THE INFLUENCE OF ENVIRONMENT AND NUTRITION ON THE DEVELOPMENT OF RYEGRASSES

by

A. SCOTT LAIDLAW B.Sc.

Thesis submitted to the University of Glasgow
for the degree of
Doctor of Philosophy by the Faculty of Science.

October, 1974

SUMMARY

The study was concerned with the influence of environmental factors such as light, temperature and nutrition on the production of tillers and inflorescences and induction of cold hardiness in ryegrasses.

Field trials were carried out to determine if nitrogen fertiliser altered the degree of flowering in perennial ryegrass varieties. Although tiller numbers increased due to nitrogen in one experiment, the percentage of flowering tillers was not markedly altered. This applied to both an early (Pax Ø tofte) and late (S23) perennial ryegrass.

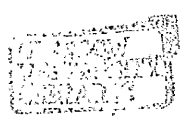
A study of nitrate effects on the production of leaf primordia, tillers and inflorescences under glasshouse conditions was carried out. Nitrate increased leaf appearance in main axes and tillers as well as increasing total primordia production and tiller bud expansion.

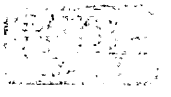
An observation from field and glasshouse experiments that tiller production in ryegrasses at the flowering stage decreased both in annual and perennial ryegrasses. This suggested that apical dominance existed in ryegrasses. Using surgical techniques, removal of the apical region and expanding leaves in annual and perennial ryegrasses increased the expansion of tiller buds. Employing tiller number and tiller bud expansion as criteria apical dominance was also found to exist in annual ryegrasses (Westerwolds and Lolium temulentum) when the main axis was flowering, but was most marked under conditions of low nitrate. Results are discussed in terms of current theories of apical dominance, particularly with relevance to grasses.

As well as apical dominance lack of cold tolerance was also a factor which limited the perennation of ryegrasses. Cold tolerance was measured by survival or regrowth after subjection to low temperatures or by the degree of damage brought about by low temperatures, measured by the amount of electrolytes released from the plants.

Hardening of perennial ryegrasses was found to be dependant on low temperatures. Long photoperiods at low, but above zero, temperatures induced hardiness at a faster rate than short photoperiods in some experiments. However at higher temperatures, the effect of daylength varied according to species and variety. Nitrogen fertiliser decreased hardiness in ryegrass in the field but the reverse was found under controlled environmental conditions. Roots were generally less responsive to hardening conditions than shoots.

Conclusions are drawn and are considered in terms of future research and, in particular, the role of perenniality in determining persistence in ryegrass.





CONTENTS

ACKNOWLEDGEMENTS

The author wishes to thank Professor M.B. Wilkins for provision of facilities within the Department of Botany. Gratitude to Dr. A.M.M. Berrie, for his supervision and guidance in the conduct of the work and preparation of the thesis is expressed. Thanks are also due to Mr C. Weatherup, Department of Agricultural Biometrics, the Queen's University of Belfast, for statistical advice and to Miss L. Townsend who typed the thesis.

INTRODUCTION

It is a common belief among practising agriculturalists that applications of high levels of nitrogenous fertiliser delay the onset and reduce the degree of flowering in grasses. The aim of this study was, initially, to determine if nitrogen fertiliser influenced flowering of grasses, particularly ryegrasses, under conditions experienced in the West of Scotland.

The intention was to study, also, the influence of nitrogen and other environmental factors on the behaviour of the grass plant and constituent tillers under controlled environmental conditions. The degree of control the apex of a grass plant exerts over the tillers, or tiller buds on the axils of its leaves has not been studied in any detail. It was considered that such a study would allow the role of flowering on tillering to be separated from that of environmental influences such as nitrogen.

Not only flowering, but other aspects of behaviour of a grass plant or tiller influenced by environment including cold hardiness were considered important. A conference held at the West of Scotland College of Agriculture in 1969 highlighted the lack of knowledge of the basic problems associated with winter kill of pastures. Therefore, it was considered that this study afforded an opportunity to determine some of the factors which influence cold hardiness of the grass plant.

SECTION 1. REVIEW OF LITERATURE

Tillering Pattern

The arrangement of possible tillers on a grass plant has been described by Mitchell (1953a), Langer (1956) and Rawson (1971). Essentially on the main axis, the coleoptile node may or may not give rise to a tiller. This has been found to be under genotypic, as well as environmental control, (Mitchell, 1953a; Patel and Cooper, 1961). Phleum pratense L. does not give rise to tillers at this site whereas Lolium perenne L. may or may not. Daylength has been found to increase the incidence of coleoptile tiller under winter conditions although not to the same extent as the plant will produce under summer conditions (Patel and Cooper, loc. cit.).

The behaviour of a tiller at this site in wheat has been found by Rawson (1971) to differ from other tillers on the main axis. Although it can appear about the same time as the tiller at the first leaf node, it does not, at earing, have the same dry weight. It is in fact, much lighter being of similar dry weight to the tiller in the axil of the third leaf on the main axis, although this tiller arose considerably later than that of the coleoptile.

Seedlings from light seed have been found by Arnott (1969) to be less able to produce a tiller in the coleoptile

axil in comparison to those from heavier seeds. Depth of sowing also influences the development of coleoptile tillers. In S24 ryegrass fewer seedlings have tillers at the coleoptile node when seed is sown at a depth of 25 mm than seedlings from seed sown at a depth of 6.2 mm.

The tillers appear progressively up the main axis under optimal nutritional conditions. These are primary tillers and, in turn tillers are produced in the axils of their leaves and are known as secondary tillers. The prophyll of these subsequent tillers may also subtend a tiller, and although they behave irregularly they are less anomalous than the coleoptile tiller, (Rawson, 1971).

As well as the coleoptile and prophyll tillers, other tillers also are influenced by their position in relation to the main axis. Cooper and Seed (1949) found that the leaf number on the secondary tillers at heading was less than the main axis, ie. the tillers flowered in a shorter time from origin than the main axis. Langer, (1956; 1957; 1959b) has found in timothy that for the same time of origin, primary tillers had heavier flowering heads than corresponding secondary tillers. This has also been found at lower positions within any one order. Langer and Ryle (1959) found the length of the panicle decreased in S48 timothy as the order was

increased. Davis and Laude (1964) found that in Bromus mollis, the 1st, 2nd and 3rd primary tillers on the main axis maintained that order with respect to tiller number, caryopsis size and number of flowering heads. When these tillers were removed and transplanted, these characters increased in each instance, but the order was not changed.

In wheat, Rawson (1971) has found that primary tillers decrease in contribution to total grain yield with increase in position (acropetally) disregarding the coleoptile tiller. The relative contribution was 22%, 20% and 8% for primary tillers number 1, 2 and 3 respectively and this was also the order of the weight of the tillers at earing.

In timothy, Langer and Ryle (1959) labelled tillers formed before July 23rd and distinguished between primary and secondary tillers. Although 85.2% of primary tillers flowered, only 44.5% of the secondaries, formed before that date, flowered. A similar situation has been found in cocksfoot and meadow fescue ie. the primary tillers although of similar age to secondary tillers, are more fertile. (Langer and Lambert, 1959).

Morphology of flowering in grasses

The morphological changes which take place at the apex of a grass plant at flowering have been described by Sharman (1945). The first sign of flowering, termed floral initiation, involves an increase in the length of the apex due to primordia being laid down rapidly. In the vegetative apex, primordia comprising the apex would develop into leaves. However, in the initiated apex, the bud primordia in the axils of the leaf primordia develop whereas the leaf primordia remain

as they are. The stage at which these bud primordia become obvious when the apex is dissected out and viewed down a microscope, is termed the "double-ridge stage".

In many temperate perennial grasses, a long photoperiod is necessary before initiation takes place (Cooper and Calder 1962). Also in most of them the plants have to be subjected to a period at low temperatures below 10°C but above 0°C (Evans, 1960) before they will respond to long days. This period of low temperature is known as vernalisation and the process which occurs during vernalisation is known as floral induction (Evans, 1964).

Annuals, on the other hand, do not generally require vernalisation to respond to the daylength which will initiate flowering, and in fact Lolium rigidum does not even require an obligate vegetative phase prior to responding to long days, unlike most grasses. So it will respond immediately after germination to the appropriate photoperiod and flower at a stage where the leaf number corresponds to the number of leaf primordia in the apex of the embryo.

After initiation of flowering the bud primordia in the apex grow out to form the axes of spikelets if the inflorescence is a spike or primary branches if the inflorescence is a panicle. These branches in turn bear primordia and form the secondary branches and so on until the order of branching is reached which bears the spikelets.

The primordia which develop into the axes of spikelets in turn give rise to the glume primordia. In the axils of the glumes the florets develop, each floret subtended by a lemma. The parts of the floret which eventually develop are the palea, the carpel, the stamens and the lodicules are the last to be formed. The apical dome of the initiated apex also becomes a spikelet. When that occurs further spikelet primordia initiation on the main axis is not possible.

Internode elongation of the inflorescence takes place simultaneously with the development of the spikelets resulting in ear emergence when the developing inflorescence appears above the sheath of the flag leaf.

Effects of date of origin on tiller behaviour

The effect of position of the tiller on its behaviour as described earlier tends to be confounded by the time of origin of the tiller. Wilson (1959) found in perennial and

Italian ryegrass, and timothy tillers formed in winter and early spring had the highest flowering potential whereas in cocksfoot, only winter formed tillers had high fertility.

With timothy Langer (1959b) observed that all tillers formed 6 weeks after the experiment commenced under high N treatment flowered, whereas, of those formed on the 12th week, only 47% flowered.

Langer and Lambert (1959) with meadow fescue and cocksfoot have found the earliest formed tillers have the greatest chance of bearing inflorescences. With cocksfoot, tillers present in November bear most of the heads in the next growing season, whereas those produced by mid-March in meadow fescue plants are the most fertile.

More recently, Lambert and Jewiss (1970), have shown that not only time of origin but also order and position of a tiller determines its fertility. Time of sowing also has been found to influence the time of earing in tillers. Langer and Ryle (1959) with S48 timothy did not find any flowering in plants sown after the middle of May, yet tillers on older plants which arose after this time were able to flower, suggesting conditions were suitable for flowering after this time.

<u>The effect of nitrogen on tiller behaviour when influenced by position and date of origin</u>

The effect of position and time of origin of tillers

on behaviour of the tillers can be influenced by nitrogen fertiliser application. Langer (1959b) found that the secondary tiller production was influenced by nitrogen more than that of primary tillers. In S48 timothy, increasing nitrogen gave rise to an increase in primary tiller number from 36 to 42 whereas secondary tillers increased from 141 to 297.

Wilson (1959) found that nitrogen delayed the decline in production of fertile tillers in Italian and perennial ryegrasses as the season progressed when grown under New Zealand conditions ie. of tillers produced in November, 2% were eventually fertile under low N conditions whereas 55% were fertile when the N level was high. Langer (1959b) also found a similar situation in S48 timothy. Tillers formed in the sixth week of the experiment were 100% fertile under a high N level but only 89% under lower N conditions. The effect was more marked on tillers formed in the 12th week. 47% were fertile under high N when harvested and none under low N had initiated apices.

When the total percentage of fertile tillers borne on a plant at any one time is considered, nitrogen has been found to have an effect in a number of instances although the effect has rarely been found to be significant. In spaced plants, Langer and Lambert (1959) found no difference in the relative percentage fertility between N treatments in S215 meadow fescue. Langer (1959b) by dissecting tillers prior to ear emergence found in S48 timothy

that 20% of the tillers at high N were initiated whereas 14% were initiated at low N, 5 weeks after the N treatments commenced. After 7 weeks 85% were initiated at high N and 68% at low N.

Therefore although nitrogen does not seem to exert a marked effect on the percentage of fertile tillers produced at any one time, it can greatly influence the total number of tillers produced and also the number of fertile tillers (Wilson, 1959; Langer, 1959b; Ryle, 1964b)

These instances so far have been concerned with spaced plants or plants in pots in greenhouses. In the field under sward or drill conditions, the effect of nitrogen on fertility is not so straight forward. Evans (1953) recorded a decline in fertile tiller number when N levels were increased in S48 timothy. However, S37 cocksfoot responded positively to N with respect to flowering tiller number. In another trial (Evans, 1954) the same effect of N was recorded in S48 and S37 as was noted in the previous trial. S23 was found to respond positively to N by producing more fertile tillers. Lambert (1964) like Evans, found S48 timothy responded poorly to nitrogen with regard to fertile tiller number under sward conditions. Lambert found meadow fescue to have a small but positive response to N.

Ryle (1966) considers that the variable response of plants under sward conditions to nitrogen could be due to the varying availability of nitrogen to the plants under these conditions.

as well as the effect of competition brought about by the appearance of new vegetative tillers and increase in size of existing tillers.

Effect of nitrogen on leaf appearance

The literature concerning the effects of nitrogen on leaf appearance is somewhat confusing. Some consider that nitrogen has no effect on the expansion rate of leaves, while others have shown definite, though small, increases in leaf expansion.

Langer (1959b) considered that in timothy, when the rate of leaf appearance was measured in the main axis and on the first two primary and secondary tillers, nitrogen had no effect. Plants which were supplied with 150 ppm N solution did not have significantly different rates of leaf expansion from those supplied with 30 ppm. Purvis, (1934) also found that there was no effect of nitrogen on the rate of leaf expansion in Petkus winter rye. Differences were recorded but were not significant. Other reports of N not having an effect on leaf expansion include Cooper (1951) and Bean ((1961) cited by Anslow (1966)).

O'Brien (1960) showed that in both cocksfoot and perennial ryegrass, those at the higher nitrogen treatments had

ahigher number of leaves per plant. However the tiller number was also greater, and when the ratio of leaf number to tiller number is calculated from the data presented it is not greatly different for the varying N treatments.

In a survey of the effects of N and temperature on the leaf expansion of seven perennial forage grasses, Ryle (1964a) found that high nitrogen treatment (150 ppm) increased the rate of leaf expansion when the seven species were considered together compared to the effect of the solution containing 15 ppm N. Russian workers have shown that nitrogen applied to soil of 80% moisture content gives rise to a higher leaf number on the main axis of grass plants compared to no N application. N treated plants have 11 leaves when the plants without N have 10 leaves. (Lebeder and Mel'nik, 1965)

This topic has been reviewed by Anslow in a paper considering environmental effects on leaf growth in grasses (1966).

Effect of nitrogen on the flowering of grasses.

The study of the role nutrition plays in the flowering of plants goes back to the time of Kraus and Kraybill (1918) who considered that the ratio of carbon to nitrogen in the plant was a factor involved in determining whether the plant remained vegetative or became reproductive. If the ratio was

low, they considered flowering was prevented whereas a high ratio enhanced flowering. Turner (1922) observed that the practice in horticulture to delay the onset of flowering was to apply nitrogenous fertiliser, as this was considered a means of increasing vegetative growth at the expense of flowering.

A comparison in C-N ratio in soya beans between those grown under long and short photoperiods was carried out by Murneek (1948). He found that up to the twelfth day of the life of the plants, when initiation had occurred in the plants under short day conditions, the C-N ratio was smaller in those initiated plants than in those under long day conditions. By the 20th day, the C-N ratio was higher in the initiated plants (Hexose sugar concentration was a measure of C, and total nitrogen a measure of N). Therefore, this experiment suggested that C-N ratio increased after floral initiation, rather than prior to it.

On the other hand, in support of the C-N theory, Grainger (1938) was able to associate delay in flowering of a late-flowering chrysanthemum cultivar to a delay in translocation. He found that translocation did not take place until 5 hours after the onset of darkness, so the short nights of summer may have prevented sufficient carbohydrate to be translocated to the apex, preventing floral initiation. Grainger agrees with Kraus and Kraybill (1918) that in tomato, the species also used by them in their studies, the

higher the C-N ratio, the more floriferous is the plant. However, Sheard (1940) has found that this does not hold for all the plants he has surveyed.

High nitrogen levels have been found to slightly favour flower induction by immersing leaves in high and low concentration of solutions of various nitrate salts, each with glucose added (Cajlachjan, 1944) and that both long and short day plants could be influenced in the same way. Application of nitrogen has been found by Withrow (1951) not to alter the critical daylength of plants grown under low N, P and K conditions.

Gott et al.(1955) have been unable to detect any significant difference in rate of attaining initiation in Petkus winter rye by varying the amount of N applied. However, in wheat, under low nitrogen conditions ear emergence was observed 47 days after sowing whereas the high nitrogen level resulted in ear emergence 14 days earlier (Halse et al. 1969). Wilson (1959) has also found nitrogen having a promotive effect on ear emergence. In perennial and Italian ryegrass ear emergence was advanced by 7 days by high nitrogen application and by 20 days in timothy. Langer (1959b) did not observe any significant effect of nitrogen on time of ear emergence in S48 timothy, although he found in another experiment that severe deficiency in

nitrogen could restrict flowering to primary tillers or prevent flowering entirely in S48 timothy, (Langer, 1959b). Severe nitrogen deficiency has also been found to prevent flowering in B4542 cocksfoot (Calder and Cooper, 1961)and S24 perennial ryegrass and S37 cocksfoot (Ryle 1964 b).

Therefore, it would seem that in grasses, the level of nitrogen applied can have an effect on flower appearance. However, rather than high levels enhancing flowering it is apparent that it is very low levels which delay or prevent initiation taking place.

The effect of nitrogen on flowering would seem to be more marked after flower initiation ie. during inflorescence development. Langer (1959b) found that the length of the apex in vegetative plants under high and low N levels was similar. However, when floral initials were apparent, the low N gave rise to a mean apical length of 3.56 mm in comparison to a mean apical length of 13.33 mm at high N. Thereafter, the high nitrogen gave rise to a greater number of florets per ear. This has also been observed by Ryle (1963). Floret number would seem to be more influenced by nitrogen than the number of branches on the inflorescence, (Ryle, 1964b). Ryle found in perennial ryegrass, meadow fescue and cocksfoot that the level of nitrogen had to be very low before the primary branch number was affected.

In relation to the effect of time of origin of tillers on inflorescence size already discussed, increasing nitrogen has a greater effect on the number of florets in the ears of those late formed tillers than on those initially formed. In timothy (Langer, 1959b; Ryle, 1964b) not only does nitrogen increase the number of florets per ear, but it also increases the weight of individual seeds formed from these plants (Lambert, 1956; Sonneveld, 1957).

Ryle, (1967) considered that as an explanation for the effects of nitrogen on inflorescence development in the grass plant coupled with the influence of light (Ryle, 1966) on this same stage of development, the demand for nutrients by the developing inflorescence must be increasing due to the rapid growth of ear and stem at this stage of development. So deficiencies in carbohydrate and/or nitrogen will obviously restrict the growth of the components of the inflorescence.

Effects of inflorescence development on tillering

It has been demonstrated in a number of instances that the rate of tillering decreases at flowering in perennial grasses. This has been shown in L. perenne (Cooper and Saeed, 1949), Bromus inermis (Lamp, 1952) and P. pratense (Langer, 1959b), and in annuals eg. barley (Aspinall, 1961; Thorne, 1962) and a number of other cereals (Skripcinskii, 1958). However,

under certain conditions the reduction in tillering at flowering can be overcome (Aspinall, 1961; Joffe and Small, 1963). Aspinall with Piroline barley was able to minimise the effect of flowering on tillering rate and also give rise to a flush of tillers after flowering by maintaining the plants on a high nutritional plain. Joffe and Small (1963) by providing the oat plant with optimal nutritional and other environmental conditions prevented flowering from permanently inhibiting tillering.

Removal of these developing inflorescences has been found to alter the tillering rate in comparison to intact plants. Canfield (1939) by removing the heads in Tobosa grass (Hilaria mutica) increased the basal area covered by the plants due to an increase in vegetative tillers at the base of the plants. Deheading crested wheat grass (Agropyron desertorum) has been shown to give rise to an increase in the number of tillers which develop (Cook and Stoddart, 1953) and Jameson and Huss (1959) found the number of elongating tiller-buds increased in little bluegrass (Agropyron scoperius) by removing elongating stems.

Lolium multiflorum has been found to have a higher number of developing tillers when cut at 2 cms from ground level than when cut at 6 cms, a greater number of developing

apices being removed at the lower cutting height (Maeda and Ohara, 1963). Bradshaw (1959) found an increase in tiller number per unit area when initiated apices and developing stems were removed in Agrostis tenuis by cutting or when flowering was suppressed by choke disease.

On the other hand, Ellison (1960) considered that clipping swards reduced tiller numbers, the reason being that tillers, with their apices removed, died. It was also for this reason that Branson (1953) considered Switch-grass (Panicum virgatum) was more susceptable to grazing that big blue stem (Andropogon gerardi) as the former had more elongating stems, the apices of which were removed at grazing. Davies (1969) has found that the tiller numbers of S24 plants clipped in May are greater than those of intact plants when counted in July, under conditions of low N fertility.

In order to explain the effects of flowering on the rate of tillering, a number of theories has been put forward, and all are more or less based on theories which have been employed to explain apical dominance, and will be discussed in the next subsection.

Apical Dominance in grasses

Although apical dominance has been studied in many dicotyledonous species (reviewed by Phillips, 1970), few

studies have been carried out on monocotyledons, particularly grasses. One reason, presumably, for this to be neglected in the Gramineae, is that the apex is rather inaccessable prior to ear emergence, for any surgical work to be carried out.

One of the first studies of apical dominance in grasses was carried out by Leopold (1949). He damaged the apices of barley and teosinte plants by puncturing the developing apex with a needle, giving rise to increased tillering in the treated plants. However, most of the other studies of apical dominance in grasses have involved plants at a more advanced stage of development, i.e. where the apex is at least initiated (Jewiss, 1972; Aspinall, 1963; Thorne, 1962).

Hypotheses to explain the mechanism of apical dominance have been reviewed by Phillips (1970). He considers that four have been put forward at various times to explain the influence of the apex on the expansion of lateral buds, viz. the nutritive theory, the direct theory, the indirect theory and the nutrient diversion theory.

It is not the intention of this review to consider these theories in detail. However they can be summarised briefly as follows: The nutritive theory was the first put forward to explain apical dominance. The early workers considered that the apex was competing with the lateral buds for nutrients, and these nutrients moved along concentration

gradients to the apex. The apex was in a preferential position as it was formed before the lateral buds, so could command the available nutrients, hence suppressing the growth of lateral buds.

The direct theory assumed auxin was produced at the apex, and entered axillary buds, preventing their expansion (Thimann, 1937) due to their higher sensitivity to auxin than the main shoot.

Snow, on the other hand, considered that due to the basipital transport of auxin, it could not enter axillary buds. Therefore it must be having an indirect effect on lateral bud growth. This became known as the indirect theory.

With the increasing accumulation of knowledge concerning nutrient transport within the plant and how it is affected by hormones the nutrient-diversion theory has won strong support in recent years to explain apical dominance. It was first put forward by Went (1936) who suggested that nutrients are directed towards high concentrations of auxin by some physiological effect, other than growth promotion. This has been extended to include the diversion of the flow of other hormones. Phillips (1970) suggests that it is the cytokinins which are directed by apically produced auxin, which regulate the growth of axillary buds.

Apical dominance in the vegetative stage of grasses

The studies so far considered, with the exception of Leopold's work in which it is not clear what state the apices were in, have involved plants in which the apex was at or beyond the double ridge stage. However, in most of the studies in dicotyledonous plants, they were in the vegetative state. The study of apical dominance in grasses has presumably been confined to the flowering stage as flowering is accompanied by a decrease in tillering, suggesting that apical dominance takes place at this stage. Also this stage is of economic importance in the sward as it affects annual production. However, as nutrient has been found to determine the degree of tillering in vegetative grass plants then this could be considered as a demonstration of apical dominance being altered in the vegetative grass plant.

During the early work on apical dominance, Snow (1929) found that removal of the expanding leaves in pea seedlings (Pisum sativum) released axillary buds from inhibition, and he postulated that the expanding leaves were the source of the "correlative inhibitor". It has been put forward that IAA is present in high concentrations in the expanding leaves eg. in Aster (Delisle, 1938) and the primary leaves of maize (van Overbeek , 1938). As IAA is considered by some workers

to be the correlative inhibitor (Thimann, 1937) then it is possible that removal of the expanding leaves reduced the amount of correlative inhibitor present.

There is some circumstantial evidence that the vegetative grass plant is under the control of apical dominance (Mitchell, 1953b; Kirby and Faris, 1970; 1972). By decreasing light intensity Mitchell found that the lateral buds in ryegrass species were suppressed. If the low light treatment was imposed early enough in the life of the plant, then total suppression of the buds was possible.

Kirby and Faris found that in barley seedlings from seeds sown at high density, showing lateral bud suppression, the tiller buds either grew out or did not when at a particular stage, rather than exhibit the type of growth pattern which would be expected if nutrient had been limiting and causing the response. They consider that this on-off effect suggests hormonal control of tiller bud expansion in the young vegetative barley plant. Morphological characteristics of the seedlings at high sowing density have been interpreted by them to suggest gibberellin had increased within the seedling.

Concerning this on-off effect, Mitchell (1953b) and Kirby and Faris (1972) found that tiller buds, when suppressed were not capable of growing out after a limited time of

suppression.

Effect of inorganic nutrition on apical dominance in grasses

Earlier in this review, the effect of inorganic nitrogen on the tiller number of grasses has been discussed. It was concluded that increasing nitrogen gave rise to higher tiller number in many sustances. Therefore it can be concluded that the elongation of tiller buds to give rise to visible tillers is increased by nitrogen application.

Gregory and Veale, (1957) found that in flax (Linum usitatissimum) apical dominance was greatest under low nitrogen levels, and could be overcome by removal of the apex. In barley Aspinall (1961) was able to overcome the effect of flowering on the tillering rate by increasing inorganic nutrient concentrations applied to the plants.

McIntyre (1964; 1965) by subjecting Agropyron repens plants to low N found the rhizomes exhibiting complete apical dominance but this could be overcome by increasing the concentration of nitrogen applied to the plants.

At low levels of inorganic nutrient, Aspinall (1963) found that removal of a proportion of the spikelets in barley increased tillering at a more pronounced rate than in plants at high nutrient level, again suggesting that apical dominance was influenced by inorganic nutrition.

Under sward conditions, however, the effect of nitrogen on cut swards at flowering is not so straight forward. Evidence of poor regrowth after cutting under high N levels in ryegrass has been put forward (Grassland Research Institute, 1969). Other instances are quoted by Williams (1970). It has been suggested that although this level of nitrogen has given rise to high growth rate, reserve assimilates may be low as light is limiting under sward conditions. So this may override any effects of the release from apical dominance (Jewiss, 1972).

Mechanism of apical dominance in grasses

Leopold (1949) was able to reproduce the effect of the apex in decapitated barley plants on tillering by replacing the apex with NAA (an analogue of IAA). He considered that this substantiated the role of IAA in apical dominance (Thimann, 1937).

However Aspinall (1963) and Thorne (1962) with flowering barley plants have been unable to repeat the effect of auxin which was observed by one of its analogues by Leopold.

More recently, Jewiss (1972) has shown that application of an antiauxin eg. TIBA or ACPI-55 at certain concentrations can give rise to a resumption in tillering when the rate is decreased at flowering in L. temulentum and Triple dirk wheat

This work however, does not attempt to assign the site of auxin production to any particular part of the plant. Therefore even if auxin is implicated in the suppression of tiller buds, the apex may not be the source.

Gibberellin has also been implicated in the suppression of tillering in grasses (Jewiss, 1972; Kirby and Faris, 1970; 1972). Jewiss found that gibberellin application gave rise to premature internode elongation in Triple Dirk wheat and concomitant suppression of tiller bud elongation. Kirby and Faris, by increasing plant density, internode elongation in barley took place and tiller buds were prevented from growing out at the base of the elongating internode.

Although at various parts of this review, carbohydrate levels have been suggested to be playing an important role in determining the fate of tiller buds, one experiment by Jewiss (1972) has shown that release of a tiller bud from inhibition is not in the first instance accompanied by increase in carbon transport into that bud in comparison to corresponding buds on control plants. So rather than C^{14} from leaf fed with $C^{14}O_2$ passing into the tiller bud in high quantities prior to the bud extending, it enters when the bud is about to extend, suggesting that events taking place in the released bud prior to extension are not initiated by increased carbo-

hydrate. As cell division takes place prior to elongation, Jewiss considers that some factor is present in the buds subsequent to TIBA application. He suggests that cytokinins, presumably from the root are directed into the bud, giving rise to cell division and is the first stage in the release of the bud from inhibition. Therefore he postulates that auxin diverts cytokininon its passage, from the roots, away from tiller buds and so inhibits their expansion. The results obtained from experiments involving gibberellin application would suggest that in those instances, gibberellin is diverting cytokinins to growth centres other than the buds eg. elongating stem.

Bioassays carried out on the xylem sap of maize have shown that at least three cytokinins and four gibberellins, as well as four inhibitor substances are present (Atkin and Barton, 1971). The plants were grown for 7 weeks at a range of temperatures. However, the work so far carried out in this field is far from conclusive.

Cold Hardiness

Gassner (1918) found that by growing winter plants at low temperatures, the hardiness of these plants was increased. He also found that the low temperatures under which the plants were growing gave rise to an increase in the soluble carbohydrate content. Gassner concluded that the high carbohydrate

content gave rise to the increase in cold hardiness.

Tumanov (1940) explained the induction of winter hardiness in terms of phasic development. He considered that while a winter plant was still in the thermophase ie. vernalisation was not complete, it was winter hardy, but if allowed to grow after the thermophase was completed, the plant was much less hardy. It was concluded that it was the completion of the thermophase rather than the effect of the photophase which was responsible for the decrease in hardiness as hardiness decreased at the end of the thermophase whether the conditions favoured the photophase or not.

Kuperman (1936) attempted to explain the effect of vernalisation on hardiness by comparing the carbohydrate levels between vernalised and non-vernalised plants. He found the vernalised plants had a lower soluble carbohydrate level, and so considered that explained the lower hardiness of vernalised plants. Tumanov and Federova, however, found that these differences in soluble carbohydrate level were not manifest before the winter, a time when there would be a difference expected if that was the cause of the differential in hardiness.

Since that time, there has been a number of studies carried out examining the effects of environment on hardening, and attempts have been made to elucidate the mechanism of

cold hardiness. As this study will be concerned only with environmental effects on the hardiness of ryegrasses, the following review will be more or less confined to these effects.

Cold hardiness of Ryegrass species

Vadation in hardiness among members of the genus Lolium was found in Holland (Wassenaer et al. 1954). The short rotation ryegrass, H.I., was found to be less hardy than Italian ryegrass. However, some of the hybrid ryegrasses bred from Dutch perennial ryegrass and Italian ryegrass were hardier than the New Zealand hybrid (H.I.). British and New Zealand perennial ryegrasses were less hardy than the Dutch type. Hay types were found to be less hardy than pasture types.

Comparing S22 Italian ryegrass and HI short rotation ryegrass to S23 and S24 perennial ryegrass, Jones (1953) found that the first two had better autumn and winter growth but, particularly under some defoliation conditions, they did not survive the winter as well as the perennial ryegrasses.

Assessing hardiness by the lack of winter "kill", Jones (1958) found S22 and Irish Italian ryegrasses were hardier than HI. Among the perennials, S24 suffered less than New Zealand Mother perennial ryegrass.

The degree of hardiness among perennial and Italian ryegrasses has been found to be correlated to the intensity

of cold requirement necessary for flowering (Wit, 1955). Therefore the hardiest cultivars were those which had the greatest vernalisation requirement for flowering. The Italians gave rise to 98% heading after 60 days refigeration of seeds compared to 86% in the early haytype perennials, 57% in the pasture types and 44% in late hay types. Also the Italians were the least hardy.

A less marked difference between Italian and perennial ryegrasses was found by Pohjakallio et al. (1963) in Scandanavia where only some of the perennial ryegrass varieties were more hardy than the Italians. Hunt (1962), however, found Manawa and S22 Italian although outyielding Presto perennial ryegrass in the establishment year were adversely affected by the following winter. So Presto outyielded Manawa and S22 in the following season.

In Switzerland, Caputa (1956) found that both perennial and Italian ryegrass did not survive well over the winters and were much less hardy than grasses such as Phleum pratense Poa pratensis, Poa trivialis, Agrostis spp. and Festuca rubra. On the other hand, both Italian and perennial ryegrasses have been recommended for growing in cold regions of Colombia (Crowder, 1959).

It would seem from those conflicting accounts of the relative hardiness of perennial and Italian ryegrasses that not

only is there variation between cultivars and species, but the region in which the plants were tested as well as the method of assessment affects the comparison.

Recently, Lorenzetti et al. (1971) found that when comparing hardiness of ecotypes of perennial ryegrass under artificial conditions, the north European ecotypes were generally more hardy than those from the Mediterranean region. They found a relationship between the degree of cold hardiness and the mean minimum temperature of the coldest month of the year in the region of occurence of each ecotype. The hardier ecotypes from North Europe have been found to exhibit a lower rate of leaf expansion than the less cold resistant ecotypes from the Mediterranean region at 5°C under controlled environment conditions (Cooper et al., 1962; Cooper, 1964). This difference in the rate of leaf area increase has been found to be due to increased cellular elongation rather than cell division (Welsh Plant Breeding Station, 1964).

Although Robson and Jewiss (1968) and Chatteree (1961) found that the net assimilation rate in North African ecotypes of tall fescue was higher than S170 in the winter, McCall and Cooper (1967) found no differences between net assimilation rates in winter between Mediterranean and North temperate ecotypes of ryegrass, cocksfoot and tall fescue. Although relative growth rate has been found to be higher in

Mediterranean types than in temperate types of the same species (Chatterjee, 1961; McCall and Cooper, 1967; Robson and Jewiss, 1968). McCall and Cooper have attributed this to a higher leaf area ratio in the Mediterranean type than higher net assimilation rate.

In the Republic of Ireland, Crowley (1969) has claimed he has been able to combine the winter hardiness of S321 and the early-spring growth characteristic of an Algerian ecotype of L. perenne. However as has been found repeatedly, S321 is one of the least hardy of the British perennial ryegrasses, and although it may be relatively hardy in the milder conditions of Eire, it could not be considered hardy by British standards.

Some of the examples of comparisons between various ryegrasses have been based on trials carried out in the field. Although this study is concerned only with cold hardiness, these examples from the field are a measure of "winter hardiness", and could be considered as a plant's ability to withstand winter conditions. These conditions not only take into account the effect of low temperature, but also other factors such as frost heaving, ice covering, susceptability to winter growing fungi not associated directly

with any physiological properties of the grass at low temperature.

There have been differences found among varieties of L. perenne in susceptability to winter fungi. Valiage has been found to be fairly resistant in Finland to Typhula spp., Sclerotinia borealis and Fusarium nivale (Jamalainen, 1954). However, when L. perenne is compared to other grasses such as Alopecurus pratensis, Poa pratensis, and Festuca pratensis with respect to resistance to these parasitic fungi, L. perenne is more susceptable (Jamalainen, 1960).

Effect of Nitrogen on Cold Hardiness in Grasses

One of the earliest studies of the effect of nitrogen on the cold hardiness of grasses was carried out by Carroll and Welton (1939). They found that increasing the amount of nitrogen applied in the autumn decreased the hardiness of the plants over the ensuing winter. However, differences in hardening between the two N levels was not obvious until November. More recently, the N response has been found to be dependent on the time of application of the fertiliser. Wilkinson and Duff (1972) by applying N fertiliser at various times in the autumn to Kentucky bluegrass found that the greatest differences in hardiness throughout the winter were

between the treatments which has not received any fertiliser and the treatment which had been given fertiliser at every date of application, the latter giving rise to less hardy tillers. Early and late applications of N were found to have less effect than mid-autumn applications, although the late applications gave rise to less hardy tillers in the spring.

Howell and Jung (1965) were able to show that at 12 out of 20 sampling dates throughout the winter, Dactylis glomerata plants which had been given low N and cut at the bloom stage (ie. less than 10% of the inflorescences at anthesis) were the hardiest of all the treatments.

Comparing the performance of a number of ryegrass strains at a range of nitrogen levels, it has been found that 580 units of N/annum have adverse effects on the hardiness of all varieties in comparison to no N applied (Breese and Foster, 1971). At intermediate levels the later high tillering strains are more resistant to the low temperatures imposed.

In Bermuda grass, winter hardiness has been found to be associated with low nitrogen levels (Kresge and Decker, 1965). Applications of potash also enhanced hardiness, the ideal ratio of N:K being 2.4:1. Calder and Macleod (1966) have shown that when total available carbohydrates and winter

hardiness in alfalfa were reduced by frequent defoliation they were increased with potassium fertiliser application. Adams and Twesky (1960) found that high N levels reduced winter survival but when potash was applied in high doses as well as the nitrogen, the deleterious effects of N were reduced. They also found that high N levels gave rise to increased growth so potassium deficiency was experienced , and they considered that the lack of hardiness under high N levels is due to low available amounts of potash relative to nitrogen at a time when the plant needs potassium for hardiness.

Kolosova (1941), however, has found that timothy, Agrostis pratensis and B. inermis had higher winter hardiness when N was included in the fertiliser rather than when potash and phosphate were applied alone.

In species other than grasses, eg. Juniperus chinensis Pillet and White (1969) have found that autumn nitrogen does not have an adverse effect on winter hardiness and, in fact, has been found to have a beneficial effect on spring growth (Meyer, 1969).

Taking turf quality and clipping weights as criteria of winter growth of P. pratensis and F. rubra , Ledeboer and Skogly, (1973) have observed autumn N increases turf quality ie. greater

cover of greenness. Also later applications of N increased the number of tillers formed over the winter. Winter survival was not adversely affected by the high N. Hanson and Juska (1961) observed that autumn fertilised Poa pratensis plants had a slower growth rate in the spring. Powell et al. (1967) observed increased winter colour with high applications of autumn N. The high N also gave rise to a slow depletion in carbohydrate over the winter. Top growth was not increased in the spring. This lack of top growth was also noticed by Ledeboer and Skogly (loc.cit.) who considered that the increase in tillering in winter increased the basal growth rather than increasing growth above the clipping level ie. lateral growth was encouraged.

Effect of defoliation on the cold hardiness of grasses

As well as fertiliser application, defoliation is the other factor which can be controlled under field contions or in agricultural practice and this factor has been found to have an influence on a plant's ability to withstand the temperate winter conditions.

Baker (1956) by delaying the last cut of the year of S24 perennial ryegrass found the ability of the grass to withstand the winter was increased. American work on Dactylis glomerata has shown that when cut at various stages of

development ie. vegetative, ear emergence and anthesis, those cut at the stage approaching anthesis were hardiest during the following winter (Howell and Jung, 1965). Thomas and Lazenby (1968a) found that the hardiness of Festuca arundinacea plants was reduced when cut 6 days prior to the cold treatment compared to intact plants. Also on dropping the temperature to 16°F survival was less in plants cut back to the level of the soil than those cut to 3" or left intact.

Grandfield (1935) found that when alfalfa was cut in autumn, the greater the growth which had taken place between cutting and the onset of winter, the greater was the yield in spring. Red clover has also been found to give smaller spring yields in the second year if the last cut of the previous year took place on September 15th or later at Wisconsin (Torrie and Hanson, 1955). It is difficult, however, to determine to what extent the amount of vegetation entering the winter period is affecting hardiness and to what extent it is merely adding directly to the yield in spring. Spring yields may in fact not be a suitable measure of hardiness in such experiments.

A survey carried out after the severe winter of 1962-63 showed that generally, the amount of herbage carried over the autumn determined the degree of damage of the ensuing

winter to the sward (Baker and David, 1963). This was applicable even when the herbage was cut at the end of autumn. There was, however, a few exceptions to this which could not be explained. The presence of herbage on swards throughout the autumn having a detrimental effect on winter survival was also seen during a comparison of Westerwolds and Italian ryegrass varieties (Baker and Ciard, 1964). Survival of the same winter of the North African ecotype Syn II was greatly improved when it was cut in late autumn ie. October (Rudman and Alder, 1964). On the other hand, claims of late defoliation or frequent defoliation reducing winter hardiness of pasture grasses have been made (Welsh Plant Breeding Station Report, 1964a).

There is a possibility that the type of frost may determine whether defoliation or abundant herbage gives rise to better hardiness. In Digitaria spp. it has been found that less damage is encountered in winter if growth remains on the swards when light frequent frosts are encountered (Prine, 1963). More severe frosts, however, cause less damage to swards where the cover has been removed.

Hardiness and defoliation have been associated with reserve carbohydrates in the roots. Fulkerson (1967) found that the system of cutting in the autumn which gave rise to the highest reserves in the roots of vernal alfalfa, gave rise to the highest cold resistance. Similar conclusions have been drawn by Calder and MacLeod (1966). They found that

when the cutting frequency was increased in alfalfa the electrical conductivity of the exudate, the total available carbohydrate, and the cold hardiness decreased.

Other examples of defoliation giving rise to a decrease in cold hardiness include oat (West, 1962) and most of the British pasture grasses (Welsh Plant Breeding Station, 1964a).

The effect of daylength and light intensity on cold hardiness of grasses

Daylength and light intensity have been shown to be factors which determine the degree of hardiness of plants. They are also responsible for controlling carbohydrate levels. However, many of those studies have not separated the effects of a photosynthetic and a physiological daylength and so in many instances, the two are confounded.

One of the first studies of daylength on hardiness was carried out by Dexter (1933) on wheat plants. He found that if the plants were grown under short day conditions prior to hardening, they were hardier than those under long days. However, long days during the hardening process, at $0^{o}C$, had a more pronounced effect on hardiness than the short day conditions, the long day being photosynthetically long as well as physiologically so.

Tysdal (1933) growing alfalfa under artificial conditions found short days had a promotive effect on hardiness during the hardening phase compared to long days. Red clover has also been found to be induced to harden under short day conditions. Polijakallio et al. (1960) compared the effect of daylength on the relative hardiness of ecotypes of red clover when grown in Finland. Varieties adapted to more northerly conditions were hardier than those from a more southerly region. However, when daylength prior to winter was shortened, the more southerly plants were hardier than those in the natural long days at that time of the year. It is considered that the short days of autumn in those places at a southerly latitude were inducing cold-hardiness.

Morley et al. (1957) studying a range of ecotypes of Medicago sativa in Australia found that all ecotypes were less winter dormant when the daylength was increased. Hardy strains of alfalfa have been shown to produce less top growth when grown in greenhouses under short days compared to less hardy types (Oakley and Westover, 1921; Coffindaffer and Burger, 1958; Seth and Dexter, 1958). Smith (1961) found less hardy ecotypes produced a high number of tall growing plants in autumn whereas the more hardy ecotypes produced a low percentage of these tall plants.

Hodgson (1964) comparing four ecotypes of alfalfa found that indigenous plants were not affected by photoperiod when grown in Alaska i.e. they were equally hardy under long and short day conditions. The varieties from California (i.e. those from a lower latitude) did not harden under any of the photoperiods employed, although there was a trace of hardiness under short days. The variety Ranger, from a position not as far south as California hardened better under short days ie. daylength similar to the area of origin in autumn. Hodgson considered that, as a consequence of this affect of daylength, hardiness is under the control of phytochrome. He, however, was basing this on the daylength effect and not on any photo-reversibility evidence which characterises an event mediated by phytochrome.

This effect of the ecotypes from more southerly latitudes being less hardy under conditions of a higher latitude has also been found in grasses. Klebesadel et al. (1964) found that in Festuca rubra ecotypes from areas over a latitude of 60°N the maximum percentage kill was 37% whereas those from latitudes less than 50° had in the same instances 100% kill when grown in the north ie. over 60°N. A similar pattern was seen in Poa pratensis ecotypes.

Altering the natural nyctoperiod in Alaska, Klebesadel (1971) has been able to demonstrate that the more southerly ecotypes of bromegrass and Kentucky bluegrass increase in hardiness when the photoperiod is shortened in the period prior to low temperatures whereas the indigenous ecotypes are much less affected by either lengthening or decreasing the photoperiod.

From these examples, it is not clear whether the daylength effect is operative before or during hardening ie. the period of low temperatures conducive to hardening.

More recent studies of the effect of daylength during the hardening period on hardiness have shown that long days during the hardening period increase hardening in a number of ecotypes of L. perenne (Lawrence et al.). During the hardening phase (2^{o}C 39 W/m^2) for two weeks) long days (16 hours) gave rise to 52% tiller survival compared to 36% survival when hardened under short days (8 hours).

Khalin (1970) in Poland has shown that the optimal photoperiod for inducing hardiness in Bromus inermis, Arrhenather um elatius, Phleum pratense and Alopecurus pratensis is of 12 to 14 hours duration and Festuca pratensis and Dactylis glomerata had an optimal daylength of 24 hours for inducing hardiness. Red clover has also been found to be hardened most efficiently at 18 to 21 hours (Sjøseth, 1964).

With respect to light intensity Lawrence et al.(1973) have shown that in the period prior to hardening short days and high light intensity give rise to greater hardiness than long days at low light intensity in a range of perennial ryegrasses. However during hardening, the longer days at low light intensity confer greater hardiness than short days at the higher intensity, the total daily energy being similar in both treatments.

The effect of morphology on hardiness

Although this study is concerned with the effects of environment on cold hardiness, there are other factors which contribute to the degree of hardiness of a plant. These factors can be related to environment and so are worthy of consideration in this review. One of these factors is morphology of the plant.

A list of instances where morphological factors are associated with cold hardiness is given by Levitt (1956). Smith in his review of cold hardiness of forage grasses and legumes also mentions some morphological factors which can be associated with the relative hardiness of plants and so can be employed as a means of determining the potential hardiness without freezing. Apical meristems, axillary meristems and the vascular transition zone have been considered three critical regions which determine the degree of survival of

barley plants after cold treatment (Olien, 1964) and studies have been carried out where the position of those critical regions are considered in relation to degree of exposure and cold hardiness of the plant.

Kolosova (1941) found that the nodes at which tillers were formed varied in depth from 10 mm to 40 mm in some forage grasses. She considered that this partly determined the ability of the grass plants to survive the winter. Bromus inermis, a hardy plant had those nodes at 20 mm below the soil surface. Dactylis glomerata and Lolium perenne (French strains), however, had their nodes at 12 and 15 mms respectively below the soil surface in those plants which survived and 8 and 10 mms in those which were killed.

The importance of the apex of cereals when damaged by low temperatures has been stressed by Livingstone and Swinbank (1950) and they considered that most of the damage to the apex was done after floral initiation. The upright growth of cereals at this stage has been considered as one of the factors which make cereals more susceptable in Australia as elongation of the internodes exposes apical and axillary meristems to the upper, colder zones, (Gott 1961). This is related to the response of the wheat variety to daylength. Some varieties become less dependant on daylength as the ear develops. To

during a mild spell in winter, despite the short days, the ear may develop, raising the growing point into more exposed regions and be damaged by the spring frosts.

Comparing spring and winter wheat, barley and oats, Tavcar (1931) found that the varieties with their "vegetative point of the principal stalk" above the level of the soil surface are less resistant to low temperatures.

Single (1961) studying the frost hardiness of various varieties of winter wheat found that the less elongated the stem, the more frost hardy was the plant. He found that the laboratory results did not always correspond to those collected in the field as some varieties were seen to be relatively hardier in the laboratory than in the field. It is suggested that these differences are due to the growth habit of some varieties which are at a stage of development where their growing points and axillary meristems are in colder zones than others in the field, whereas under the artificial conditions employed zonation does not occur.

Thomas and Lazenby (1968[c]) suggest that the differences in hardiness encountered between the three strains of Festuca arundinacea (Syn 1, Syn 2 and S170) could be partly explained by the relative position of the apex within the crown. They, however, did not carry out any dissections to determine the relative position of the apices in the three strains.

SECTION 2. MATERIALS AND METHODS

Species used. Most of the experiments carried out involved one or a number of representatives of the genus Lolium. These included the annuals L. multiflorum Lam. (var. Westervoldicum) and L. temulentum L. (Ba 3081), biennial Italian ryegrass L. multiflorum (Tetila Tetraploid) and Perennial ryegrass L. perenne L. (Pax Øtofte, Hunsballe and S23 ie. an early, medium early and late flowering perennial ryegrass cultivar respectively).

In an initial experiment laid down in plots, timothy (Phleum pratense L.) was also used. The early flowering cultivar S352 and a late flowering cultivar S48 were employed.

Seed was supplied by Messrs McGill Smith, Ayr except for L. temulentum seed (cv. Ba 3081) which was obtained from the Welsh Plant Breeding Station, Aberystwyth.

Flowering characteristics of the cultivars of L. perenne and P. pratense (days after 1st May to reach ear emergence) (from McGill Smith catalogue, 1972)

L. perenne	Pax Øtofte	(Danish)	16
	Hunsballe	(Danish)	34
	S23	(British)	44
P. pratense	S352	(British)	40
	S48	(British)	54

In one experiment, some tillers of two strains of L. perenne were obtained from the Scottish Plant Breeding Station, Pentlandfield These strains were S321 and Argo, the former having been bred at Aberystwyth, the latter in Poland.

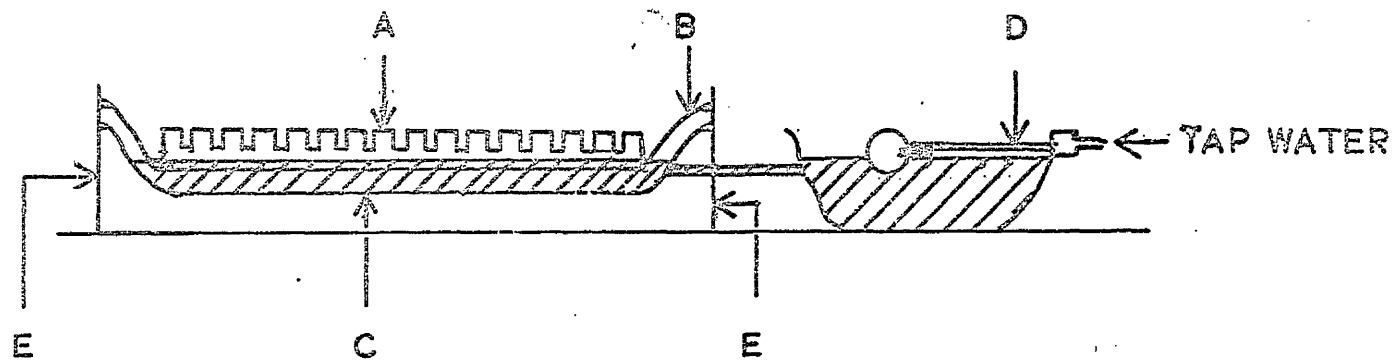
Pots and Potting Media Seeds were sown in plastic seed trays on perlite or vermiculite. Perlite was used initially but it was prone

to water logging when excess water or nutrient was applied and so vermiculite was used latterly. The medium was moistened with nutrient prior to being spread in the seed tray. When in the seed tray, it was compressed to give rise to a smooth, firm, level seed bed. After broadcasting the seeds (at a seed rate of approximately 1 seed per cm^2) they were covered with $\frac{1}{4}$ inch layer (approx.) of graded E sand. Nutrient and water were added as required.

When the seedlings had reached the desired stage, which varied depending on the experiment, they were transplanted into pots. Again the size of pot and the number of plants planted in each pot was dependant on the nature of the experiment. The rooting medium used initially was perlite but in the majority of experiments vermiculite was employed. The type of medium used in each experiment is given at the appropriate point in the Results Sections

A few experiments were carried out on "Baystrat" blocks. This is a polymer material, each block being a cube of side 2.5 cms grouped in sheets. They were placed in nutrient solution, partially immersed ie. 6 mms in the solution. The system is represented diagramatically in figure 2.1. The seedlings were planted in these blocks by making a small hole in the centre of the upper surface of each block. The root of the seedling was placed in the hole and the block was compressed slightly round the hole to secure the seedling. The nutrient solution was taken up by capillarity. The level of the nutrient solution was maintained at a constant level by a cistern which was positioned so that when the cistern was full, the level was the same as that at which the nutrient should be in the blocks. Therefore as growth continued, the concentration of nutrients decreased. However, maintaining the solution at a constant level prevented salting out, which would occur if evaporated water had not been replaced.

FIG.2.1 DIAGRAMATIC REPRESENTATION OF SIDE VIEW OF BAYSTRAT APPARATUS



A BAYSTRAT BLOCKS
B TRAY WITH PERFORATED BASE
C TRAY CONTAINING NUTRIENT SOLUTION
D CISTERN
E SUPPORTS FOR TRAYS

Blocks were placed in a tray with a perforated base, and this was inserted into another tray which contained the nutrient. this was inserted into another tray which contained the nutrient This lower tray was connected to the cistern. The nutrient was replenished at intervals depending on the experiment.

Nutrient solution. The solution used in this study was one which was used for general purposes at the laboratory. It consisted of 3 stock solutions, based on modified Hoagland's.

$Ca(NO_3)_2.4H_2O$	160 gms)	in 1 litre
KNO_3	100 gms)	
KH_2PO_4	30 gms	in 1 litre
$MgSO_4 7H_2O$	45 gms)	in 1 litre
Fe chelate (Sequestrene)	5 gms)	

500 mls of each stock solution were added to water in a tub and made up to 200 litres. The phosphate solution was not mixed with either of the other two stock solutions before being added to the water so that precipitation of insoluble phosphates did not take place. The tubs were covered to prevent algal growth on the surface of the nutrient.

