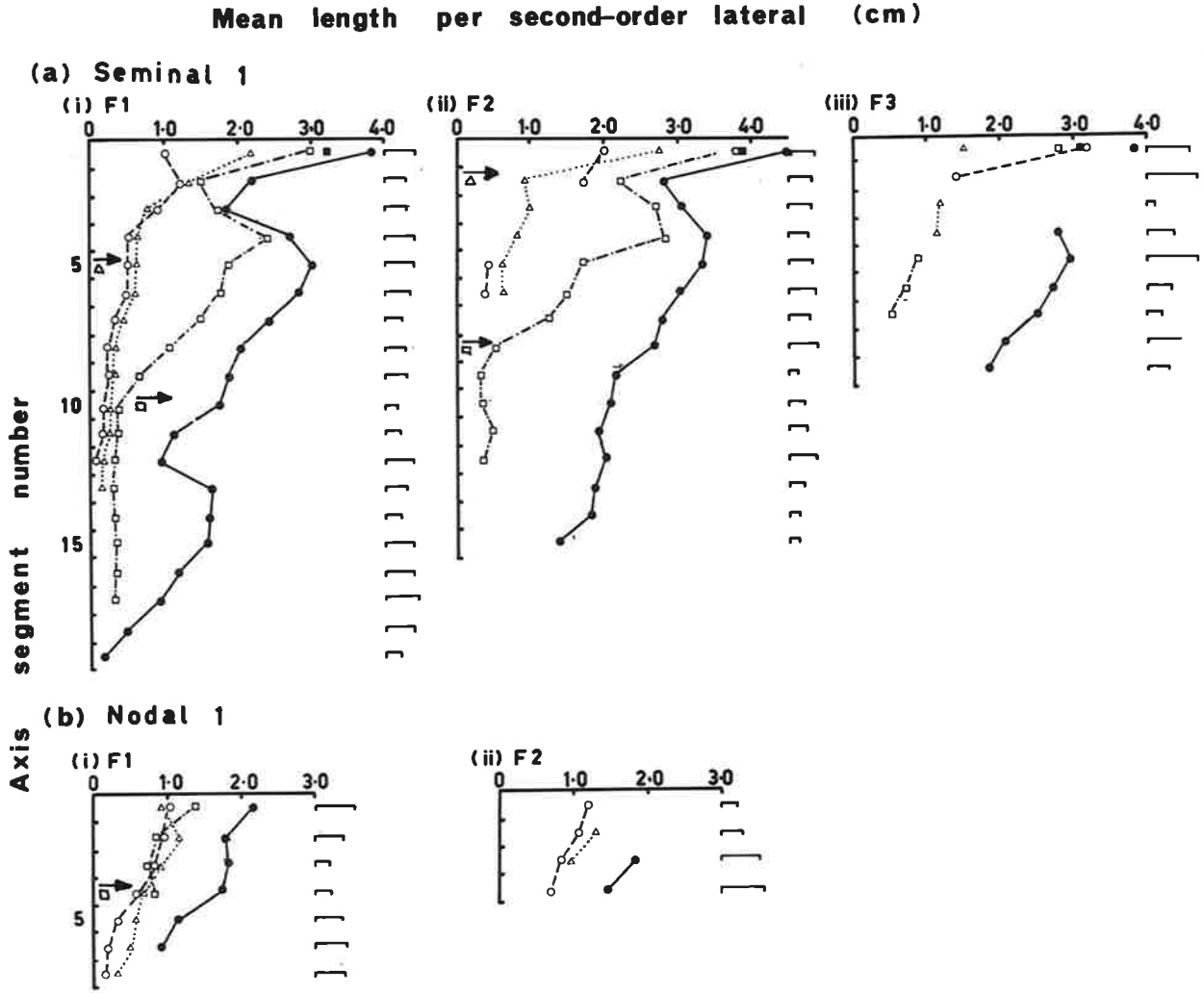


= " N_R^3

= twice the pooled standard error of the treatment means. Bartlett's test indicated homogeneity of variance over all treatments (P = 0.05)

= position of most distal first-order lateral in treatment N_R^2 when plants were supplied with high-N solution

= position of most distal first-order lateral in treatment N_R^3 when plants were supplied with high-N solution


Mean length per second-order lateral (cm)
(a) Seminal 1
(i) F1
(ii) F2
(iii) F3
0
1·0
2·0
3·0
4·0
5
10
15
segment number
(b) Nodal 1
Axis
(i) F1
(ii) F2

those on the seminal root. In segments 1 and 2, lengths were significantly greater where growth had occurred in high-N solution than where it had occurred in low-N solution. In more distal segments lengths tended to be reduced where laterals had experienced an increase in nitrogen supply during their growth, but otherwise did not differ significantly. In contrast, second-order laterals were of shorter length where growth had occurred entirely or partly in high-N solution.

The shorter length at harvest of laterals already present at the time of change from low-N to high-N solution in treatments N_R^2 and N_R^3 compared with those in treatment N_1, indicates that change to the high-N solution reduced their elongation rate subsequent to transfer. The length of a lateral is determined not only by the rate but also by the duration of its elongation. The period available for elongation at any point along a subtending root member depends upon the elongation rate of the subtending root member. The greater length of the more distal first-order and second-order laterals in treatment N_1, compared with N_R^1, results partly from the more rapid extension of the subtending root members in this treatment, and hence the greater time available for lateral elongation in corresponding segments along the axis.

Mean rates of elongation (cm per lateral per day) of the first-order and second-order laterals within each segment were calculated as $l_H/(t_H - t_i)$, where l_H is the length at harvest,

Table 5.7

Mean rates of elongation (cm per lateral per day) of first-order and second-order laterals in selected axis and first-order lateral segments of (i) the first seminal root, (ii) the first nodal root, calculated from mean lengths per segment measured at harvest and estimates of branching progression during growth (see section 5.3.5.b).

