EXPERIMENTAL STUDIES ON THE RELATIONSHIP BETWEEN SPIKELET PRIMORDIA AND GRAIN SIZE IN BARLEY

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This thesis is dedicated to my best friend Aidan C Lewis-Smith and to my parents, Joyce M C Lewis and J Denis Lewis. Without their love and continued support and encouragement this thesis would never have been completed.

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I hereby declare that I, Stella M Lewis-Smith, alone have composed this thesis and that, except where indicated, the work peresented here is my own.

ABSTRACT

This project aimed to examine the relationship between spikelet primordium size at the double ridge stage and the final grain weight in two row barley. Earlier work in this laboratory had suggested that the size of prigmordia at the double ridge stage influenced final grain size. This work was repeated to confirm the finding and, whilst the relationship held, the results were quantitativly different due to differing environmental conditions during the later stages of grain development. Subsequent work sought to manipulate primordial width at double ridge stage by altering nitrogen supply in order to determine the generality of the apparent primordium width / grain weight relationship. Results from these experiments led to investigation of the effect of tillers and the application of a growth retardant on the size of spikelets and grain.

Lowered nitrogen supply for the first 40 days after sowing reduced the size of spikelet primordia at the double ridge stage and plant dry weight with the result that smaller grain were produced. Reduced nitrogen supply for only 20 days after sowing led to reduced grain weight in *cv.* Maris Mink but not in *cv.* Proctor. Proctor had larger central florets and set larger grain than Maris Mink. Reduced nitrogen supply during the vegetative stage of mainstem apex development had no effect on spikelet size in Proctor but marginally increased spikelet size in Maris Mink. Plant dry weight was not altered by this treatment. Final grain size was reduced in Proctor but increased in Maris Mink.

The effect of altering the environment on spikelet and grain size was dependent on the timing of tiller production and the rate of spikelet development. This in turn was related to the duration of the particular environmental regime and to the specific developmental response of the cultivar being used.

The removal of tillers resulted in an increase in final grain size but caused no difference in spikelet size at the double ridge stage. Detillered Proctor set larger grain than similarly treated Maris Mink plants. Central floret size was larger in detillered plants than in those of the freely tillering plants. Uniculm plants subject to a low nitrogen regime had reduced grain yield and smaller spikelet primordia and central florets than those grown under high nitrogen conditions. It is proposed that the extent by which the yield of Proctor exceeds that of Maris Mink may be dependent upon the time at which tillers develop. The presence of tillers is shown to reduce the size of mainstem central florets but not spikelet size at stage 4. The effect of reduced nitrogen supply on spikelet primordia at stage 4 appears to be related to the reduced nitrogen supply *per se* rather than to reduced assimilate levels. Floret size is, however, probably a result of both effects.

Treatment with chloro-choline chloride, a growth retardant, caused completely different results in the two cultivars but did appear to reduce plant dry weights. In Maris Mink CCC application caused a short period of intense spikelet development and both central floret size and final grain weight were increased in mainstem apices. Tiller grain weight was substantially increased in treated plants. In Proctor CCC application caused a reduction in the rate of mainstem spikelet development. The size of spikelets and florets in Proctor were similar to those of the controls but final grain weight in mainstem and tiller ears of treated plants were reduced. In both cultivars CCC had a greater effect on apex development the earlier it was applied. Differences in grain weight in this experiment appear to be a result of altered plant growth and development rather than plant size. It is proposed that CCC has two distinct effects on the apex, a short term effect manifest regardless of application time and a long term effect only manifest if the compound is applied sufficiently early in development for the apex to still be susceptible to its action.

CONTENTS

SECTION 1. INTRODUCTION

1.1. CEREAL YIELD AND PRODUCTION

Trends in production

The significant rise in cereal prices during 1973 and 1974 transformed the profitability of cereal production (Challinor, 1981) so that it became an attractive proposition to the farming community. Farmers intensified production of cereals though the area of land under cereal production increased little (Challinor, 1981). In the past 4 decades the two fold increase in wheat and barley yields in the U.K. was due mainly to improved management and the introduction of new cultivars (Riggs, 1984). This increase in yield in barley amounted to an increase of 2.8% a year; 70% of this being due to improved crop husbandry such as the use of fertilisers, herbicides and pesticides (Silvey, 1978). In wheat new cultivars contributed to about half of the yield increase between 1947 and 1977 (Silvey, 1979).

Yield increases in new cultivars were due to an increased harvest index with the biomass remaining relatively constant (Donald and Hamblin, 1976; Riggs, 1983 & 1984; Riggs *et al*, 1981; Bingham, 1983). The harvest index in spring barley increased from 41% in cultivars of the 1950's to 50% in new cultivars (Riggs, 1983). Breeding for high grain yield and short straw indirectly increased the harvest index of new cultivars (Donald and Hamblin, 1976) though a further increase in the harvest index, to 55 - 60 %, may be possible if the leaf area index could be increased at anthesis and the grain filling period lengthened (Riggs, 1984; Austin *et al*, 1980). Hanson *et al* (1985) suggested that high biomass lines could be used as parents in a breeding programme to produce higher yielding cultivars, but the heritability of the high biomass trait was found to be low and associated with weak straw. Breeding

programmes that selected for high yield in barley have however caused a slight increase in biomass (Riggs *et al*, 1981).

Increased yield has led to huge amounts of cereal being produced such that the European Common Market (EEC) became self-sufficient for cereals and during the 1980's had surpluses of approximately 7 million tonnes of wheat and 4 million tonnes of barley (Challinor, 1981). There is an increasing deficit of cereals in third world countries and the Soviet Union has harvests that vary by up to 60 million tonnes (Challinor, 1981) suggesting an available market for this vast grain surplus. However, the price of cereals in the EEC in 1977 was 50% higher than that on the world market and although the European price has declined subsequently, it still remains some 15% higher than world prices (Challinor, 1981). These high prices have reduced the desirability of European cereal and thus the grain mountains have become a common feature of the 1980's. In spite of the increases in yield the cost of the production of a tonne of wheat has not fallen in real terms (Murphy, 1980) since the high grain prices are in part due to high imput costs. The future aim of cereal producers in the EEC must be to reduce production costs without affecting yield. A reduction in input costs would allow the EEC member states to produce grain at a more competitive price on the world market.

There is now a need for a reduction in production costs which considers of the demands of the changing standards in society. Consumers are becoming increasingly health-conscious and there is much concern about nitrates leaching off farm land into water supplies. Equally, fears have been expressed about the harmful effect of many other chemicals being added to field crops. New technology and novel methods are needed in order to both allay public concern and reduce production costs. To achieve this a greater understanding of yield determinants is needed, both in terms of genetic limitations and the requirements of plants for

nutrients and minerals. Plant growth regulators offer a new and potentially powerful tool for increasing yield by modifying plant growth and development (Wareing, 1976).

In order to optimize the timing of agro-chemical inputs, reduce the amounts of chemical required and at the same time minimize wastage and potential pollution to the environment, it is necessary to understand cereal development and growth, particularly in the early stages of development, and the associated physiological processes which have been shown to have a large influence on yield. The initiation and development of spikelet primordia can affect the number of sites available for grain set. Slow initiation of spikelet primordia seems to be associated with a concomitant increase in the number of spikelets initiated (Rawson, 1971). There is also evidance that the length of the apex at the double ridge stage of development is correlated with final grain number (Lucas, 1972).

Determinants of yield

The environment, plays a large part in determining the rate of spikelet initiation and the number produced (Cottrell *et al*, 1981). At high temperatures spikelet initiation rate is fast and final number is reduced (Rawson, 1970; Halse and Weir, 1974). At lower temperatures initiation is slower but more primordia are produced (Cottrell *et al*, 1985).

Daylength is known to affect the number and development of spikelets in barley (Aspinall and Paleg, 1963). The initiation rate and the period during which initiation occurs are faster and shorter in long than in short days suggesting that spikelet production is under photoperiodic control (Cottrell *et al*, 1981; 1982). This control seems to be mediated by gibberellins, the greater amounts produced in long days being associated with rapid initiation rates (Cottrell et al, 1982; 1983).

Low nitrogen given from the 4th day after sowing has been shown to cause a reduction in the number of spikelets per ear in 2- and 6-row barleys and this also gives rise to a reduced number of grains set (Dale and Wilson, 1978).

Genetic differences also affect early spikelet primordium development and this seems to play a role in determining final grain yield (Austin, 1980). Cultivars with fast leaf emergence rates are associated with fewer numbers of spikelets per ear in both barley (Dale and Wilson, 1978) and wheat (Holmes, 1973; Fisher, 1973). When cultivars initiate mainstem leaves before collar formation and spend a longer period in the vegetative phase (Wall and Cartwright, 1974; Pinthus, 1967) more spikelets are subsequently initiated. It has been suggested that final spikelet number may be determined solely by events that occur before the double ridge stage (Lucas, 1972; Thorne *et al*, 1968). However the results of others suggest that this is not necessarily the case (Allison and Daynard, 1976; Rawson, 1970).

The amount of tillering exhibited by a cultivar appears to affect the productivity of shoots especially mainshoots. It appears that developing tillers compete with the mainstem for assimilate and nutrients and this causes a reduction in mainstem growth and development (Aspinall, 1962; Kirby, 1973). Cultivars with fewer tillers or no tillers at all may well have increased mainstem yields. When tillers have been physically removed in wheat and barley, grain yield and the total biomass of the mainshoot has been substantially increased (Kirby and Jones, 1977; Mohamed and Marshall, 1979; Kemp and Whingwiri, 1980). Fast spikelet initiation rate in biculm wheat appears to give rise to larger ears than in freely tillering varieties (Marshall and Boyd, 1985). It has been suggested that the faster rate of spikelet initiation in biculms may be due to an increased supply of metabolites and mineral nutrients available for apical developmentas a result of the lack of competing tiller buds (Kirby and Faris, 1970).

Alternatively, the lack of tillers may cause a change in hormones, such as gibberellins which have been shown to affect spikelet initiation (Nicholls and May, 1964, Cottrell *et al*, 1982) and suppress tiller production (Jewiss, 1972; Johnston and Jeffcoat, 1977).

However a policy of reducing tiller numbers may be unwise since this would prevent recovery where the mainstem is damaged or irreversibly inhibited as a result of adverse circumstances (Evans *et al*, 1980). Tillers can contribute a large proportion of the grain yield and therefore their presence is often desirable. Early development and growth can affect final grain yield by changes in the number of tillers initiated and the number that survive to set grain. Both the environment as well as growth substances produced by the cultivar, regulate this. Tiller buds develop very early on in plant growth and some are present in the grain 24 hours after planting (Fletcher and Dale, 1974); conditions early on may well affect the chances of tiller survival and the amount of grain they will ultimately produced.

In optimal environmental conditions tiller bud formation and growth is uninhibited but in a hostile environment tillering is suppressed more than leaf production (Jewiss, 1966). High light intensity favours tiller production (Khalil, 1956; Friend, 1965 & 1966) while low light intensity markedly reduces the growth and development of tillers (Mitchell, 1953; Bean, 1964; Spietz and Ellen, 1972; Langer, 1963). A reduction in light intensity and nutrient supply due to a high plant density seems to substantially reduce tiller production (Kays and Harper, 1974; Kirby and Faris, 1972). When Lolium perenne plants were grown at high densities mainstem leaf production was unaltered but 12 weeks after planting tiller number was 3 times greater in plant populations grown at low when compared with high densities (Colvill and Marshall, 1981).

Nutrient supply alone can also affect tiller production (Ong *et al*, 1978; Troughton, 1968; Gericke, 1922; Asana *et al*, 1966). Increasing the supply of nitrogen increases tillering (Fletcher and Dale, 1974; Langer, 1959; McIntrye, 1965; Kumura, 1956) and even increased potassium and phosphorus appear to have an effect on tillering (Langer, 1959; Honya, 1961). In young barley plants where there is a delay in application of exogenous nitrogen after planting, the outgrowth of the first tiller bud is retarded (Fletcher and Dale, 1974). This influence of nitrogen on tiller bud outgrowth may be partly due to a stimulation of a plant growth regulator, for example cytokinin. When, in the absence of nitrogen, a range of cytokinins such as kinetin, BAP and zeatin were added to the roots (Sharif and Dale, 1980a) and directly to tiller buds (Jinks and Marshall, 1982; Langer *et al*, 1973; Jewiss, 1972; Clifford and Langer, 1975) bud outgrowth was stimulated.

Temperature also affects tillering (Taylor and McCall, 1936). In temperate grasses tiller production is highest at low temperatures (Evans *et al*, 1964; Rawson, 1971) and this appears to alter the rate of leaf appearance and tiller leaf emergence (Langer, 1963). Also in rice the number of emerging tillers is highest when the water temperature in which the crop is grown at a minimum of 15-16 ^{o}C at night and 31 ^{o}C in the day, the optimum being 31^{o}C both day and night (Tsunoda, 1964; Matsushima *et al*, 1964).

The amount of carbon dioxide in the atmosphere surrounding a crop appears to increase the storage capacity and yield of grain in such a way that in barley a high amount of carbon dioxide increases the number of ear-bearing tillers (Gifford *et al*, 1973) and in rice grain number and size are increased (Yoshida, 1972; Yoshida *et al*, 1973) by the same treatment.

The production of tillers is suppressed at the time when the mainstem becomes reproductive (Jewiss, 1972; Aspinall, 1961).

The developing inflorescence or the elongating stem internodes could be producing auxins since the addition of the auxin inhibitor, TIBA, at this time appears to stimulate tillering (Jewiss, 1972; Langer *et al*, 1973) and the removal of the mainstem also results in rapid tiller production (Harrison and Kaufman, 1980). It is unlikely that auxins alone regulate tillering and it has been suggested that the cytokinin:auxin ratio is important, as the outgrowth of tiller buds are promoted by a high ratio in explants (Harrison and Kaufman, 1980). Certainly auxins have been shown to inhibit the growth and development of tiller buds whereas cytokinins promote their growth and development (Johnston and Jeffcoat, 1977; Sharif and Dale, 1980a & b; Jinks and Marshall, 1982). Lately, attention has been focussed on the effects of growth retardants which interfere with gibberellin synthesis. It has been shown that early application of such retardants as chlormequat increase tillering in very young plants (Hutley-Bull and Schwabe, 1982) and the number of tillers that go on to set grain (Hofner and Kühn, 1982; Koranteng and Matthews, 1982; Hill *et al*, 1982; Child *et al*, 1983).

A large number of tillers die before they ever produce grain (Simmons et al, 1982). It is usually the last emerged tillers that die prematurely and it is thought that their death is a direct result of failure to compete for light and nutrients (Langer *et al*, 1964; Matsushima, 1957; Ishizuka and Tanaka, 1963; Spietz and Ellen, 1972). Also it has been shown that prior to death the small basal tillers have a poor assimilatory capacity (Ong et al, 1978) and are thus unable to sustain further growth. Tiller death varies through the year so that in late winter and spring the rate of tiller appearance exceeds that of death but at the onset of flowering of the most advanced shoots in summer tiller death exceeds appearance (Hebblethwaite, 1977; Langer *et al*, 1964; Colvill and Marshall, 1984). It has been suggested that barley plants with greater within-plant uniformity in early growth and development use resources more effectively thus

increasing the number of shoots going on to set grain (Matthews *et al*, 1982) and reducing the number of tiller deaths. The use of chlormequat has been shown to cause tillers to develop more synchronously and it is suggested that for this reason more go on to survive to grain set (Matthews *et al*, 1982). Increases in tiller survival without a compensatory reduction in mainstem and primary shoots grain yield is most desirable. Perhaps the way to increase yield is by the use of such growth retardants as chlormequat or by selection and breeding for cultivars with early, synchronous development of tillers. By increasing within-plant uniformity, and thus using resources more efficiently, it may be possible to reduce other agro-chemical imputs such as late nitrogen application.

The size of grain has been found to vary in different environments (Ellis and Kirby, 1980; Cottrell *et al*, 1985) and individual grain weight is larger on the mainstem than on tillers (Cottrell *et al*, 1985). Also grain weight varies with position along the spike in such a way that grains in the central spikelets are larger than both those set in basal and distal spikelets (Cottrell *et al*, 1985; Cottrell and Dale, 1984; Bremner and Rawson, 1978). Until recently, much of the work on final grain size concentrated on conditions after anthesis. It has been shown in barley that the differences in grain size within the ear appear before grain filling because carpel size at anthesis, which also varies along the spike, correlates with final grain weight (Scott *et al*, 1983). Grain size has also been found to correlate with endosperm cell number (Gleadow *et al*, 1982; Cochrane and Duffus, 1983) and in wheat a correlation between the variation in grain size with position along the ear and endosperm cell number has been found (Singh and Jenner, 1982). However, the duration and rate of grain filling has been shown to largely determine the size a grain achieves. High temperature reduces the duration of grain filling and causes smaller grains to be produced (Ford *et al*, 1976; Wattal, 1965). Low light intensity reduces the rate of grain

growth (Ford and Thorne, 1975) and while water stress increases the rate of cell division and initial grain growth (Wardlaw, 1971), final grain size is reduced due to a reduction in the duration of grain filling (Aspinall 1965). Final grain weight is also partly determined by the number of grain on each ear. When grain number is reduced by excision the weight of grain is increased (Radley and Thorne, 1981; Klinck and Sim, 1976; Bingham, 1967). Increases in grain weight do not fully compensate for the loss in grain number and this is accentuated as more grains are removed suggesting that there is a limit on the ability of those grains left to grow faster or for longer (Evans *et al*, 1980). Grain removal is more likely to cause an increase in grain weight when control weights are low (Simmons et al, 1982) and adverse conditions during early grain development can cause later grain removal to be ineffective in increasing final grain weight (Wardlaw, 1970; Jenner, 1980). Also different cultivars respond differently to grain removal (Jenner, 1979). These results support the suggestion that there may be a upper limit to grain size which is achieved depending on conditions experienced by cereal plants prior to grain filling. More recently there is evidence to suggest that these differences in grain size are set even early on in spike development, soon after spikelet primordium initiation at the double ridge stage of development (Cottrell and Dale, 1984). These findings are of considerable interest and if they were confirmed it could mean that the potential maximum size of grain may be determined early in spikelet development, and that grain size may be limited by spikelet primordia size in some circumstances.

What has to be borne in mind in work on manipulation of spikelet size and grain size is that in altering any one component of yield, compensation by other components tends to occur. It has already been shown (Darwinkel, 1979) that if one can increase the number of grain bearing ears by increasing plant populations, a decrease in number of spikelets, grain number per spikelet and dry

weight per grain occurs. Yield components would appear, therefore, not to be independent of each other. High grain no. m^{-2} is generally associated with low mean grain weight and mean grain weight is the least variable component of yield especially when cultivar differences are removed as a source of variation (Russell, 1990). The aim of the work presented here was to investigate the possibility that final grain size may be limited by the size of spikelet primordia at the double ridge stage of apex development. At the same time it was necessary to determine the extent to which any alteration in spikelet and grain size changed other components of yield.

Much expertise exists at Edinburgh on a number of aspects of barley development and growth (Dale, 1972; Dale *et al*, 1972; Felippe and Dale, 1973; Fletcher and Dale, 1974; Blenkinsop and Dale, 1974; Metivier and Dale, 1977; Dale and Wilson, 1979; Sharif and Dale, 1980a & b; Cottrell *et al*, 1981; Cottrell *et al*, 1982; Cottrell *et al*, 1983; Cottrell and Dale, 1984; Cottrell *et al*, 1985; Cottrell and Dale, 1986). Also barley development is relatively simple in comparison with other cereals such as wheat and oats. Whereas in the developing wheat ear each spikelet produces a number of florets and oats develop a panicle consisting of a main axis with subdivided branches grouped in clusters at the node of the main axis (Bonnett, 1936; 1937), in barley the ear consists of a spike where the number of spikelets at each joint of the rachis is limited (Bonnett, 1935). In two row barley development is simpler still as side spikelets develop very slowly and remain rudimentary, having no awns and being infertile (Bonnett, 1935). For these reasons two row spring barley was used throughout this study.

