EFFECT OF COLD ROOT TEMPERATURE ON GROWTH OF WHEAT

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Patricia Mary Joan Hallem

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Plant Science

May 1981

EFFECT OF COLD ROOT TEMPERATURE ON GROWTH OF WHEAT

BY

Patricia Mary Joan Hallem

A thesis submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

MASTER OF SCIENCE

ACKNOWLEDGEMENTS

I wish to thank everyone who helped and encouraged me throughout this program, especially Dr. W. Woodbury, and my husband, Tom.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF TABLES (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

ABSTRACT

Hallem, Patricia Mary Joan. M.Sc., The University of Manitoba, 1981. Effect of Cold Root Temperature on Growth of Wheat. Major Professor: W. Woodbury.

The effect of low soil temperature on the growth of spring wheat (Triticum aestivum cv. Glenlea) was studied under growth chamber conditions.

Rate of seedling emergence was reduced progressively as the soil temperature was reduced from control (16.5°-17.5°C) to 12°C to 8°C.

Low soil temperature (12°C) was found to have no effect on the dry weight of wheat roots, but these roots maintained a high fresh weight longer than controls. Morphology, as well as anatomy, indicated that roots grown at both low temperatures (12°C and 8°C) maintained living cells throughout the cold treatment.

Low soil temperature (12°C) suppressed the influence of plant nitrogen status on root growth. In the control treatment, high nitrogen plants had roots with low fresh and dry weights, while low nitrogen plants had roots with high fresh and dry weights. 12°C plants maintained similar root weights until temperature controls were removed, whereupon the root weights

fluctuated.

Root/shoot ratios were higher in 12°C plants. Adjustments in ratios at about 19 days post-emergence (controls) and 24 days post-emergence (12°C) indicated the occurrence of internal changes at about this time.

Total leaf protein was similar in both treatments; total leaf chlorophyll was greater in 12°C plants; total leaf amino acids were greater in controls. Due to a lag in senescence of leaves, 12°C plants maintained functional protein and chlorophyll longer than did controls.

Living tillers persisted to the end of the experiment in 12°C plants, whereas in control plants, tiller number fell sharply from day 24 to the end of the experiment.

12°C roots showed numerous anatomical adjustments. Root diameter and cortex radius increased. The number and radius of central metaxylem vessels increased. An external carbohydrate layer was present.

Overall, 12°C plants exhibited a lag in growth and senescence of five to ten days behind control plants. They were also reduced in height. No damage due to water stress was apparent, as may have been expected from cold soil, but the smaller plants maintained organs (leaves and roots) over an extended period. The modifications in root anatomy would decrease the resistance to water flow into the root and up to the shoot and ultimately to the developing grain. The carbohydrate layer may reduce the danger of root and soil shrinking away from each other during water stress.

INTRODUCTION

Little previous work has been done on the effect of cold root temperature on the overall growth of temperate cereals, especially by growing the plants out in a soil. Such work would be valuable in western Canada, as spring soil temperatures are cold at seeding time, relative to ambient air temperatures, and even though the soil usually contains ample moisture, the low temperature would render it less available.

The present research attempts to simulate field temperature conditions at planting time in the Red River Valley. 12°C has been called an optimum temperature for wheat root growth (Wilkinson, 1967); 8°C is a root temperature used by Clarkson (1974, 1976) in his studies. These temperatures are probably within the range of temperatures encountered by wheat roots in early spring (Neustad and Woodbury, personal communication). Plants were examined for effects of temperature stress as well as water stress induced by the cold.

It is also hoped that a thorough study of wheat plants, emphasizing their root conditions, and growing them in their natural environment, soil, would call attention to the importance of roots, and of monitoring root conditions, during experiments, to obtain a complete picture of plant growth. The information gained on biochemical components (chlorophyll, protein, amino acids) and physical characteristics, points out that 12°C soil temperature does not impair functioning of the plant, and indeed renders it more efficient in terms

of growth. Many of the results agree with the results reported by other workers studying temperate cereals: Power et al, 1963, 1970, with respect to barley; Sojka et al, 1975, and wheat; Case et al, 1964, and oats.

The most important results are the least conclusive, but hopefully, the most exciting and stimulating to future researchers. They demonstrate that wheat root anatomy, far from being a rigid characteristic, is flexible and capable of adjustment in a changing environment. Low experimental soil temperature induced a cross-sectional (especially with respect to the xylem vessel number and diameter) change towards lowered resistance to fluid uptake, and therefore greater availability of soil solution.

LITERATURE REVIEW

Soils and Plant Growth

The influence of the soil on the growth of any crop plant, that is, the direct influence on the germinating seed, and then the root system, and the indirect effect on the shoot, is of fundamental importance in the understanding of the physiology of that plant. Nevertheless, many workers ignored this relationship in their studies and explanations of plant behaviour. Hydroponics is culture without soil or microorganisms, using instead a sterile, aerated nutrient solution, or an inert medium and nutrient solution. Conclusions drawn from this type of experiment are useful only in relation to other soilless culture experiments; they cannot be used to explain phenomena observed on plants grown in soil. In fact, results of hydroculture often differ substantially from results of soil culture (Nielsen et al, 1960; Case et al, 1964; Rovira and Bowen, 1973). Other workers used a conventional soil mix to grow out their experimental material, or they planted in the field, but often they assumed that the root system and its environment had no effect on the rest of the plant. This assumption was made for the sake of simplicity, but again the conclusions drawn from any such experiment should not be applied indiscriminately to all work. Most workers failed to report the conditions under which their roots grew, or even to hold them constant while varying the environment of the tops. In such a case, it is difficult to say how much of the experimental

variation observed was actually due to changes in root growth and metabolism.

Soil Water

Soil is a very complex system, and each of its properties is irrevocably affected by all the others. Soil water influences crop growth, but also interacts with soil minerals and dissolved salts, soil gases, and the soil particles themselves, to jiggle the rhizosphere to a new but temporary equilibrium.

The soil water status is expressed in terms of soil water potential, and, as in diffusion, water moves from a higher to a lower potential. The principle components of water potential are matric potential (capillary and surface forces and hydraulic conductivity), osmotic potential, and pressure potential (hydrostatic potential). Each soil was found to be unique with respect to the relative values of each component of total water potential (Hunter and Erikson, 1952; Pawloski and Shaykewich, 1972; Ward and Shaykewich, 1972); this was important in estimating total water availability in a particular system. Water movement takes place in the soil in response to gravity, capillarity and so-called suction by the plant root. Such movement will take place as long as the soil is at a water content between saturation and permanent wilting point. Water flow through saturated soils is determined by the hydraulic force and the ease with which soil pores permit movement. The hydraulic gradient can exist in both a vertical and a horizontal direction,and is the difference in height of water above and below the soil column. Water table is an important component

of hydraulic force.It has been stated that water flows only through pores of a certain minimum diameter (Voorhees et al, 1971; Taylor, 1974; Cannell, 1977); drainage would therefore be seriously impeded in a compact soil of high bulk density. There is no available water in soils at or below permanent wilting point, and consequently, water movement is so slow as to be unable to maintain the plant. The water remaining in the soil is tightly bound in the smallest micropores and around individual particles.

The rate at which water moves through the hydrological cycle, alternating between the liquid and vapour phases, depends on the energy available at the plant leaf (stomatal cavity) or the soil surface. Water is pulled through the plant by transpirational flow; if the soil is at a water potential below wilting, or if root absorption is inadequate, the plant will wilt. At night, or on a very humid day, the plant is not transpiring. and there is minimal upward movement of water through the xylem, whereas in daylight, transpiration begins, a potential gradient is set up between the air surrounding the leaves and the external soil water, and water is essentially sucked through the plant via the xylem.

Water has a high heat capacity and high thermal conductivity and diffusivity; wet soils warmed faster and retained heat longer (Rosenberg, 1974). The intrinsic viscosity of water, measured in g/cm/sec, increases as its temperature decreased, so that the flow of cold water to roots would be slower. Changes in plant growth with a sub-normal rooting temperature may be due to water stress, as well as temperature stress.

While excess soil water could exert its effects mainly by the exclusion of air, a water deficit could influence plant growth in many ways. Germination rate was considerably lowered by extreme water tensions, although final germination per centage may not have been impaired (Pawloski and Shaykewich, 1972). The rhizosphere of the established plant was mainly confined to a volume of soil sufficient to meet the transpirational demands of the current foliage; as the soil moisture was used up, roots grew outward along moisture gradients, toward more vailable water supplies. If a part of a root was subjected to water stress, compensation by other roots maintained a constant uptake (Lawlor, 1973). If the water table dropped progressively over the growing season, the roots grew along the receding front, to very great depths. Potential danger existed where plants enjoyed an ample water supply early in growth, but a sudden drought occurred, and they were left with a shallow root system, inefficient in seeking out and utilizing distant reservoirs. Root hairs were usually absent in plants growing at low soil water potential, although laterals may be much more numerous. Roots growing in moist, aerobic soils exhibited prolific root hair growth. Root caps were often covered with a layer of mucilage, a lubricant; roots growing through dry soil showed a greater exudation of carbonaceous material (identified by ^{14}C), possibly mucilage, near the root cap area (Martin, 1977). This was perhaps a compensatory feature for the higher friction coefficient of dry, as opposed to wetted, soil particles, but the mucilage may also serve to maintain a contact between soil and root surfaces under conditions of high transpiration, where the root may have shrunk as a result of water loss (Huck et al, 1970).

Overall plant growth under water stress was reduced, but the extent is unknown. Plants that were acclimatized early to growing in soil below field capacity grew successfully and yielded respectably (Passioura, 1972). Non-acclimatized plants reacted to stress by wilting, reduced growth, tissue damage and even death. Some kinds of plants, for example. those that readily close their stomata during the hottest part of the day, and those that employ the Crassulacian acid matabolic pathway, have become adapted to water stress tolerance.

Soil Temperature

The soil temperature, with its diurnal and annual periodicity, has a most important effect on plant growth, and yet, comparatively few studies have been done using soil temperature as one of the variables, because it is so hard to separate its effects from, for example, water or oxygen stress. The daily temperature course is determined by solar and terrestrial radiation, and exhibits a maximum just after noon, and a minimum just before sunrise. The amplitude of the variation decreases with depth, to about 30 cm, where it disappears. Surface variation is small in winter, when snow insulates the ground, and on mulched or cropped land, or in wet soils.

The annual course of soil temperature, in temperate latitudes, is usually characterized by one maximum, in July or August, and by one minimum, in January or February. Amplitudes of annual oscillations also decreased with depth. On an average, the depth of penetration of annual fluctuations varies from 8-25 m(Shul'gin, 1965); below this depth, there was a layer of constant temperature. Observation of Canadian

soils has shown that heat content at 100 cm was large and constant all year; therefore the rooting volume of soil was warmed from the bottom and the top with the onset of warm weather in spring (Nielsen, 1974).

The soil structure also influenced its heat capacity. Since water has a high specific heat, but a low conductivity, relative to air, soils which retained water warmed up more slowly, but held the heat more tenaciously, especially near the surface, and therefore showed a more constant temperature throughout the growing period. Darker soils, such as those high in organic matter, had a lower albedo or reflectivity, and a greater capacity for heat absorption.

Soil temperature affected plant growth at every stage. Water absorption by seeds of wheat and corn increased with increasing temperature, reaching a maximum at 35^{o}C (Chaudhary et al, 1971), and resulting in a greater rate of germination at higher temperatures. Seedling emergence varied over a range in temperature; the emergence curve had a maximum value characteristic of the crop. 'Cool' crops, such as wheat, peas and turnips, exhibited emergence maxima at lower temperatures (15-20^{o}C) than 'warm' crops, like cotton, sorghum, rice and melon (25-30^{o}). The 'cool' crops even showed signifigant but slow germination at 5^{o}C, and very reduced germination at 35-40^{o}C, while 'warm' crops were only inhibited at 40^{o}C (Singh and Dhaliwal, 1972). The low temperatures prevented actual germination, and caused improper seedling development, whereas at high temperatures, increased respiration rate and metabolic failure occurred.

Root zone temperature affected morphology and distribution of roots. At sub-normal temperatures, roots were usually whiter, thicker

and less branched than at normal temperature; at higher temperatures, roots became more filamentous (Ketellapper, 1960; Brouwer and Hoogland, 1964; Nielsen and Cunningham, 1964; Garwood, 1968; Abdelhafeez et al, 1971). The soil temperature profile influenced to some extent the rooting pattern. Roots were more prolific in the warmer zone near the surface, and in the subsoil, fewer, thicker roots with reduced activity would be present (Nielsen, 1974). Anatomically, roots grown at sub-normal temperatures showed elongated cells, due to delayed maturation (Burstrom, 1956; Varner et al, 1963), and a suberized endodermis much closer to the root tip (Brouwer and Hoogland, 1964). Shoots of plants with cold roots showed reduced leaf area and number of stomata (Herath and Ormrod, 1965). The data suggested a water stress interaction as well. Kleinendorst and Brouwer (1970), in studies on corn, suggested that lowered root temperature induced water stress in shoots, as a result of reduced permeability. Growth could only resume as the osmotic value of the cell sap approached normal, and turgidity recovered and cell expansion resumed.

Dry matter accumulation in the laminae of rooted bean leaves was greatest at the lowest temperature rooting medium (Humphries, 1967), but wheat plants had decreased tillering and dry matter accumulation in $9^{o}C$ soil (Sojka et al, 1975), as compared to higher soil temperatures, 15.5^{o} and $22^{o}C$. While the work by Humphries on rooted bean leaves illustrated nicely the source/sink relationship between root and shoot, this approach is too simplistic to be applied to the whole plant. The root functions as a sink for materials, especially carbohydrates, translocated from the top. However, it exercises an active role as

well, that of absorption of some nutrients and manufacture of some necessary growth factors, for example, cytokinins; hence, any stimulus that reduces the metabolic rate in the root slows down these processes and indirectly affects the shoot.

In tomato, higher transpiration rates in plants at higher soil and air temperatures was noted, but upon examination of the stomatal resistance, it was shown that plants at 22°C soil temperature showed pronounced stomatal closure at midday (Abdelhafeez et al, 1971). This again suggested a water stress effect, although optimal irrigation was maintained throughout the experiment.

There is little information on the effect sub-normal root temperature had on yield, either throughout the growing season, which is not a practical approach anyway, as annual plants are only exposed to cold soils early in their life, or at any period in the growing season. Nordin (1977) examined potassium and calcium uptake in roots of wheat plants after a period of pre-cooling. His data may be cautiously applied to plants germinated and grown in cold soil. Net uptake of both ions decreased during cooling; net calcium uptake recovered completely, while net potassium ceased completely, with no recovery. These factors would certainly affect final yield. Sojka et al (1975) found that tillering in wheat is reduced as soil temperature is lowered from 21° to 9°C, and Brengle and Whitfield (1969) add that tiller reduction at 12.8°C is compensated for by more kernels per head than at 18.3°C. Shul'gin (1965) examined tillering of wheat in the USSR, and concluded that, for winter wheat, average air temperature above 10°C, and maximum soil and air temperatures of 15°C, produced the greatest tillering

increment.

These responses may have in part reflected responses of the plant to vernalization. Low temperature hastened floral induction, which was promoted by a long-day photoperiod. In winter wheat, for instance, if the seed was vernalized and grown out under long day conditions, seven leaves would be produced per tiller, and the tiller number would be reduced, as would the number of spikelets per head. Spring wheats may retain some response to vernalization. An extreme example is seen in some of the Mexican semi-dwarf wheats. These lines were selected as day-neutral, in keeping with the aims of the CIMYT program to develop wheats with wide geographic adaptability. However, if they were vernalized, a single tiller developed early and both leaf number and spikelet number was reduced (Halse and Weir, 1974).

While responses to vernalization and to photoperiod were inherited independently (Halse and Weir, 1974), it is not clear how responsive Canadian varieties may be to these factors.

Root Growth and Metabolism at Sub-optimal Root Temperature

Root metabolism at sub-optimal root temperatures was invariably affected with respect to several parameters. Unfortunately, many discrepancies exist in the literature as to the duration of the cold treatment, the actual low temperature used, the stage of growth at which the roots were subjected to a low temperature, and the shoot temperature. Also, growth conditions previous to the experiment are very important, as they establish an equilibrium of root/shoot metabolism against which experimentally induced variation will be compared; these were different in almost every lab. In addition to these complications, there was no

comprehensive study of the effect of sub-optimal root temperature for any one crop. The temperate cereals, wheat, oats, rye and barley, can be conveniently classed together, and probable applications to one crop extrapolated from work done on another. More heat-loving crops, such as beans, corn and tomatoes, were also extensively studied, and appear to have many similarities as well.

Possibly the most obvious effect of cooling of roots was the change in weight relative to that of roots grown at the so-called 'optimum' root temperature. While individual crops experienced distinct minima, optima and maxima, growth was practically suspended below a soil temperature of 4^0C for most crops (Namken et al, 1974). But as the soil (or nutrient solution) temperature optimum was attained, dry weight accumulation was maximized; in fact, it was this standard (dry weight accumulation) which was often used to define optimum root temperature. However, optimum root temperature can only be defined for specific experimental conditions; it varied greatly with age of the plant, shoot temperature and environmental conditions. For oats grown in a pot in a greenhouse, and only subjected to regulated soil temperature from the fifth day to harvest at various stages of plant growth, Nielsen et al (1960) found that root growth measured in g dry weight per pot (with 10 plants in a pot) was maximum at 41^0F (5^0C), and thereafter decreased as soil temperature increased to 54^o, 67^o, and 80^oF (12.2^o, 19.4^o, and 26.7^oC) for all treatments. Exclusion of phosphorus from the fertilizer treatments resulted in the lowest root yield, but the trends all remained the same. Barley (Power et al, 1963) responded to added phosphorus in the same way. In this experiment, nine seedlings of barley,

previously grown at 15°C to the two-leaf stage, were potted together and then subjected to 9°, 15.5° or 22°C soil temperature (air temperature was 22°C). On any given day, greatest root growth occurred at 15.5°C soil temperature, but when dry weights were compared at equal stages of morphological development (three-leaf, four-leaf, tillered, headed, soft dough, mature), it was found that prior to heading, root growth (expressed as g dry weight per cm) at 9°C was less than the other two treatments, and was maximum at 15.5°C. By soft dough stage, dry weights at 15.5° and 22°C started to decline, whereas dry weight at 9°C continued to increase, and surpassed the other two treatments. It appeared that a period of adjustment was required for the barley plant to initiate rapid growth and development. At lower soil temperatures, the adaptation period was longer, but once the period was over, growth rates were similar. For wheat as well, 15.5°C soil temperature resulted in the greatest root dry weight per plant, and root dry weight at 9°C surpassed that at 21°C (Sojka et al, 1975). In this study, seedlings were grown to the three-leaf stage at 25°C, and only exposed to the experimental temperatures for 25 days, whereupon the whole lot was harvested. Therefore, no information regarding the rate of accumulation of dry matter was obtained.

In a study of a variety of crops, Brouwer (1962) showed that optimum root temperature for dry weight accumulation was a range rather than a specific temperature, for crops such as flax, strawberries, peas and beans, but rape, maize and oats do in fact have a point optimum. He found for oats that the optimum root temperature for dry matter gain

was 20°C. His work differs from others' in that all his plants were germinated at 20°C and then grown to harvest, after from two to six weeks in a nutrient solution, instead of in soil. The crops he studied all had a root temperature optimum around 15°-30°C, which was higher that that found for temperate cereals by other workers mentioned, all of whom grew their experimental material in soil.

It is unfortunate that most experiments were terminated well before yields could be noted, because all the experimental work cited above can only be applied to yield of roots. It must be remembered that, for all the crops listed above, financial profits are measured in terms of grain or fruits, and there is an equilibrium point beyond which the root may become a liability, and may lower yield by virtue of its size and its drain upon photosynthetic products.

Brouwer (1962) determined that, for strawberries, optimum root temperature for fruit dry weight accumulation was around 25°-30°C, whereas, for root dry weight accumulation, it ranged from 10°-25°C.

Barley root yields have been maximized at 6°-13°C (Korovin et al, 1961; Williams and Vlamis, 1962; Power et al, 1963, 1964, 1970; Mack, 1965). Grain yields were best at 18°C. Yield of oat grain has been greatest at 18°-20°C (Case et al, 1964), but with ample nitrogen, phosphorus and potassium, most roots were produced at 5°C. Nielsen et al (1960) found that, at heading and maturity, yields of foliage and grain or straw increased with temperature to 67°F (19.4°C) regardless of nutrient treatment, but at 80°F (26.7°C), all yields were depressed below those at 41°F (5°C). Wheat yields have been best with root zone temperatures of about 20°C, but it has been reported that wheat produced

fewer tillers at 12.8°C than at 18.3°C. However, at 12.8°C, there were 50% more kernels per head (Brengle and Whitfield, 1969).

The optimum root temperature for yield of cotton seemed to be about 28°-30°C (Letey et al, 1961; Pearson et al, 1970). The most favourable root zone temperature for rice was quoted by Owen (1971) as being around 25°-30°C. At the time of tillering, water temperature was most important, because the growing points were submerged, but once tillering was complete, yield potential was fixed, and air temperature became critical. At high water temperature, tillering rate was increased over a shorter period, so that as root and growing point temperature was increased, yield potential was decreased. This phenomenon exists in any tillering plant where yield is measured in fruits of foliage (as in grasses and forage crops). Peas grown at a root temperature of about 21°C had the greatest yield.

Root crops needed special consideration, as their potential yield was directly affected throughout their life cycle by the soil temperature. Whereas other crops had a yield potential measured by photosynthetic versus respiration rates of tops, which could be greatly reduced by a cold root's inability to supply water, minerals and some growth hormones to the developing shoot, root crops had a yield potential determined by the immediate environment (the soil), which could be altered by reduced photosynthesis and supply of translocated carbohydrate to the subterranean heterotropic organ. Army and Miller (1959) found that turnips had an optimum root temperature of 19°C in the autumn, but in spring it was 27°C; the difference in the spring was related to more sunshine. Potato production can be related to both number and size of

tubers. Tuber number was highest at a soil temperature of 10°C, and decreased steadily with increasing temperature. However, at cooler temperatures, smaller tubers predominated. Yields of sugar beet root were greatest at 24°C soil temperature (Radke and Bauer, 1969), but percentage sucrose was greatest at about 18°C (Ito and Takeda, 1963; Radke and Bauer, 1969).

It is important to notice carefully if yield of above-ground parts is quoted in grain or fruits, or in tops or foliage. It is true that leaf area and photosynthetic capability of any plant determine its yield potential by fixing the amount of carbohydrate available for grain filling and fruit production, but top yield is not the same as fruit yield. Thus Nielsen (1974) quoted several studies on beans, coffee, citrus and sugarcane in an ambiguous manner, referring only to 'top' growth. Brouwer (1962) made it clear that he distinguished between shoots and fruits in his studies on the effect of rooting solution temperature on relative dry weight accumulation of various crop species.

Differences in root weights at various temperatures have been observed for a variety of crops; however, there is little information about what makes up this greater root mass. In some cases, roots grown in soil were impossible to clean thoroughly, so any chemical analysis of their composition would be incorrect. Nielsen and Humphries (1966) pointed out that the size of the root system was not necessarily related to the shoot requirements, and so the root system of any plant may not always absorb water and nutrients to the full extent of which it is capable. The shoot, which is a sink for root assimilates,

controlled to a certain extent their rate of uptake. One theory underlying the root/shoot ratio was that of relating nitrogen assimilated to carbohydrate fixed (Troughton, 1974). The ratio could be affected by environment, but varied as the plant grew, and so would need to be interpreted with caution. In view of this, roots could be analysed for nitrogen as well as total carbohydrate, at different temperatures, to determine their relative ability to receive and store photosynthetic products from the top. The ratio between these two components, as well as any deviation from control conditions, would help to point out any possible metabolic 'abnormalities' in cold roots.

Most studies confined themselves to an analysis of various nutrients, especially nitrogen, phosphorus, potassium and calcium, in roots grown in cold media, to get an idea of ion uptake under these conditions. Active absorption of ions depended on energy supplied by oxidation of carbohydrate through respiration, and absorption was slowed down by cold (Nielsen and Humphries, 1966). Entry of ion into the free space of roots was not temperature dependent, as it occurred mainly through passive diffusion.

Temperature had a definite regulatory effect on the rate of respiration, (Fujiwara and Suzuki, 1961), which supplied energy for ion absorption, as well as other root metabolic activities. Field studies, as well as greenhouse studies, often combined impaired aeration, achieved by flooding with water or nitrogen gas, with low soil temperature, to measure yields under conditions of reduced respiration (Luxmoore et al, 1973; Labanauskas et al, 1975). In each experiment, all done with wheat,

reducing soil oxygen, especially on a long-term basis (20 to 30 days), or increasing soil temperature to 25°C, drastically reduced grain yield. Low soil temperature (5° or 15°C), either at control aeration or under conditions of flooding, had little effect on yield reduction, in terms of grain dry weight per plant. It appeared then that cold soil did not impede the diffusion of oxygen to the respiring roots, and did not reduce respiration below that required for continued reproduction, but that it somehow reduced root metabolic rate. An increased yield might have been expected if the root functioned mainly as a sink for photosynthate, in competition with the developing grains, but no such increase was found when root respiration was impaired by cooling. However, long-term oxygen exclusion in the vicinity of the roots caused deformities, similar to the symptoms of excess shoot hormone.

The conclusions drawn in these studies pointed to a more active function for roots than just a sink for exports from the top. On the other hand, Jensen (1960) measured rates of respiration, by both carbon dioxide release and oxygen uptake, by intact roots of tomato and corn, and found that it approximately doubled between 25° and 30°C. At 25°C, Luxmoore et al (1973) found grain yields to be greatly reduced, compared to yields at lower soil temperatures. Such supra-optimal temperatures affected more than respiration rates; with biological reactions, the Q_{10} is often in the range of two to three, but may be higher with specific enzyme systems; at very high temperatures, enzyme malfunction and tissue damage can result.

Reduced plant growth rate at sub-normal root temperature has been

ascribed to nutrient deficiency. In fact, phosphorus deficiency and cold stress can both induce a characteristic purple anthocyanin pigmentation in affected plants.

Phosphorus as a soil constituent is immobile, and Sutton (1969) concluded that the soil temperature could reduce the availability and quantity of native phosphorus to plants. He suggested that the beneficial effects of fertilizer phosphate were mainly due to its increased availability and higher concentration in solution, at any soil temperature. In an analysis of oat roots harvested 43 days after emergence, Case et al (1964) found that phosphorus uptake increased as soil temperature increased from 15^{o} to 25^{o}C. In each case, phosphorus content of roots was greatest where it was applied in a more soluble form. Phosphorus content of shoots was also compared, and it evidently increased as soil temperature increased as well, showing that at higher root temperatures, phosphorus passed through the entire plant system more quickly, and accumulated in the tops.

Potassium uptake (using ^{86}Rb) was studied by Nordin (1977), and his work showed that influx into roots of intact wheat plants decreased with root cooling, and that potassium movement was restricted to the lower shoot, whereas potassium content of upper shoots decreased. Potassium influx recovered to almost normal rates 12 hours after return to normal control temperatures, whereas plants not provided with this recovery period did not resume normal potassium influx. Potassium leakage by diffusion was also reduced at low temperature, pointing to the probability that membrane permeability with respect to potassium may have been decreased by cold; upon return of plants to normal root

temperature, potassium leakage or efflux increased, so that net uptake was negative. This increase was explained by a higher permeability of the root cell membrane induced by return of roots to control temperature.