In pot experiments where nitrogen was varied, the normal solution was considered the high nitrogen level solution and low nitrogen solution had 1/5 nitrogen content of the normal solution. NO_3^- was replaced by Cl^- in the low nitrogen solution. The stock solution which replaced NO_3^- with Cl^- was:-

KCl	108 gms)	in 1 litre
$CaCl_2$	74 gms)	

In order to obtain a solution with 1/5 the nitrate concentration of the normal solution, 400 mls of the chloride solution were added

to 100 mls of the nitrate stock solution and the resulting solution replaced the 500 mls of nitrate stock solution in the normal solution.

It was considered that chloride would not accumulate sufficiently to have adverse effects on the low N treatment, as it has been reported that both Italian and perennial ryegrass have a high tolerance to chloride ions. (Cordukes and Parups, 1972).

In nutrient experiments, the solutions were applied to the pots in measured quantities generally 50 mls or multiples of 50 mls at specified intervals. This was done by cutting a plastic measuring cylinder to size so that it could be dipped into the bucket of nutrient, and when full contained 50 mls. This gave rise to efficient application of nutrient to the pots.

In those nutrient experiments, care was taken so that a minimum amount of leaching took place from the pots ie. the pots were kept below field capacity whenever possible. This meant that the plants had to be watered regularly, a small amount of water each time, they were generally watered daily but when the pots were in the greenhouse under strong sunlight in summer, they were watered twice daily.

Where nutrient was not considered a variable the plants were given nutrient solution ad libitum.

Layout of Pot Experiments The layout of pot experiments was a totally randomised arrangement, and the pots were rearranged at regular intervals, usually weekly.

Field Experiments There were four field experiments carried out in this study. These can be considered under the following headings:

(a) A comparison of tillering at different nitrogen levels of two timothy and three perennial ryegrass cultivars and the effect on the number of tillers which flower(Experiment 3.1).

(b) A comparison of tillering at different nitrogen levels of an early and late flowering perennial ryegrass cultivar and the effect of nitrogen on the time of ear emergence and number of flowering tillers. (Experiment 3.2.).

(c) A study of the effect of nitrogen on the hardiness and winter survival of L. perenne (cv. Pax Øtofte).(Experiment 6.4.e.).

(d) The effect of removing inflorescences on tiller number in Westerwolds at four nitrogen levels. (Experiment 3.3.).

(a) A comparison of tillering at different nitrogen levels of two timothy and three perennial ryegrass cultivars and the effect on the number of tillers which flower.

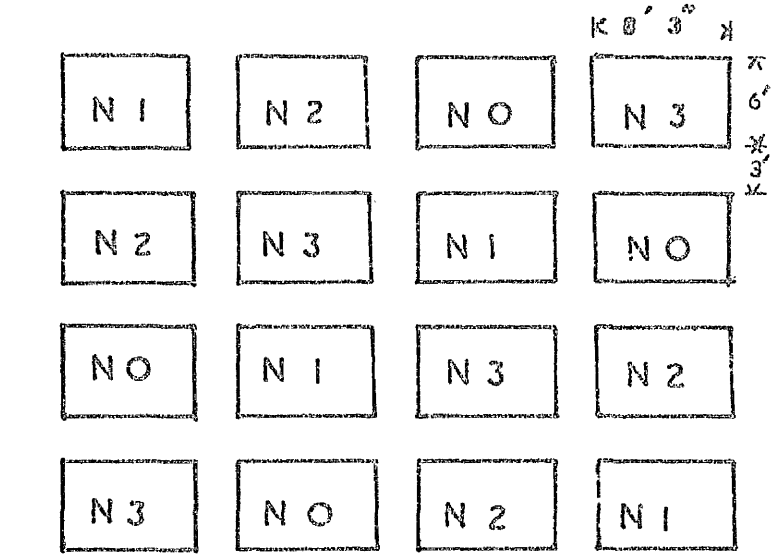
The cultivars chosen for this experiment were:-

L. perenne	Danish Pax Øtofte	(early flowering)
	Hunsballe	(medium early flowering)
	S23	(late flowering)
P. pratense	S352	(early flowering)
	S48	(late flowering)

Seeds were sown in seed trays on 4/3/70 and placed in growth cabinet type A at 20°C and 7½ hour day. On 1/4/70 the trays were transferred to a growth cabinet (type B) at +7°C and a 7½ hour day until 5/5/70. On this and the subsequent day, these seedlings were planted in pots.

The layout of the plots was of a Latin square design and is represented diagrammatically in fig. 2.2. The arrangement of species and cultivars within a plot are represented in fig. 2.3. Meadow fescue (S215) plants which had been grown from seed with the experimental plants were used as guard plants.

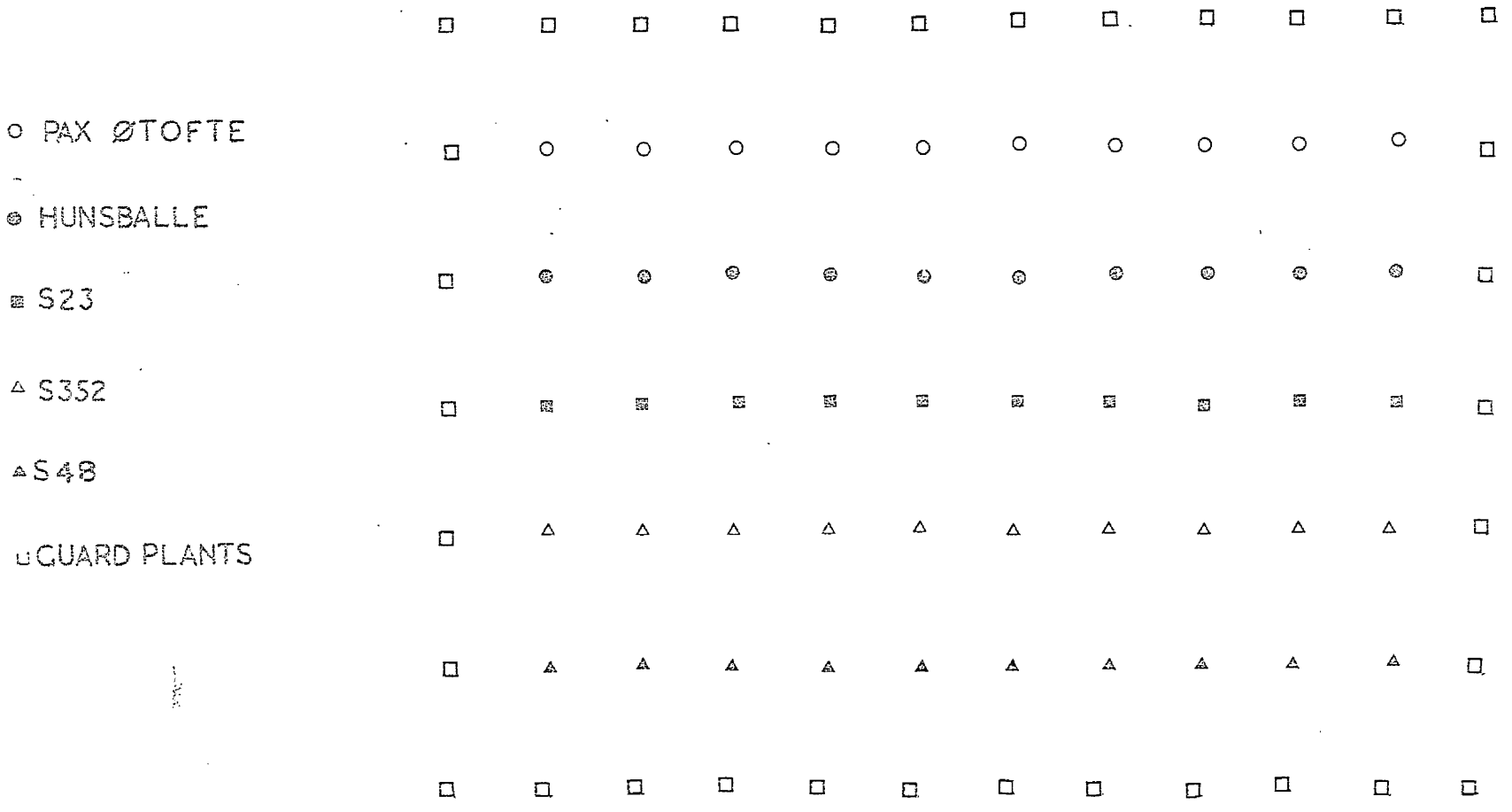
FIG.2.2-ARRANGEMENT OF PLOTS.- EXPT 3.1



UNITS OF N(EQUIV/ACRE/ANNUM)

N O	O
N 1	66·6
N 2	133·3
N 3	200

FIG.23-ARRANGEMENT OF PLANTS WITHIN A PLOT. — EXPT. 3.1



The rows of plants within each plot were planted one foot apart and the distance between each plant was 9 inches. There were 10 plants (experimental) per row with a guard plant at either end of each row. A row of guard plants along each side of the plot completed the perimeter of the plot which consisted of equally spaced guard plants surrounding the 5 rows of experimental plants.

As the design of the plots was a Latin square each plot in the first row of four plots was chosen at random to be treated with one of 4 nitrogen levels. This was repeated for the next row within the limitations that a nitrogen level could not be repeated more than once in each column (or row) of plots. Therefore after the third row had been allocated the four nitrogen levels, there was only one possible arrangement for the fourth row. The plots were separated by 3 feet wide paths.

The plots were laid down in the gardens of the Botany Research Laboratories at Garscube. Soil analysis of the area taken in March of that year had shown that a dressing of potassic fertiliser would be beneficial. A dressing equivalent to 2 cwts of muriate of potash (60% K_2O) per acre was applied to each plot immediately after the grasses were planted. For purposes of applying fertiliser the perimeter of each plot was considered to be 6 inches to the outside from the centre of each guard plant ie. equivalent to half the distance between each row. The area of the plots were calculated accordingly.

Nitrogen was applied to the plots in the form of Nitro chalk (15.5 units N) at the equivalent rates of 100, 66.7, 33.3 and 0 units per acre per application. Two applications took place viz. on 16/6/70 and 16/7/70.

Prior to the first nitrogen application, the tiller number of each plant was recorded. The tillers were again counted at a time when almost all of the plants were bearing flowers.

This was carried out two weeks after the second nitrogen application ie. on 30/7/70. Tillers were considered as either "vegetative" ie. at a stage prior to ear emergence or "flowering" when they were at or beyond ear emergence. A tiller was considered to be at ear emergence if any part of its inflorescence was visible above the leaf sheath of the flag leaf.

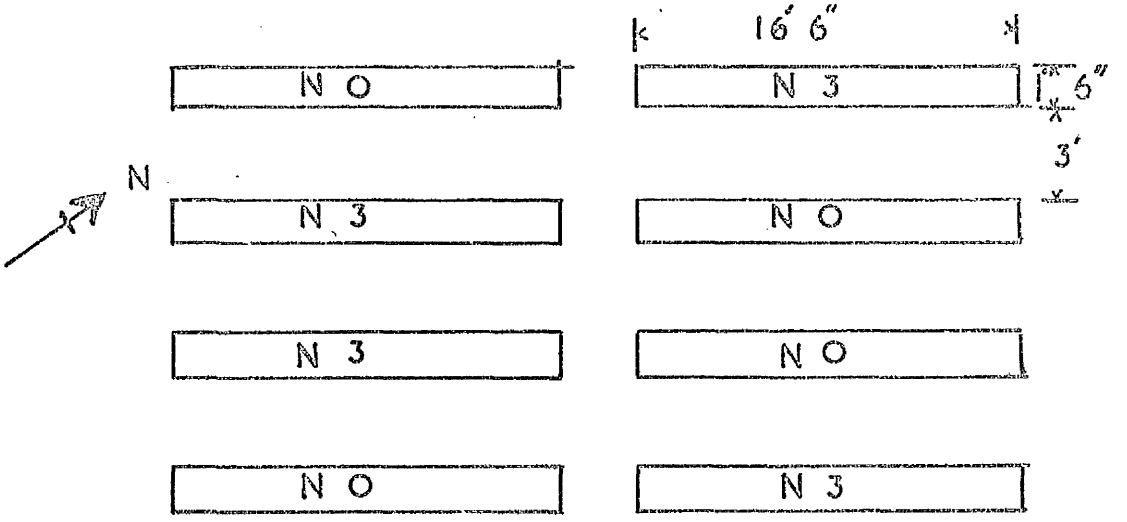
(b) A comparison of tillering at different nitrogen levels of an early and late flowering perennial ryegrass cultivar and the effect of nitrogen on the time of ear emergence and number of flowering tillers.

Seeds of L.perenne (cvs Pax Øtofte and S23) were sown on 1/8/71 in the heated greenhouse under natural illumination. On 1/9/71 they were planted in small "Jiffy pots" in a peat:sand:soil mixture (1:1:1 parts by volume). They were transferred to the unheated greenhouse on 3/9/71 to become acclimatised to the increasingly colder days of autumn before being planted out in plots on 15/9/71. They were kept in their Jiffy pots when planted out as these decompose in the soil. This allowed the plants to be planted out at this time of year without disturbance of the root system.

The layout of the plots is represented diagramatically in figure 2.4, and the arrangement within each plot in figure 2:5. The plants were planted 1' 6" apart in each row, 12 plants per row. This allowed 10 experimental plants and 2 guard plants, one at each end of the row. The rows were 1' 6" apart and there were two rows per plot, one of each cultivar. Guard plants were the same cultivar as the plants in the particular row. As there were only two rows per plot, it was considered unnecessary to have guard rows parallel to the experimental rows on either side of each plot. Plots were separated by 3' wide paths.

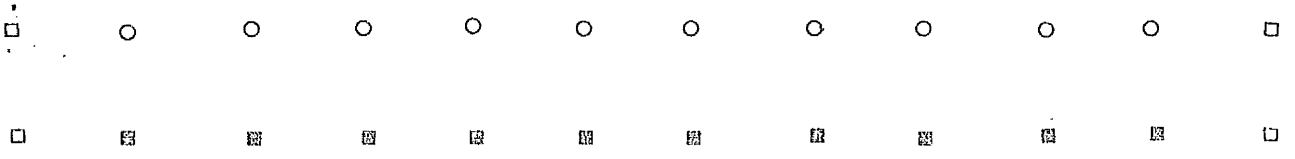
Results are described on pages 70-75.

FIG. 2.4 – ARRANGEMENT OF PLOTS.– EXPT. 3.2



	UNITS OF N(EQUIV./ACRE/ANNUM)
NO	O
N 3	300

FIG.2.5–ARRANGEMENT OF PLANTS WITHIN A PLOT–EXPT. 3.2



○PAX OTOFTE ◙S23 □GUARD PLANTS

Twice the number of plots were planted initially, as it was realised there could be a high casualty rate due to the adverse effects of the ensuing winter. As the experiment did not commence until the time the first nitrogen treatment was applied, it was considered justifiable to use the plants of half the number of plots to replace those killed in the other 8 plots. Dead plants were replaced on 13/3/72 and a tiller count was taken.

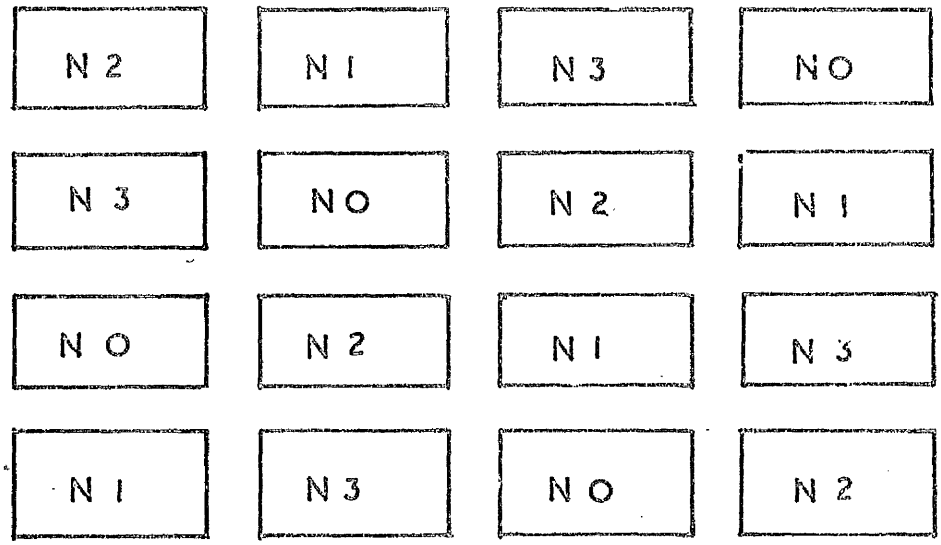
Nitrogen was applied to half the plots in the form of Nitro-chalk (15.5 units N) at the rate equivalent to 150 units/acre on 24/4/72. The four rows of two plots were considered as four blocks and the nitrogen treated plots were decided by tossing a coin to determine which of the two plots within each block received nitrogen.

Tiller counts were taken at intervals, initially weekly. The dates of tiller counts are given at the appropriate point in the Results section. As the plants flowered, the "flowering" and "vegetative" tillers were recorded. From 8/6/72 onwards harvesting was employed as the plants had grown to the extent that counting tillers was too difficult to be carried out *in situ*.

On 14/7/72, the second nitrogen application took place at the same rate as on 24/4/72 and on 15/8/72, the plants which had been harvested and counted on 14/7/72 were again harvested and tiller counts were taken. During every harvest, the vegetative and flowering tillers were weighed, when fresh and when dried at 100°C for 24 hours.

Harvesting entailed clipping plants at 1" above ground level. Except for the plants harvested on 14/7/72, all others after being harvested were not further considered. Generally 2 plants per plot were harvested at any one time.

FIG. 2.6 – ARRANGEMENT OF PLOTS.– EXPTS. 3.3 & 6.4



UNITS OF N (EQUIV/ACRE/ANNUM)

N O	O
N 1	100
N 2	200
N 3	300

(c) A study of the effect of nitrogen on the hardiness and winter survival of L. perenne cv. Pax Øtofte

Seeds of L. perenne cv. Pax Øtofte were sown on 5/3/71 in seed trays and grown in the heated greenhouse under natural day-length until 9/4/71 when they were transferred to a refrigerator at +8°C and low intensity lighting (8 hour day) for 3 weeks. This low temperature treatment was intended to vernalise the seedlings as they had initially been grown for a flowering study in which they were to be compared to Westerwolds under a number of nitrogen levels. (ie. A comparison between an annual and a vernalised perennial ryegrass). On 1/5/71 the Pax Øtofte plants were planted and Westerwolds seeds were sown in plots, the Westerwolds seeds being sown in positions which would give rise to spaced plants. As the Pax Øtofte plants did not flower (presumably due to insufficient vernalisation) they were used for a cold hardiness study and the Westerwolds for a decapitation study, the latter being described in the next subsection.

The layout of this experiment also applies to the decapitation experiment. The sixteen plots were arranged according to a latin square with four nitrogen levels and four replicates of each nitrogen level. The layout is shown diagramatically in figure 2.6. The arrangement of species within plots is represented in figure 2.7. There were 2.5 rows of Westerwolds and 2.5 rows of Pax Otofte. Each row was one foot apart and the plants within each row were one foot apart. Each row comprised 10 experimental plants and one guard plant at each end of each row. The guard plants comprised Westerwolds sown at the same time as the experimental Westerwold plants. Guard rows of Westerwolds were also sown on either side of the 5 rows of experimental plants.

A dressing of muriate of potash equivalent to 2 cwts/acre was spread on each plot at the time of planting. Nitrogen was applied to the plots in three equal dressings on 15th June, 27th July and 7th September, each application representing 100, 66.7, 33.3 and 0 units of nitrogen per acre on the appropriate plots.

The details of the experiments in which the Pax Otofte plants were involved are given at the appropriate point in the Results Section.

(d) The effect of removing inflorescences on tiller number in Westerwolds at four nitrogen levels.

The details of plot layout and nitrogen applications are given in the preceding subsection. When the seeds were sown, three were sown at each position so that after they had reached the second leaf stage, they could be selected for uniformity and only one seedling be allowed to continue to occupy the position. This was done on 18/5/71.

Decapitation took place on 27/7/71 and 12/8/71, and tiller counts were taken on 27/7/71, 12/8/71 and 28/8/71. The plants were numbered as in figure 2.7. Numbers 2, 6, 10, 14 and 18 were decapitated and numbers 4, 8, 12, 16 and 20 were left intact in each plot.

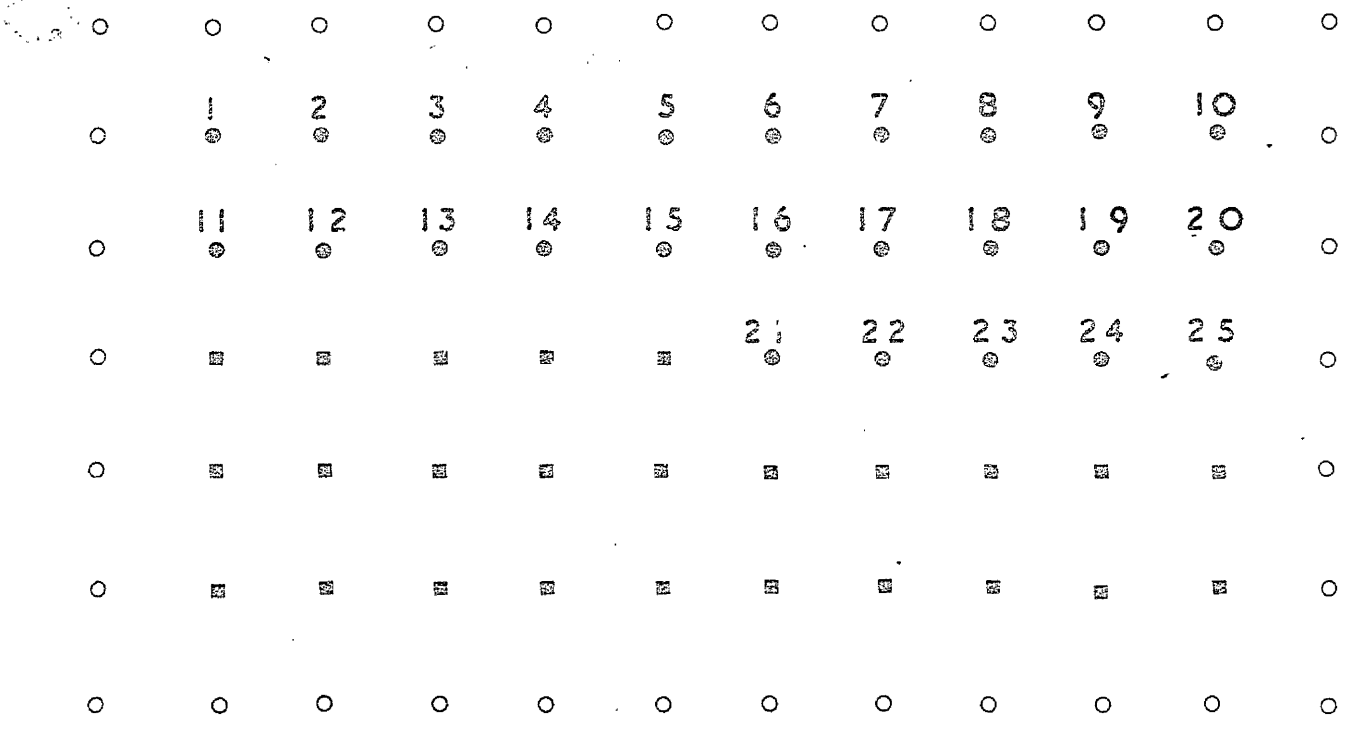
Apical Dominance

(i) Surgical Technique

(a) Expanding leaves Expanding leaves were removed by making a small incision in the expanded leaf sheaths at a point approximating to the position of the apex. The incision was made with a mounted needle which had been filed to form a microscalpel. The two most advanced expanding leaves were removed by cutting just above the apex. Occasionally, the tip of the next oldestexpanding leaf was also removed but that was exceptional.

FIG. 2.7

ARRANGEMENT OF PLANTS WITHIN A PLOT.— EXPTS. 3.3 & 6.4



(b) Developing Inflorescences Apices which were at or beyond the "double ridge" stage of development were removed in a manner similar to the expanding leaves. However, due to stem elongation, at these stages, the position of the apex was more variable. Its position was judged by touch on the upper portion of the plant to locate the youngest developed node and an incision was made longitudinally just above this node. The apex was removed at the base of the lowest developing spikelet.

(ii) Measurement of Expansion of Tiller Buds

Observations on bud expansion was more or less confined to the period when the bud was enclosed by its prophyll. At this stage the prophyll is expanding, and it was considered that measurement of this would be a reflection of tiller bud expansion. Prophyll elongation was measured either by use of a rule or, when too small to be measured in this way, the bud prophyll was measured using a stereoscopic microscope fitted with a calibrated eyepiece graticule.

There were instances where the first leaf had elongated beyond the tip of the prophyll. In these instances, not only prophyll length but also the number of buds in which this had occurred was recorded.

When the experiments were of a longer term, the tiller number was used as a measure of apical dominance.

Cold Hardiness

(i) Conditions for hardening

Depending on the cabinet being used and the nature of the experiment the temperature at which the plants were hardened varied between +5 and +7°C. The daylength was also dependent on the type of cabinet and the nature of the experiment, but was either 8 or

16 hours, ie. both daylengths were photosynthetically 8 hours but the 16 hour day was physiologically a long day.

The duration of the hardening treatment was either 10 or 14 days but again this varied. When it was different from those two periods, it is stated at the appropriate point in the text.

(ii) Assessment of Cold Hardiness

Cold hardiness was assessed by firstly exposing the plants to a range of low temperatures then measuring the damage brought about by, or assessing the ability of the plant to survive after, the cold treatment.

Damage was measured by the electrolyte release method (Dexter, 1930) and survival by tiller counts either once or at regular intervals after the temperature treatments.

(a) Low temperature treatments The manner in which the plants were subjected to low temperatures changed as the work progressed, as certain stages in the procedure were found to be unnecessary. In the initial experiments (particularly those involving plant material which had been growing outside in the field), the tillers were placed in glass jars and stood upright in water. These jars were placed in a Grant low temperature bath at about $+6^{o}C$, filled with 25% ethylene glycol. The material was kept in the dark in the jars over night at that temperature.

The following morning, samples were removed from the jars, the water was emptied out, the jars were dried and the plants were returned, and the temperature of the ethylene glycol was allowed to drop. The air temperature within the jars dropped at the rate of $2-3^{o}C$ per hour from the time of resetting the bath.

Meanwhile, the temperature within the cryostat was dropped to $0^{o}C$. This fell at a slightly faster rate than that of the water bath. When the air temperature within the jars had fallen

to $0^{o}C$, samples of the various treatments were transferred to jars within the cryostat for 1 hour, allowing 10 minutes for equilibration, before being removed. The temperature was then dropped to the next desired level, ready for the transfer of another group of samples when the air within the jars in the bath had reached this temperature, and the procedure was repeated.

Comparative observations showed that the technique using the water bath gave results no different from that obtained from material which was treated solely by reducing the air temperature in a cryostat. The procedure adopted was to place a batch of material in the cryostat, lower the temperature to the first set point and hold for 1 hour at that temperature. A sample was removed after the set exposure. The cryostat was set to the next temperature at which a sample was to be taken and the procedure repeated.

When the samples had been removed from the cryostat, they were left on a clean glass plate on a bench at room temperature to thaw, when necessary, and their hardiness was measured.

(b) Measurement of Hardiness - Electrolyte Release Method

In order that the amount of damage to each plant, or plant part, be measured the conductivity of the leachate was measured after the samples had been immersed in a known amount of distilled water for a standard length of time and was related to the total conductivity which was measured after the plants had been autoclaved ie. where it was assumed that the leachate was at maximum conductivity due to complete release of electrolytes on disruption of the cellular membranes. Therefore the greater the degree of cold damage, the narrower would be the ratio between the reading prior to autoclaving and after autoclaving.

This was done by placing the sample in a 15 x 150 mm testtube after it had thawed and immersing it in 10 mls distilled water.

After 24 hours at room temperature the water was made up to 10 mls and the conductivity measured with a soil conductivity meter.* This expressed the conductivity in CF units. These units were directly proportional to the concentration of ions within the solution as had been found when graded concentrations of sodium chloride solution had been used to calibrate the instrument.

The liquid was returned to the tube, and the conductivity cell was rinsed with distilled water ready for the liquid from the next sample. The mouth of the testtube was covered with aluminium foil and the samples were autoclaved at 15 lbs/sq inch for about 10 minutes to disrupt the cells totally, and the tubes were then cooled to room temperature.

When cool, the liquid was made up to 10 mls with distilled water and the conductivity was measured. When this technique has been used by others the total conductivity is generally measured 24 hours after autoclaving (Polwart, 1970). However, it was found that there was very little difference in total conductivity whether measured immediately after autoclaving (allowing the tubes to cool to room temperature) or 24 hours after autoclaving (Table 2.1).

Table 2.1

A comparison in mean conductivity immediately after autoclaving and 24 hours after autoclaving in grass tillers (12 replicates)

Hrs after autoclaving	0 hrs	24 hrs
CF units	4.9	5.2

The initial conductivity reading was related to the total conductivity as a proportion and expressed as the Relative Conductivity.

* Soil "CF" meter Type MC4 Electronic Switchgear (London) Ltd.

(c) Measurement of Hardiness - Survival

Survival of the treated plants was measured by planting them, after thawing, in 4" pots, 4 plants per pot generally, and placed in the heated greenhouse. Modified Hoagland's nutrient solution was applied twice per week and the plants were watered when necessary. Tillers were counted at intervals or once after a set period of time following the cold treatment.

The above applied to tillers which had been removed from the field. However, in many of the experiments, young plants were used ie. 2-4 leaf stage. When the conductivity of those plants was measured either the root or shoot or both were placed in the testtube depending on the aim and nature of the experiment. This is specified at the appropriate points in the text.

Dissection of Apices

In some experiments, it was found necessary to dissect out the apex of an axis in either the main tiller or in some instances a few of the tillers and tiller buds. The apices were examined under a binocular microscope with a zoom lens and were described by assigning them to one of the categories in Table 2.2. When an apex was considered to be at a stage intermediate to two stages 0.5 was added to the lower value.

Table 2.2

Stage	Description
1	Vegetative (few ridges).
2	Vegetative (many ridges).
3	Double ridge.
4	Triple ridge - axes of spikelets becoming obvious (particularly in the centre).
5	Reproductive primordia seen in more advanced spikelets.
6	Stamen and other reproductive primordia seen in all spikelets - lemmae developing.

Stage	Description
7	Elongation of apex becoming prominent. Lemmae covering middle spikelets.
8	Approaching ear emergence (lemmae fully covering spikelets).
9	Ear emergence ie. first signs of emergence.
10	Ear fully emerged.

The above stages were chosen as they were easily recognised. At no time was any attempt made to summate those numbers or carry out any calculations on them as they do not represent equal intervals of time. They were only used as a convenient means of describing an apex by a shorthand method.

These stages resemble those described by Jeater (1956) for L. perenne. However, stage 5 here resembles 5 and 6 in his system. Hence stages 6, 7, 8, and 9 described here correspond to stages 7, 8, 9, and 10 respectively in his system.

Examples of some of the stages are shown in figure 2.8.

Measurement of ear emergence

The development of inflorescences was related, in some experiments, to the number of leaves formed on the axis prior to flowering. This technique has been employed by Purvis (1934), and Gregory and Purvis (1937) in rye, and Cooper (1956) to identify strains of Lolium perenne. The leaf number was easily obtained as tillers were tagged according to position. By relating to tillers on the axis, the leaf number at flowering was easily counted.

Dissection of plants to count expanding leaves.

In some experiments the total number of leaves (including expanding leaves) was found. The number of expanding leaves was found by using a "microscalpal" previously described. The larger,

Fig. P.3. Some stages of development of spikelets on inflorescences prior to emergence in Westerwolds. (Approximate magnification factors are in brackets).

(a) Stage 3. Early double ridge stage (x15).

(b) Stage 4. Triple ridge stage (x15).

(c) Stage 5. Reproductive primordia becoming obvious (x8).

(d) Stage 6 (Late). Lemmae becoming obvious. Elongation underway - almost at stage 7 (x8).

(e) Stage 8. Distal spikelet with awns obvious on lemma (x8)

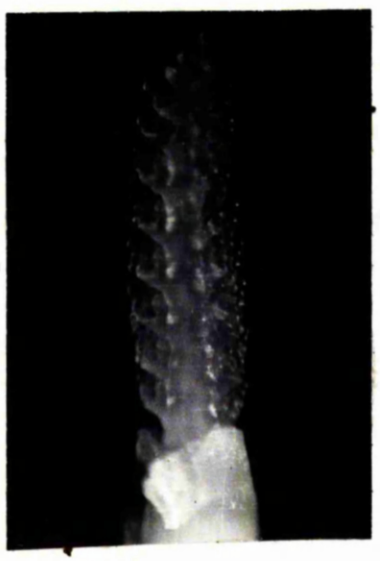
(f) Stage 8. Proximal spikelet with lemma almost enclosing florets (x8).

Fig. 2.8

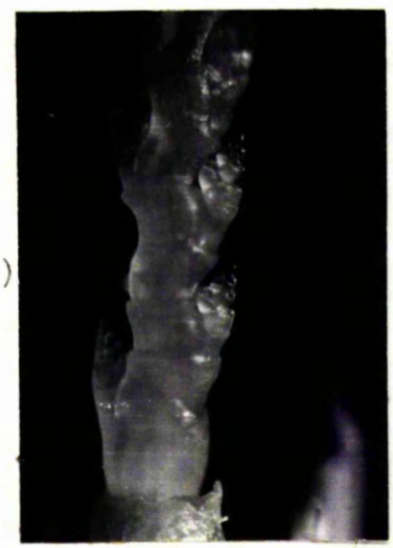
(a)

(b)

(c)

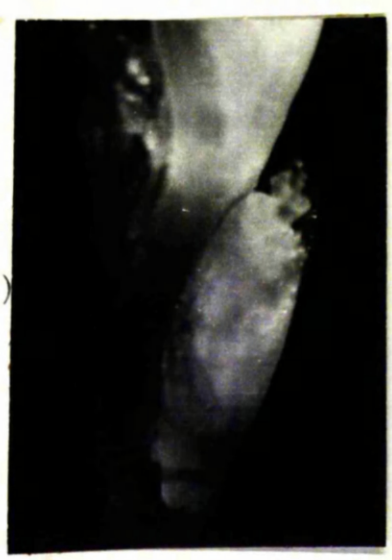


(d)



(e)

(f)



fully expanded leaves were removed by peeling off the axis until it was not feasable to peal off any more without damaging the axis, and a longitudinal incision was then made along the leaf sheaths. Examination under the binocular microscope revealed the number of leaves as they were dissected out one by one until the apex was reached. A leaf was considered to be expanding if it had reached or was beyond the stage where it was covering at least one ridge above its place of origin.

Any primordium which was at a stage less than that just described was considered a "ridge". A leaf was considered to be fully expanded when the ligule was above the top of the preceding leaf sheath.

Description of tillers

In a number of experiments where the relative number of tillers beyond the ear emergence stage was being studied, these tillers were described as "florifercus" and those not emerged "vegetative". This was, therefore not a description of the apex but of the external morphology of the tiller.

When tillers were counted, any part of the tiller which could be seen above the subtending leaf sheath was considered to be a visible tiller and was counted as a tiller.

The description of the position of a tiller was based on its order and node from which it arose. Tillers arising from axils on the main axis of a plant were considered to be primary, those arising from primary tiller axils were considered to be secondary etc.

Presentation of results and statistical analysis

Measurements from nutrient experiments in pots, apical dominance experiments and cold hardiness experiments in pots, were represented as means with standard errors included. Where means

were compared, the student's t-test was employed. Significance between means compared is denoted by the means having different superscripts, usually at the 5% level of significance. Where a group of means are represented, not all may have been compared to each other so although two means could be shown to have different superscripts this may not mean that they are significantly different. For example in cold hardiness studies where there are measurements at different temperatures, the effect of different temperature treatments cannot be compared statistically, but, of course, comparisons can be made within any one temperature treatment. However, this will be clearly specified wherever appropriate.

Where results have been recorded on a presence or absence basis, the results of the treatments are compared by a chi-squared Contingency Test employing Yate's small number correction (Fisher and Yates, 1963).

Results from experiments where plots have been laid out in a Latin square arrangement, are analysed in accordance with the procedure set down by Snedecor (1967). The means of these experiments are given at the appropriate part of the Results section and the Analysis of variance tables are included in the appendices.

Analysis of variance of means of different low temperature treatments, when tiller counts were taken at weekly intervals, were carried out and the analysis of variance tables are presented in the Appendix II.

There are instances where data have been in a form where they could only be analysed by non-parametric means eg. when apices were scored for stage of development employing the criteria described in table 2.2. In such cases the analysis took the form of a comparison between the orders of ranking of the individuals of

each sample of the two treatments. The test is known as the Mann-Witney test and is described in Snedecor and Cochran (1967) pages 130-132.

Controlled Environment Chambers

Type A These cabinets were in pairs, the same cooling heating units controlling the temperature of two of each pair. They had a single bank of lights. The bank comprised of 11 Daylight alternating with 11 Warm White 65 watt fluorescent tubes. The walls were lined with metalised Melinex. Supplementary lighting eg. when daylength was to be extended at low light intensities, could be obtained by the middle pair of tubes being operated independantly of the other 20. Full intensity radiant flux density was 22.54 W/m^2, and supplementary radiated 2.05 W/m^2. Temperature could be controlled to within $\pm 0.5^{o}C$.

Type B Cabinets of this type were manufactured by R.K. Saxton (Sax Air) Ltd. They were constructed to National Institute of Agricultural Engineering specifications and although of sophisticated design and operation, they were less accurate than type A with respect to control of temperature. Temperature fluctuation could not be reduced below $\pm 1.5^{o}C$. A double bank of lights of the same composition as type A cabinets viz. Daylight alternating with Warm White, had a radiant flux density 19.30 to 21.99 W/m^2.

Type C Whereas types A and B were housed at the Botany Department Research Laboratories at Garscube, type C was in the Main Botany Department Building. This type was of a growth room design as the platform occupied only about half of the horizontal area of the chamber. This allowed recording of plants to be done within

the chamber when necessary. This chamber was used for inducing hardiness in some of the later cold hardiness experiments as it could maintain a temperature as low as $+5^{o}C$ with a fluctuation of $\pm 0.5^{o}C$. Lights radiated 32.00 W/m^2 (radiant flux density).

Greenhouse

A Hartley greenhouse divided into bays was employed. Minimum temperature was $15^{o}C$ and light was provided by either mercury vapour lights which emitted 43.2 W/m^2 or a bank of alternating daylight and warm white fluorescent tubes emitting 11.19 W/m^2.

SECTION 3. FIELD STUDIES ON THE RELATIONSHIP BETWEEN NITROGEN, FLOWERING AND TILLER PRODUCTION.

RESULTS AND DISCUSSION

Experiment 3.1

Effects of N on tillering and flowering ; Field experiment 1.

Before embarking on a detailed study of the effects of nitrogen on the development of the grass plant, field studies were carried out in order to determine the relative effects of nitrogen on the tillering and flowering of a range of cultivars within two species. Lolium perenne and Phleum pratense were used. The cultivars of L. perenne were Pax Øtofte, Hunsballe and S23 and of P. pratense were S352 and S48. These cultivars were employed as they represented a range of heading dates within the two species. Details of these have already been recorded (page 45). Experimental details are described in pages 49-51.

The number of tillers and the relative number of flowering tillers were recorded prior to the application of N on 1/7/70 and again in the first week of August in 1970.

Total tiller number Although the plots which were to receive different N levels did not differ significantly (Table 3.1), when the tiller number was recorded prior to the first application of nitrogen, there were differences between rows of plots. This was due to the row next to the laboratory having a smaller tiller number than the other three (figure 2.2, Materials and Methods). The laboratory cast a shadow for part of each day upon this row, therefore, this may have contributed to the lower tiller number.

Species and cultivar differences were also recorded. The two P. pratense cultivars had smaller mean tiller numbers than any of the L. perenne cultivars. Pax Øtofte and Hunsballe had significantly higher tiller numbers than S23 which in turn had significantly more than S352, and S48.

The mean total tiller numbers of each of the cultivars at the second tiller count are presented in table 3.2. Although total tiller numbers are consistently lowest at the lowest N level ie. where no N was applied, the differences are not significant. Where no N has been applied, the tiller counts are consistantly lowest. Therefore it is possible that N has not been entirely ineffective.

The differences in tiller number between some of the varieties at some N levels are pronounced. There is little difference between Pax Ótofte and S23 at any one of the N levels, whereas Hunsballe has consistently lower tiller numbers than the other two cultivars. The most noticeable difference is between either of the P. pratense and L. perenne cultivars, the former having markedly lower tiller numbers.

Flowering tiller number and percentage flowering tillers

Just as the total tiller number differed only when cultivars were considered so did the total flowering tillers (Table 3.3). However S23 was more akin to S352 and S48 than the other two cultivars of L. perenne. When the percentage of flowering tillers was considered, S23 had the lowest percentage of tillers which were at the stage beyond ear emergence at all N levels except at the $N level when Pax Øtofte had a slightly lower percentage.

Table 3.1

Mean total tiller number per plant prior to N application

Species/Cultivar		Eventual Nitrogen level			
		0N	1N	2N	3N
L. perenne	Pax Øtofte	4.25	4.45	5.13	4.83
	Hunsballe	4.55	4.25	4.20	3.98
	S23	2.95	2.80	2.95	3.50
P. pratense	S352	0.43	0.28	0.33	0.40
	S48	0.75	0.33	0.68	0.68

Table 3.2

The effect of nitrogen on the mean total tiller number of a number of cultivars of L. perenne and P. pratense

Cultivars	Nitrogen levels			
	0N	1N	2N	3N
Pax Øtofte	114.0	159.5	146.0	162.5
Hunsballe	70.5	95.75	99.25	121.0
S23	127.25	141.25	142.5	141.75
S352	27.0	36.5	40.0	40.0
S48	34.25	50.25	55.5	49.75

Table 3.3

The effect of nitrogen on the mean number of flowering tillers of a number of cultivars of L. perenne and P. pratense

Cultivars	Nitrogen Levels			
	0N	1N	2N	3N
Pax Øtofte	23.0	14.5	21.0	24.0
Hunsballe	24.25	22.25	20.5	18.5
S23	12.25	14.5	9.75	13.0
S352	9.25	9.75	10.25	10.25
S48	8.75	10.75	14.5	10.75

Table 3.4

The effect of nitrogen on the mean percentage (transformed) of flowering tillers of cultivars of L. perenne and F. pratense

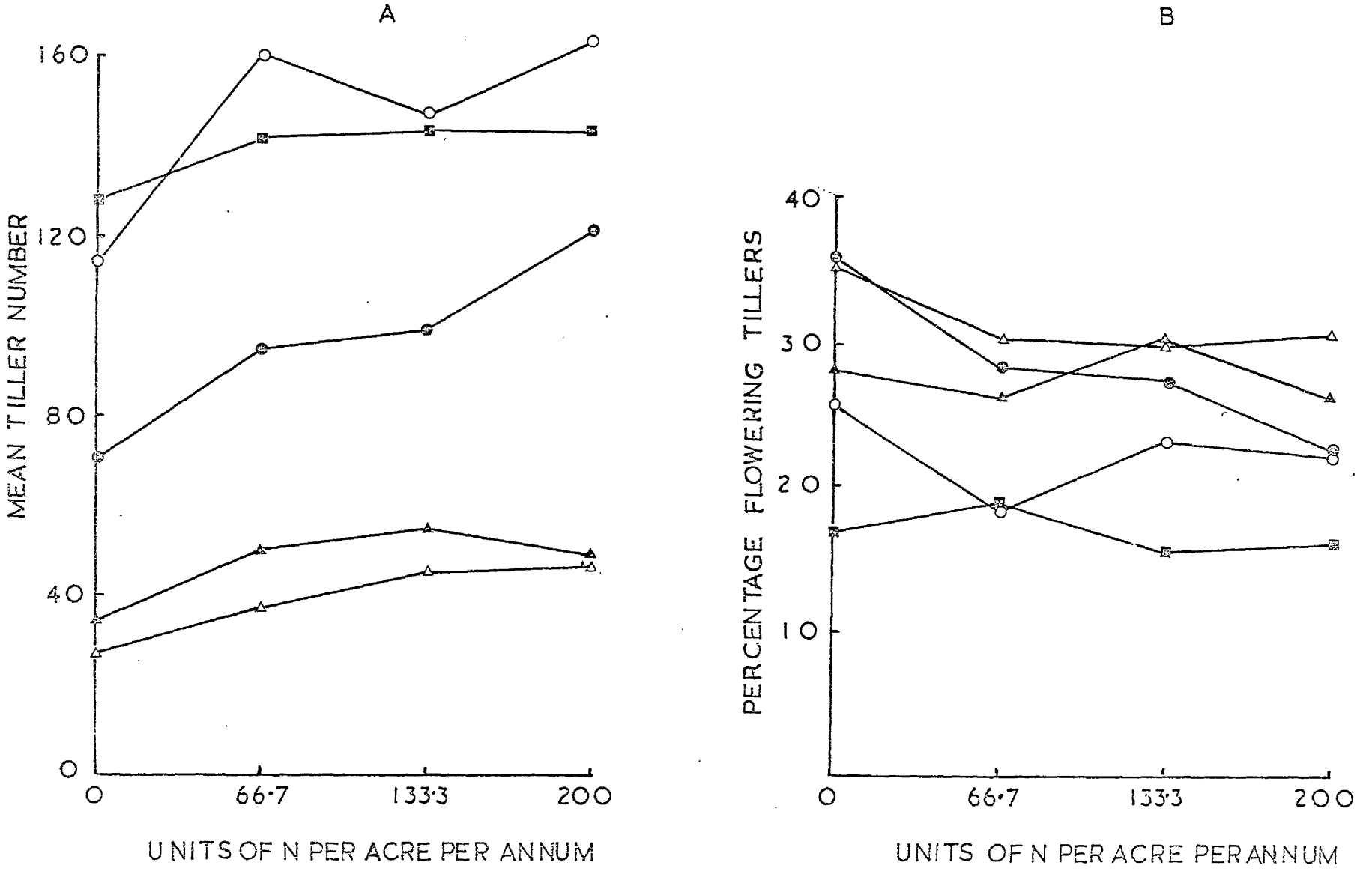
	Nitrogen levels			
	ON	1N	2N	3N
Pax Ø toffe	26.60	18.15	23.53	22.05
Hunsballe	36.20	28.38	26.98	22.61
S23	16.83	18.51	15.26	15.87
S352	35.36	30.46	29.64	30.45
S48	28.33	26.44	30.36	26.12

Fig. 2.1. Mean tiller number (A) and mean percentage flowering tillers (B) in artificially vernalised L.perenne and P.pratense on 1/8/71 at four N levels.

○ Pax Østofte
● Hunsballe
▣ S23
} L.perenne

△ S352
▲ S48
} P.pratense

FIG. 3.1


A
B
160
120
80
40
0
MEAN TILLER NUMBER
0
66·7
133·3
200
UNITS OF N PER ACRE PER ANNUM
40
30
20
10
PERCENTAGE FLOWERING TILLERS
0
66·7
133·3
200
UNITS OF N PER ACRE PER ANNUM

It was considered that recording tiller numbers of different cultivars at varying nitrogen levels in greater detail would be beneficial in explaining the difference in responses detected in the preceding experiment. In order that recordings could be made often the cultivars were reduced to Pax Otofte and S23, and the N levels to the equivalent of 0 and 300 units N/acre/ annum. Also, it was considered more satisfactory to plant the grass plants in plots prior to winter so that vernalisation would occur under field conditions. Therefore, the precedure adopted for this experiment differed considerably from that of the previous experiment. Nevertheless they were both concerned with measuring the effects of nitrogen on tillering and flowering in early and late grasses.

Experiment 3.2.

Effects of N on tillering and flowering. Field experiment 2.

Total Tiller [illegible] (Table 2.5; Fig.32)

By the end of the winter, Pax Øtofte had a greater mean number of tillers per plant than S23. Hence when nitrogen was applied on 13/3/72, there was a significant varietal difference. This difference persisted until 2/5/72. On 23/5/72, varietal differences were again recorded, but at that time S23 had the greater number of tillers and continued to have up to the final count in the primary growth phase on 14/7/72.

Differing responses due to nitrogen were detected on the first count after application ie. 23rd April, and continued to increase in tiller number throughout the duration of the experiment. The relative response of the varieties to nitrogen however, were different. S23 responded more positively to the N application than Pax Øtofte, and so significant interactions between nitrogen levels and varieties were obtained on 14/7/72.

The relative tiller numbers of plants which were recorded on 15/8/72 having regrown since 14/7/72 revealed a similar pattern to that found at the last tiller count of the primary growth cycle. However, the low N treatment of S23 gave rise to a tiller number nearer the high N treatment of Pax Otofte than in the last primary growth count. An interaction between nitrogen levels and varieties was also detected at this time, due to the greater response of S23 to N.

The first recorded significant increase in tiller number of S23 relative to that of Pax Øtofte followed the onset of ear emergence in Pax Øtofte.

The tiller numbers of S23 after the onset of flowering was somewhat erratic eg. in the high N treatment there was a sharp

Table 3.5

Tiller numbers and percentage flowering tillers at intervals in Pax Øtofte and S23 at two N levels

	Mean total tiller number				Mean percentage flowering tillers			
	Pax Øtofte		S23		Pax Øtofte		S23	
	ON	300N	ON	300N	ON	300N	ON	300N
13 March	4.8	5.2	2.8	3.1	0	0	0	0
23 April	18.0	20.3	15.9	18.1	0	0	0	0
2 May	22.2	27.5	17.8	25.5	0	0	0	0
9 May	28.6	38.0	30.6	36.8	0	0	0	0
16 May	35.4	53.9	40.0	53.4	7.58	6.26	0	0
23 May	41.4	64.6	52.6	74.1	17.78	16.97	0	0
30 May	51.1	85.0	72.5	107.0	24.10	22.34	0	0
*8 June	74.4	131.4	-	-	29.12	25.92	0	0
15 June	81.0	133.4	116.9	205.7	27.62	28.54	4.62	5.31
*19 June	82.4	168.1	143.1	292.7	31.16	26.03	14.69	13.24
*30 June	-	-	182.8	301.8	-	-	15.83	17.35
*14 July	248.1	229.7	198.9	453.9	31.48	44.06	28.82	30.39
*15 August	286.0	346.6	357.4	700.3	26.24	22.26	17.24	12.99

* Plants clipped to 1 inch above ground level.

Plants clipped on 15th August are the same

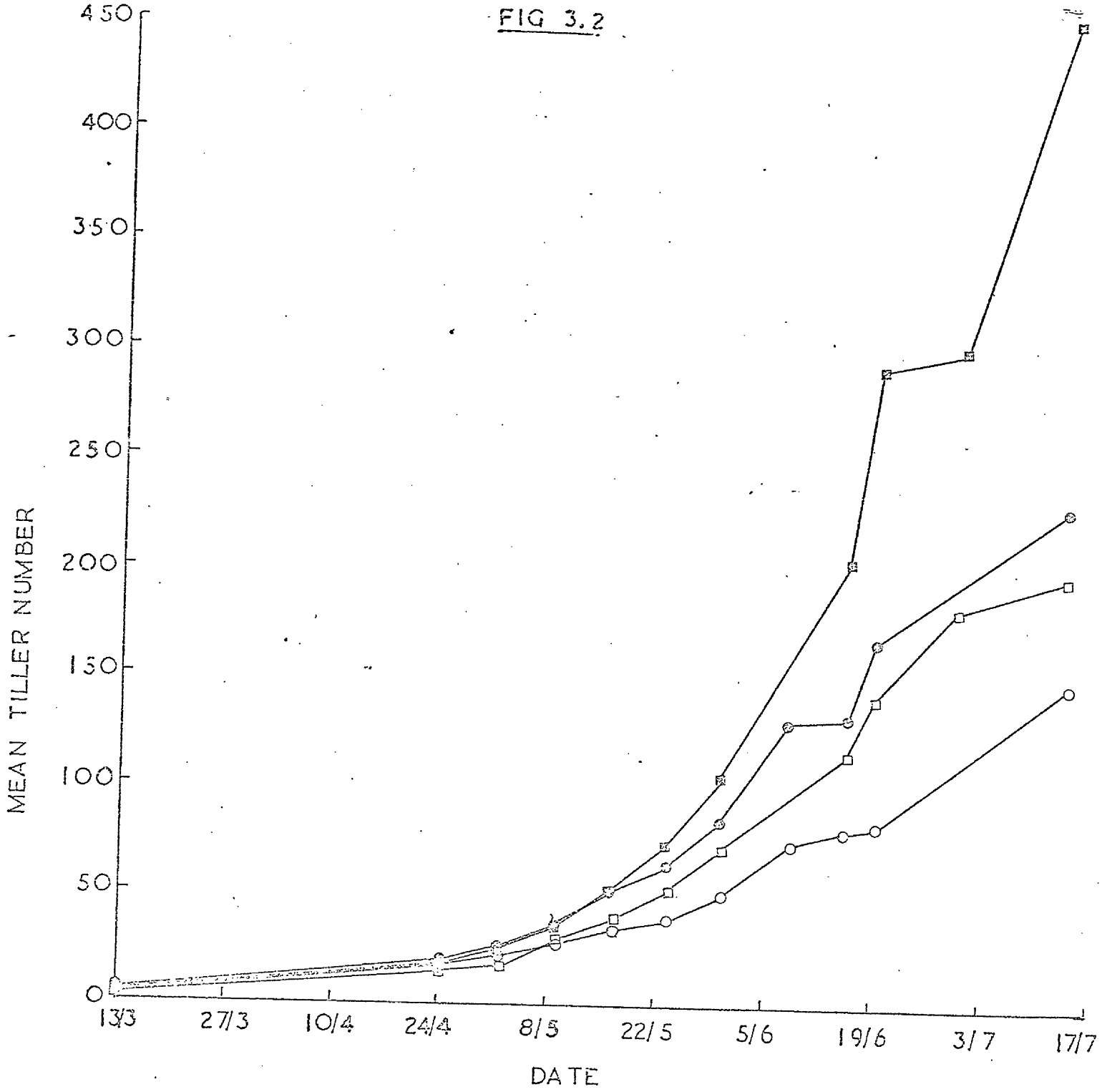
plants clipped on 14th July.