1.2. PLANT GROWTH AND DEVELOPMENT

Developmental stages in barley

In order to carry out an investigation of the sizes of spikelet primordia in the early development of the barley spike it is necessary to understand the early developmental steps of the barley spike. A detailed study of the development of the barley spike was made by Bonnett (1935) and is summarised below.

The developmental stages which have been identified in this study as being important in determining grain size are those which occur until the time of initiation of primordia cease. Two phases of development have been delineated over this period, the vegetative stage and the reproductive phase. The vegetative phase of development is regarded as having started at germination, where 24 hours after planting the barley embryo carries primordia of the coleoptile and the first 4 mainstem leaves (Dale *et al*, 1972). After germination the mainstem leaves continue to be initiated and form a succession of leaves arising at opposite sides of the apex meristem, resulting in the formation of two vertical rows of leaves. The vegetative shoot apex is conical in shape (Nicholls and May, 1963) and its size varies with time (Cottrell, 1980). The apex consists of a dome and cell division occurs at the base of this with leaf primordia appearing at the sides of the apex as small ridges. As time passes the older crescent shaped leaf primordia enclose the apex and these will later expand to form leaves. The apex remains in the vegetative phase until 3-6 leaves have emerged, depending on the cultivar and sowing date (Kirby and Appleyard, 1984). At this stage the apex begins to switch from producing leaf primordia to producing reproductive primordia. The rate of primordia production increases (Kirby, 1974) and the stem apex begins to elongate ready for spike differentiation (Bonnett, 1935). The primordia at the base of the apex develop into leaves while those which are still only small ridges situated close to

the apical meristem do not grow much more and become vestigial (Kirby and Appleyard, 1984). However the buds in the axil of these vestigial primordia each go on to develop rapidly and form upper ridges, producing a double ridge structure. The first visible double ridge structures appear on the primordia situated slightly below the middle of the spike and then others below and above this point develop double ridge structures (Barnard, 1964). Just above the flag leaf primordium and below the first spikelet there forms a distinct circular ridge and this is known as the collar of the spike (Weibe and Reid, 1961; Kirby and Appleyard, 1984). Following the development of the double ridges the spikelets differentiate further with the development of 3 mounds- a central floret and 2 lateral florets. The first spikelets to develop this triple mound structure are those which are situated in the lower central part of the spike. Two small papillae develop either side of the spikelets and these small projections are the glume initilas. Flower differentiation then occurs with the development of the lemma appearing as a ridge across each spikelet primordium. After the differentiation of the lemma, the palea differentiates but this is obscured from view by the rest of the spikelet. Gradually 3 distinct mounds develop on the tissue above the lemma and these go on to form the stamens. The pistil develops from the remaining tissue between the stamen which is undifferentiated. Following the differentiation of the stamen initials the upper central most part of the lemma develops an awn primordium which in time grows and elongates rapidly.

These distinct steps of development have been used to form a numerical scale of development by Aspinall and Paleg (1963) and this scale has subsequently been modified by Cottrell (1980). A more recent scale by Waddington *et al* (1983) has been devised but this was mainly devised to be more specific about developmental stages after pistil initiation. As the present study only measures spike development up to the time when initiation ceases the Cottrell (1980) modified scale was used. Use of this scale

also allowed direct comparisons to be made with previous work carried out at Edinburgh.

Spikelet primordia development and growth

Throughout the development of the spike the most advanced spikelets appear to occur just below the centre of the spike. Each primordium passes through each developmental stage but the time at which each enters a certain stage differs. The basal spikelets are more advanced than those at the tip, the former having been initiated first although the distal primordia do appear to develop faster than those at the base of the spike (Cottrell *et al*, 1982). This means that at any one time the spike will consist of a number of spikelets, the number depending on how many have already been initiated, at different stages of development. The numerical scale identifies the stage of the most advanced spikelets on the ear and this is then said to be the stage which the apex has reached. The ear is indeterminate and spikelet initiation ceases when the most advanced spikelet primordia reach the stamen initial stage (Nicholls and May, 1963; Cottrell, 1980), not on the production of a terminal spikelet as in wheat.

Cottrell and Dale (1984) described in detail the sizes of individual spikelets and central florets up to the end of spikelet initiation. It appears that on reaching the double ridge stage the size of individual spikelets are such that those at the base of the ear are larger than those at the tip. On reaching subsequent stages the size difference remains between the base and tip of the ear. The growth and development of primordia progress in phase with each other so that the difference between the sizes of primordia at the double ridge stage is maintained throughout subsequent stages. The smaller size of distal primordia is partly compensated for by investing more primordial tissue in the central

floret. This means that the size difference between the central florets at the tip and base of the spike is reduced.

EXPERIMENTAL STUDIES ON THE RELATIONSHIP BETWEEN SPIKELET PRIMORDIA AND GRAIN SIZE

1.3. THE AIMS AND APPROACH OF THIS PROJECT

The purpose of this project was to investigate the relationship between the size of spikelet primordia and final grain weight. The aim was to examine and extend these new ideas about how the development of spikelet primordia may affect and determine final grain weight. Two cultivars were used throughout this work and a uniculm variety was used in one experiment investigating the effect of tillers on mainstem spikelet primordia size.

Firstly it was necessary to confirm the results found by Cottrell and Dale (1984) and to determine the extent to which all the experimental results were repeatable. If the results could be confirmed a number of questions follow:

1) Can a short term change in growth conditions during spikelet primordia development affect the size of primordia and will this have an effect on final grain weight?

2) If the size of mainstem spikelet primordia and thus grain size can be altered, what effect would this have on the other component parts of the plants?

3) Do tillers affect the size of the mainstem spikelet primordia and does the number and size of these tillers affect final grain weight?

4) If it was possible to slow down the development of distal primordia without affecting their relative growth rate would their size be increased and would a more uniform set of primordia be produced?

Work Plan

The work carried out by Cottrell and Dale (1984) used freeze substitution and critical point drying to process apices ready for measuring individual spikelet primordium sizes under the scanning electron microscope. Large numbers of specimens can be processed simultaneously using this method and the preparations can be coated with gold and maintained indefinitely at room temperature in the presence of a desiccant and studied subsequently. This method of preparation was used. However this method is prone to cause tissue shrinkage. As a result there are changes in the dimensions of the specimen which may be sufficient to limit the value of any quantitative measurements made, although there is a suggestion that the degree of shrinkage might be constant for stem apices of wheat (Moncur, 1979). For this reason it was necessary to use the newer method of sample preparation for the Scanning Electron Microscopy (SEM) sample preparation- Low Temperature SEM to quantify the degree of shrinkage. No dehydration should occur when using this technique and therefore shrinkage should not a problem although a small amount of tissue expansion may result from the freezing of the tissue water. Quantitative measurements made by this technique are more reliable than measurements made following other preparative treatments (Read *et al.*, 1983; Beckett et al., 1984). However, it was only possible to use this method as a means of quantifying the former method as the procedure was slow and only allowed a single specimen to be fixed and processed at a given time. Additionally, because there was no long-term preservation there was no possibility of returning to the specimen at a later date for further observations.

The two cultivars used throughout were Proctor and Maris Mink, both have been previously investigated by others in studies on early growth and development and ear production (Dale, 1972; Felippe and Dale, 1973; Fletcher and Dale, 1977; Dale and Wilson, 1979; Sharif and Dale, 1980a & b; Cottrell, 1981; Foster and Dale, 1983; Cottrell and Dale, 1984; Cottrell *et al*, 1985; Aspinall, 1966; Kirby and Faris, 1970; Ruckenbauer and Kirby, 1973; Gallagher *et al*, 1976; Matthews *et al*, 1982). Both cultivars that had similar growth rates and ear developmental rates and it was thought possible to establish the generality of the relationship between spikelet primordia and final grain size.

Partial confirmation of the results of Cottrell and Dale (1984) led to a number of questions: Can the size of spikelet primordia at double ridge be altered experimentally? Will this have an inevitable effect on grain size? Do sub-optimal conditions pre-anthesis result in an inevitable reduction in grain size from the potential maximum, or can compensation and recovery occur if more favourable conditions follow the adverse? In an attempt to answer these, plants were subjected to different nitrogen and temperature treatments in the hope of altering the size of spikelet primordia and to determine whether this affected final grain size.

Following the confirmation of the results found by Cottrell and Dale (1984) plants were subjected to different nitrogen and temperature treatments in the attempt to alter the the size of spikelet primordia and to determine whether this affected final grain size. The results of this experiment led to attempts to determine the extent of tiller competition on mainstem spikelet primordia size and final grain weight.

Subsequently a growth retardant, chloremequat (CCC) was used to try and slow the development of distal primordia. Also CCC has been shown to increase tillering without apparently affecting

mainstem grain production. Thus the effect of CCC on spikelet primordia size and final grain size was recorded.

SECTION 2. MATERIALS AND METHODS

2.1. PLANT MATERIALS

Three cultivars of spring barley, Hordeum vulgare L., were used in these studies. Proctor and Maris Mink were used throughout; and Uniculm Compana was used in the experiments on the effect of tillers on spikelet primordia and grain size.

Maris Mink and Proctor plants were grown from grain produced at Edinburgh University in the Botany Department Greenhouses. Uniculm Compana plants were grown from grain produced at the Plant Breeding Institute, Cambridge. This grain was kindly supplied by R Giles of the Genetics Research Unit. All the grain was stored at 8°C until required for planting.

Maris Mink and Proctor are both late maturing cultivars and have relatively slow developmental rates. Below is a table showing the origin, the mean number of days to masculation (Forster and Dale, 1983), the mean number of days to the emergence of the flag leaf and the mean number of leaves (Dale and Wilson, 1978) of these two cultivars.

	origin	$\bar{x}$ no. days to flag leaf appearence	$\bar{x}$ no. leaves	$\bar{x}$ no. days to masculation
Maris Mink	UK	53.5	9.8	66.8
Proctor	UK	46.0	9.0	72.6

2.2. CULTURAL METHODS

The first experiment performed was carried out to confirm the results found by Cottrell and Dale (1984) and thus the cultural conditions differed from all subsequent experiments. The plants of this repeat experiment were grown continually in glasshouse conditions. However in subsequent experiments it was necessary that all but one of the growth conditions were constant during the development of spikelet primordia. Plants were grown in as similar conditions as was possible throughout all the experiments presented here. With one exception, in each experiment one feature of the cultural conditions was changed. In the experiment investigating the effect of tillers only the cultivar grown differed. The specific alteration of one of the cultural conditions was only maintained in the constant environment, over the period from planting to the end of spikelet initiation. After this initial period all the plants that were grown to set grain were transferred to the glasshouses.

The treatments used in the constant environment were:

1) an alteration in nitrogen supply
2) the removal of tillers and use of a uniculm cultivar
3) the application of one dose of growth retardant

Growthroom conditions

Grains of the barley cultivar in use were sown in a controlled environment at a constant temperature of 20 °C. All treatments were subjected to 16h days. Irradiance, in the wavelength range 400-700 nm, was provided by warm white fluorescent and tungsten lamps at an intensity of 400 $\pm$ 150μmol m^{-2} s^{-1}.

* (Photon flux density varied between different positions on the bench)

Greenhouse conditions

In the repeat experiment that was carried out to confirm the results of Cottrell and Dale (1984) grains of Maris Mink were sown in the greenhouse in April 1986. The mean daily sunshine hours recorded for each month over the period of both experiments were obtained from The Royal Observatory, Blackford Hill, Edinburgh, courtesy of Bob Watson.

In plants grown from seed in the growth rooms, spikelet primordia initiation stopped at day 40 under high nitrogen conditions and at this stage a number of plants were transferred from the controlled environment to the greenhouse. At this point all specific treatments stopped and plants from each treatment were transplanted into John Innes no. 1 potting compost and left to set grain.

Over the autumn and winter periods natural daylight was supplemented with irradience in the wavelength range 400-700 nm from warm white fluorescent tubes and tungsten bulbs. The greenhouse was heated during this period and maintained at 15 °C.

In the experiment involving the effects of high and low nitrogen treatments on spikelet primordia and grain development, another batch of plants was transferred to the greenhouse at day twenty, when the stage of the whole apex was at the double ridge stage, to set grain.

Growth of plants

In all the experiments carried out in the controlled environments, single, selected grains of uniform size were planted in 10 cm square plastic pots containing washed river sand F grit (British Industrial Sands Ltd.).

On transfer from the controlled environment growthrooms to the greenhouse, plants were transplanted, one plant per pot, into 14 cm square plastic pots containing John Innes potting compost no. 1

Plants grown in the controlled environment growthrooms were given 50 cm^3 of distilled water per pot at the time of planting and subsequently on alternate days. More frequent watering was sometimes needed for older plants. Mineral nutrients were supplied, in solution, on day 4 after planting and subsequently at weekly intervals until day 40. These nutrients were applied at two designated levels, high (normal) and low nitrogen (L). The two levels correspond to that given by Dale and Wilson (1978).

The high N nutrient solution contained :

N: 14.0 mg pot^{-1} as $NaNO_3$

P: 3.2 mg pot^{-1} as KH_2PO_4

K: 43.0 mg pot^{-1} as KH_2PO_4 and K_2SO_4

Mg: 2.9 mg pot^{-1} as $MgSO_4$

Ca: 10.0 mg pot^{-1} as $CaCl_2$

Fe: as versenate salt

Micronutrients

The low nitrogen nutrient solution contained a tenth of the amount of nitrogen found in the high nitrogen solution, 1.4 mg per pot, but the amounts of all the other nutrients remained the same. Unless otherwise stated, plants were supplied with the normal, high, level of nitrogen. In one experiment some plants were given low ntrogen for 12 days and then subsequently given high nitrogen until transfer.

Plants grown in, or transferred to, the greenhouse were watered once a day to the point of soil saturation. In very hot weather, when pots were liable to dry out, plants were watered twice a day.

No nutrients were supplied to the plants in the greenhouse as John Innes no. 1 potting compost contains added mineral nutrients.

Where the experiment of Cottrell and Dale (1984) was repeated two grains per pot, of Maris Mink, were sown in 14 cm square plastic pots containing John Innes potting compost no. 1 and grown continually in the glasshouse.

The removal of tillers and investigation of uniculm tiller production

To investigate the effect of tillers on mainstem primordia size and development it was necessary to remove tillers from a batch of both Maris Mink and Proctor plants. Tillers are side shoots which arise at ground level, the major ones of which arise from the base of main stem leaves. When a tiller developed above the leaf sheath from whose base it arose, it was removed by hand. In performing this operation it was necessary to make sure that the apex of each tiller was removed in order to ensure that the tiller would not develop. Despite the fact that this was always done the plants continued to produce tillers until the time of grain filling. All these tillers were removed at the time of their appearance. Damage to the plant was kept to a minimum.

When using the uniculm cultivar: Compana it was necessary to check whether this cultivar ever produced tillers or dormant tiller buds. A batch of 50 plants were grown in the greenhouse and left to set grain. Eight plants out of the fifty produced 1 to 2 tillers and thus the grain from these plants was discarded. The grain from the remaining plants was collected and a sample of 20 grain were grown up again, none of these plants produced tillers. Also another batch of 20 plants was grown and harvested 40 days after sowing to check for possible tiller bud development. None of these plants appeared to have produced tiller buds that were visible under the binocular microscope, at the time of harvest.

Grains from the true uniculm plants were thus used in the subsequent experiments, none of which when grown produced tillers.

Adding the growth retardant CCC

The the growth retardant chloro-choline chloride (C-2635 Sigma; molecular weight of 158.1) or CCC, was used to investigate the effect of a growth retardant on Proctor and Maris Mink spike growth and development. Application of CCC needed to be easy to apply and effective very soon after application if early spikelet primordia were to show any symptoms of treatment. In wheat CCC has been found to be rapidly absorbed through the roots (Belzile *et al* 1972) and thus any effect of CCC on plant growth and development might be expected to occur soon after application. Secondly, application of CCC as a root drench does not require any special equipment and a single dose can be applied simply in a measured amount of solution. Thus CCC was applied as a root drench in the concentration of 175 parts per million. Each plant was given a single dose of 50 cm^3 CCC and left for two days. After 2 days any remaining CCC was washed out of the sand. This concentration of CCC has been shown by workers at the Shell Laboratories, Sittingbourne to be in the middle of the dose range which is non-toxic to plants as well as temporarily retarding barley plant growth. CCC was applied after collar formation, during spikelet intiation and development. Four separate treatment times were used.

The application times were one dose of CCC early in mainstem spike development on:

1) day 16
2) day 20
3) day 24
4) day 28

2.3. APICAL PROCESSING

In all the experiments investigating the relationship between the size of spikelet primordia and final grain weight, apical preparation was by freeze substitution and critical point drying. Low temperature scanning electron microscopy was used to try and determine to what extent freeze substitution and critical point drying affected the whole spike and individual primordia.

Freeze substitution and critical point drying

At each harvest apices were quickly dissected, under a Vickers Binocular Microscope, placed in a Beam capsule (Agar Aids) and frozen in liquid nitrogen. The capsules were previously prepared for use by making small holes in their sides to allow the liquid nitrogen to drain freely in and out over the apices. After freezing the apices, still in the capsules, they were placed in an -80 °C freezer to equilibrate for two hours. Following this, each capsule was given a small 'lid' of Parafilm to prevent the apices floating out of the mouth of the capsules when they were placed in methanol. The methanol had been pre-chilled in flow tubes (Flow Laboratories) at -80 °C over night. The screw neck tubes were prepared by placing 3 cm of 3Å molecular sieve in the bottom of the tubes and filled with dry methanol. A plug of cotton wool was pushed down onto the molecular sieve to prevent dust from reaching the apices. The tubes were then sealed in a plastic box and stored upright for 14d at -80 °C. The box was taken through a series of different temperature treatments: -40 °C for 7d, -20 °C for 24h, 4 °C for 12h and finally at room temperature for 2h. The capsules where then placed in fresh dry methanol and left for 1h following which they were taken through a 3:1, 2:1, 1:1, 1:2, 1:3 methanol:acetone series, 1h in each, finally being transfered to 100% dry acetone for 24h.

The apices were then processed by critical point drying (Robards 1978) and mounted on Scanning Electron Microscope (SEM) stubs. These were then coated with gold in a sputter coater (SC500, Emscope) and viewed under a Cambridge 250 SEM where they were photographed.

Low temperature scanning electron microscopy

Apices were dissected out under the binocular microscope, orientated such that the spikelet primordia were in the field of view and stuck onto a cryo-stub with tissue tek. The cryo-stub was then frozen in liquid nitrogen in a slushing chamber, put under vacuum and then transferred to the cold stage on the SEM. The cold stage was kept at -180 °C. Ice accumulated on the apex when it was frozen so in order to remove it, for photographic recording, the ice was sublimed off. The ice was sublimed off by raising the temperature of the cold stage to -60 °C, the stage was then cooled again back to -180 °C. The apex was then transferred to the cryo-coating unit were it was coated with gold. The coating unit was kept at -180 °C. After coating, the apex and cryo-stub were returned to the cold stage on the SEM were the apex was photographed.

Apices that were cryo-processed as well as being processed by freeze substitution and critical point drying were placed on a small stub mounted onto the cryo-stub. This small stub plus apex was removed from the cryo-stub once they had been cryo-processed. The small stubs and apices were then processed by freeze substitution and critical point dried. Photographs were taken of the apex after each process.