Nordin (1977) also studied calcium uptake and translocation in the intact wheat plant (using ^{45}Ca). Total calcium uptake in the plants decreased during root cooling, but contrary to potassium, recovered afterwards. The effects on calcium distribution resembled those of potassium, with the exceptions that no decrease of the calcium content of the upper shoot occurred, (calcium is naturally very immobile in the plant) and the increase of calcium in the roots was low. After cooling, the rate of increase of calcium in the roots was higher than in the controls, and an increase in calcium content of the upper shoot was observed.

Several principles concerning the mechanism of uptake of phosphorus and potassium, as opposed to calcium, can be called upon to explain the differences between the ions. Firstly, phosphorus and potassium were presumed to be translocated in the symplast of the root, whereas calcium travelled through the apoplast (Nordin, 1977; Pitman, 1977; Clarkson and Sanderson, 1978). Travel through the symplast entailed movement through the cytoplasm of one cell and the plasmodesmata linking adjacent cells to the cytoplasm of the next. The presence of high densities of plasmodesmata in even State lll endodermal cells would allow ready access to the stele and thence to the rest of the plant. (State 1 endodermal cells possess suberin in transverse and radial walls; in State ll cells, a continuous layer of suberin is laid down as

lamellae between the plasmalemma and the cell wall; and in State 111 cells, a thick layer of cellulose is deposited over the suberin lamellae (Clarkson and Robards, 1975; Pitman, 1977)). Pitman (1977) reported that various reviews on symplastic movement all concluded that it was a non-active process, but active processes and hence metabolic energy were probably required for initial entry into the symplast, and for cytoplasmic streaming which moved the ions through the cell. Viscosity of cytoplasm increased as its temperature decreased (Nielsen and Humphries, 1966).

Since there is no evidence in the literature of an active efflux of potassium from the roots, it seems probable that the lowered leakage from the root was caused by a decreased cell membrane permeability for the ion at low temperature. This could also account for decreased uptake. Calcium, on the other hand, was translocated apoplastically, through the cell wall, by diffusion and passive movement in absorbed water, and by ion exchange. Nordin's (1977) experiments with calcium also indicated a lowered membrane permeability during cooling, both in terms of efflux and influx, but immediately upon return of the plants to control root temperatures, calcium influx resumed at the control rate, and efflux declined. This was possibly due to a lower calcium concentration in cooled roots than in controls. This calcium may have been strongly bound to cell walls, or at least to cell interior. Indeed, Humphries (1951) showed that the uptake of one element by excised roots was inversely proportional to the concentration of that element in the root cells.

Transpiration rate greatly affects the flow of water and solutes through the entire plant, beginning with absorption at the root/soil

interface. Therefore, it is reasonable that conditions affecting transpiration rate will affect ion uptake; conversely, factors affecting ion solution influx will affect transpiration rate. Transpiration is controlled by stomatal opening and closing, due to changes in guard cell turgidity. Potassium has a general function in the regulation of water in plant cells, and it has been shown to act during osmotic adjustments of plants placed under stress (Rains, 1976). It was found that potassium was selectively absorbed by plants, and was one of the main ions involved in protecting the plant from losing water and becoming physiologically dry. More specifically, potassium accumulation by the guard cells, and the resulting increased turgor, caused the stomata to open. Abscissic acid was able to prevent potassium accumulation by guard cells within minutes of application (Varner and Ho, 1976).

Nordin (1976a, 1976b) examined the relationship between water-stressed wheat plants and their transpiration rates upon their return to control temperatures. (In both cases, root cooling, to $1^{o}C$ for 24 hours, a very low temperature for a very short time relative to other studies done, induced water stress in the plants.) The most important observation was that transpiration rate in stressed plants was much lower than in unstressed plants, and that there was a delay in transpiration upon stimulation of the plant by light after its return to control temperature. Nordin also observed microscopically that stomatal apertures were smaller in plants with cooled roots, when they were stimulated by light, and even after 24 hours in the control chamber, stomata did not open to the extent of those in control plants, which had not been subjected to the water stress. Nordin concluded that the

decreased water flow through the plant after removal of stress could be attributed mainly to increased stomatal resistance, and not to effects on root conductivity. This stomatal resistance could be induced by reduced potassium uptake by cooled roots (Nielsen et al, 1960; Nordin, 1976b). Net potassium uptake did not return to normal in cold-stressed plants which were returned to control temperature, thereby continuing a potassium deficiency already induced by cold treatment. However, in a subsequent paper, Nordin (1977) concluded that potassium deficiency and resultant guard cell flacidity were not the reasons for impaired transpiration. Rather, water stress and/or abscissic acid was implicated.

Nordin based his conclusions that shoot rather than root influences were responsible for water stress and transpiration lag on duplicates of all his experiments, using excised tops in both control and test situations. Clarkson et al (1974) and Clarkson (1976) studied exudation by detopped barley roots held at low temperature, but they included a pre-treatment at the selected low temperature as part of the experimental regime. In principle, their conclusions contradicted those of Nordin, although they found that a low pre-treatment temperature appeared to produce an adaptation to the imposed stress, and exudation rate (although not the water potential, expressed as concentration of potassium and other inorganic ions) was increased by the adaptation period. A decreased resistance to water movement, possibly through an increased hydraulic conductivity of the cooled root, was implied. Consequently, the root did play an active part in regulating water content of the whole plant, but only after a period of adjustment.

This approach is more realistic than the 24-hour isolated treatment period of Nordin; his conclusions may have been correct, where roots had no adjustment period, and were acting as truly stressed organisms, whereas Clarkson's may have adapted, and were no longer in a state of temperature stress. However, the potassium concentration of the sap was not altered and this reinforced Nordin's theory that reduced transpiration and water stress in the plant were not due to deficiency in the guard cells. Clarkson et al (1974) began by implicating abscissic acid, and the abscissic acid/cytokinin ratio in the plant, as the reason for altered water movement in the plant as a whole, but after study, they rejected this hypothesis, and ascribed the differences in exudation rate to changes in the membrane of root cells induced by low temperature.

Clarkson's various studies were the only ones to observe influx of water and ions after a pre-treatment period. (Some of Nordin's experiments employed a post-treatment period of adjustment and recovery.) Nordin (1976a) found that water stress could be induced by subjecting roots of three-day old plants to two hours of mannitol in the nutrient solution, or to periods of cooling, or to one hour of drying. Clarkson (1976) found that a pre-treatment period of 12 to 72 hours allowed for an adaptation mechanism to be implemented; Power et al (1970) proposed that a period of adjustment was required for plants grown at soil temperature below the optimum for dry weight accumulation, whereupon their growth rate approached 'normal'. It would seem that experimental conditions which most nearly approximate natural conditions would yield the most valuable information. The information gleaned by Clarkson and

his various co-workers agreed with positions held by students of root anatomy, that water stress, for instance, when induced by cold soil, caused a structural change in the plant which allowed the plant to maintain adequate water content. A review of the anatomical and morphological literature will follow.

Nitrogen uptake and assimilation have been investigated as well, since they occur primarily through the roots. Unfavourable root temperatures, either high or low, resulted in accumulation of nitrate-nitrogen in plants, due to a reduction in nitrate-reductase activity (Younis et al, 1965; Nowakowski et al, 1965: Watschke et al, 1970). Protein synthesis, which occurred actively in the root, followed typical temperature laws, and in the cold environment, proceeded very slowly.

There is a dearth of information on the hormonal activity of the xylem sap exuded from cooled roots. Nordin (1976b) examined the effect of abscissic acid on transpiration rate of the excised shoots of plants whose roots had been subjected to cooling, but he did not analyze the roots of either experimental or control plants for changes in the concentration of endogenous abscissic acid. Clarkson et al (1974) concluded that the effects of cooling on both exudation and the accumulation of soluble carbohydrate in barley were consequences of reduced growth and the possible alteration of the relative amounts of growth substances in the root, specifically, abscissic acid and cytokinin. In a later publication, Clarkson (1976) introduced a different mechanism for altered exudation rate (membrane modification),

but did not disprove or refute the hormonal balance theory. Work done by Atkin et al (1973) on corn supplied more evidence for its validity. In analyses of xylem sap of 30- to 40-day old plants, grown in root media of 8^{o}, 13^{o}, 18^{o}, 23^{o}, 28^{o} and 33^{o}C, they found the greatest export of total cytokinin and gibberellin occurred at 28^{o}C, and the lowest export of an unidentified growth inhibitor occurred at 33^{o}C (the root temperature optimum for shoot extension growth). As the root temperature approached 8^{o}C, the sap contained more inhibitor and less cytokinin. At the lower root temperatures, root extension was restricted, root mass and branching decreased, and shoot growth was also restricted, suggesting that prolonged cold introduced an altered balance between the growth promoters (gibberellin and cytokinin) and the growth inhibitor.

In another study, on grapes, Skene and Kerridge (1967) analyzed and compared cytokinin activity in root exudate of detopped plants growing at 20^{o} and 30^{o}C. In the case of grape, 30^{o}C produced longer primary shoots, longer and thinner roots, and a more massive root system (in terms of both fresh and dry weights). Thus it appeared that, for overall plant growth, 30^{o}C was approaching the optimum. For both temperatures, major cytokinin activity occurred at the same location on a chromatogram, but sap from 20^{o}C roots contained an additional cytokinin, not present in the 30^{o}C roots. Skene and Kerridge speculated that temperature had an effect on cytokinin production or interconversion, or on the utilization of cytokinins by roots for their own growth. No conclusions were drawn.

Aside from these few studies, no information can be found reviewing root stress induced by cooling, and its effects on hormones exported from roots via the sap. Torrey (1976) made an interesting point, especially in light of the possible interaction of certain hormones, and the effect an abnormal balance may have on plant growth. Abscissic acid caused stomatal closure, and according to Torrey, abscissic acid originated in the shoot, and particularly in the chloroplast. Cytokinins caused stomatal opening, and they in their turn originated in the root. A certain mutant tomato, flacca, synthesized only about 10% of the abscissic acid that a normal plant did, but root exudate and leaves of the plant showed a much higher cytokinin content. These plants showed increased resistance to water absorption, due to the high cytokinin of roots, and abscissic acid treatment of the shoots increased root exudation rate. It was tentatively concluded that abscissic acid's effects on stomatal movement were largely localized in the leaf, whereas cytokinin's effects on stomatal activity may have originated in the root and been transported to the shoot (Tal and Imber, 1970, 1971; Tal et al, 1970). In cold-root-stressed cereals, abscissic acid concentration has not been studied, but concentration of cytokinin (in corn and grapes) was altered (Skene and Kerridge, 1967; Atkin et al, 1973). Transpiration rate was reduced in wheat (Nordin, 1976b). The mechanism, although not yet described, may be linked to a disturbed abscissic acid/cytokinin ratio in a stressed plant.

Low soil temperature has some indirect effects on root growth as well. In the natural environment of the plant, and even in greenhouse

and growth chamber studies where the soil is not sterilized, micro-organisms have a very important role in the breakdown of organic matter, and the release of mineral nutrients, into the root environment. In cooler soils, this rate of release is considerably slower, and may even stop, if the temperature range of the organism is exceeded, and it is either killed or becomes dormant.

At increasingly low temperatures, some of the physical properties of the soil phase are altered. Viscosity of liquid water increases. Solubilities of both gaseous carbon dioxide and oxygen increase. Solubility of ions is decreased as the solvent temperature decreases. All these seemingly insignificant effects combine to further limit the supply of water and nutrients to a plant which may have internal mechanisms to automatically limit absorption when its roots experience cold stress.

It is noteworthy that most studies grew plants initially at high temperature, for example, at 20^{o}C, and stressed them at a much lower temperature, for example, at 8^{o}C. This was unnatural in several ways. Firstly, the heat capacity of the soil would prevent abrupt transitions in root temperature. Secondly, in the field, surface roots (to a depth of 30 to 50 cm) would be exposed to repeated diurnal fluctuations in temperature. Thirdly, for spring planted crops, the trend is toward a gradual warming of the roots, from the surface down, rather than an abrupt cooling, as most labs employ. All the diurnal fluctuations and vertical temperature gradients would be very difficult to impose in the lab or greenhouse.

Top Growth of Plants with Roots Exposed to Cold Soil

The indirect effect that cold soil has on the growth of above-ground plant parts is varied, and by extracting information from the reports of different individuals or groups, a tentative scheme of this phenomenon can be realized.

Size, expressed as shoot dry weight, was monitored for wheat by Sojka et al (1975), and was greatest at 15°C, lowest at 9°C, and intermediate at 21°C. The reduction from maximum dry weight was 12% at 21°C, and 20% at 9°C. Barley followed the same general pattern, with maximum top dry weight (expressed in g/cm) occurring at 59°F (15°C), and declining on either side of this temperature (Power et al, 1963). In both these experiments, harvest was carried out before heading, so top dry weight comprised only stem and leaf tissue, and maybe unemerged heads, and should not be construed as an indication of grain yield. Unfortunately, studies of temperate cereals (wheat, oats, rye, barley) often quote top yield as dry weight. In view of the fact that cold temperature stress has been interpreted as water stress by many workers, a look at fresh weights as well as dry weights may yield some valuable information. Brouwer (1962) did quote fresh weights, but only for broad beans. For each leaf (first, third, fifth, seventh) fresh weight increased with root temperature to a plateau at about 20° to 30°C, where it declined sharply. It is noteworthy that Brouwer found 20° to 30°C to be the temperature range for roots of broad beans which produced the greatest relative dry weights of both roots and shoots. In other words, 20° to 30°C was optimum root temperature for broad beans.

Brouwer (1962) also reported that, at the lower root temperatures studied, the initiation of new leaves was much less retarded than the development of the leaves. This held true for all plants in his study: peas, rape, broad beans, red kidney beans, strawberries, maize, oats and flax. At $10^{o}C$, for example, the number of leaves differed only by one from the number present at temperatures ranging from 15^{o} to $30^{o}C$, but the fresh weight of the individual leaf was much less. At 5^{o} and $35^{o}C$, not only the fresh weight of the leaves, but also the number of leaves, was much smaller.

Brouwer (1962) and Power et al (1963) were in agreement with respect to the existence of a distinct optimum for maximum growth, although their explanations for it differed. Brouwer found that, shortly after planting, differences in growth at root temperatures in the middle of the range studied (15^{o} to $30^{o}C$) were very small, with only the extremes (5^{o} and 10^{o}, and $35^{o}C$) showing a direct growth-reducing effect. After a certain period of time, the optimum became more and more pronounced: with peas and red kidney beans, 29 and 20 days after germination respectively are the times necessary to establish a 10^{o} to $15^{o}C$ range for maximum fresh weight, and at 43 and 30 days after germination respectively, the optimum remained essentially the same, having narrowed to a specific temperature in the range. Although Brouwer admitted that a satisfactory physiological explanation for the growth schedule as yet remained elusive (as of 1962), at the time he attributed it to potassium uptake. Up to a temperature of $15^{o}C$, growth and potassium uptake were directly proportional, whereas at higher root

temperatures, initial growth was relatively more rapid, and available potassium more rapidly depleted, resulting in reduced amounts in the soil at later stages. Below 15°C, growth was inhibited by water deficiency.

The findings of Power et al (1963) with barley were the same: for the first few weeks after planting, plant development, expressed by plant height in cm, proceeded most rapidly at warmer soil temperatures, and thereafter declined. Growth at 59°F (15°C) was rather slow for the first week, but then increased rapidly, and soon surpassed 80°F (26.7°C). Plant development at 45°F (7.2°C) and 52°F (11.1°C) was similar to that at 59°F, except that the initial time lag of low growth rates lasted one to two weeks longer. At seven weeks after transplanting, growth rates of 80°F plants had declined, whereas those of plants at lower soil temperatures had surpassed them and showed no sign of decline. Power et al (1963) suggested that this was due to a lowering of optimum soil temperature as growth progressed, but it seems reasonable to apply Brouwer's thesis here, and attribute the decline in growth rate to depletion of available nutrient early on in the life cycle, and perhaps to crowding in the pot, reducing water and nutrient uptake per plant. There could also be an environmentally induced morphological and/or anatomical adaptation at work here. This possibility will be explored in the next section of this review.

Whatever the explanation, it appears that normal growth is in no way hampered by soil temperatures which are lower than those usually employed, and that the plants can adapt quickly and attain a normal growth rate.

Water content of plant parts was not included in most papers, and cannot be calculated because fresh weights were also omitted. However, water use data were quoted for barley by Power et al (1963, 1970), with nutrient uptake, specifically uptake of added phosphorus. Their work showed that final dry weights, grain yields and nutrient uptake of plants with roots at 9°C usually equalled or exceeded those at 15.5°C, the soil temperature considered to be optimum for barley production. Growth of 9°C plants lagged behind the others until the four-leaf stage, where it increased to approximately the growth rate at higher soil temperatures. Nutrient uptake and water use data suggested that this slow initial growth rate may have been due to restricted translocation from root to shoot, and not to reduced uptake of either water or nutrients by cool roots. Water use per g dry matter of tops was highest at 22°C soil temperature, and lowest at 9°C. In terms of leaf area, water use was lowest at 15.5°C soil temperature until after heading.

Nutrient uptake of cereals with roots exposed to a range of temperatures supported the hypothesis that reduced uptake was not to blame for slow initial growth, and that it was probably due to slower rates of metabolism and translocation which prevented the absorbed substances from reaching the tops, where their presence or absence would be revealed by chemical analyses. In fact, Nielsen et al (1960) found ions to be more concentrated in roots of lucerne grown at 5°C than at 12°C.

Case et al (1964), in a study of the influence of soil temperature and added phosphorus on oats, found that phosphorus uptake was highest at 15°C (optimum root temperature for oats), lowest at 25°C, and

intermediate at 20°C. They measured phosphorus uptake as mg per pot, with 20 plants to a pot. Nielsen et al (1960) also studied oats, and found that concentrations in shoots, on a dry weight basis, of nitrogen, phosphorus, and the cations magnesium and potassium, tended to increase with increasing temperature, from 41°F (5°C) through to 67°F (19.4°C), and to decline at 80°F (26.7°C), whereas calcium content decreased along the same temperature curves. 67°F root temperature produced the most root dry matter per top growth per pot.

Amount and availability of phosphorus in the soil appeared to have a significant effect on nitrogen uptake and assimilation, although Nielsen concluded that temperature should have little effect on uptake of phosphorus from soil rich in this element, and that this principle should apply to other elements. Therefore, at low soil temperatures, omission of supplemental phosphorus suppressed uptake of nitrogen, phosphorus and potassium (Nielsen et al, 1960), and reduced their concentration in tops.

Labanauskas et al (1975) studied nutrient uptake by wheat plants at 5°, 15° and 25°C, with respect to concentration and total amounts of a range of macro- and micronutrients in the grain. Plants grown at 25°C soil temperature yielded lower amounts of grain per pot than those grown at 5° and 15°C. Although the concentrations of most of the determined nutrients in the grain from plants grown at 25°C soil temperature were higher than from plants grown at 5° and 15°C, the total amounts per pot found in the grain of plants from the 25°C soil temperature were lower than the others. Nutrients considered were

nitrogen, phosphorus, potassium, calcium, magnesium and sodium (expressed as a per cent and as g per pot), and zinc, manganese, copper and iron (expressed as ppm and as total mg per pot). Only total sodium per pot and ppm zinc failed to show any significant differences; in every other case, the values for 5^{o} and 15^{o}C differed significantly from 25^{o}C.

Power and his co-workers (1963, 1970) studied content of nitrogen and phosphorus in barley. In terms of per cent total nitrogen in tops, the maximum amount occurred at the four-leaf stage for all soil treatments, 9^{o}, 15.5^{o} and 22^{o}C, although before the four-leaf stage, total nitrogen uptake for 9^{o}C soil temperature was markedly lower. At most stages of growth, soil temperature did not influence nitrogen uptake. In general, phosphorus content and uptake varied in a manner similar to nitrogen content and uptake, increasing until maturity for all treatments. In general, per cent total nitrogen and phosphorus in tops was highest for 9^{o}C soil temperature after the four-leaf stage, and lowest at 15.5^{o}C; total nitrogen uptake showed the opposite trends, being lowest in 9^{o}C soil treatment until the soft-dough stage, where it surpassed the other two treatments, which were similar and tended to even out at this stage.

The reasons given for these trends in nutrient uptake were the same as the explanations for the differences in dry weights at given experimental temperatures, and just as vague. It was suggested that low soil temperature had a direct effect on the physiology and metabolic activity of the plant, and effectively stifled nutrient translocation from roots to tops (in the case of phosphorus) (Case et al, 1964), or inhibited transformation of absorbed forms to forms utilizable by the plant (as in

the case of nitrogen) (Nielsen, 1974). Hence there was a buildup of ions in the roots, and top growth suffered quantitatively, although seemingly not qualitatively. It has also been suggested that cold soil has an indirect effect on plant growth by influencing the rate of breakdown of organic matter by soil microorganisms, thereby limiting the availability of the elements contained in the organic matter to roots.

If it is true that reduced growth rates at sub-optimal soil temperatures were partially or wholly due to reduced translocation of absorbed nutrients to sites of utilization in the tops, then it seems possible that translocation of photosynthate down the stem to the roots might also be affected. Fujiwara and Suzuki (1961) concluded that, with the top photosynthesizing portion of the barley plant maintained at optimum temperature for photosynthesis ($25^{o}C$), and the root at optimum temperature for respiration ($30^{o}C$), the rate of translocation of photosynthate down the stem would be maximized, and there would be minimal storage of sugars in the root, and therefore little increase in root dry weight. On the other hand, with the top temperature kept at $25^{o}C$, but root temperature lowered (in this experiment, to $15^{o}C$ minimum), translocation rate was lowered, but the root accumulated dry matter as its respiration rate was greatly reduced. These conclusions gave support to the theory that reduced plant growth at low temperatures was due to a metabolic slow-down in the roots. Respiration provided energy, and without it, active transfer of substances across the root and into the transpiration stream could not occur.

Also, it has been reported that root dry weight increased at low

temperatures; Power et al (1963) stated that low temperatures restricted top growth more than root growth, but that the opposite occurred at high temperatures. Brouwer and Hoogland (1964) provided a series of pictures of bean seedlings grown at root temperatures ranging from 5^{o} to $35^{o}C$; it is quite evident that at root temperatures approximating the optimum, root mass increased much more than tops, and at the highest temperature, root mass was drastically reduced.

Tillering and eventual grain yield are two functions of cereal growth which are closely connected. Both can be altered by soil temperatures other than the optimum. Nielsen (1974) reported that wheat grew more slowly at $12.8^{o}C$ than at $18.3^{o}C$, and produced fewer tillers. However, at the lower soil temperature, the plants produced 50% more kernels per head. Sojka et al (1975) found that tillering at every harvest date was maximized at $15^{o}C$ soil temperature, and at harvest, tillers per plant at $9^{o}C$ slightly outnumbered those at $21^{o}C$. Neither Luxmoore et al (1973) nor Labanauskas et al (1975) quoted data on number of tillers per plant, but they each found that yield of wheat grain was highest at 5^{o} and $15^{o}C$, as opposed to $25^{o}C$, both in terms of g grain per plant, number of grains per head, and mean grain weight. Barley showed the same response to soil temperature; tillers per plant and grain yield in g per plant were all greatest at $15.5^{o}C$ soil temperature, and although $22^{o}C$ produced more tillers than did $9^{o}C$, $9^{o}C$ produced a greater grain yield per plant. Nielsen (1974) sketched a temperature/yield profile for the temperate cereals barley, oats and wheat, and in each case, grain yield was best at 15^{o} to $20^{o}C$, and drastically lower as the temperature

was raised. It was suggested that the yield potential of barley at least, and probably the other cereals, decreased with rising root temperatures partly because higher temperatures hastened maturity, and did not allow for the development of all the plant factors leading to a realized maximum yield potential.

It appears to be fairly conclusive that low soil temperatures (at least to a minimum of $4^{o}C$, when all growth stops) are not injurious to plants, and merely provide an environmental stimulus which, when overcome, provides no permanent impediment to normal growth.

Anatomy and Morphology

Since the original invention of the magnifying lens made examination and description of plant cells possible, continued improvements have elevated the microscope to an instrument of great precision and power, capable of revealing even minute structural features of plant cells. Despite the fact that microscopy is so highly developed today, Percival's text (1921) is a reference text for many discussions of wheat anatomy. In his text, the short section on root structure describes the seminal root as having epidermis, cortex, endodermis, pericycle, phloem and xylem; the xylem consists of archs of protoxylem, and a central large metaxylem vessel. Other descriptions of wheat root anatomy, for example, that of Cutter (1975), have either assumed this configuration to be correct, or have not taken the trouble to examine some roots to see if there are in fact exceptions to this rule. Meyer (1976), in his observations of roots, considered principally the variations in cross-sectional diameter of the metaxylem vessel and of the whole root. He did make a note of the

periodic appearance of a di-vessel system in a seminal root, where the cross wall split a single into two. (However, the equivalent radius of the metaxylem vessel was not altered.) Passioura (1974) described the anatomy of wheat roots, and separated seminal from nodal roots. According to his discussion, the xylem of the seminal axis is dominated by a single large vessel in the centre of the stele, whereas the nodal roots are larger in diameter and have several large vessels. In his experiments on wheat growing on stored water, Passioura (1972) attributed the performance of the plants restricted to one seminal root to water conservation, because of the reduction in xylem vessel number and therefore greater resistance to water uptake. In his discussion, he cited xylem diameter as an important character to be incorporated into a breeding program, but not vessel number.

Meyer (1976) showed that the diameter of the metaxylem vessel was under genetic control. He grew wheat roots in a nutrient solution contained in growth pouches, and found that as the temperature fell from 35^{o} to 5^{o}C, there was an increase in the average radius of the metaxylem vessel. In this study, he observed no consistent increase in vessel number.

Since the root is the site of water uptake, resistance to water absorption is of great interest to practical physiologists as well as theorists and mathematicians. In his study on the resistance exhibited by root systems of white clover (Trifolium repens L) and tall fescue (Festuca arundinacea Schreb.), Burch (1979) concluded that root xylem was not the site of a major resistance to water uptake, but that, in wet soils, water movement into and through a system was predominantly

influenced by a large resistance to radial water flux through root tissues outside the xylem, and that, with drying of the soil, a root contact resistance existed due to shrinkage of root, soil, or both. Taylor and Klepper (1975) believed that the same was true for cotton, and in fact, Huck et al (1970) photographed a diurnal fluctuation in cotton root diameter, corresponding to variations in relative humidity at the leaf/air interface. Wind (1955) and Passioura (1972, 1974, 1977) studied the monocotyledon wheat, and suggested that axial resistance regulated by root xylem tissue was most important in water uptake and conservation. Landsberg and Fowkes (1978) attempted, by means of modelling mathematical manipulations based on observations and measurements by other workers, to separate the resistances of axial (xylem or vertical) and radial (cross-sectional or horizontal) components of the monocot root. They concluded that the interaction between the two must be considered, and not the independent effect of either one. Graecen et al (1976) did not study axial versus radial flow, but did attribute water flow across the root to gradients in osmotic and matric potentials within the root, and between the root and soil.

The work done by many individuals and groups on exudation rates from detopped root systems held for variable periods at different temperatures has never been attacked from the stand-point of anatomical variations induced by different temperature regimes. Brouwer and Hoogland (1964) described the endodermis of bean grown at sub-normal temperatures. Relative to that of bean grown at a normal temperature, it was suberised much closer to the tip. Clarkson (1976) found the

same feature in rye roots. Apart from this structural change, rates of water flow through detopped roots have been interpreted mainly on the basis of theory and conjecture. As was noted before, Clarkson (1976) believed the increase was due to changes in the lipids of cell membranes at low temperatures; also, an increase in the number of ion absorption sites resulted in increased water influx to maintain osmotic balance. Kaufmann (1975) reached similar conclusions when he compared xylem pressure potentials of citrus and Engelmann spruce at low soil temperatures.