(cm/lateral/day)

	Nitrogen treatment			
	N_1	N_R^1	N_R^2	N_R^3
(i) Seminal 1				
(a) First-order laterals				
Axis segment number: 1	0.21	0.22	(0.14)*	(0.20)
5	0.28	0.15	(0.14)	(0.15)
10	0.32	0.14	0.10	(0.12)
15	0.45			(0.06)
20	0.50			
(b) Second-order laterals within the 0-5 cm first-order lateral segment				
Axis segment number: 1	0.06	0.02	(0.02)	(0)
5	0.07	0.02	0.02	(0)
10	0.09		0.03	0.03
15	0.17			0.08

Table 5.7, continued

		(cm/lateral/day) Nitrogen treatment			
		N_1	N_R^1	N_R^2	N_R^3
(c) Second-order laterals within the 5-10 cm first-order lateral segment					
Axis segment number:	1	0.10	0.05	0.06	(0.05)
	5	0.12	0.04	0.04	(0.04)
	10	0.33			
(11) Nodal 1					
(a) First-order laterals					
Axis segment number:	1	0.10	0.14	(0.09)	(0.05)
	5	0.30	0.17		
(b) Second-order laterals in the 0-5 cm first-order lateral segment					
Axis segment number:	1	0.07	0.03	0.02	0.04
	5	0.06	0.02	0.03	

* Values in parentheses for treatments N_R^2 and N_R^3 are for laterals initiated before transfer from low-N to high-N solution. The rates shown were found for the period after transfer, from $(l_H - l_T)/(t_H - t_T)$, where l_H is the length at harvest, l_T is the length at transfer, t_H is the day number at harvest, and t_T is the day number at transfer. Values of l_T were assumed to be those recorded for treatment N_1 for the corresponding root segments and times.

t_H is the day number at harvest, and t_i is the day number when laterals at the mid-point of a segment commenced to elongate. t_i was determined for each axis and first-order lateral segment from graphs of the progression of branching along each axis and along the first-order laterals of each axis segment, as described in section 5.3.5.a. Calculation of mean rates of elongation is justified by the linearity of the length/time curves obtained from estimations of length during growth, as shown in Figs. 5.11, 5.12 and 5.13. The calculated mean rates of elongation are shown for selected axis segments in Table 5.7.

Similar effects of the N treatments are apparent in Table 5.7 as were shown in Table 5.6, derived from the estimates of length made during growth. In addition Table 5.7 shows that the effect of high-N on lateral elongation rate appeared to be similar when the additional N was supplied relatively late in treatment N_R^3, as when supplied earlier in treatments N_R^1 and N_R^2.

5.3.6 Length of apical unbranched zone

The length of the apical unbranched zone of the axes was related positively to their rate of elongation (Table 5.8). Accordingly, the zone was longer in low-N than in high-N solution, in the seminal than in the nodal root, and in the later stages of growth in treatment N_1. The unbranched zone decreased in length after supply of additional N. The length of the apical unbranched

Table 5.8

Influence of time of supply of additional nitrogen on the length (cm) of the apical unbranched zone of the axis of (a) the first seminal, and (b) the first nodal root, at 4 times after germination.

		Nitrogen treatment				
		N_1	N_R^1	N_R^2	N_R^3	2 x pooled standard error*
(a) Seminal 1						
Day:	27	13.0	9.9	-‡	-	2.4
	38	16.3	16.5	-	-	6.3
	52	28.0	12.9	17.0	-	2.7
	94	31.0	5.7	5.5	10.0	3.0
(b) Nodal 1						
Day:	38	13.5	9.4	-	-	3.1
	52	14.6	12.3	12.4	-	2.4
	94	5.7	2.3	5.3	4.0	3.1

* Bartlett's test indicated homogeneity of variance over all treatments (P = 0.05)

‡ Measurements were not made until N treatments had been imposed

zone of the first-order laterals showed a similar response to that of the axis. As lengths were smaller the absolute differences were relatively small. Data are shown in Table A5 (Appendix 2).

5.3.7 Diameters of root members

The diameters of root members in the two roots examined were not measured. However they were apparently greater when nitrogen was supplied at the high rate (Plates 5.3 - 5.7).

5.3.8 Derived root data

(a) Total length and number of members per root

The total length and number of members of each root at harvest were derived from the measures of branching density, length, and length of apical unbranched zone for each order of root member. The number of first-order laterals on the axis was computed by accumulating the number present in each axis segment. The number of axis segments completely filled with first-order laterals, and the number of first-order laterals in the most apical segment (incompletely filled) were determined from the branched length of the axis, obtained by subtraction of the length of the axis apical unbranched zone from the length of the axis. The number of second-order laterals per first-order lateral within each axis segment was computed analagously. The number per axis segment

was obtained by multiplying by the number of first-order laterals per axis segment, and the total number for the root by accumulating the numbers per axis segment. The total number of members per root was computed as the sum of the members of each order. The length of first-order laterals within each axis segment was computed as the product of the number in the segment and the mean length per first-order lateral. The length of second-order laterals within each first-order lateral segment was computed analagously, and the total length of the root by accumulation of the lengths of laterals in each segment and the length of the axis.

In the seminal root the effects of the nitrogen treatments on elongation of the root members greatly outweighed their effects on branching density. This resulted in a greater total length of root and number of members in the treatments receiving N at the low rate, the extent of the effect depending upon the duration of the period at the low rate (Table 5.9). Owing to the effect of nitrogen concentration on the diameters of root members (section 5.3.7), the effect of nitrogen supply on root weight may well have been different from the effect on root length (the weight per individual root was not measured). Variability between replicates largely obscured effects of nitrogen supply on the overall growth of the first nodal root. The response to additional N appeared to shift with time, tending to be opposite to that of the seminal root in the early stages of growth of the first nodal axis, but qualitatively similar in the later stages of its growth.

Table 5.9

Influence of time of supply of additional nitrogen on the total length and total number of members of all classes of (a) the first seminal and (b) the first nodal root per plant, 94 days after germination.

	Nitrogen treatment				
	N_1	N_R^1	N_R^2	N_R^3	L.S.D. (P=0.05)
(a) Seminal 1					
Total length of members (m):	256	74	76	129	31
Total number of members:	7840	4370	4910	6220	1172
(b) Nodal 1					
Total length of members (m):	29	45	34	13	n.s.
Total number of members:	1250	3350	2660	750	1467

(b) Vertical distribution of length of root members along the root axis

The total length of root members per axis segment was derived in the manner indicated in section 5.3.8.a. For the seminal root, a greater length of root members was generally present on those segments of the axis where growth had occurred in low-N than where it had occurred in high-N solution (Fig. 5.16.a). The effect was more pronounced in segments distal to those already present when nitrogen-deficiency was first established in treatment N_1 (segment numbers ≥ 5). Not only was the seminal axis shorter at high N, but the root length per segment decreased more sharply with distance from the base of the root. Accordingly, the proportions of total root length that occurred in the most basal axis segment of the seminal root for treatments N_1, N_R^1, N_R^2 and N_R^3 were 15, 42, 31 and 22%. The effect of the N treatments on the vertical distribution of length differed in the nodal root from that in the seminal. In the basal segments the overall length of the members was significantly less at low-N than at high-N supply. Late addition of N appeared to check the growth of the first nodal root (Fig. 5.16.b).