Fig. 3.2. Mean tiller number per plant of vernalised Pax Øtofte and S23 at two N levels at intervals from 13/3/72 to 14/7/72.

● Pax Øtofte
■ S23
} High nitrogen (300 units N/acre/annum)

○ Pax Øtofte
□ S23
} Low nitrogen (0 units N/acre/annum)

FIG 3.2


450
400
350
300
250
200
150
100
50
0
MEAN TILLER NUMBER
13/3
27/3
10/4
24/4
8/5
22/5
5/6
19/6
3/7
17/7
DATE

increase in tiller number between 15/6/72 and 19/6/72, yet between 19/6/72 and 30/6/72, the tiller number remained more or less constant. Whether this was due to sampling or due to some other factor such as the onset of flowering, it is not possible to conclude. The low N treatment of S23 gave rise to a gradual tailing off in tiller number from 19/6/72 to 14/7/72 ie. the period after flowering was underway.

Percentage of flowering tillers (Table 3.5; fig.3.3.)

Pax Øtofte had tillers which were at ear emergence in both N treatments on 23/5/72. These had reached this stage between 16/5 and 23/5/72. The percentage of tillers which had ears visible were similar at both N levels in Pax Øtofte at the tiller counts until 14/7/72, when the high N level had a significantly higher percentage of tillers which were flowering than the low N level.

S23 did not have any flowering tillers until 15/6/72 and these had not emerged before 29/5/72. As the percentage of flowering tillers was so low ie. about 5% at both N levels, it is unlikely that ear emergence had been taking place long before 15/6/72.

By 14/7/72, the low N treatments of S23 and Pax Øtofte and the high N treatment of S23 had similar percentages of flowering tillers. The high percentage in the high N treatment of Pax Øtofte gave rise to a significant varietal and N treatment difference. Although the interaction between N and varieties was not significant the F ratio was close to that at the 5% significance level ie. 4.29 compared to 5.12.

The reason for this high percentage flowering tillers in the high N Pax Øtofte treatment is not obvious. Perhaps the low number of vegetative tillers appearing after the onset of flowering tillers formed early in the season which were initiated was

Fig. 3.3 Percentage flowering tillers in vernalised Pax Øtofte and S23 at two nitrogen levels from 9/5/72 to 14/7/72.

● Pax Øtofte
■ S23
} High nitrogen (300 units N/acre/annum).

○ Pax Øtofte
□ S23
} Low nitrogen (0 units N/acre/annum)

FIG. 3.3

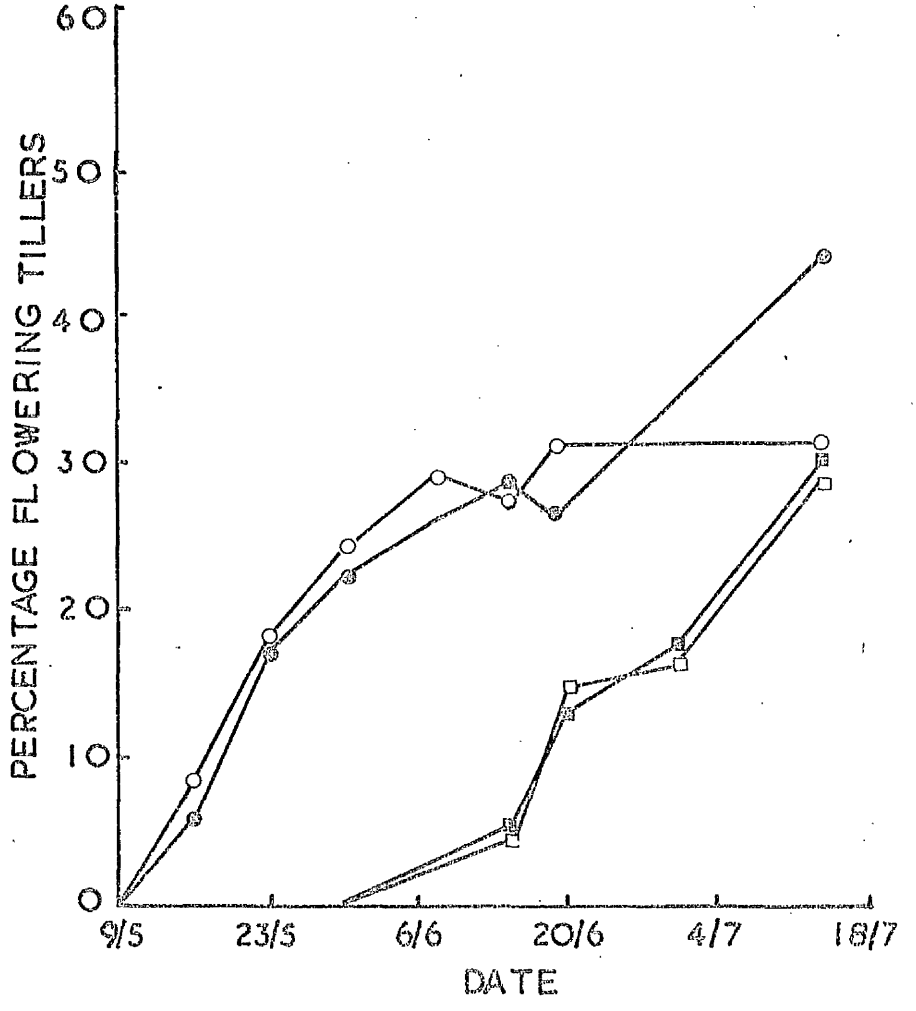
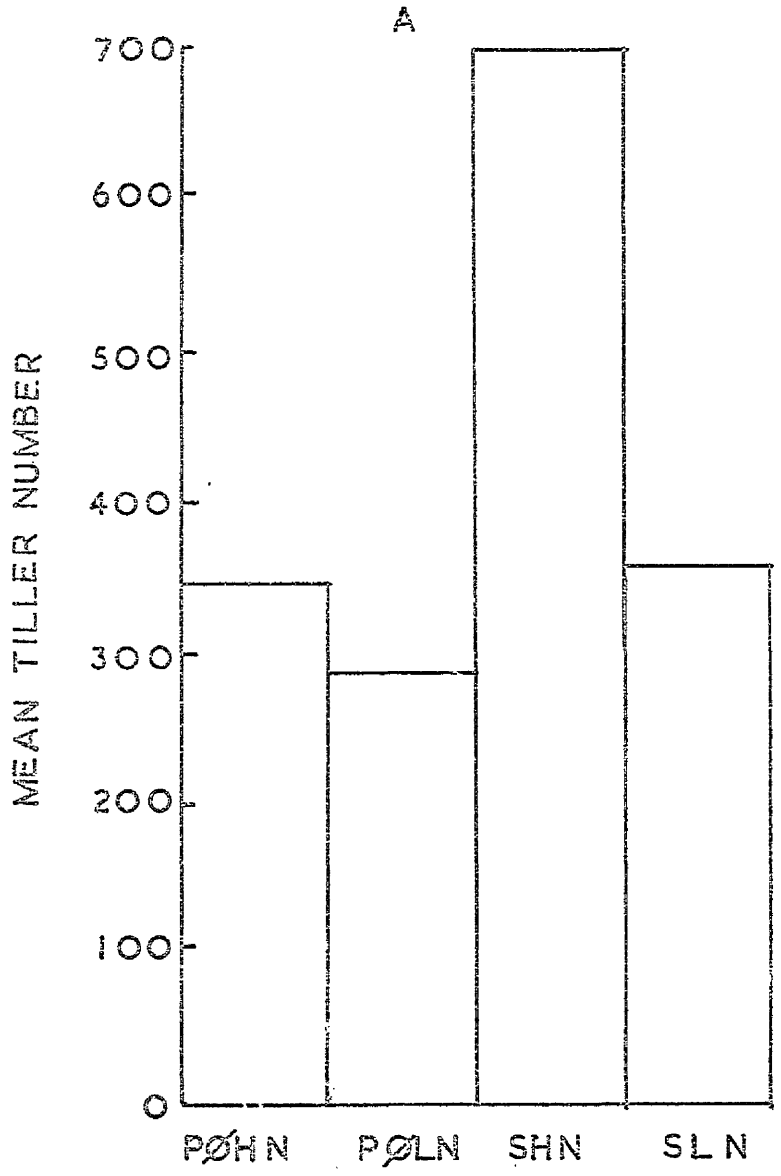

PERCENTAGE FLOWERING TILLERS
60
50
40
30
20
10
0
9/5
23/5
6/6
20/6
4/7
18/7
DATE

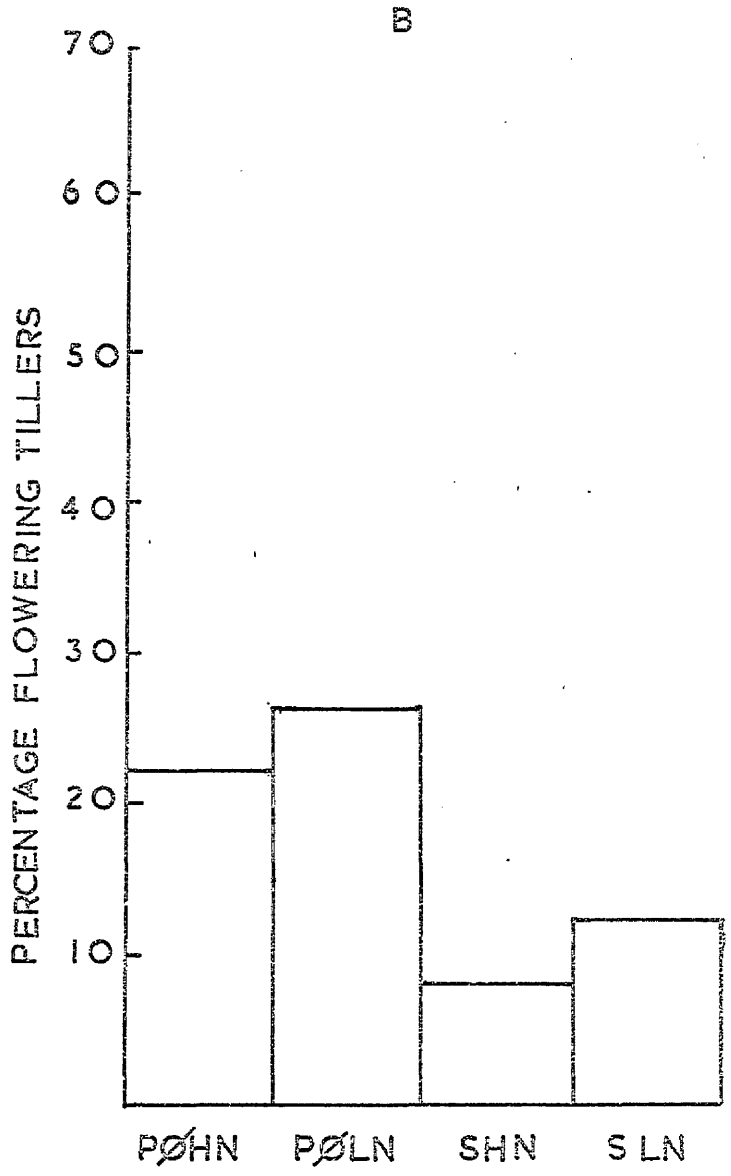
Fig. 3.4.1. Mean tiller number of spaced plants of vernalised Pax Øtofte and S23 at two N levels on 15/8/72 regrown since clipped on 14/7/72.

Fig. 3.4.2. Mean percentage flowering tillers of spaced plants of vernalised Pax Øtofte and S23 at two N levels on 15/8/72 regrown since clipped on 14/7/72.

PØHN	Pax Øtofte	High nitrogen (300 units N/acre/annum)
SHN	S23	
PØLN	Pax Øtofte	Low nitrogen (0 units N/acre/annum.
SHN	S23	

FIG. 3.4


A
MEAN TILLER NUMBER
700
600
500
400
300
200
100
0
PØHN
PØLN
SHN
SLN


B
PERCENTAGE FLOWERING TILLERS
70
60
50
40
30
20
10
PØHN
PØLN
SHN
SLN

Mean dry weight per tiller (total, vegetative and flowering) of Pax Ø tofte and S23 at intervals at two N levels (gms)

	Pax Ø tofte						S23					
	0N			300N			0N			300N		
	F	V	T	F	V	T	F	V	T	F	V	T
8 June	0.310	0.055	0.105	0.423	0.085	0.155	-	-	-	-	-	-
19 June	0.367	0.061	0.164	0.492	0.103	0.195	0.423	0.067	0.093	0.496	0.096	0.120
30 June	-	-	-	-	-	-	0.498	0.086	0.120	0.673	0.137	0.189
14 July	0.491	0.078	0.192	0.539	0.122	0.342	0.586	0.125	0.222	0.727	0.172	0.317
15 August	0.259	0.071	0.105	0.288	0.082	0.122	0.314	0.087	0.109	0.284	0.085	0.098

F = Flowering

V = Vegetative

T = Total

responsible for the high percentage compared to the low N treatment.

Cultivar differences in percentage tiller number were significant in the regrowth tiller count, Pax Ótofte having a greater percentage than S23. Therefore even when day length is not apparently the critical factor, which it would not be from mid July to mid August, the rate of initiation or floral development is slower in S23 than in Pax Ótofte.

Dry weights of total vegetative and flowering tillers (Table 3.6)

As the season progressed, the tillers during the primary growth phase appeared to become heavier, as would be expected.

The mean weight of total tillers on 19/6/72 was greater in Pax Ótofte than S23. This however was not significantly reflected in the components of the total tiller weight ie. vegetative and flowering tiller weights. The high nitrogen treatment, however did give rise to a significantly greater vegetative tiller weight than the low N level.

On 30/6/72, when S23 alone was sampled, the mean weight of the total, vegetative and flowering tillers was greater at the high N level. Heavier total and vegetative tillers were recorded at the high N levels on 14/7/72 than at the low N treatments, but the differences due to nitrogen in the flowering tillers were not significant.

Varietal differences were significant on 14/7/73 when the mean dry weight of vegetative tillers were considered. At both N levels, S23 had heavier vegetative tillers than Pax Ótofte at the corresponding N level. This held for flowering tillers. The heavier flowering tillers in S23 could be due to the longer vegetative period of S23 plants prior to flowering early in the season. The

vegetative tillers of S23 being heavier than Pax Otofte tiller may be due to the greater number of tillers in the former which were considered vegetative but were actually approaching ear emergence.

No significant differences between treatments within each group of tillers were detected in the regrowth tiller count. The weights of the vegetative tillers of each treatment were similar to those of the vegetative tillers of the respective varieties at the first harvest in the primary growth cycle. The weight of the flowering tillers, however, was consistently lower, than the first harvest in the primary growth phase. Presumably this was due to the short period of growth subsequent to clipping before flowering ensued.

Experiment 3.3.

The effect of removing inflorescences on tiller number in Westerwolds at four nitrogen levels.

In order to determine the effect of removal of the flowering heads in a grass plant on subsequent tillering this experiment was carried out. The details are presented in "Materials and Methods" page 54

Westerwolds was the ryegrass studied in this experiment as it did not require vernalisation as a prerequisite to flower so was convenient for studies involving flowering effects on tillering. It also has large tillers which lend themselves to being easily counted in comparison to perennial ryegrass when large numbers per plant are recorded.

The results are presented in table 3.7. Analysis of variance tables are in Appendix I.

Table 3.7

Mean Total tiller number, mean increase and mean percentage increase in tiller number from 27-7-71 of intact and decapitated plants at 4N levels

	Nitrogen levels (Equivalent units/acre/annum)							
	0N		1N		2N		3N	
Mean tiller number	Intact	Decap	Intact	Decap	Intact	Decap	Intact	Decap
27-7-71	28.40	32.85	34.10	28.70	34.10	39.95	32.40	34.65
12-8-71	34.70	44.60	49.85	41.80	49.1	56.25	48.15	51.65
28-8-71	41.05	51.45	61.05	56.15	66.3	75.35	68.90	71.90
Mean increase from 27-7-71								
12-8-71	6.30	11.75	15.75	13.10	15.00	16.30	15.75	17.00
28-8-71	12.65	18.60	26.95	27.45	32.20	35.40	36.50	37.25
Mean % age increase from 27-7-71 (transformed)								
12-8-71	17.54	29.85	45.35	43.17	38.06	37.70	37.43	34.04
28-8-71	41.90	50.58	79.15	74.83	83.11	75.05	87.10	86.32

Total tiller number Although decapitation has no significant effect on the total tiller number, nitrogen has an effect at both tiller counts ie. on 12/8/71 and 28/9/71. However, position of the plots also has a pronounced effect on tillering. This is particularly so at 12/8/71 when both rows and columns are affecting tiller number. From the analysis it seems that the row of plots nearest the laboratory (figure 2.6) has significantly fewer tillers than the other three plots. Also the most southerly column of plots (the columns run north west-south east, approximately) contains a significantly lower number of tillers than the other three.

The row nearest the laboratory may have been effected by the shadow of the laboratory being cast over these plots and so reducing the incident light intensity on the plots in this row. The cause of the column of plots mentioned to be giving a low tiller count is more difficult to explain. It may have been due to inherent fertility differences between the soil in this group of plots and in the other 12.

The affect of nitrogen on the total tiller numbers is greater at 28/8/71 than 12/8/71. Also, although not significant, any difference between decapitated and intact plants is greatest at the lowest N level, decapitated plants having the higher tiller number.

Increase and percentage increase in tiller number

Increase and, in particular, percentage increase, in tiller number are more meaningful measurements of treatment effects in such an experiment, as nitrogen had been applied for a considerable time prior to the commencement of the decapitation treatments, so influencing tiller number prior to decaptiation.

Increase in tiller number follows a similar pattern to that of total tiller number ie. nitrogen having an effect as well as position of plots (viz. rows and columns). Percentage increase is only significantly different at the various nitrogen levels at the later tiller count and position does not have a significant effect. The latter suggests that the factor or factors affecting tiller number in some rows and columns more than others was more effective prior to the commencement of decapitation, hence affecting total tiller number, and as a consequence increase in tiller number without affecting percentage increase.

At the later tiller count, the greatest N effect is between the ON and IN treatment. Increases to the higher N levels have small and non significant effects.

Discussion

Differences in tiller number due to nitrogen, found to be significant in Pax Øtofte and S23 in experiment 3.2 were not significant in experiment 3.1. However, there are basic differences in procedure between the two experiments which might explain the contradiction. In experiment 3.1 the plants were artificially vernalised and planted at the beginning of May in plots. This would give rise to a) a smaller amount of vegetative growth prior to flowering than would have occurred had the plants been planted prior to the previous winter as was done in experiment 3.2 and b) flowering in Pax Øtofte would be delayed due to the delay in floral initiation. If it is assumed that floral development from initiation to ear emergence on the main axis is of a minimum duration of 4 weeks, then ear emergence in Pax Øtofte would not occur until the beginning of June, at the earliest. This assumption is based on the results of Cooper (1953) for S24. Also, the critical daylength of S23 would be reached in May, (Cooper, 1951) and, although S23 has been found to develop inflorescences slowly after initiation (Evans, 1960), there would be only small differences in the dates of ear emergence between the two varieties in experiment 3.1.

In experiment 3.2, it was found that tillering rate decreased at flowering in Pax Øtofte and, and also, but to a smaller extent in S23. The reduction in differences in time of ear emergence between the two varieties in experiment 3.1 could have given rise to similar effects on tillering at around the same time. This could explain the similar tiller numbers of Pax Øtofte and S23 in experiment 3.1 when the tillers were counted at the beginning of August.

The obvious decrease in the rate of tillering in some treatments at ear emergence in experiment 3.2 in L. perenne may partly account for the decrease in production in L. perenne at flowering under spaced plant conditions found by Cooper and Sand, (1949).

American work on Bromus inermis has shown that two tillering phases occur, one prior to ear emergence and another subsequent to anthesis (Lamp, 1952). Langer has also found that a cessation in tillering occurs when flowering takes place in timothy, although high N treatment can overcome the effect.

That N can overcome the decrease in tillering at flowering is seen to an extent in S23. Although the curves in figure 3.2 are somewhat erratic from sampling date 15 June onwards, presumably due to the sampling technique, distinct differences in curve slope between the two N treatments for S23 are seen subsequent to the onset of ear emergence in S23.

The difference in response to N of the two varieties is well demonstrated by the tiller number of the regrowth where only S23 has a significantly higher tiller number at the higher N level than at the low N plain. The response of Pax Øtofte to N is positive when the percentage of flowering tillers is considered at the 14/2/72 harvest. This may be due to the high tiller number which was present early in the season when the initial flowering stimulus was perceived, and so may have been proportionately high in comparison to the final total tiller number.

The slow development of inflorescences of S23 found by Evans (1960) may explain the low percentage of flowering tillers of S23 at both N levels at the second harvest in mid-August in comparison to Pax Øtofte. It is unlikely that daylength has been insufficiently

long to promote flowering in S23. The daylength for the period of growth declined from 17 hours to 15 hours, (sunrise to sunset) (Proons Nautical Almanac, 1972). Although the critical daylength for S23 is considered to be greater than 13 hours (Cooper, 1963), in order that it be able to flower in early June, initiation would have commenced by late April. The daylength for this period is about 15 hours. Therefore it could be considered that during the period of regrowth, daylength exceeded the critical daylength of S23.

Mean tiller weights of vegetative and flowering tillers of S23 at the first harvest are heavier than the corresponding weights of Pax Øtofte tillers. However, due to a greater number of flowering tillers in Pax Øtofte, the mean total tiller weights are similar. The high flowering tiller weights in S23 are presumably due to S23 having a longer vegetative growth period prior to initiation than Pax Øtofte.

Langer (1957) has shown that in timothy tiller weight increases exponentially until ear emergence after which it appears to tail off and ultimately decrease. The significant effect of nitrogen on total tiller number at the end of the first growth cycle also agrees with the findings of Langer (loc cit.) in timothy, although the differences between N levels are less dramatic than those found by him. Increasing the N level from 6 ppm to 150 ppm Langer found a 20 fold increase in mean tiller weight when P and K were maintained at a high level.

The differences between spaced plants of these two varieties are reflected in their behaviour under sward conditions (Aldrich, 1968). Aldrich found Pax Otofte had a high spring growth in comparison to S23. Pax Øtofte (considered together with two other similar Danish varieties of

L. perenne) yielded 106% up to 1st May in comparison to S23 yielding 61%. However in the period from 2nd May to 1st July, the Danish group had a mean yield of 97% that of S24, whereas S23 gave rise to a yield of 113%. Although it can be dangerous to extrapolate from spaced plants to sward conditions, the changes in rates of tillering may be implicated, at least partly in the relative seasonal yield of these cultivars.

In order to pursue further the effect of flowering on tillering, experiment 3.3 was carried out. Despite the claims that the developing inflorescense restricts the appearance of tillers (Review of Literature, pp 16-18) the removal of inflorescences at or beyond the stage of ear emergence at fortnightly stages has no effect on tillering. This would suggest that the presence of those parts on the plant have no effect on tillering. In Westerwolds, nearly all tillers flower (Patel and Cooper, 1961) therefore although these tillers, at or beyond ear emergence have their inflorescences removed, there will be a large number of tillers with developing inflorescences prior to ear emergence remaining on the plant. As it is not clear which stage of floral development is responsible for the claimed inhibition of tillering, it is possible that a stage prior to ear emergence could create an inhibitory effect.

Although not significant there is a greater difference in percentage tillering between intact and decaptitated plants at the lowest N level than between treatments within higher N levels, the decapitated having the higher percentage increase. (Table 3.7).

Regular defoliation has been found by Hunt (1962) and Baker, et al. (1965) to allow Westerwolds to persist into the next growing season. This may be due to stimulated tillering by the removal of apices, the additional tillers perhaps being responsible for the continuation into the next season.

The range of nitrogen used in this experiment has been found to be within the range which gives rise to linearity in yield under sward conditions (Darlow, 1965). However it would seem that the optimum level for tillering is lower (Table 3.7).

Westerwolds would appear to differ from wheat in that Aspinall (1963) was able to induce in wheat an increase in tiller number by removal of the spikelets, the greater the number of spikelets removed the greater the effect. However, this was marked only at the low nutrient levels. The fertility of the soil even at the low N level in the experiment described here may have been insufficiently low in N to give rise to any significant effect decapitation may have induced. As has already been mentioned, decapitated plants had almost twice the mean tiller number of intact plants in ON treatment. Therefore it may be only at deficiency levels of nutrition that decapitation has an effect on tillering.

SECTION 4: STUDIES OF EFFECTS OF NITROGEN ON GRASS PLANT DEVELOPMENT UNDER CONTROLLED ENVIRONMENTAL CONDITIONS.

The last section dealt with effects of N on tillering of spaced plants under field conditions. However, in order to determine more fully the effect of N on the morphology of the grass plant a series of experiments was carried out under semi-controlled environmental conditions.

The first experiment was concerned with the effects of N on the tillering pattern of an annual and perennial ryegrass. It was considered that as the project was partly concerned with flowering, then annuals would be more suitable than perennials for such a study due to the former not requiring vernalisation treatment. Also annuals tend to attain the flowering state more quickly than any of the perennial ryegrasses.

The annual ryegrass used in the experiments in this section was L. multiflorum (var. Westerwoldicum, cv. Dutch). The perennial ryegrass cultivar was Pax Øtofte, which was used in experiments in the previous section.

In the field, due to the rate of development of tillers, it was not feasable to mark the tillers according to order and position. It was considered that the experiments carried out in this section would allow tillers to be marked, and N levels could be compared regarding their effects on the organisation of the tiller hierarchy in ryegrasses.

Experiment 4.1

The effect of nitrogen on the tillering pattern of grasses

In order to determine to what extent nitrogen level influenced the total primary, secondary and tertiary tiller number in rye-grasses, L. perenne ev. Pax Ötofte and L. multiflorum var. Westerwoldicum cv. Dutch were studied under two nitrate regimes. The experiment was terminated when it became obvious that some of the Dutch Westerwolds plants were approaching flowering.

Seeds of Dutch Westerwolds and Pax Øtofte were sown on 20/3/72 and the seedlings were planted in 3½" pots on 8/4/72 in vermiculite. Throughout the duration of the experiment the plants were under a 16 hour day in the heated greenhouse. On 14/4/72, no tillers were visible. Two weeks later, the tillers were counted and this was repeated at weekly intervals for a further two weeks.

Nitrate levels were administered as aliquots of 50 mls solution at two nitrate levels, ie. 1N and 1/5 N, applied once per week, the first application being on 8/4/72.

The following were measured:

(a) Total tiller number

(b) Primary tiller number

(c) Secondary tiller number

(d) Position of each tiller (node)

The tiller numbers of the two species are presented in tables 4.1 and 4.2, the frequency at which primary tillers arise from the various nodes on the main axes are in table 4.3 and the number of primary tillers bearing secondary tillers is presented in table 4.4.

Table 4.1.

Mean total tiller numbers

		Days			
		0	16	24	32
Westerwolds	HN	0	1.5 ± 0.31^{ab}	4.2 ± 0.60^{c}	7.2 ± 0.93^{e}
	LN	0	1.2 ± 0.18^{a}	2.4 ± 0.24^{b}	3.0 ± 0.31^{f}
Pax Øtofte	HN	0	2.1 ± 0.26^{b}	5.3 ± 0.54^{c}	8.2 ± 0.80^{e}
	LN	0	1.8 ± 0.13^{ab}	2.6 ± 0.14^{d}	3.3 ± 0.20^{f}

Means within the same column without a common superscript are significantly different at 5% level.

Table 4.2

Mean primary and secondary tiller numbers

	Days	0		16		24		32	
		P	S	P	S	P	S	P	S
Westerwolds	HN	0	0	1.5 ± 0.31^{ab}	0	3.3 ± 0.29^{c}	0.9 ± 0.4	4.5 ± 0.35^{e}	2.7 ± 0.79^{g}
	LN	0	0	1.2 ± 0.18^{a}	0	2.4 ± 0.24^{d}	0	2.8 ± 0.30^{f}	0.2 ± 0.17^{h}
Pax Øtofte	HN	0	0	2.1 ± 0.26^{b}	0.1 ± 0.09	3.1 ± 0.26^{c}	2.2 ± 0.33	4.0 ± 0.25^{e}	4.2 ± 0.57^{g}
	LN	0	0	1.8 ± 0.13^{ab}	0	2.6 ± 0.14^{c}	0	2.9 ± 0.31^{f}	0.4 ± 0.21^{h}

Numbers within the same column with differing superscripts are significantly different at 5% level of probability.

Table 4.3

Number of plants which have primary tillers at designated nodes on the main axis.

Treatment		Node					
Day 16		1	2	3	4	5	6
Westerwolds	HN	5^{a}	9^{c}	5^{d}	$-^{g}$	-	-
	LN	5^{a}	10^{c}	1^{e}	$-^{g}$	-	-
Pax Øtofte	HN	1^{ab}	10^{c}	11^{f}	2^{g}	-	-
	LN	$-^{b}$	12^{c}	11^{f}	$-^{g}$	-	-
Day 24							
Westerwolds	HN	5^{a}	12^{c}	11^{d}	11^{e}	3^{g}	-
	LN	6^{a}	13^{c}	10^{d}	1^{f}	$-^{g}$	-
Pax Øtofte	HN	1^{ab}	10^{c}	12^{d}	11^{e}	3^{g}	-
	LN	$-^{b}$	12^{c}	13^{d}	9^{e}	$-^{g}$	-
Day 32							
Westerwolds	HN	6^{a}	12^{c}	13^{d}	12^{e}	8^{gh}	5^{i}
	LN	6^{a}	13^{c}	12^{d}	3^{f}	-	$-^{j}$
Pax Øtofte	HN	1^{ab}	10^{c}	12^{d}	12^{e}	11^{g}	2^{ij}
	LN	$-^{b}$	12^{c}	13^{d}	10^{e}	4^{h}	$-^{j}$

Numbers in the same column recorded on the same day without a common superscript are significantly different at the 5% level.

Table 4.4.

Total number of primary tillers bearing secordary tillers within each treatment.

		Tiller number
Westerwolds	HN	20^{a}
	LN	2^{b}
Pax Øtofte	HN	24^{a}
	LN	6^{b}

Numbers without a common superscript are significantly different at 5% level.

Tiller number. At the second tiller count, the low nitrogen treatment of Westerwolds had a significantly smaller mean total tiller number than the high nitrogen treatment of Pax Øtofte. By the third count, the two low nitrogen treatments had similar total tiller numbers, and those were significantly lower than those of the two high N treatments. This was also the case at the fourth count.

When the components of total tiller number were considered, the plants of the high nitrogen treatment in both species had significantly higher primary and secondary tiller numbers at the fourth tiller count than the low N levels. The secondary tiller number was seen to be more responsive to nitrogen than the primaries eg. at the fourth count, the high N treatments had less than twice the number of primary tillers of the low N treatments, but had more than ten times the number of secondaries.

Tiller position. Westerwolds, at both N levels had a higher number of plants with tillers at node 1 on the main axis than Pax Øtofte. However, as time progressed, Westerwolds at the low N level had no plants with tillers at nodes 5 and 6 at the last tiller count, whereas 8 out of 13 of the high N level had a tiller at node 8 and 5 had one at node 6.

Pax Øtofte had a higher number of plants with tillers at node 3 at the second tiller count ie. considering both N levels together, there were 22 out of 25 plants with a tiller at that node, whereas Westerwolds had only 6 out of 26.

It would appear therefore, that although Westerwolds had more plants tillering at node one, Pax Øtofte gave rise to tillers at node 2 earlier than Westerwolds. Westerwolds was also affected more by lack of nitrogen than Pax Øtofte, as this is reflected in

the slower production of tillers, particularly at [illegible] nodes than the low N level of Pax Øtofte.

Table 4.5.

Number of plants with primary tillers bearing secondaries on each node on the main axis at the last tiller count

		Node				
		1	2	3	4	
Pax Otofte	HN	1	10^{a}	11^{c}	2 (out of [illegible]	plants)
	LN	-	3^{b}	3^{d}	- (out of [illegible]	plants)
Westerwolds	HN	-	8^{a}	9^{c}	2 (out of [illegible]	plants)
	LN	-	2^{b}	-	- (out of [illegible]	plants)

(Numbers with different superscripts within the same [illegible] are significantly different at 5% level)

Most of the plants at the high N level had secondaries borne on the primary tillers at weeks 2 and 3 in both varieties. Although 12 out of 26 Westerwold plants had tillers at node 1, only one gave rise to secondary tillers. It would seem that the development of the primary tiller at that node was severely restricted.

In Pax Øtofte, the primary tillers at nodes 2 and 3 formed at both N levels by the second tiller count. There is unlikely that the relative difference in age of the tillers at the two N levels is the only factor responsible for the relative low number of primary tillers bearing secondaries at N treatment. It is likely that nutrition is having an effect on the growth of the secondary tiller buds.

This experiment did not take into account the effect of nitrogen on the expansion of tiller buds prior to becoming [illegible]

the subtending leaf sheath. The following experiments were designed to study the effect of N on the components of tiller bud expansion.

Experiment 4.2

The effect of nitrogen on the leaf expansion of Pax Øtofte

Seedlings of Pax Øtofte at the third expanding leaf stage were potted in vermiculite in 2½" pots. Two weekly applications of 50 mls of nutrient were applied to each pot, half of the group receiving the high nitrogen solution and the other half, the low nitrogen solution. Two weeks after potting, the plants were removed and the following characters measured.

1. Total number of tillers at each node.
2. Number of expanded leaves on the main axis.
3. Number of expanding leaves on the main axis.
4. Number of leaf primordia on the apex of the main axis.
5. Total number of leaf primordia ever produced on the main axis (including 2. to 4. above).

Table 4.6
Comparison in characteristics between Pax Øtofte at high and low N treatments two weeks after commencement of treatments

	High Nitrogen	Low Nitrogen
Mean tiller No.	$2.6 \pm a$	$1.2 \pm b$
Mean No. of expanded leaves	4.0 ± 0.0^{c}	4.0 ± 0.0^{c}
Mean No. of expanding leaves	4.1 ± 0.19^{d}	3.3 ± 0.16^{e}
Mean No. of leaf primordia	2.1 ± 0.10^{f}	2.1 ± 0.10^{f}
Mean No. of total leaves and primordia	10.2 ± 0.20^{g}	9.4 ± 0.17^{h}

Numbers in the same row without a common superscript are significantly different at 5% level.

The mean tiller number of the high nitrogen treatment was approximately twice that of the low nitrogen level, when the two treatments had been administered for two weeks. Expanded leaf number was not affected by nitrogen, the two treatments bearing the same number, viz. 4. On the other hand, expanding leaf number in the high nitrogen treatment was significantly greater than in the lower N level. Although the number of ridges (leaf primordia) was the same in both treatments, the total number of leaf primordia formed was greater in the high nitrogen treatment.

This suggests that more leaf primordia have been formed under the high nitrogen level which was applied for two weeks than in the low N level, but the increase in leaf primordia is cancelled out by the increase in leaf expansion so the number of ridges in the apex has not changed.

Experiment 4.3

The effect of nitrogen on the production and expansion of leaves in Van Thoffe

Seedlings at the third expanding leaf stage were planted in 2½" pots in vermiculite and were fed 4 weekly applications of 50 mls nutrient solution, the first being applied immediately after potting. As in the previous experiment two nitrogen levels were used. The plants were kept in the heated greenhouse under natural light conditions ie. daylengths were those of May and June. Four weeks after potting, the experiment was terminated and the following measurements were recorded.

a) Number of buds expanded at each node (19 plants per treatment).

b) No. of expanded leaves on main axis.

c) No. of expanding leaves on main axis.

d) No. of leaf primordia.

e) Total leaf primordia.

Table 4.7

No. of tiller buds expanded at each node (19 plants)

Node	1	2	3	4
H.N	13^{a}	19^{c}	19^{d}	14^{e}
L.N.	3^{b}	17^{c}	18^{d}	2^{f}

Figures within the same column without a common superscript are significantly different at 5% level.

Table 4.8

Characteristics of the main axis of Pax Olofte at high and low N treatments

	High N	Low N
Mean Expanded leaf No.	5.35 ± 0.10^{a}	5.0 ± 0^{b}
Mean Expanding leaf No.	3.53 ± 0.11^{c}	3.47 ± 0.11^{c}
Mean No. of ridges	3.63 ± 0.13^{d}	2.84 ± 0.13^{e}
Mean Total leaf primordia	12.52 ± 0.15^{f}	11.32 ± 0.15^{g}

(Figures within same row without a common superscript are significantly different at 5% level).

The high nitrogen treatment has given rise to a significantly greater number of tiller buds to have expanded at nodes 1 and 4. The difference in tiller buds to have expanded at nodes 2 and 3 between the two treatments is not significant.

Considering the leaf characters, there was a greater number of leaves which had expanded over the four week period under high nitrogen than in the low N treatment. All of the low nitrogen plants had an expanded leaf number of 5 whereas higher N level gave rise to a number of plants which bore 6 expanded leaves. This occurred despite the two batches of plants at the beginning of the experiment bearing two expanded leaves and being selected for uniformity.

The small difference in expanding leaf number was not significant, unlike the ridge number of the apices in which the high nitrogen treatment gave rise to a mean ridge number of 0.8 greater than the lower level. The higher expanded leaf number and ridge number was reflected in the significantly higher total leaf primordia number at the high N level which was 1.2 primordia greater than the lower level.

Experiment 4.4

The effect of nitrogen on the leaf expansion of tiller buds in Westerwolds

10 day old seedlings (at the second visible leaf stage) were planted in Baystrat at 2 nitrogen levels ie. 1N and 1/5N. 2 weeks later, the plants were removed from the Baystrat blocks and the following characters of buds were recorded at nodes 1 and 2.

a) Expanding leaf number

b) Leaf primordia (ridges).

c) Total leaf primordia (a and b).

Table 4.9

Mean number of leaves and leaf primordia of buds at nodes 1 and 2 of Westerwolds plants at high and low N levels

	Position of bud (node)	HN	1N
Mean number of expanding leaves	1	3.23 ± 0.11^{a}	2.88 ± 0.21^{a}
	2	2.53 ± 0.13^{f}	2.31 ± 0.18^{f}
Mean number of leaf primordia	1	2.71 ± 0.12^{b}	2.13 ± 0.15^{c}
	2	2.35 ± 0.13^{g}	1.88 ± 0.14^{h}
Mean total leaves and primordia	1	5.94 ± 0.19^{d}	5.00 ± 0.16^{e}
	2	4.89 ± 0.23^{i}	4.19 ± 0.17^{j}

Means within same row without common superscripts are significantly different at 5% level.

Expanding leaves are not significantly different in buds at both N levels in buds at nodes one and two. However, the high N treatment gives rise to a greater number of leaf primordia in the apex and as a consequence this influences the number of primordia which have been laid down in the life of the bud (total leaves plus leaf primordia). This is common to buds at both nodes.

Experiment 4.5

The effect of nitrogen on expansion and stage of development of axillary buds

Westerwold seed was sown on 20/6/72. On 2/7/72, the seedlings were planted in Paystrat blocks and grown under natural illumination in a heated glasshouse, under two nitrogen levels ie. 1N and 1/5N. The nutrient was changed once ie. on 24/7/72. On 9/8/72, those whose main axes were at or just past ear emergence were chosen from each nitrogen level and dissected. The stage of development of the first four primary tillers was recorded, and it was noted whether the tiller had developed beyond the prophyll (bud) stage. Nine plants were chosen from each nitrogen level. In order to increase the number of observations in each sample, the tillers at nodes 1 and 2 were treated together, as were those at nodes 3 and 4. Contingency tables were drawn up, the criterion for separation in each instance being mentioned in the title of each table.

The number of secondary and tertiary tillers were also recorded.

Table 4.10

Tillers at nodes 1 and 2 a) whether apex is rapidly elongating
b) whether tiller is still at bud stage

	High N	Low N	Probability
a) Apices not elongating	0	1	N.S
Apices elongating	18	17	
b) Bud stage	0	0	N.S
Beyond bud stage	18	18	

Table 4.11

Tillers at nodes 3 and 4, a) whether apex is rapidly elongating
b) whether tiller is still at bud stage

	High N	Low N	Probability
a) Apices not elongating	3	10	*
Apices elongating	15	8	
b) Bud stage	5	15	**
Beyond bud stage	13	3	

When the tillers at nodes 1 and 2 of the treatments are considered, all but one of the tillers had developed beyond the double ridge stage (Table 4.10). Other separations were attempted based on stage of development to discriminate between the two nitrogen levels, but none was significant, suggesting that the apices of tillers at nodes 1 and 2 at the two nitrogen levels were similarly distributed throughout the various stages. None of the tillers was still at the tiller bud stage.

At nodes 3 and 4, however, there were significant differences between the two nitrogen levels (Table 4.11). There was a significantly greater number of vegetative apices among the tillers of the low nitrogen treatment, and also the proportion of tillers still at the bud stage at that level of nitrogen was greater than at the high nitrogen level. Unfortunately, the sample was not large enough to determine whether there was a direct association between those tillers which were still at the bud stage and those which had vegetative apices.

The Mann-Witney non-parametric test was carried out to determine if the relative ranking of individuals in the two N treatments with respect to stage of the development of the apex at each node, was higher in one N level than the other. The results are presented in table 4.12.

The conclusions from this test are similar to what was found employing the χ^2 test, viz. the apices of buds and tillers at nodes 3 and 4 are more advanced at the high N level than at the low N level. However, this test allowed the buds at each node to be considered separately.

Table 4.12

Stage of development of the apex of each tiller or bud at nodes 1 to 4

Rank order	Node 1		Node 2		Node 3		Node 4	
	HN	LN	HN	LN	HN	LN	HN	LN
1	9.1	9.1	9.0	9.0	7.0	5.0	5.0	4.0
2	9.1	9.0	8.9	8.5	6.5	4.0	4.0	2.5
3	8.9	8.9	8.9	8.5	6.5	3.0	3.0	2.5
4	8.9	7.5	8.9	7.5	5.0	3.0	3.0	2.0
5	8.5	6.0	8.0	7.0	4.0	2.5	2.5	2.0
6	7.9	6.0	7.0	6.5	3.5	2.0	2.5	1.0
7	6.5	5.0	6.0	5.0	2.5	2.0	2.5	1.0
8	6.5	5.0	5.0	2.5	2.5	2.0	2.5	1.0
9	6.0	4.0	3.5	2.0	2.0	1.0	1.0	1.0
	T=70NS		T=76NS		T=61*		T=62.5*	T at 5%=63

Analysed by Mann-Witney non-parametric test.

So far, these experiments have involved transplanted grass seedlings, and that they have been under a period of standard conditons until the different nutrient solutions are applied. The following experiment was designed to study the effects of N on the tillering of Westerwolds from germination to post ear emergence in an attempt to determine if N not only affected tillering but also influenced the flowering of the plants.

Experiment 4.6.

The effect of nitrogen on the tillering pattern of Westerwolds up to and during flowering

Two seeds of Dutch Westerwolds were sown in each of 48 Jiffy pots on 15/12/71. 24 of the group received high nitrate solution, 10 mls per 8 days while the other 24 received the same amount of low N solution. On 26/1/72 the plants were planted in $3\frac{1}{2}$" pots containing vermiculite and the amount of nutrient solution was increased to 50 mls at each application. The plants were grown in a 16 hour day in the heated greenhouse. On 2/1/72 the seedlings were thinned to one seedling per Jiffy pot, and on 6/1/72, the first tiller count was taken. This was repeated every 4th day until 7/2/72, when there was an interval of 8 days, followed by another count on the 19/2/72, ie. after a further 4 days. The final count was taken on 27/2/72. Ten plants from each treatment were taken at random and the apices and tiller buds dissected.

The following characters were recorded at each tiller count.

1) Leaf number on main axis.
2) Primary and Secondary tiller number and leaf number of each.
3) Nodes from which each tiller arose.
4) Number of flowering tillers.

After 22/1/72, the nodes and leaf numbers were not recorded.

Leaf number on the main axis (Table 4.13).

By 3/2/72 the plants in the high nitrogen treatment had a significantly higher number of leaves than those of the low nitrogen treatment and persisted to the last tiller count on 19/2/72. From 3/2/72 the leaf number increased in both treatments at a slower rate than the period previous to that date. By that time, most of the plants were at, or beyond, ear emergence.

	Mean number of leaves on the axis		Mean number of total tillers		Mean number of primary tillers		Mean number of secondary tillers		Mean number of Flowering tillers (including main axis)	
	HN	LN	HN	LN	HN	LN	HN	LN	HN	LN
6/1/72	4.21±0.12	4.04±0.04	1.29±0.18	1.21±0.13	1.25±0.14	1.21±0.13	0.04±0.01	0	0	0
10/1/72	-	-	2.25±0.21	1.92±0.08	2.08±0.14	1.92±0.08	0.17±0.10	0	0	0
14/1/72	5.22±0.26	5.25±0.10	2.48±0.24	1.96±0.10 s	2.22±0.16	1.92±0.08	0.26±0.11	0.04±0.04	0	0
18/1/72	5.96±0.10	5.91±0.12	2.88±0.28	1.96±0.14 s	2.46±0.16	1.92±0.15	0.42±0.16	0.04±0.04 s	0	0
22/1/72	6.63±0.15	6.71±0.15	4.05±0.55	2.25±0.16 s	3.17±0.16	2.08±0.12 s	0.88±0.29	0.17±0.08 s	0	0.17±0.08 s
26/1/72	7.42±0.16	7.31±0.18	5.62±0.50	2.63±0.21 s	3.54±0.16	2.46±0.11 s	2.08±0.39	0.17±0.13 s	0.08±0.07	0.42±0.10 s
30/1/72	8.17±0.21	7.50±0.22	6.13±0.63	2.96±0.19 s	3.96±0.16	2.71±0.11 s	2.17±0.50	0.25±0.12 s	0.13±0.07	0.54±0.10 s
3/2/72	8.91±0.23	7.83±0.22 s	7.53±0.68	3.29±0.26 s	4.20±0.19	2.96±0.14 s	3.33±0.58	0.33±0.18 s	0.52±0.16	0.71±0.11
7/2/72	9.08±0.23	8.08±0.27 s	8.12±0.67	3.37±0.29 s	4.29±0.19	3.04±0.16 s	3.83±0.57	0.33±0.21 s	1.21±0.25	1.00±0.14
15/2/72	9.33±0.27	8.13±0.34 s	9.49±0.90	3.75±0.31 s	4.37±0.22	3.25±0.20 s	5.12±0.75	0.50±0.20 s	2.20±0.32	1.45±0.19 s
19/2/72	9.37±0.29	8.16±0.34 s	10.33±0.40	4.82±0.41 s	5.00±0.20	3.62±0.25 s	5.33±0.73	1.20±0.31 s	2.37±0.34	1.75±0.18

Table 4.13

Mean numbers of leaves and tillers at two N levels in Westerwolds under long days

(S denotes significant difference between high and low N treatments)

Mean number of tillers (Table 4.13)

At the last tiller count, the mean total tiller number of the high nitrogen treatment was greater than twice that of the low nitrogen treatment. When the components of the total tiller number were considered, the secondary tillers were seen to be more affected by the N levels than the primaries eg. on 19/2/72 the primary tiller number of the treatments was 5.00 and 3.62 at the high and low N levels respectively whereas the respective mean secondary tiller numbers were 5.33 and 1.20.

Mean number of flowering tillers (Table 4.13)

When the main axes were first seen to be at ear emergence, the low N level had a greater mean number of flowering tillers. This was due to a higher number of plants at this level having reached ear emergence than those at the higher N level. By 3/2/72, the difference was not significant. On 15/2/72 the high N had a greater number of flowering tillers. This presumably was due to the greater number of tillers per plant which had been formed earlier in the experiment.

Dissection of plants

On 4/2/72, 10 plants from each of the N levels were dissected. Due, however, to the variation in stage of development of the main axes six from each N level were chosen. All had their main axis at the stage where anthesis had taken place and the seed was beginning to fill out. It was considered that due to the large variation which existed even within those plants at the same stage, the 12 plants were considered together in most instances for the remainder of the study.

Number of primary tillers in relation to the number of leaves on the main axis (Table 4.14)

The mean number of leaves on the main axes was significantly

higher in the high N treatment. The mean number of primary tillers on the main axis was also greater in the high N levels.

Table 4.14

Leaf number and uppermost node bearing a tiller on the main axis of plants at the anthesis stage at two N levels

	HN	LN
Mean leaf No. on main axis	$9.33^{a} \pm 0.37$	$7.0^{b} \pm 0.46$
Mean uppermost node bearing a primary tiller	5.17 ± 0.57	3.83 ± 0.49

Means in the same row without a common superscript are significantly different at 5% level.

However, the difference between the leaf number and the uppermost node bearing a primary tiller ranged from 3 to 4. Therefore discounting the flag leaf, the node of which, when dissected did not appear to bear any buds, there were two or three nodes which bore tiller buds but did not expand despite these nodes having been in existence for a considerable length of time.

Relative proportion of vegetative apices in tillers and tiller buds

Due to the large variation among plants within the two N treatments, the two treatments were considered together. When the relative number of primary, secondary, tertiary and quaternary tillers and buds which were vegetative were considered, all of the primary tiller and buds were florally initiated. Among the secondary tillers, less than 10% of the buds and tillers were still vegetative. Considering the tertiary and quaternary tillers together (there were only four in the latter order), over 40% were still vegetative.

When the distribution of vegetative apices was considered in the twelve plants dissected, about 20% of the vegetative

tillers and buds were of the second order, the remainder being tertiary and quaternary.

Therefore the majority of tillers and buds which were still vegetative were found among the tillers of the lower orders. Although this may have been partly due to those tillers being younger, position may also have been influential in determining the relative fertility of the apices. The primary tillers which had not expanded ie. were still within their prophyll and had not expanded beyond the subtending leaf sheath were initiated whereas the tillers and buds of the lower orders, some beyond the bud stage were in many instances still vegetative. Therefore, time of appearance of a tiller did not seem to be the only factor determining the stage of the apex.

Table 4.15

Relationship between orders of tillering with respect to vegetative apices.

Order of tillering.	No. of vegetative apices within each order.	Mean percentage of apices within each node still vegetative.
Primary	0	0
Secondary	10	8.99 ± 2.97
Tertiary and Quaternary	38	43.58 ± 7.81

Discussion

Effect of nitrogen on tillering order. In both Pax Otofte and Westerwolds, (Expt. 4.1) the secondary tiller number was affected more by the nitrogen treatment than primary tiller number eg. at the last tiller count, the primary tillers at the high N treatment were less than twice that of the low N treated plant. However the number of secondary tillers at high N level was greater than 10 times the number at the low N level. This is similar to what was found in timothy (Langer, 1959b). On a longer term basis, (Expt. 4.6) the relative difference between high and low N treatments with respect to affect on primary and secondary tiller number persisted (Table 4.13).

Fewer primaries at the low N level supported secondary tillers. Most of the secondaries at the high N level were supported by tillers on the main axis at nodes 2 and 3. Table 4.3 shows that at node 2, there were primary tillers . . . . in most of the plants at both N levels in both species at the second count ie. day 16. In Pax Otofte this was also the case for tillers at node 3 at both N levels. Therefore by day 32, it would be expected that secondary tillers would be visible on those tillers. However it would appear that the low N treatments reduces the number of secondary tillers on the primary tiller at node 2, (Table 4.4) and node 3 in Pax Øtoftø. This would suggest that N has a direct affect on the production of secondary tillers.

As primary tillers are less affected by the N levels than secondaries with respect to numbers expanding, it is possible that they are in a preferential position for any available nutrients within the plant. This has been considered at another point in this study when concerned with tillering at the flowering stage. Work with C^{14} has shown that some tillering sites have a greater chance of receiving their quota of carbohydrate

than others when carbohydrate is limited (Ryle, 1971). This may also apply to the distribution of nitrogen. Alternatively the low N could be reducing the availability of carbohydrate due to reducing a) leaf size (Ryle, 1964) and b) the assimilation of carbohydrate per unit area of leaf (Ryle and Hesketh, 1969).

That the secondary tillers are subject to a lower nutritional plane may explain the lower contribution of secondaries to grain yield than primaries appearing at the same time (Langer, 1956; 1957; 1959; Rawson, 1971). Also, the lower primary tillers appear to be less affected than the uppermost primary tillers. Again this may be explained in terms of relative preferential position for nutrient, and has been shown that in Bromus mollis (Davis and Laude, 1964) and in wheat (Rawson, 1971) that the primary tillers at the higher nodes yield less than those at the lowermost positions.