Tyree (1973) believed that the regulation of osmotic potential was the key. He presented a model whereby increased water flux through excised roots was an electro-osmotic phenomenon to maintain internal salt concentration. Excision would disrupt the normal rate of movement, as the pump would now be actively pumping anions to the exudate, and the resulting electrogenic potential difference in the xylem sap would cause cations to be drawn in through the root to the exudate. Enhanced water flow was mainly an osmotic reaction to the increased salt flux, and was temperature-independent.

Glass (1978) called the increased exudation rate and potassium ion efflux a response to wounding. He found a two-hour recovery period; at four hours after excision, exudation had increased beyond control levels, and at six hours, it was back to normal. He criticized papers which reported increased exudation rate, based on measurements taken immediately after excision.

Neither of these workers concerned himself with root anatomy, or with a temperature effect.

Gross physical appearance of root systems grown at below-normal

root temperature has been thoroughly described and photographed by Brouwer and Hoogland (1964); for the variety of crops studied, the effect of low temperature was the same. 'Cold' roots were shorter, thicker, more fleshy and white; laterals appeared far back from the tip, and hairs were not numerous. 'Warm' roots, in comparison, were long, thin, fibrous and yellow, and possessed abundant root hairs and laterals close to the apex.

Burstrom (1965) examined the correlation between root cell elongation and root temperature. In an analysis of wheat root growth, it was found that the real rate of stretching of individual cells or segments increased rapidly up to 30°C, but the grand period of elongation was very much shortened with increasing temperature, so final cell length decreased. Consequently, final root length may decrease. The total growth in length of roots had an optimum around 20°C, and decreased as the temperature varied in either direction. At optimum temperature, cell division was more rapid but of shorter duration than at lower temperatures, and cold roots experienced delayed maturation and therefore a greater degree of elongation (Nielsen, 1974).

Another structural feature of some roots is the external mucigel layer, composed of carbohydrate, and discussed by Martin (1977) and by Balandreau and Knowles (1978), among others. An extensive review of the root mucigel has been presented by Balandreau and Knowles (1978). Detailed studies using electron microscopy and autoradiography showed that the root cap cells were very active in the production of mucigel, As the root extended through the soil, mechanical abrasion caused

sloughing of the outer cells of the root cap, and release of the mucigel, which could act to lubricate the soil/root interface. It was estimated that between three (wheat) and ten (corn) tonnes per hectare per year of carbon were transferred to the soil in this way (Balandreau and Knowles, 1978); these figures far exceed grain yield in these crops. While the root cap was once thought to be the source of the mucigel, later work has shown it is secreted by epidermal and root hair cells on the older portions of the root.

It has been suggested that the mucigel may have a role in maintaining contact between the root and the soil surface, when root shrinkage occurs during periods of rapid transpiration. The evidence, however, is indirect, being based on the observation that soil particles cling more tenaciously to the roots of plants grown under dry conditions.

The mucigel probably has an important influence on the microflora of the rhizosphere. It would provide nutrients to those organisms which are able to degrade it, and to metabolize the constituent sugars. In addition, it may slow down diffusion of organic materials which 'leak' from the root cells. The resulting increase in concentration of these materials at the root surface may selectively favour development of microbes, which are able to associate with the mucigel.

Apparently, no one has studied the variability of the mucigel layer of roots subjected to varying temperatures. This topic will be featured in the Results and Discussion of this thesis.

The effect of cold root temperatures on plant growth has been attributed to a water stress effect. Relatively few studies have been done, correlating cold roots and overall plant performance, and

therefore, an examination of those done on dry versus wet soil may provide some clues. Passioura (1972, 1974, 1977) felt that a plant could function best in times of water stress if it had a small, shallow root system, even a single seminal root of small diameter. Such a root system would automatically restrict the intake of water in times of plenty, such as early in the season, when vegetative growth is taking place, assuring a supply later, for grain growth, when rain is not likely to fall. His experiments on wheat plants grown on stored water back up this theory.

On the other hand, Hurd (1974) and Hurd and Spratt (1975) have found that those varieties which thrive in 'dry' boxes were those which could quickly produce an extensive root system, especially ones which branched freely and grew deep, to take advantage of all available water. In their natural habitat, these roots would be following a falling water table throughout the growing season.

It is interesting to note that, while these two philosophies are diametrically opposed, they have both been developed in response to very similar climatological conditions, namely, the arid, drought-prone wheat-growing plains of western Canada and Australia.

It is important that the inherent genetic variability of the wheat plant can be exploited by the plant itself, to optimize the use of any water present in its rooting environment, in response to a temperature or moisture stimulus. This variability has been recognized and used by MacKey (1973) and by Hurd (1974), in breeding and development of new and more drought-resistant cultivars.

MATERIALS AND METHODS

Physical Features and Biochemical Determinations

Materials

Glenlea wheat, Triticum aestivum, all of one seedlot, was chosen as the test material. Glenlea was selected because it is commonly grown in Manitoba, and because it is a relatively high yielder (Manitoba Zonation Trials, 1971-75, 1974-78).

The seeds were treated with Captan, a powdered fungicide, prior to planting. Soil mix used for the first replication was 1 perlite: 1 sand: 1 unsterilized soil; for the second, third and fourth replications, Turface was substituted for perlite. The soil mix was chosen instead of a native soil for reproducibility in future work, in this and other labs. Preliminary determinations of the field capacity of each soil mix allowed it to be maintained at approximately 120% of field capacity for replication 1, and 150% of field capacity for the three others. It was necessary to eliminate moisture stress from the experiment, and the seemingly high water contents resulted in no flooding or pooling of water at the bottom of the pots.

No analysis of soil nutrients was conducted, nor was seed nitrogen determined.

Pots used were three-litre capacity, rectangular plastic Frig-O-Seal containers. Their shape and depth allowed maximum use of space in the water bath, while maintaining constant, brisk water flow around each

pot. The water boxes, made of wood and lined with plastic, were deep enough to contain the pots plus water up to the level of the soil in each pot.

Experimental soil temperatures were maintained by a Jewel 'Aquachiller' water pump/refrigeration unit. In the control, treatment temperature was allowed to fluctuate without any regulation about the ambient air temperature. The soil surface was covered with white styrofoam packing chips to insulate against possible temperature variation induced by overhead lighting and ambient air temperatures in the growth cabinet. The styrofoam chips also reduced surface drying.

Methods

Seeds were planted in soil that had already been brought to experimental moisture content and temperature, and thinned to a maximum of six seedlings per pot, at about one week after planting.

Experimental temperatures for replications 1 and 2 were 8^{o}, 12^{o} and control (16.5^{o}-17.5^{o}C); in the third and fourth replicates, 8^{o}C was omitted. 12^{o}C was chosen because it approximates the temperature near the soil surface at about May 1, when wheat is sown in the Red River Valley, surrounding Winnipeg. Also, according to Wilkinson (1967), cereal roots in the Red River Valley region follow the 12^{o}C isotherm into the soil throughout the growing season. 8^{o}C was selected because it would definitely impose a stress on plant growth, and because other studies, for example by Clarkson and his co-workers, used an experimental temperature close to 8^{o}C. The control was

regulated by the maintenance of growth cabinet air temperatures at $20^{o}/15^{o}C$, day/night, a regime which is commonly used in growing wheat indoors. With this regime in use, it should be possible to equate control results with data quoted in the literature where root temperature is not monitored, but where air temperature is maintained at about the same level as in this work. The light intensity was approximately 210 microeinsteins m^{-2} sec^{-1}, and measured by a Li-cor quantum radiometer/photometer, model LI-185A.

Moisture content was maintained by weight. Fertilizer in the form of 20-20-20 (nitrogen:phosphorus:potassium), diluted ½ tablespoon in a gallon of water, was added during the growing season, when all the plants had reached the two-leaf stage in any one treatment. Since one of the desired observations was the effect of stress on the plants, this single application of fertilizer was deemed sufficient, since it resulted in a pronounced nitrogen deficiency, and hastened senescence in the lower leaves especially.

Experimental temperatures were imposed until 24 days after 50% emergence of the plants in any one treatment, when the 'Aquachiller' was turned off. Soil temperatures were then allowed to attain control values; this took about 24 hours. This was done because, in the field, the soil temperature has by this time (mid- to late-May) risen with the arrival of warmer days and nights.

Plants were harvested for destructive examination at intervals of five days, beginning at 14 days after 50% emergence of the treatment, and continuing through 19, 24, 29, 34 and 39 days after 50% emergence.

Each treatment consisted of two boxes containing a number of Frig-O-Seal containers; at each sampling date, one randomly selected Frig-O-Seal container was removed from each box, and all the plants contained therein (approximately six in each container, or 12 in all) pooled to comprise the replicate sample.

Harvest was begun at 14 days post-emergence because, by this time, the young plant is dependent on photosynthetic products from the top, and has depleted the stored resources in the seed. Any results thus obtained should reflect the true effect of cold on the root's ability to support plant growth.

Various physical parameters of the plants were recorded:

Reps 1 and 2: leaf length
leaf dry weight (oven-dried)
leaf fresh weight
root fresh weight
root dry weight (oven-dried)

Reps 3 and 4: leaf length
leaf fresh weight
leaf dry weight (freeze-dried)
leaf area
plant height
plant fresh weight
plant dry weight (freeze-dried)
root fresh weight
root dry weight (oven-dried)

In all replications, only the first, third and fifth leaves were examined.

Various biochemical assays were conducted on the leaf tissue. All photometric determinations were done on the same Bausch and Lomb 'Spectronic 20'. All the original data obtained by physical measurement and by biochemical assay are tabulated in the appendix of this thesis; diagrams in the text are usually illustrative of interactions between factors.

Chlorophyll:

In replications 1 and 2, a portion of fresh tissue was ground immediately in 80% acetone (Arnon, 1949), centrifuged to sediment leaf tissue, made up to constant volume, and absorbance read at 683 nm. In replications 3 and 4, chlorophyll was extracted from freeze-dried tissue in the same manner. Results are expressed in relative chlorophyll units.

Amino Acids:

In replications 1 and 2, a portion of fresh tissue was boiled in 80% ethanol for 10 minutes to extract free amino acids (Tetley and Thimann, 1974). The extract was diluted 1:10 with distilled water, and a ninhydrin test (Yemm and Cocking, 1955) done on an aliquot. Proline and hydroxyproline were determined by reading absorbance at 440 nm; all other amino acids were determined by reading absorbance at 570 nm. In replications 3 and 4, the same procedure was followed using dried leaf tissue. Absolute amounts of amino acids were determined from standard curves of valine and hydroxyproline.

Protease

In replications 1 and 2 only, a portion of fresh tissue was frozen immediately in Tris extraction buffer, pH 6.8 (Peoples and

Dalling, 1978), and thawed and ground at a later date for assay. In no case was the tissue left frozen for more than a week. The freezing and thawing disrupts cell membranes for a more complete release of enzyme. The ground mixture was centrifuged, filtered through Sephadex 20 to remove endogenous amino acids, and an aliquot of the filtrate incubated with an aliquot of casein solution in a water bath at 50°C. The reaction was stopped by TCA, and activity determined by a ninhydrin test on the supernatant of the centrifuged mixture (Peoples and Dalling, 1978), in the same manner as for amino acid determination. Protease activity was expressed as mg total amino acids released, from the standard curve with valine and hydroxyproline.

Protease determination was not carried out for replications 3 and 4, because the results obtained from the first two were very confusing, and it was felt that a thorough look at the other components (amino acids, protein, chlorophyll) would yield results that would be beneficial to future studies and interpretations of leaf protease.

Reducing Sugars:

This assay was only conducted on replications 1 and 2, as there seemed to be little difference between treatments with respect to reducing sugar content. An aliquot of the same 80% ethanol extract as was used for amino acid determination was used for reducing sugar determination, and a Somogyi test done (Nelson, 1944). Absorbance was read at 500 nm. Absolute amounts of sugar were calculated from a standard curve with glucose.

Protein:

In replications 1 and 2, an aliquot of the same filtrate used

for protease determination was reacted with an aliquot of commercial coomassie blue dye reagant, and the absorbance read at 595 nm (Bio Rad Technical Bulletin #1051). Absolute protein content was determined from a standard curve using the protein standard supplied in the dye kit. In replications 3 and 4, dried leaf tissue was ground in a mortar and pestle with the same Tris extraction buffer, centrifuged and filtered as in replications 1 and 2, and the same protein determination done.

Since no destruction of any cellular components under consideration should occur during freeze-drying, or during freezing in buffer and thawing, the values obtained for all replications are comparable, regardless of the method by which they were obtained.

Anatomical Observations

Materials

Three varieties of wheat, Triticum aestivum, were chosen, all of which are widely grown on the Canadian prairies: Glenlea, a high-yielding utility wheat, of which two seed lots were selected, and Sinton and Neepawa, two hard red spring wheats, of which one seed lot of each was selected.

In a plastic cup, 150 g of soil (1 unsterilized soil: 1 sand: 1 Turface) was mixed with water to bring it to 50% field capacity, which was thereafter maintained by weight. The soil was covered with white styrofoam chips, for insulation, and the cups floated in wooden boxes full of water at the desired experimental temperatures, until soil temperature was at the proper level.

Experimental temperature, regulated by a Jewel 'Aquachiller', was

12°C, with a non-regulated control, which fluctuated around 16.5°-17.6°C. These temperatures were chosen for the same reasons given in the previous Materials and Methods section.

Methods

Seeds were surface-sterilized with Teramine (diluted 250x) and planted five per cup. One week following 50% emergence of the seed lot, all the plants in the lot were harvested whole, and the roots stained by immersing them whole (still attached to the plant) in 0.5% methylene blue, and placing the plants in a stream of moving air for about 1.5 hours.

The primary seminal root was chosen for examination; it was cut off at the seed and cross-sectional examination begun at the cut surface. For hand sectioning, the root piece was held in a styrofoam packing chip, and cut with a stainless steel razor blade. Sections were placed on a glass slide coated with Haupt's adhesive and 3% formalin, heated to dry, then covered with glycerin and a cover slip. Examination and measurement were done under a light microscope.

A limited examination of roots was done on the first replication of the first part of this work. Immediately after harvest, several randomly chosen roots from plants at 14, 24 and 34 days post-emergence were placed in buffered glutaraldehyde and embedded in methacrylate. The roots from 14 days post-emergence from all three temperature treatments were sectioned, made into slides, and stained: Toluidine blue was used for xylem tissue, and periodic acid/Schiff's for carbohydrate.

RESULTS AND DISCUSSION

Seedling Emergence

The appearance of the emergent seedlings was identical and normal in all three temperature treatments. Emergence proceeded at about a similar rate following a three-day lag behind controls in the case of 12°C soil, and about a seven-day lag in 8°C soil (Figure 1). These data support data reported by Blackshaw (1979) for wheat. The final per cent emergence declined slightly as the soil temperature falls.

Root Growth

Figures 2, 3, 4 and 5 illustrate the fresh and dry weights, per cent water and fresh/dry weight ratio of roots for the average of four replicates for the two treatments, control and 12°C soil, and two replicates for 8°C soil.

It has been observed that wheat roots follow a 12°C isotherm down into the soil over the growing season, either as the result of coincidence, or because 12°C may be an optimum temperature for the extension of wheat roots (Wilkinson, 1967). In the Red River Valley, the mean daily temperature at the soil surface reaches 12°C at about mid-May; at this time, a steep temperature gradient exists, with rapid warming of only the few surface cm. Other studies in this laboratory indicate that by the time the coleoptile emerges (seed planted at 2.5 cm depth), the roots may extend 15 cm or more into the soil (Neustad

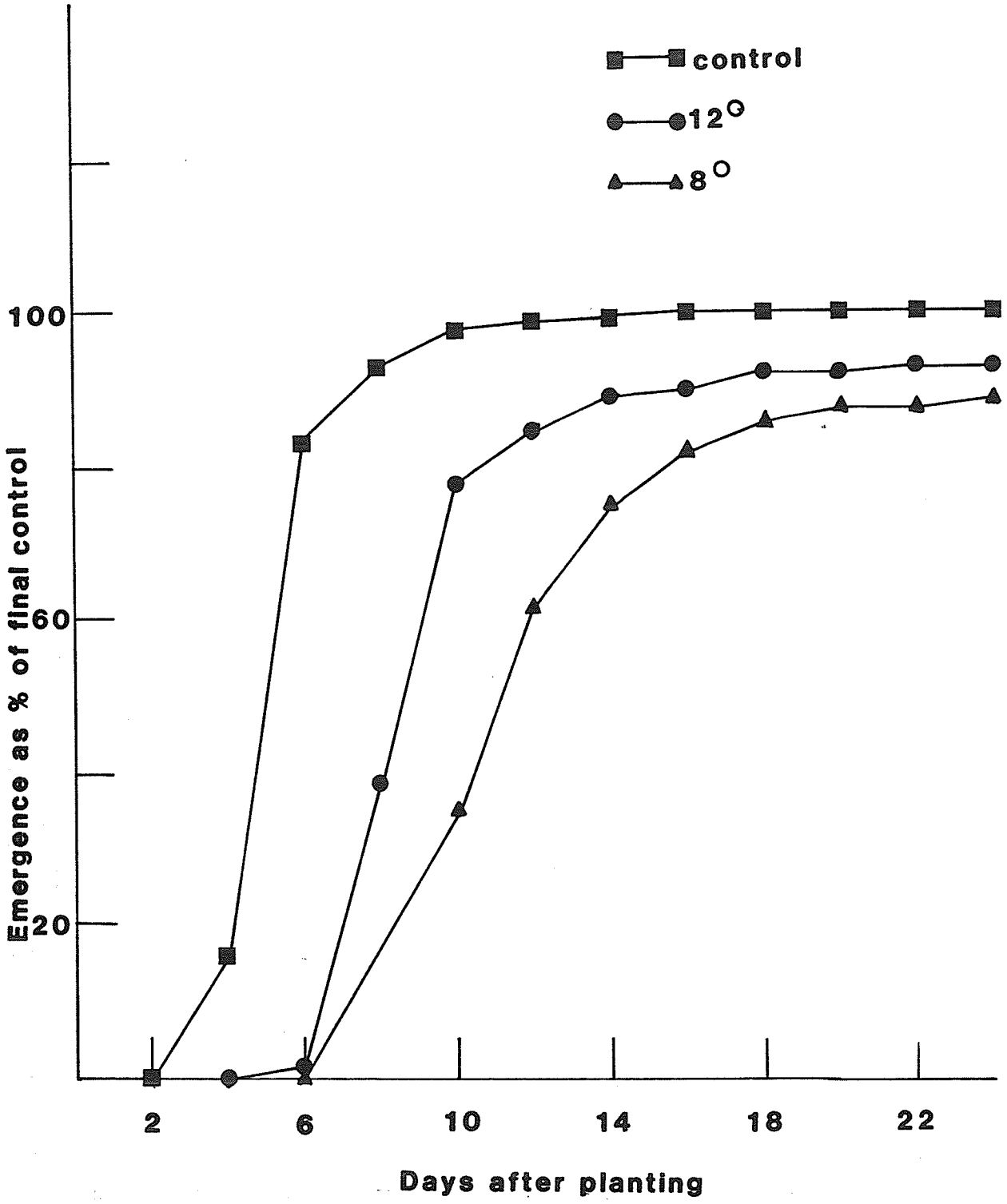

Figure 1. The effect of soil temperature on seedling emergence.

and Woodbury, unpublished). Thus, it is possible that, in the field, seedling roots are exposed to the range of temperatures used in these experiments.

Root growth rate, expressed as fresh weight increase, shows that 12°C roots grow at a greater rate, and to a greater size at 39 days post-emergence, than do controls (Figure 2). The dry weight curves for the two treatments are almost identical (Figure 3).

Martin (1977) fed ^{14}C to shoots and monitored its appearance in roots and in soil. He found a reasonably consistent loss of ^{14}C from roots at two atmospheric growth temperatures, and over a range of plant ages; total ^{14}C recovered from soil amounted to roughly 20% of photosynthate, and 40% of total ^{14}C translocated to roots. Corresponding to this loss of carbon was a cortical disintegration, which occurred in sterilized and unsterilized soils. Cortical death appears in control roots in the present research, and not in 12°C roots. Several physical features manifest this.

The cortex comprises 2/3 to 3/4 of the root volume (Rovira and Bowen, 1973). Cell death and turgor loss are accompanied by a loss of water and a reduced fresh weight. Fresh weight of 12°C roots reaches a greater maximum, but dry weights of roots from both treatments are almost identical. Cortical death is followed by a loss of cell material to the soil, specifically, of 40% of the total carbon translocated to roots (Martin, 1977). This means that control plants may require 1.4 times the measured dry weight as incoming photosynthate to maintain the root system, but the lost carbon is not recoverable.

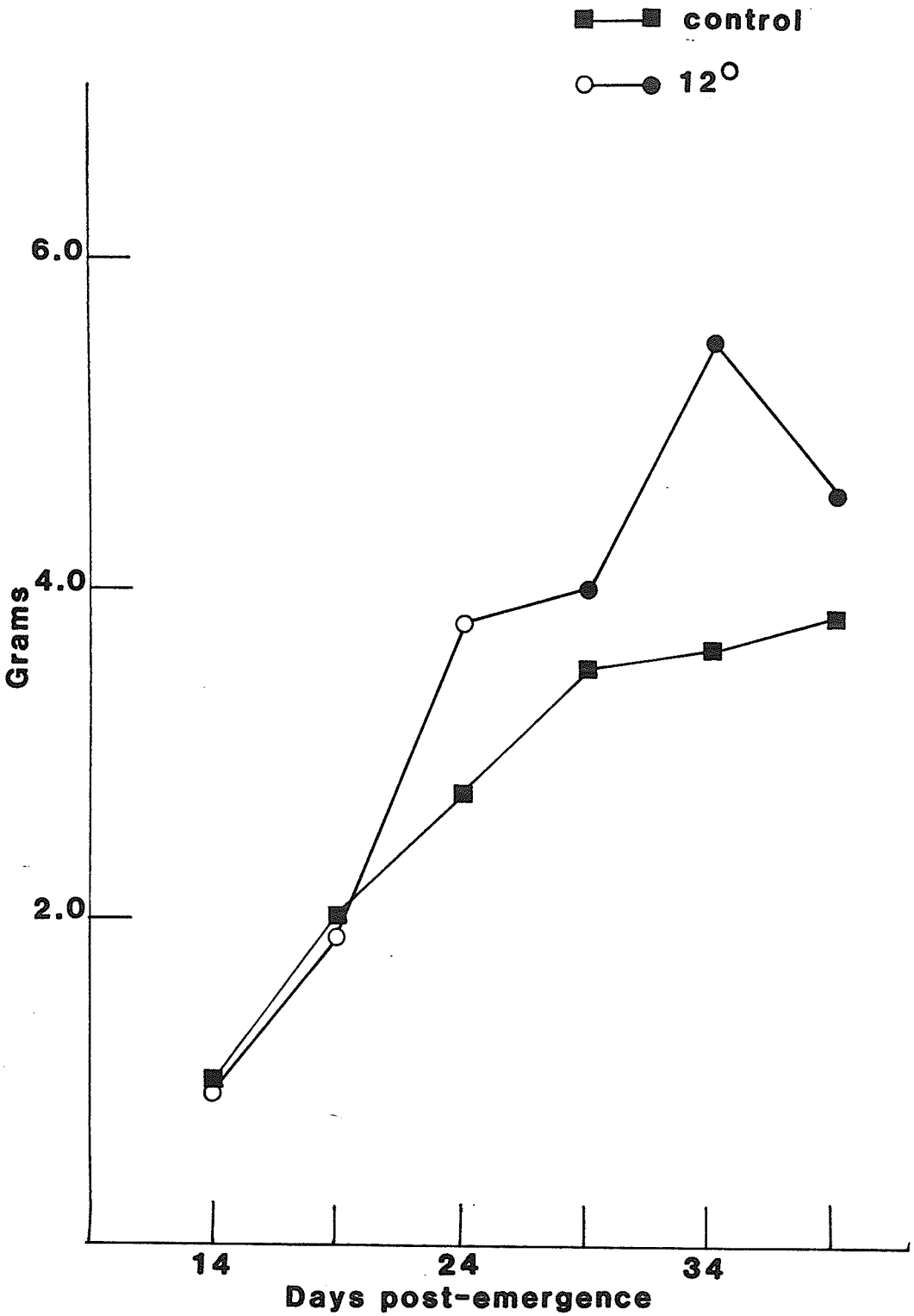

Figure 2. The effect of soil temperature on root fresh weight. (Shaded figures indicate soil temperature control removed.)

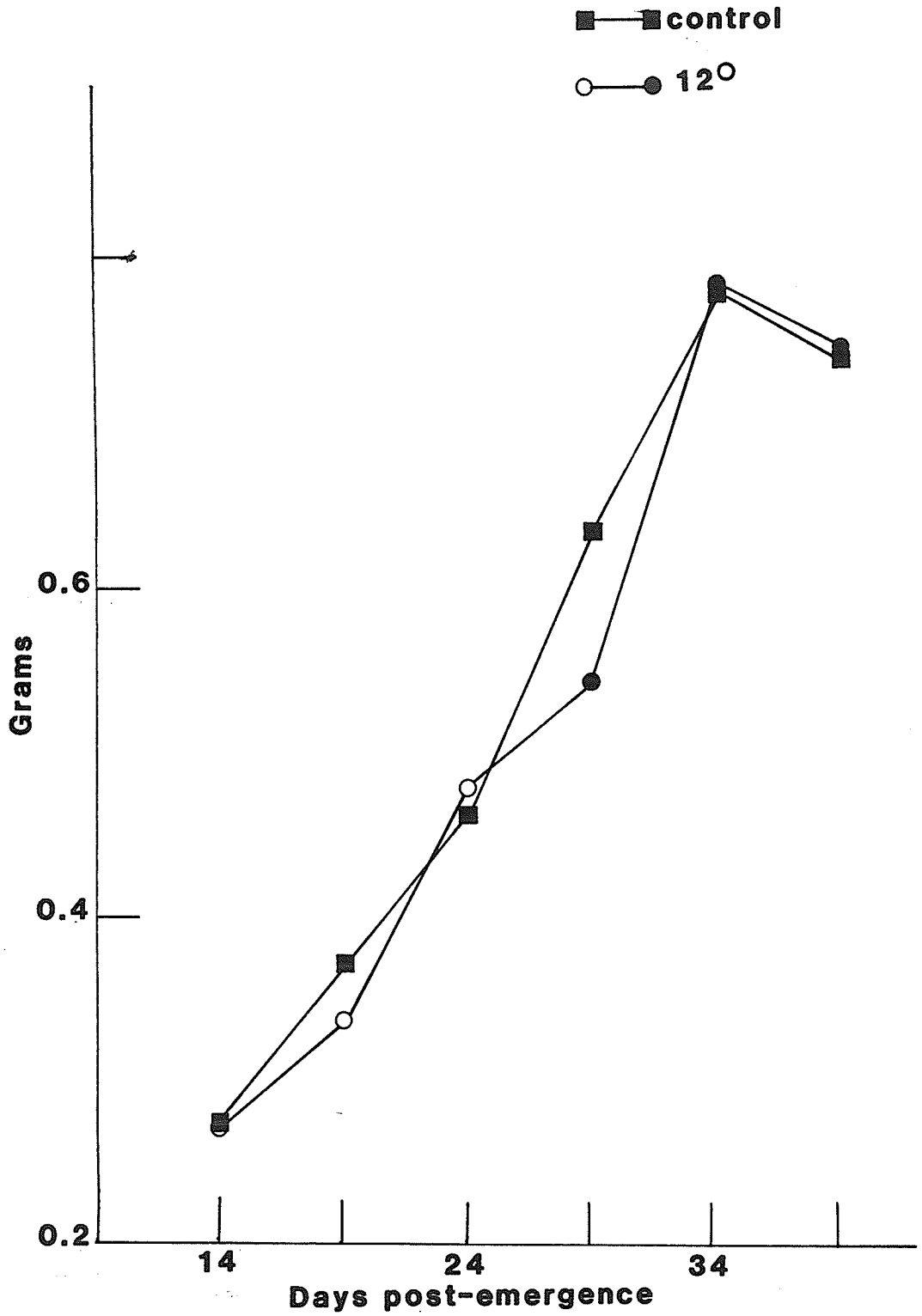

Figure 3. The effect of soil temperature on root dry weight. (Shaded figures indicate soil temperature control removed.)