(c) The contribution to root length of different member classes

The total length of each order of root members per root was derived in the manner indicated in section 5.3.8.a. In the

Figure 5.16

Influence of time of supply of additional nitrogen on the total length of members of all classes per axis segment, at harvest:

(a) first seminal root

(b) first nodal root

●——————● = treatment N_1

○— — —○ = " N_R^1

△·····△ = " N_R^2

□— · —□ = " N_R^3

|——————| = twice the pooled standard error of the treatment means. Bartlett's test indicated homogeneity of variance over all treatments (P = 0.05)

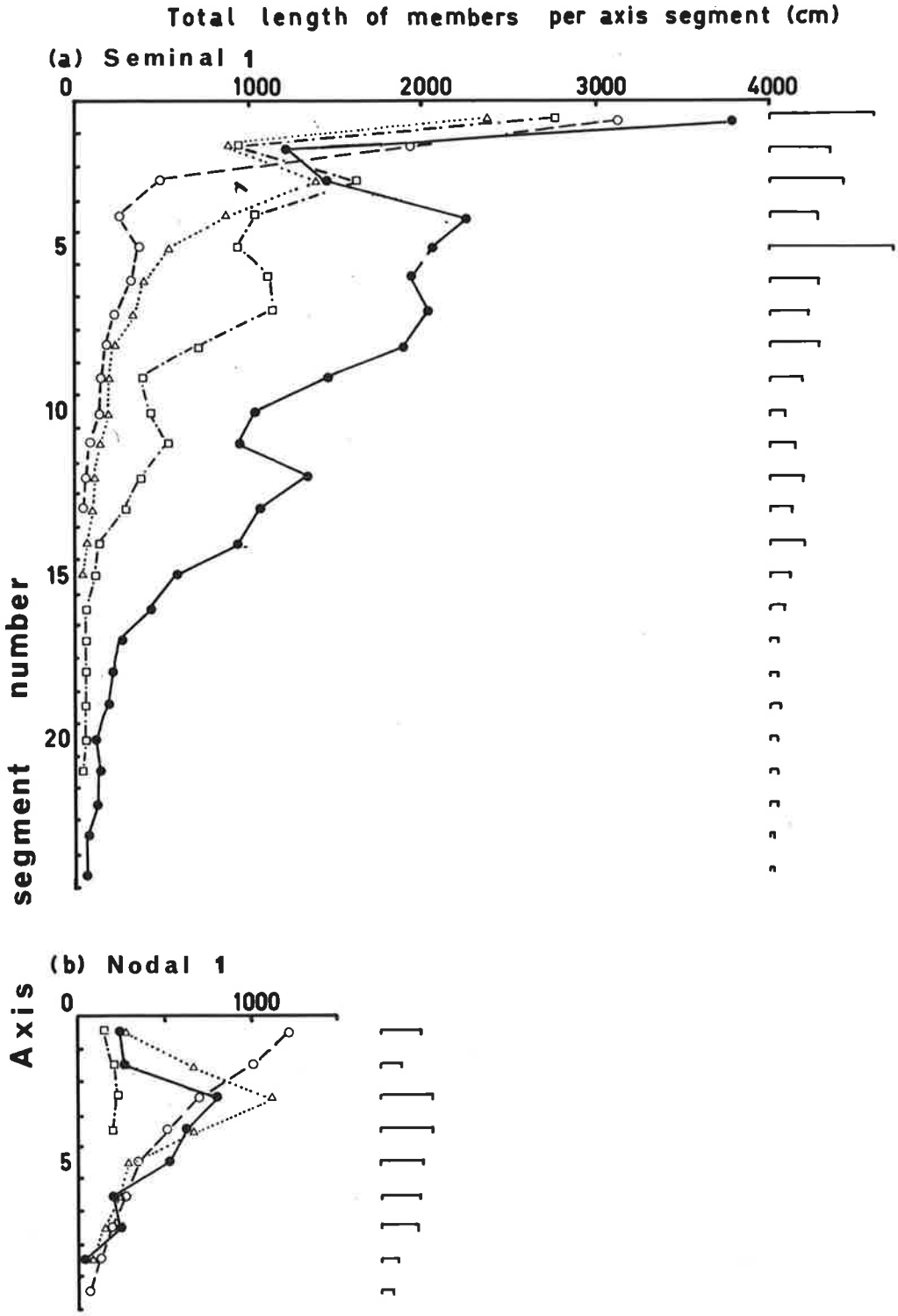

Total length of members per axis segment (cm)
(a) Seminal 1
0
1000
2000
3000
4000
5
10
15
20
segment number
(b) Nodal 1
Axis
0
1000
5

Table 5.10

Influence of low and high nitrogen supply throughout growth (treatments N_1 and N_R^1) on the percentage of total root length at harvest comprised of axis, first-order, and second-order laterals.

		Seminal 1			Nodal 1		
		Axis	First-order lateral	Second-order lateral	Axis	First-order lateral	Second-order lateral
(a) Axis segments:							
A1*	N_1	0.2	10	90	4.0	39	57
	N_R^1	0.3	15	85	0.8	27	72
A5	N_1	0.4	19	81	1.8	50	48
	N_R^1	2.7	54	43	2.9	72	25
A10	N_1	0.9	36	63			
	N_R^1	6.7	82	11			
(b) Whole Root:							
	N_1	1.0	27	72	2.6	43	54
	N_R^1	1.6	34	64	2.0	44	54

* A1 = 0–10 cm, A5 = 40–50 cm, A10 = 90–100 cm axis segment

seminal root particularly, the second-order laterals contributed most of the length in well developed zones of the axis - Table 5.10. The contribution of the second-order laterals declined, and that of the first-order laterals increased from the base towards the apex of each axis, the trend being steeper at the high rate of N supply.

Laterals of higher than second-order were not measured comprehensively on the roots. However they appeared to contribute a relatively small amount of root length (see Plates 5.3 - 5.7). Measurements of third-order lateral length were made at harvest on selected axis segments of the first replicate of treatments N_1 and N_R^1. The segments are those shown in Plates 5.3 - 5.6. The lengths of third-order lateral in the 0-10 cm, 40-50 cm and 90-100 cm axis segments of the first seminal root, expressed as a percentage of the total of axis, first-order and second-order lateral length in the segment, were 10.5, 2.4 and 0.5% for treatment N_1, and 19.2, 0 and 0% for treatment N_R^1. The percentage lengths in the 0-10 cm and 40-50 cm axis segments of the first nodal root were 4.4 and 0% for treatment N_1, and 6.7 and 0% for treatment N_R^1.

5.4 Discussion

Nitrogen deficiency at the low level of N supply was not established until nearly half-way through the experiment, shortly before the first deferred applications of additional nitrogen (N^2 treatments). Interpretation of the growth responses is complicated by this situation, since early-formed members of the shoot and root system were initiated and commenced growth under conditions of near sufficiency at both 'low' and 'high' levels of supply. The degree of nitrogen deficiency established at low N supply after early growth is indicated by the shoot growth data obtained (Table 5.3; Figs. 5.5, 5.6; section 5.3.2).