2.4. GROWTH MEASUREMENTS

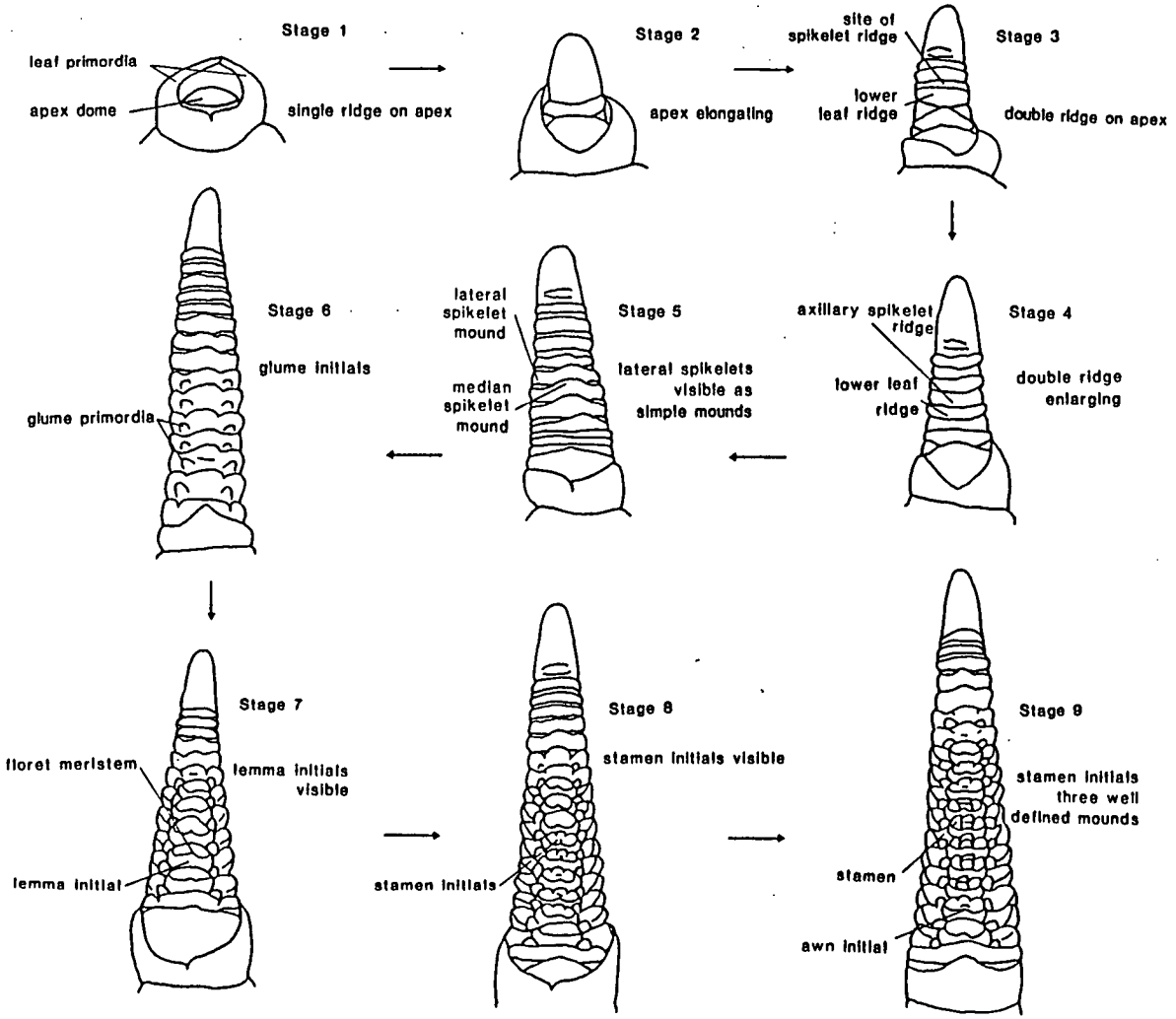
Apex development (Figure 1)

The designated stage of apex development was determined by the stage of the most advanced spikelet. The stage of the most advanced spikelet was recorded at each harvest. Harvests were taken from the time of the initiation of reproductive primordia until day 40. The scale used to score the development of the spikelet primordia is one used by Cottrell (1980) which was modified from Aspinall and Paleg (1963). The scale is set out below and illustrated in Figure 1.

Scale of apex development

Single ridge on side of apex- apex not elongating	1
Apex elongating	2
Double ridge on apex	3
Double ridge enlarging	4
Lateral spikelets visible - triple mound	5
Glume initials visible	6
Lemma initials visible	7
Stamen initials just visible	8
Stamen initials well defined as three distinct mounds	9
Awns longer than spikelets	10
Barbs visible on awns	11

Figure 1. The developmental stages of two row barley as defined by Aspinal and Paleg and modified by Cottrell 1980. Each stage is determined by the most advanced spikelet primordia on the apex and each apex consists of primordia at this stage as well as less advanced primordia. (not to scale)


Stage 1
leaf primordia
apex dome
single ridge on apex
Stage 2
apex elongating
Stage 3
site of spikelet ridge
lower leaf ridge
double ridge on apex
Stage 4
axillary spikelet ridge
lower leaf ridge
double ridge enlarging
Stage 5
lateral spikelet mound
median spikelet mound
lateral spikelets visible as simple mounds
Stage 6
glume initials
glume primordia
Stage 7
floret meristem
lemma initials visible
lemma initial
Stage 8
stamen initials visible
stamen initials
Stage 9
stamen initials three well defined mounds
stamen
awn initial

Primordia widths at double ridge stage 4 (Figure 2, 3)

The size of individual spikelets were obtained from measurements made on photographs taken on the SEM. The width of the upper part of the ridge known as the axillary 'spikelet' ridge was determined for each sucessive primordium up the spike (Figure 2). In two row barley spikelet primordia are initiated alternately on each side of the apex insuch a way that the first spikelet initiated is on the opposite side from the second and so on .

The primordia were numbered from the collar upwards and, in all the experiments, the mean of the sizes of the first two spikelets to be initiated has been calculated and used in graph plots where it is presented as the width of spikelet 1.5. The same has been repeated for each successive spikelet pair so that the mean of the sizes of spikelets 3 and 4 is presented as the value for spikelet number 3.5 and so on up the spike.

Figure 2. The spike of a 20d old plant indicating the parameter used to measure the width of a spikelet primordium at the double ridge stage (4). Each primordium at this stage consists of 2 parts (the axillary spikelet ridge and the lower leaf ridge) and are initiated on alternate sides of the spike.

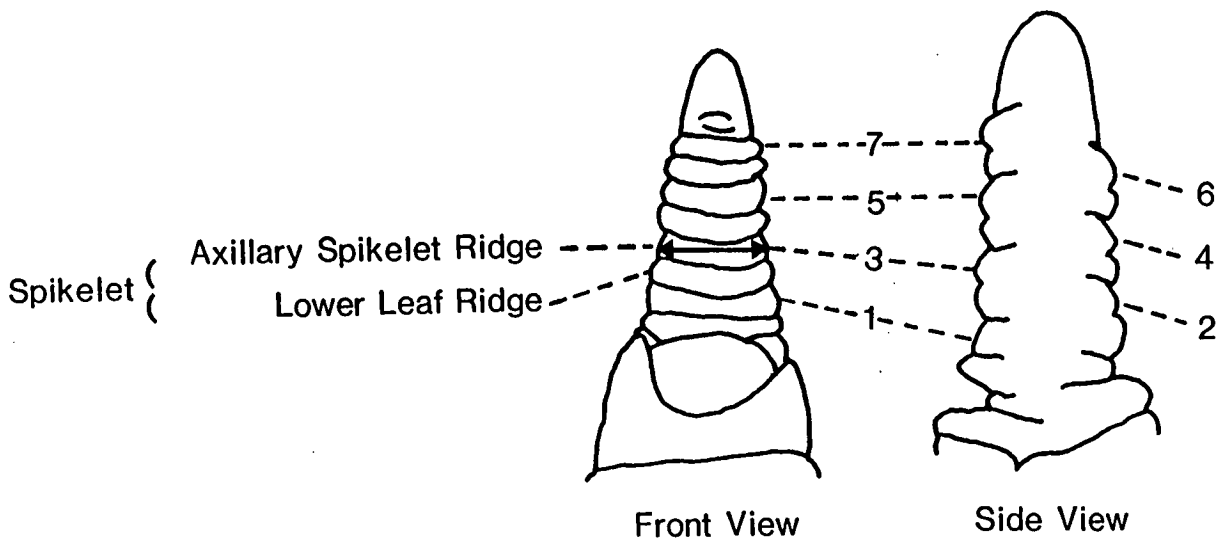
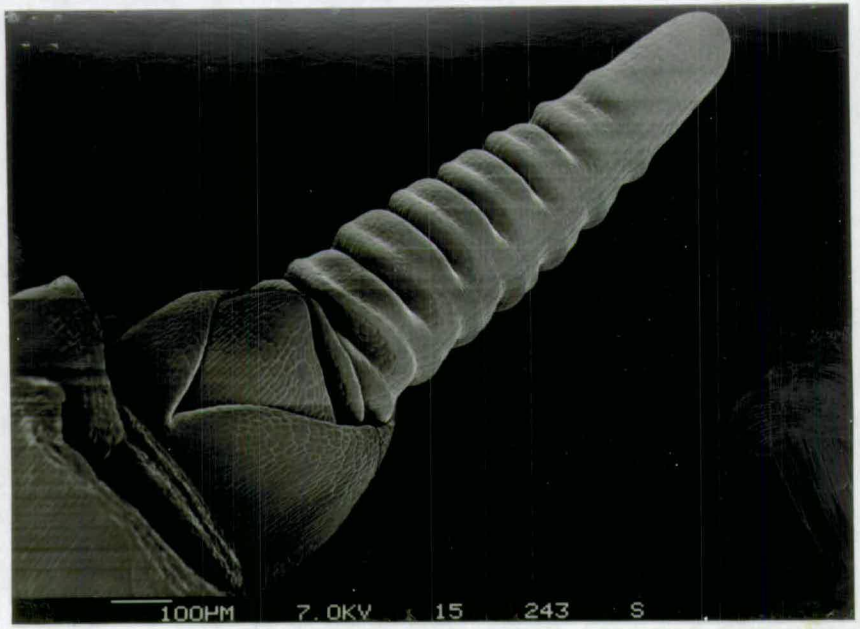
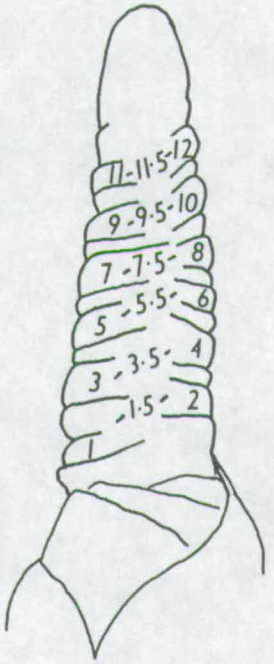

Spikelet
Axillary Spikelet Ridge
Lower Leaf Ridge
7
5
3
1
6
4
2
Front View
Side View

Figure 3. The side view of a spike at the double ridge stage of development (4) showing how spikelet primordia are initiated from the base of the spike upwards. The primordia are numbered accordingly, in order of their initiation. A mean of each pair of primordia initiated is shown, and width measurement of these pairs were meaned to the give width data from the base to the tip of the spike.





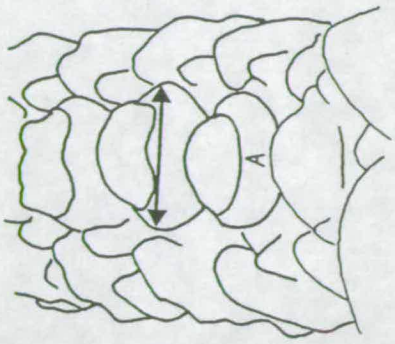
Side View

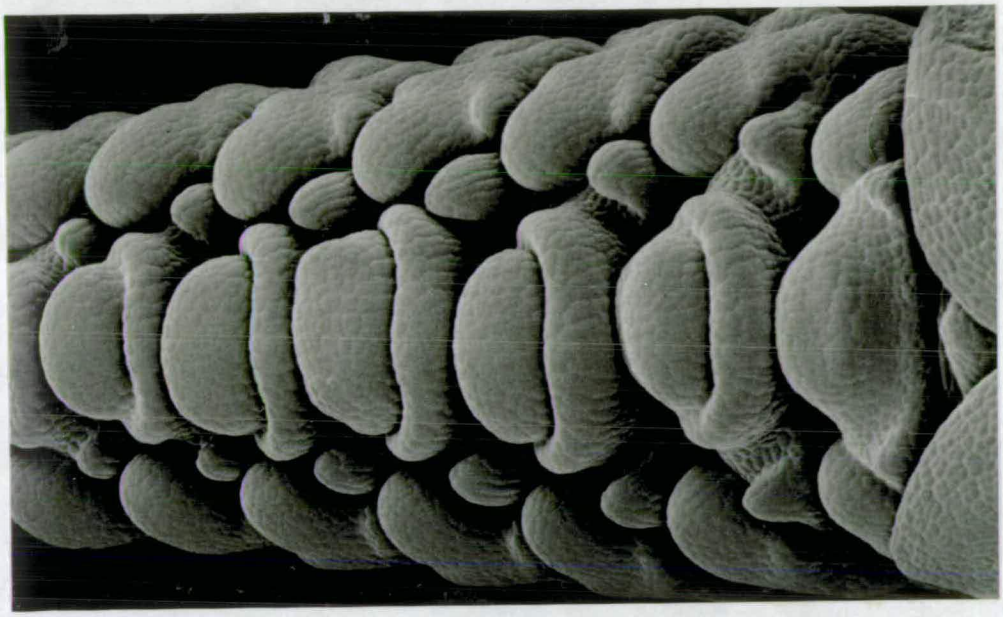
Floret widths at stage 7 and 9 (Figure 4, 5)

In all the cultivars the widths of whole primordia were only measured at double ridge stage (stage 4) whilst for stages 7 (Figure 4) and 9 (Figure 5) the widths of the central florets of appropriate primordia were recorded.

At any one harvest spikelets along the ear were at different stages of development with the most advanced spikelets being located in the mid to basal positions. For this reason measurements of both central florets and spikelet widths at stage 4 were collected from spikes of very different ages. It was also not possible to obtain a uniform sample of spikelets and central florets at given positions and stages. Apical spikelets develop faster than basal spikelets and therefore spend less time at any one developmental stage so that there were fewer samples available for distal than for basal spikelets. Some developmental stages are shorter in duration than others and because harvests were taken at regular intervals the size of spikelet samples varied according to which stage was being measured. In each experiment there was variability between plants which caused more spikes to be sampled which were at one stage of development than at another.

Figure 4. Micrographs and diagram of a spike at stage 7 (lemma initials visible) indicating the parameter used to measure the width of the central florets (eg. A) at this stage of development.





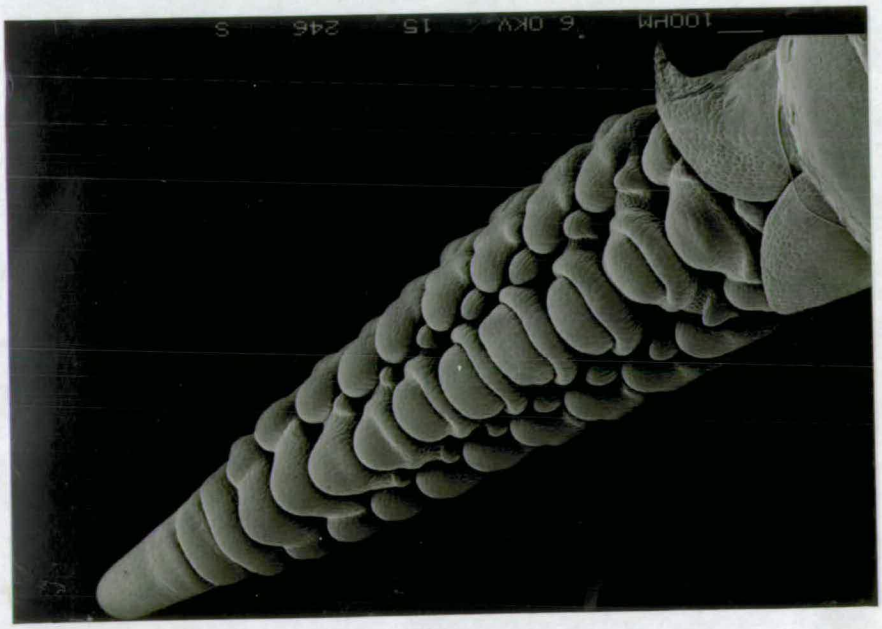
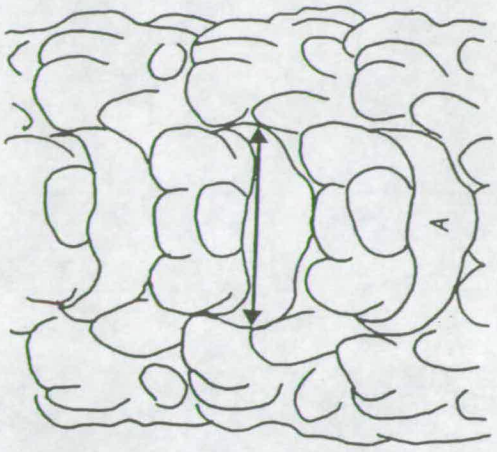
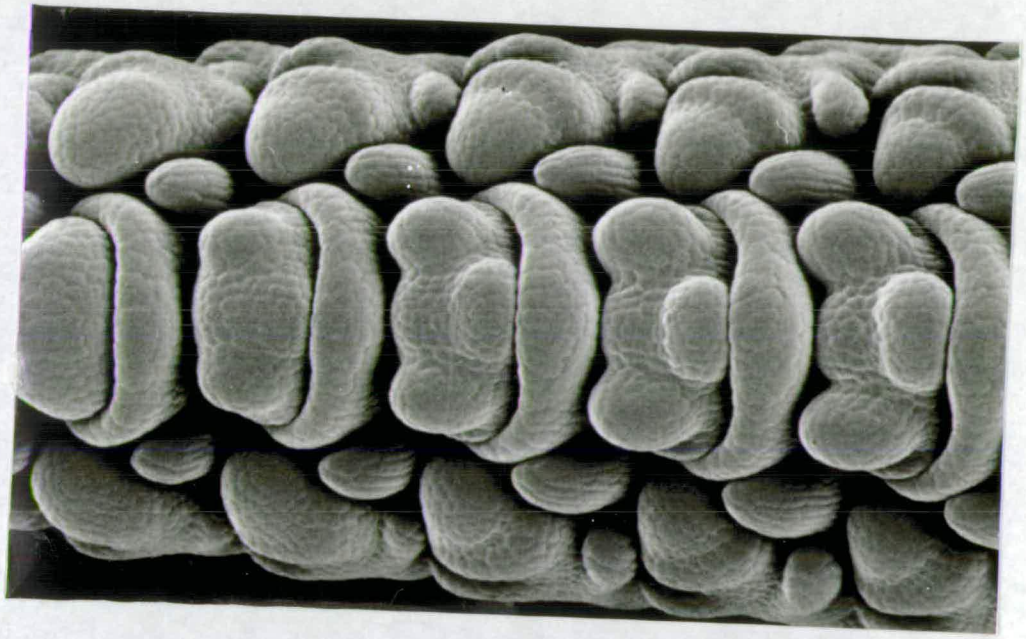
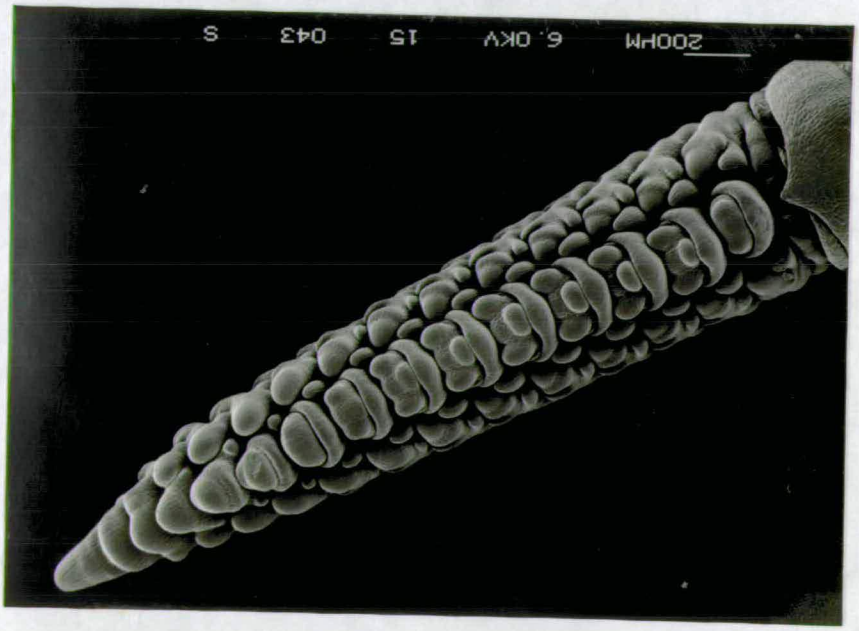

100HM
6.0KV
15
246
S

Figure 5. Micrographs and diagram of a spike at stage 9 (stamen initials visible as 3 distinct mounds) indicating the parameter used to measure the width of the central florets (eg. A) at this stage of development.