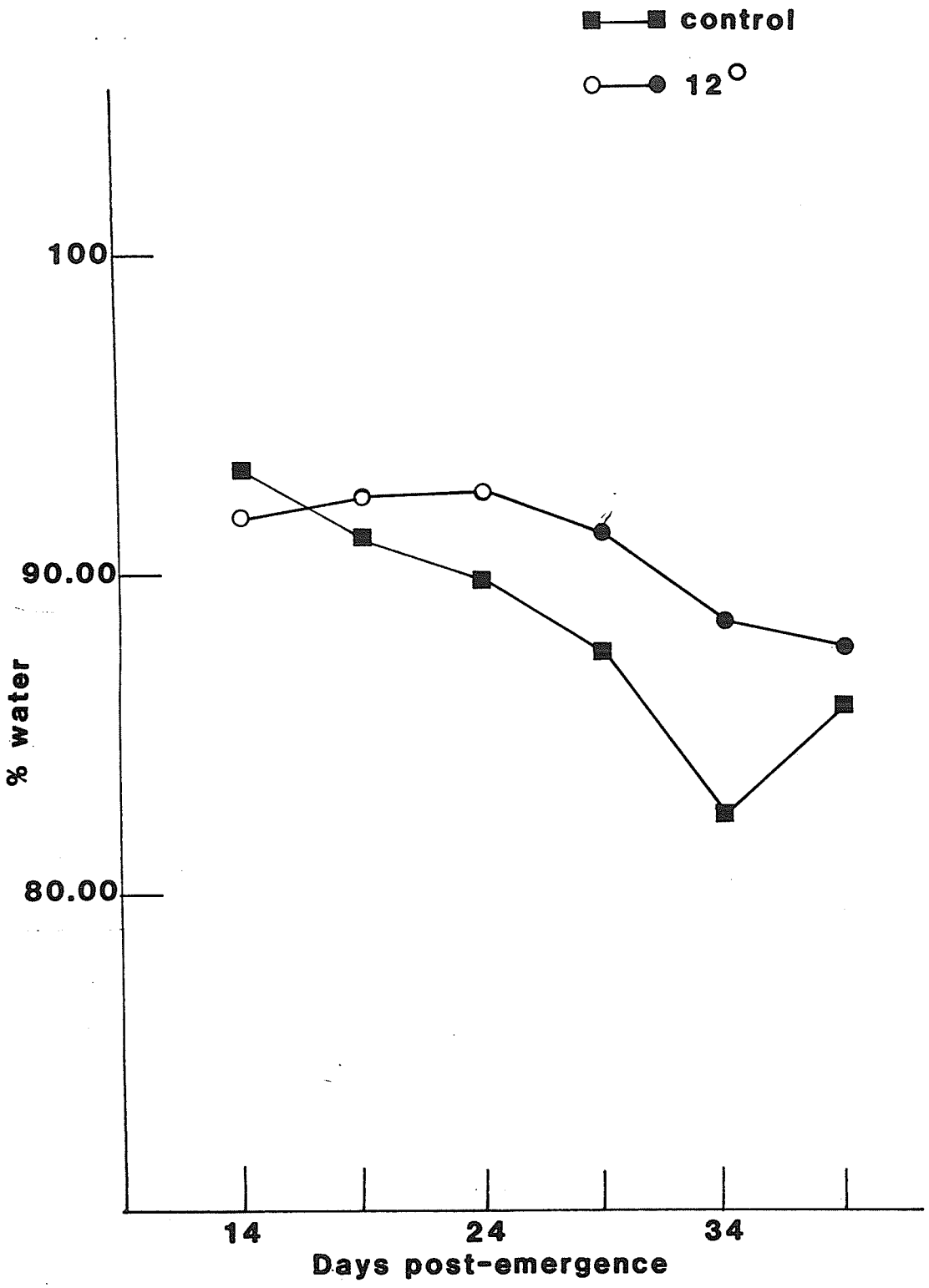

Figure 4. The effect of soil temperature on root per cent water content. (Shaded figures indicate soil temperature control removed.)

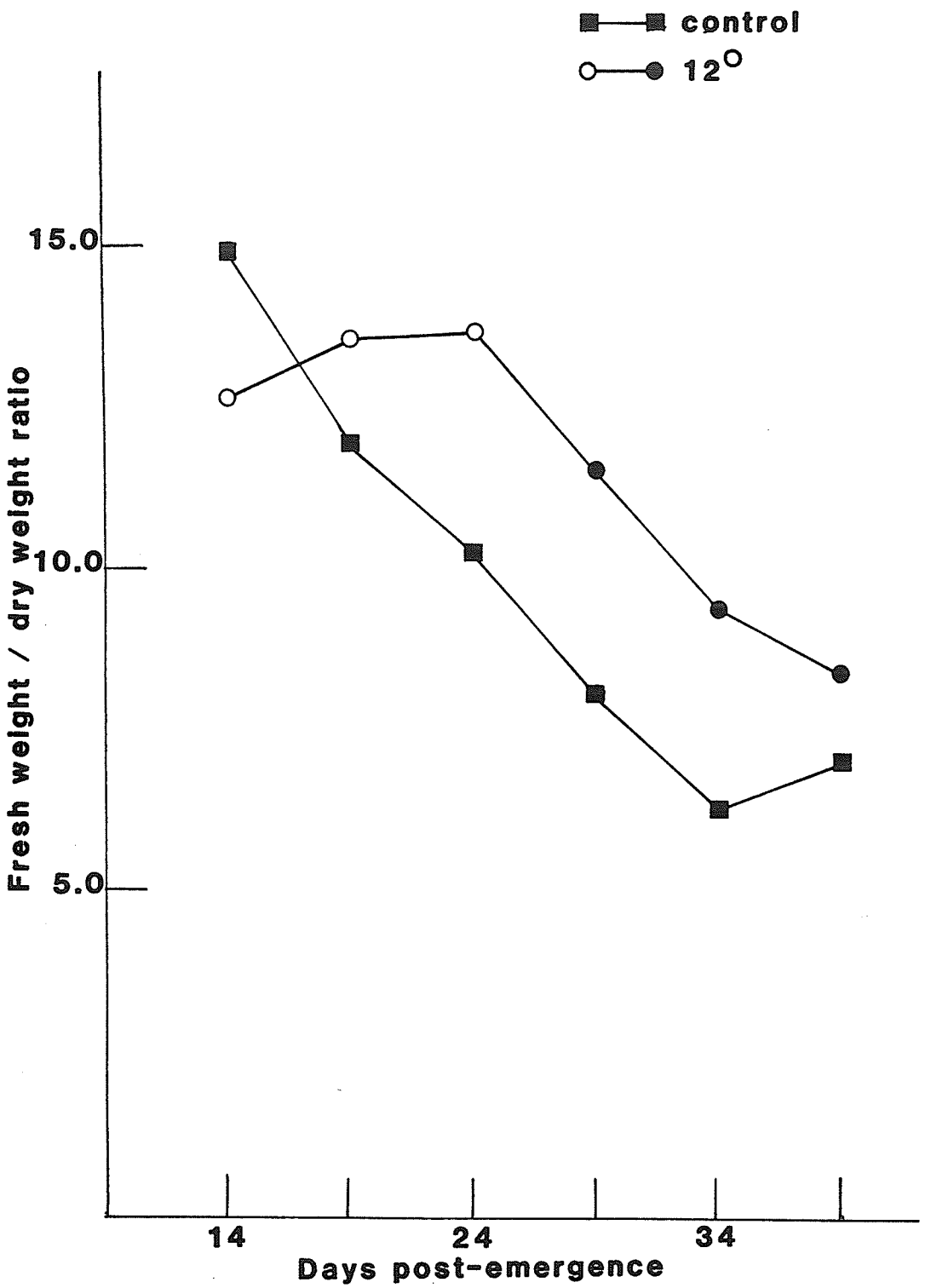

Figure 5. The effect of soil temperature on root fresh weight / dry weight ratio. (Shaded figures indicate soil temperature control removed.)

Control roots are yellowish brown and wiry; both 12°C and 8°C roots are white and fleshy. This indicates water retention and the maintenance of living cells and cytoplasm by the low temperature roots, as opposed to non-living cells, which have lost their contents in control roots.

Finally, actual examination of root cross-sections (of all three temperature treatments) revealed that the cortex of control roots had begun to break down within a cm of the apex, whereas there was no sign of breakdown in 12°C and 8°C roots.

The trends in per cent water content in control and 12°C roots complement the fresh weight results. Initially, per cent water and fresh weight are lower in cold-stimulated roots, but surpass controls by 24 days post-emergence. Per cent water, like other parameters (mainly in leaves) reaches a similar maximum value in both temperature treatments, and declines at a similar rate, but after a lag of one to two sampling periods (5 to 10 days) for 12°C roots. The decline in per cent water also follows the temperature shift to control values. This lag phenomena was also seen by Power and his co=workers (1963, 1970) in their various experiments investigating the growth of barley under cold soil conditions.

The fresh weight/dry weight ratio follows a similar pattern of identical rate of decline, but after a lag in 12°C roots (Figure 5). The controls show a continuous decline, right from the first sampling date, whereas 12°C roots maintain a constant ratio up to 24 days post-emergence.

The curves describing fresh weight, dry weight, per cent water content and the fresh weight/dry weight ratio for the first two replicates (three experimental temperatures) were not as smooth as those for all four replicates. The same trends are present, and again, $12^{o}C$ soil emerges as a very favourable temperature for wheat roots.

The fresh weight of the $12^{o}C$ roots surpassed that of the other two treatments at every sampling date, and increased early in the experiments at a very sharp rate. Fresh weights of the other two root systems are equal up to day 29, when controls drop off sharply, with a subsequent return to the same values as on day 29. $8^{o}C$ roots continue to increase, and by the end of the experiment, approach $12^{o}C$ fresh weight.

Dry weights of $12^{o}C$ roots are slightly better overall than controls (except on day 29), and $8^{o}C$ roots are definitely lagging, although again, by the end of the experiment, they are still increasing.

The data for per cent water content and fresh weight/dry weight ratios are similar in all three treatments, but the per cent water content is much less sensitive, and responds to stimuli with fluctuations of similar shape but much smaller magnitude than do fresh and dry weight. Note that the decline in fresh weight/dry weight ratio is very large, going from 15/1 to 4/1. Loss of turgor of cortical cells (which represent the greatest part of root volume), upon their death or senescence, would be expected to result in a large loss in the water-holding capacity of the root, as is seen in the data. Death of cells within the stele would have little influence. The per cent water data would be expected to be less sensitive to changes

in water-holding capacity, since water turns up in both terms of the ratio $\left(\frac{\text{Fresh weight - Dry weight}}{\text{Fresh weight}} \times 100\right)$, and cortical death would influence both terms simultaneously.

Fresh weight/dry weight ratio shows a constant decline in control roots (except for day 34). The cold-treated root systems both increase to about day 29, when they both decline, 12°C very sharply, and 8°C much more gradually.

The decision at the beginning of the research to remove the temperature controls at day 24 was perhaps unfortunate. It was thought that allowing the soil to warm up at this point would mimic field soil conditions; however, it appears that many internal changes occur in the plants at about this time, independent of external stimuli. Growth analysis data of root and shoot (Figures 6, 7 and 8) help to illustrate this. Analysis of fresh weights show that, in root and shoot, the overall growth rates are similar for each treatment, except for the rise in root fresh weight of 12°C plants at day 34. The composite curve (root plus shoot) has the same pattern for both treatments.

Interpretation of dry weight data proves more complicated, until the composite curve is examined in conjunction with the separate curves. In control plants, the trends for dry weights are the same as for fresh weights, that is, root and shoot growth rates increase approximately in synchrony. However, for the first three sampling periods in 12°C plants, the slope of the composite (root plus shoot) curve is zero, showing no apparent change in growth rate, until day 24, when the

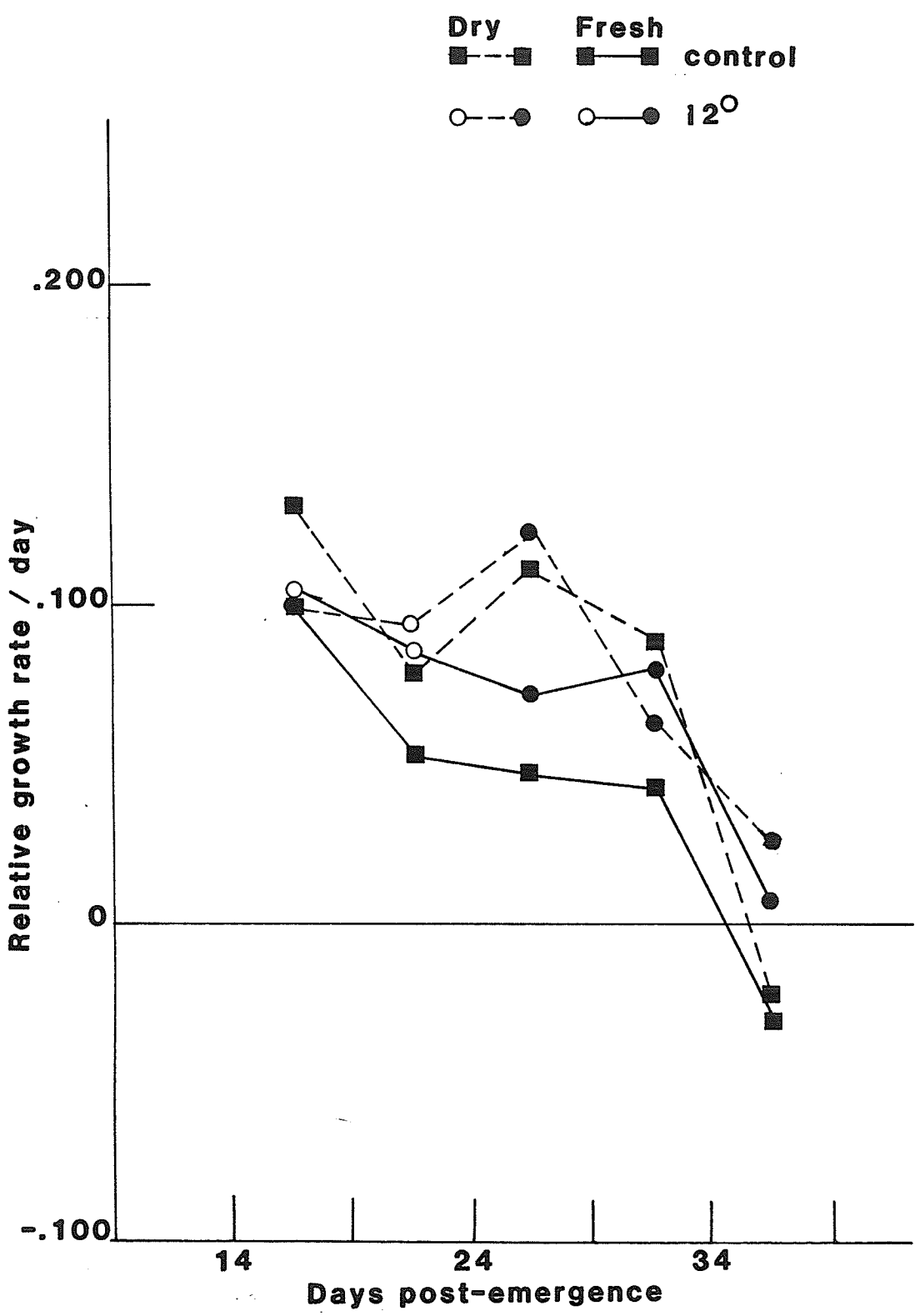

Figure 6. Relative growth rates of shoot fresh and dry weights. (Shaded figures indicate soil temperature control removed.)

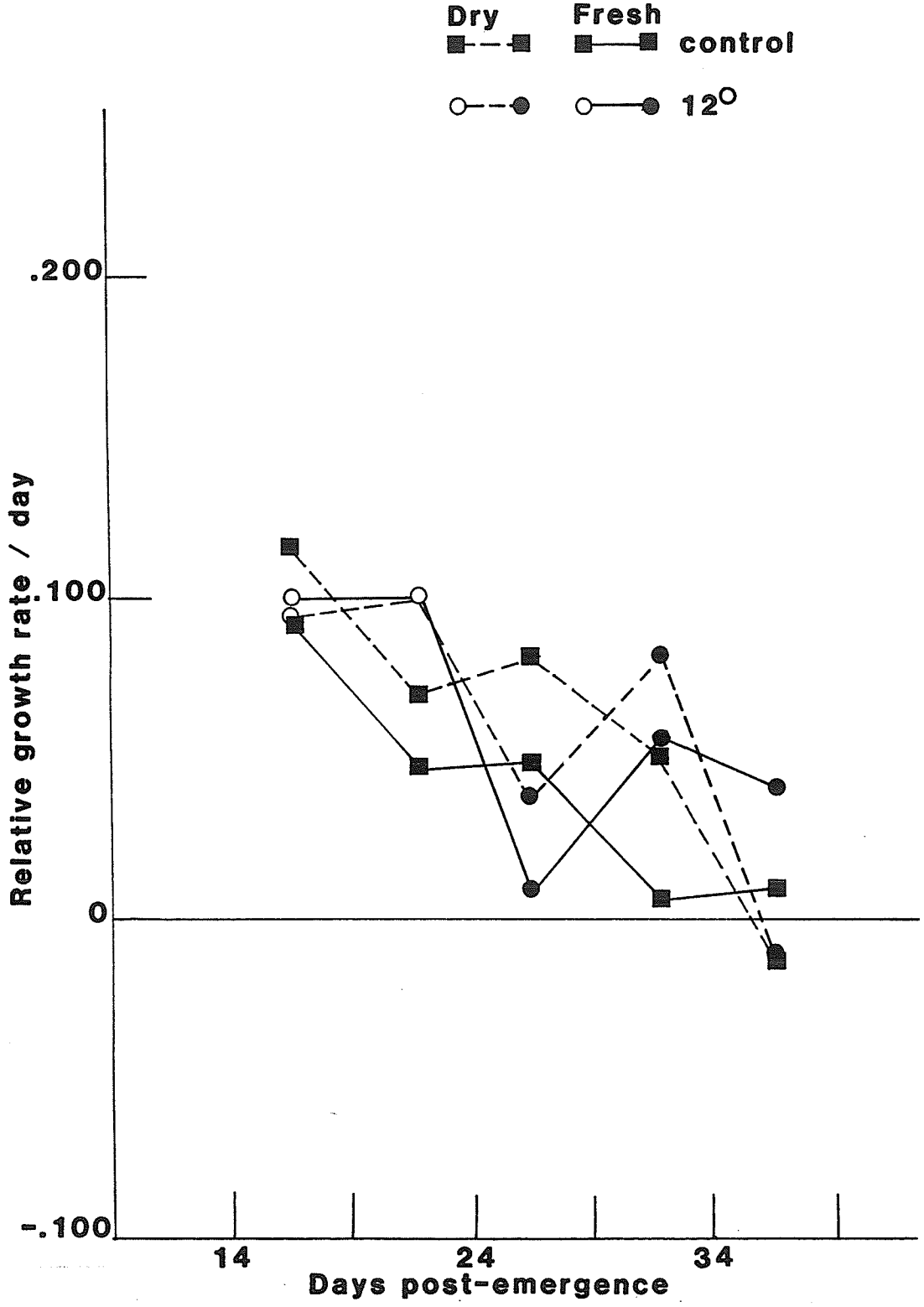

Figure 7. Relative growth rates of root fresh and dry weights. (Shaded figures indicate soil temperature control removed.)

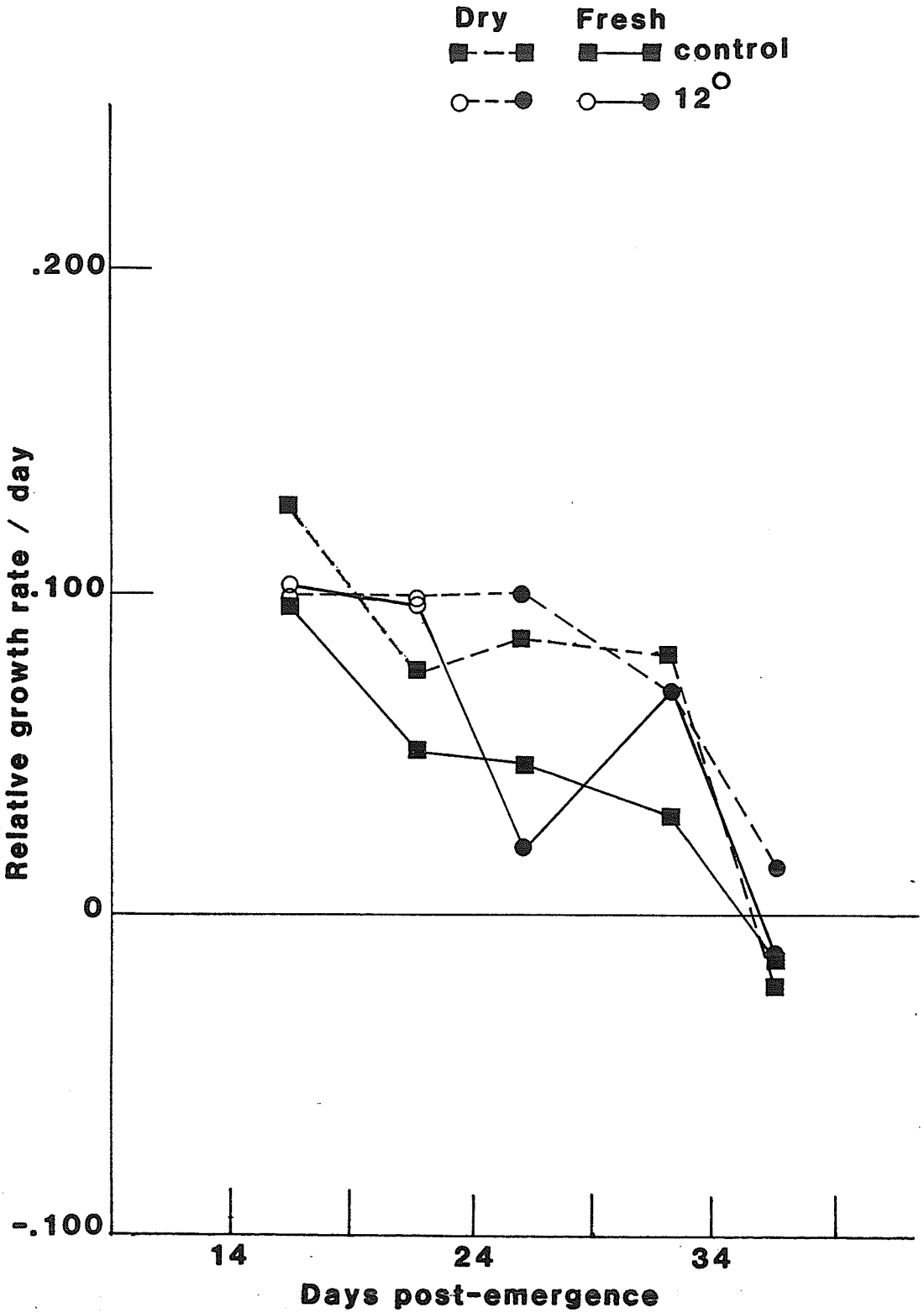

Figure 8. Relative growth rates of total (root plus shoot) fresh and dry weights. (Shaded figures indicate soil temperature control removed.)

composite curve drops sharply. At this point, the shoot growth rate increases abruptly, while the root drops just as sharply. It appears that, at about day 24, there is a transient shift to the shoot, which depresses the supply of growth materials to the root. Following day 24, the overall dry weight plunges, as do those for root and shoot, as the plants mature and senescence sets in. The fall in composite growth rate towards the end of the experiment is noted in control and 12°C curves.

The onset of senescence noted at 24 days post-emergence is early, even in stressed plants. Deliberately-induced nutrient stress, as well as crowding in the pots, were the main causes of this premature senescence.

It may come as no surprise that growth analyses of the total fresh and dry weights of the three leaves examined (leaves 1, 3 and 5) show the same general trends as the data for root growth analysis, only with a delay of again about one sampling period (Figure 9). The plant, while functioning as a whole organism, appears to display its overall cycles independently in at least some of its component parts.

In these experiments, soil temperature control was only imposed through 24 days post-emergence (only the early growth stages were studied). It may be that young roots show different susceptibility to soil temperature than do older roots. Also, the seminal and nodal (adventitious) roots may not show identical responses. In addition, as the season advances, the steepness of the temperature

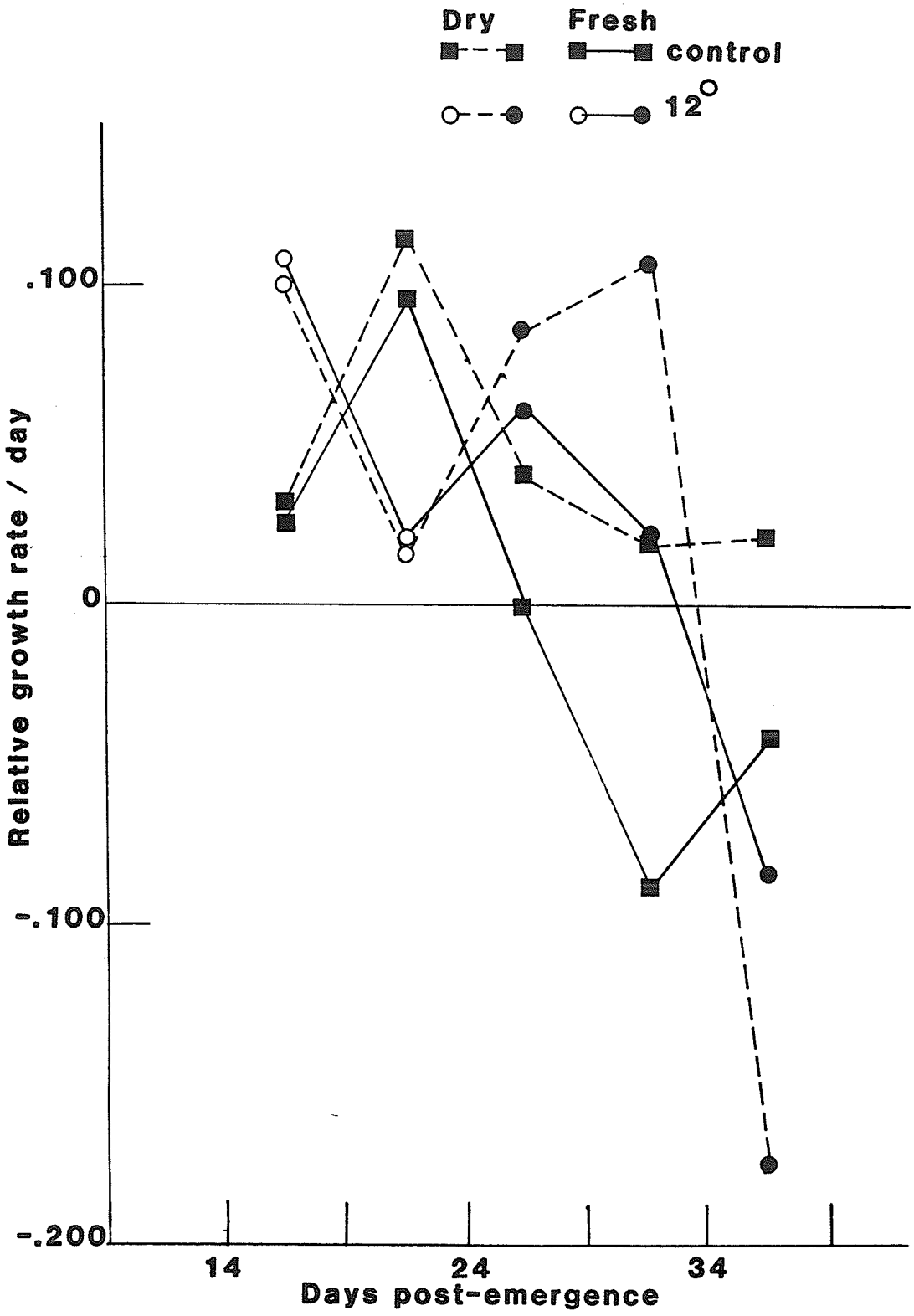

Figure 9 . Relative growth rates of total leaf fresh and dry weights. (Shaded figures indicate soil temperature control removed.)

gradient (12^{o} to 8^{o}C) decreases exponentially as the 12^{o}C isotherm moves down. Thus, unless 12^{o}C is a sharply defined optimum for root growth, the roots may lose track of the 12^{o}C isotherm as it becomes more diffuse.(Wilkinson, 1967).