Experiment 4.6 confirmed the effect of N on tillering order in Westerwolds, ie. the secondary tiller number was affected more by nitrogen than the primary tiller number. In this experiment, primary tillers production decreased near the end of the experiment, presumably due to the onset of flowering.

Effect of nitrogen on leaf expansion

That N increases the number of expanding leaves on the main axis (Expt. 4.2) agrees with the findings of Ryle (1964) for a number of perennial grasses. He found the number of expanding leaves in both high and low N treatments to be less than that found in this study. In a heated greenhouse, he found a mean of 1.13 activity growing leaves at high N and 1.08 under low N conditions, whereas the mean numbers of expanding leaves found in this experiment were 4.1 and 3.3 at high and low N respectively. However, Ryle did not clearly state the criterion he employed for distinguishing an expanding leaf from a leaf primordium. Therefore

what was considered a small expanding leaf in this experiment may have been considered by him to be a leaf primordium.

When plants were allowed to grow under the two N levels for 4 weeks (Expt. 4.3) the number of expanding leaves was not significantly different between the two N levels. However, the expanded leaf number was greater at the high N level, as was the number of ridges. This effect of N on the number of expanded leaves has also been found by Ryle (1964) in seven species of perennial grasses, Cooper, (1951) in ryegrass and by Bean (1961).

When allowed to grow beyond ear emergence, Westerwolds has a lower number of leaves on the main axis at the low N level after ear emergence is underway (Expt. 4.6, table 4.13). This is presumably due to a greater number of plants having flowered at the low N level earlier than those at the higher level of N.

There have also been reports that N has no effect on leaf expansion eg. Langer (1959), with timothy, Purvis (1934) with winter rye, and Davies (1972) with perennial ryegrass. The reasons for these differences are not obvious. Both Langer and Purvis employed N levels which differed widely, therefore it is unlikely that there were insufficient differences in N levels between treatments. Perhaps ryegrass responds in this way to varying N whereas timothy and rye do not.

Leaf primordia number on the main axis being affected by nitrogen agrees with the findings of Langer (1959) who found that high N could give rise to apical lengths of four times those at plants given one third the level of N. This increase in primordia number prior to floral initiation has been found in timothy to be reflected in the number of spikelets eventually formed (Langer, 1959 ; Ryle, 1963). In ryegrass, however, as well as cocksfoot and meadow fescue, the transition to the

flowering state does not reflect so markedly the effect of N on the primordia number prior to initiation (Ryle, 1964).

Effect of nitrogen on flowering

As already stated, in experiment 4.6, low nitrogen initially gave rise to a significantly greater number of flowering tillers than high nitrogen. This effect was not apparent in Expt. 3.2 in the field (Expt. 3.2) where, except for one instance when Pax Otofte had a higher proportion of flowering tillers at high N level than at the low N level, nitrogen level did not influence the proportion of tillers which were beyond ear emergence. Therefore there may be either a difference in response to nitrogen between L. perenne and L. multiflorum, or the difference in N levels in the field may not be as wide as that under controlled conditons. A similar effect of N on flowering has been observed in rye by Berrie (pers. comm.) under controlled environmental conditons. The reason for this is not apparent, and, in fact, the opposite effect is seen in some ax illary tillers as will be discussed under the heading "Effect of nitrogen on stage of development of buds".

Effect of nitrogen on leaf expansion of axillary tillers

In experiment 4.3, the low N level is seen to have a significantly lower number of tillers out of 19 plants in the treatment at nodes 1 and 4, than the high N treatment. This prompted a further study of the effects of N on tiller bud expansion, and the role of expanding leaves in bud expansion, thus experiment 4.5 was carried out. In this experiment, the buds at nodes one and two had a greater number of leaf primordia at the high nitrogen level than those under low N conditions. Little attention has been paid to tiller buds when environmental effects on tillering have been studied. As a consequence there does not seem

to the comparable studies on other grasses. These data show that the initial stages of tiller bud expansion are affected by the different N levels.

No significant difference was detected between the N levels with respect to number of expanding leaves. Nevertheless, this could have been due to insufficient time between the beginning of the N treatments and dissection for differences to be manifest. There were differences between the two N treatments but they were not significant. In experiment 4.5 a greater number of tillers are beyond the bud stage at nodes 3 and 4 at the high N level. This effect is also seen at the higher nodes of Pax Ötofte and Westerwolds at the high N level in experiment 4.1.

Therefore nitrogen influences the expansion of tiller buds on the main axis by increasing leaf primordia, and by increasing the rate of leaf expansion so possibly increasing the number of expanding leaves.

Effect of nitrogen on stage of development of buds

That lack of nitrogen has a retarding effect on the development of the apex of tillers at node 3 and 4 in experiment 4.6 would suggest that mineral nutrition has an effect on morphogenesis of apices. There have been instances where nitrogen has been depleted to the extent where the apex on the main axis has been prevented from becoming florally initiated. Cooper and Calder (1961) demonstrated this with cocksfoot. Ryle (1964) also quotes instances of where nutrient can be sufficiently low to depress flowering in the main axis of ryegrass. This however, has already been discussed in the section dealing with the experiments carried out in the field (Expt. 3.2).

Concerning the effect of nitrogen on the fertility of tillers, it has been shown in the previous section that Pax Ötofte had a significantly higher percentage of tillers at the ear emergence stage when at high N relative to those plants at the low N level.

This has also been found in timothy (Langer, 1959).

In those instances quoted, only tillers which were visible have been considered. However in this experiment, buds as well as expanding tillers were taken into account. This gives a more valid comparison between N levels as it takes into account the position and order of the tillers under study whether they be tillers or buds. Unfortunately, there were too few tiller buds to determine if there was a relationship between plants with buds and buds with apices still at the vegetative stage. Therefore it is not possible to conclude whether the lack of nitrogen was indirectly influencing the fertility of the tillers by preventing expansion or whether lack of nutrition directly delayed the morphogenetic change of the apices. As the fertility of visible tillers is increased by the addition of nitrogen, as was found in Pax Øtofte in experiment 3.1, it is likely that N has a direct effect, although delay in expansion may also be contributory.

Relative fertility of primary, secondary and tertiary tillers and buds

Experiment 4.6 demonstrates that the higher the order of tillering, the greater is the percentage fertility in Westerwolds. This is found when buds and tillers are considered. In timothy (Langer, 1959; Lambert and Jewiss, 1970) and meadow fescue (Lambert and Jewiss loc.cit.) primary tillers are fertile before secondaries, which in turn are fertile before tertiaries. Langer and Ryle (1959) found that in S48 timothy 85.2% of primary tillers formed before 23rd July flowered whereas only 44.5% of secondaries formed before this time were eventually fertile.

Presumably, due to Westerwolds being an annual and so the apices of the tillers and buds are initiated rapidly (Hämmer, 1961)

the percentage fertility of the various orders would be expected to be high. This is found to be the case as the fertility of the plants chosen for dissection is:-

primaries, 100%, secondaries 91.0% and tertiary and quaternaries 56.42%

It would seem that under the nitrogen regimes employed in experiments in this section, not only is leaf appearance affected by nitrogen level, but so also are morphological changes of apices of tillers. There seems to be a contradiction in effect of nitrogen between the apices of the main stem and apices of the tillers and buds. High N levels delay the appearance of flowers on plants initially (Expt. 4.6). However after initiation, apices of tillers at given positions on the main axis are more advanced at high than at low nitrogen levels. (Expt. 4.5).

As a result of the apparent reduction in number of tillers appearing at flowering in Lolium perenne (Expt. 3.2) and in Westerwolds (Expt. 4.6), it was considered that apical dominance may be a factor implicated in the control of tillering in grasses. This has been discussed in the "Literature Review" pages 18-26. The following section comprises a series of experiments designed to demonstrate whether apical dominance exists in grasses.

SECTION 5. STUDY OF THE ROLE OF THE APICAL REGION OF A GRASS PLANT ON TILLER DEVELOPMENT.

RESULTS AND DISCUSSION

Experiment 5.1

Effects of expanding leaves on tiller bud enlargement

Removal of expanding leaves

Seedlings of Pax Øtofte and S23 at the third visible leaf stage had their expanding leaves removed ie. the third and fourth leaves of each plant. Ten seedlings of each strain were subjected to this treatment, another ten remaining intact. The 40 seedlings were planted in Baystrat and one week later, the experiment was terminated.

In order to count the tiller buds, the leaf sheaths were pulled back at nodes 1 and 2. The tiller buds greater than 1 mm were counted, and the results are presented in Table 5.1. The effect of removing expanding leaves is demonstrated in Figure 5.1.

Table 5.1

Mean number of expanding buds per plant at nodes 1 and 2

(Expanding bud = bud > 1 mm)

	Intact	Defoliated
P.0	0.3^{a}	1.6^{b}
S23	0.1^{a}	0.9^{d}

Means within the same row without a common superscript are significantly different at 5% level.

Removal of the expanding leaves in both S23 and Pax Øtofte results in a significant increase in the number of tiller buds per plant greater than 1 mm.

This experiment was repeated with L. temulentum. Seedlings at the third visible leaf stage were used. 14 had their expanding leaves removed, while 14 remained intact. The seedlings were planted in Paystrat and one week later the experiment was terminated.

In this experiment buds greater than one mm at nodes 1 and

2 were recorded. Those buds which had reached the stage where their first leaf was appearing above the tip of the prophyll were also counted.

Table 5.2

Effects on buds of removing expanding leaves on main axis

Data from 14 plants per treatment

	Intact		Defoliated	
	Node 1	Node 2	Node 1	Node 2
Expanding tiller buds within prophyll	5^a	$-^a$	2^a	$-^a$
Expanding buds ; 1st leaf thro' prophyll	1^b	1^b	12^c	7^b
Total tiller buds > 1mm	6^d	1^d	14^e	7^d
Mean No. of buds per plant	0.5 ± 0.17^f		1.5 ± 0.21^g	

Numbers in the same row without a common superscript are significantly different at 5% level.

Although there is no significant difference between the two treatments in number of expanding buds within their prophyll, there is a significantly greater number in the defoliated treatment which have expanded beyond their prophyll. The total and mean expanding tiller bud number are also greater in the defoliated treatment.

The mean tiller bud number when compared to the results in the previous experiment follow a similar trend. Therefore the effect of removing the expanding leaves is similar in two cultivars of L.perenne and in L.temulentum.

So far, only buds greater than 1 mm have been considered. The following experiment was carried out to find the effects of removal of the young expanding leaves on the expansion of all tiller buds at nodes 1 and 2. As well as Pax Ø tofte, Westerwolds was also used in this experiment.

The seedlings which were at the third visible leaf stage were treated as in the previous experiments, ten plants of each species being defoliated, and ten remaining intact. They were planted in Baystrat and after seven days, the length of each bud was measured in the 40 plants at nodes 1 and 2.

Table 5.3

Mean prophyll lengths (mm) of buds on plants intact and plants with expanding leaves removed

	Intact		Defoliated	
	Arithmetic mean	Trans mean	Arith mean	Trans mean
West. Node 1	0.83	0.256 ± 0.024^{a}	4.34	0.665 ± 0.094^{b}
West. Node 2	0.95	0.280 ± 0.035^{a}	5.74	0.700 ± 0.122^{b}
P.O. Node 1	0.39	0.159 ± 0.012^{c}	2.84	0.421 ± 0.128^{ab}
P.O. Node 2	0.43	0.152 ± 0.014^{c}	0.76	0.242 ± 0.023^{a}

Transformed means without a common superscript are significantly different at 5% level.

Removal of expanding leaves in Westerwolds has a similar effect on expansion of tiller buds at nodes 1 and 2 as in Pax Øtofte. Although the tiller buds of Westerwolds in the defoliated treatment are larger than Pax Øtofte buds, the buds of the intact plants of Westerwolds are also bigger. Therefore for node 1, there is approximately the same proportionate increase in tiller bud size. For node 2 buds however, the Westerwold plants have responded better than Pax Øtofte buds to defoliation.

Therefore the same general pattern of response by tiller buds when the expanding leaves are removed is demonstrated in two species of ryegrass. The degree of variation in response, however, seems to differ between the species.

No account has been taken of the effect of time on the expansion of tiller buds in intact or defoliated plants. The following experiment was designed to determine the rate of expansion of tiller buds in defoliated plants relative to that of intact plants.

Experiment 5.2

Effect of time on tiller bud growth

Pax Øtofte seedlings were used, just as in previous experiments. 24 had their expanding leaves removed, and 24 remained intact. 8 seedlings were dissected at this stage and the prophyll length of the bud at node 1 was measured in each plant. The 48 seedlings were planted in Baystrat and at four day intervals, 8 plants were removed for each treatment. The prophyll of buds at node 1 was measured and the number of buds which had expanded beyond their prophyll was counted.

Table 5.4.

Prophyll lengths (mm) in intact plants and plants with expanding leaves removed

	Intact		Defoliated	
Days	Arith mean	Trans mean	Arith mean	Trans mean
0	0.26	0.1006 ± 0.0088	0.26	0.1006 ± 0.0088
4	0.38	0.1373 ± 0.0146^{a}	0.68	0.2070 ± 0.0133^{b}
8	0.53	0.1791 ± 0.0206^{c}	2.94	0.5449 ± 0.0845^{d}
12	0.55	0.1890 ± 0.0127^{e}	5.39	0.6921 ± 0.1323^{f}

Means within the same row without a common superscript are significantly different at 5% level

Fig. 5.1 Mean prophyll length of buds of Pax Øtofte plants at node 1 when plants are intact (-⊙-) or expanding leaves are removed (-▣-) at third leaf stage, at intervals up to 12 days subsequent to commencement of treatments.

FIG. 5.1

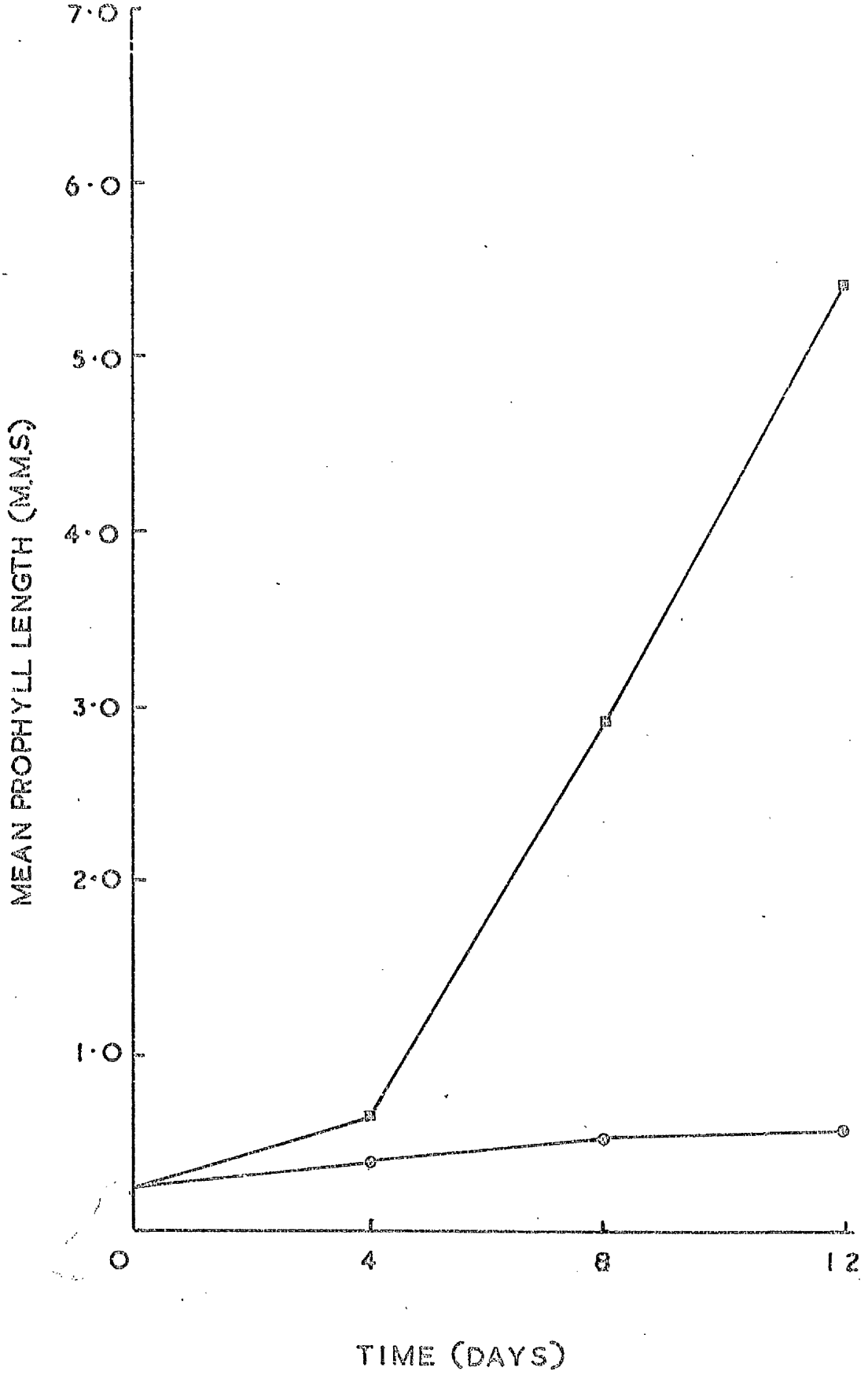


Table 5.5

No. of plants (out of 8) which had buds at node 1 with 1st leaf above prophyll tip

Days	Intact	Defoliated
0	0	0
4	0	0
8	0	3
12	0^{a}	5^{b}

Plant numbers within the same row without a common superscript are significantly different at 5% level.

Four days after the removal of the expanding leaves, the tiller buds were significantly larger than in the controls, (Table 5.4; Fig. 5.1). This difference increased with time, and 12 days after the commencement of the treatments, the defoliated plants had a prophyll length on buds at node 1 of greater than 9 times the length of the corresponding prophyll on the control plants.

The number of buds which had expanded to the extent where their first leaf was appearing above the tip of the prophyll is recorded in Table 5.5. 8 days after the start of the treatments, the defoliated plants have a few at this stage and by the 12th day, the number of buds at this stage is significant. None of the control buds had reached this stage by day 12.

Experiment 5.3a

Effects of the light passing through the incision on axillary buds

Seedlings of Westerwolds which were at the fourth visible leaf stage were chosen. 75 were selected and the following treatments were imposed on the plants ie. 15 plants per treatment.

1) Intact controls
2) Intact, but with a tightly fitting cylinder of aluminium foil about 6 mms high surrounding the base of the leaf sheaths.
3) Incised. An incision made in the sheaths about 4 mms long extending from the lowest internode in a longitudinal direction.
4) Defoliated. An incision made as in 3) and the young expanding leaves removed ie. leaves 4 and 5, occasionally a little part of 6.
5) Defoliated. With a cylinder of foil as in 2) which covers the incision and preventing most of the incident light from reaching the tiller buds.

The seedlings were planted in Baystrat. 8 days later the plants were dissected and the expansion of buds at nodes 1, 2 and 3 was measured by measuring the prophyll lengths.

Table 5.6

Mean prophyll length (arithmetic and transformed (mm))

	Node 1		Node 2		Node 3	
Treatments	Arith mean	Trans mean	Arith mean	Trans mean	Arith mean	Trans mean
1 Intact	2.28	0.4430 ± 0.0673^{a}	2.59	0.4341 ± 0.1051^{dc}	1.83	0.3214 ± 0.0983^{e}
2 Intact (covered)	1.10	0.3018 ± 0.0467^{a}	1.33	0.3169 ± 0.0446^{c}	0.69	0.2232 ± 0.0183^{e}
3 Incised	4.70	0.6817 ± 0.0818^{b}	3.23	0.5146 ± 0.1120^{cd}	0.86	0.2570 ± 0.0375^{e}
4.Defoliated	2.71	0.5278 ± 0.0526^{ab}	4.51	0.6216 ± 0.0664^{d}	0.92	0.3405 ± 0.0554^{f}
5 Defoliated (Covered)	2.84	0.5399 ± 0.0600^{ab}	4.95	0.6253 ± 0.1035^{d}	3.30	0.4454 ± 0.1060^{f}

Means without a common superscript are significantly different at 5% level.

The means of the prophyll lengths of the various treatments of nodes 1, 2 and 3, (Table 5.6) are presented along with appropriate controls, ie. the covered treatments may be compared with the covered control (2)and the non covered with the non covered control (1). While the intact plants which were covered with foil (2)have mean prophyll lengths less.than the appropriate control (1)the values do not differ significantly. It can be concluded that covering does not effect bud expansion but because of the consistently lower value of the covered material it might be concluded that this treatment should not be thought of as being without any effect.

The incised plants (3)have prophylls which are larger than the controls at nodes 1 and 2. The defoliated (covered) treatment(5) has significantly longer prophylls at node 1 than in the intact covered. Defoliation does not give rise to any significant difference in prophyll length when compared to the intact plants but were consistently longer than the intact treatment.

As the defoliated plants did not respond in the same way as the defoliated (covered) treatment, the experiment was repeated in order to determine if the responses obtained in this experiment were reproducible.

Experiment 5.3b

The experiment was carried out as before except it was decided to change the rooting medium. It was considered that the algal growth on the Baystrat may have been detrimental to some of the treatments. Therefore vermiculite in Jiffy pots was used. The treatments were the same as in the previous experiment, 10 plants this time being subjected to each treatment. The experiment was terminated after 8 days. The plants were dissected and the prophyll of buds at nodes 1, 2 and 3 were measured.

Table 5.7

Arithmetic and transformed means of prophyll length (mm)

	Node 1		Node 2		Node 3	
Treatment	Arith mean	Trans mean	Arith mean	Trans mean	Arith mean	Trans mean
1.Intact	0.69	$.2217 \pm .239^{a}$	1.69	$.3277 \pm 0.0982^{c}$	2.07	$.3377 \pm .1036^{e}$
2 Intact (covered)	).63	$.2106 \pm .0125^{a}$	0.68	$.2234 \pm 0.0136^{c}$	0.64	$.2102 \pm .0260^{e}$
3 Incised	5.52	$.7281 \pm .0902^{b}$	9.45	$.8914 \pm 0.1189^{d}$	7.49	$.7648 \pm 1326^{f}$
4 Defoliated	3.22	$.5985 \pm .0521^{b}$	4.22	$.6476 \pm 0.0792^{d}$	2.61	$.5083 \pm .0697^{fe}$
5 Defoliated (covered)	2.26	$.4769 \pm .0635^{b}$	4.84	$.6749 \pm 0.1014^{d}$	2.28	$.4591 \pm 0.0736^{fe}$

Means within the same column without a common superscript are significantly different at 5% level in tables 5.6, 5.7 and 5.8.

Table 5.8

Number of tiller buds (out of 10) which have expanded beyond the prophyll

	Node 1	Node 2	Node 3
Intact	0^{a}	0^{c}	0^{e}
Intact (covered)	0^{a}	0^{c}	0^{e}
Incised	5^{b}	7^{d}	5^{f}
Defoliated	4^{ab}	3^{cd}	1^{fe}
Defoliated (covered)	3^{ba}	6^{d}	1^{fe}

Numbers (within the same column) without a common superscript are significantly different at 5% level.

In both experiments, the prophyll lengths of the covered control plants are not significantly different from the intact controls. However as in the previous experiment the consistently lower value of the covered prophylls suggest that covering may be having an effect on prophyll length. In the second experiment defoliated plants have larger prophylls than controls at nodes 1 and 2. The difference between the prophylls of defoliated covered and the covered intact plants is marked in both experiments, the former having the larger prophylls. In Table 5.6, the difference is significant at the first two nodes and in Table 5.8, the prophylls of buds at all 3 nodes are longer in the defoliated covered treatment.

The incised treatment also has an effect in both experiments. Although in the first experiment this treatment has larger prophylls than control at node 1 in the second experiment, the effect is positive at all 3 nodes.

When the number of buds which have expanded beyond the prophyll are considered in the second experiment (Table 5.8)

incised is the most effective treatment as the number of buds which have expanded beyond this stage is significant at all of the 3 nodes under consideration. The defoliated treatment has an effect at node 1 and the defoliated covered, an effect at node 2.

Experiment 5.4

The wounding effect of incision on the expansion of axillary buds

Seedlings of L. multiflorum in which the second leaf was almost fully expanded were selected and 10 were subjected to each of the following treatments.

1) Intact control.

2) Defoliated - young leaves removed.

3) Incision made on the leaf sheath of leaf number one but expanding leaves left intact.

The seedlings were planted in vermiculite after the treatments were administered and the experiment was terminated 8 days later. The prophyll of the tiller bud at node 1 was measured. The first leaf of each bud was also measured i.e. the distance from the node of the leaf to its tip. The node was easily seen under the dissecting microscope after the prophyll had been removed.

Table 5.9

Lengths of prophyll and first leaf of buds at node 1 (mm) (arithmetic and transformed means)

	Prophyll		1st leaf	
	Arith mean	Trans mean	Arith mean	Trans mean
Control	0.81^{a}	$0.285 \pm .013$	0.57^{c}	0.1950 ± 0.009
Defoliated	1.78^{b}	$0.41 \pm .055$	1.14^{d}	0.2898 ± 0.038
Incised	0.71^{a}	$0.208 \pm .021$	0.43^{c}	0.1531 ± 0.015

Means without a common superscript in the same column are significantly different at 5% level

The mean prophyll length of the defoliated treatment is significantly greater than the mean prophyll length of the buds of either the intact or incised plants (Table 5.9). The incised

plants, however, are not significantly different from the intact.

When the first leaf of the buds is considered, the same pattern is seen, the defoliated plants having buds with longer first leaves than either the intact or incised treatments and the incised and intact treatments being similar to each other. This is contrary to the response due to incision found in previous experiments. This will be considered in the discussion.

Experiment 5.5

Removal of apices and expanding leaves in L. multiflorum (Westerwolds)

Seeds of L. multiflorum (Westerwolds) were sown on 14/4/72, under a 16 hour day. 3 weeks later, the seedlings were planted in 3½ inch pots in vermiculite and transferred to a growth cabinet at 20°C and a daylength of 16 hours. The plants throughout the course of the experiment were watered with Hoaglands solution.

One week after the plants had been placed in the growth cabinet, when they had five visible leaves, the plants were removed from their pots ensuring minimal disturbance of roots. 30 had their apices removed (including the expanding leaves immediately surrounding the apex), and another 30 remained intact. (The apices ranged from vegetative to beyond the double ridge stage). The plants were repotted and returned to the growth cabinet. At this stage none of the plants had tillers visible above the subtending leaf sheaths. Tiller counts were taken 5 and 10 days after decapitation. By the tenth day, a few of the intact plants had reached ear emergence stage.

Table 5.10

Mean tiller number per plant

Days	Decapitated	Intact
0	0	0
5	1.2 ± 0.11^{a}	0.1 ± 0.08^{b}
10	1.5 ± 0.09^{c}	0.5 ± 0.18^{d}

Means in the same row without a common superscript are significantly different at 5% level.

Within 5 days of removing the apical region of the stems of Westerwolds, the number of tillers on the decapitated plants was significantly greater than in the intact plants, and this trend still persisted by the tenth (Table 5.10). By this time a few of the intact plants were at the stage where the distal spikelets on the inflorescence of the main axis were appearing above the leaf sheath of the flag leaf. A few of the intact plants by this time also had started to tiller.

This experiment involved both vegetative and initiated apices, but irrespective of physiological stage, by day 5 all of the decapitated plants had tillers which emerged above the subtending leaf sheath.

An experiment was designed to study the effect of removal of the developing inflorescence on the expansion of tillers in Lolium. So that all of the plants would be at the same physiological stage when the treatments commenced, the inbreeding L. temulentum species was employed.

Experiment 5.6

Removal of the developing inflorescence and tiller expansion

38 day old L. temulentum plants were used in this experiment. They were at the fifth visible leaf stage and had been under continuous light in a growth cabinet at 25°C. The apices were just prior to ear emergence stage, and were removed in 10 of the plants. Another 10 were left intact.

Subsequent to the removal of the apices, both sets of plants were planted in $3\frac{1}{2}$" pots in perlite and were returned to the growth cabinet. They were fed 50 mls. nutrient solution every 3 days and watered regularly.

Five tiller counts were taken viz. at day 0, 5, 10, 15 and 20. At the second count, ie. 5 days after decapitation, the intact plants were at ear emergence, and by the 15th day, anthesis had occurred.

Table 5.11

Mean tiller number per plant

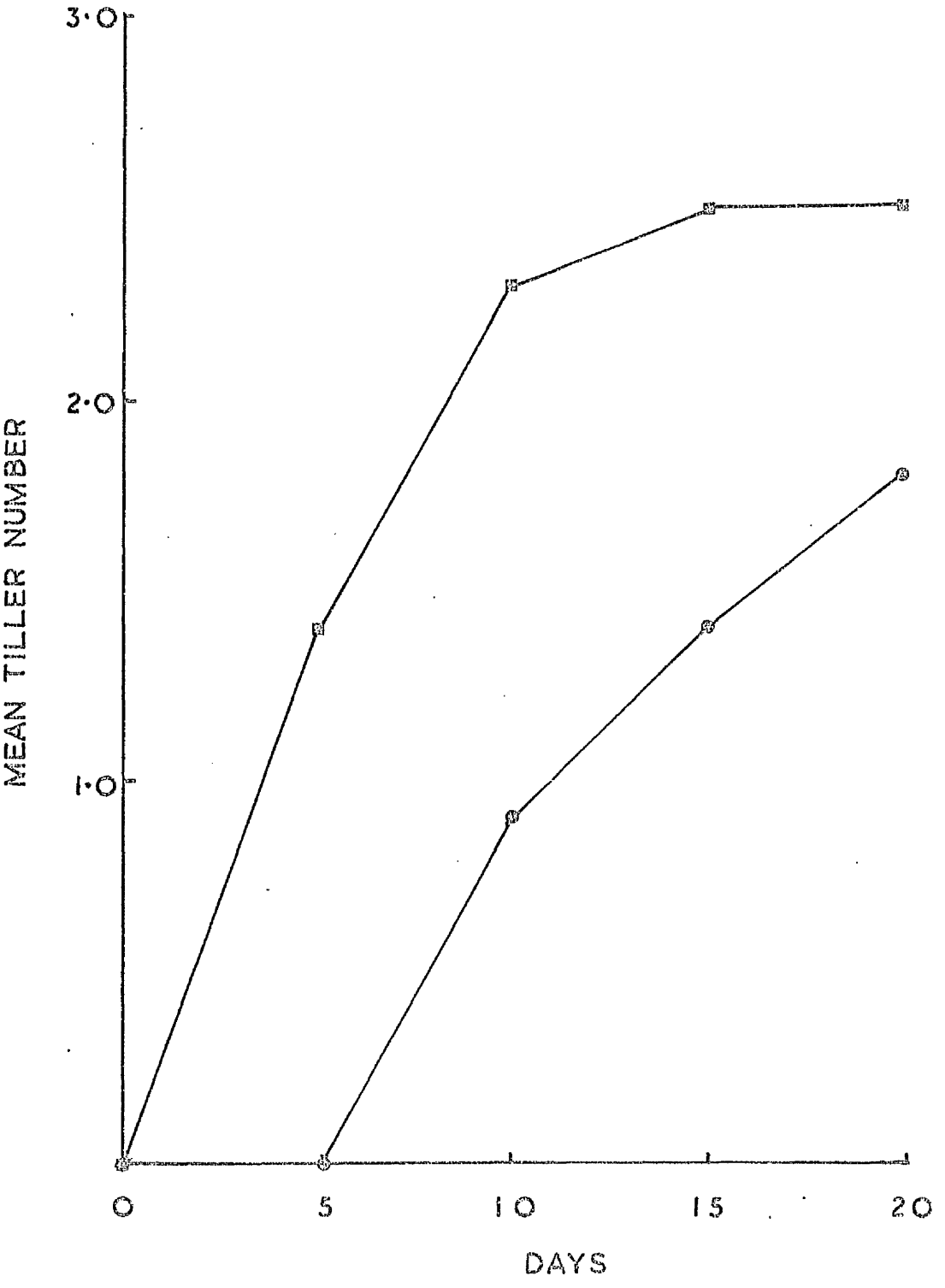
Days	Decapitation	Intact
0	0	0
5	1.4 ± 0.32^{a}	0^{b}
10	2.3 ± 0.26^{c}	0.9 ± 0.36^{d}
15	2.5 ± 0.22^{e}	1.4 ± 0.42^{f}
20	2.5 ± 0.22^{g}	1.8 ± 0.44^{g}

Means within the same row without a common superscript are significantly different at 5% level.

Decapitation ie. removal of the developing inflorescence had a significant effect on tiller production until day 20 (Table 5.11; Fig. 5.2). Due to rapid increase in tiller number in the intact treatment, by day 20 the difference between the

Fig. 5.2 Mean tiller number of *Lolium temulentum* plants at intervals after commencement of treatments either intact (●) or with developing inflorescence removed (▣).

FIG. 5.2



two treatments was not significant. By this time the seed was beginning to fill out on the heads of the main axes of the intact plants.

Therefore although removal of the initiated apices prior to ear emergence accelerated tiller production, the effect was not permanent. In fact very little effect was seen on tiller numbers between day 10 and 20 in the decapitated plants ie. 2.3 tillers per plant at day 10 and 2.5 at day 20.

In order to determine what effects decapitation was having on the tiller buds prior to appearance above the subtending leaf sheath, and to what extent the position of the bud on the main axis influenced the effect, the following experiment was carried out.

Experiment 5.7

The effect of removing the apex on the elongation of tiller buds in L. te mulentum

Seedlings of L. te mulentum, which had been growing in the heated greenhouse under a daylength of 16 hours were selected when they were 15 days old. They were at the stage where they had two visible leaves. They were potted in vermiculite and after 4 weeks, when they were at the fifth visible leaf stage 48 plants were subjected to one of the following three treatments (16 plants per treatment).

1) Apices were removed by surgery. They were at the stage where the stamen primordia were beginning to expand.
2) An incision was made through the leaf sheaths as in 1) but the apex was left intact.
3) The plants were left intact.

The plants were repotted, and returned to their former conditons. 10 days later the experiment was terminated, and the prophyll lengths of buds at nodes 1, 2, 3 and 4 were measured. The number of buds which had expanded beyond their prophyll was also recorded in each treatment.

Table 5.12

Mean prophyll length of tiller buds at nodes 1 to 4 (in mms) Arithmetic mean

Node	Control	Incised	Decapitated
1	0.67	2,20	3.50
2	0.83	8.49	10.32
3	1.23	15.26	17.94
4	4.13	17.20	15.97

Table 5.13

Mean prophyll length of tiller buds at nodes 1 to 4 (Transformed data)

Node	Control	Incised	Decapitated
1	0.197 ± 0.021^{a}	0.373 ± 0.072^{b}	0.514 ± 0.085^{b}
2	0.260 ± 0.023^{a}	0.859 ± 0.111^{c}	1.031 ± 0.034^{c}
3	0.335 ± 0.026^{b}	1.166 ± 0.056^{d}	1.246 ± 0.044^{d}
4	0.496 ± 0.048^{b}	1.157 ± 0.094^{d}	1.173 ± 0.095^{d}

Table 5.14

No. of buds at each node, out of 16 per treatment beyond the prophyll stage

Node	Control	Incised	Decapitated
1	0^{a}	3^{a}	5^{a}
2	0^{a}	11^{c}	14^{c}
3	0^{a}	13^{c}	14^{c}
4	1^{a}	14^{c}	13^{c}

Means without a common superscript are significantly different at 5% level in tables 5.13 and 5.14.

The prophyll lengths of the buds at the first four nodes in the three treatments 10 days after the plants had been decapitated are presented in table 5.12. The transformed means are in table 5.13, with standard errors of the means.

At all four nodes the incised and decapitated plants had significantly longer prophylls than the intact plants. In all of the treatments, the mean prophyll length at node one was the smallest. The prophyll lengths were progressively longer up to node three in the decapitated and incised treatments. Node 4 had a prophyll of similar length to node 3. In the intact plants however, the mean prophyll at node 4 was significantly longer than that of the bud at node 3.

The incision treatment was similar to the decapitated plants in response. In that treatment, the developing inflorescence had died in most instances, ie. it was brown and shrivelled when the experiment was terminated. Two were still living and in those plants, the tiller buds were similar in size to the intact plants. This would suggest that the incised plants are behaving as though decapitated due to to the death of the apex.

The number of buds, out of sixteen, which had expanded beyond the prophyll at each node is similar in the incised and decapitated treatments. At each node they have a significantly greater number of expanding buds than the intact treatment.

Although only a few buds at node 1 in the incised and decapitated treatments had expanded beyond the prophyll stage, the majority of buds at the other three nodes in both treatments had a leaf appearing above the prophyll stage in the intact treatment.

Experiment 5.8

The effect of removing the initiated apex of the main axis in L. multiflorum (Westerwolds) on tillering under two nitrogen levels

24 day old seedlings of Westerwolds kept in a heated greenhouse at natural daylengths, at the third or fourth visible leaf stage were selected for leaf sheath elongation. This was considered to be the first sign of the plants' approaching the flowering stage. 40 were planted each in 2½" pots in vermiculite, prior to which 20 of the plants had their apices removed. The apices were at the double ridge stage, or were at the stage immediately prior where a greater number of primordia were being laid down in comparison to pure vegetative. 10 of the intact plants were subjected to the low nitrate level as were 10 of the decapitated plants. The other ten of each treatment were given the high nitrogen solution.

The solutions were applied at the rate of 50 mls. per week in one application. Although the plants were watered every day, care was taken that the water did not leak through the vermiculite by ensuring as little water as possible drained completely through the rooting medium.

Tillers were counted at intervals, and at the end of the experiment the plants were dissected to determine the true position of each tiller on the plant.

Fig. 5.4 demonstrates the difference in tillering between the high N decapitated treatment and the low N intact treatment, 12 days after commencement of the treatments.

The progress in tillering is represented graphically in Fig. 5.3.

Fig. 5.3 Mean tiller number of Westerwolds plants at intervals after commencement of treatments at two N levels with initiated apex removed or plants left intact.

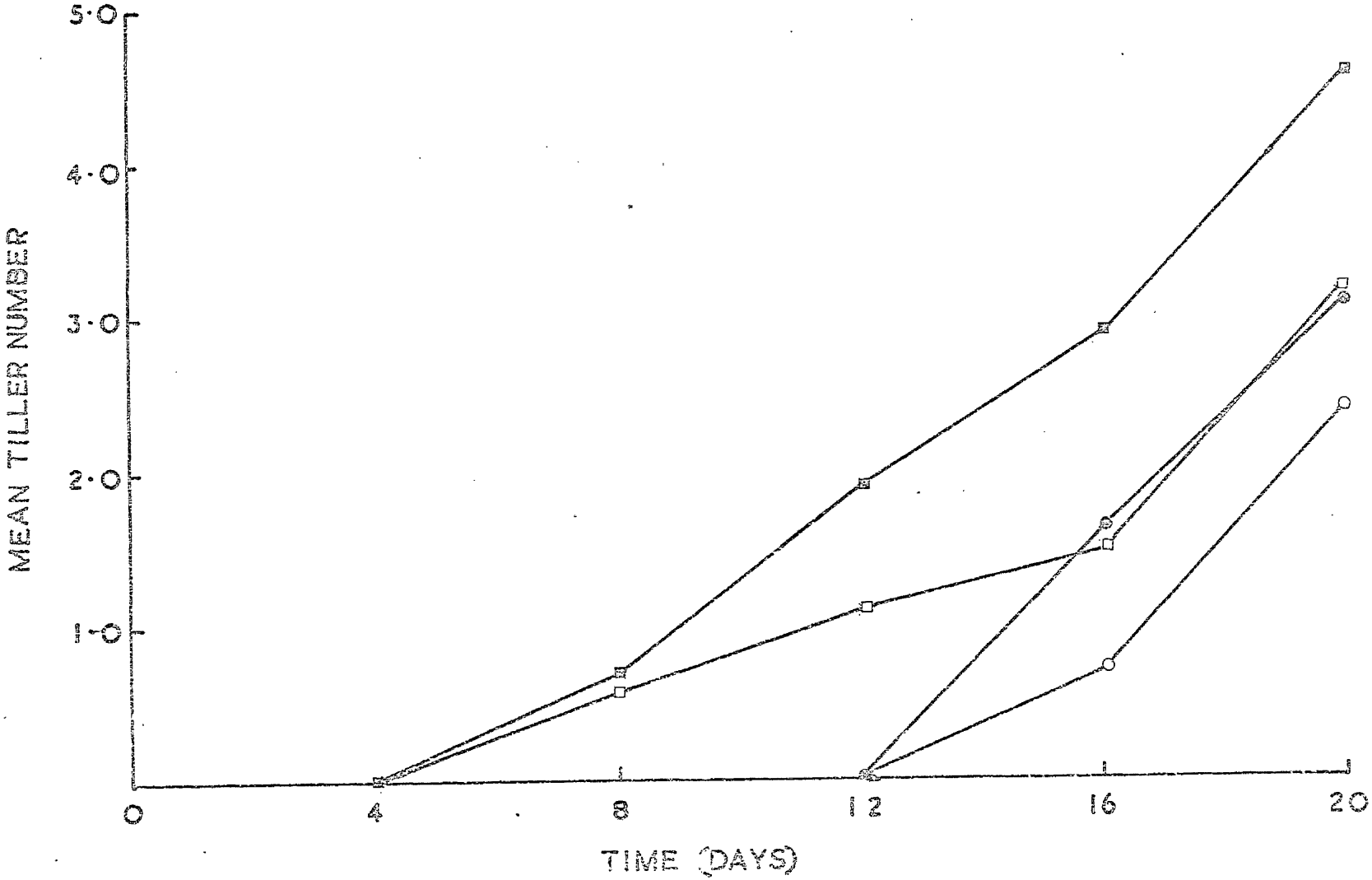
- ⊡ Decapitated
- ⊛ Intact

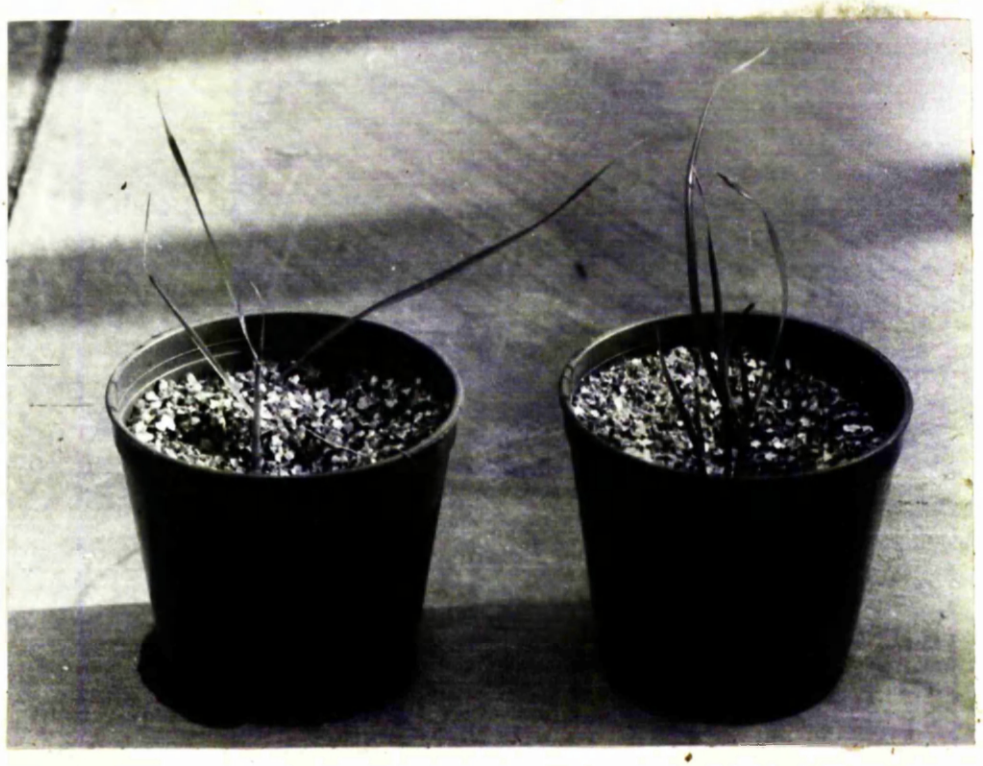
} High N (50 mls HN solution per week)

- □ Decapitated
- ○ Intact

} Low N (50 mls LN solution per week)

FIG. 5.3





Low N
Intact

High N
Decapitated

Fig. 5.4. Typical effects of decapitation and high N application on Westerwolds (right hand side) in experiment 5.8 12 days after decapitation compared to intact low N treatment.

Table 5.15

Mean tiller number at intervals after decapitation

	High Nitrogen		Low Nitrogen	
Days	Decap	Intact	Decap	Intact
0	0	0	0	0
4	0	0	0	0
8	0.7 ± 0.21^{a}	0^{b}	0.6 ± 0.22^{a}	0^{b}
12	1.9 ± 0.18^{c}	0^{d}	1.1 ± 0.18^{e}	0^{d}
16	2.9 ± 0.35^{f}	1.3 ± 0.33^{hg}	1.5 ± 0.27^{g}	0.7 ± 0.21^{h}
20	4.6 ± 0.43^{i}	3.1 ± 0.35^{j}	3.2 ± 0.78^{ij}	2.4 ± 0.64^{j}

Means in the same row without a common superscript are significantly different at 5% level.

Table 5.16

Mean number of primary and secondary tillers 20 days after decapitation

	High Nitrogen		Low Nitrogen	
	Decap	Intact	Decap	Intact
Primary tillers	$2.1^{a} \pm 0.18$	$2.7^{a} \pm 0.34$	$1.8^{a} \pm 0.33$	$2.2^{a} \pm 0.36$
Secondary tiller	$2.5^{b} \pm 0.31$	$0.4^{c} \pm 0.16$	$1.4^{d} \pm 0.37$	0.2 ± 0.13^{c}

Means within the same row without a common superscript are significantly different at 5% level

Table 5.17

Number of primary tillers at nodes 1 and 2 which have a visible leaf number of 3 and over

	Decapitated	Intact
High N	15^{a}	3^{b}
Low N	8^{bc}	3^{b}

Numbers without a common superscript are significantly different at 5% level.

The mean tiller number of the decapitated plants was significantly greater than in the intact plants within 8 days of the apices being removed in both nitrogen treatments (Table 5.15). Tillering did not commence in the intact plants until after the 12th day.

By the 12th day, the high nitrogen plants which had been decapitated had a significantly greater number of tillers than the low nitrogen decapitated plants. However, by the 20th day, the high nitrogen intact, the low nitrogen intact and the low nitrogen decapitated treatments had similar tiller numbers. The high nitrogen decapitated treatment had a significantly larger tiller number than the intact plants at both nitrogen levels.

At day 20, the decapitated treatments at both nitrogen levels had a greater number of secondary tillers than the corresponding intact treatments (Table 5.16). The high nitrogen decapitated treatment also had a higher secondary tiller number than the low nitrogen intact plants. All treatments had a similar number of primary tillers.

This increase in secondary tillers could have been due to the direct effect of removal of the apices or possibly due to the removal of the apices inducing early expansion of primary tillers and so indirectly inducing early secondary tiller expansion. To determine which of those two possibilities were responsible, the number of tillers at nodes one and two which had 3 or more leaves was counted. This was considered to be a measure of the proportion of primary tillers which had arisen soon after the apices were removed. Only the high nitrogen treatment had a significantly greater number of primary tillers at nodes one and two in the decapitated treatment relative to the intact plants (Table 5.17). Although the low nitrogen level

had a greater number in the decapitated group than the intact plants with three or more leaves, the difference was not significant. (These results were statistically tested by applying a χ^2-test in the form of a contingency table. This was employed by considering the maximum number of tillers at nodes one and two to have 3 or more leaves in each group to be 20, ie. 10 plants and 2 tillering sites.) Despite the low nitrogen treatment not having significantly different tiller numbers with 3 or more leaves between the two treatments, the decapitated plants did have a mean of 1.1 tillers at day 12 when the corresponding intact treatment had none. Therefore, this would suggest that the primary tiller had arisen earlier in the decapitated plants than in the intact group.

Experiment 5.9

Removal of elongating and flowering tillers of Westerwolds at two nitrogen levels

Westerwolds seed was sown on 20/3/72 in vermiculite under natural light conditions in the heated greenhouse. On 7/4/72 ie. 20 days later, 48 seedlings were selected and planted on each in $3\frac{1}{2}$" pots in vermiculite. 24 received high nitrogen solution at the rate of 50 mls per week, the other 24 receiving the same amount of low nitrogen solution.

On 19/5/72, when the plants were flowering, the main tiller approaching anthesis stage, 12 from each treatment were cut at a height of 4 cms from the vermiculite surface. Another 12 from each nutrition treatment remained intact. Cutting at this height removed the emerged inflorescences, a number of developing apices, and portions of the elongating stem.

The plants were placed in a 16 hour daylength cabinet (8 hours full intensity and 8 hours supplementary lighting). Tiller counts were taken at intervals during the month after transfer to the long day cabinet when the experiment was terminated.

Table 5.18

Mean tiller number

	High Nitrogen		Low Nitrogen	
Days	Intact	Decap	Intact	Decap
0	6.25 ± 0.43^{a}	6.45 ± 0.48^{a}	2.54 ± 0.57^{b}	2.23 ± 0.30^{b}
7	9.50 ± 1.03^{c}	9.75 ± 0.95^{c}	3.00 ± 0.30^{d}	4.23 ± 0.34^{c}
14	10.83 ± 1.35^{f}	10.42 ± 1.14^{f}	3.46 ± 0.27^{g}	4.38 ± 0.31^{h}
21	11.67 ± 1.34^{j}	11.67 ± 1.16^{j}	3.69 ± 0.29^{k}	4.53 ± 0.37^{k}
28	12.5 ± 1.37^{l}	12.95 ± 1.21^{l}	4.69 ± 0.38^{m}	5.31 ± 0.46^{m}

Table 5.19

Mean tiller increase from day 0

	High Nitrogen		low Nitrogen	
Days	Intact	Decap	Intact	Decap
0	0	0	0	0
7	3.25 ± 0.94^{a}	3.33 ± 0.78^{a}	0.46 ± 0.27^{b}	2.0 ± 0.24^{a}
14	4.58 ± 1.23^{d}	4.0 ± 0.91^{d}	0.92 ± 0.25^{e}	2.15 ± 0.30^{d}
21	5.42 ± 1.24^{g}	5.35 ± 0.95^{g}	1.15 ± 0.37^{h}	2.31 ± 0.32^{j}
28	6.25 ± 1.26^{j}	6.50 ± 1.16^{j}	2.15 ± 0.39^{k}	3.08 ± 0.35^{k}

Means in the same row without a common superscript are significantly different at 5% level in tables 5.18 and 5.19.

Clipping the plants in the low nitrogen treatment gave rise to a significantly higher tiller number than the intact plants when tiller numbers were counted 7 and 14 days after clipping (Table 5.18). This is demonstrated more clearly when the increase from the time of clipping is recorded (Table 5.19; Fig. 5.5). The clipped treatment at low nitrogen level gives rise to a significantly greater increase in tiller number than the intact plants at days 7, 14 and 21 after clipping.

Irrespective of how the data for the high nitrogen level is considered, the tiller number for clipped and intact plants is similar.

Fig. 5.5 Mean increase in tiller number subsequent to commencement of treatments of Westerwolds plants at two N levels, either clipped at day 0 or left intact. 50 mls nutrient solution applied per week.