As in Experiment A, there was a contrast between the growth response obtained to foliar-supplied and root-supplied nitrogen. In this experiment no significant response was obtained to foliar-supplied urea, of either the shoot or root system. Although the rate and method of foliar application were similar to Experiment A, a greater loss of nitrogen from the foliage following application appears to have occurred in this experiment (section 5.3.1). In Experiment A loss of urea from the shoot was correlated with the rate of application. The rate of foliar application per plant in this experiment, applied in two dressings, exceeded the highest rate used in Experiment A (74 cf. 50 mg N $plant^{-1}$). This may have been responsible for the greater apparent

losses in this experiment. No further discussion of the result can be offered beyond what has been presented already in section 3.4.

In contrast to foliar-applied nitrogen, plants responded rapidly to additional N supplied by the root path, at every stage of application. Leaf area of the plant increased as a result of (i) increase in the size of existing tillers, through increase in the size of leaves still having the ability to enlarge at the time of application, and (ii) increase in the number of tillers. Increase in plant dry weight accompanied increase in leaf area. The responses were characteristic of plants to which nitrogen has been applied during the vegetative phase of growth (see sections 2.1.2.a and b, and 2.2.2).

The adjustment of root weight ratio of the plants to a lower value following addition of nitrogen was characteristic (Brouwer 1965). If the value of 0.2 for treatment N_R^3 at harvest is assumed to be the interrupted half-way stage, adjustment occurred at every time of nitrogen addition. It appears that the rate of dry weight gain of the root system of high-nitrogen plants fell below that of low-nitrogen plants during the period of adjustment (plant root weight for treatment N_R^3 was significantly lower than that for treatment N_1 at harvest), and only thereafter increased to a higher rate commensurate with the enhanced rate of plant dry weight gain. This pattern of response has been demonstrated previously for maize plants (Brouwer, Jenneskens and Borggreve 1961). It is

interesting that the period of adjustment of root weight ratio coincided approximately with the lag between supply of additional N and the emergence of additional nodal roots.

Additional nitrogen stimulated the production of nodal roots. These accompanied, with a time delay, the additional tillers produced at the higher level of nitrogen. Increase in nodal root number as the plant develops is well known (Troughton 1962), and an association between tillering rate and nodal root production has been shown previously (Pinthus and Eshel 1962; Pinthus 1969). The increase observed in this experiment occurred as a result of both the increased number of tillers producing nodal roots and an increase in the number of nodal roots produced by each tiller. Nodal root number was increased both on tillers produced before and on tillers produced after the time of additional nitrogen supply.

At the nitrogen concentrations used in the latter half of the experiment (0.02 and 0.7 mM), the axis and the first- and second-order laterals of the first seminal root elongated more rapidly and branched less densely in the low- than in the high-nitrogen solution. Past studies of the influence of nitrogen supply on root morphology of cereals in nutrient solution have been confined to relatively young plants, and therefore the lower orders of root member. In solutions of uniform concentration over the whole root system, seminal axes have most commonly shown

the same elongation and branching response to nitrogen as in this experiment (Bosemark 1954; Wiersum 1958; Brouwer, Jenneskens and Borggreve 1961; Brouwer and Loen 1962; Williams 1968). Recently Drew, Saker and Ashley (1973), using barley, found that the elongation response of seminal axes altered with time. Although for the first few days of growth axes elongated more rapidly in low- (0.01 mM) than in high- (1.0 mM) nitrogen solution, subsequently elongation rate was independent of the nitrogen concentration, and towards harvest (34 days from germination) was less at the lower concentration. The authors concluded, in accord with a hypothesis of Brouwer and Loen (1962), that axis elongation rate is not determined by the ambient nitrate concentration but by the overall nitrogen status of the plant.

A fuller hypothesis than that proposed by Brouwer and Loen would seem to be necessary, to account for the conflicting observations recorded in the literature, and those of this experiment. It would appear that elongation rate is influenced by both the ambient nitrogen concentration and the overall plant nitrogen status, and the rate observed depends upon the interaction of the two. The ambient nitrogen concentration acts directly on the rate of cell division (Williams 1968) and mean cell length (Bosemark 1954), while the plant nitrogen status acts indirectly, via the current assimilation rate of the shoot system and the consequent supply of assimilate to the root. Under conditions

of moderate nitrogen deficiency where the supply of assimilates does not restrict extension (given the capacity of root members to vary in diameter), the direct influence is expressed - that is, enhanced elongation rate under conditions of low nitrogen concentration; but where deficiency becomes severe a reduced assimilate supply overrides the direct influence and restricts elongation. Thus the response depends upon the degree of nitrogen deficiency present.

A second qualification arises, because the response of laterals to ambient nitrogen concentration apparently depends upon whether the concentration over the root system is uniform or not. Under conditions of non-uniform nitrogen concentration contrasting results to those of Experiment C have been obtained. The stimulation of root growth in or around zones locally enriched with nitrate or ammonium in soil is well known (Nobbe 1862; Crist and Weaver 1924; Weaver 1926; Gliemeroth 1953; Passioura and Wetselaar 1972). Examining the effects of localization of supply in solution culture, employing similar nitrogen concentrations to those of Experiment C, Drew, Saker and Ashley (1973) showed that elongation rate and branching density of first- and second-order laterals were increased approximately two-fold in a short zone of nitrate enrichment along the seminal axis, relative to equivalent laterals on axes supplied uniformly at a lower concentration of nitrate. A similar response of first-order

laterals on seminal axes of young wheat plants has been shown by Hackett (1972).

Under non-uniform conditions assimilates are, in the long-term, preferentially directed to the nitrogen-enriched root members, as evidenced by the preferential root growth in this zone and the reduced growth of the remainder of the root (Drew, Saker and Ashley 1973). Perhaps under these circumstances the direct effect of ambient concentration is soon overridden by the influence of the assimilate supply, in the equivalent but opposite way that, as suggested earlier, extreme nitrogen deficiency may exert an overriding influence on root extension. Where no preferential distribution of assimilates can occur - that is, in a uniform environment - the enhanced assimilate supply under conditions of nitrogen abundance may not be sufficient, spread over the whole root system, to override a direct effect of ambient concentration on root growth.

A possible alternative explanation for the contrasting results in Experiment C and of Drew, Saker and Ashley (1973) might lie in the use of ammonium nitrate in the former case and nitrate alone in the latter. The continuous decline in pH in Experiment C suggests a predominant absorption of ammonium over nitrate. No other reports are known of the detailed growth response of roots to uniform variation of ambient ammonium nitrate concentration. However, the response of the seminal axes in

Experiment C to additional ammonium nitrate was similar to that reported in previous experiments in which nitrate alone has been employed (Bosemark 1954; Wiersum 1958; Brouwer, Jenneskens and Borggreve 1961; Brouwer and Loen 1962; Williams 1968). The decline in pH of the nutrient solution following replenishment was more rapid at the high-N level, and the pH was always lower in the high-N solution (Fig. 5.3). The lowest value recorded in the reservoirs was 4.3. This may have been low enough to inhibit root extension.