Interaction of Nitrogen Status and Root Temperature

Brouwer (1966) reviewed the influence of various environmental factors on root/shoot ratio. He pointed out that low nitrogen and low root temperature would favour root growth and depress shoot growth. When the nitrogen of the leaves (protein plus amino acids) is considered, it is seen that the plants in the first two replicates were growing at a higher nitrogen status than plants in replicates 3 and 4 (Figure 10). The reason for this difference is not clear, since soil nitrogen data were not obtained. However, if the root growth data are now compared for the high and low nitrogen plants as replicates 1 and 2 versus 3 and 4, it becomes apparent that, in the control plants, root growth was influenced by nitrogen; root growth was greater in the low nitrogen plants, as Brouwer suggested (Figures 11 and 12). For the 12^{0}C plants, there is very little difference in root growth across the four replicates, as long as the root temperature was held at 12^{o}C. After the temperature shift, root growth was strongly depressed in the high nitrogen plants, whereas the temperature shift had little influence on the low nitrogen plants.

Brouwer's analysis indicated that an increase in temperature of the soil, or in nitrogen status, should depress root growth and promote shoot growth. It would seem that, for wheat at least, low

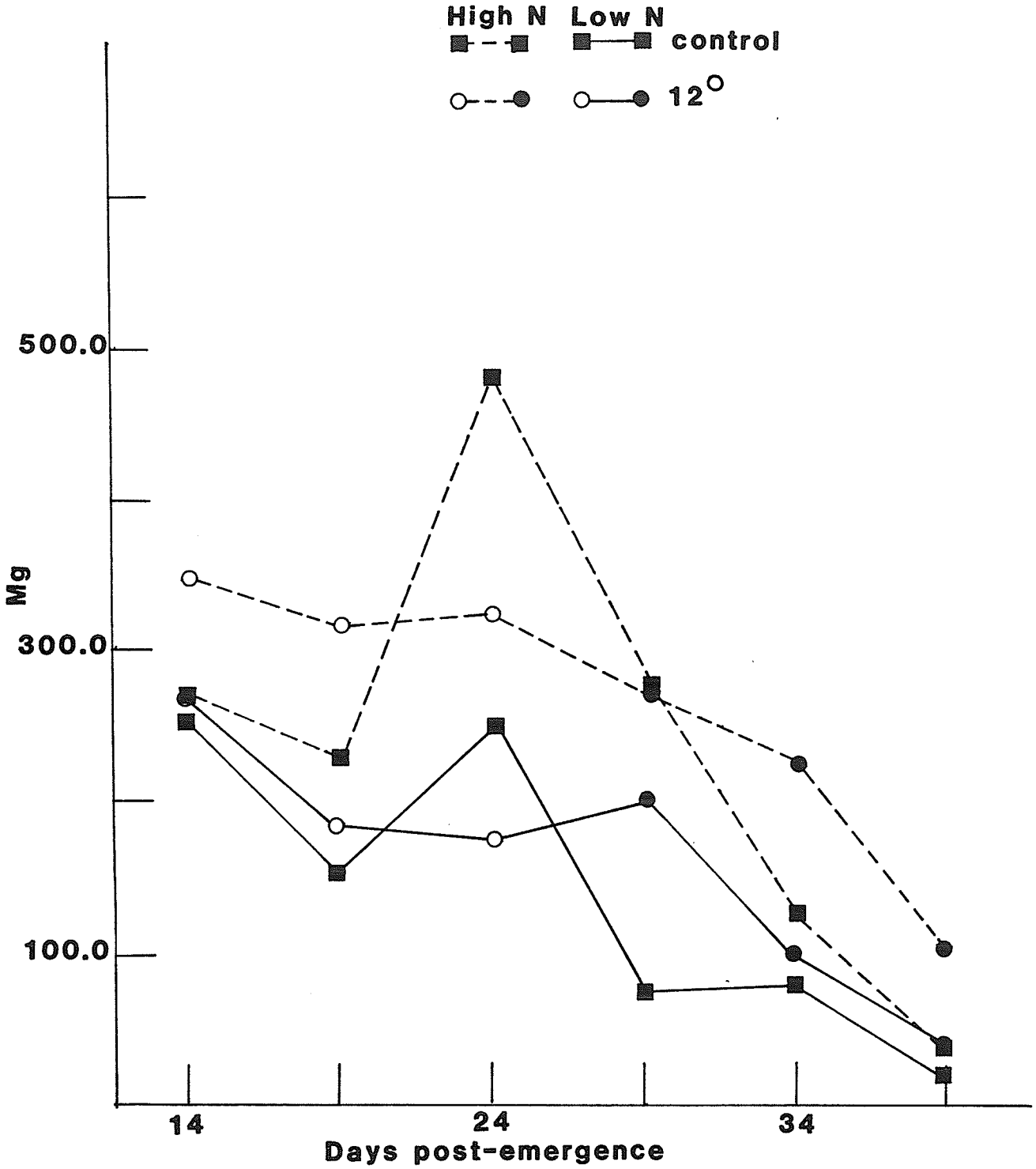

Figure10. Total leaf nitrogen: reps 1 and 2 (high N) vs reps 3 and 4 (low N). (Shaded figures indicate soil temperature control removed.)

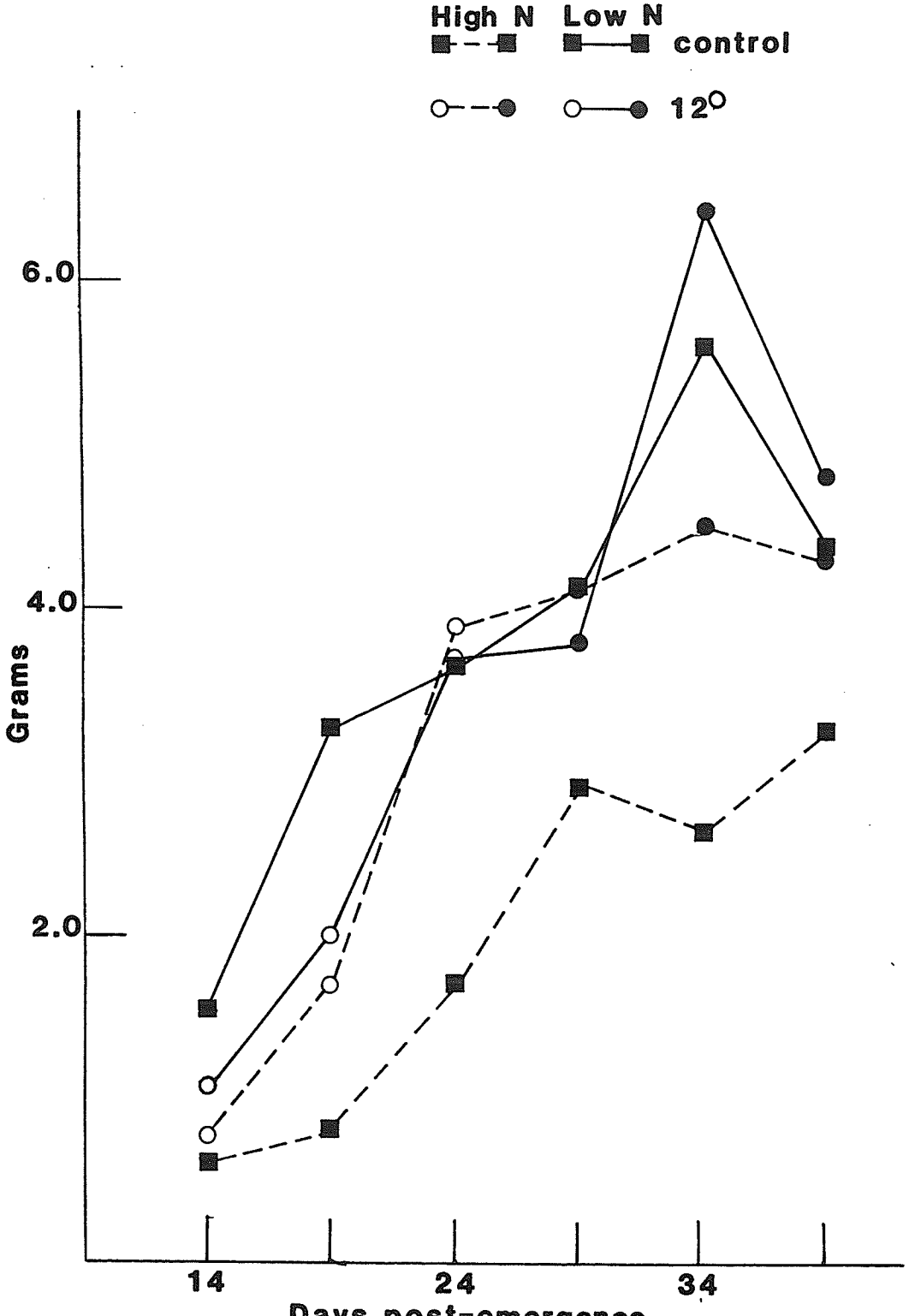

Figure 11. Root fresh weight and plant nitrogen status. (Shaded figures indicate soil temperature control removed.)

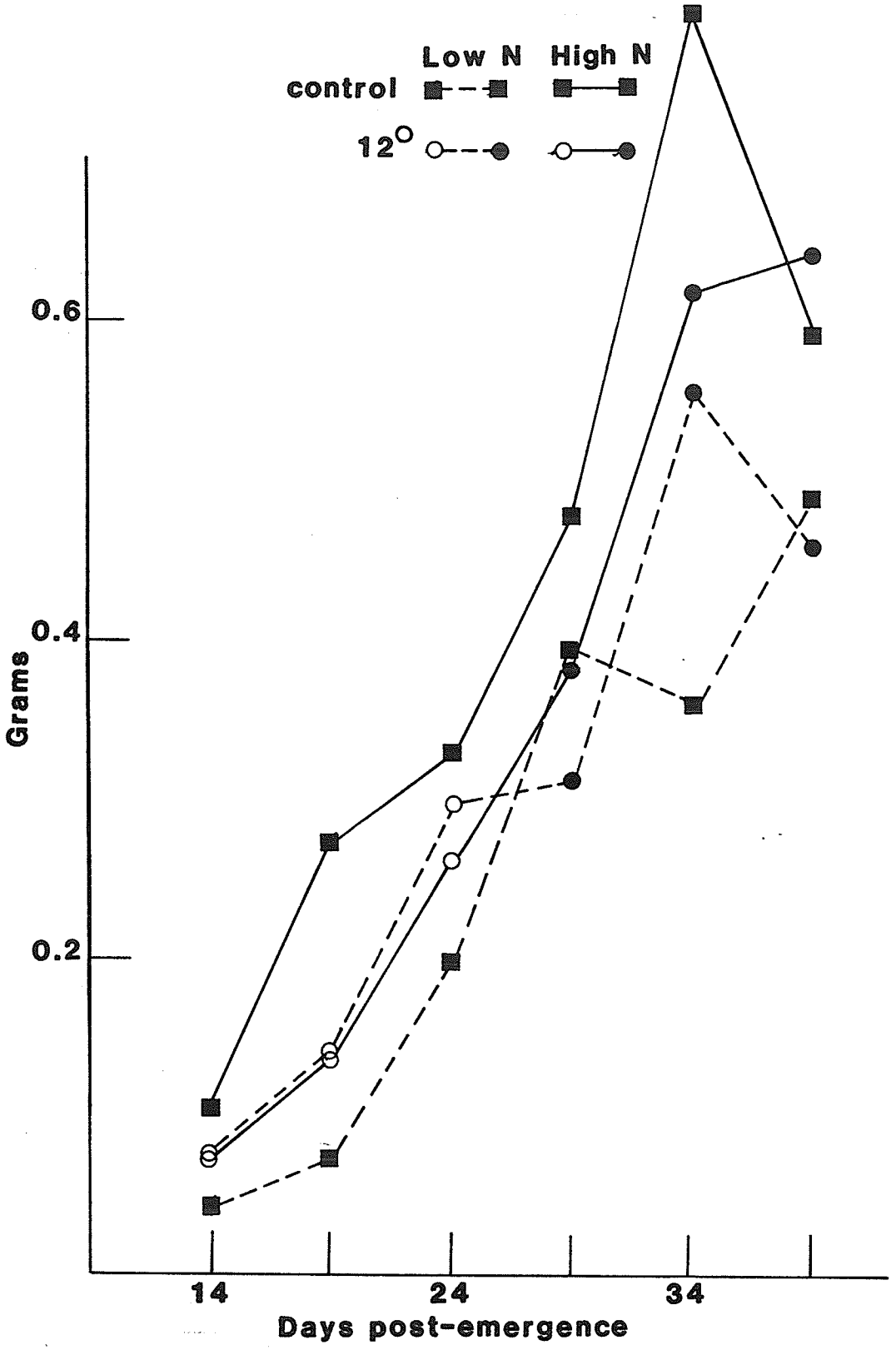

Figure 12. Root dry weight and plant nitrogen status. (Shaded figures indicate soil temperature control removed.)

root temperature overrides the effect of nitrogen status, where root growth is considered. At the higher temperatures, either throughout plant growth in the control plants, or after the temperature jump (in 12°C plants), nitrogen status of the plants becomes the dominant influence.

This interpretation must remain speculative at this point. It is based upon a limited number of experiments, and the cause of the change in nitrogen status is unknown. In addition, we do not have data for shoot growth and total plant nitrogen across the four replicates. However, if roots at 12°C are insensitive to the nitrogen status of the plant, the observation has important implications for nitrogen fertilizer and date of planting.

Root and Shoot Growth Correlations

In replicates 3 and 4, total fresh and dry weight for root and shoot were obtained for root temperatures of 12°C and control. Various combinations of these data can be used to look at growth correlations between these two organs.

The root/shoot ratio (fresh weight and dry weight, Figure 13) of the control plants shows a more or less continuous decline, from day 14 through day 29, after which the value seems to stabilize. On the other hand, the 12°C roots show an abrupt decline in the ratio at about day 24.

The ratio is higher for the 12°C plants, largely as the result

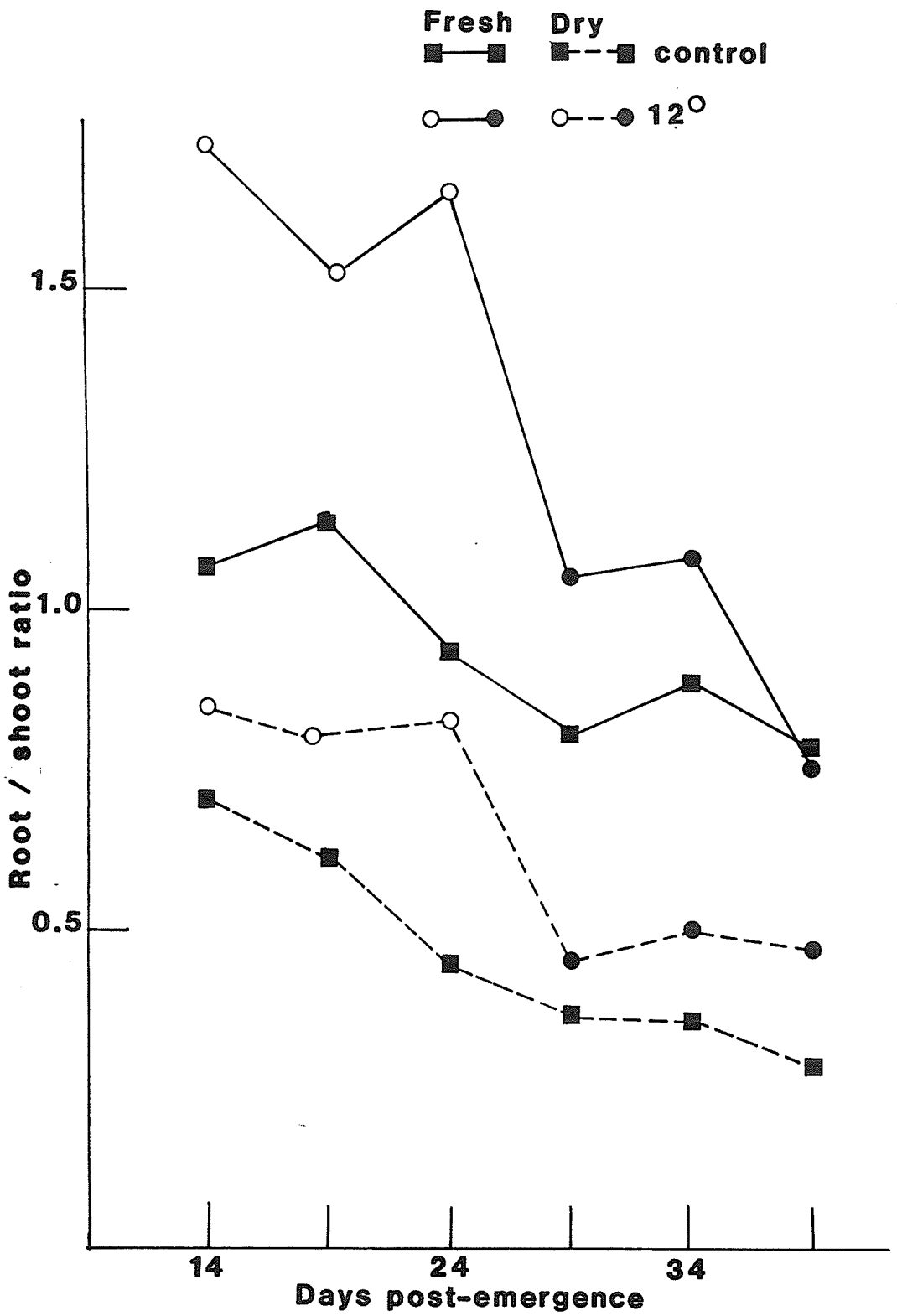

Figure 13. The effect of soil temperature on root / shoot ratio. (Shaded figures indicate soil temperature control removed.)

of depressed shoot growth. In 12°C plants, at day 24, there is an inhibition of root growth and a surge in shoot growth; after day 29, the curve shifts back to the same slope as before. Note that the control plants show a shift in the root/shoot ratio at day 19, and recovery seems to occur at day 24. For roots at both temperatures, there is an abrupt loss of both fresh weight and dry weight following day 34 (Figures 2 and 3). In the control plants, this is accompanied by an abrupt decline in shoot weight. This reinforces the theory that metabolic changes occur within the plant at about days 19 to 24, and that these processes are not much influenced by the root temperature. Floral initiation and jointing probably occur at about these points, and both would impose a heavy demand on reserves. Plant height shows an abrupt increase, beginning at day 24, indicating the onset of jointing (Figure 14).

Leaf Nitrogen and Chlorophyll

The individual leaf patterns for total nitrogen give the expected overall pattern. The total rises early in the life of the respective leaf, reaches a plateau, and then falls as senescence progresses and the leaf eventually becomes non-functional. Both treatments, 12°C and control, show these trends.

Chlorophyll per leaf also expresses the life cycle of the leaf quite clearly, rising early in the experiment and falling as the leaf yellows and senesces.

The relationship between these two parameters is significant.

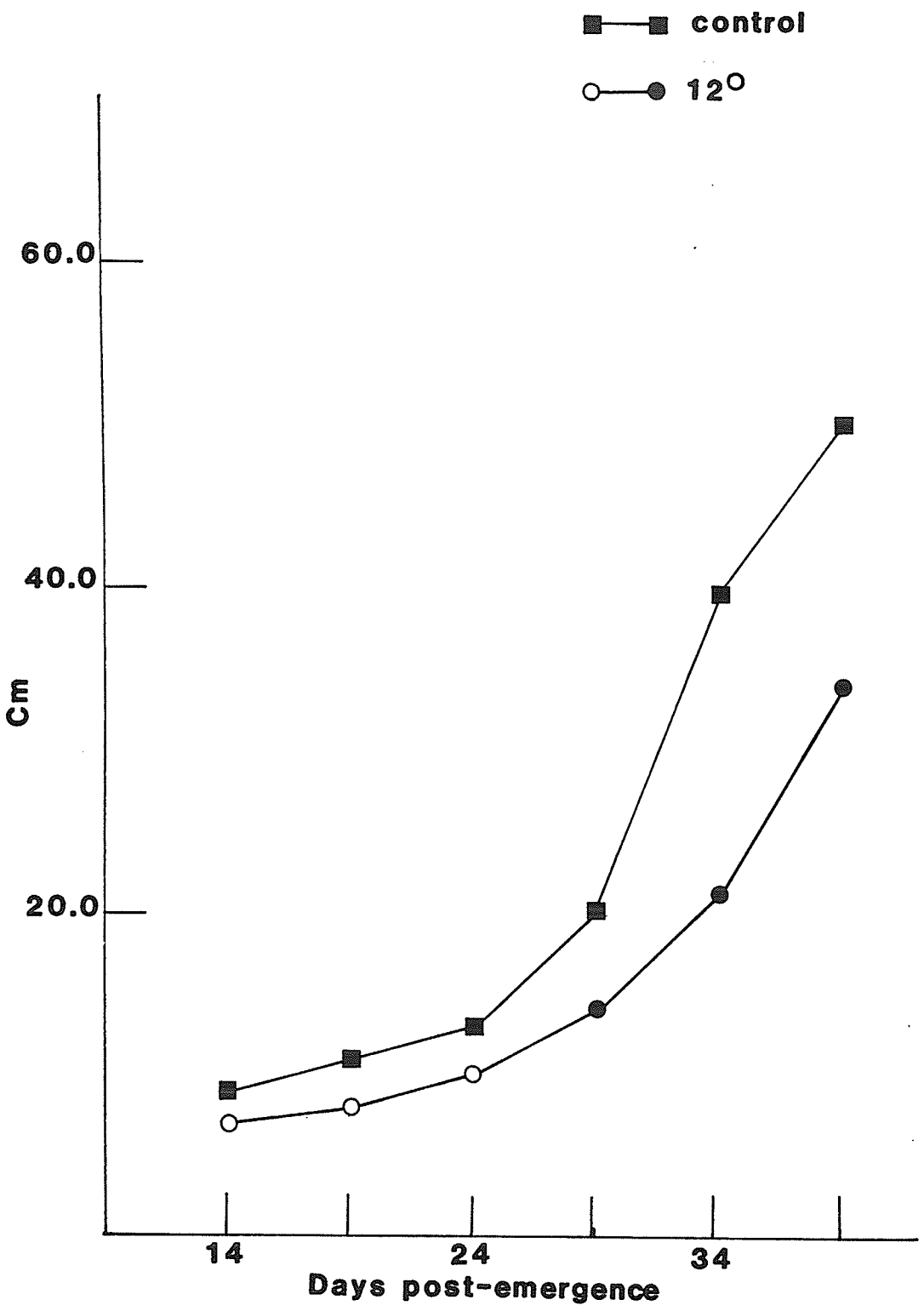

Figure 14. The effect of soil temperature on plant height . (Shaded figures indicate soil temperature control removed.)

The greatest protein component of the leaf is RuDP carboxylase, or Fraction 1 protein, which is contained in the chloroplast. Usually the first visible sign of senescence is yellowing, indicative of chloroplast disruption and chlorophyll breakdown. But before this, the protein component of the leaf has started to decrease, and the constituent amino acids are being exported from the dying organ. Therefore, loss of leaf protein precedes loss of leaf chlorophyll.

Figure 15 gives the total protein per leaf (leaf 1 plus leaf 3 plus leaf 5). The total protein is nearly equivalent for each treatment, as expressed by a similar area under each curve, but the 12^{o}C plants hold a more constant amount of leaf protein over a longer period (the plateau from about 19 to 34 days post-emergence). The controls peak sharply at day 24, and fall again just as sharply. This means that the 12^{o}C plants' protein is functional, at least in the first, third and fifth leaves, for a longer period, whereas the control plants' protein is probably being exported to subsequent leaves, and, by the end of the experiment, to the developing grain.

This functional protein is important in terms of functional chloroplasts, for photosynthesis to be continued in each leaf as long as possible. And indeed, the 12^{o}C leaves develop and senesce more slowly than do the control leaves.