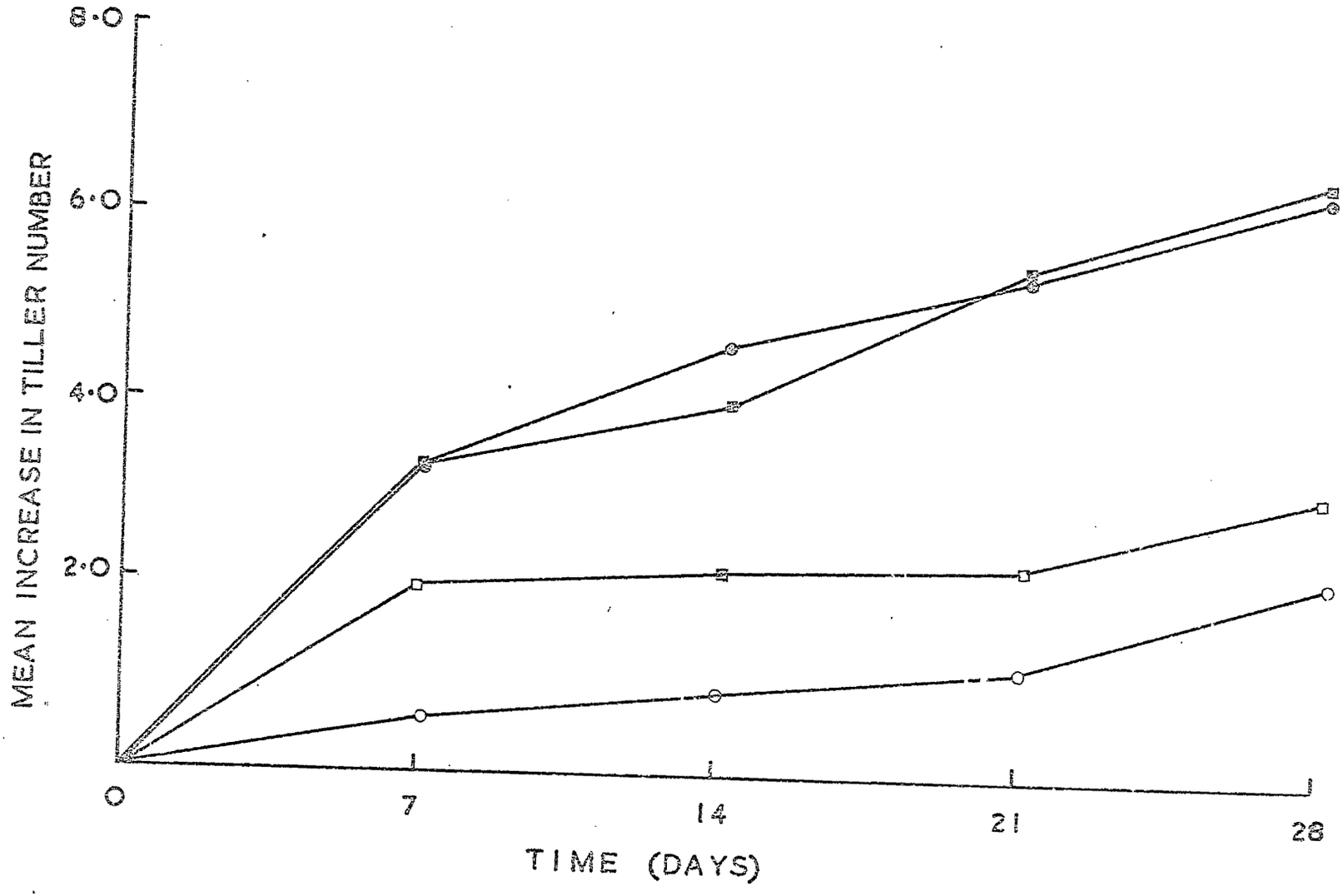
⊙ Intact, High N

◦ Intact, Low N

▣ Clipped, High N

□ Clipped, Low N

FIG. 5.5


8·0
6·0
4·0
2·0
0
MEAN INCREASE IN TILLER NUMBER
7
14
21
28
TIME (DAYS)

Experiment 5.10

The effect of decapitation on tiller bud expansion and development in L. temulentum.

L. temulentum plants which had been growing in the heated greenhouse under natural daylengths (May-June) were selected when they were 5 weeks old. 27 were chosen, and were subjected to the following treatments, 9 plants per treatment.

1) Incised and apex removed, the apex being at the double ridge stage.

2) Incised, but apex left intact.

3) Plants left intact.

The plants were planted in vermiculite and fed Hoaglands solution.

10 days later, the plants were dissected and the following recorded:

a) Length of prophyll at nodes 1 and 2.

b) Number of expanding leaves in buds at nodes 1 and 2.

c) Number of unexpanded leaf primordia in buds at nodes 1 and 2

d) Number of apices in 9 buds at each node ie. 1 and 2, which were florally initiated ie. at the double ridge stage.

e) Number of expanding tillers ie. those beyond the prophyll stage.

Table 5.20

Characteristics of buds at nodes 1 and 2

Node	Node	Intact	Incised	Decapitated
Mean Prophyll length (mm)	1	1.79	6.52	15.71
	2	1.12	6.81	10.73
Transformed Prophyll length	1	0.4074 ± 0.0637^{a}	0.6531 ± 0.1078^{a}	1.1861 ± 0.0694^{b}
	2	0.3218 ± 0.0230^{d}	0.7849 ± 0.1126^{e}	1.0309 ± 0.0677^{f}
Mean expanded leaf Primordia	1	3.10 ± 0.26^{g}	4.89 ± 0.70^{h}	6.67 ± 0.69^{h}
	2	4.56 ± 0.66^{i}	6.56 ± 0.29^{j}	7.33 ± 0.37^{j}
Mean No. of expanding leaves	1	2.11 ± 0.11^{k}	2.67 ± 0.16^{l}	3.0 ± 0.23^{l}
	2	2.10 ± 0^{m}	2.44 ± 0.17^{n}	2.78 ± 0.14^{n}
No. of expanding tillers	1	0^{o}	2^{o}	8^{p}
	2	0^{q}	1^{q}	5^{r}
No. of buds at double ridge stage	1	0^{s}	2^{st}	6^{t}
	2	0^{u}	5^{v}	7^{v}

Means within the same row without a common superscript are significantly different at 5% level.

The prophyll lengths of the buds at node 1 in the decapitated treatment are significantly longer than in the intact and incised treatments, the latter two having mean prophyll lengths which do not differ significantly. At node 2, the decapitated treatment has the longest mean prophyll, but the incised treatment has a longer prophyll than the intact (Table 5.20).

The incised and decapitated treatments have similar mean unexpanded leaf primordia and mean number of expanded leaves at both nodes and in each case are significantly higher than the intact means. The mean number of expanding tillers has a higher value in the decapitated treatment than in the other two at both nodes. The number of buds at the double ridge stage is higher in the decapitated treatment and incised treatment than in the controls at node 2. At node 1, the decapitated treatment has a higher number at the double ridge stage than the control, the incised treatment not being significantly different from either of the other two treatments.

The incised treatment assumes an intermediate position between the other two treatments for all the characters considered at both nodes. There are instances where the similarity is towards the decapitated and in other instances, towards the intact. This effect may be due to a lag in the time taken for the apex to die due to drying out, and so tending to give rise to decapitation effects but later than the decapitated plants.

The positive effect of decapitation on the number of leaf primordia on the apex may have been due to the morphogenetic change to the flowering stage which was manifest in the decapitated treatment. This change in the decapitated plants' buds from vegetative to initiated apices indicates the bud is not only induced to elongate after decapitation but also is able to undergo these morphological changes associated with the apices of a plant already induced to flower.

Discussion

Removal of expanding leaves. All of these experiments demonstrate that by removing the expanding leaves in young ryegrass plants the buds in the axils of expanding leaves expand at a faster rate in comparison to intact plants. This effect is not restricted to L. perenne (Tables 5.1, 5.4 and 5.5) but is also seen to exist in L. temulentum (Table 5.2) and L. multiflorum (Westerwolds) (Table 5.3)).

When L. temulentum was used, not only was the number of tiller buds greater than one mm. recorded, but so also was the number of buds which had their first leaf expanding beyond the tip of the prophyll. A significantly greater number of buds were at this stage in the defoliated treatment suggesting that not only was prophyll induced to elongate faster but the induction of the expansion of the first leaf was also promoted.

The first part of Experiment 5.1 considers buds which are at the stage where they are at least one mm long. In the latter part of the experiment involving Pax Øtofte and Westerwolds, however, all buds at nodes one and two were taken into account ie. the length of all the buds were recorded.

Measurement of the buds involved measuring the length of the prophyll and counting the number of buds which had their first leaf protruding above the tip of the prophyll. It was considered that measurement of the prophyll was preferable to measuring the extremities of the bud as prior to the first leaf expanding through the prophyll, the prophyll itself would be measured. However, after the first leaf appeared above the prophyll, the length measured would be from the base of the prophyll to the tip of the first leaf. Also, in some experiments, the tiller buds had given rise to visible tillers and more than one leaf was

appearing above the prophyll. So the upper extremity of the tiller would be difficult to determine. Therefore, as the project was concerned with the early expansion of tiller buds, it was considered that the prophyll length was a suitable measure of expansion.

When all of the buds at nodes 1 and 2 are considered in Pax Otofte expansion of the buds in the defoliated plants is greater than in the intact treatments. This is also true of Westerwolds. When a time course on tiller bud expansion was carried out, the buds on the treated plants had significantly longer prophylls than the intact controls by the fourth day after the treatments commenced, and by the twelfth day, significantly more buds had passed the prophyll stage than the controls.

The results of these experiments suggest that removal of the expanding leaves by surgery accelerates the expansion of tiller buds in at least three species of ryegrass. There are, however, other possibilities which could be responsible for the elongation of the buds. These include the effect of light passing through the incision and impinging on the buds and/or the wounding effect of the incision.

These possibilities were studied further, and it was found that the exclusion of light by enclosing the base of the plant in aluminium foil did not prevent the expansion of the buds when the expanding leaves were removed. The foil seemed to have excluded the light from the part of the plant it enclosed as it assumed a chlorotic appearance by the end of the experiment. The variation among replicates was greater in the first experiment in which light was excluded compared to the second. This may explain the relative lack of response to the treatments in the former experiment.

The positive effect of the incision in these two experiments would suggest that injury to the sheath causes elongation of the tiller buds. However, it was noted that in the incised treatment when the expanding leaves were left intact, that the expanding leaves grew out of the incision in many instances rather than continuing to expand up the column formed by the expanding leaf sheaths. This gave rise to disfiguration of those leaves as well as an increase in the rate of expansion of the tiller buds at the lower nodes. It was also noted that in the incised plants in which this did not occur, the tiller buds were similar to those in the control plants. However, in the experiment where the plants were selected with their oldest expanding leaf almost fully expanded, the incision was seen to have no effect on the buds and the expanding leaves did not grow out of the incision. This was due to the presence of the oldest expanding leaf functioning as a guide for the younger leaves. So in the other two experiments the effect of the incision was presumably indirect.

The removal of expanding leaves, therefore, is responsible for the increase in the expansion rate of tiller buds. This suggests that under the circumstances in which the plants were grown the presence of the expanding leaves exert an inhibitory effect on the growth of axillary buds.

The removal of expanding leaves inducing elongation of axillary buds has been found in pea (Snow, 1929) and dwarf bean (White, pers. comm.). However, in those plants apical dominance is apparent for a considerable time, ie. a large number of leaves are visible on the main axis before any axillary buds grow out under "natural conditions". Grasses, on the other hand, can have tillers visible above the subtending leaf sheath of the first leaf when the third leaf is expanding. The plants in the experiments carried out in this study were kept in a seed tray under very

densely sown conditions until the treatments commenced. Kirby and Faris (1972) found that when barley seedlings were grown under such conditions, the elongation of the tiller buds was reduced. Mitchell (1953) observed inhibition of tiller bud expansion in ryegrass when the plants were grown under low light conditions. Therefore it is possible that the plants used in the experiments in this study were exhibiting apical dominance due to the conditions under which they were grown.

Nevertheless, when removed from these conditions and the various treatments commenced, the removal of the expanding leaves allowed the plants to overcome this apical dominance effect sooner than the intact plants. In fact, there is evidence in the time course experiment that the tiller bud at the first node of the intact plants was permanently suppressed, hardly expanding throughout the twelve days the experiment was continued after the treatments commenced.

The presence of the expanding leaves inhibiting the elongation of axillary buds has been concluded to be due to the expanding leaves being the source of correlative inhibitor. There is strong evidence to suggest that the inhibitor, or at least one of them is indole-acetic acid. Young leaves have been reported to have high levels of IAA. Delisle (1938) found young leaves of Astor are high in IAA and considered them to be the main source. Van Overbeck (1938) found that the primary leaf in maize seedlings was rich in IAA ie. the first leaf still within the coleoptile.

The terminal meristem including expanding leaves has been found to be a sink for carbohydrate assimilated in the youngest expanded leaf. (Ryle 1970a; 1970b; 1972). In *L. perenne* and *L. temulentum* Ryle found that in the early vegetative stage, the apical region, including expanding leaves claimed 15 to 20% of

the labelled carbon, when the youngest fully expanded leaf was fed $C^{14}O_2$. Therefore it is possible that the removal of most of the "terminal meristem" sink allows a greater amount of assimilate to be available for the expanding tiller buds. In light of low intensity, there is a greater proportion of the labelled carbon retained in the fed leaf than in light of a higher intensity (Ryle, 1970b) but the terminal meristem contains a similar amount of ^{14}C under both light intensities. Therefore less ^{14}C is found in the roots and tiller buds in the low light treatment.

However, in the experiments described in this study, the plants in both intact and defoliated treatments were grown under high light conditions after the treatments commenced. Therefore it is difficult to imagine that carbohydrate was limiting after the plants had been under those conditions for a period. It is more likely that the removal of the leaves has removed a factor which was preventing the tiller buds from expanding. This is particularly so when the time course experiment is considered. After the fourth day, the tiller buds in plants in which the young leaves had been removed were significantly larger than the intact. Throughout the course of the experiment, the buds of the intact plants hardly expanded at all, despite the plants being under high light conditons. Therefore it is more likely that the buds in the intact plants were inhibited from elongating by some factor other than carbohydrate, produced in the expanding leaves.

That the expanding leaves in grasses have no influence on tillering has been put forward by Jameson and Huss (1959) in the introduction to their paper. They quote the work of Mitchell (1953b) and state "Mitchell (1953) found that young leaves of ryegrass (Lolium spp.) were not effective in inhibiting lateral buds". Mitchell, however, did not show that the young leaves were not effective, and did not claim this. He did however conclude "Although portions of leaves at all stages of maturity were cut off

by the defoliations, it cannot be inferred that there was direct removal of any major centre of auxin production... Thus cutting at the one inch level will have removed no stem apices and little if any expanding leaf tissue."

Therefore although Mitchell was able to show that by cutting to the height of one inch the elongation of tiller buds was reduced, without removing expanding leaves and apices, this is not evidence that those parts of the plant have no effect on tillering.

Removal of apices. In the experiment where the apical region (morphological apex and small expanding leaves surrounding the apex) of Westerwolds plants were removed, a significantly greater number of tillers were borne on the decapitated plants. Some of the apices of these plants were initiated when the treatments commenced, as was obvious when the experiment progressed since the inflorescences of some of the plants were visible above the leaf sheath of the flag leaf.

This experiment resembles the type of surgical experiments carried out in dicotyledons ie. where apex and expanding leaves are removed, and the results are similar to what would be expected in dicotyledons ie. the axillary buds grow out after decapitation. However, due to the plants approaching the flowering state, it was considered that the flowering apex may have an effect on the elongation of tiller buds. In the experiment where L. temulentum at the stage just prior to ear emergence was decapitated, the treated plants gave rise to an increase in tiller number compared to the intact plants. However by day 20 the two treatments were not significantly different.

The results of this experiment show that removal of the developing inflorescence gives rise to tiller production. However, it only accelerates what eventually happens in the intact plants. This effect of the reproductive axis on tillering is similar to what has been found in perennial grasses (Lamp, 1952; Langer, 1959) and in barley, (Aspinall, 1961). In all of those instances, the initiation of inflorescences gave rise to a temporary decrease in tillering. However, the"flush" of tillers formed after decapitation is seen to be controlled, as, between day 10 and day 20, the decapitated plants only show an increase of 0.2 tillers per plant.

Therefore, it would seem that the inhibitory effect of the reproductive axis on tiller bud expansion comes to an end at some point after the ears have emerged, probably about anthesis. Also, as the increase in tillering brought about by decapitation only lasts for a limited time, it is possible that the apices of these newly expanded tillers are inhibiting further expansion. Although no dissections were carried out to determine the state of those apices, L. temulentum., being an annual, would be initiated at a low leaf number, (Rümmer, 1963). Therefore this would allow the intact plants to approach the tiller number of the decapitated plants in the long term. This effect of the flushes of tillers on floral initiation is seen in barley (Aspinall, 1961). When nutrient was applied weekly, there were two flushes of tillering and this was reflected in the periods of ear emergence ie. there were two periods, following each of the periods of tillering. Removal of the developing inflorescence in Andropogon cristatum gives rise to the elongation of one, or occassionally two axillary buds at the lower nodes of the axes (Cook and Stoddart, 1953).

The lag in flowering, has been found in barley not to be due to the lack of buds (Aspinall loc. cit) but due to these buds not expanding during flowering. In the second period of tillering only about one third of these buds did expand, another third dying before becoming visible above the subtending leaf sheath. In the experiments reported in this study, lack of buds also does not seem to be the reason for the inhibitory effect of flowering on tiller production, as the final tiller number of the inhibited plants approaches that of the decapitated plants.

Effect of nitrogen on apical dominance in grasses. When the expansion of tiller buds at the four lowest nodes was examined prior to emergence from the subtending leaf sheath it was found that the prophyll lengths were significantly longer and number

of buds appearing above the prophyll were significantly higher in the decapitated plants at each node.

Tiller buds at nodes one and two in the intact plants were small ie. mean length was less than 1 mm. This was in comparison to the buds at node 4 which were five times longer. The decapitated plants also have smaller prophylls at nodes one and two than at nodes three and four. This would suggest that 1) the older buds have been under greater suppression and 2) because of the length of time they have been suppressed, the less they grow out when the apex is removed.

This is similar to what was found by Mitchell (1953b) when he transferred ryegrass plants from low light intensity to conditions of high light intensity. The buds which were suppressed the longest were less likely to grow out when placed in more conducive conditons. Likewise in this experiment, though it is unlikely light was the factor limiting tiller bud growth, the tiller buds at the lower nodes were less responsive to decapitation of the main axis.

In the incised treatment, it was found that the tiller buds were similar to those in decapitated plants. However, as has been pointed out, the developing inflorescences had died in all but two of the plants, and in those two plants the tiller buds were more like those of the intact than the decapitated plants. It is more than likely that the incision treatment was affecting the growth of buds indirectly, by allowing the developing inflorescences to become dehydrated, than directly by giving rise to a wounding response.

Varying the level of nitrogen applied to intact and decapitated Westerwolds plants, the intact plants at both N levels initially have lower tiller numbers than decapitated plants after the decapitation treatments commence. However, by day 20 the high nitrogen decapitated treatment is the only treatment with a

significantly higher tiller number than the others. It is significantly higher than the other treatments at all tiller counts after day 8. This suggests that the combination of decapitation and high nitrogen application gives rise to higher tiller number and so presumably has overcome apical dominance.

Although there are marked differences between the two N levels in the decapitated treatments, the intact plants are not affected in this way, at least not significantly. This is contrary to what has been found by Aspinall (1963). He was able to show that the degree of release from apical dominance in barley by removing the developing spikelets, was more noticable at the low nutrient level than the high nutrient level, suggesting that at the high level, apical dominance was less marked.

One explanation for the results of this experiment could be that the plants, prior to decapitation had been kept in the seed tray under severe nutrient stress, both inorganic and carbohydrate. Having been released from these conditions, and the apex having priority over the other meristems for any available nutrient, the tiller buds in these two intact treatments will be low in nutrient in comparison to the apex. However, in the decapitated treatment, where the apex is not depriving tiller buds of nutrient, the tiller number in those treatments could be a reflection of the amount of available nutrient, particularly in the long term, as is the case ie. at day 8 the tiller numbers are not significantly different, but from then up to at least day 20 they are.

Although by day 20, the mean primary tiller number of each treatment is similar, it is the secondary tillers which are responsible for the differences in tiller number between treatments. Both the decapitated treatments have significantly higher tiller numbers than the corresponding intact plants. This, however,

could be due to the primary tillers of the decapitated plants expanding before the intact plants. The number of primary tillers at nodes 1 and 2 with greater than three leaves substantiate this, particularly at the high N level.

The results of this experiment suggest strongly that nutrition is implicated in apical dominance. Although this experiment, as has already been pointed out, does not allow the same conclusions to be drawn as from that of Aspinall's the second nutritional experiment does. In that experiment it is found that although clipping the plants at the high nitrogen level has no effect, there is a positive effect on tiller number when those at the low level are clipped.

One of the differences between this experiment and the preceding one is that the plants had been grown under the different nitrogen levels for a considerable period prior to the commencement of the clipping treatments. It is possible that the plants at the high N level were under optimal nutritional conditons, and so there may have been sufficient nutrient available to satisfy the requirements of the developing inflorescences but insufficient to allow optimal development of tiller buds. By clipping ie. removing the developing flowering axes, the sinks would be reduced, at least temporarily, hence allowing more tiller buds to expand after clipping. That the difference between intact and decapitated (or clipped) plants is temporary could be explained as has been mentioned in reference to a previous experiment, by the formation of developing inflorescences in the expanding tillers. These would exert an inhibitory influence on the remaining buds. Hence the intact plants would be able to attain a similar tiller number before they also were restricted in the same way from producing further tillers.

When the inflorescences which were at or beyond ear emergence were removed in the field in spaced plants of Westerwolds at a number of nitrogen levels, the increase in tiller numbers over the period was not significantly different to that of intact plants. This could be explained by the fact that the inflorescences prior to ear emergence were exerting an effect, preventing any increase in tiller number over the intact plants. Alternatively, the N levels, even when no N was applied was not sufficiently low to give rise to the effects found in the greenhouse/growth cabinet experiment. Earlier experiments in this study had shown that the developing inflorescences in Westerwolds at stages prior to ear emergence did inhibit the elongation of axillary buds. Also the N levels used in this plot experiment were seen to have an effect on the tiller numbers. Therefore the former explanation is probably the more reasonable, although the latter cannot be discounted.

Nitrogen has been seen to overcome apical dominance effects in flax, Linum usitatissimum (Gregory and Veale, 1957) and in rhizomes of Agropyron repens (McIntyre, 1964; 1965). Inorganic nutrients in total have also been found to decrease apical dominance effect in barley (Aspinall, 1961; 1963). Therefore the results of the clipping experiment are in agreement with this effect.

Although current theories in apical dominance involve the diversion of nutrients and hormones by other hormones within the plant, as already discussed in the Literature Review, the results of these experiments do not elucidate the mechanism of apical dominance in grasses. They do however agree with what has been postulated in studies in the changes in tiller populations over a number of seasons ie. in tall fescue (Festuca arundinacea) (Robson, 1968), meadow fescue (Festuca pratensis) and timothy (Phleum pratense) (Langer, Ryle and Jewiss, 1964). Robson (1969)

considered that the relative competition between vegetative and reproductive tillers changed as the season progressed, and so there were various times of the year when tiller death occurred in spaced plants, presumably due to this competition. Langer, Ryle and Jewiss (1964) consider that the decrease in tillering at flowering is due to a) increased competition for assimilate by the developing inflorescence and b) hormonal control of flowering apex on the elongation of tiller buds. Certainly Ryle (1970a; 1970b; 1972) has shown that the developing inflorescence and elongating stem are a large sink for assimilate from the flag leaf. Therefore other parts of the plant particularly meristems will be deprived during flowering.

However, Ryle (1964) found that under low N conditions, branching in the developing apex was reduced in perennial ryegrass. Therefore the apex was influenced by N level. It is difficult, nevertheless, to determine whether the effect of N was direct or whether it was influencing assimilate level. It has been well established that high nitrogen levels increase the mean leaf area and so presumably the effective photosynthetic area (Ryle, 1964). Also low nitrogen reduces the photosynthetic ability per unit area of leaf in maize (Ryle and Hesketh, 1969). Therefore low N may be giving rise to a reduction in the level of assimilate within the plant, hence increasing competition among the various "sinks".

Morphological changes in tiller buds freed from suppression

Although it has been shown that nitrogen, or total inorganic nutrients can overcome the effect of flowering on tiller production, the amount of nutrient required has to be at a high level, (Aspinall, 1961). From a study of the allocation of minerals within the grass plant particularly at flowering, it has been found that developing barley grains compete strongly with tiller

buds and that it takes a very high amount of nutrients to be applied to overcome this strong competitive effect of the developing inflorescence (Langer, 1972). Therefore some mechanism must be operating within the grass plant which controls the allocation of nutrients to the various plant parts. So the partitioning of nutrients among the various meristems cannot be explained simply on the basis of relative competitive strength between the plant parts for limited amounts of nutrient.

In the experiments where the developing tiller buds were dissected subsequent to decapitation in Lolium temulentum, it is seen that decapitation increases a) the total primordium number, b) the number of expanding leaves, c) the proportion of plants with buds at the double ridge stage and d) prophyll length.

The unexpanded leaf primordium number could be due to the transformation of the apices to the flowering state, as this figure includes the primordia which subtend the spikelet primordia, and it has been found that the rate at which primordia are laid down is increased immediately prior to the attainment of the double ridge stage

A number of important points emerge from the results of this experiment. First of all, it could be concluded that the general activity of the buds is increased by decapitation ie. prophyll elongation, leaf expansion and primordium formation are increased. Secondly morphogenetic changes are induced to take place in the buds of the decapitated plants, particularly the transition from the vegetative to the flowering state. This suggests that there could be more to the decapitation effect than just increased availability of nutrients (organic and/or inorganic) for the tiller buds. That the apex has been transformed in a number of the decapitated plants could implicate the role of plant hormones in the apical dominance of grasses.

Cell division rather than cellular elongation in lateral buds is increased in grass plants when they are released from suppression either with application of TIBA or naturally, (Jewiss, 1972). Jewiss argues that cytokinins are capable of increasing cellular division. Also as application of kinetin to suppressed axillary buds frees them from suppression he considers cytokinins could be the regulator within the grass plant which is responsible for the release of the buds from inhibition.

The morphogenetic change in the buds of decapitated grasses would suggest that the release from the suppressed state was not entirely due to nutrition. It could be considered that the buds were dormant and that the removal of the apex released them from this dormancy giving rise to increased cell division, elongation of the prophyll (presumably due to cellular expansion), and a visible change in the morphology of the apex.

Jewiss considers that the elongating stem of a flowering axis could be playing a role in the inhibition of lateral buds. In some of the experiments in this study, most of the apices which were removed were too small to remove solely without removing the uppermost expanding leaf ie. they were removed at a point below the uppermost node. Branson (1953) has found that removal of the developing inflorescence below the lowest spikelet but above the highest node in Andropogon does not inhibit the elongation of the stem. However, if the apex is removed below the uppermost node, the stem does not continue to elongate. In the experiment where the developing inflorescence was removed in L. temulentum just prior to ear emergence, the developing apex was removed above the topmost node, and observations although not quantified, substantiated what had been found by Branson viz. the stem elongated after the apex was removed. This would

suggest that the elongation of the stem was not controlled by the developing inflorescence. As a consequence, it is possible that the source of inhibition imposed by the flowering axis is not the expanding stem but is the young inflorescence. However, this is inconclusive as the stem length was not recorded in any plants.

So far the influence of flowering on tiller bud behaviour has been studied in annual grasses. Annuals by definition generally complete their life cycle within one year, ie. they lack perennniality. Nevertheless by studying annuals and the characteristics which they possess which is responsible for them being annuals, information about perennniality can be deduced. The preceding experiments have shown that when the main axis flowers tiller bud expansion is inhibited and that tiller buds as well as tillers can be florally initiated. These are factors which may prevent a grass plant from perennating. However, even if after flowering some tillers and buds remain vegetative, in temperate conditions they also have to be able to withstand the low temperature conditions of winter. The following section is concerned with a study of some of the factors which may influence the ability of a grass plant to withstand low temperatures.

SECTION 6. STUDY OF SOME FACTORS INFLUENCING COLD HARDINESS IN RYEGRASSES.

RESULTS AND DISCUSSION

The effect of some environmental factors on cold hardiness of ryegrasses

It is generally believed that short days and lower temperatures of autumn are responsible for the onset of hardiness of plants allowing them to withstand the low temperatures of winter. It is also considered that under field conditions, hardiness is reduced if N has been applied in the autumn. These factors important in field conditions were considered to merit further study. In this section, use has been made of controlled environmental facilities to study in more detail the effects of daylength, temperature and nitrogen nutrition on the induction of hardiness.

It should be emphasised that the following study is not concerned with winter hardiness but with cold hardiness which, as already mentioned, is just one component of the former.

Most cold hardiness studies of plants under artificial conditions involve the plants being subjected to low temperatures while still in their rooting medium . In order to ensure that the roots reach the desired low temperature, the plants generally have to be kept under the low temperature conditions for a number of days. This method, however, imposes certain limitations on experiments due to a) plants in pots take up so much room that in a small chamber the number of plants tested at any one time is small and b) the length of time at any one temperature is long and so the risk of temperature fluctuation is increased.

The following experiment was designed to test if short term treatment of bare roots was comparable to long term cold treatment of plants with roots in soil.

Experiment 6.1

Comparison of amount of damage caused by two methods of freezing

Pax Øtofte and S23 plants which had been growing in the heated greenhouse and were at the fourth visible leaf stage were transferred to a cold cabinet at +5°C under long days (16 hrs). They were grown in 3½" pots, 4 plants/pot.

After one week at +5°C, 3 pots of each strain were placed in the refrigerator as were 12 plants of each strain which had been removed from their pots. The temperature was dropped to -5°C. After 1 hour at this temperature, the plants which were not in pots were removed from the refrigerator and the degree of low temperature damage assessed by the electrolyte release method.

The plants which were in pots were kept in the refrigerator for 3 days at -5°C, in darkness. At the end of this period, they also were assessed for damage. The results of the amount of damage caused by the two systems were compared. (Table 6.1).

Table 6.1

Mean Relative Conductivity of Plants at Various Treatments

	Control	-5°C loose	-5°C in pots
Pax Øtofte	.24± 0.020	.75± .016	.74± .022
S23	.27± 0.009	.72± .030	.76± .021

No significant difference in damage was found when the two methods of freezing the plants were compared, which gave rise to a relative conductivity of about 0.75. Therefore the results obtained by the technique employed in this project ie. by removing the plants from their pots and subjecting them for 1 hour to the appropriate temperature can be compared to those of other workers who kept their plants in pots and subjected them to a longer period at low temperature.

Artificially assessing the hardiness of plants can be carried out in a number of ways (Dexter, 1956; Smith, 1964). The two methods used in this study were electrolyte release and tiller or plant survival (Materials and Methods pages .). Preliminary experiments had shown that when mean relative conductivity of plants subjected to a range of low temperatures was plotted against the appropriate low temperature, there was usually a sharp increase in the slope of the curve between two temperatures. Plotting percentage survival against temperature also gave rise to a steep slope in the curve between the same two temperatures. The following experiment demonstrates such a relationship between survival and mean relative conductivity.

Experiment 6.2

Comparison of Survival and Electrolyte Release as Methods in Assessing Cold Hardiness in Pax Øtofte

Plants with three visible leaves were used in this experiment. They had been sown on 1/11/71 in a heated greenhouse under natural light conditions. As the aim of the experiment was to compare damage and ability to survive at a range of low temperatures, there was no need for the plants to be hardened.

On 29/4/71, 100 were removed from the seed tray and were subjected to the following temperatures, 20, 0, -2, -4, -6°C. 10 plants were removed at each temperature and their damage assessed by the electrolyte release method. Another 10 were used to measure their ability to survive at each temperature by counting the number of plants living 10 days after the cold treatment.

Table 6.2

Relative conductivity and % dead plants after low temperature treatments

	Rel. Cond.	% dead
+20°C	0.18 ± 0.02	0
0°C	0.17 ± 0.02	0
-2°C	0.33 ± 0.02	0
-4°C	0.75 ± 0.03	80
-6°C	0.82 ± 0.03	100

The results presented in table 6.2 are not conducive to statistical comparison. However, it is obvious from both percentage dead plants and relative conductivity figures that there is a large increase in both when the plants are subjected to a temperature of between -2 and -4°C (Fig. 6.1). These results suggest that when a relative conductivity reading less than 0.40 is obtained, deaths are not encountered, but when the mean relative conductivity is greater than 0.70 the percentage survival is about 20.

Experiment 6.3

Comparison in hardiness of Pax Øtofte, S23 and Tetila tetraploid

Seedlings of these three cultivars when at the third expanding leaf stage were transferred from the heated greenhouse under natural daylight conditons (March-April) to a growth cabinet at 16 hours daylength and +7°C. They remained under these conditions for 2 weeks and then were subjected to a range of low temperatures ie. +7, 0, -4, -6 and -8°C.

12 plants of each cultivar were removed at each temperature and planted in 3, 4" pots (ie. 4 plants/pot). The tillers were counted immediately after the cold treatment and at weekly intervals thereafter for 3 weeks. The relative tiller numbers for each treatment are presented in table 6.3.

Table 6.3

Comparison between Tetila tetraploid, Pax Øtofte and S23

Relative tiller numbers at a series of intervals after subjection to a range of low temperatures

(Day 0 = 100)

Low temperature treatment		Days subsequent to low temperature treatment: Day 7	Day 14	Day 21	Probability: Day 7	Day 14	Day 21
+7°C	T	158.53	199.13	370.63			
	P	130.80	188.800	331.93	NS	NS	NS
	S	120.43	151.47	243.73			
0°C	T	227.300	285.63	348.17			
	P	141.500	275.80	372.73	NS	NS	NS
	S	194.43	270.03	407.93			
-4°C	T	158.33	196.10	250.00			
	P	154.67	307.10	409.00	NS	*	NS
	S	130.57	202.50	326.00			
-6°C	T	0.00	0.000	0.000			
	P	109.67	139.10	248.53	***	***	***
	S	26.97	21.17	27.13			
-8°C	T	0	0	0			
	P	0	0	0	NS	NS	NS
	S	0	0	0			

Fig. 6.1 Comparison between percentage of plants killed (⊙) and Relative Conductivity Values (⊡) after subjection to a range of low temperatures.

FIG. 6.1

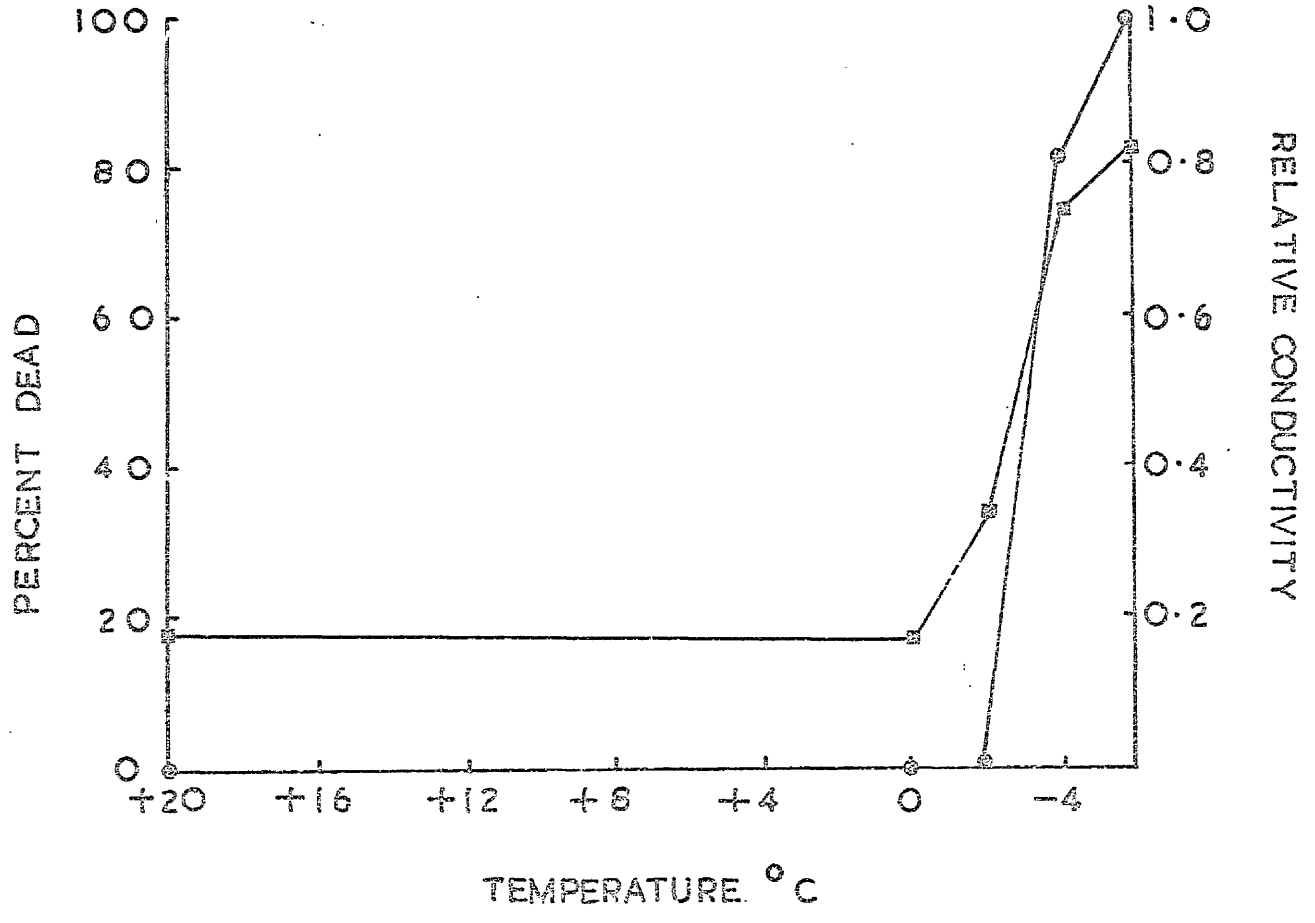

100
80
60
40
20
0
PERCENT DEAD
1·0
0·8
0·6
0·4
0·2
RELATIVE CONDUCTIVITY
+20
+16
+12
+8
+4
0
−4
TEMPERATURE °C

Fig. 6.2.A Comparison between hardened Pax Øtofte (left) and S23 plants 14 days after subjection to $-4^{o}C$.

Fig. 6.2.B Effect of low temperatures on hardened S23 plants at +7, -4, -6 and $-8^{o}C$ 14 days after subjection to low temperatures.

Fig. 6.2.A.

Pax Øtofte S23

Fig. 6.2.B

The varieties have similar tiller numbers at all tiller counts at +7°C and 0°C. At -4°C, however, varietal differences appear. At day 14 Pax Øtofte has a significantly higher relative tiller number than S23 and Tetila tetraploid. But at day 21 although there are large differences between the varieties, they are not significant. A demonstration of the comparative effect of -4°C on Pax Øtofte and S23 can be seen in Fig. 6.2.A.

After the plants had been at -6°C the Pax Øtofte plants have a significantly higher relative tiller number on day 7 than S23 plants which in turn have a significantly higher number than those of Tetila tetraploid. The significant difference between S23 and Tetila tetraploid is not persistent and so by day 14, the difference is not significant. The effect of the temperature range employed on S23 ryegrass is shown in the photograph in Fig. 6.2.B. The relative tiller number of Pax Øtofte plants at days 14 and 21 increases and so they remain significantly higher than the S23 and Tetila tetraploid plants.

-8°C is too low a temperature for any of the plants to survive and so obviously the varietal differences are not manifest at this temperature.

These results suggest that Pax Øtofte is more cold hardy than S23, which in turn is only marginally, if at all, more hardy than Tetila tetraploid under the hardening conditions employed in this experiment.

Experiment 6.4. Effect of N on hardiness in the field

Experiment 6.4.a

Degree of hardiness of Pax Øtofte tillers in autumn of a season's growth under different Nitrogen regimes

Samples of tillers were taken on 5/9/71 from the field and were subjected to low temperature treatment. The temperatures at which samples were removed were +6°C, +2°C, -2°C and -6°C. The

results of the mean relative conductivity of the samples are presented in table 2.3. There were 4 tillers per nitrogen treatment at each temperature.

Table 6.4.

Mean relative conductivities at various low temperatures of Pax Øtofte at 4 N levels

Temp °C	ON	1N	2N	3N
+6	0.24 ± 0.07^{a}	0.19 ± 0.04^{a}	0.20 ± 0.04^{a}	0.23 ± 0.07^{a}
+2	0.32 ± 0.05^{b}	0.25 ± 0.03^{b}	0.31 ± 0.08^{b}	0.37 ± 0.04^{b}
-2	0.73 ± 0.02^{c}	0.65 ± 0.06^{c}	0.65 ± 0.05^{c}	0.67 ± 0.05^{c}
-6	0.80 ± 0.04^{e}	0.90 ± 0.01^{d}	0.93 ± 0.02^{d}	0.88 ± 0.05^{ed}

Means within the same row without a common superscript are significantly different at 5% level.

There were no significant differences in mean relative conductivity between any of the nitrogen levels at any one temperature except at -6°C at which temperature the lowest nitrogen level has a mean relative conductivity significantly less than that of the 100 and 200 units N treatment (table 6.4.). To what extent this result is meaningful is debatable as a relative conductivity of 0.80 represents a high degree of damage. So from the aspect of survival, the results may not have been as above as at -6°C, percentage kill could have been total.

Experiment 6.4.b

In mid-October, procedure similar to experiment 6.4.a was repeated. Tillers were collected on 11/10/71 and were subjected to +6°C and -3°C. Sufficient material was collected so that as well as relative conductivity being measured, so also could the number of adventitious roots formed after a period in nutrient solution. Tillers which had obviously slight stem elongation

were selected, as there would be a greater chance of there being nodes, hence potential sites for root formation. Five tillers were tested in each treatment by each test.

The tillers which were kept to determine the degree of root formation were placed in 25 x 150 mm test tubes containing 10 mls of nutrient solution. Roots were counted after one week, and again, one week later. The results of the relative conductivities and root counts are given in table 6.5.

Table 6.5

Assessment of damage at a range of low temperatures

Test	Temp.	0N	1N	2N	3N
Mean relative conductivity	+6	0.19±0.01	0.18±0.03	0.19±0.04	0.12±0.04
	-3	0.63±0.10	0.81±0.02	0.75±0.03	0.81±0.06
Mean root count, after 1 week	+6	3.6 ±0.68	3.4 ±0.51	2.6 ±0.24	2.25±0.66
	-3	0	0	0	0
Mean root count after 2 weeks	+6	5.4 ±0.86	5.4 ±0.97	3.2 ±0.20	4.5 ±1.17
	-3	0.6 ±0.40	0	0	0.2

Relative conductivity

The relative conductivities in table 6.5. show that at a small subzero temperature, the tillers are damaged quite severely, the least damaged being the 0N treatment. Due, however, to the high variation the 0.63 value of the 0N treatment is not significantly less than the higher values of the other three treatments at $-3^{\circ}C$.

Adventituous roots

The number of adventituous roots formed after subjection to the two temperatures also demonstrates the effect of $-3^{\circ}C$. None of the treated plants had produced nodal roots after a period at the

low temperatures whereas allthe nitrogen treatments which were at +6°C have a mean root number of 2.25 or over. Two weeks after the cold treatment, the ON and 3N levels had a few roots visible but were still appreciably lower than the control plants.

Experiment 6.4.c

Comparison in hardiness between plants at low temperature and plants under natural conditions in autumn

On 5/9/72, 8 plants from the ON treatment and 8 from the 3N level were planted in 6" pots and brought into growth cabinets (Type A) at +7°C. 4 of each treatment were placed in a cabinet at 8 hours daylength (8 hours full intensity). The plants were kept under those conditions for one month after which samples were taken. Four plants from each of the two N levels in the plots were chosen at this time, and tillers were removed from them.

10 tillers per N treatment were removed at +6°C, 0°C and -6°C. 5 of the tillers were employed in relative conductivity measurements, and 5 were repotted and the number of living tillers were counted three weeks later. The results are presented in table 6.6.

Table 6.6

Mean relative conductivity and tiller number after subjection to low temperature treatment

		Outside		Short days/low temperatur	
		ON	3N	ON	3N
Mean relative conductivity	+6°C	0.47 ± 0.05	0.51 ± 0.09	0.38 ± 0.09	0.38 ± 0.04
	0°C	0.40 ± 0.07	0.58 ± 0.08	0.59 ± 0.07	0.46 ± 0.06
	-6°C	0.85 ± 0.04^{a}	0.83 ± 0.04^{a}	0.57 ± 0.05^{b}	0.67 ± 0.06^{ab}
Mean tiller number 3 weeks after cold treatment.	+6°C	1 ± 0^{c}	1.8 ± 0.37^{d}	2.2 ± 0.58^{de}	2.8 ± 0.20^{e}
	0°C	1.3 ± 0.4^{f}	1.4 ± 0.37^{f}	2.0 ± 0.32^{fg}	2.8 ± 0.20^{g}
	-6°C	0.2 ± 0^{h}	0	2.0 ± 0.45^{j}	0.4 ± 0.40^{hi}

Means within the same row without a common superscript are significantly different at 5% level.

Table 6.7

Mean relative conductivity and tiller number when 2N levels are considered together in each environmental treatment

		ON + 3N	
		Outside	SD/LT
Mean relative conductivity	+6°C	0.49 ± 0.04^{a}	0.34 ± 0.05^{a}
	0°C	0.49 ± 0.05^{b}	0.52 ± 0.05^{b}
	-6°C	0.84 ± 0.03^{c}	0.62 ± 0.04^{d}
Mean tiller No. 3 weeks after cold treatment	+6°C	1.4 ± 0.22^{e}	2.5 ± 0.31^{f}
	0°C	1.7 ± 0.26^{g}	2.4 ± 0.22^{g}
	-6°C	0.1 ± 0^{h}	1.2 ± 0.39^{i}

Means within the same row without a common superscript are significantly different at 5% level.

Table 6.8

Mean relative conductivity and tiller number when two environmental treatments within one N level are considered together

		Outside + SD/LT treatment	
		0N	3N
Mean	+6°C	0.39 ± 0.05^{a}	0.44 ± 0.05^{a}
relative	0°C	0.50 ± 0.05^{b}	0.52 ± 0.02^{b}
conductivity	-6°C	0.71 ± 0.06^{c}	0.75 ± 0.04^{c}
Mean tiller No.	+6°C	1.6 ± 0.34^{d}	2.3 ± 0.18^{d}
3 weeks after	0°C	1.8 ± 0.25^{e}	2.3 ± 0.18^{e}
cold treatment	-6°C	1.1 ± 0.38^{f}	0.2 ± 0.20^{g}

Means within the same row without a common superscript are significantly different at 5% level.

Outside 0N and 3N The two nitrogen levels are not significantly different at any of the temperatures at which hardiness was tested when relative conductivity was the criterion for hardiness. The mean tiller numbers three weeks after the low temperature treatments had been imposed reveal differences at the +6°C treatment. The high nitrogen level has a greater mean tiller number. At -6°C, all the plants of the high nitrogen treatment are dead, whereas the low nitrogen level has a mean tiller number of 0.2. The difference in this instance is very small, although significant. Considering that the mean tiller number at +6°C is small in the low nitrogen treatment, the relative difference in tiller number at -6°C will be larger than the actual.

Short days - low temperature, 0N and 3N Mean relative conductivities of the two nitrogen levels at any one temperature are similar. However, the mean tiller number three weeks after the low temperature treatment of -6°C is significantly larger at the

low nitrogen treatment, suggesting that the plants at this nitrogen level have been less affected by the low temperature than the high nitrogen.

Whether relative conductivity or tiller number is taken as the means of gauging hardiness, the low nitrogen treatment under the short day-low temperature conditions is more hardy than the plants which had been kept outside, irrespective of nitrogen level.

Outside and short day-low temperatures Considering both nitrogen levels together, the short day-low temperature treatment has significantly smaller mean relative conductivity at -6°C. The smaller effect of -6°C on the short day-low temperature treatment is also reflected in the mean tiller number which is higher under those conditions than for plants which had been grown outside. Although the mean tiller number of plants which had been subjected to +6°C was higher in the short day-low temperature treatment, -6°C has reduced this by just over 50% whereas in the outside treatment the low temperature (-6°C) has reduced the mean tiller number by over 90%.

ON and 3N Niether relative conductivity nor mean tiller number reveal any significant differences between high and low nitrogen treatments. However when the tiller number after subjection to -6°C is compared to that of the control plants, the mean decrease in tillering caused by the low temperature is 31.3% in the low nitrogen treatment and 91.3% in the plants at the high nitrogen level. Therefore the high nitrogen treatment has given rise to a decrease in hardiness.

These experiments described are concerned with the hardiness of Pax Otofte in the autumn and early winter. In order to determine the effects of nitrogen on the cold hardiness in the middle of winter, the following experiment was carried out.

Experiment 6.4.d

The effect of nitrogen on hardiness of Pax Øtofte in mid winter

As in the previous experiment, only the two extreme nitrogen treatments were employed ie. ON and 3N. On 29/12/71, 40 tillers from each of the two nitrogen levels were brought in from the plots. The ambient temperature at the time the tillers were collected was +4°C, and so the tillers were placed in 4°C, this being the first temperature at which the tillers were tested. The other temperatures at which samples were tested were 0°C, -4°C, -8°C and -12°C.

The mean relative conductivities for each treatment are presented in table 6.9.

Table 6.9

Relative Conductivities	ON	3N
+4°C	0.10±0.01	0.10±0.01
0°C	0.08±0.01	0.14±0.04
-4°C	0.35±0.05	0.36±0.03
-8°C	0.69±0.03^{b}	0.79±0.01^{a}
-12°C	0.79±0.01	0.84±0.03

Means within the same row without a common superscript are significantly different at 5% level.

Down to -4°C, the relative conductivities for each treatment are fairly low, with very little difference between the two nitrogen levels. However, at -8°C, the difference of 0.10 units between the two nitrogen treatments is significant, the low nitrogen treatment having the lower 'mean relative conductivity. This suggests that the damage inflicted on the tillers at -8°C is less in the low nitrogen treatment than at the higher level of nitrogen.

Experiment 6.4.e

An estimate of the effect of winter on the survival of tillers of Pax Stoffe under a range of nitrogen levels

The intention of this experiment was to assess the effect of a winter in the West of Scotland on the survival and regrowth of L. perenne tillers under a range of nitrogen levels. The results of the previous experiment had suggested that hardiness is greater at low nitrogen level. This study was undertaken as an extension of those previous experiments.

The same plots as were employed in the previous experiments were used. 3 plants from each plot were uplifted (i.e. 12 plants per nitrogen level) on 23/3/72. By this time, spring growth had commenced. The tillers of each plant were placed in order of the following categories.

1. Overwintering tillers still living. These were distinguished by bearing dead leaves lower down their axis, suggesting that they had been present prior to the winter.
2. Dead tillers. A tiller was considered dead if it did not have any green leaves.
3. Regrowth tillers. These were identified by bearing only green leaves and were found in the axils of the older overwintering tillers.

From these groups the following were calculated.

Mean number of overwintering tillers, mean number of regrowth tillers, mean number of dead tillers, mean number of living tillers, (regrowth and overwintering), and mean total overwintering tillers (dead and living).

The dead tillers were also expressed as a percentage of the total overwintering tiller. This was considered to represent

Table 6.10

The effect of nitrogen on tiller numbers subsequent to winter

	0N	1N	2N	3N	Se (diff)
Living overwinter	236.8	196.3	340.8	259.7	33.40
Regrowth	51.3	44.5	71.7	95.0	19.03
Living (Total)	288.1	240.8	412.5	354.7	52.34
Dead	74.1	70.7	81.4	161.0	25.29
Total overwinter	310.9	267.0	422.2	420.7	35.90
Total tiller no.	362.2	311.5	493.9	515.7	62.03

Table 6.11

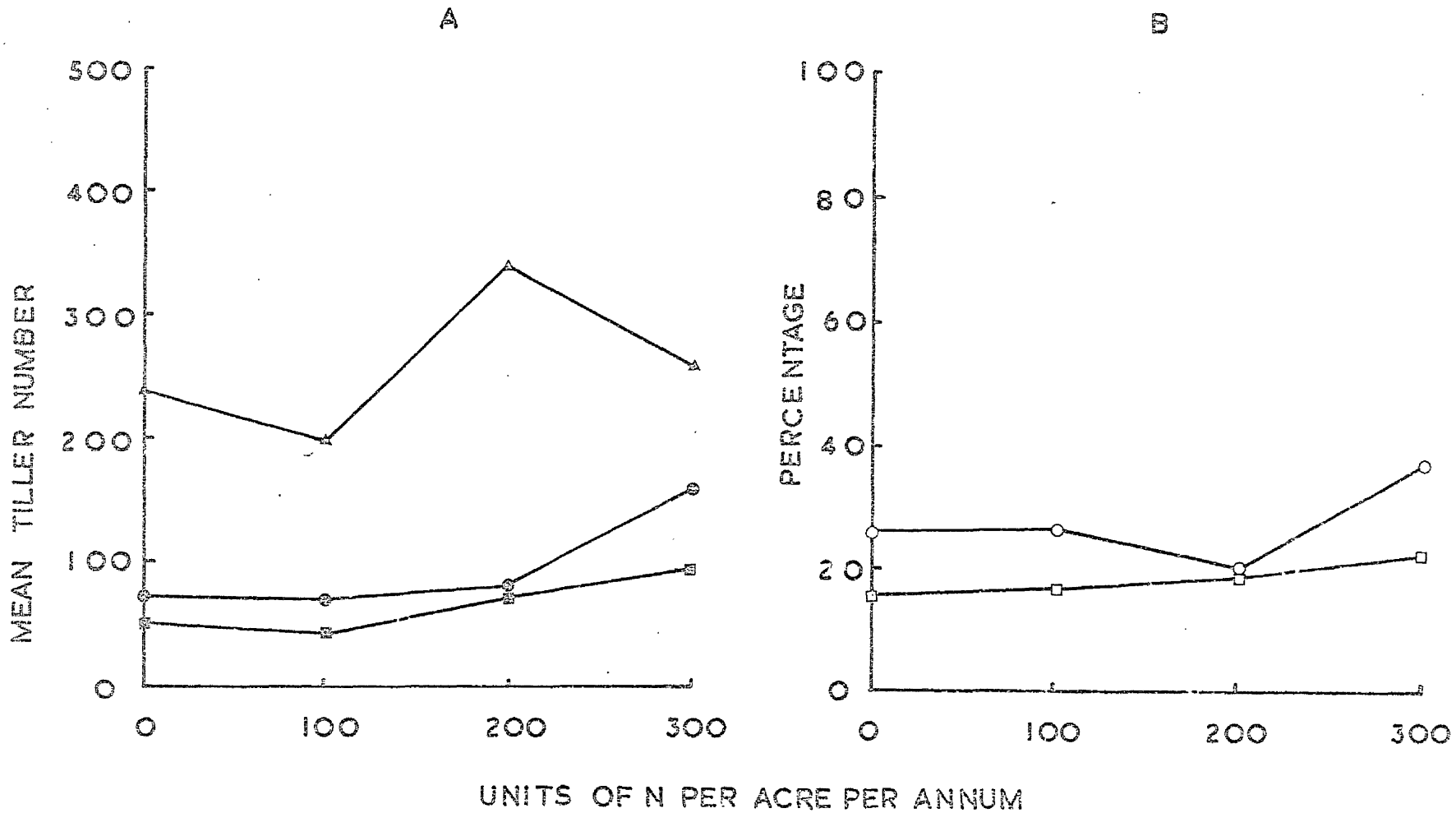
The effect of nitrogen on percentage of tillers relative to those overwintering or total per plant

	0N		1N		2N		3N		
	Arith	Trans	Arith	Trans	Arith	Trans	Arith	Trans	Se (diff)
% Dead of O.W.	25.4	30.165	26.1	29.955	20.1	26.390	36.6	37.190	2.5481
% Living of O.W.	74.6	59.835	74.0	60.100	79.1	63.048	63.4	52.810	2.5501
% Regrowth of O.W.	15.6	23.193	16.7	27.640	19.9	25.253	22.7	28.075	3.2573
% Dead of total	22.1	27.978	23.2	27.610	17.5	24.385	31.5	34.130	2.8559

Fig. 6.3.A Mean total number of tillers per plant which have survived the winter (▲), appeared in Spring (■) and died before Spring (○) of Pax Øtofte plants at four N levels.