A




200HM
6.0KV
15
043
S

Leaf and tiller emergence

At each harvest for apices a note was taken of how many tillers and leaves had emerged in order to gain a general view of the plants growth and development. Leaves were numbered in the sequence of their appearence so that the first leaf to appear was called L1, the second to appear L2 and so on. Tillers develop as axillary buds at the base of mainstem leaves or other tillers and so were numbered and labelled according to which part of the plant the tiller subtended; as set out below.

Tc tiller developing at the axil of the coleoptile

T1 tiller developing at the axil of the first leaf on the mainstem

T2 tiller developing at the axil of the second leaf on the mainstem

T3 tiller developing at the axil of the third leaf on the mainstem

T4 tiller developing at the axil of the fourth leaf on the mainstem

T5 tiller developing at the axil of the fifth leaf on the mainstem

2° tillers developing at the axils of the primary tillers

3° tillers developing at the axils of the secondary tilers

Determination of plant and tiller dry weight

The dry weights of plants and individual tillers were determined in all the experiments except in the case of the experiment where tillers were removed. At each harvest for mainstem apices five plants were placed in separate aluminium trays. The trays were then placed in a drying oven which was kept at 80 °C. These were left to dry for 48h and then weighed to determine total plant dry weight. The developing tillers were also weighed separately to determine their individual dry weight.

Determination of final grain weight

The individual grain weights were determined for the first pair of spikelets on the ear. The mean of this value was calculated and is presented as the value of the grain weight for grain number 1.5, in a manner similar to that used to present the spikelet width data. The same procedure was used for each successive spikelet pair such that the mean of the grain weights for positions 3 and 4 is presented as the grain weight for spikelet 3.5, and so on. Ears were removed from the plants only when the whole plant had died. Grains were stripped from the ears, dried and then bagged ready for weighing.

2.5. STATISTICAL ANALYSIS

Space in the controlled environment was limited. This feature coupled with the long duration and variety of treatments used made it impossible to use large replication. For this reason sampling occurred at regular, frequent intervals and at each harvest the number sampled was kept small. In each treatment 5 plants were examined at each harvest and 15 plants were grown along side the plants being harvested finally at the end of treatment being transferred to the glasshouses to set grain.

All the data was expressed as the means of the individual values. Standard statistical methods were used to calculate means and to determine whether or not there was any significant difference between two sets of data. 95% confidence limits were calculated and used (Parker 1980) to determine whether there was a significant difference between mainstem primordium widths or grain weights of different treatments. When it was necessary to determine the difference between more than two sets of data Analysis of Variance: Double classification was used (Parker 1980). The use of this method determined whether or not there was a difference between a set of treatments and whether there was a difference between different components of a specific treatment. For example - the null hypothesis for grain yield was that there was no difference between grain weight of different treatments and there was no difference between different shoots in plants from the same treatment.

In the experiment where the two methods of apical processing were compared regression analysis was used to examine the data. All the spikelet primordia width data that was plotted against the final grain weight data had 2nd order polynomial curves fitted. The most basal two primordia developed irregularly and were slow to develop. Grain was generally not set in these two basal positions but occasionally small, ill formed grain were set. Due to these features these basal positions were omitted when fitting the curve. In the experiments where floret size appeared to have more relation with final grain size, 2nd order polynomial curves were fitted to these data as well. Where possible all experiments were set out as randomised blocks and the appropriate methods of analysis used.

SECTION 3. THE EFFECT OF DIFFERENT PROCESSING METHODS ON THE DIMENSIONS OF BARLEY SPIKELET PRIMORDIA

3.1. INTRODUCTION

Critical Point Drying (CPD) is one of the most common methods of preparing soft tissue specimens for ambient temperature scanning electron microscopy (ATSEM). The technique uses freeze substitution (FS) with methanol (Robards, 1978), followed by a treatment to replace the methanol with acetone. Finally, the acetone is replaced with liquid carbon dioxide in a pressure chamber where at its critical point (at 31.1 °C and 73 atm.) the carbon dioxide is vapourised (Horridge & Tamm, 1969), leaving apices intact, dehydrated and ready for SEM analysis. Large numbers of specimens can be processed similtaneously and the preparations can be coated with gold and maintained indefinitely at room temperature in the presence of a desiccant and studied subsequently. With this treatment there appears to be little in the way of tissue damage (Beckett *et al.*, 1984) although an irregular shrinkage of various fungal structures has been reported (Read *et al.*, 1983). Shrinkage is an inevitable consequence of the dehydration of biological material which contains a high percentage of water and, in plants, the situation is further complicated by a relaxation of the cell wall with very small losses of turgor as the specimen is processed. As a result there are changes in the dimensions of the specimen, well documented for animal systems (e.g. Boyde *et al.*, 1981) which may be sufficient to limit the value of any quantitative measurements made for plants, although there is a suggestion that the degree of shrinkage might be constant for stem apices of wheat (Moncur, 1979).

The newer method of SEM sample preparation, described as low temperature SEM (LTSEM) and reviewed by Echlin *et al.* (1978), utilises the rapid freezing of fresh, fully hydrated material as a means of specimen fixation provided the material is maintained below -130 °C, enabling a very high standard of speciman preservation to be obtained. Because no dehydration need occur, speciman shrinkage is not a problem although a small amount of tissue expansion, less than 2.7% of linear dimension may result from the volume expansion freezing of the tissue water by about 9% on freezing. Quantitative measurements of speciman linear dimensions made by this technique are likely to be more reliable than measurements made following other preparative treatments (Read *et al.*, 1983; Beckett et al., 1984). However, the procedure is slow as only a single specimen can be fixed and processed at a given time, and this has the consequence of limiting replication. Additionally, because there is no long-term preservation of it there is no possibility of returning to the specimen at a later date for further observations.

Although a number of studies have examined the effects of freeze-drying and CPD on animal and plant tissues there have been no direct comparisons of the effects of CPD and LTSEM on higher plant material. In the present study, a comparison has been made of the dimensions of spikelet primordia on the stem apices of spring barley following LTSEM and FS/CPD to assess the extent of shrinkage and to determine whether any shrinkage following FS/CPD is independent of primordium size and position on the ear. Such information would enable LTSEM to be used for the calibration of measurements obtained by FS/CPD, so that the advantages of speed and replication provided by FS/CPD could be combined with the accuracy of measurement afforded by the former method.

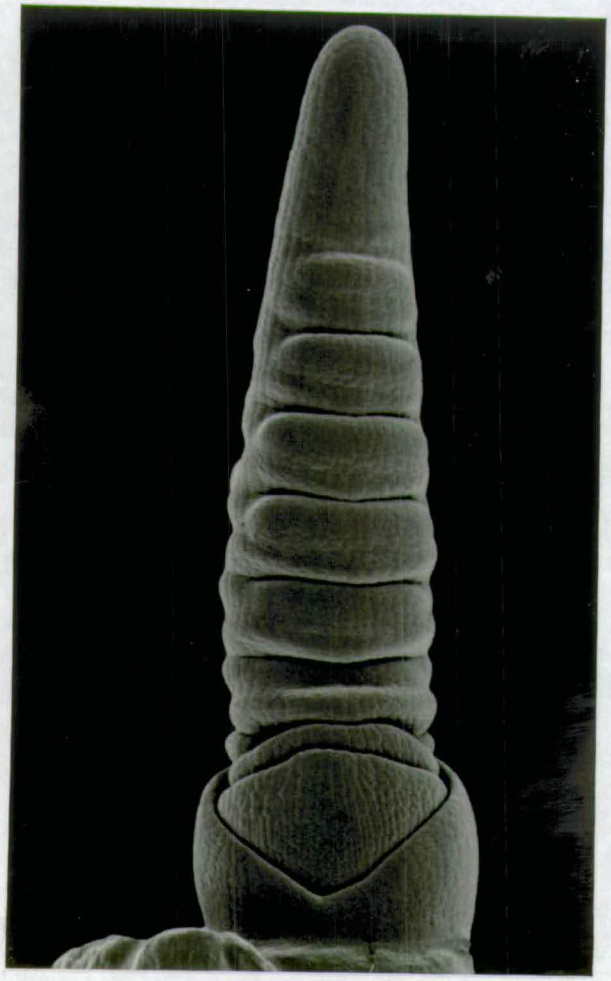
3.2. RESULTS AND DISCUSSION

The visual appearance of the same apex viewed after LTSEM and again after FS/CPD is shown in Figure 6. The loss of detail following FS/CPD is evidant.

When the widths of spikelet primordia at the double ridge stage on apices processed by FS/CPD were compared with those of spikelets on apices prepared by LTSEM, the degree of shrinkage shown by the CPD material was found to be more-or-less constant for any one spike, regardless of primordium size or position on the spike. There was a highly significant (r= 0.96) correlation between the sizes of primordia at corresponding positions on the apices prepared by LTSEM or FS/CPD (Figure 7a). The average extent of shrinkage of CPD material, determined from this regression line, was 7.1%. The mean of the shrinkage values determined for primordia at each of six positions on 10 apices examined by LTSEM or FS/CPD was 10.6% (Figure 7b).

Figure 6. Micrographs showing the visual appearence of the same apex photographed while being viewed by LTSEM (micrograph A) and then after processing by FS/CPD being viewed under ambient temperature SEM (micrograph B).

A



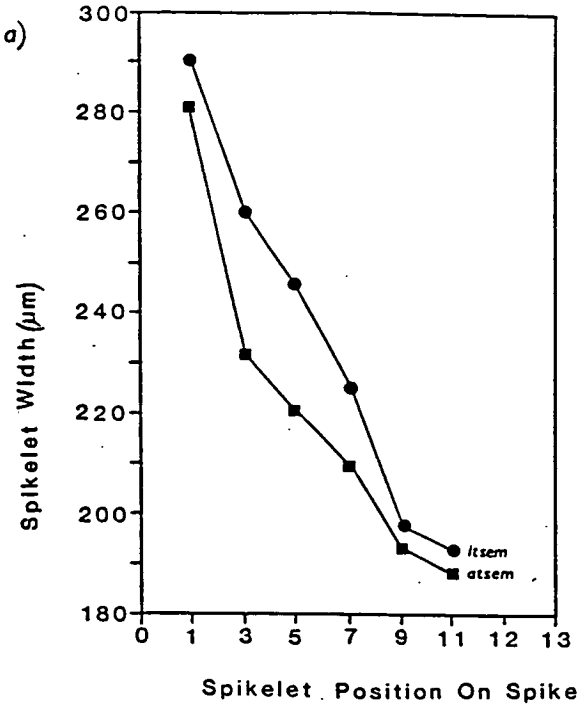
100 µm

B

100 µm

Figure 7a. The mean width of spikelet primordia at the double ridge stage (4) of development, at various positions along the spike, for apices processed and viewed by LTSEM (●) or processed by FS/CPD and then viewed by ATSEM (■).

Figure 7b. The mean extent of shrinkage of material processed by FS/CPD and viewed by ATSEM as determined by plotting ATSEM primordia widths at stage 4 against LTSEM primordia widths at stage 4, and fitting a regression line ($y = 0.87x + 16.77$; $r = 0.96$).


a)
300
280
260
240
220
200
180
Spikelet Width (µm)
0 1 3 5 7 9 11 12 13
Spikelet Position On Spike
ltsem
atsem

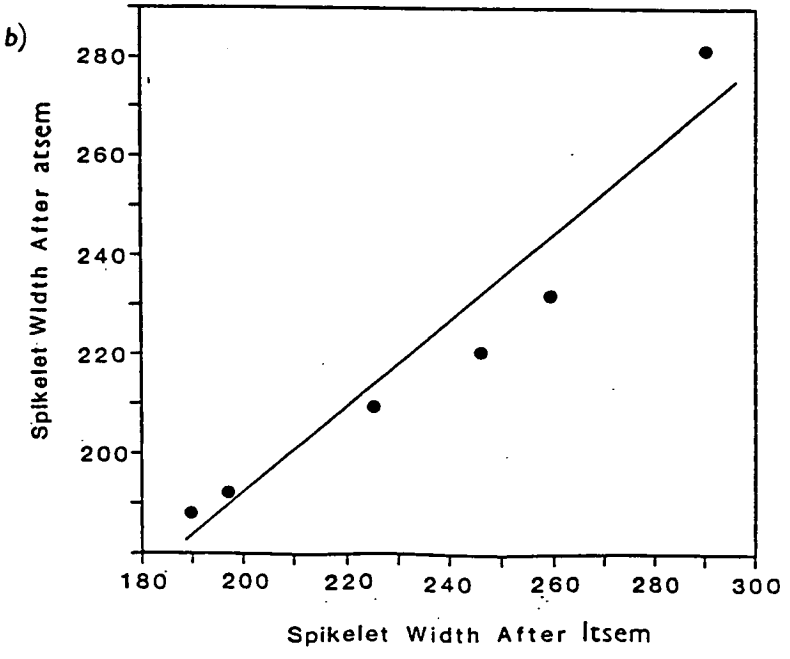
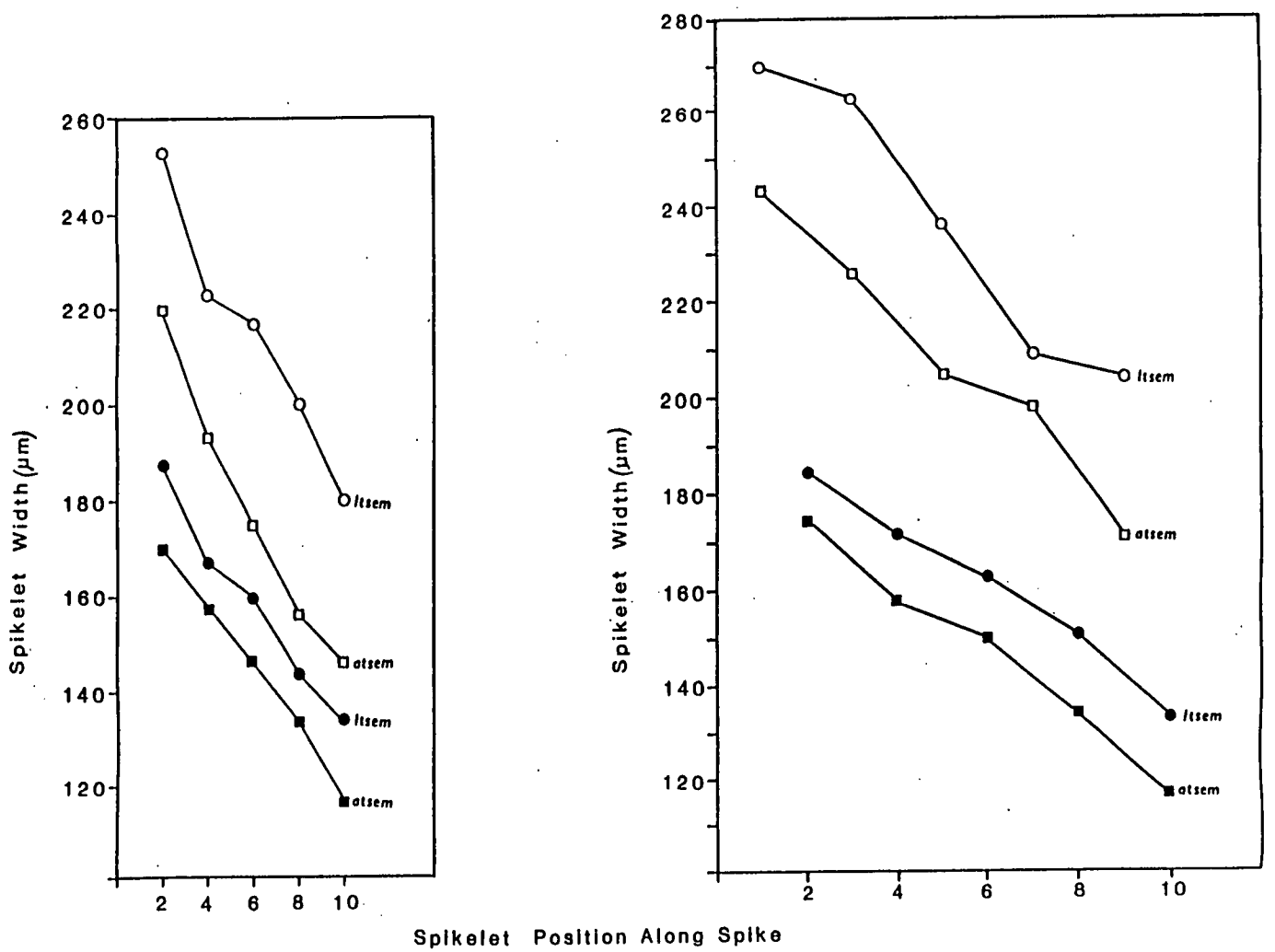

b)
280
260
240
220
200
Spikelet Width After atsem
180 200 220 240 260 280 300
Spikelet Width After ltsem

Figure 8. Spikelet primordia widths of 4 apices processed first by LTSEM and then subsequently by FS/CPD. The open symbols represent the widths of apices processed by LTSEM and the corresponding closed symbols represent the same primordia after processing by FS/CPD.


Spikelet Width (µm)
260
240
220
200
180
160
140
120
2
4
6
8
10
ltsem
atsem
ltsem
atsem
Spikelet Position Along Spike

Measurements were also obtained using primordia on four apices that had been subjected first to LTSEM and then subsequently FS/CPD (Figure 8). The mean shrinkage of each primordium on a given apex, as well as the shrinkage value determined for that apex by regression analysis are presented in Table 1. As in the previous comparison, primordia examined by LTSEM were larger than those processed by FS/CPD by about 10% of linear dimensions. Comparison of shrinkage estimates obtained from direct measurements on the same primordia and by the regression method showed the latter approach giving slightly higher values when meaned for all the apices (10.6% ±(se) 1.64 compared with 11.5% ± 1.90). Interestingly, the coefficient of variation is high at about 36-42% indicating differences between apices in their response to the two treatments. The reasons for this are not clear but may well be linked to differences between specimans in the rate and direction of freezing during cryofixation for LTSEM (cf.Jeffree et al.,1987) which could induce conformational changes in shape of the primordia, although we have no direct evidence for this.