Amounts of amino acids generally coincide with amounts of protein (Figure 16), although the actual total amino acids per plant is much greater in controls. Again, this could be an indication of the overall faster growth rate of control plants. Where metabolism

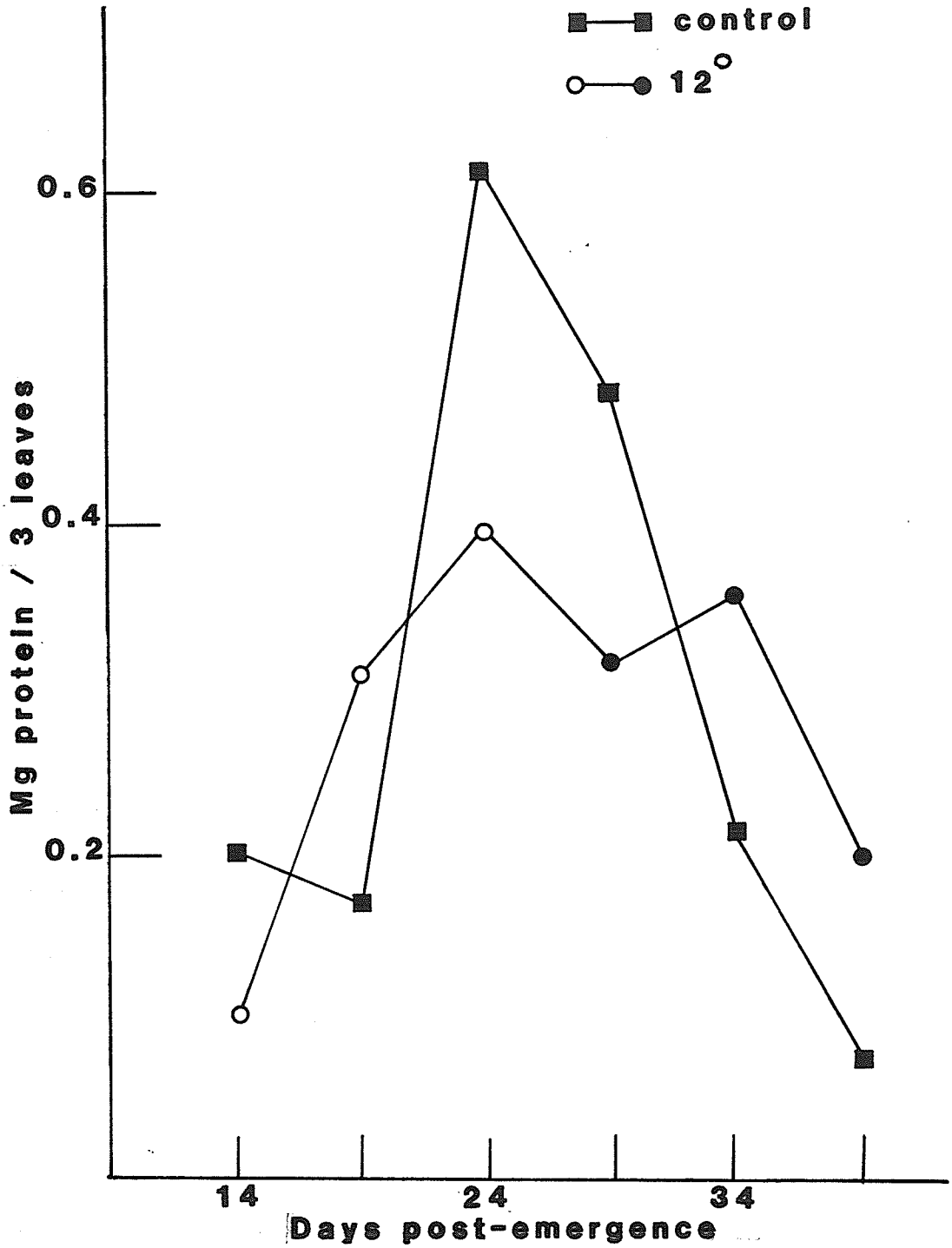

Figure 15. The effect of soil temperature on total protein / 3 leaves (Shaded figures indicate soil temperature control removed.)

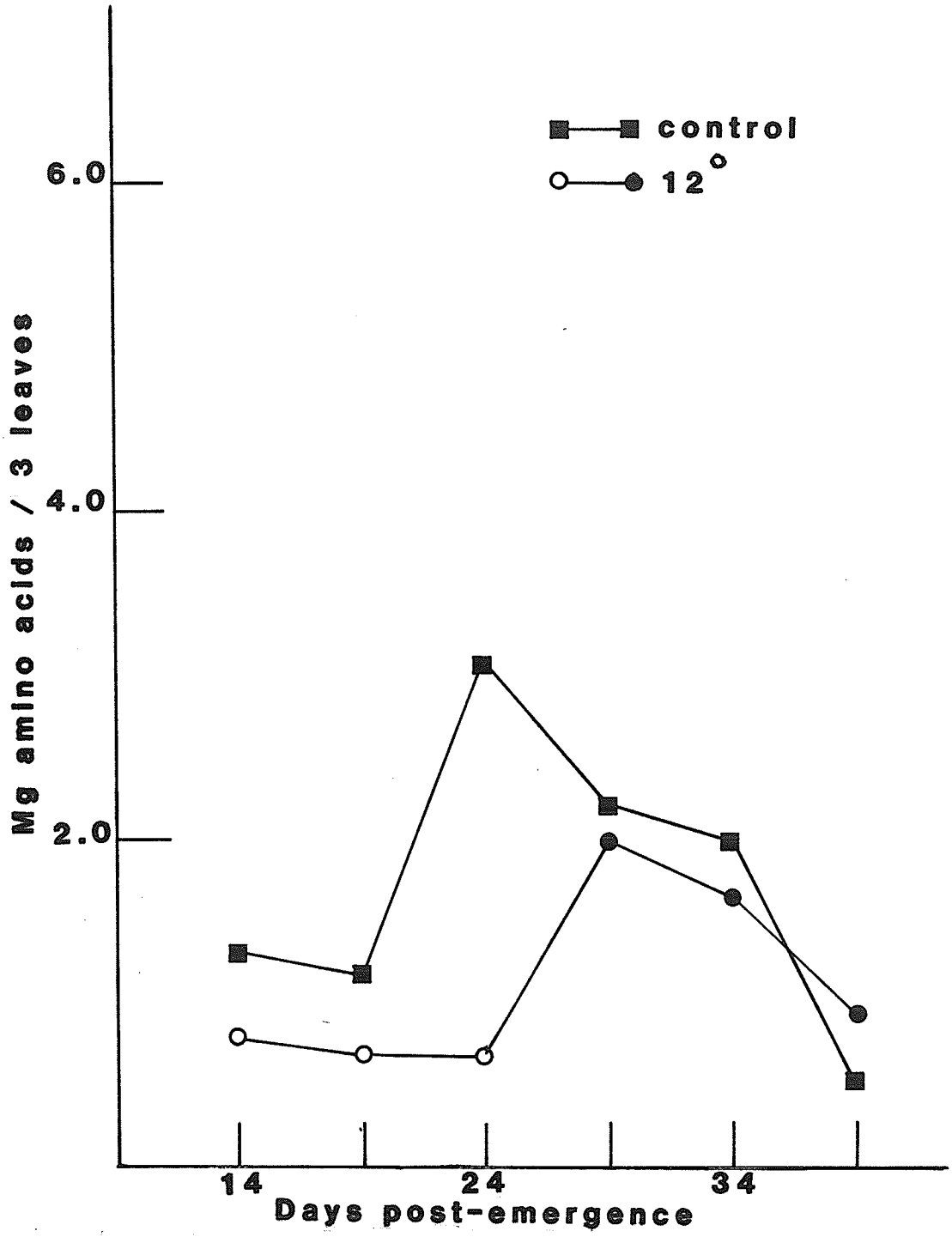

Figure 16. The effect of soil temperature on total amino acids / 3 leaves. (Shaded figures indicate soil temperature control removed.)

is proceeding at a greater rate, at any given time, relatively more amino acids are in transit between products; in 12°C plants, the respective product proteins last slightly longer. Overall, due to the greater amounts of amino acids in controls, there is more organic nitrogen in control plants.

The relationship between amino acids and protein is expressed as a ratio, amino acids per total leaf nitrogen (amino acids plus protein), in Figure 17. It is noteworthy that the curves are very similarly shaped, showing the same general pattern early in the experiment, with a sudden upward shift in the ratio (in favour of amino acids), at day 24 in 12°C plants, and at day 29 in controls. The control ratio has been constant to day 29, rising probably as a result of the senescence of the large fifth leaf, and the export of organic nitrogen. 12°C plants, conversely, have shown a less constant ratio, falling to day 24. (The decline in the ratio indicates an increase in protein relative to amino acids). The jump at day 24 shows mobilization of some of the protein, but since the total protein is constant, the amino acids are immediately reused, appearing as leaf protein in newly-manufactured leaves.

The chlorophyll content per g dry weight leaf, in the 12°C treatment, is greater in leaf 1, the same as or greater in leaf 3, and nearly the same in leaf 5; 12°C soil temperature delays chlorophyll synthesis. Overall, the amount of chlorophyll in leaf 1 plus leaf 3 plus leaf 5 of 12°C plants is greater than in the corresponding leaves of control plants, and, like protein, it persists longer (Figure 18).

Increased chlorophyll content of 12°C plants may indicate an

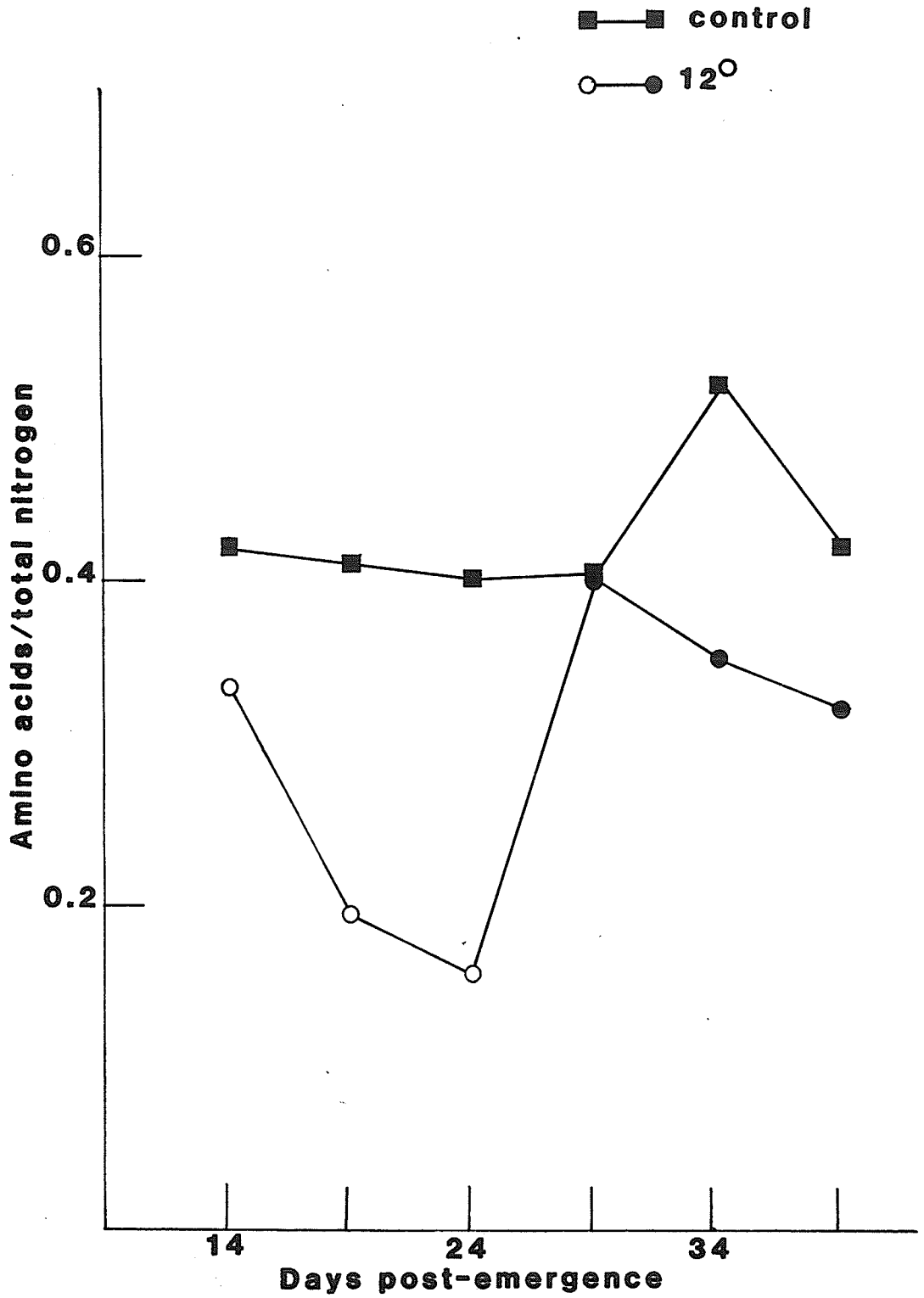

Figure 17. The effect of soil temperature on the ratio amino acids / total leaf nitrogen. (Shaded figures indicate soil temperature control removed.)

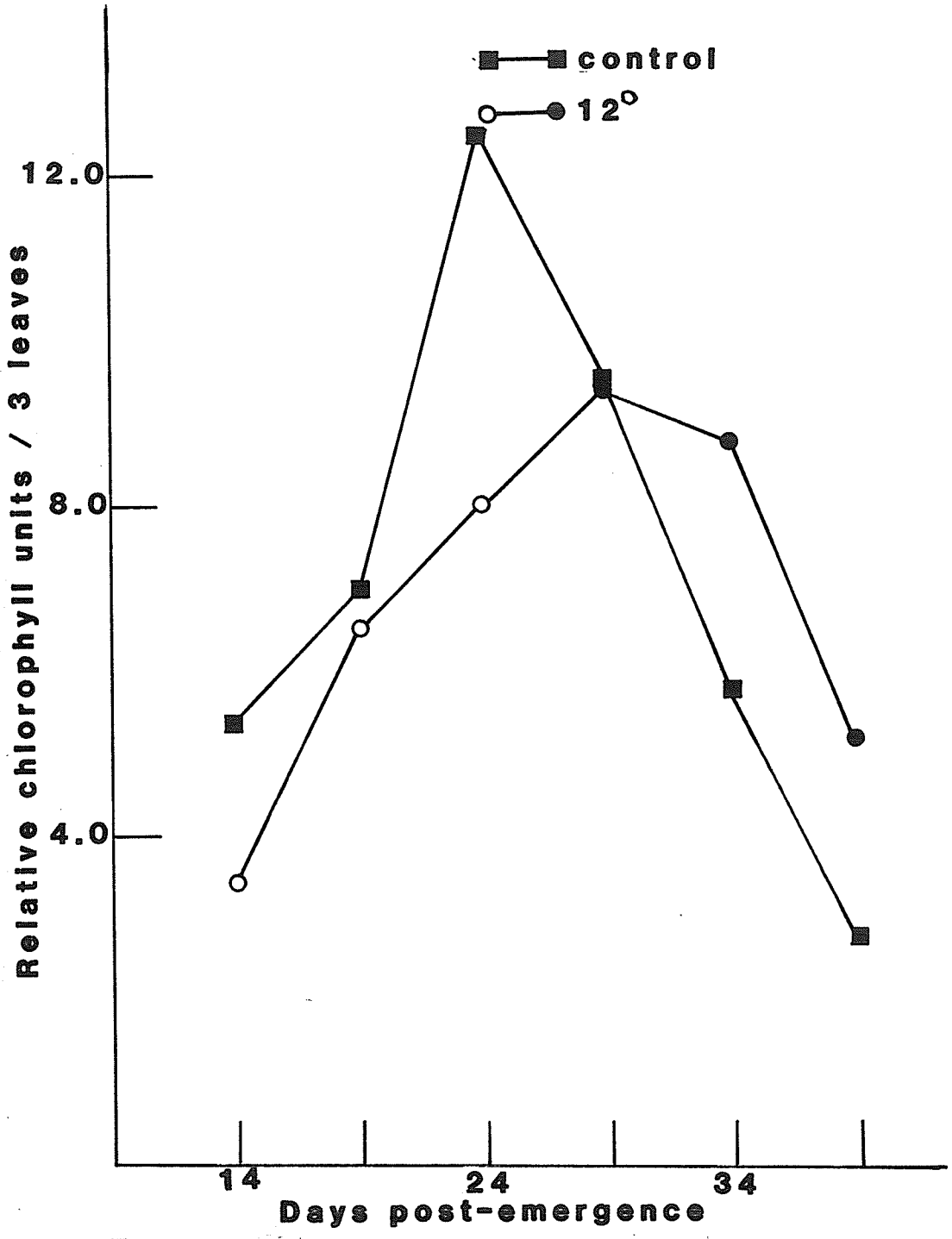

Figure 18. The effect of soil temperature on total chlorophyll / 3 leaves. (Shaded figures indicate soil temperature control removed.)

increased light-trapping potential, but the dark reactions would still be limiting. The chlorophyll molecule is not involved in these dark reactions, but enzymes, including RuDP carboxylase, are. Hence, a higher chlorophyll/protein ratio does not ensure a higher rate of photosynthesis. Soluble protein was characterized in this experiment, and it may be taken to represent the RuDP carboxylase component. Even though the 12°C plants contain more chlorophyll, there is no concommitant rise in total leaf protein, so the overall rise in photosynthesis may not rise significantly. However, unless leaf proteins are identified individually, it is impossible to say if RuDP carboxylase is changing relative to the other components.

Maximum leaf area seems not to be affected by cold root temperature (Figure 19). The light absorptive area persists longer. Also, the leaves in 12°C plants maintain functional chlorophyll and protein (photosynthetis apparatus) for a longer time. Net photosynthesis may indeed increase in 12°C plants because of this physical adjustment.

Tillering

The cold soil stimulus results in a greater number of tillers per plant in the 12°C treatment than in the control. The control tillers begin to die rapidly by day 29, and by the end of the experiment, fewer than one living tiller per plant remains (Figure 20). In 12°C plants, by means of a slower rate of growth from germination on (as illustrated by the characteristic 'lag' in many parameters already discussed), have conserved their resources, having been given only tap water since the beginning of the experiment, and can still

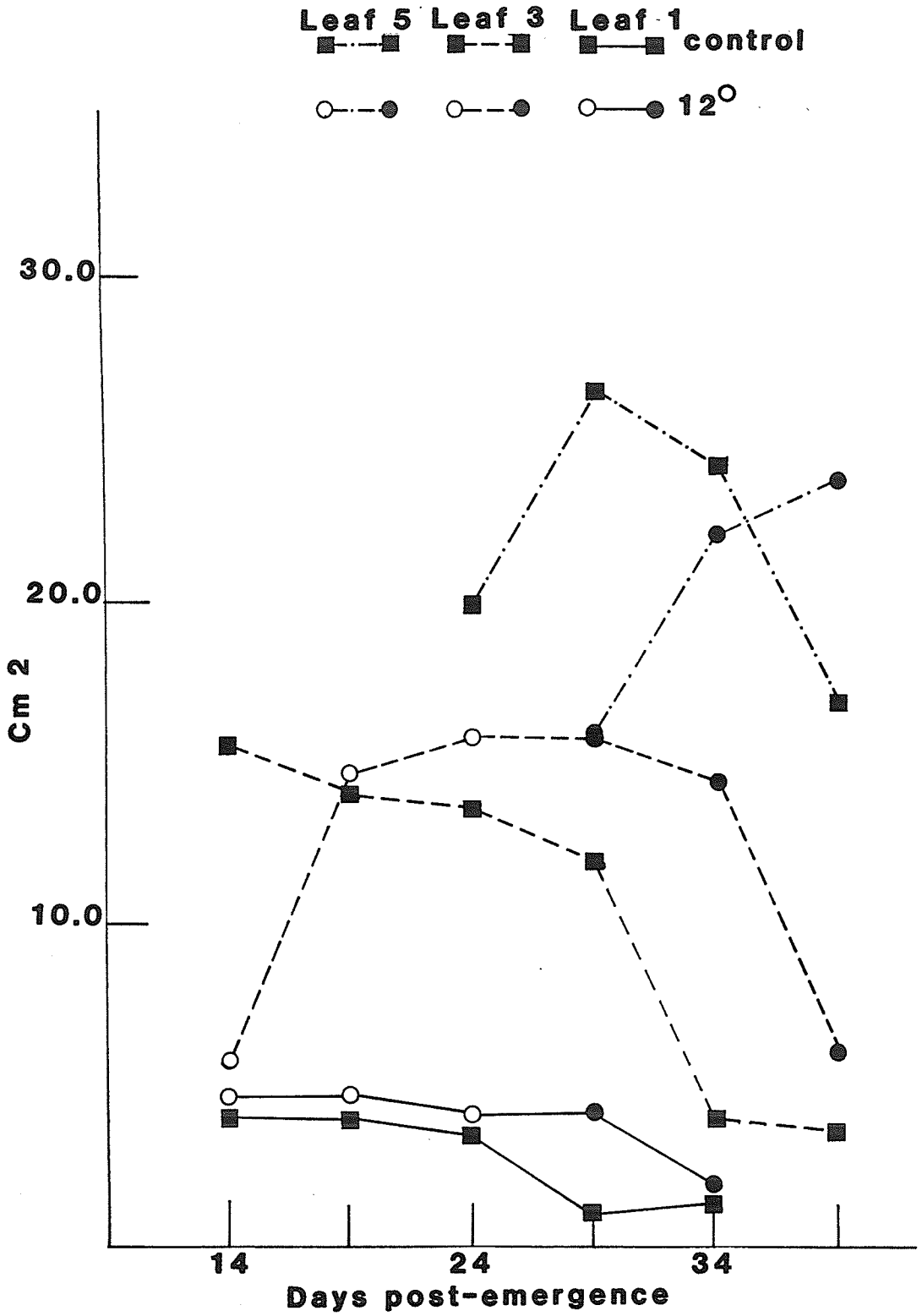

Figure 19. The effect of soil temperature on leaf area. (Shaded figures indicate soil temperature control removed.)

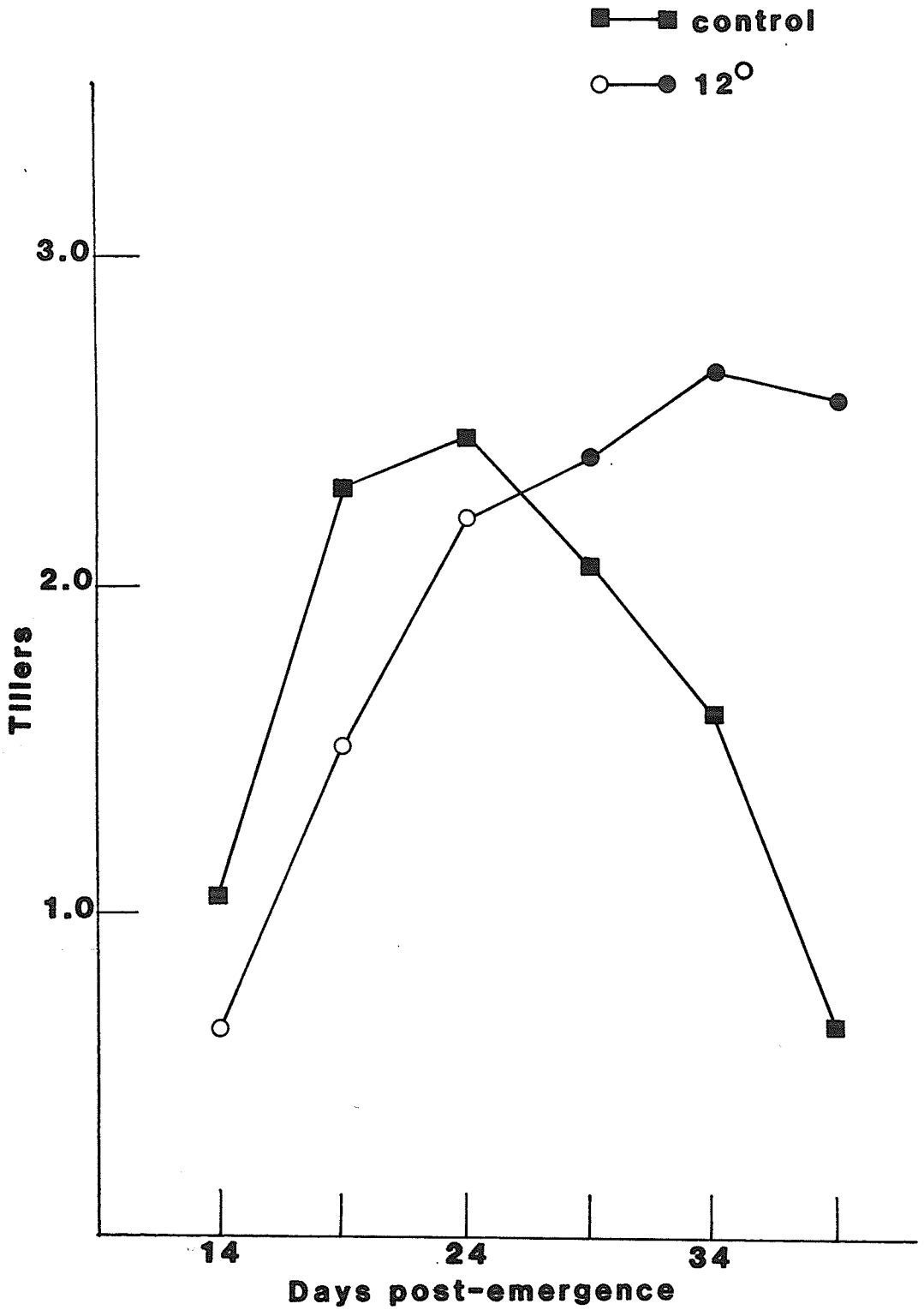

Figure 20. The effect of soil temperature on tillers per plant. (Shaded figures indicate soil temperature control removed.)

support tillers at the final sampling date. None of the tillers in either treatment appeared to have reproductive structures. They serve primarily as proof of the efficiency of 12°C plants as opposed to controls, despite the fact that all the plants were probably deficient with respect to several nutrients.

Root Anatomy

With respect to vascular tissue modifications, an increase in root diameter and in cortex radius is noted in the 12°C treatment (Tables 1 amd 2). Meyer's study (1976) indicated that, in moist soil, the axial resistance in roots was limiting to water flow up to the shoot, but in drier soils, as in soils where the water which is present is of limited availability, radial resistance in soil becomes limiting. As the root diameter increases, so does its surface area, as well as root volume. Similarly, the volume of the soil cylinder adjacent to the root surface, from which water is extracted, is also increased. This enlargement of root diameter would decrease the relative resistance of the soil to water uptake by the plant in two ways: firstly, by increasing the actual amount of reserve water adjacent to the root, and secondly, by lowering the radial resistance of the soil, as a higher concentration of moisture leads to a greater hydraulic conductivity. In addition to supplying the plant with a more constant supply of water, the larger reserve in the soil may prevent or delay root shrinkage, which occurs in times of water stress.

The number of distinct central metaxylem vessels, separated from

TABLE 1. Seedling root anatomy: 12°C root temperature. (Measurements in mm.)

	Glenlea (lot 1)	Glenlea (lot 2)	Sinton	Neepawa	Average
Sample size	17	28	27	26	Total 98
Root diameter	.61±.10 (16.4%)	.60±.07 (11.7%)	.63±.02 (3.2%)	.62±.06 (9.7%)	.62
Stele diameter	.24±.06 (25.0%)	.24±.05 (20.8%)	.26±.06 (23.1%)	.24±.06 (25.0%)	.25
Cortex radius	.19±.05 (26.3%)	.18±.04 (22.2%)	.19±.04 (21.1%)	.19±.04 (21.1%)	.19
Inner vessel diameter per root	.071±.02 (28.2%)	.077±.02 (26.0%)	.115±.02 (17.4%)	.092±.02 (21.7%)	.089
Number of vessels	1.12±.33 (29.5%)	1.18±.39 (33.1%)	1.48±.94 (63.5%)	1.50±.76 (50.7%)	1.32
Number of archs	6.88	7.18	7.11	7.15	7.08
Number of cells across cortex radius	5.53 .25 (4.5%)	5.61 .67 (10.2%)	5.89 .51 (8.7%)	6.04 .72 (11.9%)	5.77
Resistance per root (mm^{-4}) (Poiseuille)	3.94×10^4	2.85×10^4	$.57 \times 10^4$	1.40×10^4	2.19×10^4

Values in brackets are coefficients of variation.

Poiseuille equation: $R\ 1/r^4$, where R=resistance, r=vessel radius. $R_{total}=(1/r_1^4 + \ldots 1/r_n^4)/n$, where n=number if distinct radii in a treatment.

TABLE 2. Seedling root anatomy: control root temperature. (Measurements in mm.)