Fig. 6.3.B Dead tillers (○) and regrowth tillers (□) expressed as a percentage of mean number of tillers surviving over the winter, of Pax Øtofte plants at four N levels.

FIG. 6.3.


A
B
MEAN TILLER NUMBER
PERCENTAGE
500
400
300
200
100
0
100
80
60
40
20
0
0
100
200
300
0
100
200
300
UNITS OF N PER ACRE PER ANNUM

measure of the tillers from the previous year which did not survive.

In order to determine if the nitrogen of the previous year influenced regrowth the following year, the regrowth tiller number was expressed as a percentage of the overwintering tillers. Dead tillers were included in this as some gave rise to regrowth tillers despite the fact that they did not bear any apparently living leaves.

The results are presented in tables 6.10 and 6.11 and in Fig. 6.3.

The analyses of variance for these data are in Appendix II None of the treatments is significantly different despite the apparent trends which seem to exist. The standard errors are large in most instances, due to wide variation between plants within the same treatment.

The numbers of tillers which were living after the winter tends to fluctuate throughout the nitrogen range. Comparing the ON and 3N treatments, there is very little difference, but the 1n treatment has just over half of the tillers which survived the winter in comparison to the 2N level.

Regrowth, shows an upward trend with increasing nitrogen, with one exception ie. the 1N level where it is slightly less than ON. The ON treatment in this instance has just over half the number of regrowth tillers of 3N level.

When the total living tiller numbers are considered, the two lower N levels have a mean of about 100 less tillers than the two higher N levels.

Dead tillers are twice as frequent in the highest nitrogen level than in the ON treatment. There is very little difference between the dead tiller number of treatments ON, 1N and 2N.

The total over wintering tiller number is considered to be a measure of the number of tillers which entered the winter. Considering the two lower nitrogen treatments together, they have a mean of about 130 tillers less than the 2N and 3N treatments. This pattern also exists in the total tiller numbers, where the two lower N levels together have a mean of about 170 tillers less than the two higher levels.

Due to the dependance of one group of tillers on another eg. regrowth being dependant on tillers which enter the winter, dead tillers being dependant on total tiller number etc., the tiller numbers of one group expressed as a percentage of those of overwintering group have been calculated and are presented in table 6.11, along with dead tillers expressed as a percentage of the total.

The percentage of dead tillers expressed as a percentage of the overwintering tiller number is considered as a measure of the degree of damage caused by the winter conditions on the tillers which entered the winter. There are no clear cut trends throughout the range of nitrogen treatments. Although the 3N level has the highest percentage kill, the 2N level has the lowest. The percentage of dead tillers is of course the balance.

Experiment 6.5

The effect of nitrogen on the hardiness of S321 and Argo at the end of October in the East of Scotland

30 tillers at each of two nitrogen levels of Argo and S321 were collected at the Scottish Plant Breeding Station at Pentlandfield, on October 27th 1971. As much root as possible was included on each tiller when it was cut from the plant. The tillers were brought to Glasgow in a polystyrene box with ice, to keep the temperature at approximately the same level as it was in the field when the tillers were cut (+7°C).

The treatments to which the plants had been subjected are outlined. They were sown on 27th July 1970 and planted out on 7th September.

The plots were of 12 plants per cultivar, 9 inches apart. The cutting frequency and the fertiliser treatment of the two nitrogen levels were:-

Cut	Date	Fertiliser as units of N : P : K	
		N1 (Low N)	N2 (High N)
	15/3	20 : 40 : 40	80 :150:150
1	1/5	20 : 0 : 0	60 : 0: 0
2	26/5	0 : 0 : 0	60 : 0: 0
3	21/6	20 : 40 : 40	60.:150:150
4	13/7	0 : 0 : 0	60 : 0: 0
5	24/8	20 : 0 : 0	60 : 0: 0
	23/9	20 : 0 : 0	60 : 0: 0
Tillers collected	27/10	Nil	Nil

The tillers were subjected to +7°C, -3°C and -7°C. 5 were used for conductivity studied at each treatment, another 5 were planted in the heated greenhouse and the tiller number after three weeks was recorded.

The results are presented in tables 6.12 and 6.13

Table 6.12

Mean relative conductivities at low temperatures

	Argo		S321	
	N1	N2	N1	N2
+7°C	0.16 ± 0.03^{a}	0.16 ± 0.03^{a}	0.20 ± 0.02^{a}	0.13 ± 0.02^{a}
-3°C	0.37 ± 0.04^{b}	0.47 ± 0.09^{db}	0.34 ± 0.03^{b}	0.52 ± 0.04^{dc}
-8°C	0.41 ± 0.05^{e}	0.48 ± 0.07^{fe}	0.51 ± 0.04^{fe}	0.65 ± 0.05^{f}

Table 6.13

Mean relative conductivities at each temperature when both N treatments of Argo are compared to both of S321 and when one N level of both cultivars is compared to other N level

	S321	Argo	N1	N2
	1N + 2N	1N + 2N	Argo + S321	Argo + S321
+7°C	0.16 ± 0.02^{ab}	0.18 ± 0.02^{ab}	0.21 ± 0.02^{b}	0.14 ± 0.02^{a}
-3°C	0.43 ± 0.04^{cd}	0.42 ± 0.03^{cd}	0.36 ± 0.03^{c}	0.49 ± 0.05^{d}
-8°C	0.58 ± 0.04^{e}	0.44 ± 0.04^{f}	0.46 ± 0.04^{f}	0.57 ± 0.50^{f}

Means within the same row in tables 6.12 and 6.13 without a common superscript are significantly different at 5% level.

Table 6.14

Number of plants out of 5 which survived after 3 weeks

	Argo		S321	
	1N	2N	1N	2N
+7°C	5^{a}	5^{a}	5^{a}	5^{a}
-3°C	4^{b}	4^{b}	2^{b}	3^{b}
-8°C	2^{c}	3^{c}	0^{c}	0^{c}

Table 6.15

Number of plants (out of 10) which survive when the two N levels of each cultivar are treated together and the two cultivars at each N level are considered as one treatment

	Argo	S321	N1	N2
	1N + 2N	1N + 2N	Argo + S321	Argo + S321
+7°C	10^{a}	10^{a}	10^{a}	10^{a}
-3°C	8^{b}	5^{b}	6^{b}	7^{b}
-8°C	5^{c}	0^{d}	2^{cd}	3^{cd}

Means within the same row in tables 6.14 and 6.15 without a common superscript are significantly different at 5% level.

When the tiller numbers of plants which are surviving after three weeks are considered, the nitrogen treatments do not have an effect on the hardiness of the plants, neither when the cultivars are treated separately or together. Grouping the two nitrogen treatments (table 6.15) of the one cultivar, the difference between cultivars is significant at -8°C, Argo having a higher number of plants alive 3 weeks after low temperature treatments (-8°C).

Experiment 6.6

The effect of nitrogen on the hardiness of grasses under controlled environment conditions

In the previous experiments involving the effects of nitrogen on hardiness, the nitrogen was applied to plants in the field. In this experiment, however, nitrogen was applied in the form of nutrient solution, and the plants grown in an inert rooting medium viz. vermiculite. This experiment was carried out with a view to establishing a system where nitrogen effects could be studied in more detail under more controlled conditions than could be achieved in the field or in soil in cabinets.

The seedlings which were planted in 2" pots were fed 50 mls nutrient solution per week. They were grown in the heated greenhouse for 3 weeks until they were transferred to the short day cabinet at +7°C. The plants were subjected to the hardening conditions for 2 weeks, then the hardiness was tested at +7, 0, -2, -6, -10 and -12°C.

The relative conductivities at each of those temperatures are presented in table 6.16, and Fig. 6.4.

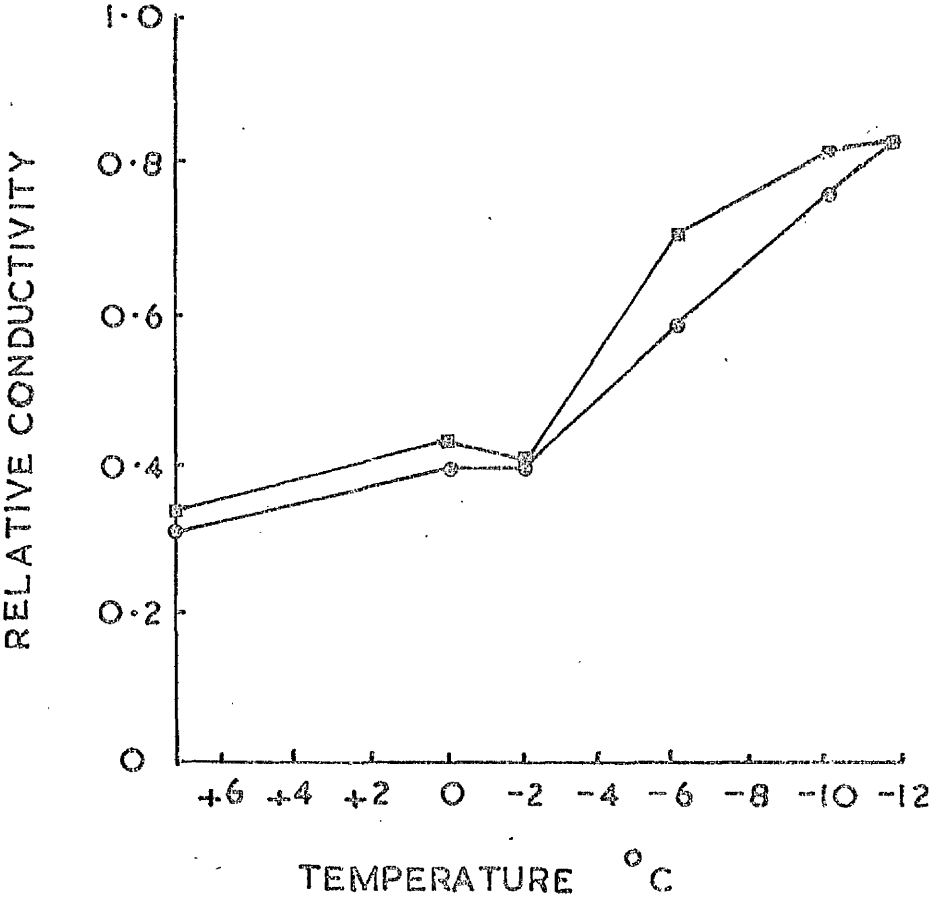
At -6°C, the low nitrogen treatment has a significantly higher mean relative conductivity than the high nitrogen.

These results are in conflict with what was found with plants treated with different nitrogen levels in the field ie. high nitrogen level decreases the hardiness of the plants.

There are two possibilities which could explain this apparent contradiction. The first is that the chloride ions may have an adverse effect on hardiness. Considering that 4/5 of the nitrate in the low nitrogen treatment is replaced by chloride, there is a possibility that there may be physiological effects of the chloride.

Fig. 6.4. Comparison in relative conductivity between Pax Otofte plants at high N (⊙) and low N (⊠) levels, hardened for two weeks at +7°C and subjected to a range of low temperatures.

FIG. 6.4


1·0
0·8
0·6
0·4
0·2
0
RELATIVE CONDUCTIVITY
+6 +4 +2 0 -2 -4 -6 -8 -10 -12
TEMPERATURE °C

The second possibility is that at very low nitrogen levels, the hardiness of a plant is impaired. Therefore in those instances it is possible that the high nitrogen is low enough to be considered a moderate nitrogen level, at which the optimum rate of hardiness is obtained when the plants are subjected to hardening conditions. If this second possibility were the case either a too low or too high level of hardiness would have a detrimental effect on the rate of hardening.

Therefore as this is in contrast to the situation in the field, it is considered that as a means of studying hardiness factors under different nitrogen levels, it is unsuitable.

Table 6.16

Mean relative conductivity of hardened plants at two N levels after subjection to a range of low temperatures

	LN	HN
+7°C	0.35 ± 0.05	0.32 ± 0.03
0°C	0.41 ± 0.06	0.39 ± 0.04
-2°C	0.41 ± 0.06	0.39 ± 0.04
-6°C	0.72 ± 0.05^{a}	0.59 ± 0.04^{b}
-10°C	0.82 ± 0.01	0.77 ± 0.03
-12°C	0.84 ± 0.02	0.84 ± 0.02

Means within the same row without a common superscript are significantly different at 5% level.

Experiment 6.7

The effect of temperature on the induction of hardiness of Pax Øtofte and S23

Plants of Pax Øtofte and S23 at the fourth expanding leaf stage were subjected to the following treatments.

1. 24 of each cultivar were placed in the heated greenhouse under 16 hour daylength for 10 days.

2. 24 of each cultivar were transferred to a growth cabinet (Type A) at +7°C for 10 days, under a 16 hour daylength.

Prior to transfer, all of the plants had been grown in the heated greenhouse under 16 hour daylength.

When the plants had been under the two conditions for 10 days they were subjected to +7°C, a sample of 12 of each cultivar was removed, then the temperature was dropped to -7°C. The plants were then repotted and grown in the greenhouse for 3 weeks when the number of plants surviving were scored.

Table 6.17

Number of plants surviving 3 weeks (out of 12 plants)

		+7°C	-7°C
PØ	HT	12^{b}	1^{a}
	LT	12^{b}	10^{b}
S23	HT	12^{b}	1^{a}
	LT	12^{b}	7^{ab}

Number of plants surviving after 3 weeks (out of 24 plants - S23 + PØ considered together)

		+7°C	-7°C
PØ + S23	HT	24^{b}	2^{a}
	LT	24^{b}	17^{b}

Numbers without a common superscript are significantly different at 5% level.

The number of plants surviving after three weeks although Pax Øtofte has a greater number of plants surviving at LT than S23 the difference is not significant (Table 6.17). Also, the low temperature, when both strains are treated together has a positive effect on the induction of hardiness, as would be expected (Table 6.18).

It has been assumed so far that short days have a positive effect on the induction of hardiness. The validity of this assumption was tested in a number of experiments which were designed to determine what effect day length had on the promotion of cold hardiness in ryegrass, particularly at low temperatures.

Experiment 6.8

The effect of daylength at low temperature on the induction of cold hardiness

Pax Ǿtofte plants at the 5th/6th expanding leaf stage which had been growing in the heated greenhouse under natural daylength conditions (ie. daylengths of January and February), were transferred to two growth cabinets. These cabinets were at 7°C, one at a 16 hour day (Type A) (8 hours full intensity plus 8 hours supplementary lighting), the other at 8 hours daylength. After 2 weeks under those conditions the plants were subjected to the following temperatures and samples of 12 plants/photoperiodic treatment removed at each ie. +7, 0, -2, -4, and -6°C.

Hardiness was assessed by the number of tillers each plant bore after 3 weeks in the heated greenhouse.

Table 6.19

Mean tiller numbers of plants 0 weeks and 3 weeks after cold treatment

	0 weeks		3 weeks	
Temp	LD	SD	LD	SD
+7°C	2.9±0.26	2.8±0.30	4.8±0.35	4.9±0.45
0°C	2.3±0.37	3.2±0.44	4.4±0.51	5.3±0.59
-2°C	2.3±0.35	2.0±0.30	4.8±0.72	4.1±0.68
-4°C	2.5±0.31	2.4±0.51	5.0±0.92	4.9±0.93
-6°C	2.5±0.24	2.3±0.23	3.3±0.92	3.1±0.55

The tiller number at day 0, and 3 weeks after cold treatments are presented in table 6.19. Only at -6°C is there a definite reduction in tillering, and this applies to both treatments ie. long and short days. Therefore after 2 weeks at different day-

lengths, at +7°C, the plants are of similar hardiness. This level of hardiness seems high, ie. after subjection to -6°C, the plants were able to tiller, although a few deaths were observed.

Experiment 6.9

The effect of time on the induction of hardiness of Pax Øtofte under long and short day conditions

Pax Øtofte seedlings at the second visible stage were transferred from the heated glasshouse, under 16 hour daylength to 2 growth cabinets (Type A), one at 8 hours, the other at 16 hours daylength, at 6°C. 10 plants were sampled at a number of low temperatures on the day of the transfer. This was repeated for each daylength treatment one week later, and again after a further fortnight.

The temperatures employed to test the hardiness of the plants were +6, 0, -4, -8 and -12°C and on the third week under the different daylengths, -2 and -6°C were also employed.

The results of the various treatments are presented in table 6.20 and figure 6.5.

Table 6.20

Mean relative conductivities of plants at a number of low temperatures at 0, 1 and 3 weeks under long and short days.

	0 weeks	1 week		3 weeks	
°C		LD	SD	LD	SD
+6	0.14 ± 0.01	0.26 ± 0.03^{b}	0.19 ± 0.02^{b}	0.32 ± 0.03^{c}	0.30 ± 0.04^{c}
0	0.16 ± 0.02	0.26 ± 0.03^{e}	0.24 ± 0.02^{e}	0.25 ± 0.04^{f}	0.31 ± 0.01^{f}
-2	-	-	-	0.33 ± 0.03^{g}	0.36 ± 0.04^{g}
-4	0.71 ± 0.04	0.48 ± 0.06^{i}	0.70 ± 0.03^{j}	0.33 ± 0.02^{k}	0.35 ± 0.02^{k}
-6	-	-	-	0.78 ± 0.02^{l}	0.81 ± 0.04^{l}
-8	0.89 ± 0.02	0.89 ± 0.03^{n}	0.93 ± 0.03^{m}	0.78 ± 0.03^{o}	0.83 ± 0.03^{o}
-12	0.95 ± 0.02	0.86 ± 0.03^{q}	0.86 ± 0.04^{q}	0.87 ± 0.03^{r}	0.87 ± 0.09^{r}

Means within each row at each week without a common superscript are significantly different.

The results in table 6.20 show that after one week at the two daylengths, the mean relative conductivity of the plants under longdays is significantly lower than that of the short day plants at $-4^{o}C$. At $-8^{o}C$, however, the difference is not significant.

After 3 weeks at the two daylengths, the difference in mean relative conductivities of the treatments at any of the temperatures employed is not significant. The temperatures -2 and $-6^{o}C$ were included to make the comparison more accurate.

Therefore, it would seem that the long day treatment has a relatively faster promotive effect on cold hardiness at $+6^{o}C$ than short days, but by three weeks they have both attained a similar level of hardiness. Comparing the results at $-4^{o}C$ between 1 week and 3 week time interval after the cold treatment, the relative conductivities of the two treatments similar to the control ie. the relative conductivities at $+6^{o}C$ This suggests the degree of hardiness is quite far advanced.

The findings of this experiment were taken further in the next experiment where the relative hardiness of roots and shoots of Pax Otofte were studied under long and short days.

Fig. 6.5. Mean relative conductivity of Pax Øtofte plants hardened under a range of hardening conditions and for varying periods and subjected to a range of low temperatures.

—●— 0 weeks hardening

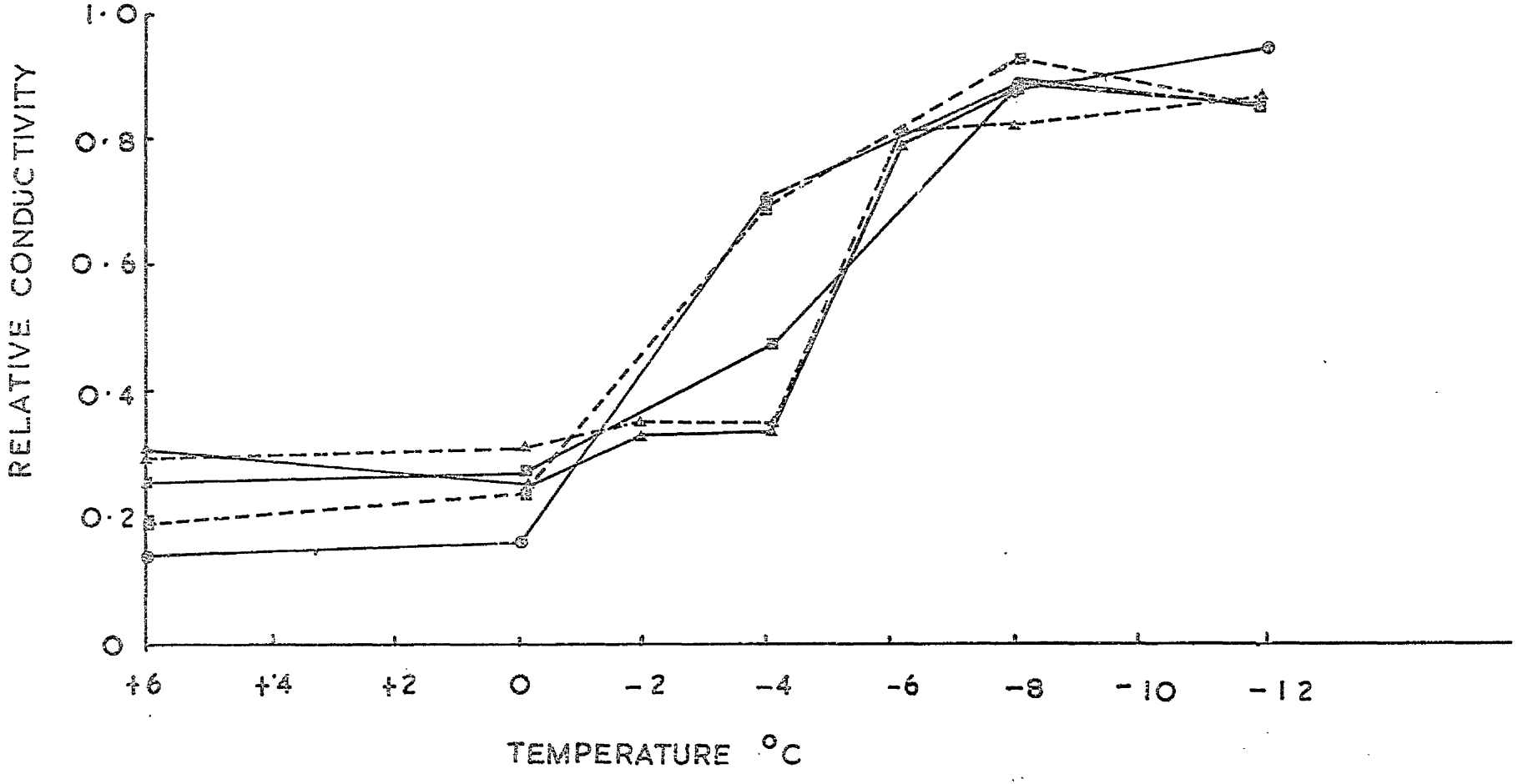
—■— 1 week at +6°C and 8 hour day.

--■-- 1 week at +6°C and 16 hour day.

—▲— 3 weeks at +6°C and 8 hour day.

--▲-- 3 weeks at +6°C and 16 hour day.

FIG. 6.5


RELATIVE CONDUCTIVITY
1·0
0·8
0·6
0·4
0·2
0
+6
+4
+2
0
-2
-4
-6
-8
-10
-12
TEMPERATURE °C

Experiment 6.10

The relative leaching rate of electrolytes from roots and shoots

Before any experiments were carried out on the relative hardiness of roots and shoots, it was considered necessary, by virtue of the difference in cell type constituting roots and shoots , to compare the relative leaching rates of roots and shoots, both undamaged and damaged.

Plants of Pax Øtofte which had been growing in the heated greenhouse were used in this experiment. They were at the fourth expanding leaf stage. 10 were removed from their pots, their roots were washed, the roots were detatched from the shoots at the first node, and the roots and shoots were each placed in test tubes, in the normal way.

Another 10 were left intact and were placed in the refrigerator which was dropped to -4°C. They were kept in the refrigerator for 1 hour at -4°C, then were separated into root and shoot and were treated in the same manner as the controls.

As soon as the plant material was placed in the distilled water, the tubes were shaken (10 rapid shakes) and the conductivity was measured. This was repeated at the following times thereafter.

30 mins, 1 hour, 2 hours, 3 hours, 8 hours, 24 hours. After 24 hours, the total conductivity was found and so the relative conductivity at each point was calculated. (Table 6.24; Fig.6.

In roots and shoots at both temperatures, the increase in relative conductivity after 3 hours is low and the increase from 8 hours to 24 hours is also low. Therefore, it would seem that the rate of leaching by the 24th hour after the cold treatment is inconsequential.

The relative conductivity of the liquid in which the root at the control temperature was immersed is much higher than that

Fig. 6.6 Relative conductivity of roots and shoots at intervals after immersion in 10 mls distilled water.

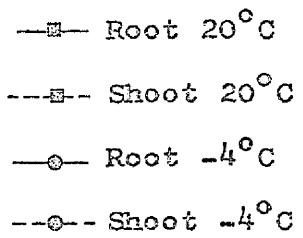


FIG. 6.6

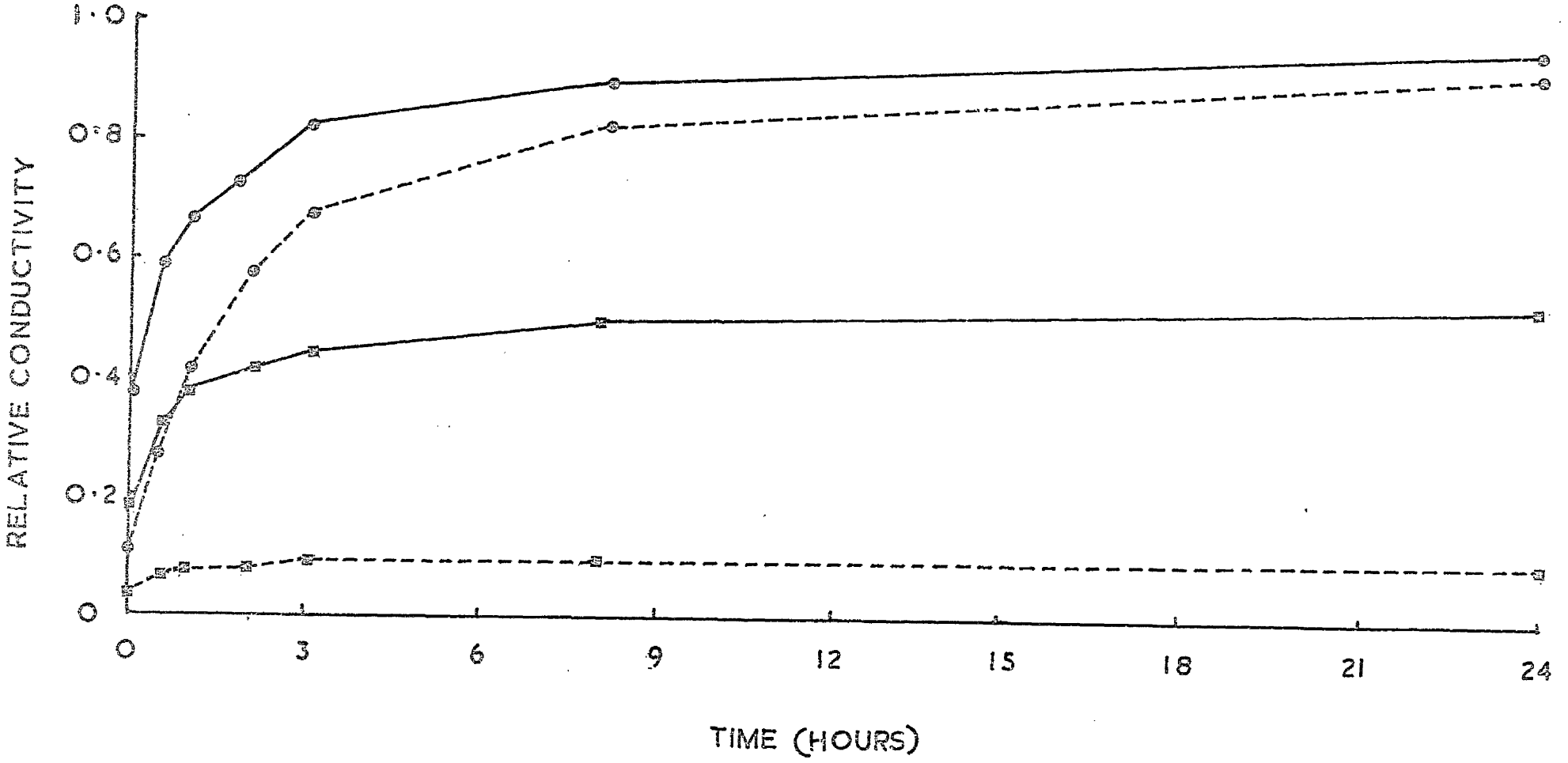

1·0
0·8
0·6
0·4
0·2
0
RELATIVE CONDUCTIVITY
0
3
6
9
12
15
18
21
24
TIME (HOURS)

Table 6.21

Time course of relative conductivities of unhardened Pax Øtofte roots and shoots at 20°C and -4°C

		0 hours	0.5 hours	1 hour	2 hours	3 hours	8 hours	24 hours
20°C	Root	0.19±0.01	0.31±0.01	0.38±0.02	0.41±0.02	0.44±0.04	0.50±0.04	0.53±0.04
	Shoot	0.04±0.01	0.06±0.01	0.07±0.01	0.07±0.01	0.09±0.01	0.10±0.02	0.10±0.02
-4°C	Root	0.37±0.03	0.59±0.04	0.67±0.05	0.74±0.04	0.83±0.04	0.90±0.02	0.95±0.01
	Shoot	0.09±0.01	0.28±0.01	0.41±0.02	0.57±0.02	0.68±0.02	0.83±0.01	0.92±0.01

of the shoot after 24 hours leaching. This would suggest that the root loses a high proportion of electrolytes when not damaged by low temperature.

Therefore any comparison of roots and shoots has to take into account this difference in relative conductivity at control temperatures. This can be achieved by applying the formula which is discussed in the context of the following experiment.

Experiment 6.11

The relative hardiness of roots and shoots in unhardened Pax Øtofte plants

The last experiment was concerned more with the rate of leaching of electrolytes from roots and shoots rather than the hardiness of the two plant parts when subjected to a range of temperatures. In this experiment, Pax Øtofte plants were grown in a greenhouse to the fifth expanding leaf stage. They were removed from their pots, the roots washed and were placed in the cryostat. The following temperatures were employed to test the hardiness of the roots and shoots,

$+20^{\circ}C$, $+5^{\circ}C$, $0^{\circ}C$, $-5^{\circ}C$.

At each temperature, a sample of 10 plants was removed, the roots were detatched from the shoots at the first node, and the relative conductivity was calculated.

The mean relative conductivities are represented in table 6.22.

Table 6.22

Mean relative conductivities of roots and shoots after subjection to a range of low temperatures

Temperature	Root	Shoot
Standard	0.47 ± 0.02	0.11 ± 0.03
$+5^{\circ}C$	0.54 ± 0.04	0.11 ± 0.03
$0^{\circ}C$	0.62 ± 0.04	0.40 ± 0.04
$-5^{\circ}C$	0.93 ± 0.04	0.89 ± 0.01

Just as in the previous experiment, after 24 hours of leaching at room temperature, the roots have given rise to a higher relative conductivity than shoots at standard temperatures. Therefore so that the results for root and shoot can be compared, the following conversion is made.

1. The mean initial conductivity for the samples at the standard temperature is calculated and expressed as a proportion of the mean total conductivity.

2. As this portion of the total conductivity is assumed to be a measure of leaching due to causes other than low temperature damage, this will apply to all treatments. Therefore the total conductivity for each sample in each treatment is multiplied by this proportion.

3. The result is subtracted from the total conductivity and from the initial conductivity.

4. The remaining portion of the initial conductivity is divided by the remaining portion of the total conductivity.

This new relative conductivity is the ratio of the amount of damage caused by the low temperature to the maximum amount of damage which could possibly be caused by low temperatures.

An example of conversion is demonstrated overleaf. The results of this experiment subsequent to conversion are presented in Table 6.22a.

Table 6.22a
Mean converted relative conductivities of roots and shoots after subjection to a range of low temperatures

Temperature	Root	Shoot
Standard	0	0
45°C	0.15±0.20	0.02±0.02
0	0.28±0.20	0.32±0.13
-5°C	0.87±0.03	0.75±0.04

By excluding that part of the total leachate which was independant of injury due to low temperatures, results for roots and shoots for any one temperature treatment are approximately comparable.

Example of conversion of conductivity data

Mean conductivity of plant part of standard temperature (a) = 0.3CF units.

Mean total conductivity of plant part at standard temperature (b) = 1.4CF units.

Ratio $\frac{(a)}{(b)}$ = 0.21 = R

Initial, total, converted initial, converted total and converted relative conductivity after samples have been subjected to low temperature (CF units)

Initial Conductivity I.C.	Total Conductivity T.C.	Converted Initial Conductivity IC-RxTC	Converted Total Conductivity TC-RxTC	Converted Relative Conductivity
1.1	1.5	0.78	1.18	0.66
1.0	1.2	0.75	0.98	0.79
0.9	1.1	0.67	0.87	0.77
0.9	1.0	0.69	0.79	0.87
1.0	1.1	0.77	0.87	0.89
1.1	1.5	0.78	1.18	0.66
1.0	1.7	0.64	1.34	0.48
1.2	1.7	0.84	1.34	0.63
1.3	1.5	0.98	1.18	0.83
0.9	1.0	0.69	0.78	0.87

Although errors are high for means of roots at higher temperatures (ie. where the means are low), these means are relatively unimportant in studying hardiness, the higher mean values being those which differ when cold injury differs.

Experiment 6.12

The effect of daylength at low temperatures on the hardiness of Pax Øtofte and S23 roots and shoots

In this experiment, only one sub-zero temperature was employed. From a previous experiment, it was considered that $-4^{o}C$ was important in distinguishing daylength effects on hardiness.

The plants in this instance were at the third expanding leaf stage and were subjected to hardening condition in a cabinet (Type C) at 16 hours daylight at $+5^{o}C$. Short days were obtained by transferring the plants in the short day treatment into a box at 5 pm, and covering the cardboard box with black polythene. They were removed into the open cabinet at 9 am each morning. The box was kept in the cabinet and thermometer readings within the box revealed that the temperature inside the box was similar to that within the cabinet ie. $+5^{o}C$. The hardening period lasted for 10 days, after which the plants from each daylength were subjected to $+5^{o}C$ and $-4^{o}C$.

The results are presented in table 6.23

Table 6.23

Mean relative conductivity (converted)

		Long Days		Short Days	
		Root	Shoot	Root	Shoot
P.Ø.	+5	0	0	0	0
	-4	$0.78^{b}\pm0.09$	0.44 ± 0.05^{a}	$0.65^{ab}\pm0.12$	$0.62^{b}\pm0.05$
S23	+5	0	0	0	0
	-4	0.69 ± 0.16^{ab}	0.44 ± 0.06^{a}	0.69 ± 0.13^{b}	0.63 ± 0.06^{b}

Means at the same temperature without a common superscript are significantly different at 5% level.

In both cultivars of Lolium perenne, 10 long days have a promotive effect on hardiness relative to the same number of short days. It is the shoots which are influenced in the cultivars rather than the roots.

A more meaningful comparison of hardiness can be made if a number of low temperatures are employed to test the hardiness of the plant parts of cultivars. The next experiment was designed with this in mind.

Experiment 6.13

The relative hardiness of roots and shoots of Pax Øtofte and S23 Perennial ryegrass

Seedlings of those two cultivars of perennial ryegrass were placed in long days for two weeks at +5°C (Cabinet Type C). At the end of this period the plants were at the third or fourth expanding leaf stage. In order to test the relative hardiness of the roots and shoots of the two cultivars, the plants were subjected to a range of low temperatures viz. +5, 0, -2.5, -5 and -7.5°C. Samples of 7 plants/treatment were removed at each temperature and the roots were separated from the shoots at the first node. The relative conductivities were calculated.

The relative conductivities at +5°C and 0°C were bulked as the means were not significantly different so the sample from which the portion of the total conductivity due to factors other than low temperature damage was calculated was increased in size.

The converted mean relative conductities are presented in table 6.24 and figure 6.7.

At -2.5°C, the shoots of Pax Øtofte had a lower relative conductivity than the roots of Pax Øtofte and S23, as well as the shoots of S23. This was also the case at -5°C, but the shoots of S23 had a lower relative conductivity than the roots of either cultivar. At -7.5°C, there was no significant difference between the mean relative conductivities of the shoots, but the difference between roots and shoots was significant for both cultivars, the shoots having the lower relative conductivities.

These results suggest that at the lower sub-zero temperatures, Pax Øtofte is hardier than S23, when shoots are considered, but in

Table 6.24

Mean converted relative conductivities of roots and shoots of Pax Øtofte and S23

	Pax Øtofte		S23	
	Root	Shoot	Root	Shoot
+5 0)	0	0	0	0
-2.5	0.33 ± 0.08^{a}	0.06 ± 0.02^{b}	0.58 ± 0.04^{c}	0.38 ± 0.07^{a}
-5.0	0.74 ± 0.03^{d}	0.19 ± 0.05^{e}	0.67 ± 0.06^{d}	0.40 ± 0.03^{f}
-7.5	0.89 ± 0.04^{g}	0.50 ± 0.04^{h}	0.92 ± 0.06^{g}	0.64 ± 0.04^{h}

Numbers within rows without a common superscript are significantly different at 5% level.

Fig. 6.7 Mean relative conductivity (A) and mean converted relative conductivity (B) of roots and shoots of hardened Pax Øtofte and S23 plants after subjection to a range of low temperatures.

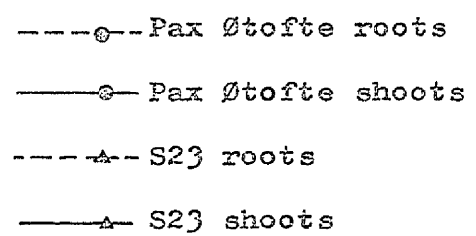


FIG. 6.7 A

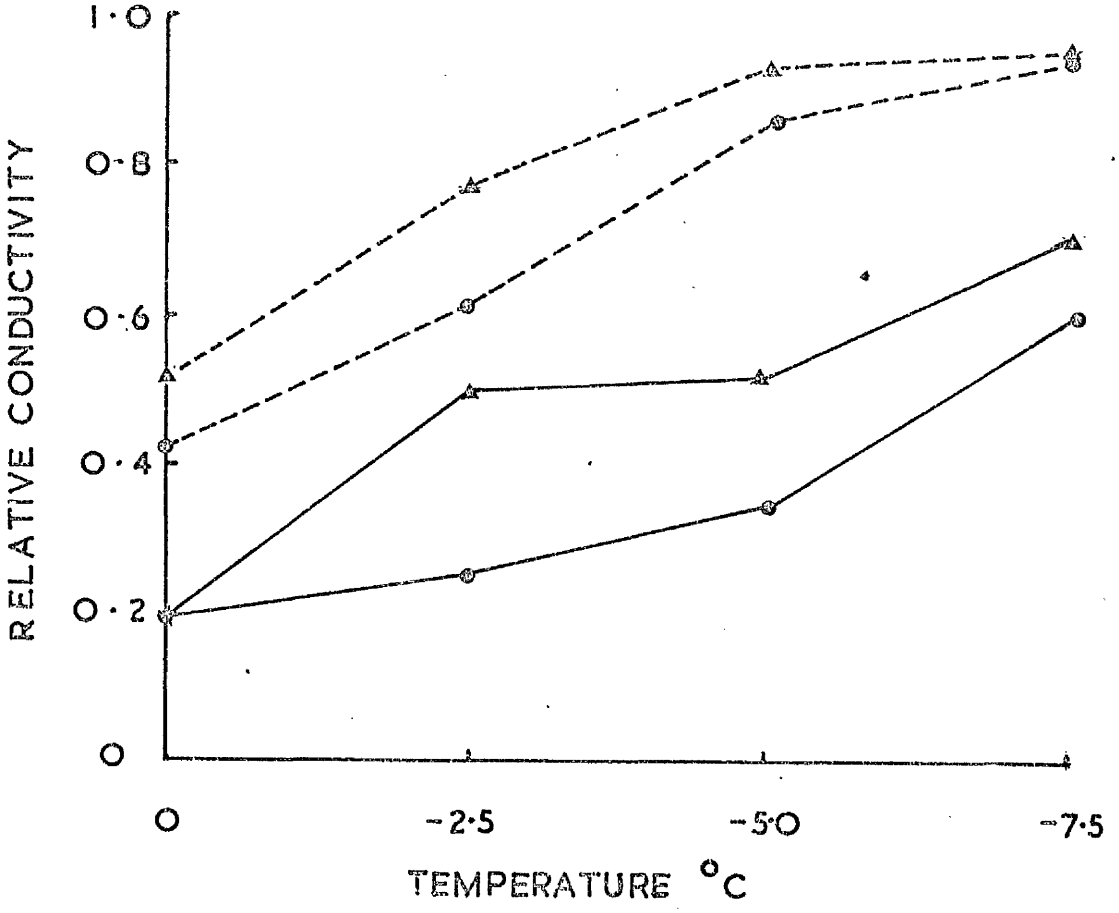
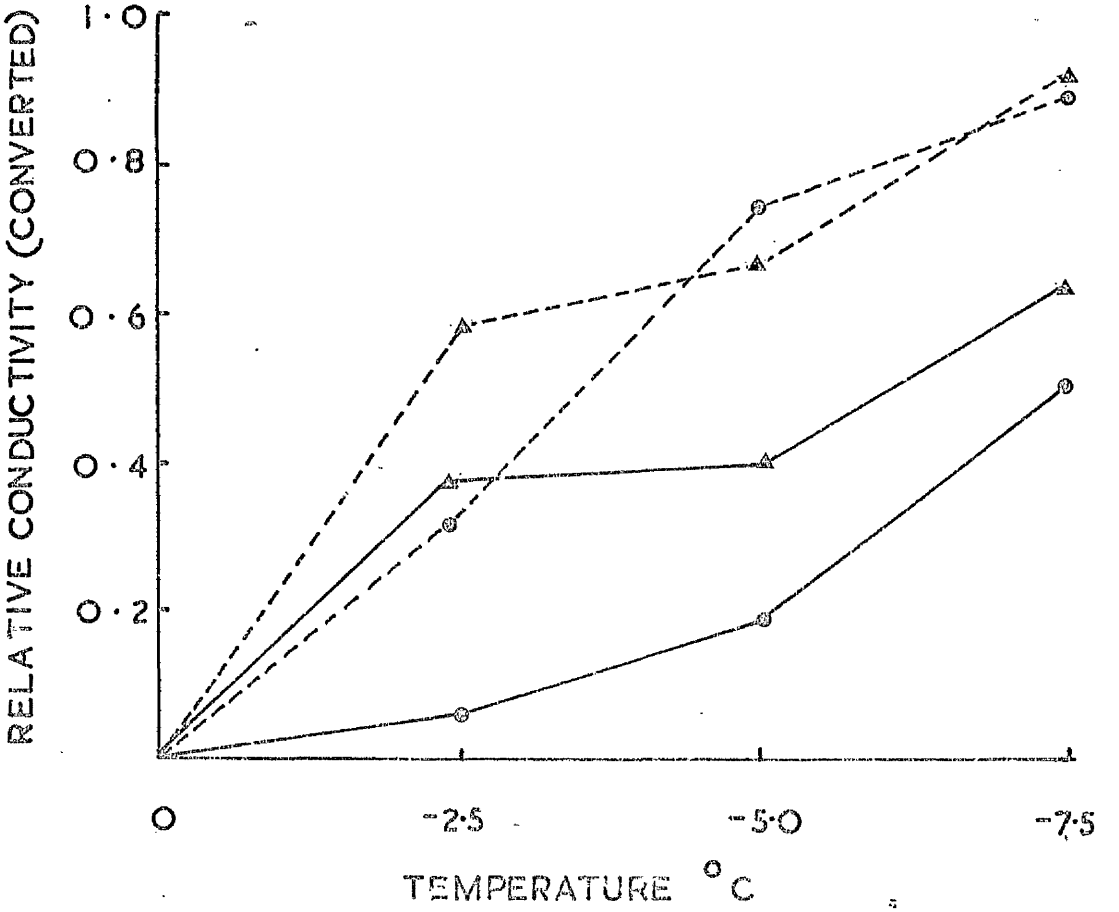


FIG. 6.7 B



both cultivars, the shoots are more hardy than the roots even at the low temperature of -7.5°C.

This distinction between S23 and Pax Øtofte was not manifest in the previous experiment at -4°C treatment. This may have been due to the hardening periods being less in the previous experiment. The results of this experiment are what would be expected if the results from experiment 6.3 were considered ie. where Pax Øtofte were found to be more hardy than S23.

Experiment 6.14

The effect of daylength at low temperatures on hardiness of roots and shoots of Pax Øtofte

Seedlings of Pax Øtofte which had been growing in the heated greenhouse were planted in $2\frac{1}{2}$" pots and when they were at the second expanding leaf stage a number was transferred at $+7^{\circ}C$ at two daylengths in growth cabinets, type A. The two daylengths were 8 and 16 hours. A few were left in the greenhouse under a 16 hour daylength brought about by a bank of fluorescent tubes emitting 11.2 Wm^{-2}.

After 10 days under these conditions, the plants were subjected to a range of low temperatures, samples of six plants per treatment at each temperature being removed ie. $+7$, 0, -2, -4 and $-6^{\circ}C$.

The results after conversion are presented in table 6.25 and figure 6.7.

Shoots At $-2^{\circ}C$, the mean converted relative conductivities for the shoots of the various treatments are similar. However, at $-4^{\circ}C$ the plants hardened at $+7^{\circ}C$ under a 16 hour day have a significantly lower mean relative conductivity than those hardened at $+7^{\circ}C$ under an 8 hour day and those remaining in the greenhouse. At $-6^{\circ}C$, the two treatments which involved hardening at low temperatures have significantly lower mean relative conductivities than those remaining in the greenhouse.

Between $-4^{\circ}C$ and $-6^{\circ}C$, the plants in the $+7^{\circ}C$, 16 hour daylength hardening period have risen markedly ie. by 0.45 units, whereas the plants hardened at the $+7^{\circ}C$, 8 hour daylength have only risen by 0.03 units.

Table 6.25

Mean converted relative conductivities of roots and shoots after different hardening regimes

Hardening condition	Long days, high temp.		Long day, Low temp.		Short days, Low temp.	
Plant part	Root	Shoot	Root	Shoot	Root	Shoot
Low temperature treatment						
+7°C) 0°C)	0	0	0	0	0	0
-2°C	0.18 ± 0.114^{a}	0.28 ± 0.057^{a}	0.35 ± 0.016^{a}	0.023 ± 0.046^{a}	0.21 ± 0.080^{a}	0.23 ± 0.093^{a}
-4°C	0.51 ± 0.115^{ab}	0.63 ± 0.057^{b}	0.51 ± 0.105^{ab}	0.18 ± 0.059^{c}	0.31 ± 0.081^{ec}	0.53 ± 0.043^{b}
-6°C	0.78 ± 0.091^{gf}	0.83 ± 0.010^{f}	0.82 ± 0.084^{gf}	0.63 ± 0.028^{g}	0.78 ± 0.083^{gf}	0.56 ± 0.048^{g}

Means without a common superscript in the same row are significantly different at 5% level.

Roots At $-2^{o}C$, as in the case of shoots, the roots have similar values in all treatments, but unlike the shoots at $-4^{o}C$ the roots do not have significantly different values for relative conductivity in any of the hardening treatments. This situation persists at $-6^{o}C$.

Roots and shoots When these are compared within treatments the long day, low temperature hardening treatment gives rise to shoots with lower relative conductivities than roots at $-4^{o}C$. This situation is reversed in the short day, low temperature hardening period where the roots are significantly less damaged than the shoots. At $-6^{o}C$ the roots and shoots within the hardening treatments have similar relative conductivities. The shoots of hardened plants, however, have significantly lower relative conductivities than those of plants which continued growth in the greenhouse.

Long days, therefore, have a promotive effect on the hardiness of shoots when hardening temperature is $+7^{o}C$. Roots of plants hardened under long days are not significantly different from roots of plants of unhardened plants, although in plants hardened under short days are hardier than the shoots.

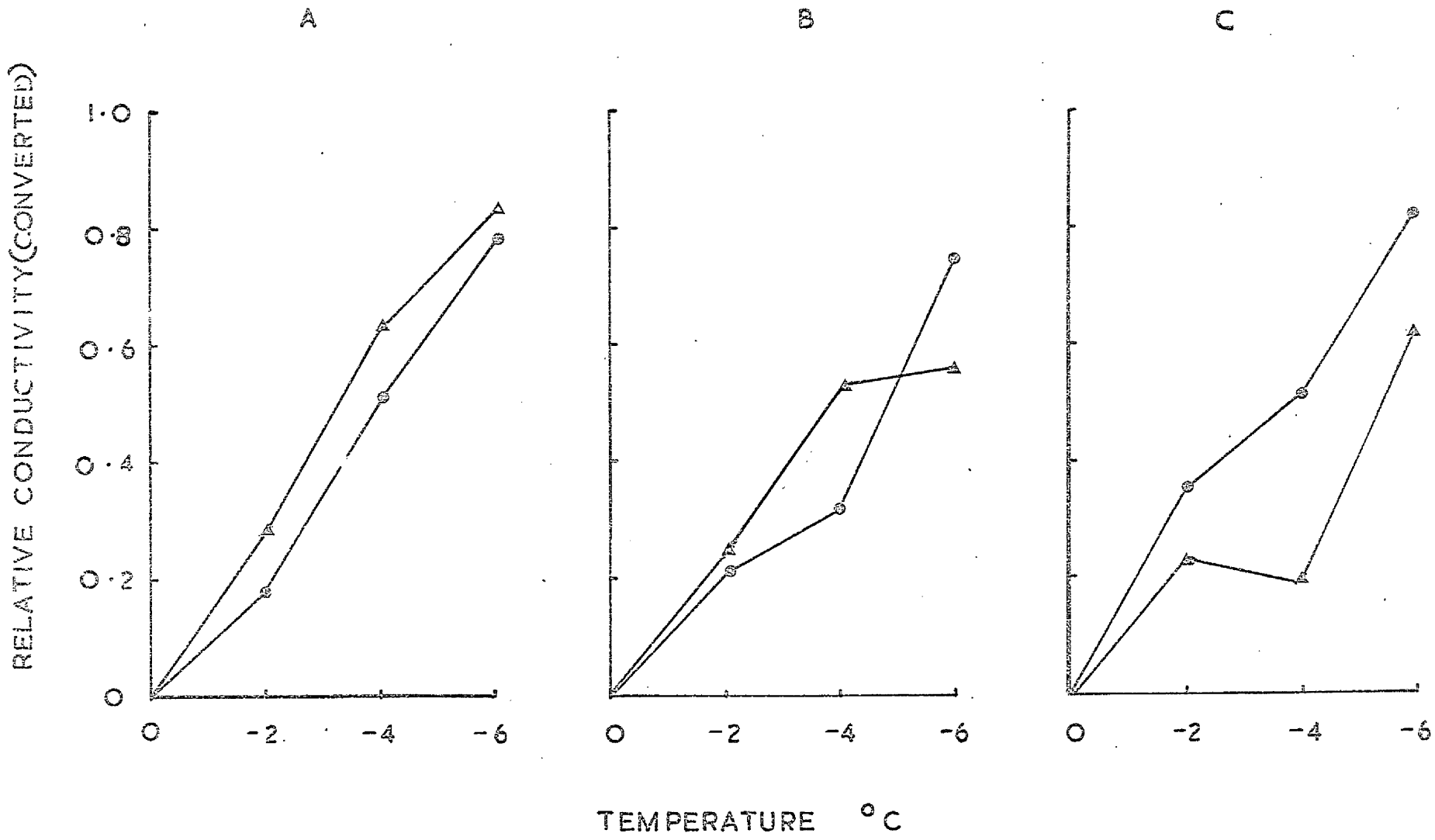
These studies of the effects of daylength and temperature on hardiness were carried out at temperatures which, although above zero, were, nevertheless low. It has been shown by Klebesadel (1964) that the daylength during the growing season has an effect on the hardiness of lucerne plants in the subsequent winter. To see if such hardening conditions could vary the degree of hardiness of ryegrasses, an annual, biennial and two perennial ryegrasses were exposed to the following experimental conditions.