The growth response of the first nodal root to nitrogen supply differed from that of the first seminal root. Although no significant differences between the levels of supply were observed, elongation rate and branching density of the members tended to be greater in the high- than in the low-nitrogen solution. Elongation rates of the axes, but not of the first-order laterals, tended to be lower than those of the seminal roots, particularly in the nitrogen-deficient plants. First-order lateral branching density tended to be higher.

The absence of consistent treatment differences was partly due to a marked, apparently random, variability in the vigour of first nodal roots. At harvest the first nodal roots of replicate plants were found to be either large or small, large roots being about twice the overall length of small ones. The root studied was in each case the first nodal root to appear.

The precise point of origin at the crown was not examined closely, other than to confirm the nodal rather than seminal origin, and the tiller of origin. It is possible that in some plants the first nodal root arose from the coleoptile node, and that this differed in growth habit from the first root arising from a true node of the stem. Roots arising from the coleoptile node of wheat are known to resemble the seminal roots in general morphology (Troughton 1962). Plants of the cultivar Halberd have been grown under similar conditions to those of Experiment C since that experiment was completed; of 15 plants grown, 5 were shown, by sectioning of the base of the plant, to have root axes arising from the coleoptile node at 21 days from germination (Barley, pers. comm.).

The two roots selected for detailed study appeared to be representative of the seminal and nodal root systems (Table 5.5). The lengths of the seminal axes not examined in detail at harvest apparently showed a similar response to nitrogen supply as the first seminal root. The first seminal root was invariably the largest at harvest, while the others were of successively decreasing length. This pattern has been observed previously, and associated with the respective origins of the roots within the seed (Troughton 1962). The first nodal root was invariably one of the largest at harvest, commensurate with the relative period of its development.

The relative lengths of the seminal and nodal roots

were much influenced by nitrogen supply. With low nitrogen supply the total length of the first seminal root was nine times the length of the first nodal, while under an abundant supply the length of the seminal root was one-and-a-half times that of the nodal. This was due more to a reduction in seminal root length under nitrogen deficiency than to an enhancement of nodal root length under nitrogen sufficiency. The contrast would probably be more extreme for the total lengths of the seminal and nodal systems, where the factor of the relative numbers of nodal roots would have added to the effect (Fig. 5.5, Table 5.5). An increase in relative contribution of the nodal system to the root length of the plant with increasing vigour of the shoot system was observed by Pavlychenko (1937), where the effects on shoot growth were due to plant spacing. A similar gross effect might be expected to arise from the influence of nitrogen supply on shoot growth, although it does not seem to have been recorded previously. Reports of the relative importance of the seminal and nodal systems in cereals vary (Troughton 1962). The seminal system has usually been observed to form the greater part of total root length, but not to the extent indicated here.

The classes of seminal root member studied (axis, first- and second-order laterals) showed a qualitatively uniform, though quantitively distinct, response to ambient nitrogen concentration. Similar phenomena have been observed for barley root members of

different order with respect to overall nutrient concentration (May, Chapman and Aspinall 1965; May et al. 1967). The response of laterals to phosphorus and potassium deficiency was found by Hackett (1968), however, to differ from that of the axes. The constant rate of elongation of the axes, and probably also of the first-order laterals, over long periods of time is interesting. The values of elongation rate and branching density measured accord broadly with values previously reported in, or derivable from, the literature (Barley 1970; Lungley 1973).

The reduced branching and elongation of second-order laterals near the base of the first-order laterals in both seminal and nodal roots, and of first-order laterals near the base of the nodal axis, in a homogeneous environment, is noteworthy. A similar pattern is evident for second-order laterals in the data of Schuurman and de Boer (1970), and was observed for first-order laterals by Hackett (1972). The phenomenon provides further evidence of internal mechanisms regulating the morphological development of the root, of the kind proposed by Riopel (1968) and Yorke and Sagar (1970).

Measurements of root member diameters were not made in the experiment, but the greater diameter of members elongating in high-nitrogen solution was obvious (see Plates 5.3 - 5.7). As well as being about twice the diameter of equivalent root members grown in low-nitrogen solution, members grown in high-nitrogen

solution were noticeably more fragile to handle. At any one level of nitrogen a correlation was apparent between the diameter of root members and their elongation rate. The longer members had the greater diameter. The phenomenon has been noted (Hackett 1972) and discussed (Hackett and Rose 1972) before. The correlation holds for the members of one order and for different orders of member of one root, but not for corresponding root members under different nitrogen treatments (cf. Hackett 1972; Drew, Saker and Ashley 1973).

A correlation was also apparent at any one level of nitrogen between elongation rate and length of the apical unbranched zone of members. This suggests that branching behind the apex may be a function of time after cell differentiation, rather than of distance from the apex as assumed in the simulation model of root growth (Appendix 1).

The effects of nitrogen concentration on total root length were dominated by the effects on elongation rate. Although low-nitrogen solution decreased branching density, total root length was increased because of the positive effect on elongation rate. The increase in elongation rate was of a greater magnitude than the decrease in branching density, and this difference was amplified over successive orders of member. Enhanced elongation influenced not only the individual lengths of the root members but also the number of members, because branching occurred over

an increased length.

The observation of root growth over an extended period emphasizes the exponential nature of increase in root length, and the dominant contribution of higher order laterals to root length in well developed root systems (in this experiment second-order laterals). Part of the importance of the seminal root system at any time derives simply from its greater period of growth. Similarly it should be noted that the contribution to root length of the numerous nodal roots which appear later in development is relatively small (see Table 5.5). Cursory examination of the plant crown near the soil surface is liable to be deceptive. Insofar as higher-order members dominate the root length of plants when they are growing at high rates, in the later stages of development, the study of root growth in small plants, and in particular of the growth of axes alone, is of restricted value. Similarly, the study of nutrient absorption in roots (Bowen and Rovira 1967; Russell and Sanderson 1967; Rovira and Bowen 1968; Clarkson, Sanderson and Russell 1968; Grasmanis and Barley 1969; Clarkson and Sanderson 1970) should extend from the root axes to the less conspicuous, more difficult, but numerically more important higher order members.