	Glenlea (lot 1)	Glenlea (lot 2)	Sinton	Neepawa	Average
Sample size	16	23	17	17	Total 73
Root diameter	.55±.08 (14.5%)	.58±.06 (10.3%)	.57±.08 (14.0%)	.58±.06 (10.3%)	.57
Stele diameter	.22±.04 (18.2%)	.24±.06 (25.0%)	.22±.04 (18.2%)	.25±.06 (24.0%)	.23
Cortex radius	.17±.04 (23.5%)	.17±.04 (23.5%)	.18±.04 (22.2%)	.16±.03 (18.8%)	.17
Inner vessel diameter per root	.068±.04 (58.8%)	.068±.01 (14.7%)	.065±.02 (30.8%)	.085±.02 (23.5%)	.072
Number of vessels	1.19±.40 (33.6%)	1.09±.50 (45.9%)	1.24±.44 (35.5%)	1.35±.49 (36.3%)	1.22
Number of archs	7.25	7.13	6.88	6.94	7.05
Number of cells across cortex radius	5.75 .45 (7.8%)	5.43 .51 (9.4%)	6.00 .94 (15.7%)	5.35 .49 (9.2%)	5.63
Resistance per root (mm^{-4}) (Poiseuille)	4.69×10^4	4.69×10^4	5.62×10^4	1.92×10^4	4.23×10^4

Values in brackets are coefficients of variation.

each other by cells, not just by a cross-wall, is greater in 12°C roots, giving rise to an overall increase in the total central metaxylem vassel diameter per root. This structural modification is shown in Figure 21.

The present results show changes in metaxylem anatomy in roots grown at 12°C as follows: one, an increase in the number of metaxylem vessels, and two, an increase in vessel radius. It is also shown that varieties differ in vessel number and size. Tables 1 and 2 contain all the information describing these parameters of seminal roots.

The dogma that the seminal roots of wheat have one metaxylem vessel, whereas the nodal (adventitious) roots have several, has stood for many years (since Percival, 1921). Thus, Ponsana (1975) examined the distribution of roots in monoliths of field soil. Since it was not possible to trace individual roots down the profile, the seminal roots were identified on the basis of metaxylem anatomy. On this basis, any root that had several vessels would be classified, perhaps incorrectly, as a nodal root. Passioura (1972) grew wheat plants in lengths of sewer pipe filled with soil. Development of nodal roots was prevented by a small aluminum pan on the surface; the seminal roots were threaded through a small hole in the bottom of the pan. He indicated that the seminal roots had only one metaxylem vessel. Meyer (1976) grew seedlings of a number of varieties in a nutrient solution contained in growth pouches. A small number of his plants (less than 12%) showed a double vessl with a common wall, However, the effective vessel radius was not changed by this configuration. Roots of seedlings of a durum wheat also showed

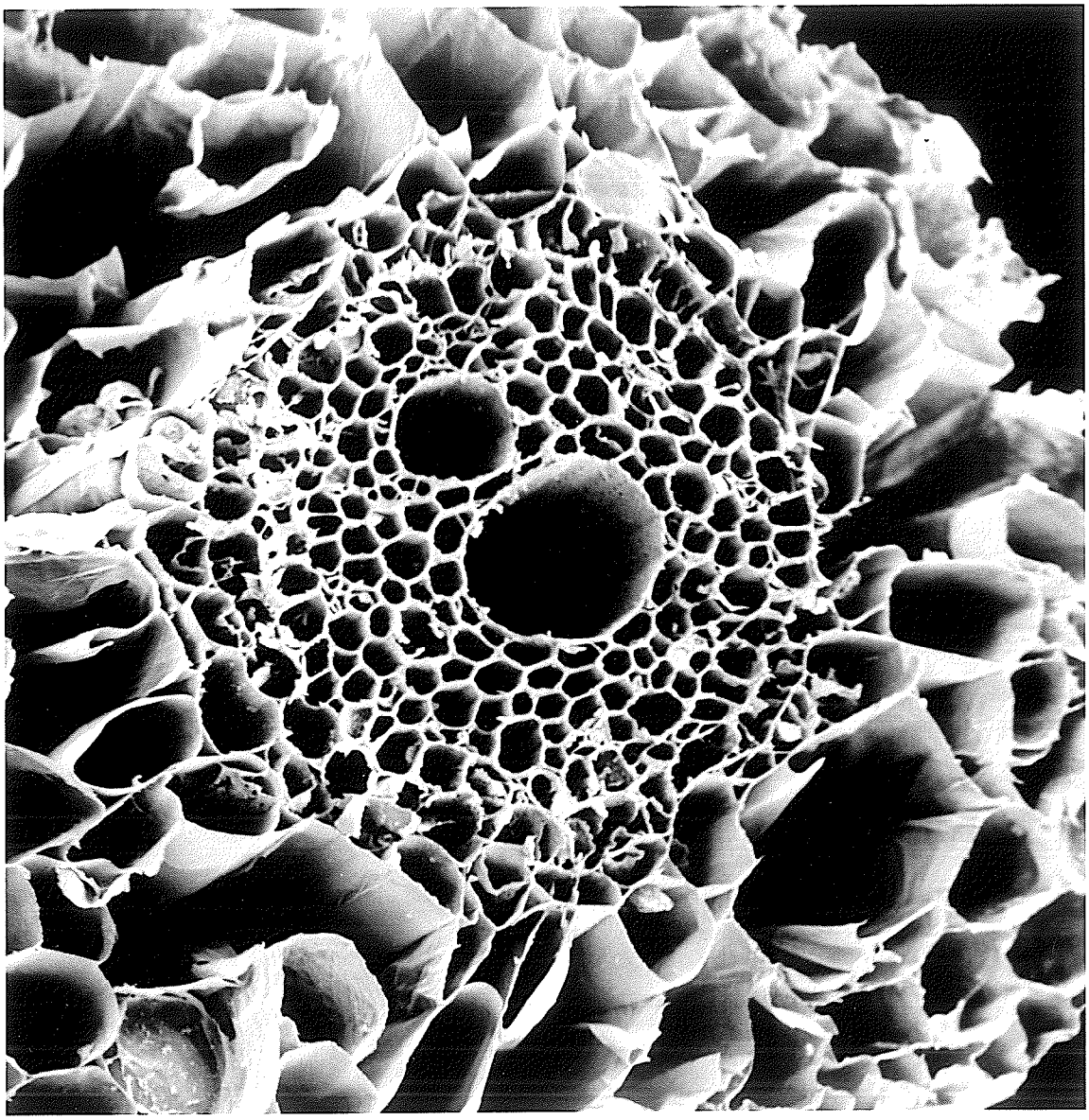

Figure 21. Central metaxylem vessels in wheat root grown at 12°C soil temperature (200x).

several vessels (Woodbury, personal commumication). Seminal roots of barley having several metaxylem vessels have been observed (Volkmar, personal communication). Barley roots are considered to show the same distinct differences between seminal and nodal roots as do wheat roots (Briggs, 1978).

The present results show that, under some conditions, seminal roots can develop several central metaxylem vessels. This is clearly within the genetic capability of the plant, since the pattern is characteristic of nodal roots. It would appear that environmental conditions may modify the pattern of differentiation in the root. However, both Passioura and Meyer noted large variations within varieties; breeding for desirable characteristics would be made much more difficult, due to the necessity of removing this variability to get a real view of environmental effect.

Passioura (1972, 1976) suggested that the metaxylem vessel of seminal roots of the wheat plant may constitute a limiting resistance to water flow. The plant can develop a maximum of six seminal roots, but five is most common (Meyer, 1976). Seminal root primordia differentiate during grain development, and the number of primordia can be reduced by stress conditions at this time. Seed size is a factor; seminal root number decreased from 5.2 to 4.2 as seed size decreased from 59 to 20 mg. Germination temperature had a slight effect on the number of seminal roots developed; between 10^{o} and $35^{o}C$, the number of roots increased from 5.0 to 5.3, but a large variety x temperature interaction was evident. Meyer studied 24 varieties at different temperatures. The mean vessel radius decreased from 35 nm

at 10°C, to 30 nm at 27°C. Varieties had a mean vessel radius (5 cm from the root base) ranging from 27 to 36 nm. However, in a pot study, it was found that vessel radius was not constant over the root length; it increased from 26 nm at 1 cm, to 45 nm at 105 cm below the base of the plant.

The external carbohydrate layer of roots also shows modifications induced by the cold soil temperature. In cross-sections of roots grown at 12°C soil temperature, in comparison with those grown at 8°C and at control temperature, a very distinct layer of carbohydrate, characterized by positive staining with periodic acid/Schiff's stain, was noted. This carbohydrate layer, although not biochemically identified, is possible mucilage. It was found on the roots preserved from the first replicate of the first part of the experiment, which were embedded in plastic. The roots of seedlings in the second part of the research were exposed to the stain, but without a protective coating around them, the outer layer was destroyed in sectioning. There would also be a loss of mucilage to microbes under non-sterile conditions, as well as a sloughing off of mucilagenous tissue to the washing solution.

SUMMARY AND CONCLUSIONS

As the soil temperature dropped from 16.5°-17.5° to 12° to 8°C, the pre-emergence lag phase was prolonged; there was little effect on rate og growth. Since growth would be expected to decline with lower temperature, it seemed that biological and physiological changes may have occurred during the lag phase, which compensated for the temperature effects.

Once seedling growth was established, soil temperature had a differential effect on growth of root and shoot. Shoot growth was depressed by low temperature, although the plants appeared normal and healthy. However, root growth was only depressed at 8°C; fresh and dry weights of 12°C plants equalled or surpassed those of plants grown at higher temperatures.

The importance of this increased efficiency of growth and metabolism, despite what has been previously considered a negative influence, is most clearly understood by observing the plants at heading. When the controls were beginning to head, at about day 34, and by day 39, there was relatively little green tissue left on the top for grain filling; only the top leaves were not senescent. 12°C plants headed about one sampling period behind controls, and even then, there was much green tissue left to ensure adequate grain filling.

The root as well was conservative of the photosynthate translocated down from the shoots. There was no wastage through dead cells and

loss of cell contents; the root tissue remained intact and functional. The thick mucigel layer may be construed as waste, but it could aid in supplying water to roots, and in promoting symbiosis with soil microflora.

In general, the low soil temperature favoured an increase in root/shoot ratio. However, it appeared that there may have been a critical temperature located between 8^{o} and $12^{o}C$, below which depression of root growth occurs. In other experiments, a shift in plant growth rate occurred at about the four-leaf stage in plants at the lowest soil temperature, with the result that, although maturity was delayed, the accumulated weight of roots, shoots, and grain eventually equalled that of plants grown at the higher soil temperatures. This growth compensation was not seen in the present study, because the experiment did not continue through to maturity.

The lag phenomena which had been observed before was present in this series of experiments. The question: was it obligatory, due to the inability of the cold-stimulated plants to surpass this growth rate because of a shortage of water and nutrient from the cooled roots; or, was it voluntary, due to an adaptation to the cold stimulus by implementing a different growth schedule. It would appear voluntary from these results. Were it merely a thermodynamic response to cold, it could be expected that the growth rate after day 24 would take off as the temperature control was lifted, and adjust itself to the control rate. This did not occur. In addition to this, basic anatomical features were altered irreversibly, and the plant seemed to benefit.

This adaptability is exciting in terms of breeding; the mechanisms for stress adaptation were already present in the wheat plant. A plant should be capable of being moulded to almost any environment, after the physical features which best suit that environment are ascertained. It must be concluded that wheat (and other temperate cereals) has evolved a number of mechanisms which allow it to compensate for low temperature during germination, emergence and early growth. Experimentally, these compensations will only be seen if time is allowed for the processed to occur.

The observed effect of nitrogen status on plant growth may be important. Increased nitrogen usually shifts the root/shoot ratio in favour of vegetative growth; low temperature has the opposite effect. In our experiments, where nutrient supply was limiting, as indicated by rapid leaf senescense and mobilization of leaf nitrogen, lower soil temperature appeared to abolish the effects of nitrogen on root growth. If this occurs under field conditions, low temperature may offset the effects of fertilizer nitrogen.

Further work should focus on the priority which nitrogen, water, temperature, and light exert on root and shoot development. The means by which the genetic pattern controlling yield, protein and drought tolerance modifies the response pattern should be examined. It would also be most helpful to discover how the death of the cortex is influenced by these factors, and if the exudation of carbonaceous material in turn influences the rhizosphere microflora.

Perhaps the most significant results of this research pertain

to root anatomy. The cold-stimulated root reacted to decreased water availability by self-imposed adaptation, to overcome this limitation in several ways. More numerous and larger central metaxylem vessels lowered resistance to upward solute flow. A greater total radius of central metaxylem gave less resistance to flow, as well as a greater surface area for entry into the vessels from the root external to the stele. An intact cortex facilitated flow through living cells, instead of by a tortuous route around air pockets in a dead root. A greater whole-root radius made contact with a greater amount of soil water. In addition to all these inner modifications, the carbohydrate outer layer resisted dehydration, and helped prevent shrinkage and separation of soil and root, and the formation of air pockets between them.

Most importantly, previous assumptions that the seminal root has only one central metaxylem vessel, while the nodal roots have more than one, are not valid.

Statistical analyses done on the root anatomy data are not conclusive of the signifigance of differences between treatments overall, although some varieties show signifigance with respect to some parameters (Tables 1 and 2). However, close examination of all the changes in 12^{o}C plants points out that these roots are all modified in the same direction, that is, all towards lowered resistance to water uptake. While statistics is a useful tool in interpretation of results, in this case, statistical results may only discourage further work on wheat plants with their roots

subjected to temperature stress, and this would be an unfortunate misuse of statistics. My hope is that future workers will be excited by the revolutionary information furnished by the series of experiments described in this thesis, and will overcome the handicaps of seemingly insignificant results, and a messy and tedious experimental method, to study roots, whether stressed or growing under optimal conditions, in much greater detail.

The present results suggest that 12°C may be optimum for wheat root extension and growth, and for the growth of shoots attached to roots growing along a 12°C isotherm. There is an adaptation to the cold stimulation, as these plants were grown from initial imbibition and germination at the experimental temperature. This is not to be confused with a recovery mechanism mentioned by some workers, who subjected plants grown at normal temperatures to sudden periods of cold; recovery implies damage, and the plants in this research were not damaged by cold. The 'lag' period noted here is not a recovery period, but a valuable growth adaptation, to facilitate very efficient growth.

The plant that deveolps when roots grow at 12°C soil temperature, while the tops develop at 16.5°-17.5°C air temperature, has an overall smaller shoot, with an extensive and viable root system, which combine to make the best of environmental adversities (low nitrogen, water stress, crowding) and benefits (12°C soil).

LITERATURE CITED

Abdelhafeez, A.T., H. Harssema, G. Veri and K. Verkerk. 1971. Effects of soil and air temperature on growth, development and water use of tomatoes. Netherlands Journal of Agricultural Science. 19:67-75.

Army, T.J. and E.V. Miller. 1959. Effect of lime, soil type and soil temperature on phosphorus deficient soils. Agronomy Journal. 51:376-378. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, polyphenol-oxidase in Beta vulgaris. Plant Physiology. 24:1-15.

Atkin, R.K., G.E. Barton and D.K. Robinson. 1973. Effect of root growing temperature on growth substances in xylem exudate of Zea mays. Journal of Experimental Botany. 24:475-487.

Balandreau, J. and R. Knowles. 1978. The rhizosphere. In Interactions Between Non-Pathogenic Soil Microorganisms and Plants. (T.R. Donnmergues and S.V. Krupa, eds.) Elsevier Scientific, Amsterdam.

Bio-Rad Labs Technical Bulletin #1051. 1977.

Blackshaw, R.E. 1979. Environmental factors affecting green foxtail (Setaria viridis) competition in spring wheat. M.Sc. Thesis, University of Manitoba, Winnipeg, Manitoba.

Brengle, K.G. and C.J. Whitfield. 1969. Effect of soil temperature on the growth of spring wheat with and without wheat straw mulch. Agronomy Journal. 61:377-379. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Briggs, D.E. 1978. Barley. Chapman and Hall, London.

Brouwer, R. 1962. Influences of temperature of the root medium on the growth of seedlings of various crop plants. Mededeling 175 van het IBS. 175:11-18.

Brouwer, R. 1966. Root growth of cereals and grasses. In The Growth of Cereals and Grasses. (F.L. Milthorpe and D.J. Ivins, eds.) Butterworths, London.

Brouwer, R. and A. Hoogland. 1964. Responses of bean plants to root temperatures. 11.Anatomical aspects. Mededeling 236 van het IBS. 236:23-31.

Burch, G.J. 1979. Soil and plant resistance to water absorption by plant root systems. Australian Journal of Agricultural Research. 30:279-292.

Burstrom, H. 1956. Temperature and root cell elongation. Physiologia Plantarum. 9:682-692.

Cannell, R.Q. 1977. Soil aeration and compaction in relation to root growth and soil management. In Applied Biology Vol. 11. (T.H. Coaker, ed.) Academic Press, London.

Case, V.W., N.C. Brady and D.J. Lathwell. 1964. The influence of soil temperature and phosphorus fertilizers of different water-solubilities on the yield of phosphorus uptake by oats. Soil Science Society of America Proceedings. 28:409-412.

Chaudhary, T.N., N.Y. Singh and G. Singh. 1971. Water absorption by seeds as affected by soil temperature. Plant and Soil. 35:189-192.

Clarkson, D.T. 1976. The influence of temperature on the exudation of xylem sap from detached root systems of rye and barley. Planta. 132:297-304.

Clarkson, D.T. and A.W. Robards. 1975. The endodermis, its structural development and physiological role. In The Development and Function of Roots. (J.G. Torrey and D.T. Clarkson, eds.) Academic Press, London.

Clarkson, D.T. and J. Sanderson. 1978. Sites of absorption and translocation of iron in barley roots: Tracer and microauto-radiographic studies. Plant Physiology. 61:731-736.

Clarkson, D.T., M.G.T. Shone and A.V. Wood. 1974. The effect of pretreatment temperature on the exudation of xylem sap by detached barley root systems. Planta. 121:81-92.

Cutter, E.G. 1975. Plant Anatomy: Organs. Edward Arnold Ltd, London.

Fujiwara, A. and M. Suzuki. 1961. Effects of temperature and light on the translocation of photosynthetic products. Tohuku Journal of Agricultural Research. 12:363-367.

Garwood, E.A. 1968. Some effects of soil water conditions and temperature on the roots of grasses and clovers. 11.Effects of variation in the soil water content and in soil temperature on

root growth. Journal of British Grassland Society. 23:117-127. In Nielsen, K.J. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Glass, A.D.M. 1978. Influence of excision and aging upon K^+ influx into barley roots: Recovery or enhancement? Plant Physiology. 61:481-483.

Graecen, E.L., P. Ponsana, and K.P. Barley. 1976. Resistance to water flow in the roots of cereals. In Water and Plant Life: Problems and Modern Approaches. (O.L. Lange, L. Kappen and E.-D. Schulze, eds.) Springer-Verlag, Berlin.

Halse, N.J. and R.N. Weir. 1970. Effects of vernalization, photoperiod and temperature on phenological development and spikelet number of Australian wheat. Australian Journal of Agricultural Research. 21:383-393.

Herath, W. and D.P. Ormrod. 1965. Some effects of water temperature on the growth and development of rice seedlings. Agronomy Journal. 57:373-376. In Nielsen, K.F. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Huck, M.G., B. Klepper and H.M. Taylor. 1970. Diurnal variations in root diameter. Plant Physiology. 45:529-530.

Humphries, E.C. 1951. The absorption of ions by excised root systems. 11.Observations on roots of barley grown in solutions deficient in phosphorus, nitrogen or potassium. Journal of Experimental Botany. 2:344-379. In Nielsen, K.F. and E.C. Humphries. 1966. Effects of root temperature on plant growth. Soils Fert. 29:1-7.

Humphries, E.C. 1967. The effect of different root temperatures on dry matter and carbohydrate changes in rooted leaves of Phaseolus spp. Annals of Botany, New Series. 31:59-69.

Hunter, J.R. and A.E. Erikson. 1952. Relation of seed germination to soil moisture tension. Agronomy Journal. 44:107-109.

Hurd, E.A. 1974. Phenotype and drought tolerance in wheat. Agricultural Meteorology. 14:39-55.

Hurd, E.A. and E.D. Spratt. 1975. Root patterns in crops as related to water and nutrient uptake. In Physiological Aspects of Dryland Farming. (U.S. Gupta, ed.) Oxford and IBH, New Delhi.

Ito, T. and T. Takeda. 1963. Studies on the effect of temperature on the sugar production in sugarbeet. 1.The effect of temperature on the growth. Crop Science Society of Japan Proceedings. 31: 272-276. In Nielsen, K.F. 1974. Root and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University of Virginia Press, Charlottesville.

Jensen, G. 1960. Effects of temperature and shifts in temperature on the respiration of intact root systems. Physiologia Plantarum. 13:822-830.

Kaufmann, M.R. 1975. Leaf water stress in Engelman spruce: Influence of the root and shoot environments. Plant Physiology. 56:841-844.

Ketellapper, H.J. 1960. The effect of soil temperature on the growth of Phalaris tuberosa L. Physiologia Plantarum. 13:641-647. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University of Virginia Press, Charlottesville.

Kleinendorst, A. and R. Brouwer. 1970. The effect of temperature of the root medium and of the growing point of the shoot on growth, water content and sugar content of maize leaves. Netherlands Journal of Agricultural Science. 18:140-148.

Korovin, A.I., Z.F. Sycheva and Z.A. Bystrow. 1961. Effect of soil temperature on utilization of phosphorus by plants. Dokl. Akad. Nauk. 137:458-461. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University of Virginia Press, Charlottesville.

Labanauskas, C.K., L.H. Stolzy and E.J. Luxmoore. 1975. Soil temperature and soil aeration effects on concentrations and total amounts of nutrients of 'Yecora' wheat grain. Soil Science. 120:450-454.

Landsberg, J.J. and N.D. Fowkes. 1978. Water movement through plant roots. Annals of Botany. 42:493-508.

Lawlor, D.W. 1973. Growth and water absorption of wheat with parts of the roots at different water potentials. New Phytology. 72: 297-305.

Letey, J., L.H. Stolzy, G.B. Blank and O.R. Lunt. 1961. Effect of temperature on oxygen diffusion-rates and subsequent shoot growth and mineral content of two plant species. Soil Science. 92:314-321. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Luxmoore, R.J., R.A. Fischer and L.H. Stolzy. 1973. Flooding and soil temperature effects on wheat during grain filling. Agronomy Journal. 65:361-364.

Mack, A.R. 1965. Effect of soil temperature and moisture on yield and nutrient uptake by barley. Canadian Journal of Soil Science. 45:337-346. In Nielsen, K.F. 1974. Roots and root temperatures In The Plant Root and its Environment. (N. Carson, ed.) University of Virginia Press, Charlottesville.

MacKey, J. 1973. The wheat root. In 4th International Wheat Genetics Symposium. Columbia.

Manitoba Zonation Trials. 1971-75, 1974-78. Prepared by the Dept. of Plant Science, University of Manitoba, Winnipeg, Manitoba.

Martin, J.K. 1977. Effect of soil moisture on the release of organic carbon from wheat roots. Soil Biology and Biochemistry. 9:303-304.

Meyer, W.S. 1976. Seminal roots of wheat: Manipulation of their geometry to increase the availability of soil water and to improve the efficiency of water use. PhD Thesis, University of Adelaide, Adelaide, South Australia.

Namken, L.N., C.L. Wiegand and W.O. Willis. 1974. Soil- and air-temperatures as limitations to more efficient water use. Agricultural Meteorology. 14:169-181.

Nelson, N. 1944. Photometric adaptation of the Somogyi method for the determination of glucose. Journal of Biological Chemistry. 153:375-380.

Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University of Virginia Press, Charlottesville.

Nielsen, K.F. and R.K. Cunningham. 1964. The effects of soil temperature, form and level of nitrogen on growth and chemical composition of Italian ryegrass. Soil Science Society of America Proceedings. 25:369-372. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Nielsen, K.F., R.L. Halstead, A.J. MacLean, R.M. Holmes and S.J. Bourget. 1960. The influence of soil temperature on the growth and mineral composition of oats. Canadian Journal of Soil Science. 40:255-264.

Nielsen, K.F. and E.C. Humphries. 1966. Effects of root temperature on plant growth. Soils Fert. 29:1-7.

Nordin, A. 1976a. Water flow in wheat seedlings after small water deficits. Physiologia Plantarum. 37:157-162.

Nordin, A. 1976b. Effects of water stress and abscissic acid on transpiration regulation in wheat. Physiologia Plantarum. 38:233-239.

Nordin, A. 1977. Effects of low root temperature on ion uptake and ion translocation in wheat. Physiologia Plantarum. 39:305-310.

Nowakowski, T.Z., R.K. Cunningham and K.F. Nielsen. 1965. Nitrogen fractions and soluble carbohydrates in Italian ryegrass. 1.Effects of soil temperature, form and level of nitrogen. Journal Science Food and Agriculture. 16:124-134. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Owen, P.C. 1971. The effects of temperature on the growth and development of rice. Field Crop Abstracts. 24:1-8. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Passioura, J.B. 1972. The effect of root geometry on the yield of wheat growing on stored water. Australian Journal of Agricultural Research. 23:745-752.

Passioura, J.B. 1974. The effect of root geometry on the water relations of temperate cereals (wheat, barley, oats). In Structure and Function of Primary Root Tissues. (J. Kolek, ed.) Veda, Bratislava.

Passioura, J.B. 1977. Grain yield, harvest index and water use of wheat. Journal of Australian Institute of Agricultural Science. pp. 117-120.

Pawloski, M.C. and C.F. Shaykewich. 1972. Germination of wheat as affected by soil water stress. Canadian Journal of Plant Science. 52:619-623.

Pearson, R.W., L.F. Ratliff and H.M. Taylor. 1970. Effect of soil temperature, strength and pH on cotton seedling root elongation. Agronomy Journal. 62:243-246. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Peoples, M.B. and M.J. Dowling. 1978. Degredation of ribulose-1,5-biphosphate carboxylase by proteolytic enzymes from crude extracts of wheat leaves. Planta. 138:153-160.

Percival, J. 1921. The Wheat Plant. Duckworth and Co., London.

Pitman, M.G. 1977. Ion transport into the xylem. Annual Review of Plant Physiology. 28:71-88.

Power, J.F., D.L. Grunes, W.O. Willis and G.A. Reichman. 1963. Soil temperature and phosphorus effects upon barley growth. Agronomy Journal. 55:389-392.

Power, J.F., G.A. Reichman and W.O. Willis. 1964. Soil temperature effects upon phosphorus availability. Agronomy Journal. 56:545-548. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Power, J.F., D.L. Grunes, G.A. Reichman and W.O. Willis. 1970. Effect of soil temperature on rate of barley development and nutrition. Agronomy Journal. 62:567-571.

Radke, J.F. and R.E. Bauer. 1969. Growth of sugarbeets as affected by root temperatures. Part 1. Greenhouse studies. Agronomy Journal. 61:860-863. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Rains, D.W. 1976. Mineral metabolism. In Plant Biochemistry. (J. Bonner and J.E. Varner, eds.) Academic Press, New York.

Rosenberg, N.J. 1974. Microclimate: The Biological Environment. John Wiley and Sons, Inc., New York.

Rovira, A.D. and G.D. Bowen. 1973. The influence of root temperature on ^{14}C assimilate profiles in wheat roots. Planta. 114:101-107.

Shul'gin, A.M. 1965. The Temperature Regime of Soils. Translated from Russian by Prof. A. Gourevitch. Sivan Press, Jerusalem.