Fig. 6.8 Mean converted relative conductivity of roots and shoots of Pax Øtofte unhardened (A), hardened under 8 hour daylength and +7°C (B) hardened under 16 hour daylength and +7°C (C).

◎ Roots

▲ Shoots

FIG. 6.8


A
B
C
RELATIVE CONDUCTIVITY (CONVERTED)
1·0
0·8
0·6
0·4
0·2
0
0
-2
-4
-6
TEMPERATURE °C

Experiment 6.15

The effect of daylength on the hardiness of an annual, Italian and Perennial ryegrasses at a temperature conducive to growth

Seedlings of Dutch Westerwolds, Tetila tetraploid, Pax Øtofte and S23 were transferred to growth cabinets (Type A) at 8 and 16 hours daylength at 20°C. Prior to this they had been grown in a heated greenhouse under natural daylengths (March-April).

4 weeks later, they were removed from the cabinets and subjected to the following range of temperatures: 20°C, 0, -2 -4, -8°C. Ten plants were removed at each temperature and were planted in 4" pots, 5 plants per pot. They were allowed to continue growth in the heated greenhouse, and weekly tiller counts were taken for 3 weeks, and the rate of tillering was used as a measure of survival ability at the low temperatures.

Due to the large differences between species in tiller number at the time of low temperature treatment, the tiller number at each weekly count relative to the tiller count at day 0 is used as a comparison between treatments and between species. These are represented in table 6.26.

After a period at 0°C long days appear to have a promotive effect on the hardiness of S23. At -4°C, although the means are not significant, there is a suggestion that short days have promoted hardiness except in Tetila tetraploid. Temperature of -8°C gives rise to complete kill in a number of treatments. However, some Westerwolds tillers having been in short days recover after being at -8°C as do some Pax Øtofte plants which had been in long days.

Table 6.26

Relative tiller numbers at weekly intervals after subjection to a range of low temperatures (tiller number at day 0 is 5)

Hardening daylength

		16 hr - photoperiod			8 hr - photoperiod		
	Variety	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
20°C	Westerwolds	6.41	10.70	20.54	6.16	16.08	18.08
	Tetila	6.67	14.67	18.33	5.00	11.00	16.50
	Pax Øtofte	6.00	8.71	13.00	5.87	9.32	12.54
	S23	6.50	12.50	20.25	5.78	13.25	17.25
0°C	Westerwolds	4.76	13.63	15.54	6.00	9.50	12.66
	Tetila	6.17	9.17	14.50	5.25	7.25	11.25
	Pax Øtofte	7.83	14.00	17.42	4.92	10.50	15.08
	S23	6.25	11.75	20.00	5.50	8.33	12.13
-2°C	Westerwolds	5.33	12.59	16.67	4.75	13.71	15.91
	Tetila	6.67	13.08	17.50	4.58	14.62	16.08
	Pax Øtofte	7.39	4.08	16.88	5.00	12.12	13.71
	S23	5.50	12.00	17.25	5.75	14.92	19.58
-4°C	Westerwolds	1.50	3.33	4.25	3.13	10.95	15.08
	Tetila	4.25	9.00	14.58	4.75	4.25	9.00
	Pax Øtofte	1.75	2.29	3.12	5.17	14.66	17.91
	S23	1.00	1.50	3.50	2.00	4.00	7.00
-8°C	Westerwolds	0	0	0	4.60	6.99	11.03
	Tetila	0	0	0	0	0	0
	Pax Øtofte	2.33	2.58	3.83	0	0	2.75
	S23	0	0	0	0	0	0

Westerwolds would appear to be the hardiest of the species and varieties tested at -8°C when the relative tiller numbers are considered. It appears that short days are responsible for this hardiness. As already stated, the means at -4°C are not significantly different. Nevertheless, the relative tiller number of Westerwold plants having been under short day conditions, when subjected to -4°C is almost four times that of plants which were in long days prior to low temperature treatment, when scored 21 days later. This again suggests that short days have promoted hardiness in Westerwolds.

The pattern of low temperature effects in Pax Øtofte is not so clear. At -4°C, the long day treatment has less than one fifth the relative tiller number of the short day treatment. However at -8°C it gives rise to higher relative tiller numbers in the long day than the short day treatment.

This experiment was concerned with photoperiod effects on hardiness. The following experiment was carried out to determine if light intensity, prior to the hardening influenced the eventual cold hardiness of the grass plant.

Experiment 6.16

The effect of light intensity prior to hardening on the cold hardiness of Pax Otofte seedlings

Seedlings of Pax Otofte which, when at the third/fourth visible leaf stage, were potted and placed in two growth cabinets (Type A). Both were at 20°C but one had a light intensity of 22.54 W/m^2, the other, 4.09 W/m^2. Two weeks later, the plants were transferred to the growth cabinet at 5°C under a 16 hour day and remained under those conditions for two weeks. They were subsequently tested for hardiness at +5°C, -2, -5 and -8°C.

The results of the conductivity measurements are presented in table 6.27.

Table 6.27

Mean relative conductivity (10 plants/ treatment)

	High Light	Low Light
+5°C	.34±0.04	.41±0.04
-2°C	.34±0.03	.35±0.02
-5°C	.70±0.04	.65±0.05
-8°C	.73±0.05	.64±0.05

At any of these temperatures, the two treatments are similar ie. light intensity does not have an effect on the release of solutes of the plants.

The light intensity difference, however, had an effect on plant growth. Plants which were left in the cabinet when others were transferred to the short day conditon were removed at the same time as the hardened plants were tested, that is, they had been under the two light intensity conditions for 4 weeks.

These were separated into roots and shoots, and dry weights were taken (table 6.28).

Table 6.28

	D Wt of Roots	D Wt of Shoots
High light intensity	2.279 ± 0.024 mgs[a]	7.20 ± 0.73 mgs[c]
Low light intensity	1.36 ± 0.259 mgs[b]	4.25 ± 0.45 mgs[d]

The low light intensity has given rise to significantly smaller roots and shoots, the higher light intensity treatment having mean root and shoot weights of almost twice those of the low light treatment.

It should be noted however, that those plants had been growing under these light conditons for twice as long as the plants used in the low temperature treatments. Nevertheless, the plants which were used in the experiment were morphologically different, the low light plants having paler foliage, longer leaf sheaths and had evidence of slight etiolation.

The argument that carbohydrate level is important in determining the degree of hardiness of a plant is not confirmed in the previous experiment. However, damage may not be the important criterion of assessment of hardiness when considering the influence of carbohydrate level on the cold tolerance of plants. Survival may be a more important gauge of hardiness in this instance as utilisation of reserves may be a determining factor in the recovery and regrowth of a plant which has been subjected to low temperatures.

The following experiment involving clipping and its effect on hardiness was carried out to determine whether there was evidence to substantiate this argument. It was appreciated that

clipping and low light intensity may not have identical effects on carbohydrate levels. Nevertheless, clipping has been shown to reduce the level of carbohydrates in the plant (Cranfield, 1935; West, 1962; Harum pers. comm.), particulary in the stubble.

Experiment 6.17

The effect of clipping on the cold hardiness of Pax Øtofte and Tetila tetraploid

Seedlings of Pax Øtofte, and Tetila tetraploid were planted in 4" pots, (4/pot) and when most of the plants were at the 7th expanding leaf stage, and had been tillering for some time. 8 pots of Pax Øtofte and Tetila tetraploid were clipped at a level 3.5 cms from the vermiculite surface. Another 8 pots of Pax Øtofte and Tetila tetraploid were left intact. One week later, the plants were transferred from the heated greenhouse to the growth cabinet (Type C) at +5°C and 16 hour daylength. They were kept under those conditions for three weeks.

The plants were tested for cold hardiness at the following temperatures ie. +5°C, 0°C, -5°C and -10°C and were then repotted (4 plants/pot) and were placed in the greenhouse. Tiller counts were taken immediately after the cold treatments and at weekly intervals for 3 weeks.

Table 6.29

Relative increase in tiller number at weekly intervals after subjection to low temperatures (Day 0 = 100)

	Intact		Defoliated	
Day 7	Pax Ø toffe	Tetila tetraploid	Pax Ø toffe	Tetila tetraploid
+5°C	107.85	123.10	134.15	134.60
0°C	115.950	119.950	105.70	109.150
-5°C	109.40	111.85	111.550	102.50
-10°C	0	0	0	0
Day 14				
+5°C	133.98	128.05	138.54	142.92
0°C	130.85	116.25	105.7	111.65
-5°C	122.70	158.25	116.85	50.25
-10°C	0	0	0	0
Day 21				
+5°C	152.45	134.75	171.00	175.40
0°C	133.35	114.75	120.05	153.35
-5°C	128.05	155.10	119.15	46.50
-10°C	0	0	0	0

The results in table 6.29 reveal that there are no varietal differences with respect to pattern of the tiller production after L.T. treatment when relative tiller number is considered in intact plants. The analysis of variance shows that defoliation reduces the combined hardiness of the two cultivars at -5°C at all tiller counts after day 0. Defoliation also has a significant effect on relative tiller number at day 7 after being at 0°C, but at subsequent counts, the difference is not significant.

Significant interactions at -5°C when examined more closely reveal that it is Tetila totraploid which has a significantly lower relative tiller number in defoliated plants than in those which remain intact. Therefore, tetila tetroploid is influenced by defoliation whereas Pax Øtofte is not, under the conditions of this experiment.

At -10°C, the plants of both species do not recover and no tillers survived as a consequence. It would seem, therefore, that -5°C is a suitable temperature to differentiate effects.

In order to study further the differences in hardiness between perennial and Italian ryegrass, S23 and Tetila tetraploid were employed to this end in the following experiment. In experiment 6.3 it had been demonstrated that after 2 weeks of hardening, these two cultivars were similar in hardiness, although the perennial ryegrass cultivar was slightly more hardy than Tetila tetraploid at -6°C when survival was employed as the criterion for assessing hardiness.

Experiment 6.18

The relative hardiness of S23 and Tetila tetraploid

3 week old seedlings at the 3rd visible leaf stage (2 expanded leaves) having been growing in the heated greenhouse under natural light conditions (in July) were transferred to the growth cabinet (Type C) at 5°C under long days. A number of plants (15) were removed at intervals and subjected to low temperatures. On the day the transfer took place, and two weeks later, the temperatures employed to test hardiness were +5°C and -4°C, and after 3 weeks under the low temperature conditions, the range was +5, -2, -4 and -6°C. At this time, the roots and shoots were considered separately.

The relative conductivity of the treatments at day 0 and after 14 days hardening are given in table 6.30. The relative conductivities after 3 weeks hardening are in table 6.31.

Table 6.30

Mean Relative Conductivity for each treatment at each temperature at 0 weeks and 2 weeks after transfer to hardening conditions

	0 weeks		2 weeks	
	+5°C	-4°C	+5°C	-4°C
S23	0.45 ± 0.03^{a}	0.86 ± 0.03^{b}	0.43 ± 0.01^{c}	0.75 ± 0.02^{d}
T.T.	0.44 ± 0.03^{a}	0.93 ± 0.02^{b}	0.39 ± 0.02^{c}	0.85 ± 0.02^{e}

Means in the same column without a common superscript are significantly different at 5% level.

The mean relative conductivity for S23 at -4°C after 2 weeks is significantly lower than that for Tetila tetraploid, whereas at the beginning of the hardening treatment the difference in

hardiness was not significant. After 3 weeks at low temperatures (table 6.31) when the roots and the shoot were considered separately the two species had similar mean relative conductivities at each of the temperatures at which hardiness was tested.

Therefore it would seem that S23 hardens at a faster rate than Tetila tetraploid but after three weeks at +5°C under long days, they are both at similar levels of hardiness at the various temperatures at which hardiness was tested.

Absolute rather than converted figures are used to represent relative conductivity as the data for roots were too variable after preliminary conversion to give meaningful results. This was due to the converted figures being negative in a number of instances, particularly at the low temperatures. The shoots under hardening conditions for three weeks have similar levels of hardiness in the two species. This contrasts with the relative conductivity figures after two weeks where at -4°C the Tetila tetraploid plants had suffered greater damage than S23.

Table 6.31. Mean relative conductivity after 3 weeks at +5°C

	Roots		Shoots	
Low temp. treatment	S23	T.T.	S23	T.T
+5°C	0.79±0.03	0.74±0.02	0.29±0.02	0.26±0.03
-2°C	0.75±0.03	0.81±0.04	0.34±0.03	0.40±0.03
-4°C	0.86±0.04	0.92±0.04	0.54±0.04	0.51±0.02
-6°C	0.96±0.03	0.94±0.04	0.79±0.02	0.77±0.03

Discussion

In experiment 6.1 removal of plants from pots and subjection of those plants to a subzero temperature for one hour gave rise to a similar mean relative conductivity as that for plants remaining in their pots and kept at the same temperature for 72 hours. Therefore by removing plants from their pots, they are capable of being affected by low temperature to the same extent as being left in pots but much more quickly. Lorenzetti et al. (1971) have found that if plants are left in pots for a period of 72 hours at -8°C it is sufficient for a temperature effect to differentiate varieties of lolium perenne. Thomas and Lazenby (1968c) found 12 hours at -9°C was sufficient to drop the temperature of the soil in pots to approximately that of the air. Both of the instances referred to involved plants in soil boxes. However in experiment 6.1, 3.5 inch pots were used ie. of less bulk than soil boxes. Therefore 72 hours would be a sufficiently long period to assess the effect of temperature on the plants.

Although electrolyte release is a method far removed from hardiness assessment in the field, based on survival, yield or visual means, some workers have found the two to be highly correlated. Emmert and Howlett (1953) found that degree of hardiness of some apple varieties in the field in autumn corresponded closely with the percentage release of electrolytes after subjection to cold treatment. However, although it may be desirable to assess the ability of a plant to withstand winter conditions by rapid means in the laboratory, this may not always be possible. The laboratory method is usually an assessment of cold hardiness, whereas the ability of a plant to

withstand winter conditons also involves other factors as has already been mentioned. Nevertheless, it would seem that cold hardiness is a major factor in winter hardiness (Lorenzetti et al., 1971). The limitations of laboratory techniques in assessing winter hardiness have been discussed by Dexter (1956).

Therefore as most of the experiments in this study have been concerned with cold hardiness rather than winter hardiness, the electrolyte release method has been compared to survival of plants after they have been subjected to low temperatures rather than to plants which have overwintered in the field. As a consequence experiment 6.2 was carried out. It was confirmed that plant death and relative conductivity were associated. The temperature range which gave rise to high percentage kill corresponded to that which was responsible for high relative conductivity values ie. a temperature between $-2^{\circ}C$ and $-4^{\circ}C$ in unhardened Pax Øtofte plants.

The relative hardiness of an early and late perennial ryegrass and an Italian ryegrass cultivar

At -4°C and -6°C, Pax Øtofte has a greater ability to survive than the other two cultivars. However, in a preliminary experiment carried out under sward conditions, little difference was found between Pax Øtofte and S23 when percentage cover was the criterion of assessing winter hardiness. In that experiment, both cultivars had 18 out of 32 assessments greater than 50% cover. However, as winter hardiness involves more than just ability to withstand low temperatures, there may be other factors which compensate for the relatively lower cold hardiness of S23. Alternatively, the stage of growth may have an effect on cold hardiness in the field. In this experiment, the plants were at an early developmental stage in comparison to plants in the sward.

The regional trials of the national Institute of Agricultural Botany have shown S23 to be hardier than Pax Øtofte. However, it is possible that lack of winter hardiness has been confounded with lack of persistency, a characteristic of Pax Øtofte. Therefore the results from these controlled environment experiments have to be interpreted with caution when attempting to relate the findings of such experiments to the field situation. This has been found by Lorenzetti et.al. (1971) with a range of ryegrasses. Although a number (under controlled conditions) corresponded in hardiness to their behaviour in the field, there were some exceptions eg. the Northern European types were less hardy than expected in the field, though they had the greatest tolerance under controlled conditions.

The apparent lack of hardiness in the Italian ryegrass cultivar is in accordance with field observations (Hunt, 1963;

wit, 1955) and this has been one of the characteristics which have been considered to contribute to its biennial habit. Therefore in comparison to Pax Stoffe, the results are similar to what could be expected in the field. While S23 would seem to be the variety which is behaving differently to its performance in the field, the data of experiment 6.3 do not offer any explanation for this.

Hardiness in Autumn

In September, the damage bought about by low temperatures was severe. It is unlikely that the differences in relative conductivity at -6°C are real. The damage is high even in the least damaged plants. Therefore it is unlikely that at -6°C any of the plants would have survived.

By mid-October, there is a suggestion that the low nitrogen treatment is influencing hardiness ie. the relative conductivity at -3°C at the low nitrogen level is the lowest and the number of plants bearing adventitious roots is the highest at the lowest nitrogen level. Wilkinson and Duff (1972) at Michigan comparing high and low levels of nitrogen on the induction of hardiness throughout the year have found that even when hardiness is not induced, the high nitrogen levels have greater relative conductivity than low nitrogen.

Comparison in hardiness between short days and low temperatures to natural conditions in September

Low nitrogen gives rise to greater survival and lower relative conductivity at -6°C in the short day-low temperature treatment compared to the low nitrogen treatment, under outside conditions. This suggests that under low nitrogen conditions, short days and low temperatures have a promotive effect on

hardiness of Pax Øtofte in comparison to natural conditions encountered in September in the West of Scotland.

High nitrogen treatment is less affected by the artificial conditions imposed. By considering the high nitrogen treatments together at -6^{o} and comparing to the total low nitrogen treatments at $-6^{o}C$, the two N levels do not differ significantly. However grouping the two nitrogen treatments together in the outside treatment and comparing to the two nitrogen levels under short day, low temperature conditions, the difference is significant at $-6^{o}C$. This, therefore, suggests that the low temperature, short daylength conditions hasten the induction of hardiness in comparison to the natural conditions in September (daylength of 12-13 hours).

There does not seem to be any comparable literature on the comparison of artificial and natural conditions for inducing hardiness under varying N levels. However regarding the effect of nitrogen on the rate of hardening, where the relative hardiness has been monitored throughout the year, in particular the autumn period, there is a time when the low nitrogen is hardening at a faster rate than the high nitrogen treatment. (Wilkinson and Duff loc.cit.). Even in midwinter when, presumably the plants are at their hardiest, the Pax Øtofte plants at low nitrogen level have lower relative conductivities at $-8^{o}C$ when sampled at the end of December. Therefore, the nitrogen effect would seem to persist at this point in winter.

Assessment of hardiness over the winter period in the West of Scotland

The difference in results between treatments in the experiments are not significant irrespective of which criteria were employed to assess effect of the previous winter on the hardiness of the plant. From an examination of the means for each treatment it can be seen that the differences between treatments are high in some instances, eg. mean number of dead tillers per plant at 0N is 74.08 whereas at 3N it is 161.00. The reason for these large differences in means not being significant could be due to the high degree of variation between blocks within treatments. This may have been due to insufficient replication and also the difficulty in interpreting which class a tiller should be placed. Although a Latin square may not have been the best design in retrospect, as the plots were laid out in an area which was partially surrounded by a building, and walls, it was considered that gradients could have been created along and across the plots. So a Latin square seemed most suitable experimental design to adopt under those circumstances.

The number of dead tillers are not only highest at the 300 units of N level but the percentage of dead tillers at this level is also highest. However, the 200 units of N/acre treatment is the lowest at 17.5%.

Regrowth tiller number is highest at the 3N level as is the percentage of regrowth tillers when related to the tillers which have survived over the winter. It was considered that the regrowth tiller number was dependant on the number of tillers surviving over the winter, hence the regrowth was presented in this way.

Autumn supplied N has been found to increase winter tiller production in P.pratense (Ledeboer and Skogly, 1973; Powell et al., 1967) without winter death of tillers being drastically increased, the temperature in some instances being as low as -23°C (Powell et al., loc. cit.).

Although tiller death is higher at the high nitrogen level in the experiment carried out, the effects are much less than those found by McCloud and Creel (1958). In Bermuda grass, they counted more than 304,000 plants per acre when no nitrogen had been applied the previous year. 244lbs/acre of N the previous year gave rise to only 76,000 tillers at the end of the winter ie. less than $\frac{1}{4}$ the number in the low N treatment.

The plants in this experiment were not clipped, therefore the adverse effects of nitrogen on the soluble carbohydrate content of the plant, (Reviewed by Davidson, 1968) may not be so severe, resulting in a less marked effect on tiller survival than may have occurred had the plants been lower in carbohydrates. Ledeboer and Skogly (1972) and Powell et al. (1967) argue that with N being available over the winter due to autumn application, the plants tend to grow more under the low temperature conditions of winter than low N treated plants. Therefore photosynthesis is allowed to continue and as respiration is low at these low temperatures, they consider that N actually can enhance the carbohydrate status for a period over the winter. Wilkinson and Duff (1972) found nitrogen applied in mid-autumn had a greater detrimental effect on the hardiness of P.pratensis than early or late autumn applications. This also could be explained on the basis of time of N application in relation to rate of plant growth. If growth is stimulated when the plant is growing at a rapid rate, then it could be considered that the

high N is giving rise to a depletion of carbohydrates in the roots, as the carbohydrate otherwise destined for the roots could be utilised for new shoot growth. So less carbohydrate reserves are possessed by the plant when entering the winter phase.

Although 3N gave rise to a higher regrowth tiller number, than the other N levels, it suffered most with respect to winter damage of existing tillers.

Therefore, there are possibly two effects of N on the hardiness of grasses.

1. the detrimental effect on survival of tillers.
2. the positive effect on regrowth in spring.

2N treatment gives rise to the lowest percentage of over-wintering tillers to die whereas 3N has the highest level of tiller death. 3N also, however, has the highest tiller regrowth. Therefore, it would seem that the nitrogen effect on hardiness with which it has been associated is prominent between 200 and 300 units of N per acre per annum.

Nitrogen effects on cold hardiness of S321 and Argo

Nitrogen has given rise to decreased hardiness in S321 whereas Argo is not affected. Argo is also hardier than S321 at the time of year the tillers were taken for samples (ie. in October). The relative low cold hardiness of S321 is in agreement with what has been found when S321 has been compared to other L.perenne cultivars, (Iorenzetti, et al., 1971). On the other hand Argo has been bred in Poland and has been selected to withstand the rigorous winters of that country.

Smith (1964) states that high levels of nitrogen are more likely to have an adverse effect on plants which are not inherently very frost hardy. He, however does not state any

references to substantiate the claim. Nevertheless the results comparing S321 and Argo would agree with his hypothesis.

If it is assumed that the conditions at the end of October in the East of Scotland tend towards the optimum for inducing hardiness in S321 whereas Argo may require conditions similar to autumn conditions in Poland for optimum hardening of that cultivar, then this may explain why N has only an effect on S321. It is possible that the effects of nitrogen on the induction of hardiness will only be manifest when the plants are under conditions which are similar to or tend towards the optimum for hardiness of that cultivar. This is reinforced by the experiment in which Pax Øtofte was compared under natural September conditions and under artificial hardening conditions (Experiment 6.4.c). The nitrogen effect was manifest in the latter situation only ie. conditions which had a positive effect on the induction of hardiness.

The results of such experiments are contrary to what has been found in the field in the previous experiments. Where different N levels were applied in nutrient solution, the low nitrogen level had a consistently adverse effect on the induction of hardiness.

The two nutrient solutions ie. low N and high N, could be considered very low and normal respectively as the 'low' N solution was only $\frac{1}{5}$N of the 'high' level, the latter having an N level equivalent to the normal Hoaglands concentration. Therefore the low N could be considered to be at deficiency levels. This level of N could be having an adverse effect on hardiness, so the N effect could be real.

However, chloride was used to replace the nitrate in the $\frac{1}{5}$N solution. Therefore the effect of Cl^- cannot be discounted. It is possible that the plant, having access to high concentrations of Cl^- took up Cl^- and this could have had a physiological effect. *L. perenne* and *L. multiflorum* have been found to have a high tolerance to Cl^- ions when yield is measured (Cordukes and Parups, 1972). However, the effect of Cl^- may be physiological and could be influencing the permeability of the cells or some other effect in hardiness.

Effects of low temperature on the induction of hardiness of ryegrass

Low temperature ie. 7°C, has a pronounced effect on the induction of hardiness in ryegrass. The two cultivars under test are seen however, to behave differently. Pax Øtofte, after 10 days at +7°C, survives better than S23 after having been frozen to -7°C.

That low temperatures are capable of inducing hardiness has been well documented (reviewed by Levitt, 1956), and they have been seen to induce hardiness in ryegrasses. (Iorenzetti et al., 1971). Although temperatures above, but close to, 0°C are generally employed in inducing hardiness, eg. Iorenzetti et al. using +2°C to harden ryegrass plants, +7°C does have an inducing effect on hardiness.

Tysdal (1933) with alfalfa, has found that temperatures during hardening do not have to be as low as +2°C, though hardening is more rapid at the latter.

The effect of daylength on the induction of hardiness in Pax Øtofte

When Pax Øtofte was subjected to +7°C under 16 hour daylength for 2 weeks, the degree of hardiness was similar to that of the cultivar under 8 hour daylength, other conditions being the same. The total number of live tillers 3 weeks after the cold treatment was a measure of hardiness.

In the following experiment, when relative conductivity was the criterion for hardiness 3 weeks of hardening was also similar for long and short days. However, after one week under the two daylengths at +7°C, the long days gave rise to a lower relative conductivity than the short days at -4°C.

This promotive effect of long days on hardiness has also been found by Lawrence et al. (1973) studying four varieties of L.perenne. During hardening, when the daylength was 8 hours, at 39.0 W/m^2 light input, mean tiller survival was 34%. 14 days after freezing, whereas a 16 hour daylength at 39.0 W/m^2 input gave rise to 52% survival. It could be argued that total light input over the period of hardening was greater in the long day. However, when the long day unput was 19.5 W/m^2 ie. half the input of light at any one time but giving equal doses of light at 16 hours and 8 hours the percentage survival was still higher than in the short days ie. 40%. Khalin (1970) has found that long days also have an important effect on the induction of hardiness on a number of Polish ecotypes of grass species. The ecotypes of Festuca pratensis and Dactylis glomerata having optimum daylengths of 24 hours for the induction of hardiness. A Norwegian worker has found that long days promote hardiness in red clover (Sjøseth, 1964; 1971).

Dexter (1933) has also found that a 15 hour day at 0°C has a greater effect on the promotion of hardiness than a 7 hour

day under the same conditions, in wheat. This reference has been quoted in a number of instances as evidence of short days having a promotive effect on hardiness. However, it was the phase prior to hardening in which Dexter found short days had a greater effect on hardiness that long days.

It is not obvious what adaptive advantage long days at low temperatures promoting hardening bestows on the plant. Hardening takes place in autumn when the days are shortening and temperatures are dropping, therefore it would be expected that short days would have been more effective than long days at low temperatures.

In some grass species, short days seem to induce hardiness in autumn. Klebesadel (1971) by decreasing the daylength in the autumn, has been able to induce southern ecotypes of grass and Kentucky bluegrass to survive under northern conditions in Alaska. A similar situation has been found in southern ecotypes of red clover.

Short days prior to the hardening period ie. during the period of growth have been shown to increase hardiness (Dexter, 1933; Lawrence et al., 1973). It is possible that this effect was responsible for the behaviour of those southern ecotypes grown in the north. Days were shortened in autumn when the plants were still growing. Therefore this may correspond to the period prior to the low temperature phase in which under controlled environment conditions, short days increased hardiness. Therefore there could be two daylength responsive phases in the hardening process, accumulation of carbohydrates will advance the hardiness of plants, ie. long days during low temperatures. Breese and Foster (1972), basing their views on the data of Lawrence et al., consider that the increase in hardiness in long days is due to the length of time over which the plant can accumulate carbohydrate. However,

in Experiment 6.9 the long day comprised 16 hours which consisted of 8 hours at full intensity (22.54 W/m^2) lighting and the other 8 hours at supplementary lighting (2.03 W/m^2) ie. supplementary lighting was too low to have a photosynthetic effect. It is more likely, from these results that the long day effect is physiological rather than photosynthetic. Klebesadel (1964) concluded that phytochrome was involved in the induction of hardiness. However, he was of the opinion that it was the short days rather than long days which induced the effect.

In the experiment carried out to determine the effect of daylength on the hardiness of some ryegrass species at higher temperatures ie. 20°C (Experiment 6.15), when tested at -8°C, the short day effect is seen to be significantly greater than that of long days. This is mostly due to the pronounced effect of short days on the hardiness of Westerwolds. At -4°C, although not significant, short days also have a pronounced effect on survival of Westerwolds ie. greater than three times the number of live tillers 3 weeks after the cold treatment are borne on the short day plants than those having been under long days.

Regarding the other species and cultivars, the effect of daylength is somewhat confused and no pattern due to daylength emerges.

Perhaps the effect of daylength during the growing phase on hardiness is not manifest unless succeeded by a period at low temperatures, as was found by Lawrence et al. .

The behaviour of Westerwolds after being at -8°C is difficult to explain. It is unlikely that the plants of this treatment did not receive a similar chilling treatment to the others, as they were a) divided into two batches and randomly distributed among the other treatments and b) only the centre of the chamber was used for chilling ie. all the treatments were within a small

area in the cryostat, so temperature gradients would have been minimal. Therefore, short days may have, in fact, been responsible for inducing hardiness. Westerwolds, if autumn sown, is capable of withstanding British winters (Hunt, 1962). Being an annual, differing from the other three cultivars tested in this experiment, it may have a different inherent hardening system, and so may be capable of hardening under short days. The ecotype differences found in Brome grass and Poa pratensis (Klebesadel, 1971) already discussed, may be akin to this.

The relative hardiness of roots and shoots

Eula and Smith (1954) compared hardiness of different species of legumes by measuring electrolyte release due to damage (specific conductivity) without relating to total electrical conductivity. The different types of tissue which could have had a) differing rates of exosmosis and b) differing total conductivities were not taken into account. When damage to roots and shoots were considered in this study, allowances were made for these.

The differing rates of exosmosis were taken into account by carrying out a time course to ensure that when conductivity was measured the rate of rapid exosmosis had been passed by root and shoot (experiment 6.10). To allow for the different release of electrolytes of roots and shoots at standard temperatures, the relative conductivity of these plant parts was converted (page 189). These procedures were considered sufficient to compare the relative damage of roots and shoots under the conditions imposed in the appropriate experiments.

The hardiness of roots was found to be less than that of the shoots in Pax Ø tofte and S23. This has also been found in Poa pratensis and Agrostis stolonifera (Beard and Olien, 1963). In these species when they had been submerged in an ice sheet in winter for 60 days, the shoots were living at the end of this period whereas the roots had died.

Thomas and Lazenby (1968c) found that the hardiness of shoots in three races of F. arundinacea was less than that for the shoots. This was found by subjecting the roots and shoots independantly to low temperatures, and then scoring the plants for survival. However the possibility of adventitious roots being formed could have

compensated for any root damage, or death which could have occurred Beard and Olien (loc.cit.) found that the plants of Poa pratensis and Agrostis tenuis under the ice sheet were able to survive due to adventitious root formation, replacing the roots which had died. The same may have occurred in the races of Festuca arundinacea.

The relative lack of hardiness of roots in other species has been found in Taxus cuspida (Mityga and Lanphear, 1971) and Juniperus chinensis (Pillet and White, 1969a). So the findings in the experiments involving S23 and Pax Øtofte are in agreement with these findings.

That the roots are less hardy than the shoots could be due to a) an inherent inability to attain a comparable level of hardiness of the shoots or b) the environment of the soil limits the rate of hardening, or c) the root material could be seminal, so may not be a true reflection of nodal root behaviour.

Certainly the temperature of the soil drops at a lower rate than the air, even in pots in growth chambers (Thomas and Lazenby, 1968c). So under controlled environmental conditions, the length of time the roots are at the desired hardening temperature will be less. However, in Juniperus chinensis, by December in Minosota the roots have been found to be unable to withstand temperatures lower than $-10^{\circ}C$ whereas the shoots survived at temperatures as low as $-39^{\circ}C$ (Pillet and White, loc.cit.). By December, it would be expected that the roots would have been at a temperature conducive to hardening for a considerable time. This would suggest they were unable to attain a greater degree of hardening, despite being in an environment suitable for hardening.

Also in the experiments reported in this study, the roots of the hardened plants were not hardier than those of the control

plants, again suggesting that they have not responded to the hardening conditions in the same way as the shoots. The plants employed in these experiments were at the 3rd-4th leaf stage. Many of those wouldhave nodal roots present prior to hardening as nodal root appearance has been found to be first observed 2-3 weeks after germination (McGill, 1974). Therefore, nodal roots would be present prior to hardening.

The comparison between Pax Øtofte and S23 confirms the results of previous experiments ie. that Pax Øtofte under the hardening conditions used in this study is more hardy than S23. It would seem that the difference in hardiness of the plants is due to the shoots rather than the roots as the roots of the two cultivars are similar in hardiness.

Also, the effect of daylength on hardiness found in earlier experiments is confirmed and is found to affect the shoots rather than the roots of Pax Øtofte, the plants under long days having hardier shoots than those under short days.

It would seem that the most important effects of low temperature in determining whether a grass plant lives or dies is due to the hardiness of the shoots. As has been shown by Beard and Olien, (1963) in grasses roots can be replaced as long as the shoots are living. However, they have found these newly formed roots are unable to support the growth of the shoots if the plants are placed in conditions which allow rapid growth. This may also account for the peculiar behaviour of some of the varieties found by Iorenzetti et al. (1971) which only survived for a short time after they had been subjected to low temperature conditions ie. roots may have been killed by the low temperatures and the adventitious roots formed after freezing may have been unable to support the growth of the shoots when placed in the greenhouse.

The effect of light intensity prior to hardening on the hardiness of Pax Otofte

Unlike the effect of light intensity prior to hardening found by Lawrence et al. on the hardiness of ryegrass strains, light intensity was found to have no effect on hardiness of Pax Otofte. However, the method of hardiness assessment in this experiment was the Electrolyte release method whereas Lawrence et al, were using the survial of the grasses after freezing as a measure of hardiness. They found mean tiller survival ranged from 3o to 49% depending on photoperiod as well as light intensity (intensity ranging from 19.5 W/m^2 to 78.1 W/m^2). However, in another experiment where the growing phase had a light intensity of 9.8 - 14.6 W/m^2 and was compared to an intensity of 48.8 W/m^2, there was no difference in hardiness, but the latter treatment had a lower light intensity treatment during the hardening period. At the end of the hardening period the percentage water soluble carbohydrate in both treatments was similar

It would be expected that had light intensity during the growing phase been important in hardiness, the two levels employed in Experiment 6.16 ie. 22.03 W/m^2 and 4.09 W/m^2 would give rise to different effects, particularly when at the latter intensity, plants were exhibiting symptoms of etiolation.

Although there is no evidence to substantiate it, there is a possibility that the effect of low light intensity may not be due to increasing the amount of damage by the low temperature but rather it could be due to lack of carbohydrate having a detrimental effect on regrowth, hence reducing the percentage survival.

The effect of defoliation on cold hardiness

The decrease in hardiness associated with defoliation in Pax Ø tofte and Tetila tetraploid is in agreement with what has been found in alfalfa (Granfield, 1935), oat (West, 1962), red clover (Torrey and Hanson, 1955), tall fescue (Thomas and Lazenby, 1968a), most posture grasses grown in Britain (Welsh Plant Breeding Station (1964a). Other instances of defoliation decreasing hardiness are reviewed by Smith (1964).

Due to this experiment having been carried out under controlled environmental conditions, the results cannot be compared directly to experiments involving defoliation effects on hardiness in the field due to the differences in microclimate which are created by defoliation in the field situation and the multitude of factors which affect overwintering. Therefore this experiment is more a measure of the effect of removal of leaves on hardiness rather the effect of clipping on the ability of a sward to withstand winter conditions.

The time of cutting prior to the cold treatment has been considered important in determining the effect of clipping on hardiness (Granfield, 1935; West, 1962; Thomas and Lazenby, 1968a). West found that six days after clipping, the carbohydrate level in the oat plants was at a minimum, and when the low temperature treatment was applied at this point, the clipped plants were noticably less hardy than the intact plants. In this experiment involving Pax Ø tofte and Tetila tetraploid, the plants were transferred to hardening conditions one week after clipping. Therefore if ryegrass is similar to oat, the carbohydrate level in the clipped plants would be low, hence entering the hardening phase containing a low level of carbohydrates. However, it is possible that during the hardening phase, carbohydrate

built up within the plants. Lawrence et al. found that the percentage water soluble carbohydrate after hardening could be thrice the level prior to hardening but was dependant on the light intensity during hardening. It cannot be assumed, therefore, that in this experiment, defoliation has given rise to a decrease in total carbohydrate causing a decrease in hardiness. Nevertheless there may be relatively less in defoliated than in the intact plants.

The role of water soluble carbohydrate in the induction of hardiness is not clear and differences in levels between hardy and non hardy plants has been argued to be due to differences in utilisation. Treharne and Eagles (1970) consider that hardy plants have a greater content of water soluble carbohydrate due to storage, whereas non hardy or less hardy plants utilise them for leaf expansion.

Comparison in hardiness between Lolium perenne (S23) and Lolium multiflorum (Tetila tetraploid)

The higher degree of hardiness of S23 compared to Tetila tetraploid after one week under hardening conditions may be a reflection of their relative cold hardiness in the field. Emmert and Howlett (1953) found apple varieties which were considered hardiest were more hardy than the less hardy varieties in the autumn. However by mid winter, the various varieties had similar degrees of hardiness. This has also been found in winter wheat varieties (Suneson and Kiesselbach, 1934). National Institute of Agricultural Botany rank S23 slightly higher than Tetila tetraploid for hardiness in the field (Aldrich, 1968). Therefore, rate of hardening in autumn may be a factor determining relative winter damage due to low temperatures.

In experiment 6.4., S321 was less hardy than Argo at the end of October in the East of Scotland. S321, as already stated is one of the least hardy perennial ryegrasses to be bred in Britain. Unfortunately no measurements of hardiness were made in mid winter to determine whether the differences in hardiness persisted. Tetila tetraploid, however, has been found to attain a similar level of hardiness to S23 under artificial environmental conditions having earlier been less hardy. If this were to occur in the field, then it is possible that it is the late autumn frost which is responsible for damage to least hardy grasses rather than the colder conditions of mid winter.

SECTION 7. GENERAL CONCLUSIONS.

Nitrogen and flowering The lack of response to spring nitrogen with respect to flowering by either early or late perennial ryegrass in the field would suggest that, in spaced plants at least, under the range of nitrogen levels likely to be applied in practice, flowering would be unaffected. Certainly the number of flowering heads was greater at the higher nitrogen levels, but the proportion remained similar at high and low N levels. Further study under sward conditions would determine whether this only applies to spaced plants.

Westerwolds under controlled environmental conditions appeared to flower earlier under low nitrogen conditions. It is possible that range of nitrogen level is important in determining the degree of influence on flowering so that in the field a soil would have to be of a very low N-status before flowering was affected.

Despite low-N treatment advancing flowering in the main axis of plants, when the apices of tillers and tiller buds were examined in plants at both N levels which had reached the flowering stage at similar times, the apices of axillary tillers on high-N plants were generally more advanced than apices of corresponding tillers and buds on low-N plants. low nutrient status within the plant may be giving rise to increased competition between apices and other meristems, hence decreasing the rate of development of axillary tillers.

Nitrogen and tillering Except for experiment 3.1, when nitrogen was applied in varying amounts tiller number per plant increased with nitrogen application. Under controlled environmental conditions secondary tiller number was affected more than primary tillers by nitrogen level. Nitrogen also appeared to promote expansion of tiller buds which would otherwise have remained dormant,

or died. This increase in tiller bud expansion was associated with faster prophyll expansion, a greater number of leaves expanding, and a higher number of leaf primordia laid down. This is presumably what would happen under sward conditions when nitrogen is applied.

Flowering and tillering Flowering appeared to exert an influence on the inhibition of tiller bud expansion in perennial ryegrass, particularly Pax Stofte (Experiment 3.2). Nitrogen appeared to compensate for the effect in S23.

Removal of the developing inflorescences in I. temulentum and Westerwolds gave rise to a temporary increase in tillering. Nitrogen also appeared to be able to compensate, at least partly, for this influence of the apex on tiller bud growth in this instance. Evidence was put forward which may implicate plant hormones in the inhibition of tiller bud expansion by the developing inflorescence viz. when tiller buds were freed from inhibition by removal of the developing inflorescence in I. temulentum, the apices of the buds underwent morphogenic change compared to the intact plants whose buds remained vegetative. Further work is necessary to determine to what extent nutrition and hormones are implicated within the plant in the control of expansion of tiller buds by the developing inflorescence. Also the evidence presented was not conclusive regarding the part of the flowering axis which controlled bud expansion. Further work involving measurement of the internodes subsequent to decapitation would have to be carried out to determine this.

Influence of the "apical region" on tiller production Evidence has been presented suggesting that the apical region of vegetative, or newly initiated plants of Westerwolds exerts some control over tiller bud expansion, when the plants have been grown from seeds densely sown. The removal of expanding leaves, from vegetative plants grown under such conditions gave rise to the release of till· buds otherwise suppressed at low nodes. Therefore the grass plant can be considered to resemble dicotyledonous plants in which apical dominance has been found to exist during the vegetative stage.

This phenomenon of apical dominance which could be considered to have been induced in perennial ryegrass because of the dense conditions under which they were grown, may be an explanation for the inability of the first tiller bud to develop, or take a long time to expand in some grasses. An example of this is tall fescue. Tall fescue establishes poorly. Perhaps research directed along such lines may elucidate and overcome this establishment problem in an otherwise useful grass species.

Nitrogen and cold hardiness Under field conditions, nitrogen was found to reduce cold hardiness of grasses;

a) when hardiness was induced artificially in Pax Øtofte in autumn.

b) in Pax Øtofte when tested in mid winter.

However, under controlled environmental conditions the reverse was found to be the case viz. low nitrogen reduced cold hardiness. Further work should involve a wider range of N levels under controlled conditions to determine if there is an

optimum level of nitrogen below and above which hardiness is adversely affected.

Temperature, light and defoliation and cold hardiness The following have been found in this study:

a) Low temperatures around 5°C induced hardiness in comparison to 20°C.

b) Cold hardiness induced by low temperature was achieved at a faster rate under long days.

c) These long days (16 hours) were not necessarily higher light energy than short days (8 hours).

d) Light intensity sufficiently low to give rise to etiolation effects on the grass plants during hardening did not increase the amount of damage brought about by sub-zero temperatures compared to plants hardened under higher light intensity conditions.

e) Defoliation prior to hardening decreased recovery of Tetila Italian ryegrass after subjection to sub-zero temperatures relative to intact plants but did not appear to affect Pax Ø toftе.

f) Roots were influenced, on the whole, less by hardening conditions than shoots. This would suggest that damage by low temperatures in the field may not be due entirely to lack of hardiness in above ground plant parts.

It could be argued that insufficient levels of environmental factors were employed. It would have been desirable to have been able to impose a wide range of levels within an experiment but limitations on the availability of facilities did not always allow this. Hence levels which were judged extreme were usually chosen.

Due to the complexity of the environment under field conditions it would also have been desirable to impose a number of variables on plants within an experiment. However, this can be taken to the extent where in order to handle the results, a mathematical model has to be applied. Nevertheless, an understanding of the degree of interaction between components of the environment is important in extrapolating from controlled environment conditions to the field. Therefore, there is scope for further research in flowering, tillering, and cold hardiness under more complex systems.

Flowering, tillering and ability to withstand low temperatures are factors of perenniality under temperate conditions. Perenniality is considered an important component of persistency of a grass sward, but the contribution of those factors to persistency has not been quantified. A study based on close observation of the behaviour of individual tillers in ryegrass swards of cultivars of varying persistency over a period of years may give rise to a clearer picture of the role of flowering, tillering and hardiness in determining productivity of a grass sward from year to year.

BIBLIOGRAPHY

Adams, W.E. & Twersky, M. (1960). "Effect of Soil Fertility on Winter Killing of Coastal Bermudagrass." Agron. J. 52, 325-326

Adams, W.E. & Stelly, M. (1962) "Fertility Requirements of Coastal Bermuda-grass and Crimson Clover Grown on Cecil Sandy Loam." J. Range Mgmt. 15, 84-7.

Aldrich, D.T.A. (1968). "A Summary of Performance Trial Results for 35 Grass Varieties in Comparison with the Recommended Varieties" in N.I.A.B. Farmers Leaflet No.16 1968/69" J. Natn. Inst. agric. Bot. 11, 293-301.

Anslow, R.C. (1966). The rate of appearance of leaves on tillers of the Gramineae. Herb. Abstr. 36, 149-55.

Arnott, R.A. (1969). The effect of seed weight and depth of sowing on the emergence and early seedling growth of perennial ryegrass (Lolium perenne). J. Br. Grassed Soc. 24, 104-100.

Aspinall, D. (1961). The control of tillering in the barley plant. 1. The pattern of tillering and its relation to nutrient supply. Aust. J. Biol. Sci. 14, 493-505.

Aspinall, D. (1963). The control of tillering in the barley plant. 2. The control of tiller-bud growth during ear development. Aust. J. Biol. Sci. 16, 285-304.

Atkin, R.K. & Barton, G.E. (1971). Role of endogenous growth regulators in the effect of soil temperature on shoot growth in Gramineal. A. Rep. Grassld. Res. Inst. 1970, 59-60.

Baker, H.K. (1956). Note on the influence of previous management on the death of perennial ryegrass during winter. J. Brit. Grassld Soc. 11, 235-7.

Baker, H.K. & David, G.L. (1963). Winter damage to grass. Agriculture, Lond. 70, 380-382.

Baker, H.K. & Chard, J.R.A. (1964). Comparison of different varieties of Italian and Westerwold ryegrasses for early bite. Exps. Prog. 16 Grassld Res. Inst. 1962-3, 57-58.

Baker, H.K., Chard, J.R.A., Davies, J. & Aldrich, D.J.A. (1965). A comparison of ryegrass varieties for early bite production. J. Br. Grassld Soc. 20, 151-155.

Barlow, C. (1965). The use of special-purpose grasses for silage production. 1. A comparison of varieties. J. agric. Sci., Camb. 64, 439-447.

Bean, E.W. (1961). Ph.D. Thesis. University of Reading.

Beard, J.B. & Olien, C.R. (1963). Low temperature injury in the lower portion of Poa annua L. crowns. Crop. Sci. 3, 362-363.

Bömmer, D. (1961). Z. Pl. Zuch. 46, 105. Cited by D.M. Calder in Milthorpe and Ivins (1966), 59-73.

Bradshaw, A.D. (1959). Population differentiation in Agrostis tenius Sibth. 2. The incidence and significance of infection by Epichloe cyphina. New Phytol. 58, 310-315.

Branson, Farrel A. (1953). Two new factors affecting resistance of grasses to grazing. J. Range Mgmt. 6, 165-171.

Breese, E.L. & Foster C.A. (1971). Breeding for increased winter hardiness in perennial ryegrass. Rep. Welsh Pl. Breed. Stn. 1970, 77-86.

Bula, R.J. & Smith, D. (1954). Cold resistance and chemical composition in over-wintering alfalfa, red clover, and sweet clover. Agron. J. 46, 397-401.

Cajlachjan, M.C. (1944). Nitrogenous food as a factor increasing the rate of flowering and fruiting in plants. Compt. Rend. Acad. Sci. U.R.S.S. 43, 75-79. (Biol. Abstr. 19, 17379.)

Calder, D.M. & Cooper, J.P. (1961). Effect of spacing and nitrogen level on floral initiation in cocksfoot. Nature, Lond., 191, 195

Calder, F.W. & MacLeod, L.B. (1966). Effect of cold treatment on alfalfa as influenced by harvesting system and rate of potassium application. Can. J. Pl. Sci. 46, 17-26.

Canfield, R.H. (1939). The effect of intensity and frequency of clipping on density and yield of black grama and tobosa grass. U.S.D.A. Tech. Bull. 681, 32pp.

Caputa, J.L. (1956). (The winter of 1955-6. Its grievous consequences on crops in French Switzerland. 4. Forage plants).
Rev. rom. Agric. 12, 101-103.
(Herb. Abstr. 27, 43).

Carroll, J.C. & Welton, F.A. (1939). Effect of heavy and late applications of nitrogenous fertiliser on the cold resistance of Kentucky bluegrass.
Pl. Physiol., Lancaster, 14, 297-308.

Chatterjee, B.N. (1961). Analysis of ecotypic differences in tall fescue and their agronomic significance.
Thesis for Ph.D. Univ. Reading.

Coffindaffer, B.L. & Burger, O.J. (1958). Response of alfalfa varieties to daylength.
Agron. J. 50, 389-92.

Cook, C.W. & Stoddart, L.A. (1953). Some growth responses of crested wheatgrass following herbage removal.
J. Range Mgmt. 6, 267-70.

Cooper, J.P. (1951). Studies on growth and development in Lolium. II. Pattern of bud development of the shoot apex and its ecological significance.
J. Ecol. 39, 228-270.

Cooper, J.P. (1952). Studies on growth and development in Lolium. III. Influence of season and latitude on ear emergence.
J. Ecol. 40, 352-379.

Cooper, J.P. (1956). Developmental analysis of populations in the cereals and herbage grasses. I. Methods and techniques.
J. agric. Sci. 47, 262-279.

Cooper, J.P. (1964). Climatic variation in forage grasses. I. Leaf development in climatic races of Lolium and Dactylis.
J. appl. Ecol. 1, 45-62.

Cooper, J.P. & Saeed, S.W. (1949). Studies on growth and development in Lolium. I. Relation of the annual habit to head production under various systems of cutting.
J. Ecol. 37, 233-259.

Cooper, J.P. & Calder, D.M. (1962). Flowering responses in herbage grasses.
Rep. Welsh Pl. Breed Sta. 1961, 20-2.

Cooper J.P., Ford, M.A. & Thomas, R.L. (1962). Light and temperature responses for vegetative growth.
Rep. Welsh Pl. Breed. Sta. 1961, 16-17.

Cordukes, W.E. & Parups, E.V. (1971). Chloride uptake by various turf species and cultivars.
Can. J. Pl. Sci. 51, 485-488.

Crowder, L.V. (1959). (Recommendation on growing pastures and fodder crops in a cold climate.)
Agric. trop. Bogota, 15, 35-51.
(Herb. Abstr. 30, 129.)

Crowley, J.G. (1969). Interpopulation hybrids.
Research Report An Foras Talimtais 1969, 19-20.

Davidson, J.L. (1968). Growth of grazed plants.
Proc. Aust. Grassids. Conf. 125-137.

Davies W. (1969). The influence of management on tiller development and herbage growth.
Welsh Pl. Breed. Sta. Technical Bulletin No. 3.

Davies, W. (1972). The pattern of reproductive development and the leafiness of spring and early summer regrowths in two contrasting varieties of ryegrass.
J. agric. Sci., Camb., 78, 27-35.

Davis, L.A. & Laude, H.M. (1964). The development of tillers in Bromus mollis L.
Crop Sci. 4, 477-80.

Delisle, A.L. (1938). Morphogenetical studies in the development of successive leaves in Aster, with respect to relative growth, cellular differentiation, and auxin relationships.
Am. J. Bot. 25, 420-430.

Dexter S.T. (1956). The evaluation of crop plants for winter hardiness.
In Advances in Agronomy, Vol. 8. By A.G. Norman 203-239.

Dexter, S.T. (1930). A method for determining hardiness in plants.
Science N.S. 71, 220-221.

Dexter, S.T. (1933). Effect of several environmental factors on the hardening of plants.
Pl. Physiol. Lancaster, 8, 123-139.

Ellison, L. (1960). Influence of grazing on plant successions of rangeland.
Botan. Rev. 26, 1-78.

Evans, L.T. (1960). Influence of temperature on flowering in species of Lolium and in Poa pratensis.
J. agric. Sci., Camb. 54, 410-416.

Evans, T.A (1953). Management and manuring for seed production in cocksfoot and timothy.
J. Br. Grassld. Soc. 8, 245-266.

Evans, T.A. (1953). The effect of nitrogen application at different dates on the seed yield of pedigree grasses.
J. Br. Grassld Soc. 9, 53-60.

Fisher, R.A. & Yates, F. (1963). Statistical tables for biological, agricultural, and medical research.
6th ed. Oliver and Boyd, Edinburgh.

Fulkerson, R.S. (1967). Alfalfa root reserve studies.
Ann. Report, April 1, 1965 to March 31, 1966.
Page 64, Agricultural Research Institute of Ontario (Herb. Abstr. (1967). 2046).

Gassner, G. (1918). Zeitschr. Bot. 10, 417-30.
Cited by Whyte (1948).

Gott, M.B. (1961). Flowering of Australian wheats and its relation to frost injury.
Aust. J. agric. Res. 12, 547-565.

Gott, M.B., Gregory, F.G. & Purvis, O.N. (1955). Studies in vernalisation of cereals. XIII. Photoperiodic control of stages in flowering between initiation and ear formation in vernalised and unvernalised Petkus Winter Rye.
Ann. Bot. N.S. 21, 87-126.