Thus FS/CPD appears to result in linear dimensions approximately 10% smaller than those obtained with LTSEM. The true degree of shrinkage resulting from FS/CPD will be marginally less than this figure due to the fact that LTSEM is likely to give dimensions slightly larger than the *in vivo* values though the effect of primordium expansion caused by freezing is likely to be very small. 1 kg water occupies 1.003 m^3 whilst 1 kg ice occupies 1.087 m^3, a volume expansion of 8.37%. Assuming a model "primordium" to be a hemisphere of free water, the degree of linear expansion on freezing will be only 2.03%, an amount which may well be difficult to detect above background variation. On the other hand, the measured shrinkage of by different apices after FS/CPD (5.9 - 16.8%; Table 1) is much larger than this and would certainly be significant, and detectable, for a primordium of 200 µm width. While for comparative purposes, this amount

of shrinkage can probably be tolerated, the use of measurements from shrunken FS/CPD specimens to derive the tissue volume could lead to major underestimates, of the order of 33%.

Table 1. Different apex shrinkage values

	APEX NUMBER				
	1	2	3	4	5
Mean of shrinkage values for individual primordia(%)	8.5	8.4	16.7	11.5	8.1
Shrinkage values obtained by regression analysis(%)	9.7	10.6	16.8	14.5	5.9

Since the degree of shrinkage appears to be independent of primordium size the CP values can be used to 'correct' those obtained by CPD to give sizes much closer to in vivo ones although because of apex-to-apex variation such correction cannot be completely adequate.

SECTION 4. REPEATING THE EXPERIMENT OF COTTRELL AND DALE (1984)

4.1. INTRODUCTION

Cottrell and Dale conducted their experiments under glasshouse conditions, growing 2 plants per 14 cm^2 pot in John Innes No. 1 compost. The procedure was followed as exactly as possible, using the same cultivar, Maris Mink, but with the difference that this experiment was conducted between April and July 1986 whereas Cottrell and Dale conducted their experiment between August and November 1983. As will be shown these seasonal differences appear to be important. Fuller details of cultural methods are given in the Material and Methods chapter.

4.2. RESULTS

Mainstem spikelet size (Figures 9a, b, c)

Cottrell and Dale found systematic differences in primordial sizes from the base to apex of the spike at all stages examined. This has been confirmed and the data show spikelets at the base of the spike to be twice as wide as those at the tip at stage 4. Quantitatively the widths of primordia at stage 4 were closely similar between the two experiments.

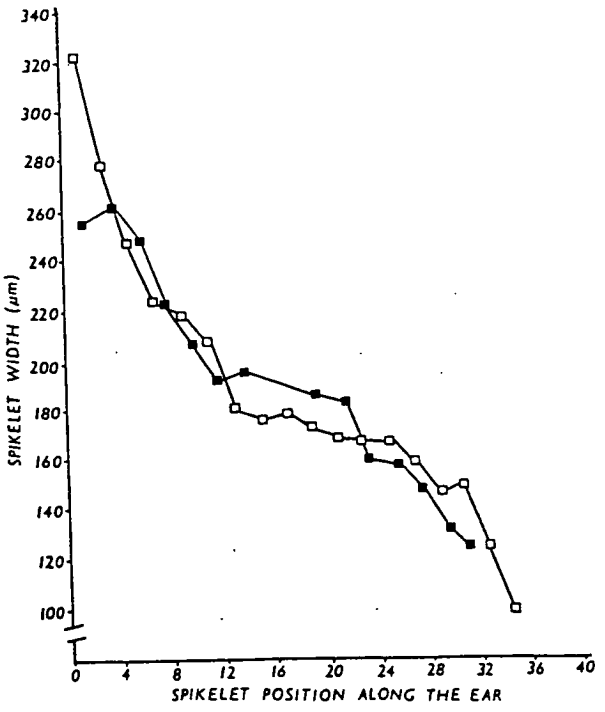
At stage 7 and 9 variation in widths of the central floret along the spike were qualitatively similar in this experiment, the largest florets being between spikelets 3 and 5 in the repeat experiment and 6 in the Cottrell and Dale experiment with a progressive decrease in size toward tip and base of the spike. However, quantitatively there was a difference between the two experiments. The repeat experiment had larger central florets at stage 7 and at stage 9.

Figure 9a. The mean width of spikelet primordia at the double ridge stage of development, at various positions along the spike, for plants grown by Cottrell and Dale (□) and for plants grown by Lewis-Smith (■).

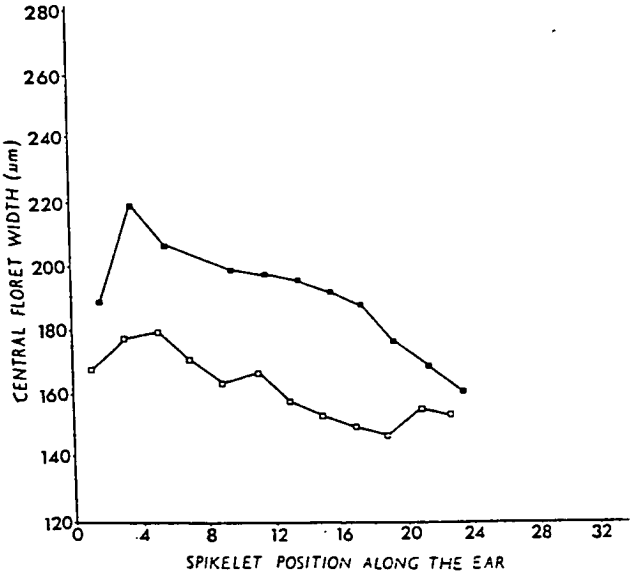
Figure 9b. The mean width of the central floret at stage 7 (lemma initials), at various positions along the spike, for plants grown by Cottrell and Dale (□) and for plants grown by Lewis-Smith (■).

Figure 9c. The mean width of the central floret at stage 9 (stamen initials visible as 3 distinct mounds), at various positions along the spike, for plants grown by Cottrell and Dale (□) and for plants grown by Lewis-Smith (■).

a)


SPIKELET WIDTH (μm)
340
320
300
280
260
240
220
200
180
160
140
120
100
0
4
8
12
16
20
24
28
32
36
40
SPIKELET POSITION ALONG THE EAR

b)


CENTRAL FLORET WIDTH (μm)
280
260
240
220
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180
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0
4
8
12
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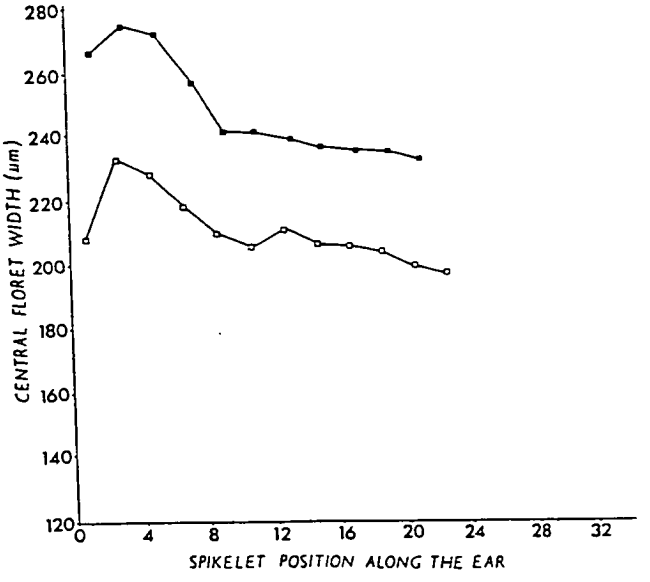
20
24
28
32
SPIKELET POSITION ALONG THE EAR

c)


CENTRAL FLORET WIDTH (μm)
280
260
240
220
200
180
160
140
120
0
4
8
12
16
20
24
28
32
SPIKELET POSITION ALONG THE EAR

Another difference was that the enlargement of the distal spikelets between stages 7 and 9 was greater in the present experiment than in that of Cottrell and Dale's.

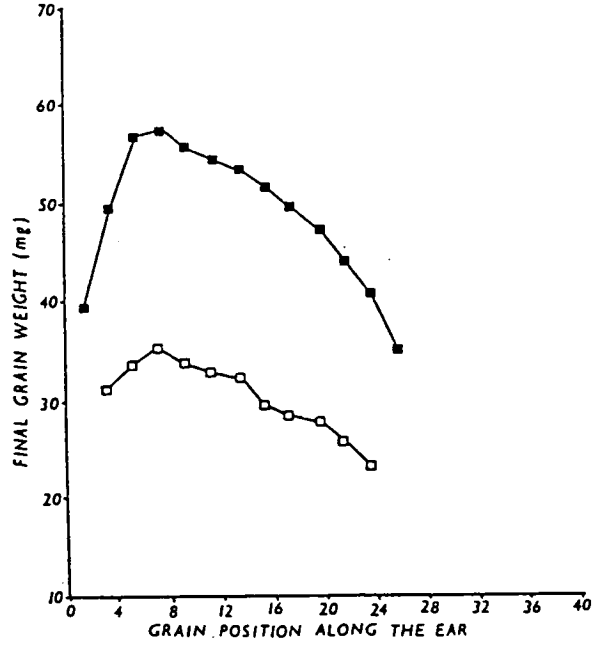
Mainstem final grain weight (Figure 10a, b)

In both experiments, grain weight gradually increased from positions 2 to 7 and then decreased with ascending position along the ear until position 24 or 26 was reached depending upon the experiment. Spikelets above these positions did not set grain. Grain weight at all positions along the ear were much smaller in the Cottrell and Dale experiment than in the repeat. A non-linear correlation was found between the width of the spikelet at double ridge stage and the final weight of the grain it produced, with the exception of spikelets 3 and 5. The shape of the fitted curve in both experiments was the same but the position relative to the y axis was shifted upwards due to the increase in overall grain size in the present experiment. Since spikelet widths were similar there was no fixed relation between absolute sizes of primordia at double ridge stage and final grain weight between runs.

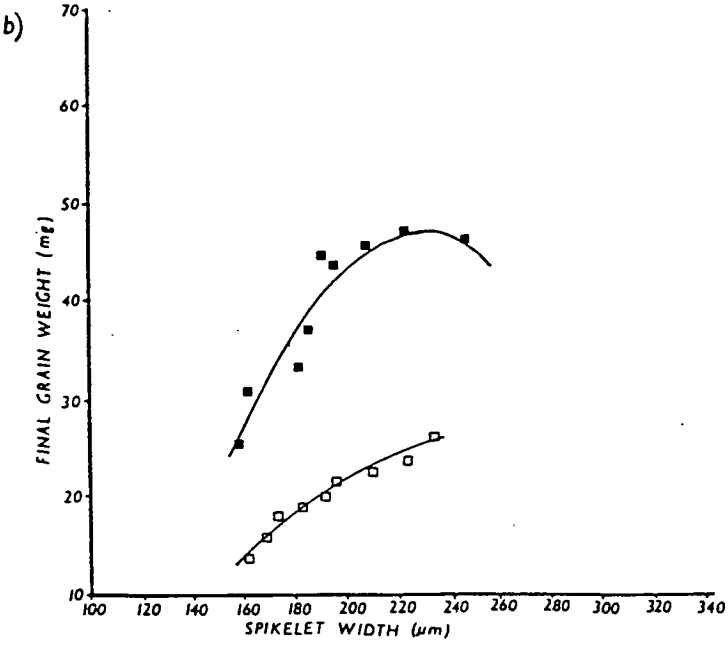
Figure 10a. The mean weight of grain set at various positions along the ear for plants grown by Cottrell and Dale (□) and for those grown by Lewis-Smith (■).

Figure 10b. The relationship between final grain weight and the width of spikelets at the double ridge stage at various positions along the ear for plants grown by Cottrell and Dale (○) ($y = -98.27 + 1.16x - 2.56x^2$; $r^2 = 0.974$, $n = 9$) and for plants grown by Lewis-Smith (●) ($y = -174.60 + 2.03x - 4.45x^2$; $r^2 = 0.904$, $n = 9$).

a)


FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
0
4
8
12
16
20
24
28
32
36
40
GRAIN POSITION ALONG THE EAR

b)


FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
100
120
140
160
180
200
220
240
260
280
300
320
340
SPIKELET WIDTH (μm)

Mean daily sunshine hours per month (Table 2)

The data for sunshine hours pertaining to the two experimental periods shows that during the repeat experiment the total solar radiation experienced by the plants was greater than for the initial experiment. Most significant perhaps is the observation that during the later months of growth, and hence during grain filling, the hours of sunshine in the second experiment were approximately twice those encountered during the similar phase of plant growth in the initial study.

Table 2. Mean daily sunshine hours for the months August to November 1983 and April to July 1986

1983	$\bar{x}$ sunhine hrs.	1986	$\bar{x}$ sunshine hrs.
August	6.03	April	3.59
September	3.76	May	6.35
October	3.38	June	6.53
November	2.42	July	4.99

4.3. DISCUSSION

Comparison of the results of the repeat experiment and those of Cottrell and Dale confirmed that there were differences in spikelet primordium size along the apex and that these differences were reflected in final grain weight along the spike. There was also a correlation between sizes of spikelets at stage 4 and final grain weight in both experiments. However the relationship was quantitatively different in the two experiments. In the later stages of primordial development the central florets were larger and grain weights were heavier in the repeat experiment, raising the question "Why were ~~the~~ the grain produced by Cottrell and Dale smaller than those of the repeat experiment?"

The soil content, plant density and watering regime in the two experiments were the same but the seasons in which they grew were different. Cottrell and Dale (1984) grew their plants in autumn, while the repeat experiment plants were grown in the spring. Total light quantity was lower over the autumn since although the greenhouse was heated and supplementary light was supplied, the light intensity was not as high as that available in the lenghtening days of spring.

Aspinall (1966) showed that although different photoperiods did not affect the time plants took to reach stage 4, they did affect the time taken to reach later stages. He suggested that this indicated that the later stages of development were more susceptible to control by the availabilty of products of photosynthesis than stage 4. Whether or not it is assimilate availability or some photoperiodic effect that caused this result is not important in the present context. What is important is that the size of primordia at stage 4 appear to be influenced less by the difference in seasonal photoperiod than the later stages. The difference in light experienced by the two experiments did not affect the size of spikelet primordia at stage 4 but may well have affected the later stages especially as floret size was larger in the spring grown plants. Altering conditions during grain filling such as by shading (Willey and Holliday, 1971a & b) and by partial defoliation (Lucas and Asana, 1968) resulted in reduced grain yields suggesting that in some cases yields may indeed be limited by photosynthate supply. The widths of spikelet primordia were the same in the two experiments so if grain size were to have been set at this early stage the grain weights should have been the same in both cases. However actual grain sizes between the two experiments were different. Clearly, the size of spikelet primordia at stage 4 is not the sole determinant of grain size. It could be that spikelet primordium size at stage 4 determines the maximum potential for grain size and that later stages determine the actual size attained. If this

were the case then the reason the grain weights in the Cottrell and Dale (1984) experiment were smaller than those in the repeat experiment could be because they had never reached their maximum potential due to photosynthate limitation. There is, however, no evidence to suggest that because the grain weights attained in the latter experiment were larger, that they would therefore represent that maximum potential size. The true size of the maximum could never be determined because one could never be sure that environmental conditions throughout subsequent developmental stages were optimal for grain production.

It could be that the size of spikelet primordia at stage 4 only determines individual grain sizes in relation to their position on the spike. At stage 4 the mid-basal primordia are larger than the proximal primordia and this relationship is maintained through to grain set. The actual size of grain could be determined by later stages of development but mid-basal grain would always be larger than those at the tip of the ear. Alternatively the relationship between the size of spikelet primordia at stage 4 and grain size could result from a combination of both these suggestions.

The question therefore remains, to what extent the size of spikelet primordia at stage 4 may influence the size of the final grain produced.

SECTION 5. EFFECT OF VARYING NITROGEN SUPPLY

5.1. INTRODUCTION

The effect of nitrogen on various aspects of plant growth and development is well documented and suggests that nitrogen supply may control the photosynthetic system, both in size and activity, thus controlling assimilate production (In barley: Dale and Felippe 1977; Metivier 1976; Dale 1979; in wheat: Ryle and Hesketh 1969). It has also been shown that nitrogen supply affects the growth and development of the mainstem apex (In barley: Dale et al 1972; Dale 1976; Dale and Wilson 1978; in wheat: Beveridge et al 1965; Langer and Hanif 1973; Single 1964). If nitrogen supply has such wide ranging effects on the plant it may well also affect the size of spikelet primordia and thus perhaps final grain size. Nitrogen supply is easy to manipulate in plants grown in a controlled environment.

During which phase of plant development is nitrogen supply, ie. during the supply of nitrogen over the vegetative phase or early on in the reproductive phase starting at $\pm$ d16, important in determining spikelet primordia size? Two cultivars, Maris Mink and Proctor, were used in the experiments to see if they showed a similar response to varying nitrogen supply. Plants were grown initially in controlled conditions in 10 cm square pots filled with sand. Two approaches were taken: the first was to supply mineral nutrients in solution to plants from sowing on into the reproductive phase. Half of the batch of plants of each cultivar received high nitrogen solution (H= 50 cm^3 solution containing 14 mg sodium nitrate) and the other half received low nitrogen solution (L= 50 cm^3 solution containing 1.4 mg sodium nitrate). Two batches of plants from each cultivar and treatment were subsequently transferred to the greenhouse to set grain, one batch

being transferred 20 days after sowing and the other 40 days after sowing.

The second approach was to supply a low level of nitrogen to plants during the vegetative phase and then give high nitrogen when the plant started to produce reproductive primordia on the mainstem. In this experiment plants were given L solution 4 days after sowing and another dose a week later, then from d14 until transfer to the greenhouse at d40 they were supplied with H solution (L/H). The result of this, and the H40 experiment, where the plants had H solution from sowing onwards, were compared. Spikelet primordia and early tiller growth data were only taken upto d28 after sowing as any affect on the double ridge stage would be seen over this early period.

For convenience the treatments used have been abbreviated as indicated in Table 3.

Table 3. Table of treatments and abbreviations

Nitrogen treatment	Transfer time	Maris Mink	Proctor
High	Day 20	(M)H20	(P)H20
	Day 40	(M)H40	(P)H40
Low	Day 20	(M)L20	(P)L20
	Day 40	(M)L40	(P)L40
Low/High	Day 40	(M)L/H	(P)L/H

5.2. RESULTS

5.2.1. THE EFFECT OF VARYING NITROGEN SUPPLY DURING SPIKELET PRIMORDIA INITIATION

In this section, for the sake of clarity the results of the two cultivars have been presented separately. However, comparisons between the two cultivars are made.

Maris Mink

Mainstem initiation and development of spikelets (Table 4, Table 5)

Plants given high nitrogen and transferred at day 40 (H40) initiated more primordia than those given low nitrogen (L40). When the plants were transferred to the greenhouse at d20 the whole apex was still at the double ridge stage and had only initiated a small number of spikelet primordia. Subsequently all new spikelets were initiated and developed in the greenhouse, after the ending of the specific nitrogen conditions. Growthroom grown plants had initiated all their spikelet primordia within the first 40 days.