Singh, N.T. and G.J. Dhaliwal. 1972. Effect of soil temperature on seedling emergence of different crops. Plant and Soil. 37:441-444.

Skene, K.G.M. and D.H. Kerridge. 1967. Effect of root temperature on cytokinin activity in root exudate of Vitis vinifera L. Plant Physiology. 42:1131-1139.

Sojka, R.E., L.H. Stolzy and M.R. Kaufmann. 1975. Wheat growth related to rhizosphere temperatures and oxygen level. Agronomy Journal. 67:591-596.

Sutton, C.D. 1969. Effect of low soil temperature on phosphate nutrition of plants - a review. Journal Science of Food and Agriculture. 20:1-3. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Tal, M. and D. Imber. 1970. Abnormal stomatal behaviour and hormonal imbalance in flacca, a wilty mutant of tomato. 11.Auxin- and abscissic acid-like activity. Plant Physiology. 46:373-376.

Tal, M. and D. Imber. 1971. Abnormal stomatal behaviour and hormonal imbalance in flacca, a wilty mutant of tomato. 111.Hormonal effects on the water status in the plant. Plant Physiology. 47:849-850.

Tal, M., D. Imber and C. Itai. 1970. Abnormal stomatal behaviour and hormonal imbalance in flacca, a wilty mutant of tomato. 1.Root effect and kinetin-like activity. Plant Physiology. 46:367-372.

Taylor, H.M. 1974. Root behaviour as affected by soil structure and strength. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Taylor, H.M. and B. Klepper. 1975. Water uptake by cotton root systems: An examination of assumptions in the single root model. Soil Science. 120:57-67.

Tetley, R.M. and K.V. Thimann. 1974. The metabolism of oat leaves during senescence. 1.Respiration, carbohydrate metabolism and the action of cytokinins. Plant Physiology. 54:294-303.

Torrey, J.G. 1976. Root hormones and plant growth. Annual Review of Plant Physiology. 27:435-459.

Troughton, A. 1974. The growth and function of the root in relation to the shoot. In Structure and Function of Primary Root Tissue. (J. Kolek, ed.) Veda, Bratislava.

Tyree, M.T. 1973. An alternative explanation for the apparently active water exudation in excised roots. Journal of Experimental Botany. 21:33-37.

Varner, J.E., L.V. Balce and R.C. Huang. 1963. Senescence of cotyledons of germinating peas. Influence of axis tissue. Plant Physiology. 38:89-92. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Varner, J.E. and D. Tuan-Hua Ho. 1976. Hormones. In Plant Biochemistry. (J. Bonner and J.E. Varner, eds.) Academic Press, New York.

Voorhees, W.B., M. Amemiya, R.R. Allmaras and W.E. Larson. 1971. Some effects of aggregate structure and heterogeneity on root growth. Soil Science Society of America Proceedings. 35:638-643.

Ward, J. and C.F. Shaykewich. 1972. Water absorption by wheat seeds as influenced by hydraulic properties of soil. Canadian Journal of Soil Science. 52:99-105.

Watschke, T.L., R.E. Schmidt and R.E. Blaser. 1970. Responses of some Kentucky bluegrasses to high temperature and nitrogen fertility. Crop Science. 10:372-376. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Wilkinson, G.E. 1967. How soil temperature affects plant growth. North Dakota Farm Research. 24:12-14.

Williams, D.E. and J. Vlamis. 1962. Differential cation and anion absorption as affected by climate. Plant Physiology. 37:198-202. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

Yemm, E.W. and E.C. Cocking. 1955. The determination of amino acids with ninhydrin. Analyst. 80:209-213.

Younis, M.A., A.W. Pauli, H.L. Mitchell and F.C. Stickler. 1965. Temperature and its interaction with light and moisture in nitrogen metabolism of corn (Zea mays L.) seedlings. Crop Science. 5:321-326. In Nielsen, K.F. 1974. Roots and root temperatures. In The Plant Root and its Environment. (N. Carson, ed.) University Press of Virginia, Charlottesville.

TABLE 3. Leaf fresh and dry weights (average of four replications).

	Control						12°C					
	Leaf 1		Leaf 3		Leaf 5		Leaf 1		Leaf 3		Leaf 5	
Days post-emergence	Fresh (g)	Dry (g)	Fresh (g)	Dry (g)	Fresh (g)	Dry (g)	Fresh (g)	Dry (g)	Fresh (g)	Dry (g)	Fresh (g)	Dry (g)
14	.109	.014	.288	.037	-	-	.101	.018	.083	.012	-	-
19	.101	.014	.356	.048	-	-	.098	.017	.301	.044	-	-
24	.087	.011	.342	.051	.436	.078	.098	.017	.353	.048	-	-
29	.031	.011	.314	.045	.514	.118	.084	.014	.330	.054	.238	.048
34	.015	.011	.127	.039	.452	.111	.041	.012	.322	.050	.369	.092
39	-	-	.065	.044	.425	.100	-	-	.139	.038	.374	.092

TABLE 4. Leaf length (average of four replications) and area (average of two replications).

	Control						12°C					
	Leaf 1		Leaf 3		Leaf 5		Leaf 1		Leaf 3		Leaf 5	
Days post-emergence	Lgth (cm)	Area (cm^2)	Lgth (cm)	Area (cm^2)	Lgth (cm)	Area (cm^2)	Lgth (cm)	Area (cm^2)	Lgth (cm)	Area (cm^2)	Lgth (cm)	Area (cm^2)
14	12.7	4.27	25.0	15.58	-	-	11.6	4.63	10.7	5.18	-	-
19	11.4	4.19	30.1	14.13	-	-	11.1	4.69	23.6	14.73	-	-
24	10.6	3.56	30.0	13.69	31.3	19.90	11.4	4.17	27.3	15.79	-	-
29	11.2	1.35	30.9	11.81	36.0	26.59	11.1	4.14	27.2	15.77	22.2	15.85
34	11.3	1.46	30.7	3.97	33.2	24.29	10.4	2.02	27.0	14.41	26.9	22.11
39	-	-	30.7	3.60	35.2	16.66	-	-	26.1	6.17	29.5	23.76

TABLE 5. Leaf content of relative chlorophyll units and mg total protein per g dry weight leaf tissue (average of four replications).

	Control						12°C					
	Leaf 1		Leaf 3		Leaf 5		Leaf 1		Leaf 3		Leaf 5	
Days post-emergence	Chl.	Pro.	Chl.	Pro.	Chl.	Pro.	Chl.	Pro.	Chl.	Pro.	Chl.	Pro.
14	119.5	35.0	101.3	40.9	-	-	117.4	56.0	107.7	48.4	-	-
19	117.5	29.0	112.9	27.6	-	-	119.8	50.0	102.4	52.6	-	-
24	68.0	22.6	100.5	34.0	83.8	53.1	102.7	39.5	130.8	66.8	-	-
29	15.1	4.1	32.6	13.7	68.3	34.0	51.1	11.6	82.0	26.4	89.1	33.7
34	8.6	-	13.3	8.4	45.9	16.1	18.3	7.7	42.5	15.7	69.1	29.5
39	-	-	7.8	-	23.6	7.6	-	-	11.6	4.9	58.5	18.8

TABLE 6. Leaf content of mg amino acids and mg imino acids per g dry weight leaf tissue (average of four replications).

	Control						12°C					
	Leaf 1		Leaf 3		Leaf 5		Leaf 1		Leaf 3		Leaf 5	
Days post-emergence	A.A.	I.A.	A.A.	I.A.	A.A.	I.A.	A.A.	I.A.	A.A.	I.A.	A.A.	I.A.
14	28.6	77.0	26.7	88.3	-	-	25.7	70.2	27.0	72.4	-	-
19	19.9	57.5	19.5	53.0	-	-	13.4	59.0	11.5	35.1	-	-
24	22.4	61.6	25.3	57.3	25.8	63.8	7.9	41.4	11.8	43.6	-	-
29	10.6	12.8	11.5	36.4	13.3	38.7	13.8	32.7	16.7	61.0	16.7	46.8
34	-	-	14.5	55.1	12.4	33.5	6.7	14.4	11.4	40.7	10.8	125.2
39	-	-	-	-	5.5	8.2	-	-	3.5	11.6	7.7	28.9

TABLE 7. Shoot fresh and dry weights (average of two replications).

	Control		12°C	
Days post-emergence	Fresh (g)	Dry (g)	Fresh (g)	Dry (g)
14	1.470	.152	.632	.086
19	2.876	.448	1.329	.170
24	3.911	.732	2.313	.323
29	5.105	1.306	3.660	.855
34	6.486	2.340	6.043	1.250
39	5.596	2.082	6.289	1.420

TABLE 8. Plant height (average of four replications).

	Control	12°C
Days post-emergence	Cm	Cm
14	9.2	7.1
19	11.1	8.2
24	12.9	10.1
29	20.0	14.0
34	39.5	21.2
39	49.9	33.7

TABLE 9. Root/shoot ratios, fresh and dry weights (average of two replications).

	Control		12°C	
Days post-emergence	Fresh	Dry	Fresh	Dry
14	1.061	.709	1.724	.851
19	1.136	.611	1.522	.805
24	.931	.447	1.651	.827
29	.803	.361	1.047	.446
34	.878	.354	1.073	.497
39	.777	.280	.762	.460

TABLE 10. Root fresh and dry weights (average of four replications).

	Control		12°C	
Days post-emergence	Fresh (g)	Dry (g)	Fresh (g)	Dry (g)
14	1.101	.074	.921	.073
19	2.042	.172	1.862	.137
24	2.669	.261	3.774	.277
29	3.515	.434	3.942	.343
34	3.626	.578	5.465	.583
39	3.799	.537	4.541	.547

TABLE 11. Number of tillers per plant (average of four replications).

Days post-emergence	Control	12°C
14	1.05	.65
19	2.28	1.52
24	2.44	2.21
29	2.06	2.38
34	1.59	2.65
39	.63	2.56

TABLE 12. Leaf fresh weight from plants with roots exposed to 8°C soil temperature (average of two replications).

Days post-emergence	Leaf 1	Leaf 3	Leaf 5
14	.080	-	-
19	.082	.066	-
24	.081	.169	-
29	.078	.220	-
34	.056	.219	.203
39	.021	.214	.222

TABLE 13. Leaf dry weight from plants with roots exposed to 8°C soil temperature (average of two replications).

Days post-emergence	Leaf 1	Leaf 3	Leaf 5
14	.020	-	-
19	.020	.011	-
24	.019	.033	-
29	.019	.037	-
34	.014	.041	.041
39	.012	.038	.049

TABLE 14. Relative chlorophyll units per g dry weight leaf tissue from plants with roots exposed to 8°C soil temperature (average of two replications).

Days post-emergence	Leaf 1	Leaf 3	Leaf 5
14	84.6	-	-
19	88.5	116.7	-
24	83.5	80.3	-
29	66.3	101.7	-
34	22.8	56.5	78.7
39	4.8	42.8	90.6

TABLE 15. Mg total protein per g dry weight leaf tissue from plants with roots exposed to 8°C soil temperature (average of two replications).

Days post-emergence	Leaf 1	Leaf 3	Leaf 5
14	50.84	-	-
19	39.33	76.61	-
24	39.01	38.43	-
29	24.49	50.85	-
34	8.68	28.29	49.44
39	-	13.58	35.56

TABLE 16. Mg imino acids per g dry weight leaf tissue from plants with roots exposed to 8°C soil temperature (average of two replications).

Days post-emergence	Leaf 1	Leaf 3	Leaf 5
14	28.90	-	-
19	33.41	-	-
24	40.57	59.51	-
29	20.69	21.26	-
34	40.89	61.97	74.06
39	-	30.49	50.77

TABLE 17. Mg amino acids per g dry weight leaf tissue from plants with roots exposed to 8°C soil temperature (average of two replications).

Days post-emergence	Leaf 1	Leaf 3	Leaf 5
14	6.41	-	-
19	9.67	-	-
24	10.53	14.79	-
29	1.68	9.11	-
34	2.31	12.82	14.97
39	-	6.10	8.18

TABLE 18. Root fresh and dry weights from plants with roots exposed to 8°C soil temperature (average of two replications).

Days post-emergence	Fresh	Dry
14	.359	.046
19	.593	.090
24	1.616	.128
29	2.693	.217
34	3.055	.269
39	3.954	.366

TABLE 19. Rate of emergence, expressed as per cent of final control emergence (control and 12°C, average of four replications, 8°C, average of two replications).

Days after planting	Control	12°C	8°C
1	0	0	0
2	0	0	0
3	0	0	0
4	16.4	0	0
5	54.2	0	0
6	83.0	1.8	0
7	88.1	13.7	0
8	92.7	38.6	0.6
9	96.8	62.2	17.1
10	97.9	77.6	35.0
11	99.2	82.5	46.4
12	99.2	84.5	61.5
13	99.5	86.6	71.7
14	99.5	89.0	74.6
15	99.5	89.3	81.2
16	100.0	89.9	81.8
17	100.0	92.1	84.6
18	100.0	92.1	85.6
19	100.0	92.1	87.8
20	100.0	92.1	87.8
21	100.0	92.1	87.8
22	100.0	92.7	87.8
23	100.0	92.7	88.4

TABLE 20. Protease activity as mg total amino acids released per g dry weight leaf tissue: Leaf 1 (average of two replications).

Days post-emergence	Control	12°C	8°C
14	19.145	8.525	14.087
19	21.884	8.084	7.390
24	6.898	22.372	13.719
29	36.129	11.394	7.593
34	-	14.417	7.543
39	-	-	-

TABLE 21. Protease activity as mg total amino acids released pre g dry weight leaf tissue: Leaf 3 (average of two replications).

Days post-emergence	Control	12°C	8°C
14	12.072	4.903	-
19	17.897	3.529	14.560
24	8.074	1.243	12.893
29	44.252	6.224	11.651
34	14.861	14.567	16.651
39	-	12.819	14.340

TABLE 22. Protease activity as mg total amino acids released per g dry dry weight leaf tissue: Leaf 5 (average of two replications).

Days post-emergence	Control	12°C	8°C
14	-	-	-
19	-	-	-
24	14.130	-	-
29	39.318	3.641	-
34	15.919	1.232	6.952
39	11.269	1.058	3.631

TABLE 23. Reducing sugars as mg per g dry weight leaf tissue: Leaf 1 (average of two replications).

Days post-emergence	Control	12°C	8°C
14	15.928	17.946	18.210
19	15.124	16.581	11.184
24	9.791	17.933	20.107
29	21.857	22.306	22.312
34	-	38.129	42.735
39	-	-	-

TABLE 24. Reducing sugars as mg per g dry weight leaf tissue: Leaf 3 (average of two replications).

Days post-emergence	Control	12°C	8°C
14	14.148	18.868	-
19	9.874	22.555	9.000
24	10.926	17.258	10.578
29	46.368	17.645	10.251
34	34.984	37.262	18.791
39	-	37.480	24.214

TABLE 25. Reducing sugars as mg per g dry weight leaf tissue: Leaf 5 (average of two replications).

Days post-emergence	Control	12°C	8°C
14	-	-	-
19	-	-	-
24	12.214	-	-
29	18.478	19.497	-
34	11.822	20.908	17.549
39	23.102	27.010	8.969

TABLE 26. Analysis of variance of leaf fresh weight: Leaf 1. Data transposed to $\log_{10}$ (1+x).

	DF	SS	MS	
Replicate	3	.0001	.00003	
Temperature	1	.00042	.00042	10.00**
Date	4	.00569	.00142	33.81**
TxD	4	.00080	.00020	4.76**
Error	27	.00113	.000042	
Total	39	.00813		

TABLE 27. Analysis of variance of leaf fresh weight: Leaf 3. Data transposed to $\log_{10}$ (1+x).

	DF	SS	MS	
Replicate	3	.0025	.0008	
Temperature	1	.0002	.0002	2.86-
Date	5	.0465	.0093	132.86**
TxD	5	.0328	.0066	94.29**
Error	33	.0024	.00007	
Total	47	.1055		

TABLE 28. Analysis of variance of leaf fresh weight: Leaf 5. Data transposed to $\log_{10}$ (1+x).

	DF	SS	MS	
Replicate	3	.0077	.0027	
Temperature	1	.0115	.0115	11.5**
Date	2	.0006	.0003	0.3^{-}
TxD	2	.0062	.0031	3.1*
Error	15	.0155	.0010	
Total	23	.0415		

TABLE 29. Analysis of variance of leaf dry weight: Leaf 1. Data transposed to $\log_{10}$ (1+x).

	DF	SS	MS	
Replicate	3	.00	.00	
Temperature	1	.00003	.00003	15.0**
Date	4	.00002	.000005	2.5*
TxD	4	.00	.00	.0^{-}
Error	27	.00006	.000002	
Total	39	.00011		

TABLE 30. Analysis of variance of leaf dry weight: Leaf 3. Data transposed to $\log_{10}$ (1+x).

	DF	SS	MS	
Replicate	3	.00028	.00009	
Temperature	1	.00	.00	0.00^{-}
Date	5	.00055	.00011	157.14**
TxD	5	.00075	.00015	214.29**
Error	33	.00001	.0000007	
Total	47	.00159		

TABLE 31. Analysis of variance of leaf dry weight: Leaf 5. Data transposed to $\log_{10}$ (1+x).

	DF	SS	MS	
Replicate	3	.0025	.0008	
Temperature	1	.0011	.0011	42.30**
Date	2	.00028	.00014	5.39*
TxD	2	.00062	.00031	11.92**
Error	15	.0004	.000026	
Total	23	.0049		

TABLE 32. Analysis of variance of root fresh weight. Data transposed to $\log_{10}$ (1+x).

	DF	SS	MS	
Replicate	3	.24	.08	
Temperature	1	.055	.055	5.73*
Date	5	1.11	.222	23.13**
TxD	5	.069	.014	1.46-
Error	33	.316	.0096	
Total	47	1.79		

TABLE 33. Analysis of variance of root dry weight. Data transposed to $\log_{10}$ (1+x).

	DF	SS	MS	
Replicate	3	.0153	.00051	
Temperature	1	.0002	.0002	.26-
Date	5	.1792	.0358	47.11**
TxD	5	.0018	.0004	.53-
Error	33	.0250	.00076	
Total	47	.2215		

TABLE 34. Analysis of variance of root/shoot ratio (fresh weight).

	DF	SS	MS	
Replicate	1	.0032	.0032	
Temperature	1	.801	.801	16.62**
Date	5	1.315	.263	5.46**
TxD	5	.134	.027	.56-
Error	11	.530	.0482	
Total	23	2.783		

TABLE 35. Analysis of variance of root/shoot ratio (dry weight).

	DF	SS	MS	
Replicate	1	.0008	.0008	
Temperature	1	.2102	.2102	19.46**
Date	5	.6195	.1239	11.47**
TxD	5	.0514	.0103	.95-
Error	11	.1192	.0108	
Total	23	1.0011		

TABLE 36. Analysis of variance of root per cent water.

	DF	SS	MS	
Replicate	3	37.29	12.43	
Temperature	1	68.17	68.17	13.42**
Date	5	324.65	64.93	12.78**
TxD	5	65.10	13.02	2.56*
Error	33	167.62	5.08	
Total	47	662.83		

TABLE 37. Analysis of variance of leaf length: Leaf 1.

	DF	SS	MS	
Replicate	3	11.147	3.716	
Temperature	1	1.191	1.191	.06-
Date	4	8.606	2.152	.10-
TxD	4	4.686	1.172	.05-
Error	27	588.74	21.805	
Total	39	614.37		

TABLE 38. Analysis of variance of leaf length: Leaf 3.

	DF	SS	MS	
Replicate	3	110.72	39.91	
Temperature	1	419.49	419.49	35.43**
Date	5	761.66	152.33	12.87**
TxD	5	185.18	37.04	3.13*
Error	33	390.61	11.84	
Total	47	1867.66		

TABLE 39. Analysis of variance of leaf length: Leaf 5.

	DF	SS	MS	
Replicate	3	166.17	55.39	
Temperature	1	438.62	438.62	2.57-
Date	2	44.4	22.2	.13-
TxD	2	81.42	40.71	.24-
Error	15	2563.64	170.84	
Total	23	3293.25		

TABLE 40. Analysis of variance of leaf area: Leaf 1.

	DF	SS	MS	
Replicate	1	.523	.523	
Temperature	1	2.31	2.31	1.23^{-}
Date	4	140.94	35.24	18.75**
TxD	4	6.54	1.64	.87^{-}
Error	29	54.40	1.88	
Total	39	204.71		

TABLE 41. Analysis of variance of leaf area: Leaf 3.

	DF	SS	MS	
Replicate	1	3.8	3.8	
Temperature	1	14.32	14.32	9.06**
Date	5	296.89	59.38	37.58**
TxD	5	229.87	45.97	29.10**
Error	11	33.26	3.02	
Total	23	578.14		

TABLE 42. Analysis of variance of leaf area: Leaf 5.

	DF	SS	MS	
Replicate	1	.64	.64	
Temperature	1	20.46	20.46	1.54^{-}
Date	2	34.43	17.22	1.30^{-}
TxD	2	162.85	81.43	6.13^{*}
Error	5	66.4	13.28	
Total	11	284.78		

TABLE 43. Analysis of variance of shoot fresh weight. Data transposed to $\log_{10}$ (1+x).

	DF	SS	MS	
Replicate	1	.0006	.0006	
Temperature	1	.0610	.0610	42.32^{**}
Date	5	.5476	.1095	76.00^{**}
TxD	5	.0107	.0021	1.48^{-}
Error	11	.0159	.0014	
Total	23	.6357		

TABLE 44. Analysis of variance of shoot dry weight. Data transposed to $\log_{10}$ (1+x).

	DF	SS	MS	
Replicate	1	.00	.00	
Temperature	1	.078	.078	16.60**
Date	5	.951	.1902	40.47**
TxD	5	.052	.0104	2.21⁻
Error	11	.052	.0047	
Total	23	1.133		

TABLE 45. Analysis of variance of plant height. Data transposed to $\log_{10}$ x.

	DF	SS	MS	
Replicate	1	.00084	.00084	
Temperature	1	.1536	.1536	16.17**
Date	5	1.5082	.3016	31.75**
TxD	5	.0186	.0037	.39⁻
Error	11	.1042	.0095	
Total	23	1.7854		

TABLE 46. Analysis of variance of number of tillers per plant.

	DF	SS	MS	
Replicate	3	2.7	.9	
Temperature	1	1.24	1.24	5.39*
Date	5	12.04	2.41	4.35**
TxD	5	10.27	2.05	8.91**
Error	33	7.6	.23	
Total	47	33.85		

TABLE 47. Analysis of variance of relative chlorophyll units per g dry weight leaf tissue: Leaf 1.

	DF	SS	MS	
Replicate	3	1419.51	473.17	
Temperature	1	2634.14	2634.14	3.84-
Date	4	75396.15	18849.04	27.46*
TxD	4	2535.07	633.77	.92-
Error	27	18531.25	686.34	
Total	39	100516.12		

TABLE 48. Analysis of variance of relative chlorophyll units per g dry weight leaf tissue: Leaf 3.

	DF	SS	MS	
Replicate	3	4671.96	1557.32	
Temperature	1	3923.56	3923.56	10.50**
Date	5	81980.62	16396.12	43.87**
TxD	5	4842.01	1210.50	3.24*
Error	33	12332.9	373.724	
Total	47	10775.05		

TABLE 49. Analysis of variance of relative chlorophyll units per g dry weight leaf tissue: Leaf 5.

	DF	SS	MS	
Replicate	3	8442.88	2814.29	
Temperature	1	4158.77	4158.77	6.28*
Date	2	5725.21	2862.61	4.32*
TxD	2	230.06	115.03	.17-
Error	15	9929.47	661.96	
Total	23	28486.39		

TABLE 50. Analysis of variance of mg amino acids per g dry weight leaf tissue: Leaf 1.

	DF	SS	MS	
Replicate	3	1806.42	602.02	
Temperature	1	212.06	212.06	2.06^{-}
Date	3	1014.57	338.19	3.28*
TxD	3	318.62	106.21	1.03^{-}
Error	21	2166.09	103.15	
Total	31	5517.76		

TABLE 51. Analysis of variance of mg amino acids per g dry weight leaf tissue: Leaf 3.

	DF	SS	MS	
Replicate	3	472.91	157.63	
Temperature	3	186.60	186.60	3.71^{-}
Date	3	140.55	46.85	.93^{-}
TxD	3	373.32	124.57	2.48^{-}
Error	21	1055.79	50.28	
Total	31	2229.57		

TABLE 52. Analysis of variance of mg amino acids per g dry weight leaf tissue: Leaf 5.

	DF	SS	MS	
Replicate	3	18.100	6.033	
Temperature	1	12.842	12.842	3.17^{-}
Date	2	283.691	141.846	35.01**
TxD	2	23.958	11.979	2.96^{-}
Error	15	60.778	4.052	
Total	23	399.369		

TABLE 53. Analysis of variance of mg imino acids per g dry weight leaf tissue: Leaf 1.

	DF	SS	MS	
Replicate	3	5722.48	1907.49	
Temperature	1	15.50	15.50	.03^{-}
Date	3	10864.16	3621.39	6.13**
TxD	3	1688.02	562.67	.95^{-}
Error	21	12413.66	591.13	
Total	31	30703.82		

TABLE 54. Analysis of variance of mg imino acids per g dry weight leaf tissue: Leaf 3.

	DF	SS	MS	
Replicate	3	1727.541	575.847	
Temperature	1	258.76	258.76	.27^{-}
Date	3	148.007	49.336	.05^{-}
TxD	3	2473.683	824.561	.85^{-}
Error	21	20475.331	975.016	
Total	31	25083.321		

TABLE 55. Analysis of variance of mg imino acids per g dry weight leaf tissue: Leaf 5.

	DF	SS	MS	
Replicate	3	21664.723	7221.574	
Temperature	1	9666.92	9666.92	2.53^{-}
Date	2	14999.792	7499.896	1.97^{-}
TxD	2	8129.993	4064.996	1.07^{-}
Error	15	57231.805	3815.454	
Total	23	111693.23		

TABLE 56. Analysis of variance of mg total protein per g dry weight leaf tissue: Leaf 1.

	DF	SS	MS	
Replicate	3	3158.753	1052.918	
Temperature	1	2218.445	2218.445	3.19^{-}
Date	3	6532.411	2177.470	3.13*
TxD	3	246.029	82.010	.12^{-}
Error	21	14625.706	696.462	
Total	31	26781.344		

TABLE 57. Analysis of variance of mg total protein per g dry weight leaf tissue: Leaf 3

	DF	SS	MS	
Replicate	3	13578.036	4526.012	8.52*
Temperature	1	2906.48	2906.48	6.41*
Date	4	8748.63	2187.16	.76^{-}
TxD	4	1034.029	258.507	
Error	27	9216.112	341.337	
Total	39	35483.287		

TABLE 58. Analysis of variance of mg total protein per g dry weight leaf tissue: Leaf 5.

	DF	SS	MS	
Replicate	3	6393.202	2131.067	
Temperature	1	395.443	395.443	3.42^{-}
Date	2	1702.452	851.226	7.35**
TxD	2	215.109	107.555	.93^{-}
Error	15	1736.325	115.755	
Total	23	10442.531		

TABLE 59. Analysis of variance of rate of emergence.

	DF	SS	MS	
Replicate	3	.171	.057	
Temperature	1	4.334	4.334	287.02**
Date	21	90.167	4.294	284.37**
TxD	21	12.431	.592	39.21**
Error	129	1.948	.015	
Total	175	109.051		