Grainger, J. (1938). Studies upon the time of flowering of plants. I. The relation of nocturnal translocation to the time of flowering.
Ann. appl. Biol. 25, 1-19.

Grandfield, C.O. (1935). The trend of organic food reserves in alfalfa roots as affected by cutting practices.
J. agric. Res. 50, 697-709.

Grassland Research Institute (1969). Plant and crop interaction with the environment.
A. Rep. Grassld. Res. Inst. 1968, 51-53.

Gregory, F.G., & Veale, J.A. (1957). A reassessment of the problem of apical dominance.
Symp. Soc. Exp. Biol. XI, 1-20.

Halse, N.G., Greenwood, E.A.N., Lapins, P. & Boundy, C.A.P. (1969). An analysis of the effects of nitrogen deficiency on the growth and yield of a Western Australia wheat crop.
Aust. J. agric. Res. 20, 987-998.

Hanson, A.A. & Juska, F.V. (1961). Winter root activity in Kentucky bluegrass (Poa pratensis L.). Agron. J. 53, 372-4.

Hodgson, H.J. (1964). Effect of photoperiod on development of cold resistance in alfalfa. Crop Sci. 4, 302-305.

Howell, John H. & Jung, G.A. (1965). Cold resistance of Potomac orchardgrass as related to cutting management, nitrogen fertilisation and mineral levels in the cell sap. Agron. J. 57, 525-529.

Hunt, J.V. (1962). Comparing yield persistence and nitrogen response of varieties of Italian and Westerwolds ryegrass 1957-1960. Exp. Rec. 3 Grassld Husb. Dep. W. Scotl. agric. Coll. pp.31.

Hunt, J.V. (1971). Productivity of Italian and perennial ryegrass mixtures. J. Br. Grassld. Soc. 26, 41-49.

Jamalainen, E.A. (1954). Overwintering of cultivated plants under snow. Pl. Prot. Bull. F.A.O. 2, 102-5.

Jamalainen, E.A. (1960). Low-temperature parasitic fungi of grassland and their chemical control in Finland. Proc. 8th internat. Grassl. Congr. 1960, 194-196.

Jameson, D.A. (1964). Effects of defoliation on forage plant physiology. In "Forage Plant Physiology and Soil Range Relationships" Ed. H. Stelley. Madison, Am. Soc. Agron. 67-80.

Jameson, Donald A. & Huss, Donald L. (1959). The effect of clipping leaves and stems on number of tillers, herbage weights, root weights, and food reserves of little bluestem. J. Range Mgmt. 12, 122-126.

Jeater, R.S.L. (1956). A method for determining developmental stages in grasses. J. Br. Grassld Soc. 11, 139-146.

Jewiss, O.R. (1972). Tillering in grasses - its significance and control. J. Br. Grassld Soc. 27, 65-82.

Joffe, A. & Small, J.G.C. (1963). A tendency to perennation in the cereals. Nature 198, 768.

Jones, Ll. J. (1958). Winter "burn".
Rep. Welsh Pl. Breed Stn. 1950-56, 100.

Jones, M. (1955). Grass - its production and use all the year round.
J. Br. Grassld Soc. 10, 107-113.

Khalin, G.A. (1970). (Frost resistance of some perennial herbage plants in relation to growth condition).
Trudy prikl. Bot. Genet. Selek. 43, 64-92.
(Herb. Abstr. 41, 2901).

Kirby, E.J.M. & Faris, D.G. (1970). Plant population induced growth correlations in the barley plant main shoot and possible hormonal mechanism.
J. exp. Bot. 21, 787-798.

Kirby, E.J.M. & Faris, D.G. (1972). The effect of plant density on tiller growth and morphology in barley.
J. agric. Sci., Camb. 78, 281-288.

Klebesadel, L.J. (1971). Nyctoperiod modification during late summer and autumn affects winter survival and heading of grasses.
Crop Science 11, 507-511.

Klebesadel, L.J., Wilton, A.C., Taylor, R.L. & Koranda, J.J. (1964). Fall growth behaviour and winter survival of Festuca rubra and Poa pratensis in Alaska as influenced by latitude of adaption.
Crop Sci. 4, 340-341.

Klebs, G (1913). Uber das Verhaltris der Au Benwelt gur Entwicklung der Pflangen.
S.B. Akad. Wiss. Heidelberg, Abt. B, Abh. 5 1-47.
Cited by Gott, et.al. (1955).

Kolosova, A.V. (1941). (Winter hardiness of perennial herbage plants).
Vert-Seljskohoy. Nank Kormodobyvance 69-72.
(Herbage Abstracts,).

Kraus, E.J. Kraybell, H.R. (1918). Vegetation and reproduction with special reference to the tomato.
Oregon Agric. Coll. Exper. Sta. Bull. 149
In "An Introduction to the Principles of Plant Physiology" by W. Stiles. 1950. Methuen and Co. Ltd. London. 464-465.

Kresge, C.B. & Decker, A.M. (1965). Nutrient balance in Midland Bermudagrass as affected by differential nitrogen and potassium fertilisation.
Proc. IX Int. Grassld Congr., Brazil.

Kuperman, F.M. (1936). Ukr. Inst. Zern. Gospod. 4, 147-80. Cited in History of Research in Vernalisation, by R.O. Whyte, in Nurneck and Whyte (1948) 1-38.

Lambert, J.P. (1956). Seed production studies. 1. The effect of nitrogenous fertiliser, applied at different rates and dates on seed production in cocksfoot (Dactylis glomerata L.). N.Z. Jl. Sci. Technol. 37, 432-442.

Lambert, D.A. (1964). The influence of density and nitrogen in seed production stands of S48, timothy (Phleum pratense L.) and S215 meadow fescue (Festuca pratensis L.). J. Agric. Sci. Camb. 63, 35-42.

Lambert, D.A. & Jewiss, O.R. (1970). The position in the plant and the date of origin of tillers which produce inflorescences. J. Br. Grassld Soc. 25, 107-112.

Lamp, H.F. (1952). Reproductive activity in Bromus inermis in relation to phases of tiller development. Bot. Gaz. 113, 413-38.

Langer, R.H.M. (1956). Growth and nutrition of timothy (Phleum pratense). 1. The life history of individual tillers. Ann. appl. Biol. 44, 166-87.

Langer, R.H.M. (1957). Growth and nutrition of timothy (Phleum pratense) 2. Growth of the plant in relation to tiller development. Ann. appl. Biol. 45, 528-41.

Langer, R.H.M. (1959a). Growth and nutrition of timothy (Phleum pratense L.). 4. The effect of nitrogen, phosphorus and potassium supply on growth during the first year. Ann. appl. Biol. 47, 211-21.

Langer, R.H.M. (1959b). Growth and nutrition of timothy (Phleum prantense L.). V. Growth and flowering at different levels of nitrogen. Ann. appl. Biol. 47, 740-751.

Langer, R.H.M. (1972). "How Grasses Grow". Studies in Biology No. 34. Edward Arnold, London. 22-23.

Langer, R.H.M. & Lambert, D.A. (1959). Ear bearing capacity of tillers arising at different times in herbage grasses grown for seed. J. Brit. Grassld Soc. 14, 137-140.

Langer, R.H.M. & Ryle, G.J.A. (1959). The effect of time of sowing on flowering and fertile tiller production in S48 timothy.
J. agric. Sci. 53, 145-50.

Langer, R.H.M., Ryle, M. & Jewiss, O.R. (1964). The changing plant and tiller populations of timothy and meadow fiscue swards. 1. Plant survival and the pattern of tillering.
J. appl. Ecol. 1, 197-208.

Lawrence, T., Cooper, J.P. & Breese, E.L. (1973). Cold tolerance and winter hardiness in Lolium perenne. II. Influence of light and temperature during growth and hardening.
J. agric. Sci., Camb. 80, 341-348.

Ledeboer, T.B. & Skogly, C.R. (1973). Effects of various nitrogen sources, timing and rates on quality and growth rate of cool-season turfgrasses.
Agron. J. 65, 243-244.

Leopold, A.C. (1949). The control of tillering in grasses by auxin.
Am. J. Bot. 36, 437-440.

Levitt, J. (1956). The Hardiness of Plants.
Academic Press Inc. New York.

Livingstone, J.E. & Swinbank, J.C. (1950). Some factors influencing the injury to winter heads by low temperatures.
Agron. J. 42, 153-157.

Lorenzetti, F., Tyler, B.F., Cooper, J.P. & Breese, E.L. (1971). Cold tolerance and winter hardiness in Lolium perenne. 1. Development of screening techniques for cold tolerance and survey of geographical variation.
J. Agric. Sci., Camb. 76, 199-209.

MacColl, D. & Cooper, J.P. (1967). Climatic variation in forage grasses. 3. Seasonal changes in growth and assimilation in climatic races of Lolium, Dactylis and Festuca.
J. Appl. Ecol. 4, 113-27.

Maeda, S. & Ehara, K. (1963). (Physiological and ecological studies on clipping of herbage plants. III. Influence of clipping on the tiller number and regrowth in Itlian ryegrass).
Proc. Crop Sci. Soc. Japan 30, 313-317. (1962).
(Biol. Abstr. 42, 11840 (1963)).

McGill, F. (1974). Ph.D. Thesis. Queen's University of Belfast.

McIntyre, G.I. (1964). Influence of nitrogen on bud and rhizome development in Agropyron repens.
Nature 203, 1084.

Mc Intyre, G.I. (1965). Some effects of the nitrogen supply on the growth and development of Agropyron repens. Weed Res. 5, 1-12.

Mitchell, K.J. (1953a). Influence of light and temperature on the growth of ryegrass (Lolium spp.). 1. Pattern of vegetative development. Physiologia. Pl. 6, 21-46.

Mitchell, K.J. (1953b). Influence of light and temperature on the growth of ryegrass (Lolium spp.). 2. The control of lateral bud development. Physiologia Pl. 6, 425-443.

Mityga, H.G. & Lamphear, F.L. (1971). Factors influencing the cold hardiness of Taxus cuspidata roots. J. Am. Soc. Hort. Sci. 96, 83-87.

Morley, F.H.W., Daday, H. & Peak, J.W. (1957). Quantitative inheritance in lucerne Medicago sativa L. I. Inheritance and selection for winter yield. Aust. J. agric. Res. 8, 635-651.

Murneek, A.E. (1948). History of research in photoperiodism. In Murneek and Whyte. (1948). 39-61.

Murneek, A.E. & Whyte, R.O. (1948). Vernalisation and Photoperiodism. A symposium. Chronica Botanica Co. Mass.

Oakley, R.A. and Westover H. L. (1921) Effect of the length of day on seedlings of alfalfa varieties and the possibility of utilising this as a practical means of identification. J. agric. Res. 15, 599-608.

Olien, Charles, Robert. (1964). Freezing processes in the crown of Hudson Barley, Hordeum vulgare (L., emend. Lam.) Hudson. Crop Sci. 4, 91-5.

Patel, A.S. & Cooper, J.P. (1961). The influence of seasonal changes in light energy on leaf and tiller development in ryegrass, timothy and meadow fescue. J. Br. Grassld Soc. 16, 299-308.

Pillet, H. (1971). Comparison of cold hardiness levels of root and stem tissue. Can. J. Pl. Sci. 51, 193-195.

Phillips, J.D.J. (1970). Apical Dominance. In "Physiology of Plant Growth and Development". Ed. M.B. Wilkins. 165-202. McGraw-Hill. London.

Pillet, N.E. & White, D.B. (1969a). Soil-air temperature relationships and cold acclimation of container grown Juniperus chinensis "Hertyi". J. Am. Soc. Hort. Sci. 94, 453-456.

Pillet, N.E. & White, D.B. (1969b). Effect of soil nitrogen and soil moisture levels on the cold acclimation of container grown Juniperus chinensis "Hertyi". J. Am. Soc. Hort. Sci. 94, 457-461.

Pohjakallio, O., Salonen, A. & Antila, S. (1960). Effect of day-length on winter hardiness of clover. Maatalous ja Koetoiminta 14, 104-111. (Herb. Abstr. 30, 1760.).

Pohjakallio, O., Salonen, A. & Antila, S. (1963). The wintering of cultivated grasses at the experimental farms Viik (60° 10'N.) and Muddusniemi (69° 5'N.). Acta Agric. scand. 13, 109-130.

Powell, A.J., Blaser, R.E. & Schmidt, R.E. (1967). Effect of nitrogen on winter root growth of bentgrass. Agron. J. 59, 529-530.

Prine, G.N. (1963). Measurement of meteorological elements of the microclimate. University of Florida. Agricultural Experiment Stations. Annual Report for the fiscal year ending 30th June, 1963. 51-52.

Purvis, O.N. (1934). An analysis of the influence of temperature during germination on the subsequent development of certain winter cereals and its relation to the effect of length of day. Ann. Bot. 48, 919-955.

Purvis, O.N. & Gregory, F.G. (1937). Studies in vernalisation of cereals. I. A comparative study of vernalisation of winter rye by low temperature and by short days. Ann. Bot., N.S. 1, 569-591.

Rawson, H.M. (1971). Tillering patterns in wheat with special reference to the shoot at the coleoptile node. Aust. J. biol. Sci. 21, 829-841.

Robson, M.J. (1968). The changing tiller population of spaced plants of S170 tall fescue (Festuca arundinacea). Jnl. Appl. Ecol. 5, 575-590.

Robson, M.J. & Jewiss, O.R. (1968). A comparison of British and North African varieties of tall fescue (Festuca arundinacea). 3. Effects of light, temperature and daylength on relative growth rate and its components. J. Appl. Ecol. 5, 191-204.

Rudman, J.E. & Alder, F.E. (1964). The comparative winter production of the North African fescues (Syn I & Syn II), S170 tall fescue and S22 Italian ryegrass. Exps. Prog. 16 Grassld Res. Inst. 1962-63. 88-89.

Ryle, G.J.A. (1963). Studies in the physiology of flowering of timothy (Phleum pratense L.). 4. Effects of shoot age and nitrogen level on the size of the inflorescence. Ann. Bot., Lond. 27, 467-79.

Ryle, G.J.A. (1964a). A comparison of leaf and tiller growth in seven perennial grasses as influenced by nitrogen and temperature. J. Br. Grassld. Soc. 19, 281-290.

Ryle, G.J.A. (1964b). The influence of date of origin of the shoot and level of nitrogen on ear size in three perennial grasses. Ann. appl. Biol. 53, 311-323.

Ryle, G.J.A. (1966). Physiological aspects of seed yield in grasses. In "The growth of grasses and cereals". Proceedings of the Twelfth Easter School in Agricultural Science, University of Nottingham, 1965. Edited by F.L. Milnthorpe and J.D. Ivins. 106-18.

Ryle, G.J.A. (1967). The effects of shading on inflorescence size and development in temperate perennial grasses. Ann. appl. Biol. 59, 297-308.

Ryle, G.J.A. (1970a). Distribution patterns of assimilated ^{14}C in vegetative and reproductive shoots of Lolium perenne and L. temulentum. Ann. appl. Biol. 66, 155-167.

Ryle, G.J.A. (1970b). Assimilate partitioning in an annual and a perennial grass. J. appl. Ecol. 7, 217-27.

Ryle, G.J.A. (1972). Uptake of carbon and supply of assimilates to meristems of L. temulentum. Ann. Bot. 36, 497-

Ryle, G.J.A. & Hesketh, J.D. (1969). Carbon dioxide uptake in nitrogen-deficient plants. Crop. Sci. 9, 451-454.

Seth, J. & Dexter, S.T. (1958). Root anatomy and growth habit of some alfalfa varieties in relation to wilt resistance and winter hardiness. Agron. J. 50, 141-144.

Single, W.V. (1961). Studies on frost injury to wheat. I. Laboratory freezing tests in relation to the behaviour of varieties in the field.
Aust. J. agric. Res. 12, 767-782.

Sjøseth, H. (1964). (Experiments with different cutting times of the aftermath.).
Forskn. Fors. Landbr. 15, 109-16.
(Herb. Abstr., 34, 1571.).

Skripeinskii, VV, (1958). (The relation between the work of organs of vegetative propogation in perennial and annual cereals and the process of seed formation).
Dokl. Aked. Nauk uzbek. S.S.R. 118, 1046.
Cited by Evans, L.T. in "Grasses and Grasslands".
Ed. by C. Barnard. Macmillan and Co., London.
126-53.

Smith, D. (1961). Association of fall growth habit and winter survival in alfalfa.
Can. J. Pl. Sci., 41, 244-251.

Smith, D. (1964). Winter injury and survival of forage plants.
Herb. Abstr. 34, 203-209.

Snedecor, G.W. & Cochran, W.G. (1967). Statistical methods.
6th Ed. Ames, Iowa State University Press.

Snow, R. (1929). The young leaf as the inhibiting organ.
New Rhytol. 28, 345-358.

Sonneveld, A. (1957).
Jaarb. Inst. biol. scheik. Ondery. Landbyewass for 1957, 21.
Cited by Ryle (1966).

Suneson, C.A. & Kiesselbach, T.A. (1934). Differential varietal responses of winter wheat to time of planting.
J. Amer. Soc. Agron. 26, 294-296.

Tavcar, A. (1930). Winterfestigkeit und genetisch bedingte Tieflage der Vegelationspunkte an Getrudepflangen.
Z. Pflangengücht. 15, 63-74.
Cited by Levitt (1956).

Thimann, K.V. (1937). On the nature of inhibitions caused by auxim.
Am. J. Bot. 24, 407-412.

Thomas, W.D. & Lazenby, A. (1968a). Growth cabinet studies into cold-tolerance of Festuca arundinacea populations.
I. Effects of low temperature and defoliation.
J. agric. Sci., Camb. 70, 339-345.

Thomas, W.D. & Lazenby, A. (1968b). Growth cabinet studies into cold-tolerance of Festuca arundinacea populations.
II. Responses to pretreatment conditioning and

to number and duration of low temperature periods.
J. agric. Sci., Camb. 70, 347-353.

Thomas, W.D. & Lazenby, A. (1968c). Growth cabinet studies into cold-tolerance of Festuca arundinacea populations. III. Cold tolerance of roots and shoots.
J. agric. Sci., Camb. 70, 355-360.

Thorne, G.N. (1962). Survival of tillers and distribution of dry matter between ear and shoot of barley varieties.
Ann. Bot., Lond. 26, 37-54.

Torrie, J.H. & Hanson, E.W. (1955). Effects of cutting first year red clover on stand and yield in the second year.
Agron. J. 47, 224-228.

Treharne, K.J. & Eagles, C.F. (1970). Effect of timperature on photosynthestic activity of climatic races of Dactylis glomerata L.
Photosynthetica 4, 107-117.

Tumanov, I.I. (1940). The physiological basis of winter hardiness in cultivated plants. Siljhozgiz, Leningrad.
Herb. Rev. 8, 214-223.

Tysdal, H.M. (1933). Influence of light, temperature, and soil moisture on the hardening process in alfaalfa.
J. Agr. Research 46, 483-515.

van Overbeek, J. (1938). Auxin distribution in seedlings and its bearing on the problem of bud inhibition.
Bot. Gaz. 100, 133-166.

Wassenaar, R. and others (1954). (Frost damage to crop varieties in the winter 1953-54)
LandbVoorl. 11, 413-423.
(Herb. Abstr. 25, 136).

Welsh Plant Breeding Station (1964a). Developmental genetics of leaf production.
Rep. Welsh Pl. Breed. Stn. 1963, 16-18.

Welsh Plant Breeding Station (1964b). Winter hardiness in pasture varieites.
Rep. Welsh Pl. Breed. Stn. 1963, 70-71.

Went, F.W. (1936). Algemeine Betrachtungen über das Auxin-Problem.
Biol. Zbl. 56, 449-463. (Cited by Phillips (1970)).

West, S.H. (1962). Effect of age, regrowth and height of clipping on freeze damage in oats.
Proc. Soil Crop Sci. Soc. Fla. 22, 186-190.

Wilkinson, James F. & Duff, D. Thomas (1972). Effects of late fertilisation on cold resistance, colour and growth of Kentucky Bluegrass.
Agron. J. 64, 345-348.

Williams, R.D. (1970). Tillering in grasses cut for conservation, with special reference to perennial ryegrass. Herb. Abstr. 40, 383-388.

Wilson, J.R. (1959). The influence of time of tiller origin and nitrogen level on the floral initiation and ear emergence of four pasture grasses. N.Z. J. Agric. Res. 2, 915-932.

Withrow, A.P. (1945). Butler Univ. Bot. Studies 7, 40-64. Cited by Murneek in Murneek and Whyte (1948). 83-90.

Wit, F. and others (1955). (Vernalisation in perennial and Italian ryegrass (Lolium perenne and L. multiflorum) Landbouwk. Tydschr. Wageningen 67, 137-147. (Herb. abstr. 25, 890).

ADDENDUM

The following references have been cited in the text but have not been included in the Bibliography.

Evans, L.T. (1964). Reproduction In "Grasses and Grassland". Ed. by C. Barnard. McMillan and Co. Ltd. New York. 126-153.

Harun, R. (1974). Personal communication. Also Ph.D. Thesis, Queen's University of Belfast.

Polwart, A. (1970). PhD. Thesis, University of Glasgow.

Sharman, B.C. (1945). Leaf and bud initiation in the Gramineae. Bot. Gaz. 106, 269-289.

APPENDIX I.

Experiment 3.1

Total tillers (Prior to N application)	d.f.	s.s.	m.s.	F		s.e.(diff)
Total	79	299.8089				
Columns	3	1.1264	0.3754	2.5840	NS	0.1204
Rows	3	8.0184	2.6728	18.4077	**	0.1204
Nitrogen levels	3	0.8044	0.2681	1.8464	NS	0.1204
Error	6	0.8717	0.1452			
Varieties	4	258.6632	64.6658	112.4231	***	0.2681
Vars x Nitrogen	12	2.7217	0.2268	0.3942	NS	0.5363
Error	48	27.6131	0.5752			

Total tillers 1-8-70

Total	79	236699.95				
Columns	3	1191.85	397.28	0.256	NS	12.4557
Rows	3	9446.95	3148.98	2.029	NS	12.4557
Nitrogen levels	3	9298.05	3099.35	1.997	NS	12.4557
Error	6	9308.70	1551.45			
Varieties	4	162402.075	40600.52	47.301	***	10.3582
Vars x Nitrogen	12	3851.825	320.78	0.374	NS	20.7164
Error	48	41200.50	858.34			

Flowering tillers 1-8-70

Total	79	5188.3875				
Columns	3	92.6380	30.88	0.658	NS	2.1663
Rows	3	234.7375	78.246	1.667	NS	2.1663
Nitrogen levels	3	15.4375	5.146	0.109	NS	2.1663
Error	6	281.574	46.929			
Varieties	4	1918.95	479.738	10.233	***	2.4208
Vars x Nitrogen	12	394.75	32.896	0.702	NS	4.8415
Error	48	2250.30	46.881			

Percentage Flowering tillers (Transformed data)

Total	79	4694.556				
Columns	3	42.439	14.146	0.710	NS	1.4114
Rows	3	321.292	107.097	5.376	*	1.4114
Nitrogen	3	313.093	104.364	5.239	*	1.4114
Error	6	119.523	19.92			
Varieties	4	2203.669	550.917	20.001	***	1.8555
Vars x Nitrogen	12	372.424	21.035	1.127	NS	3.7111
Error	48	1322.114	27.544			

Experiment 3.2

	d.f.	s.s.	m.s.	F		s.e. (diff)
Tiller number 13-3-72						
Total	15	23.49938				
Blocks	3	0.51188	0.17063	0.25655	NS	0.5766
Varieties	1	16.60562	16.60562	24.96826	***	0.4077
N Levels	1	0.39063	0.39063	0.58734	NS	0.4077
Interaction	1	0.00563	0.00563	0.00846	NS	0.5766
Error	9	5.98563	0.66507			
23-4-72						
Total tiller number						
Total	15	82.85438				
Blocks	3	19.00688	6.33563	2.23255	NS	1.1912
Varieties	1	18.27563	18.27563	6.43996	*	0.8423
N Levels	1	20.02562	20.02562	7.05663	*	0.8423
Interaction	1	0.00563	0.00563	0.00198	NS	1.1912
Error	9	25.54063	2.83785			
2-5-72						
Total tiller number						
Total	15	144.2975				
Blocks	3	45.7875	15.2625	5.32464	*	1.1972
Varieties	1	1.6900	1.6900	0.58959	NS	0.8465
N levels	1	64.0000	64.0000	22.32774	**	0.8465
Interaction	1	7.0225	7.0225	2.44995	NS	1.1972
Error	9	25.7975	2.86639			
9-5-72						
Total tiller number						
Total	15	457.08937				
Blocks	3	117.17688	39.05896	4.16675	*	2.1649
Varieties	1	0.68063	0.68063	0.07261	NS	1.5308
N Levels	1	244.14063	244.14063	26.04456	***	1.5308
Interaction (VxN)	1	10.72563	10.72563	1.14419	NS	2.1649
Error	9	39.36562	9.37396			

16-5-73

	d.f.	s.s.	m.s.	F		s.e. (diff)
Total tiller number						
Total	15	1429.6175				
Blocks	3	195.0225	65.0075	3.44061	NS	3.0736
Varieties	1	17.2225	17.2225	0.91152	NS	2.1734
N-Levels	1	1020.8025	1020.8025	54.02739	***	2.1734
Interaction (VxN)	1	26.5225	26.5225	1.40374	NS	3.0736
Error	9	170.0475	18.89417			
Percentage of flovering tillers						
Total	15					
Blocks	3	2.6023	1.260	1.2598	NS	0.3391
Varieties	1	1.7420	0.843	92.7314	***	0.2398
N-Levels	1	191.546	92.731	0.8435	NS	0.2398
Interaction (VxN)		1.742	0.843	0.8433	NS	0.3391
Error	9	2.066	0.230			
23*5-72						
Total Tiller number						
Total	15	3050.4575				
Blocks	3	107.2125	35.7375	0.61291	NS	5.3994
Varieties	1	426.4225	426.4225	7.31327	*	3.8180
N-Levels	1	1989.1600	1989.1600	34.11467	***	3.8180
V x N	1	2.8900	2.8900	0.04956	NS	5.3994
Error	9	524.7725	58.30806			
Percentage flowering tillers						
Total	15	1224.7019				
Blocks	3	5.4777	1.8259	1.4097	NS	0.8047
Varieties	1	1206.1729	1206.1729	931.2638	***	0.5690
N-Levels	1	0.6972	0.6972	0.5382	NS	0.5690
V x N	1	0.6972	0.6972	0.5382	NS	0.8047
Error	9	11.6569	1.2952			
30-5-72						
Total tiller number						
Total	15	7715.2700				
Blocks	3	353.7100	117.90333	1.32381	NS	6.6732
Varieties	1	1887.9025	1887.9025	21.19717	**	4.7187
N-Levels	1	4671.7225	4671.7225	52.45361	***	4.7187
V x N	1	0.3600	0.3600	0.00404	NS	6.6732
Error	9	801.575	89.06389			

	df	ss.	ms.	F		s.e.(diff.)
Percentage flowering tillers						
Total	15	2202.0842				
Blocks	3	13.4686	4.4895	1.5715	NS	1.1952
Varieties	1	2156.6736	2156.6736	754.9263	***	0.8451
N-Levels	1	3.1152	3.1152	1.0904	NS	0.8451
V x N	1	3.1152	3.1152	1.0904	NS	1.1952
Error	9	25.7116	2.8568			
8-6-72						
Total tiller number (Pax Øtofte only)						
Total	7	8641.46				
Blocks	3	1750.57	583.52	3.55	NS	12.8180
N-Levels	1	6497.97	6497.97	39.54	**	9.0637
Error	3	492.92	164.30			
Percentage flowering tillers						
Total	15	3193.6052				
Blocks	3	60.1529	20.0509	2.1957	NS	2.1368
Varieties	1	3028.8512	3028.8512	331.6889	***	1.5109
N-Levels	1	10.2080	10.2080	1.1178	NS	1.5109
V x N	1	10.2080	10.2080	1.1178	NS	2.1368
Error	9	82.1857	9.1316			
15-6-72						
Total tiller number						
Total	15	46763.16938				
Blocks	3	5444.44187	1814.81396	1.64117	NS	23.5138
Varieties	1	17404.20563	17404.20563	15.73898	**	16.6268
N-Levels	1	13800.37562	13800.37562	12.47997	**	16.6268
V x N	1	161.92563	161.92563	0.14643	NS	23.5138
Error	9	9952.22063	1105.80229			
Percentage flowering tillers						
Total	15	2275.8340				
Blocks	3	56.8396	18.8465	2.1356	NS	2.1006
Varieties	1	2137.2129	2137.2129	242.1798	***	1.4853
N-Levels	1	2.6082	2.6082	0.2955	NS	1.4853
V x N	1	0.0484	0.0484	0.0054	NS	2.1006
Error	9	79.4249	8.8249			
19-6-72						
Total tiller number						
Total	15	134742.89437				
Blocks	3	20939.60688	6979.86896	3.15070	NS	33.2817
Varieties	1	34400.97562	34400.97562	15.52854	**	23.5337
N-Levels	1	55377.85562	55377.85562	24.99746	***	23.5337
V x N	1	4086.40562	4086.40562	1.84460	NS	33.2817
Error	9	19938.05062	2215.33896			

	d.f.	s.s.	m.s.	F		se(diff)
Percentage flowering tillers						
Total	15	1212.1667				
Blocks	3	62.8006	20.9335	0.7973	NS	3.2630
Varieties	1	856.1476	856.1476	32.6114	**	2.5619
N-Levels	1	43.3622	43.3622	1.6517	NS	2.5619
V x N	1	13.5792	13.5792	0.5172	NS	3.2630
Error	9	236.2773	26.2530			
30-6-72 (S23 only)						
Total tiller number						
Total	7	62313.875				
Blocks	3	13583.375	4527.79167	3.25010	NS	37.3246
N-Levels	1	44551.125	44551.12500	31.97927	*	6.3925
Error	3	4179.375	1393.12500			
Percentage flowering tillers						
Total	7	116.6582				
Blocks	3	101.1623	33.7207	9.3285	NS	1.4142
N-Levels	1	4.6513	4.6513	1.2867	NS	0.8021
Error	3	10.8446	3.6148			
14-7-72						
Total tiller number						
Total	15	238009.33752				
Blocks	3	4340.07250	1446.69083	0.88569		28.5780
Variety	1	75652.50250	75652.50250	46.31585		***20.2077
N-Levels	1	113265.90250	113265.90250	69.34346		***20.2077
V x N	1	30050.22250	30050.22250	18.39730		** 28.5780
Error	9	14700.65753	1633.40417			
Percentage of flowering tillers						
Total	15	941.8104				
Blocks	3	99.2869	33.0956	1.1715	NS	3.7583
Varieties	1	266.8322	200.2225	9.4454	*	1.4142
Nitrogen levels	1	200.2225	266.8322	7.0875	*	1.4142
V x N	1	121.2201	121.2201	4.2910	NS	3.7583
Error	9	254.2487	28.2498			
15-8-72						
Total tiller number						
Total	15	543570.44				
Blocks	3	13189.19	4396.39	0.37	NS	77.0771
Varieties	1	180625.00	180625.00	15.20	**	54.5018
N-Levels	1	163014.06	163014.06	13.71	**	54.5018
V x N	1	79806.25	79806.25	6.71	*	77.0771
Error	9	106935.94	11881.77			

	d.f.	s.s.	m.s.	F		s.e.(diff)
Percentage flowering tillers						
Total	15	728.9926				
Blocks	3	5.6842	1.8947	0.0501	NS	4.2287
Varieties	1	333.7929	333.7929	8.8390	*	2.9901
N-Levels	1	72.0156	72.0156	1.7892	NS	2.9901
V x N	1	0.0756	0.0756	0.0020	NS	4.2287
Error	9	321.8715	35.7635			
Mean tiller dry weights						
8-6-72 (Pax Otofte only)						
Total tillers - mean dry weights						
Total	7	0.0172				
Blocks	3	0.0034	0.0011	0.3793	NS	0.0538
N-Levels	1	0.0050	0.0050	1.7241	NS	0.0381
Error	3	0.0088	0.0029			
Vegetative tillers - mean dry weight						
Total	7	0.0038				
Blocks	3	0.0001	0	0	NS	0.0245
N-Levels	1	0.0018	0.0018	0.0003	NS	0.0173
Error	3	0.0019	0.0006			
Flowering tillers - mean dry weight						
Total	7	0.0522				
Blocks	3	0.0027	0.0009	0.1125	NS	0.0894
N-Levels	1	0.0253	0.0253	3.1625	NS	0.0447
Error	3	0.0242	0.0080			
19-6-72						
Total tillers - mean dry weight						
Total	15	0.044489				
Blocks	3	0.007231	0.002410	1.59	NS	0.0275
Varieties	1	0.021389	0.021389	14.146	**	0.0194
N-Levels	1	0.002186	0.002186	1.445	NS	0.0194
V x N	1	0.000068	0.000068	0.045	NS	0.0275
Error	9	0.013615	0.001512			
Vegetative tillers - mean dry weight						
Total	15	0.008930				
Blocks	3	0.001372	0.000457	2.135	NS	0.0103
Varieties	1	0.000020	0.000020	0.093	NS	0.0073
N-Levels	1	0.005402	0.005402	25.24	***	0.0073
V x N	1	0.000213	0.000213	0.995	NS	0.0103
Error	9	0.001923	0.000214			

	d.f.	s.s.	m.s.	F		s.e.(diff.)
Flowering tillers - mean dry weight						
Total	15	0.109866				
Blocks	3	0.014487	0.004829	0.616	NS	0.0626
Varieties	1	0.001640	0.001640	0.209	NS	0.0443
N-Levels	1	0.022801	0.022801	2.911	NS	0.0443
V x N	1	0.000441	0.000441	0.056	NS	0.0626
Error	9	0.070497	0.007833			
30-6-72						
Total tillers - mean dry weight						
Total	7	0.014875				
Blocks	3	0.003794	0.001265	2.33	NS	0.0234
N-Levels	1	0.009453	0.009453	17.44	*	0.0165
Error	3	0.001628	0.00542			
Vegetative tillers - mean dry weight						
Total	7	0.006486				
Blocks	3	0.000462	0.000154	0.63	NS	0.0156
N-Levels	1	0.005305	0.005305	21.83	*	0.0110
Error	3	0.000729	0.000243			
Flowering tillers - mean dry weight						
Total	7	0.098982				
Blocks	3	0.032913	0.010971	6.83	NS	0.0401
N-Levels	1	0.061250	0.061250	38.13	**	0.0283
Error	3	0.004819	0.001606			
14-7-72						
Total tillers - mean dry weight						
Total	15	0.107186				
Blocks	3	0.027361	0.009120	1.92	NS	0.0487
Varieties	1	0.004761	0.004761	1.00	NS	0.0345
N-Levels	1	0.032220	0.032220	6.79	*	0.0345
V x N	1	0.000100	0.000100	0.02	NS	0.0487
Error	9	0.042732	0.004748			
Vegetative tillers - mean dry weight						
Total	15	0.021578				
Blocks	3	0.001752	0.000584	2.41	NS	0.0110
Varieties	1	0.008464	0.008464	34.98	***	0.0078
N-Levels	1	0.009120	0.009120	37.69	***	0.0078
V x N	1	0.000064	0.000064	0.26	NS	0.0110

	d.f.	s.s.	m.s.	F		s.e.(diff.)
Flowering tillers - mean dry weight						
Total	15	0.240664				
Blocks	3	0.031250	0.010416	1.02	NS	0.0714
Varieties	1	0.080089	0.080089	7.86	*	0.0505
N-Levels	1	0.03629	0.03629	3.56	NS	0.0505
V x N	1	0.001333	0.001333	0.13	NS	0.0714
Error	9	0.091702	0.010189			
SE						
15-8-72 (Regrowth)						
Total tillers - mean dry weight						
Total	15	0.004624				
Blocks	3	0.000811	0.000811	2.82	NS	0.0120
Varieties	1	0.000380	0.000380	1.32	NS	0.0085
N-Levels	1	0.000049	0.000049	0.17	NS	0.0085
V X N	1	0.000784	0.000784	2.72	NS	0.0120
Error	9	0.002600	0.000288			
Vegetative tillers - mean dry weight						
Total	15	0.002624				
Blocks	3	0.000449	0.000149	0.86	NS	0.0093
Varieties	1	0.000371	0.000371	2.14	NS	0.0066
N-Levels	1	0.000086	0.000086	0.50	NS	0.0066
V x N	1	0.000162	0.000162	0.93	NS	0.0093
Error	9	0.001556	0.000173			
Flowering tillers - mean dry weight						
Total	15	0.041056				
Blocks	3	0.000605	0.000202	0.0529	NS	0.0437
Varieties	1	0	0	0	NS	0.0309
N-Levels	1	0.002550	0.002550	0.6679	NS	0.0309
V x N	1	0.003541	0.003541	0.9274	NS	0.0437
Error	9	0.034360	0.003818			
Experiment 3.3						
Total tiller number 27-7-71						
Total	31	2495.715				
Rows	3	746.695	248.899	6.821	*	
Columns	3	549.745	183.249	5.022	*	
Nitrogen levels	3	211.885	70.629	1.936		
Error	6	218.950	36.492			
Decapitation	1	24.500	24.500	0.531	NS	
Decapitation x Nitrogen	3	190.190	63.397	1.374	NS	
Error	12	553.750	46.146			
Total tiller number 12-8-71						
Total	31	6160.27875				
Rows	3	1845.40375	615.13458	15.50	**	3.1538
Columns	3	1304.89375	434.96458	10.94	**	3.1538
Nitrogen levels	3	707.59375	235.86458	5.93	*	3.1538
Error	6	238.72750	39.78792			

	d.f.	s.s.	m.s.	F		se.(diff.)
Decapitation	1	39.16125	39.16125	0.28	NS	4.1278
Decapitation x Nitrogen	3	388.75375	129.58458	0.94	NS	5.8375
Error	12	1635.47500	136.31217			

Increase in tiller number from 27-7-71

Total	31	1419.01875				
Rows	3	282.71375	94.23792	8.890	*	1.6276
Columns	3	222.73000	74.24330	7.006	*	1.6276
Nitrogen levels	3	208.81375	64.60458	6.568	*	1.6276
Error	6	63.58120	10.59680			
Decapitation	1	1.05725	1.05725	0.023	NS	2.3941
Decapitation x Nitrogen	3	89.82375	29.94125	0.653	NS	3.3859
Error	12	550.30500	45.85875			

Percentage increase in tiller number from 27-7-71 to 12-8-71

Total	31	5449.615				
Rows	3	318.901	106.30	.509	NS	7.2239
Columns	3	265.017	88.339	.423	NS	7.2239
Nitrogen levels	3	1610.393	536.797	2.571	NS	7.2239
Error	6	1252.459	208.743			
Decapitation	1	8.757	8.757	0.059	NS	4.2885
Decapitation x Nitrogen	3	228.616	76.205	0.517	NS	6.0645
Error	12	1765.475	147.122			

Total tiller number 28-8-71

Total	31	17655.57875				
Rows	3	3778.33375	1259.44458	11.067	**	5.3327
Columns	3	2942.32400	980.77430	8.6217	*	5.3327
Nitrogen levels	3	3136.44375	1045.48125	9.1905	**	5.3327
Error	6	682.53720	113.75620			
Decapitation	1	172.05125	172.05725	0.31158	NS	8.3079
Decapitation x Nitrogen	3	317.69375	105.89792	0.19178	NS	11.7492
Error	12	6626.19505	552.18292			

Increase in tiller number from 27-7-71 to 28-8-71

Total	31	9257.549				
Rows	3	1507.674	502.558	8.032	*	3.9534
Columns	3	1151.854	383.951	6.141	*	3.9534
Nitrogen levels	3	2132.504	710.834	11.369	**	3.9534
Error	6	375.137	62.522			
Decapitation	1	53.561	53.561	0.354	NS	4.3431
Decapitation x Nitrogen	3	39.814	13.271	0.081	NS	6.1422
Error	12	1810.945	150.911			

Percentage increase in tiller number from 27-7-71 to 28-8-71

Total	31	20015.362				
Rows	3	81402.822	467.607	0.926	NS	11.2299
Columns	3	2825.683	941.894	1.867	NS	11.2299
Nitrogen level	3	7637.635	2545.878	5.046	*	11.2299
Error	6	3026.765	504.460			
Decapitation	1	9.968	9.968	0.02	NS	7.0719
Decapitation x Nitrogen	3	311.182	103.727	0.259	NS	10.0013
Error	12	4801.307	400.108			

APPENDIX II.

Experiment 6.3

Comparison in cold hardiness between Pax Ytoffe, S23 and Tetila tetraploid - relative tiller number

	d.f.	s.s.	m.s.	F		s.e.(diff.)
	Day 7, +7°C					
Total	8	6332.09				
Variety	2	2328.22	1164.1			
Error	8	4003.87	667.31	1.744	NS	14.91
	Day 14, +7°C					
Total	8	25933.70				
Variety	2	3772.67	1886.33			
Error	6	22161.03	3693.51	0.51	NS	35.08
	Day 21, +7°C					
Total	8	66519.84				
Variety	2	25380.54	12690.27			
Error	6	41139.30	6856.55	1.85	NS	47.81
	Day 7, 0°C					
Total	8	17964.08				
Variety	2	11243.80	5621.90			
Error	6	6720.29	1120.05	0.052	NS	19.32
	Day 14, 0°C					
Total	8	12168.80				
Variety	2	373.31	186.65			
Error	6	11795.49	1965.92	0.911	NS	25.6

	d.f.	s.s.	m.s.	F		s.e.(d.f.)
		Day 21, 0°C				
Total	8	21952.0				
Variety	2	5414.62	2707.31			
Error	6	16537.38	2756.23	0.98	NS	30.31
		Day 7, -4°C				
Total	8	3324.00				
Variety	2	1365.24	682.62			
Error	6	1958.76	326.46	2.09	NS	10.43
		Day 14, -4°C				
Total	8	31812.20				
Variety	2	23303.12	11651.56			
Error	6	8509.08	1418.18	8.22	*	21.74
		Day 21, -4°C				
Total	8	65486.23				
Variety	2	38251.34	19125.67			
Error	6	27234.89	4539.15	4.213	NS	38.9
		Day 7, -6°C				
Total	8	20110.86				
Variety	2	19593.27	9796.63			
Error	6	517.59	86.27	113.56	***	5.36
		Day 14, -6°C				
Total	8	35189.04				
Variety	2	33705.11	16852.55			
Error	6	1483.93	247.32	68.14	***	9.08

	d.f.	s.s.	m.s.	F		s.e.(diff)
	Day 21, $-6^{\circ}C$					
Total	8	118489.77				
Variety	2	111523.00	55761.50			
Error	6	6966.77	1161.13	48.02	***	19.67

Day 7, 14 and 21, $-8^{\circ}C$

All values are zero.

Experiment 6.4.d

	d.f.	s.s.	m.s.	F	s.e.(diff.)
	Mean tiller number				
Total	15	287,680.71			
Columns	3	10,608.36	3,536.12	0.30	
Rows	3	91,121.63	30,373.88	2.63	
Nitrogen	3	116,685.61	38,895.20	3.37 NS	
Error	6	69,265.11	11,544.19		
	Mean dead tillers				
Total	15	47,673.78			
Columns	3	5,326.91	1,775.64	0.69	
Rows	3	13,248.36	4,416.12	1.73	
Nitrogen	3	13,743.70	4,581.23	1.79 NS	
Error	6	15,354.81	2,559.14		
	Mean living tillers				
Total	15	187,696.80			
Columns	3	17,780.59	5,926.84	0.55	
Rows	3	50,709.63	16,903.21	1.57	
Nitrogen	3	53,462.82	17,820.94	1.63 NS	
Error	6	65,743.76	10,757.29		
	Mean overwintering living tillers				
Total	15	92,291.14			
Columns	3	9,464.34	3,154.78	0.71	
Rows	3	11,560.24	3,853.41	0.86	
Nitrogen	3	44,490.12	14,830.04	3.32 NS	
Error	6	26,776.44	4,462.74		

	d.f.	s.s.	m.s.	F	se(diff)
Mean no growth tillers					
Total	15	32,669.47			
Columns	3	3,196.45	1,065.48	0.74	
Rows	3	14,581.77	4,860.59	3.36	
Nitrogen	3	6,201.00	2,067.00	1.427 NS	
Error	6	8,690.25	1,448.38		
Mean total overwintering tillers					
Total	15	153,393.84			
Columns	3	11,047.60	3,682.53	0.71	
Rows	3	38,004.03	12,668.01	0.25	
Nitrogen	3	73,438.57	24,479.52	4.753 NS	
Error	6	20,903.64	5,150.61		
Percentage dead of overwintering tillers					
Total	15	739.6484			
Columns	3	127.8606	42.6202	1.64	
Rows	3	210.6199	70.2066	2.70	
Nitrogen	3	245.3398	81.7799	3.149 NS	
Error	6	155.8281	25.9714		
Percentage living of overwintering tillers					
Total	15	726.0384			
Columns	3	147.6106	49.2035	1.89	
Rows	3	195.9688	65.3229	2.51	
Nitrogen	3	226.3792	75.4597	2.901 NS	
Error	6	156.0798	26.0133		

	df	s.s.	m.s.	F	se(diff)
	Regrowth tiller as a percentage of overwintering tillers				
Total	15	808.3167			
Columns	3	118.3380	39.4460	0.93	
Rows	3	362.4187	120.8062	2.85	
Nitrogen	3	72.8323	24.2774	0.57 NS	
Error	6	254.7277	42.4546		
	Percentage dead of total tiller number				
Total	15	745.9062			
Columns	3	128.8081	42.9360	1.32	
Rows	3	222.5749	74.1916	2.27	
Nitrogen	3	198.7704	66.2568	2.031 NS	
Error	6	195.7528	32.6255.		

Experiment 6.15

	d.f.	s.s.	m.s.	F	s.e.(diff.)	
Day 7, +6°C						
Total	15	6.904				
Ryegrasses	3	0.493	0.165	0.434	NS	0.435
Daylength	1	1.918	1.918	5.059	NS	0.308
Ryeg. x Dayl.	3	1.459	0.486	1.283	NS	0.616
Error	8	3.034	0.379			
Day 14, +6°C						
Total	15	134.718				
Ryegrasses	3	49.224	16.408	3.108	NS	1.625
Daylength	1	2.364	2.364	0.448	NS	1.149
Ryeg. x Dayl.	3	40.900	13.633	2.583	NS	2.298
Error	8	42.230	5.279			
Day 21, +6°C						
Total	15	240.556				
Ryegrasses	3	105.877	35.292	2.433	NS	2.693
Daylength	1	15.016	15.016	1.035	NS	1.905
Ryeg. x Dayl.	3	3.596	1.199	0.083	NS	3.809
Error	8	116.067	14.508			
Day 7, 0°C						
Total	15	22.537				
Ryegrasses	3	2.055	0.685	0.604	NS	0.753
Daylength	1	2.806	2.806	2.474	NS	0.532
Ryeg. x Dayl.	3	8.604	2.868	2.529	NS	1.065
Error	8	9.072	1.134			

	d.f.	s.s.	m.s.	F		s.e.(diff.)
Day 14, 0°C						
Total	15	130.494				
Ryegrasses	3	38.610	12.870	2.181	NS	1.718
Daylength	1	42.023	42.023	7.120	*	1.215
Ryeg. x Dayl.	3	2.648	0.883	0.150	NS	2.429
Error	8	47.214	5.902			
Day 21, 0°C						
Total	15	151.422				
Ryegrasses	3	31.546	10.515	2.510	NS	1.447
Daylength	1	66.790	66.789	15.939	**	1.024
Ryeg. x Dayl.	3	19.564	6.521	1.556	NS	2.047
Error	8	33.522	4.190			
Day 7, -2°C						
Total	15	28.361				
Ryegrasses	3	3.277	1.092	0.599	NS	0.955
Daylength	1	8.151	8.151	4.470	NS	0.675
Ryeg. x Dayl.	3	2.345	0.782	0.429	NS	1.350
Error	8	14.588	1.823			
Day 14, -2°C						
Total	15	125.700				
Ryegrasses	3	1.451	0.484	0.036	NS	2.601
Daylength	1	3.285	3.285	0.243	NS	1.839
Ryeg. x Dayl.	3	12.702	4.834	0.313	NS	3.679
Error	8	108.262	13.533			

	d.f.	s.s.	m.s.	F		s.e.(diff.)
Day 21, -2°C						
Total	15	139.742				
Ryegrasses	3	20.436	6.812	0.538	NS	2.515
Daylength	1	2.288	2.288	0.180	NS	1.779
Ryeg. x Dayl.	3	15.798	5.266	0.416	NS	3.557
Error	8	101.219	12.652			
Day 7, -4°C						
Total	15	54.177				
Ryegrasses	3	20.665	6.888	3.074	NS	1.059
Daylength	1	10.709	10.709	4.779	NS	0.749
Ryeg. x Dayl.	3	4.878	1.626	0.726	NS	1.497
Error	8	17.926	2.241			
Day 14, -4°C						
Total	15	389.551				
Ryegrasses	3	72.489	24.163	2.509	NS	2.175
Daylength	1	78.721	78.721	8.174	*	1.552
Ryeg. x Dayl.	3	161.296	53.765	5.583	*	3.103
Error	8	77.045	9.631			
Day 21, -4°C						
Total	15	796.059				
Ryegrasses	3	96.841	32.280	0.807	NS	4.472
Daylength	1	138.474	138.474	3.463	NS	3.162
Ryeg. x Dayl.	3	240.837	80.279	2.007	NS	6.324
Error	8	319.907	39.988			

Experiment 6.17

	d.f.	s.s.	m.s.	F		s.e.(diff.)
Day 7, +5°C						
Total	7	2250.655				
Species	1	123.245	123.245	0.378	NS	12.760
Defoliation	1	714.420	714.420	2.192	NS	12.760
Sp. x Defol.	1	109.520	109.520	0.336	NS	12.760
Error	4	1303.470	325.867			
Day 14, +5°C						
Total	4	3613.680				
Species	1	1.360	1.360	0.002	NS	20.35
Defoliation	1	189.950	189.950	0.229	NS	20.35
Sp. x Defol.	1	108.200	108.200	0.131	NS	20.35
Error	4	3314.170	828.540			
Day 21, +5°C						
Total	7	9580.620				
Species	1	88.445	88.445	0.047	NS	30.610
Defoliation	1	1752.320	1752.320	0.935	NS	30.680
Sp. x Defol.	1	244.205	244.205	0.130	NS	30.610
Error	4	7490.650	1873.913			
Day 7, 0°C						
Total	7	326.689				
Species	1	27.751	27.751	1.437	NS	3.107
Defoliation	1	221.551	221.551	11.474	*	3.107
Sp. x Defol.	1	0.151	0.151	0.008	NS	3.107
Error	4	77.235	19.309			

	d.f.	s.s.	m.s.	F		s.e.(diff.)
Day 14, 0°C						
Total	7	1658.089				
Species	1	37.411	37.411	0.155	NS	10.994
Defoliation	1	442.531	442.531	1.831	NS	10.994
Sp. x Defol.	1	211.151	211.151	0.873	NS	10.994
Error	4	966.995	241.749			
Day 21, 0°C						
Total	7	5287.915				
Species	1	108.045	108.045	0.123	NS	20.955
Defoliation	1	320.045	320.045	0.364	NS	20.955
Sp. x Defol.	1	1346.805	1346.805	1.534	NS	20.955
Error	4	3513.020	878.255			
Day 7, -5°C						
Total	7	214.575				
Species	1	21.780	21.780	0.865	NS	3.549
Defoliation	1	25.920	25.920	1.029	NS	3.549
Sp. x Defol.	1	66.125	66.125	2.625	NS	3.549
Error	4	100.750	25.188			
Day 14, -5°C						
Total	7	12924.029				
Species	1	482.051	482.051	2.593	NS	9.642
Defoliation	1	6480.911	6480.911	34.855	**	9.642
Sp. x Defol.	1	5217.311	5217.311	28.059	**	9.642
Error	4	742.755	185.939			

	d.f.	s.s.	m.s.	F		s.e.(diff.)
Day 21, -5°C						
Total	7	14033.320				
Species	1	1039.680	1039.680	3.712	NS	11.835
Defoliation	1	6903.125	6903.125	24.643	**	11.835
Sp.xDefol.	1	4970.045	4970.045	17.743	*	11.835
Error	4	1120.470	280.118			
Day 7, -8°C						
Total	15	42.308				
Ryegrasses	3	14.929	4.976	170.136	***	0.121
Daylength	1	1.357	1.357	46.401	***	0.085
Ryeg. x Dayl.	3	25.787	8.596	293.872	***	0.171
Error	8	0.234	0.029			
Day 14, -8°C						
Total	15	99.795				
Ryegrasses	3	32.574	10.858	7.378	*	0.858
Daylength	1	4.851	4.851	3.296	NS	0.607
Ryeg. x Dayl.	3	50.596	16.865	11.459	**	1.213
Error	8	11.774	1.472			
Day 21, -8°C						
Total	15	253.188				
Ryegrasses	3	87.429	29.143	9.351	**	1.248
Daylength	1	24.751	24.751	7.942	*	0.883
Ryeg. xDayl.	3	98.077	32.692	10.490	**	1.765
Error	8	24.932	3.116			