Table 4. Time course of mainstem primordia initiation for MH40 and ML40 treatments up to day 36

		Mean number of primordia present at different days from sowing							
		20	22	24	26	28	30	32	34
MH40	$\bar{x}$n	7.0	11.0	11.5	12.4	17.0	24.2	-	25.4
	range	6-8	10-12	7-14	11-14	16-18	21-29	-	23-26
ML40	$\bar{x}$n	-	5.0	7.0	10.3	11.3	14.7	16.0	16.8
	range	-	4-6	5-8	8-12	10-14	14-15	15-18	15-17

Table 5. Time taken for the most advanced primordia to reach stage 4,7 and 9 and the position on the spike of the largest spikelet and grain

	Time in days to			position of largest primordia			largest grain
	stage 4	stage7	stage9	stage 4	stage 7	stage 9	
MH40	20	30	40	1.5	3.5	5.5	9.5
ML40	28	38	-	1.5	3.5	-	9.5

Plant dry weight, leaf and tiller emergence (Table 6, 7)

Plants grown in high nitrogen for 40 days increased substantially in dry weight between day 14 and day 36. Plants grown in low nitrogen conditions increased slowly in dry weight over the same period. Initially, between d14-24, the differences in dry weight between treatments were small even though H40 plants had two emerged tillers and L40 plants had none. However, by d36 L40 plants were 1467mg lighter than H40 plants. No tillers emerged in L40 plants between days 14-36 although three tillers had emerged in the other treatment, T3 emerging on day 32. Leaf emergence was also slowed by the low nitrogen treatment. On day 32 only five leaves had emerged in the low nitrogen treated plants compared with seven leaves in the other set. L40 plants produced 7 leaves on the mainstem whereas H40 produced 9. The leaves on L40 plants were chlorotic and the lower ones had turned yellow by d40; the mainstems in these plants were also thin and stunted.

Table 6. Time course in days from sowing of mean plant dry weight (mg) of Maris Mink plants treated with high and low nitrogen, including 95% confidence limits in brackets

	plant treatment:	
	HN	LN
14	110.9(10.8)	99.7(13.4)
16	163.2(15.2)	126.6(12.6)
18	186.3(22.4)	140.9(18.5)
20	254.4(30.1)	147.7(21.7)
22	254.8(27.8)	166.5(33.2)
24	335.7(39.2)	218.0(28.8)
26	416.6(44.1)	231.6(19.9)
28	590.9(47.9)	271.9(31.6)
32	1472.9(63.5)	400.4(36.2)
34	1576.8(66.7)	426.5(39.1)
36	1883.2(72.9)	416.7(28.8)

Table 7. Time course in days from sowing for leaf and tiller emergence in high and low nitrogen treated plants

	days from sowing:										
	16	18	20	22	24	26	28	30	32	34	36
HN											
	L3----------			L4------		L5------		L6--	L7--------		
	----------			T2----------					T3--------		
LN											
	----L3----------						L4------		L5--------		

Mainstem spikelet size (Figure 11a, b, c; Table 8)

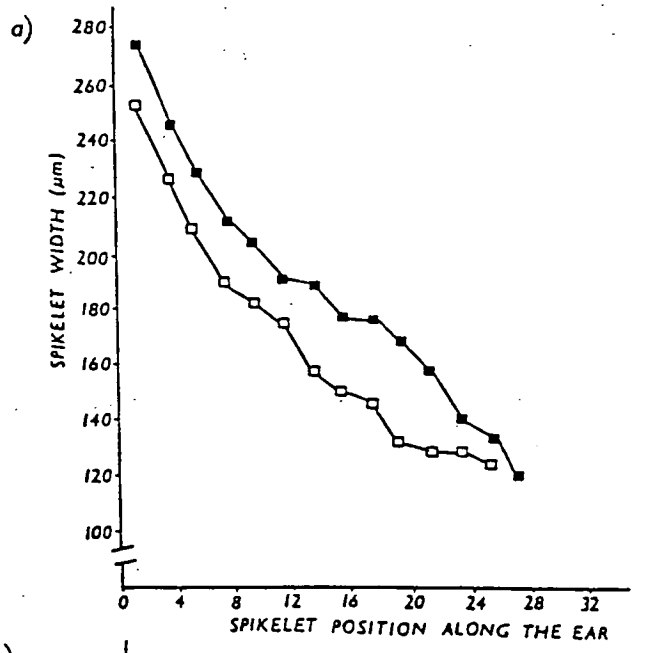
For plants in the L40 set the spikelet primordia were smaller than the equivalent spikelet primordia in the H40 set at the double ridge stage (Figure 11a), and this difference was found to be significant (Table 8). This trend was maintained throughout subsequent stages (Figure 11b) and at stage 7 the sizes of central florets were significantly smaller in L40 than the equivalent florets in H40 plants (Table 8). No data are available for the size of florets at stage 9 in L40 plants as the most advanced spikelet primordia reached this stage after final measurements were taken.

In plants in the H40 set the more distal the spikelet position the more that spikelet had grown by stage 9 in relation to its size at stage 7 (Figure 11b & c).

Figure 11a. The mean width of spikelet primordia at the double ridge stage of development, at various positions along the spike, for MH40 treated plants (■) and for ML40 treated plants (□).

Figure 11b. The mean width of the central floret at stage 7 (lemma initials), at various positions along the spike, for MH40 treated plants (●) and for ML40 treated plants (○).

Figure 11c. The mean width of the central floret at stage 9 (stamen initials visible as 3 distinct mounds), at various positions along the spike, for MH40 treated plants (■).


a)
SPIKELET WIDTH (μm)
280
260
240
220
200
180
160
140
120
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4
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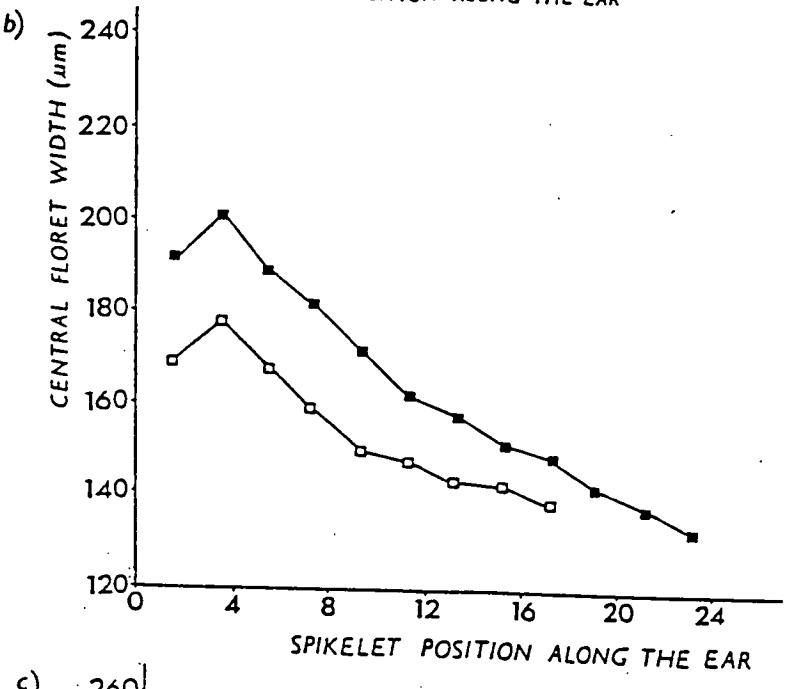
12
16
20
24
28
32
SPIKELET POSITION ALONG THE EAR


b)
CENTRAL FLORET WIDTH (μm)
240
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SPIKELET POSITION ALONG THE EAR

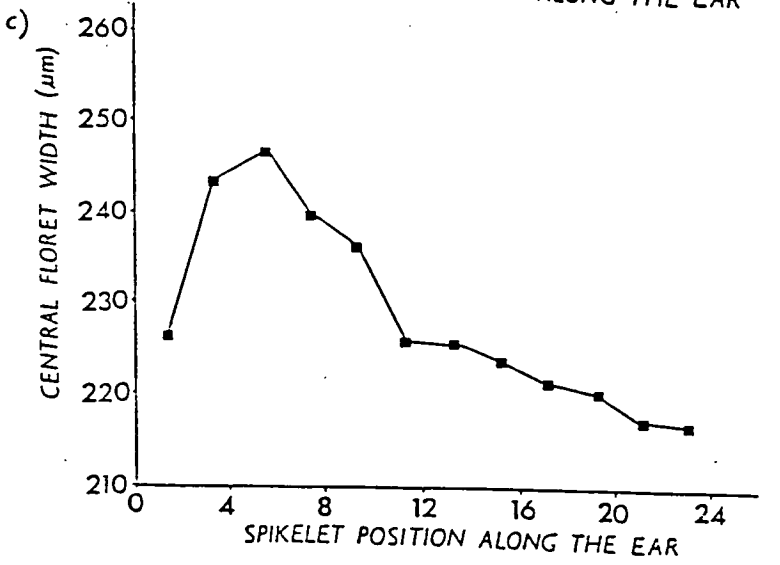

c)
CENTRAL FLORET WIDTH (μm)
260
250
240
230
220
210
0
4
8
12
16
20
24
SPIKELET POSITION ALONG THE EAR

Table 8. The 95% confidence limits (cl) for spikelet primordia widths at different positions along the spike at stage 4 and floret widths at different positions along the spike at stage 7 in Maris Mink plants at different nitrogen treatments

Spikelet size and cl at stage 4

	HN		LN	
	$\bar{x}$	cl	$\bar{x}$	cl
1.5	277.6	14.1	261.0	13.8
3.5	244.2	12.4	229.1	8.1
5.5	231.0	10.4	210.1	8.3
7.5	213.2	9.6	192.5	8.4
9.5	206.0	8.0	185.2	8.7
11.5	192.5	10.6	178.4	7.0
13.5	191.5	11.3	162.8	5.8
15.5	178.3	9.9	153.0	4.7
17.5	178.7	11.8	149.1	10.1
19.5	170.6	8.6	136.7	9.7
21.5	159.6	9.6	132.5	23.1
23.5	142.9	7.6	132.7	8.2
25.5	136.6	8.4	128.4	17.3

Floret size and cl at stage 7

	HN		LN	
	$\bar{x}$	cl	$\bar{x}$	cl
	194.1	8.0	170.8	22.8
	203.8	8.9	179.5	12.0
	191.1	7.0	169.2	14.2
	183.8	7.3	160.0	13.5
	173.7	6.2	150.9	12.5
	163.2	8.3	148.0	13.2
	159.1	7.7	144.7	11.6
	152.8	5.4	144.3	11.8
	150.9	4.7	140.5	10.5

Tiller production during mainstem spikelet production (Table 9)

Tiller production and development was markedly reduced in plants of the L40 set. Tillers did not emerge until after transfer to the compost. In the H40 set T1 started to emerge before the mainstem apex became reproductive. Dry weight gain by the tillers was initially small and then showed a sharp increase.

Table 9. Dry weight in mg of developing tillers from d18 to d34 in Maris Mink plants that were transferred to the greenhouse at d40 (MH40)

	Day from sowing:								
	18	20	22	24	26	28	30	32	34
T1	9.1	10.5	13.7	19.1	24.0	53.6	57.7	-	114.1
T2	-	1.1	6.5	11.5	15.7	18.0	31.2	-	49.7
T3	-	-	-	-	-	1.9	5.7	-	10.4

Final grain weight (Tables 10, 11, 12, 13; Figure 12a, b, c)

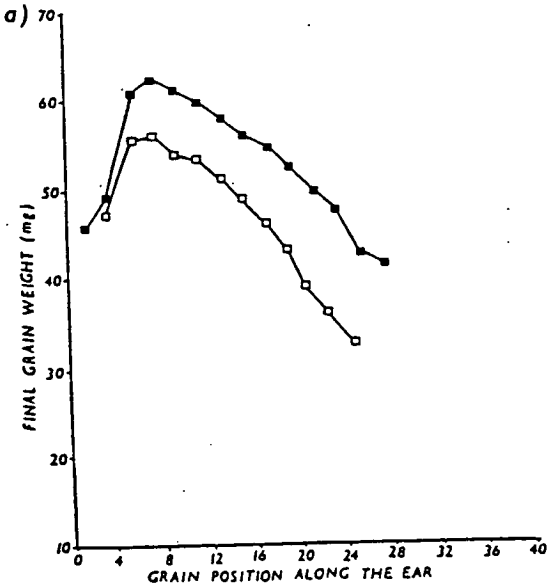
The final grain weight of the mainstem (MS) in L40 plants was significantly lower than that in the H40 plants (Table 10). The size of primordia appeared to influence the size of grain produced so that small primordia led to the production of small grain in low nitrogen treated plants. Plants transferred at d20 also produced significantly smaller grain in the L20 treatments than the H20 treatments (Table 10) but produced larger grain overall when compared with the d40 transferred plants.

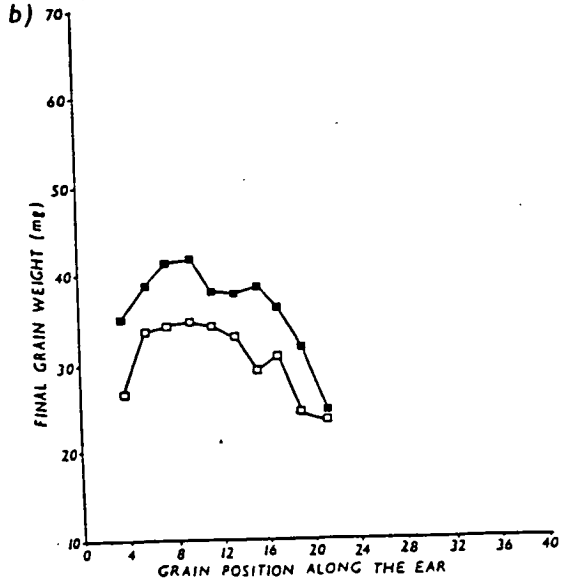
Plants transferred at d20 set more grain than those transferred at d40 and set the largest grain nearer the base of the ear (Table 11).

Figure 12a. The mean weight of grain set at various positions along the ear for MH20 treated plants (■) and for ML20 treated plants (□).

Figure 12b. The mean weight of grain set at various positions along the ear for MH40 treated plants (■) and for ML40 treated plants (□).

Figure 12c. The relationship between final grain weight and the width of spikelets at the double ridge stage at various positions along the ear for MH40 treated plants (■) ($y= -230.38 +2.58x - 6.12x^2$; $r^2= 0.927$, $n= 9$) and for ML40 treated plants (□) ($y= -5.92 + 1.30x - 3.51x^2$; $r^2= 0.899$, $n= 9$).


a)
FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
0 4 8 12 16 20 24 28 32 36 40
GRAIN POSITION ALONG THE EAR


b)
FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
0 4 8 12 16 20 24 28 32 36 40
GRAIN POSITION ALONG THE EAR

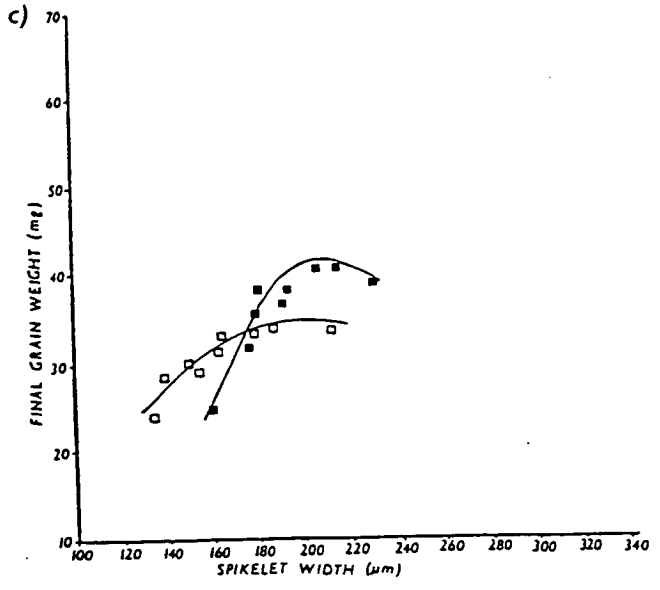

c)
FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
100 120 140 160 180 200 220 240 260 280 300 320 340
SPIKELET WIDTH (μm)

Table 10. The 95% confidence limits (cl) for grain weight at different positions along the spike in Maris Mink plants at different nitrogen treatments

Final grain weight along spike at different treatments

	H20		L20		H40		L40	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
3.5	49.7	5.3	47.6	4.3	35.0	4.3	27.0	3.5
5.5	61.3	2.4	56.2	2.5	39.1	4.9	33.9	4.9
7.5	62.6	1.9	56.5	3.5	41.8	4.7	34.2	4.7
9.5	61.8	1.9	54.6	2.2	41.9	3.9	34.9	4.3
11.5	60.5	1.9	54.2	1.8	38.0	14.7	34.3	4.5
13.5	58.8	1.8	51.8	1.9	37.8	3.9	33.0	4.3
15.5	56.5	1.8	50.0	1.9	38.5	3.7	28.9	3.5
17.5	55.3	1.8	46.8	1.8	35.8	3.7	30.1	4.9
19.5	53.0	1.3	43.9	1.9	31.5	4.1	28.5	6.5
21.5	50.2	1.6	41.8	2.7	28.9	4.3	24.0	12.5
23.5	47.9	1.6	35.8	2.5	28.3	9.8	-	-

Table 11. The position of grains set and the position and weight (mg) of the largest and smallest grains set on the mainstem of Maris Mink.

	Grain set:	largest grain:		smallest grain:	
	position	position	weight	position	weight
H40	3.5-21.5	9.5	41.9	21.5	24.6
L40	3.5-21.5	9.5	34.9	21.5	24.0
H20	1.5-27.5	7.5	62.6	27.5	41.9
L20	3.5-25.5	7.5	56.5	25.5	33.0

There was a correlation between primordia width at double ridge stage and grain weight. Small primordia at the tip of the apex gave rise to small grain and the large primordia at the centre of the spike gave rise to the largest grain.

For convenience sake the terms used to describe the different aspects of grain yield have been abbreviated as set out below:

mean individual grain weight $\bar{x}G$
mean number of grains per ear $\bar{x}N$
total grain weight per ear ΣG
total grain weight per plant* TGW
*(includes secondary and tertiary tillers)

In H40, H20 and L40 plants values of $\bar{x}G$ on the MS was higher than that on any of the tillers. Conversely, in L20 plants the $\bar{x}G$ values for T1 and T2 was higher than that for MS ears. In all but Tc, the values of $\bar{x}G$ in the L20 plants were higher than those on any of the ears set on H and L40 plants (Table 12).

In plants of the H20, H40 and L20 sets values of ΣG were significantly larger on the MS and primary tiller ears than for plants of the L40 set. The plants of the L20 set had larger ΣG those of the H40 set. The increase in ΣG shown by plants of the L20 set was a reflection of larger mean individual grain weight as there was little difference between treatments in $\bar{x}N$. In all treatments the mean individual grain weight was significantly higher in the mainstem than in the tiller ears but there was no significant difference between the first 3 primary tillers in any of the treatment (Table 13).

Table 12. Total grain weight per ear (mg), mean individual grain weight per ear (mg), mean grain number for mainstem and tiller ears and total grain weight per plant under different environmental regimes

		Treatment:			
		MH40	ML40	MH20	ML20
MS	x̄G	36.5	30.5	53.5	47.7
	x̄N	19.9	16.1	21.5	19.4
	ΣG	726.4	491.1	1150.3	925.4
Tc	x̄G	27.4	-	49.7	39.4
	x̄N	14.9	-	13.2	11.1
	ΣG	408.3	-	656.0	437.3
T1	x̄G	29.6	20.1	52.4	50.5
	x̄N	19.7	8.3	11.0	15.0
	ΣG	583.1	166.8	576.4	757.5
T2	x̄G	25.2	18.2	51.3	49.9
	x̄N	15.6	7.2	12.5	15.6
	ΣG	393.1	131.0	641.3	778.4
T3	x̄G	26.5	15.9	50.1	46.7
	x̄N	12.6	6.6	9.3	10.7
	ΣG	333.9	104.9	465.9	499.7
T4	x̄G	25.1	-	45.1	38.1
	x̄N	13.6	-	4.5	7.5
	ΣG	341.4	-	203.0	285.8
T5	x̄G	24.6	-	-	-
	x̄N	11.6	-	-	-
	ΣG	285.4	-	-	-
	TGW	3324.7	893.8	4011.8	3942.2

Table 13. Results from two way analysis of variance for total grain weight per ear (ΣG) in the different treatments and different ear positions within Maris Mink plants, including least significant difference values (LSD)

Source	d.f.	variance ratio (F)	signif.	LSD
Nitrogen treatment	3,9	17.8	***	248.8
Ear position	3,9	10.4	**	248.8

Proctor

Mainstem spikelet primordia initiation and development (Tables 14, 15)

PH40 and PL40 initiated more primordia over the 14d period from d20 to d34 than did the corresponding treatment for Maris Mink. PH40 took longer to get from stage 4 to 7 than MH40 but reached stage 9 sooner. The spikelet primordia passed through the various stages sooner in PL40 than in ML40.