The implications of the observations from the experiment for the field situation are important. However, the extrapolation of results from solution culture to field conditions is restricted

by important differences between the two environments. In particular, the mechanical resistance offered to root extension by the soil is not present in solution culture, and, in contrast to the stirred solution, the soil profile tends to be heterogeneous with respect to nutrient concentration. Fertilizer nitrogen top-dressed in the field is at first localised in the upper part of the soil profile. Despite such limitations, the following two hypotheses may be proposed: (1) The rate of extension of seminal axes will be greater when the soil is deficient in nitrogen, provided the deficiency is not too severe. Hence, when the application of nitrogen to a moderately deficient soil is deferred, the seminal root system may extend deeper in the soil profile than under a sowing time application. If lateral production occurs at usual rates behind the apices of the axes an increased root density at depth will result. (2) The seminal system will form a larger proportion of the root system under a low than under a high nitrogen supply. Since the seminal system tends to be more extensive deeper in the soil than the nodal system (Passioura 1972) this may result in a greater proportion of root length occurring at depth under a deferred than under a sowing time application of nitrogen. It has to be remembered, however, that any application of nitrogen to the soil surface is likely to stimulate growth in this region, possibly at the expense of growth below the enriched zone.

An indication as to the influence these growth effects

might have on the root distribution could be obtained by using the root growth model described in Appendix 1. Assigning input values to the model as accurately as permitted by limited knowledge of the system, the relevant root growth attributes could be manipulated and the effects on the derived root distribution obtained. However this has not been pursued further, in the absence of attribute values measured under more pertinent conditions. K. Parameswaran (pers. comm.) is endeavouring to obtain data for wheat grown at field densities in a sandy red brown earth at Roseworthy, South Australia.

6 General discussion and conclusions

It was shown in Chapter 1 that a stratagem of deferment of application of nitrogen might have advantages over the customary practice of application at sowing time for the wheat crop in southern Australia. The stratagem would enable the selection of seasons of relatively high early rainfall and sites of evident nitrogen deficiency for the use of nitrogen. These are the circumstances known to favour large responses in grain yield (Cornish 1950; Russell 1968a, b). The success of the stratagem is dependent upon the nature of the crop's response to deferred application of nitrogen. The main purpose of this study has been to examine the growth response.

It is intended to consider the growth response of wheat to time of nitrogen application firstly in the case where water is not a limiting factor to growth, and secondly where it may be, as in southern Australia.

Survey of the literature (Chapter 2) showed that varied results have been obtained from applications of nitrogen to wheat at different stages of growth. No consistent pattern is immediately discernible. The reason is undoubtedly that the response of a crop to an application of nitrogen is governed by a number of factors, which vary from one situation to another.

A factor of major importance is the proportion of the

applied nitrogen that is recovered by the crop. Crop recoveries of applied nitrogen have been shown to vary considerably, falling generally within the wide range 20 to 80% (Allison 1966). Nitrogen fertilizer is normally applied to the soil. Incomplete, variable recovery arises because two conditions are required for nutrient uptake by roots from the soil, which are met to varying degrees in different situations: (i) presence of the nitrogen in the root environs for the period of uptake, (ii) a sufficient soil water content for transfer to the root surface (Olsen and Kemper 1968). A number of mechanisms of nitrogen fertilizer loss from the soil are known which contribute to poor and varied crop recovery (Allison 1966). The importance of soil water content in contributing to variable uptake has been emphasized by Bartholomew (1971). Variable recovery of applied nitrogen undoubtedly accounts for much of the variation in yield response in experiments. It is often not determined, however. When interpreting results, it cannot be assumed that the amount of fertilizer applied is the amount the crop received.

When the variable of recovery of fertilizer has been taken into account, it has been shown that a nitrogen deficient wheat plant is capable of a grain yield response to nitrogen applied at any stage during the vegetative phase of development (Watson 1936; Thorne 1962). In this context the vegetative phase has been taken as the period from germination to the commencement

of stem extension, which leads to ear emergence. This conclusion is of key importance for the stratagem of deferment of application of nitrogen.

The ability of the plant to respond to relatively late application of nitrogen results from its pattern of development. Grain yield is the product of a number of components, which are each determined at different stages in growth. The components exhibit considerable plasticity of expression, and those that are determined late in development can respond to a late increase in the nitrogen supply. The plant is capable of an increased number of ears in response to nitrogen application throughout the vegetative phase, either through an increase in the number of tillers or in tiller survival (section 2.2.2.a). When nitrogen is applied in the latter half of the vegetative phase, spikelet number in the ear of early-formed tillers is likely to be already determined; but a response may be produced in floret number per spikelet, floret fertility, and grain size, as these attributes are determined respectively near the end of the vegetative phase, early in and during the course of the reproductive phase. All yield components of ears on tillers initiated in response to the application of nitrogen may be influenced by the improved nitrogen supply, although the shorter period available for the development of later-formed tillers reduces their potential influence on the grain yield.

The restriction of yield response to application of nitrogen during the vegetative phase reflects the indirect nature of the influence of nitrogen on grain yield (Watson 1952). Increase in grain yield results chiefly from the increase in carbon assimilation that follows from an increase in leaf area. Since the rate of leaf growth and the propensity for tillering either decrease markedly or terminate at the end of the vegetative phase (section 2.2.2.d), there is little scope for increase in leaf area beyond this time.

Where recovery of fertilizer-nitrogen by the plant has not been variable in time, the grain yield response of wheat to nitrogen applied within the vegetative phase has not been found to decline with lateness of application (Watson 1936; Thorne 1962). This result is surprising, as the potential for increase in leaf area declines as the vegetative phase progresses, and the number of unexpanded leaves on existing tillers whose size may be influenced, and the time available for leaf development on initiated tillers, decrease. Similarly, the potential for increase in the grain yield components decreases. The yield components of existing tillers become progressively determined, and the time available for development of the yield components of initiated tillers shortens.

The following reasons may explain why grain yield response is sustained with relatively late application of nitrogen. It has

been shown that the last-formed (flag) leaf and the ear of the tiller provide most of the assimilate for grain filling (Thorne 1965). An appreciable potential for response may remain until late in vegetative development, because these organs do not undergo enlargement until the end of the vegetative phase. Secondly, the rate of development (both ear and leaves) of later-formed tillers may be more rapid than of early-formed tillers, either because of rising temperature, or because of the large leaf area and more extensive root development later in the vegetative phase. Thus, the ability of later-formed tillers to influence grain yield may be greater than would be expected from the period available for their development. Thirdly, recovery of nitrogen following application tends to be rapid, and completed in a relatively short period after application when moderate rates are supplied (Viets 1965). Enhancement of the nitrogen supply tends to be transient. Thus, there may be little difference in the nitrogen content of the plants at the time of flag leaf and ear expansion resulting from an early or a delayed application of fertilizer. The total nitrogen content of the plant often represents the nitrogen supply determining growth during the later phase, because nitrogen uptake after flowering is often limited and nitrogen used in preceding growth is largely remobilized for further utilisation (Williams 1955).