Table 14. Time course of mainstem primordia initiation for PH40 and PL40 treatments up to day 36.

		Mean number of primordia present at different days from sowing								
		20	22	24	26	28	30	32	34	36
PH40	x̄n	8.6	10.0	13.5	21.5	22.0	-	29.0	29.3	40.0
	range	7-11	9-13	10-16	18-24	16-28	-	25-31	28-32	37-43
PL40	x̄n	-	9.0	10.0	15.6	22.3	-	-	24.8	-
	range	-	6-11	9-11	10-21	20-25	-	-	21-27	-

Table 15. Time taken for the most advanced primordia to reach stage 4, 7 and 9 and the position on the spike of the largest spikelet and grain in Proctor

	Time in days to			position of largest primordia			largest grain
	stage 4	stage7	stage9	stage 4	stage 7	stage 9	
PH40	20	32	36	1.5	3.5	7.5	9.5
PL40	24	27	-	1.5	3.5	-	9.5

Plant dry weight, leaf and tiller emergence (Table 16, 17)

Between d16-34 plants given low nitrogen had smaller dry weights than plants given high nitrogen and the differences increased with time (Table 16). Both leaf and tiller emergence were delayed by the application of low nitrogen solution (Table 17). H40 plants produced 9-10 leaves compared with L40 plants which produced 7. Like ML40, plants all the leaves produced by the PL40 plants were pale green with the lower ones turning yellow with time. Mainstem shoots were also short and thin.

On d20, when a batch of plants was placed in the greenhouse and the nitrogen regimes stopped, the difference in dry weight between L20 and H20 was 336.1mg. In both treatments, all the leaves had been produced by d20. L20 plants had 3 emerged leaves and 4 unemerged leaves and H20 had 4 emerged and 5 unemerged. Compared with L40 plants, L20 plants had larger, darker green leaves following transfer. Some 6 days after transfer the first tiller emerged in L20 plants. Tiller emergence was faster and more tillers were produced in L20 plants than in L40 plants, presumably because of the earlier exposure to the high nitrogen status of the compost to which they were transferred.

Table 16. Time course in days from sowing of mean plant dry weight (mg) of Proctor plants treated with high and low nitrogen, including 95% confidence limits in brackets

	plant treatment:	
	HN	LN
16	403.9(44.9)	212.8(29.5)
18	588.5(51.3)	290.1(34.1)
20	619.4(57.7)	283.3(25.8)
22	678.6(68.1)	320.7(42.7)
24	856.1(60.8)	381.4(45.5)
26	1059.9(82.3)	402.9(51.4)
28	1393.0(77.9)	460.6(58.1)
30	1394.4(85.6)	531.0(66.3)
34	1699.2(91.1)	539.8(68.2)

Table 17. Time course in days from sowing for leaf and tiller emergence in high and low nitrogen treated Proctor plants

	days from sowing:									
	16	18	20	22	24	26	28	30	32	34
HN										
	--------L4------L5--L6--L7----------									
	----------------T3------------------T4									
LN										
	------------L4------L5------L6--------									
	------------------------T1----------T2									

Mainstem spikelet size (Figures 13a, b, c; Table 18)

The width of spikelet primordia at double ridge stage in L40 plants was significantly lower than that in the H40 plants for any given primordium (Figure 13a, Table 18). There was no significant difference between Maris Mink and Proctor at this stage (Table 8, 18).

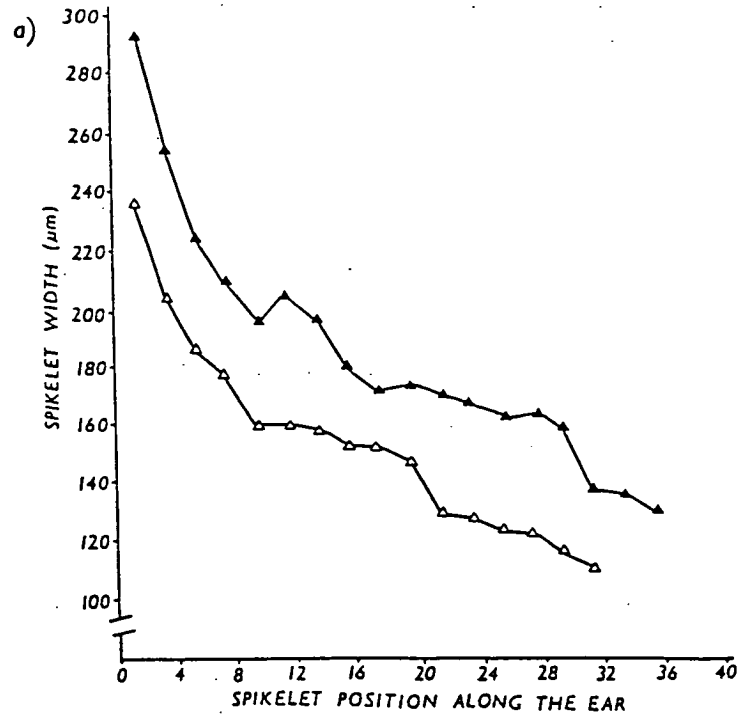
There was a significant difference at stage 7 in the size of the central florets, PH40 having larger central florets than PL40 (Figure 13b, Table 18). There was no significant difference in floret size at between the two cultivars. The most advanced spikelet primordia in PL40 had not yet reached stage 9 by d40. When the two cultivars were compared, Maris Mink at stage 9 had smaller central florets than Proctor. The largest spikelet primordia at stage 7 was situated at position 3.5 in both treatments and cultivars, but at stage 9 the largest primordia in PH40 (Figure 13c) was positioned higher up the spike than in MH40 (Figure 11c).

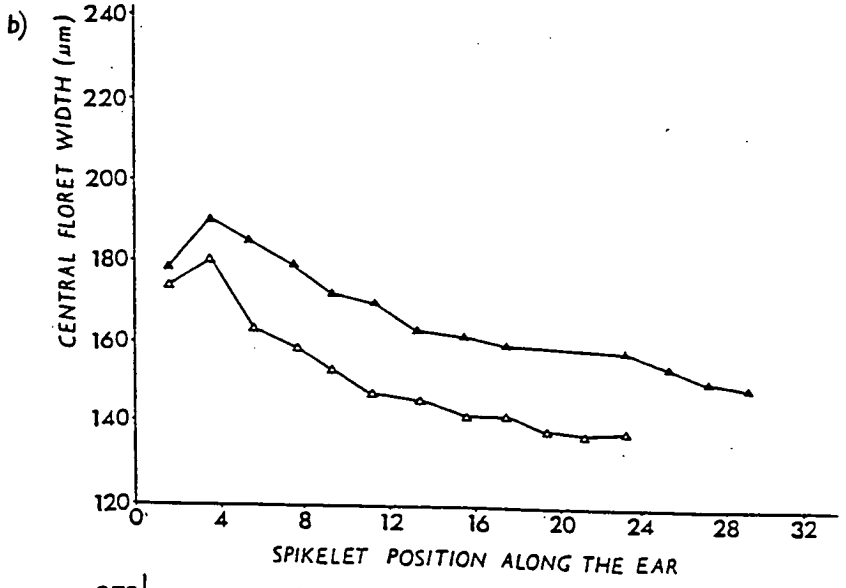
In PH40, as in MH40 (as found by Cottrell and Dale, 1984), the size variation between different primordia on the spike decreased by stage 9. In MH40 there was an increase in growth of central florets on the most distal primordia whilst in PH40 it was the central florets between positions 5 and 12 which grew the fastest between stages 7 and 9 resulting in a shift of the position of the largest primordia distally along the apex.

Figure 13a. The mean width of spikelet primordia at the double ridge stage of development, at various positions along the spike, for PH40 treated plants (▲) and for PL40 treated plants (△).

Figure 13b. The mean width of the central floret at stage 7 (lemma initials), at various positions along the spike, for PH40 treated plants (▲) and for PL40 treated plants (△).

Figure 13c. The mean width of the central floret at stage 9 (stamen initials visible as 3 distinct mounds), at various positions along the spike, for PH40 treated plants (▲).


a)
SPIKELET WIDTH (µm)
300
280
260
240
220
200
180
160
140
120
100
0
4
8
12
16
20
24
28
32
36
40
SPIKELET POSITION ALONG THE EAR


b)
CENTRAL FLORET WIDTH (µm)
240
220
200
180
160
140
120
0
4
8
12
16
20
24
28
32
SPIKELET POSITION ALONG THE EAR

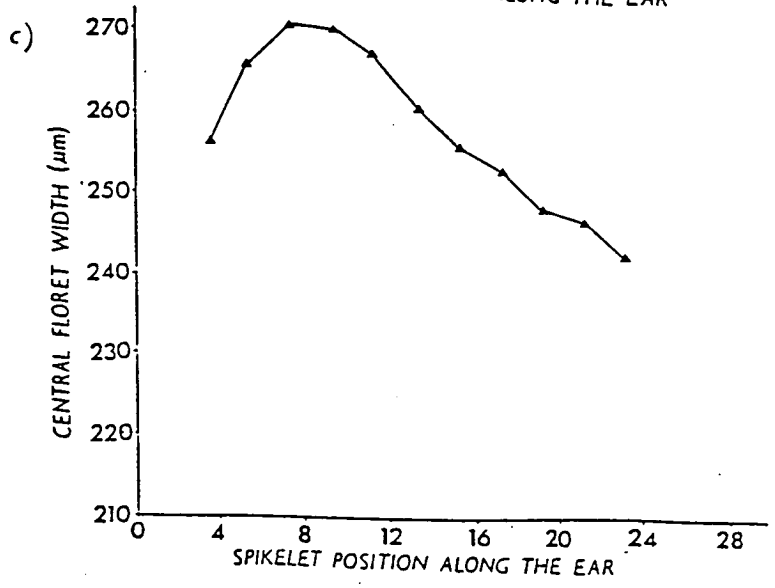

c)
CENTRAL FLORET WIDTH (µm)
270
260
250
240
230
220
210
0
4
8
12
16
20
24
28
SPIKELET POSITION ALONG THE EAR

Table 18. The 95% confidence limits (cl) for spikelet primordia widths at different positions along the spike at stage 4 and floret widths at different positions along the spike at stage 7 in Proctor plants at different nitrogen treatments

Spikelet size and cl at stage 4

	HN		LN	
	$\bar{x}$	cl	$\bar{x}$	cl
1.5	295.1	17.3	239.6	12.6
3.5	255.3	14.7	204.3	7.1
5.5	225.2	12.5	189.8	6.4
7.5	210.5	11.5	179.8	7.1
9.5	195.0	10.9	162.1	7.2
11.5	205.6	9.5	161.4	11.2
13.5	198.7	9.2	160.7	9.1
15.5	179.6	10.1	155.2	8.6
17.5	172.7	12.7	154.6	6.6
19.5	174.9	13.4	149.3	5.9
21.5	170.4	8.8	130.2	7.2
23.5	167.5	9.8	129.7	9.4
25.5	163.4	7.1	124.0	13.4
27.5	163.7	18.2	123.6	3.8
29.5	161.8	18.2	117.7	17.7

Floret size and cl at stage 7

HN		LN	
$\bar{x}$	cl	$\bar{x}$	cl
180.1	21.9	174.8	9.1
191.1	12.0	181.8	11.8
187.9	9.8	164.3	11.6
180.0	7.7	159.4	9.9
174.3	10.2	154.1	9.8
170.1	7.6	147.9	6.2
164.2	10.9	147.3	10.9
163.3	8.1	142.9	15.0
160.6	6.2	142.8	11.7

Tiller production during mainstem spikelet production (Table 19)

In the PH40 treatment, dry weight gain in T1 was small between d18 and d28 and then increased rapidly. T2 started to grow two days after T1. In PH40, T1 and T2 had already emerged by the time the apex had switched to producing reproductive primordia and the bud of T3 started to grow when the apex switched to producing reproductive primordia.

Table 19. Dry weight in mg of developing tillers from d18 to d34 in Proctor plants that were transferred to the greenhouse at d40

	Day from sowing:								
	18	20	22	24	26	28	30	32	34
T1									
PH40	47.4	69.1	71.0	90.5	137.7	209.6	263.8	-	339.1
PL40	0.7	0.9	1.3	3.1	4.7	6.8	14.6	-	7.5
T2									
PH40	14.6	21.1	25.5	37.8	88.0	105.0	120.2	-	152.3
PL40	-	-	0.2	0.5	0.7	2.6	3.7	-	7.5
T3									
PH40	0.7	2.9	4.5	5.3	15.9	38.6	40.6	-	62.7
T4									
PH40	-	-	-	-	0.4	3.5	4.1	-	4.6

The main difference between the two cultivars is that tiller production starts earlier in Proctor.

Final grain weight (Table 20, 21, 22, 23; Figures 14a, b, c)

For PL40 plants mainstem final grain weight was significantly lower than that in the PH40 plants, for any given position (Figure 14a, Table 20). PL20 produced slightly smaller grain than PH20 (Figure 14b) but produced larger grain overall when compared with the d40 transferred plants (Table 20, 21). Proctor plants set more grain than the corresponding Maris Mink plants.

In both low and high nitrogen treatments there was a correlation between the width of spikelet primordia at double ridge stage and the final grain weight (Figure 14c). These results are similar to those found in the MH40 set and in both cultivars the small primordia that developed in the L40 treatment produced small grain when compared with the H40 treatment.

In all P treatments, values of $\bar{x}G$ for the MS ears were higher than that for any of the tillers irrespective of time of transfer. Values of $\bar{x}G$ for P20 plants were higher than those for P40 plants. The plants given PL40 plants had by far the smallest $\bar{x}G$ values (Table 22).

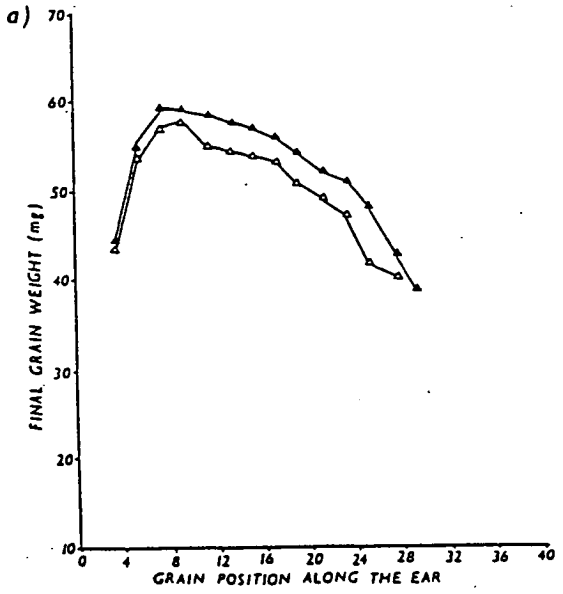
The values of $\bar{x}N$ were smallest in PL40. PL40 had its largest $\bar{x}N$ on the MS where as in PH20 and PH40 T1 and T2 produced larger $\bar{x}N$ than the MS. PH40 produced more $\bar{x}N$ on the T1 and T2 than those on PH20.

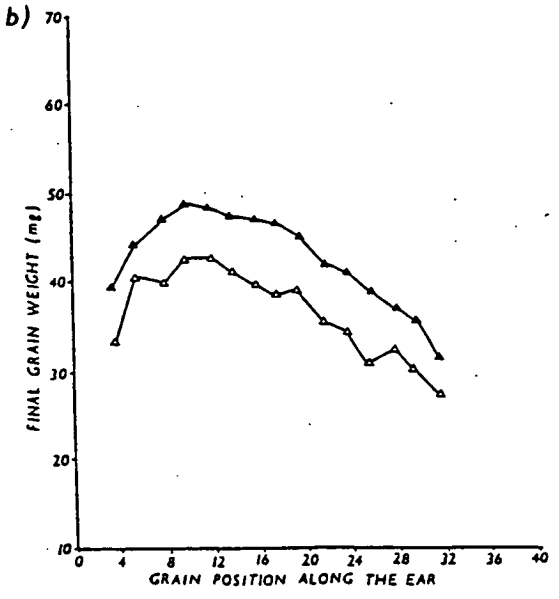
Values of ΣG for MS and primary tillers in PH20, PH40 and PL20 were significantly larger than those in the PL40 set. This was attributable partly to an increase in $\bar{x}G$ and partly to an increase in $\bar{x}N$. There was no significant difference in ΣG between the tillers in different treatments (Table 23).

Figure 14a. The mean weight of grain set at various positions along the ear for PH20 treated plants (♣) and for PL20 treated plants (▲).

Figure 14b. The mean weight of grain set at various positions along the ear for PH40 treated plants (♣) and for PL40 treated plants (▲).