Application of nitrogen to wheat after the end of the vegetative phase does not increase grain yield, but the concentration

of nitrogen in the grain usually increases (section 2.2.2.f). It is evident that nitrogen absorbed by the plant, which is not utilised in a vegetative growth response, is translocated to the grain. Application of nitrogen at any stage of development may increase the grain nitrogen content, depending on the amount recovered and the grain yield response, but the likelihood increases with increasing deferment. Applications are potentially effective as late as the middle of the grain-filling period (Finney et al. 1957). Late-applied nitrogen that increases grain nitrogen content rather than yield appears to be largely assimilated into protein (Reeves 1954; Finney et al. 1957), in a similar way to nitrogen that is remobilised from the plant tissues in the normal course of development (Woodman and Engledow 1924). At the present time no additional remuneration is given to the Australian grower for grain of higher than standard protein content. However, the possibility of increasing the crop grain protein level during the course of the season by late application of nitrogen fertilizer remains of potential value.

The growth response of a crop to application of nitrogen depends upon its state of nitrogen deficiency, and thus upon the native mineral nitrogen supply from the soil. Nitrogen is the nutrient most commonly limiting the rate of growth of crop plants, and the dependence of high grain yield on nitrogen supply is well known (Viets 1965). It appears that, provided the crop is nitrogen-deficient before treatment, and the nitrogen applied can

be recovered, grain yield response may be obtained from application of nitrogen until a late stage of vegetative development. Where these conditions have been fulfilled in field experiments, yield responses from deferred applications of nitrogen within the vegetative phase have been substantial, sometimes surpassing those obtained from sowing applications (e.g. Mason, Rowley and Quayle 1972), as occurred also in Experiment B.

Where nitrogen is lost from the soil following application, recovery by the crop from deferred application is potentially greater than from sowing time application, because the greater root development then allows more rapid uptake. The implications of this phenomenon have been discussed by Srivastava (1970). This factor, coupled with that of variation in fertilizer loss at different times of application, is responsible where graater yield response is obtained from deferred than from sowing time application of nitrogen (Mason, Rowley and Quayle 1972). In southern Australia this advantage tends to be offset by an increasing likelihood of drying of the topsoil as the season advances. For reasons indicated earlier (p. 214), recovery of nitrogen applied to a dry soil surface is dependent upon following rain, the likelihood of which decreases as the season advances (Trumble 1948). Drying of the topsoil before application of fertilizer in unfavourable seasons may account for some of the poor responses obtained from deferred application of nitrogen in previous experiments in southern Australia (Chapter 1).

It would be of advantage to a stratagem of deferment of application if a path of supply were available which was independent of rainfall for its effectiveness. Cursory examination of the literature suggests that foliar absorption provides such a path, particularly for nitrogen in the form of urea (section 2.1.2). Foliar uptake of urea solution has been shown in certain circumstances to be rapid, quantitative, and unimpeded by drying on the leaf surface (Wittwer, Bukovac and Tukey 1963). However, in Experiments A and C foliar uptake was poor. Substantial losses of urea from the foliage following application were measured, and little growth response was obtained. Presumably, either poor absorption or assimilation of the urea were responsible. A similar result has recently been obtained with wheat elsewhere (Alkier, Racz and Soper 1972). Closer examination of the literature shows that the supposition that foliar uptake represents an effective path of nitrogen fertilizer supply to cereal plants is not well founded (section 2.1.2). Much attention has been given to the foliar absorption of urea, but studies have seldom extended to observation of the subsequent growth response. Relatively little work has employed gramineous species. When these circumstances are taken into account, together with ambiguities of technique in certain instances, it becomes apparent that the results obtained here do not conflict with previous evidence (section 3.4). It appears probable that nitrogen that is 'foliar-applied' to field crops

is predominantly taken up from the soil, either from the material not intercepted by the foliage or from that washed off the foliage by subsequent rainfall. The poor growth responses obtained in southern Australia in the past from deferred applications of nitrogen as foliar spray (Reuter 1967; Russell 1969) may be partly accounted for by ineffectiveness of foliar-applied urea. Recovery may be dependent on rain following application, and thus suffer from the disadvantages of nitrogen application to the soil discussed previously. Volatile loss of urea from the leaf surface may also occur (section 3.4). In view of the potential advantage of foliar application of urea in southern Australia, further work to ascertain the reasons for its ineffectiveness on wheat should be undertaken.

It should be noted that the wheat crop is better able to utilize urea applied as foliar spray after ear emergence, and late sprays generally increase grain nitrogen content (section 2.2.2.f). Apparently the ears absorb urea from solution more efficiently than do the leaves (Petinov and Pavlov 1960).

The cultivars used in Experiment B were shown to differ in responsiveness to deferred application of nitrogen. This response was not due to differences in the recovery of applied nitrogen. Clearly it is important to test new fertilizer practices on a range of cultivars. Established cultivars have been bred for adaptation to existing fertilizer practice, and suitability for a new practice is likely to be fortuitous. Part of the failure of deferred

applications of nitrogen to produce worthwhile yield responses in previous trials in southern Australia (Chapter 1) may be due to the chance use of an unsuitable cultivar. In two series of such trials in South Australia (Reuter 1967; Russell 1969) the cultivar 'Gabo' was employed, which in Experiment B showed a relatively poor response to delayed application of nitrogen. In earlier experiments in South Australia (Birks and Cole 1930; Richardson and Gurney 1935) the cultivars were not of sufficient interest even to be named.

The attributes that might lend adaptability to a stratagem of deferment of nitrogen application, which might be sought in a cultivar, may be suggested from earlier conclusions. The most suitable cultivar in Experiment B, 'Halberd', showed superiority in all grain yield components rather than in any one component. Since most grain yield components are not determined until late in the vegetative phase this was not surprising. The maintenance of high values of all other yield attributes by 'Halberd', despite an increase in ear number, suggests a relatively large contribution of grain from the tillers initiated in response to nitrogen application. The number and the rate of development of late-formed tillers initiated in response to nitrogen application, and the responsiveness of unexpanded leaves on existing tillers, particularly of the flag leaf, seem likely to be of importance in a stratagem of deferment of application of nitrogen.

It was shown in Chapter 1 that in southern Australia the water relations of the wheat crop are of critical importance in relation to response to nitrogen application. The results of recent investigations suggest that reserves of water below 75 cm in the soil are not utilised efficiently by the crop, at least in some situations, because of insufficient root development in depth (Walter 1971 ; Schultz 1971). The results of Experiment C were interpreted to show that deferred application of nitrogen might allow more extensive root development lower in the soil profile than application at sowing. This was because of greater seminal root growth, and higher rates of seminal root elongation. This hypothesis needs to be tested in soil culture.

The premise that in a water-scarce environment the yield of the crop will be benefited by greater root development to enlarge the water supply is contrary to that proposed recently by Passioura (1972). Passioura showed that the restriction of seminal root growth to one seminal root can lead to a greater grain yield in a water-scarce environment than if unrestricted root growth is permitted. This happened because relatively unrestricted root growth and water use exhausted the water supply before crop development was complete, causing a depression in grain yield. Premature depletion of soil water is often induced in low rainfall seasons in southern Australia by the application of nitrogen, which increases the rate of water use (Chapter 1). Although it is

important to avoid premature exhaustion of the crop water supply, restriction of the water use in good seasons may also limit the potential yield of the crop. The stratagem proposed here seeks to realise the greater yield potential in relatively wet seasons, when water supply is not likely to be heavily depleted before anthesis. In such seasons, more rapid extraction of water at depth through deeper root development would be beneficial, insofar as turgor could be maintained for longer periods.

A stratagem of deferment of the decision whether or not to apply nitrogen permits conservation of water in two ways: in the event of a dry early season leading to a decision to withhold fertilizer a relatively low water use results from the reduced growth of the crop, and this is appropriate to the relatively low supply of rainfall; where N fertilizer is applied late, the time course of leaf development is such that water tends to be conserved early in the season, relative to a sowing time application of fertilizer.

Benefits to yield resulting from relatively low plant density under conditions of limited water supply have been demonstrated (Du Plooey and Le Rouk 1968; Pelton 1969). However, this was not found in Experiment B, where the crop did not develop leaf area rapidly enough to experience significant water stress in the particular season. At reduced plant density the relative importance for grain yield of tillers initiated in response to

nitrogen application is increased. The interplay of plant density and deferment of nitrogen application warrants further study in a range of seasons.

Careful examination of the agronomic aspects of the stratagem of deferment of nitrogen fertilizer application has shown that the potential exists for it to succeed. An insight has been gained into the nature of the growth response. Probable reasons for disappointing results obtained in some previous field experiments have been discerned. More pertinent field trials now need to be designed, taking account of the conclusions reached in this study.

Appendix 1

"The growth of root systems - a numerical computer simulation model"

D.R. Lungley (1973) - Plant and Soil 38, 145-159.

Lungley, D. R. (1973). The growth of root systems — A numerical computer simulation model. *Plant and Soil, 38*(1), 145-159.

Appendix 2

Tabulated data

Table A1

Tiller number per sq. metre, Experiment B:

(a) main effects of Sowing rate, at four harvests;

(b) main effects of Cultivar, at four harvests;

(c) Sowing rate x Cultivar interaction, harvest 1;

(d) Sowing rate x Cultivar interaction, harvest 3.

		Harvest			
		1	2	3	4*
(a) Sowing rate:	S_1	43	66	111	100
	S_2	149	199	233	197
L.S.D.(P=0.05)		32.2	22.2	16.7	31.1
(b) Cultivar:	M	10	128	238	171
	Q	72	111	137	119
	H	113	150	152	144
	P	107	142	156	122
L.S.D.(P=0.05)		8.3	15.6	11.7	16.7

* Number of ears per sq. metre at harvest 4

Table A1, continued

(c) Harvest 1

		Cultivar				L.S.D. (P=0.05)
		M	G	H	P	
Sowing rate:	S_1	45	32	49	49	11.5
	S_2	165	120	180	168	
L.S.D.(P=0.05)			48.2			

(d) Harvest 3

Sowing rate:	S_1	122	83	107	107	17.7
	S_2	189	194	189	203	
L.S.D.(P=0.05)			30.6			

Table A2

Total shoot dry weight per hectare (kg), Experiment B: main effects of Sowing rate at four harvests.

		Harvest			
		1	2	3	4
Sowing rate:	S_1	42	83	1383	2360
	S_2	100	278	1971	2832
L.S.D.(P=0.05)		28	n.s.	215	n.s.

Table A3

Shoot dry weight per hectare (kg), Experiment B:

(a) Sowing rate x Cultivar interaction, harvests 1 and 2;

(b) Sowing rate x Nitrogen interaction, harvests 1 and 2.

(a)

		Cultivar				L.S.D. (P=0.05)
		M	G	H	P	
Sowing rate:	S_1	17	13	17	25	13
Harvest 1	S_2	78	50	72	61	
L.S.D.(P=0.05)		33				
	S_1	99	72	83	72	33
Harvest 2	S_2	351	223	238	228	
L.S.D.(P=0.05)		199				

(b)

		Nitrogen treatment				L.S.D. (P=0.05)
		N_o	N^1	N^2	N^3	
Sowing rate:	S_1	11	22	17	17	14
Harvest 1	S_2	61	89	55	61	
L.S.D.(P=0.05)		33				
	S_1	72	99	89	67	61
Harvest 2	S_2	200	372	239	217	
L.S.D.(P=0.05)		206				

Table A4

Shoot nitrogen uptake per hectare (kg), Experiment B

Sowing rate x Nitrogen interaction, harvests 1 and 2.

		Nitrogen treatment				L.S.D. (P=0.05)
		N_o	N^1	N^2	N^3	
Sowing rate:	S_1	0.4	1.1	0.7	0.6	0.9
Harvest 1	S_2	1.7	3.4	1.8	1.8	
L.S.D.(P=0.05)			5.0			
	S_1	2.8	4.4	4.2	2.2	3.1
Harvest 2	S_2	6.1	12.2	8.9	7.2	
L.S.D.(P=0.05)			11.7			

Table A5

Influence of time of supply of additional nitrogen to the roots on the mean length of the apical unbranched zone of first-order laterals (cm), within selected axis segments, at 4 times after germination (Experiment C):

(a) first seminal root

(b) first nodal root

		Time of N addition				
		N_1	N_R^1	N_R^2	N_R^3	2 x pooled standard error ‡
	Day					
(a) Seminal 1						
0 - 10 cm axis segment	27	4.1	3.3	- *	-	1.4
	38	3.8	4.6	-	-	1.2
	52	4.5	2.2	2.6	-	0.9
	94	3.2	1.2	0.9	2.2	0.6
40 - 50 cm axis segment	52	7.8	1.5	4.3	-	1.0
	94	2.5	0.9	0.7	1.7	0.5
90 - 100 cm axis segment	94	2.8	1.6	0.9	0.8	0.5
(b) Nodal 1						
0 - 10 cm axis segment	52	2.3	2.1	2.2	-	1.2
	94	1.6	1.3	0.7	1.8	0.9
40 - 50 cm axis segment	94	3.0	1.2	-	-	1.1

‡ Bartlett's test indicated homogeneity of variance over all treatments (P=0.05)

* Measurements were not made until N treatments had been imposed

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