Figure 14c. The relationship between final grain weight and the width of spikelets at the double ridge stage at various positions along the ear for PH40 treated plants (♣) ($y= -301.95 + 3.54x - 8.92x^2$; $r^2= 0.904$, $n= 13$) and for PL40 treated plants (▲) ($y= -75.58 + 1.36x - 3.96x^2$; $r^2= 0.962$, $n= 14$).


a)
FINAL GRAIN WEIGHT (mg)
GRAIN POSITION ALONG THE EAR


b)
FINAL GRAIN WEIGHT (mg)
GRAIN POSITION ALONG THE EAR

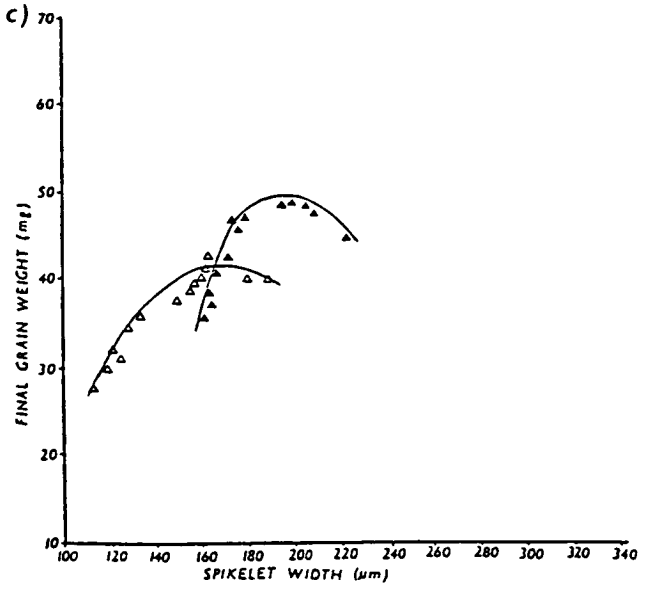

c)
FINAL GRAIN WEIGHT (mg)
SPIKELET WIDTH (μm)

Table 20. The 95% confidence limits (cl) for final grain weight at different positions along the spike in Proctor plants at different nitrogen treatments

	Final grain weight along spike at different treatments							
	H20		L20		H40		L40	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
3.5	44.5	4.5	44.1	3.1	39.7	3.3	34.0	4.7
5.5	55.2	3.7	54.0	2.2	44.3	3.7	40.4	3.7
7.5	59.9	3.5	57.4	3.7	47.6	3.7	40.2	3.1
9.5	59.5	3.5	57.9	3.7	49.1	2.2	42.7	3.1
11.5	58.9	3.7	55.2	3.7	48.6	2.2	41.7	2.9
13.5	58.4	3.5	54.6	3.1	47.5	2.4	40.9	2.4
15.5	57.4	3.7	54.2	3.5	47.3	2.4	39.8	2.4
17.5	56.3	2.2	53.5	3.1	46.6	1.9	38.6	2.7
19.5	54.1	2.2	51.4	3.1	45.3	2.2	38.6	2.9
21.5	52.1	2.2	49.1	3.7	42.7	2.7	36.0	3.1
23.5	51.2	2.2	46.5	5.9	41.4	2.7	34.6	2.9
25.5	47.8	3.9	41.7	6.2	38.9	2.3	31.3	3.5
27.5	42.9	2.7	-	-	37.2	3.5	32.1	4.9
29.5	38.8	3.7	-	-	35.5	1.4	29.8	6.1

Table 21. The position of grains set and the position and weight (mg) of the largest and smallest grains on the mainstem of Proctor

	Grain set:	largest grain:		smallest grain:	
	position	position	weight	position	weight
PH40	3.5-31.5	9.5	49.1	31.5	31.0
PL40	3.5-31.5	9.5	42.7	31.5	27.3
H20	3.5-29.5	7.5	59.9	29.5	38.8
PL20	3.5-27.5	9.5	57.9	27.5	41.2

Table 22. Total grain weight per ear (mg), mean individual grain weight per ear (mg), mean grain number per ear for mainstem and tiller ears and total grain weight per plant under different environmental regimes

		Treatment:			
		PH40	PL40	PH20	PL20
MS	x̄G	43.5	36.0	52.9	50.5
	x̄N	17.6	16.2	18.9	18.9
	ΣG	765.6	583.2	999.8	954.5
Tc	x̄G	-	-	43.2	42.3
	x̄N	-	-	15.2	14.3
	ΣG	-	-	656.6	604.9
T1	x̄G	42.5	25.2	48.2	45.1
	x̄N	27.1	10.5	23.6	18.1
	ΣG	1151.8	246.6	1137.5	816.3
T2	x̄G	41.3	20.0	45.4	41.2
	x̄N	24.2	9.5	20.4	17.7
	ΣG	995.3	190.0	926.2	729.2
T3	x̄G	38.7	16.0	46.3	40.3
	x̄N	17.6	7.5	18.8	17.6
	ΣG	681.1	120.0	870.4	709.3
T4	x̄G	37.2	-	39.5	39.9
	x̄N	16.3	-	19.5	15.2
	ΣG	606.4	-	770.3	606.5
	TGW	4200.2	1139.8	5969.8	4858.8

Table 23. Results from two way analysis of variance for total grain weight per ear (ΣG) in the different treatments and different ear positions within Proctor plants, including the least significant difference values (LSD)

Source	d.f.	variance ratio (F)	signif.	LSD
Nitrogen treatment	3,9	19.1	**	304.7
Ear position	3,9	2.6	ns	

5.2.2. REDUCED NITROGEN SUPPLY OVER THE VEGETATIVE PHASE OF GROWTH

Mainstem spikelet primordia initiation and development (Tables 24, 25).

After the ending of the low nitrogen treatment and the introduction of high nitrogen Maris Mink plants produced more primordia than those plants given high nitrogen from sowing. However, in Proctor fewer primordia were produced in the plants given low nitrogen until d14 than in those given high nitrogen from sowing, and fewer primordia were initiated in PL/H compared with ML/H. L/H plants had slightly delayed apical development such that ML/H took two days longer to reach stage 4 than MH plants and PL/H plants took 4 days longer than PH plants to reach stage 4.

Table 24. Time course of mainstem primordia initiation for L/H and H treatments up to day 28.

		Number of primordia present at different days from sowing					
		18	20	22	24	26	28
ML/H	x̄no	6.0	7.0	8.0	14.4	15.6	19.9
	range	4-9	6-10	6-11	12-17	12-20	18-22
PL/H	x̄no	5.4	7.3	7.8	13.6	17.0	17.4
	range	5-7	6-9	6-10	11-15	14-18	16-21
MH	x̄no	-	7.0	11.0	11.5	12.4	17.0
	range	-	6-8	10-12	7-14	11-14	16-18
PH	x̄no	-	8.6	10.0	13.5	21.5	22.0
	range	-	7-11	9-13	10-16	18-24	16-28

Table 25. Time taken for the most advanced primordia to reach stage 4 and the position on the spike of the largest spikelet and grain.

	Time in days to stage 4	largest primordia at stage 4	largest grain
ML/H	22	1.5	7.5
PL/H	24	1.5	11.5
MH	20	1.5	9.5
PH	20	1.5	9.5

Plant dry weight, leaf and tiller emergence (Tables 26, 27)

On addition of high nitrogen solution Maris Mink plants responded faster than Proctor to the increase and initially showed a larger dry weight gain (Table 26). However on d24 Proctor plants dry weight gain sharply increased so that by d28 they were heavier than Maris Mink plants. Leaf and tiller emergence was slightly faster in Maris Mink than in Proctor plants (Table 27).

Table 26. Time course in days from sowing of mean plant dry weight of Maris Mink and Proctor plants treated with low nitrogen for the first 14d ~~and those treated with low nitrogen for d40~~, including 95% confidence limits in brackets

	plant treatment:	
	ML/H	PL/H
18	215.1(23.5)	194.8(9.8)
20	274.5(23.7)	227.6(26.1)
22	339.3(50.2)	248.1(49.0)
24	342.8(29.8)	257.5(39.4)
26	419.0(88.8)	437.7(96.2)
28	516.2(159.4)	609.3(92.1)

Table 27. Time course in days from sowing for leaf and tiller emergence in plants treated with low nitrogen for 14d

	days from sowing:					
	18	20	22	24	26	28
M	--------L5----------					L6
	--------T2------			T3----		
P	L4----------			L5------		L6
	----Tc------			T2--------		

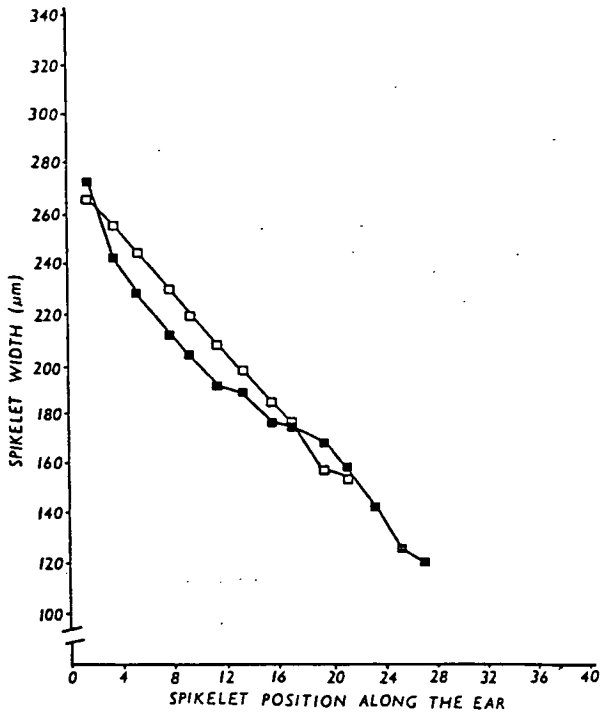
Mainstem spikelet size (Figure 15a, b; Table 28)

In both Maris Mink and Proctor, there was no significant difference in spikelet primordia widths at double ridge stage between plants in L/H sets and plants in the H sets.

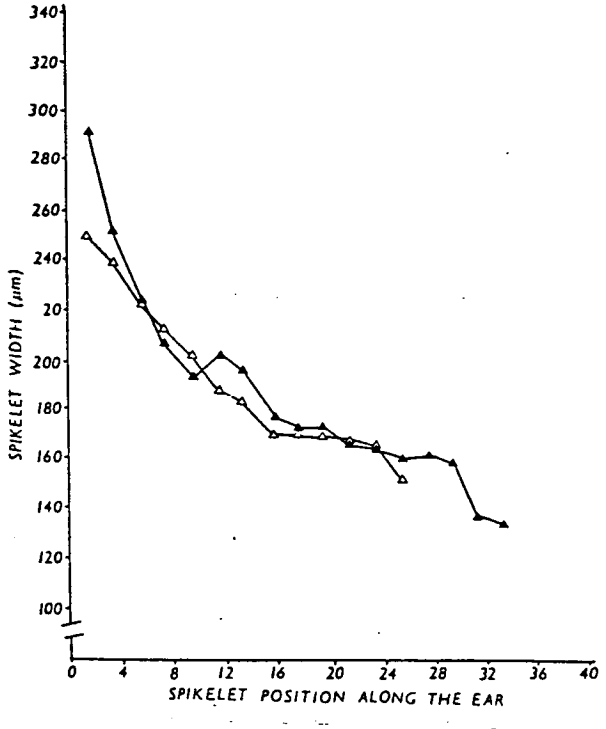
Figure 15a. The mean width of spikelet primordia at the double ridge stage of development, at various positions along the spike, for MH treated plants (□) and for ML/H treated plants (■).

Figure 15b. The mean width of spikelet primordia at the double ridge stage of development, at various positions along the spike, for PH treated plants (▲) and for PL/H treated plants (△).

a)


SPIKELET WIDTH (µm)
340
320
300
280
260
240
220
200
180
160
140
120
100
0
4
8
12
16
20
24
28
32
36
40
SPIKELET POSITION ALONG THE EAR

b)


SPIKELET WIDTH (µm)
340
320
300
280
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240
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120
100
0
4
8
12
16
20
24
28
32
36
40
SPIKELET POSITION ALONG THE EAR

Table 28. The 95% confidence limits (cl) for spikelet primordia widths at different positions along the spike at stage 4 for plants L/H treated plants and H treated plants

Spikelet size at stage 4 at different positions along the spike

	ML/H		MH		PL/H		PH	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	269.2	21.4	277.6	14.1	253.8	16.3	295.1	17.3
3.5	258.7	19.9	244.2	12.4	242.8	16.1	255.3	14.7
5.5	247.3	19.9	231.0	10.4	224.6	14.1	225.2	12.5
7.5	232.4	19.0	213.2	9.6	215.9	15.9	210.5	11.5
9.5	221.2	18.0	206.0	8.0	204.4	13.5	195.0	10.9
11.5	210.4	18.0	192.5	10.6	189.3	14.9	205.6	9.5
13.5	198.3	14.5	191.5	11.3	185.4	14.1	198.7	9.2
15.5	186.0	14.9	178.3	9.9	172.4	17.8	179.6	10.1
17.5	180.8	13.1	178.7	7.6	172.5	35.1	172.7	12.7
19.5	159.8	16.9	170.6	8.4	170.9	11.4	174.9	13.4
21.5					170.9	15.7	170.4	8.8

Tiller production during mainstem spikelet production (Table 29)

In ML/H plants T1 started to make a rapid dry weight gain from a smaller dry weight than the MH plants. Tc started to increase in dry weight rapidly around the time the most advanced spikelet primordia on the mainstem were entering the triple mound stage. T2 developed more slowly in the ML/H set than those in the MH set.

In contrast to the ML/H treatment, plants of the PL/H treatment showed delayed growth of T1 when compared with that of the PH plants. T2 was even further delayed, beginning to grow 8 days after the mainstem had begun to produce spikelet primordia whereas in the PH plants T2 had started to grow at the time the apex switch from producing vegetative to reproductive primordia.

Table 29. Dry weight in mg of developing tillers from d18 to d28 in Maris Mink and Proctor plants supplied with low nitrogen over the vegetative stage of plant development and in plants given high nitrogen from sowing

	18	20	22	24	26	28
Tc						
ML/H	2.1	5.0	14.1	20.5	40.5	46.2
T1						
ML/H	10.0	10.9	26.7	41.2	60.3	70.5
PL/H	10.2	10.5	36.2	44.5	88.2	142.8
MH	9.1	10.5	13.7	19.1	24.0	53.6
PH	47.4	69.7	71.0	90.5	137.7	209.6
T2						
ML/H	-	-	-	3.5	7.9	11.6
PL/H	-	-	-	2.0	6.2	17.5
MH	-	1.1	6.5	11.5	15.7	18.0
PH	14.6	21.1	25.5	37.8	88.0	105.0
T3						
ML/H	-	-	-	-	-	3.2
PL/H	-	-	-	-	1.3	5.7
MH	-	-	-	-	-	1.9
PH	0.7	2.9	4.5	5.3	15.9	38.6

Tiller growth in the ML/H set was faster than in the MH set, for example in the ML/H set T1 had increased in dry weight by 60.5 mg between d18-28 whereas T1 in the MH set over the same period had only increased in weight by 44.5 mg. However in the PL/H set tiller growth was slower than in the PH set, for example T1 in the PL/H set had only increased in dry weight by 132.6 mg as compared to an increase in dry weight of 162.2 mg in the PH set over the same period.

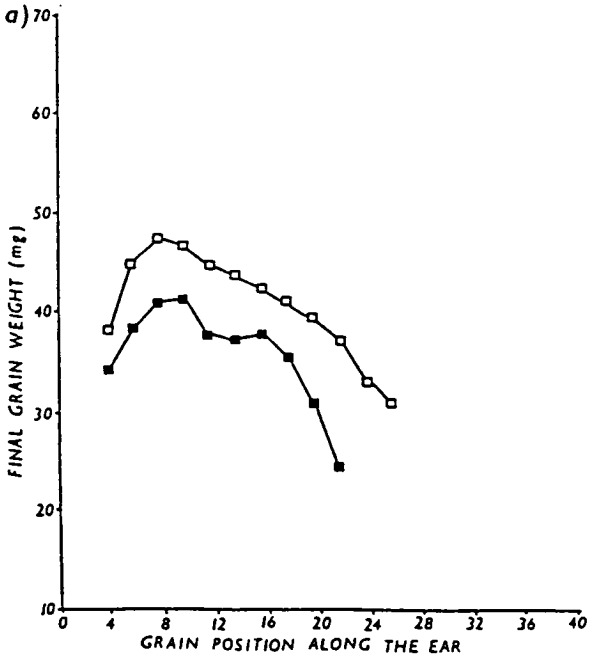
Final grain weight (Tables 30, 31, 32, 33; Figures 16a, 16b; 17a, 17b)

ML/H produced significantly larger mainstem grain on all positions when compared with the MH plants (Figure 16a, Table 30). More grain were also set in ML/H (Table 31) and perhaps this could be related to the faster initiation of spikelets. As found before there was a correlation between the width of the spikelet at double ridge stage and the final weight of the grain it produced (Figure 16b). The curve for the ML/H set was shallower than that for MH reflecting the smaller differences between the largest and smallest grains in ML/H.

Plants in the PL/H set had slightly smaller grains than in the PH set but the difference was not significant (Figure 17a, Table 30). Grain were smaller in the PL/H set than in the ML/H set but the former set more grain and showed a smaller difference between the largest and smallest grain. The usual correlation between the width of the spikelet at double ridge stage and the final weight of the grain it produced was found. The shape of the curves in PL/H and PH sets are similar. The main difference between the PL/H and ML/H sets was that in the PL/H set the grain was smaller than in H set, whereas in ML/H the grain weight was larger than in MH.

Figure 16a. The mean weight of grain set at various positions along the ear for MH treated plants (●) and for ML/H treated plants (○).

Figure 16b. The relationship between final grain weight and the width of spikelets at the double ridge stage at various positions along the ear for MH treated plants (●) ($y= -230.38 + 2.58x - 6.12x^2$; $r2= 0.927$, $n= 9$) and for ML/H treated plants (□) ($y=-25.80 + 0.61x - 1.28x^2$; $r^2= 0.963$, $n= 9$).


a)
FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
0
4
8
12
16
20
24
28
32
36
40
GRAIN POSITION ALONG THE EAR

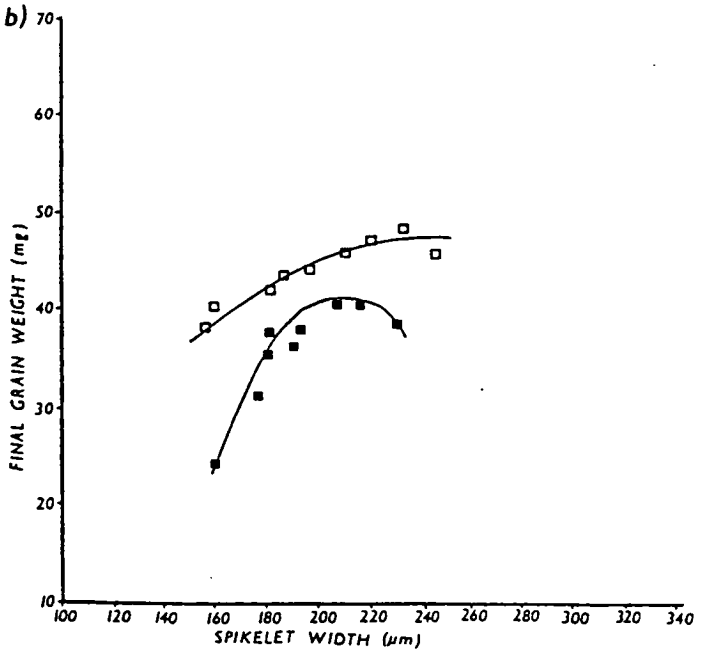
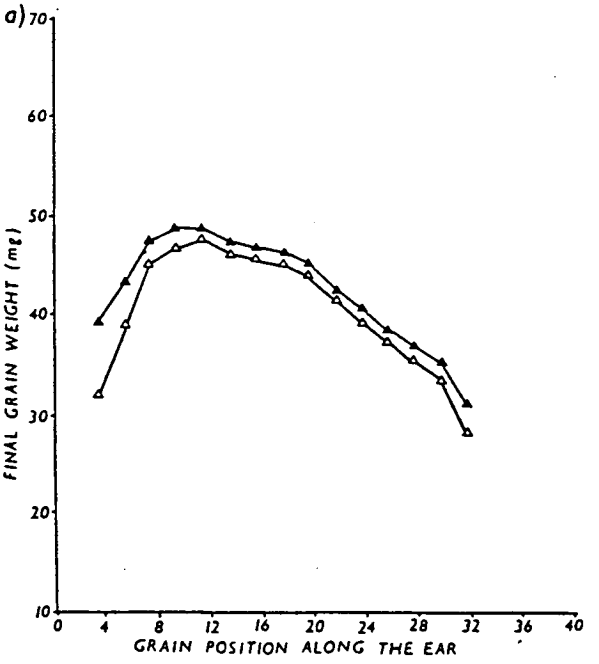

b)
FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
100
120
140
160
180
200
220
240
260
280
300
320
340
SPIKELET WIDTH (μm)

Figure 17a. The mean weight of grain set at various positions along the ear for PH treated plants (▲) and for PL/H treated plants (△).

Figure 17b. The relationship between final grain weight and the width of spikelets at the double ridge stage at various positions along the ear for PH treated plants (▲) ($y = -301.95 + 3.54x - 8.92x^2$; $r^2 = 0.904$, $n = 13$) and for PL/H treated plants (△) ($y = -163.43 + 2.13x - 5.37x^2$; $r^2 = 0.794$, $n = 10$).


a)
FINAL GRAIN WEIGHT (mg)
70
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40
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20
10
0
4
8
12
16
20
24
28
32
36
40
GRAIN POSITION ALONG THE EAR

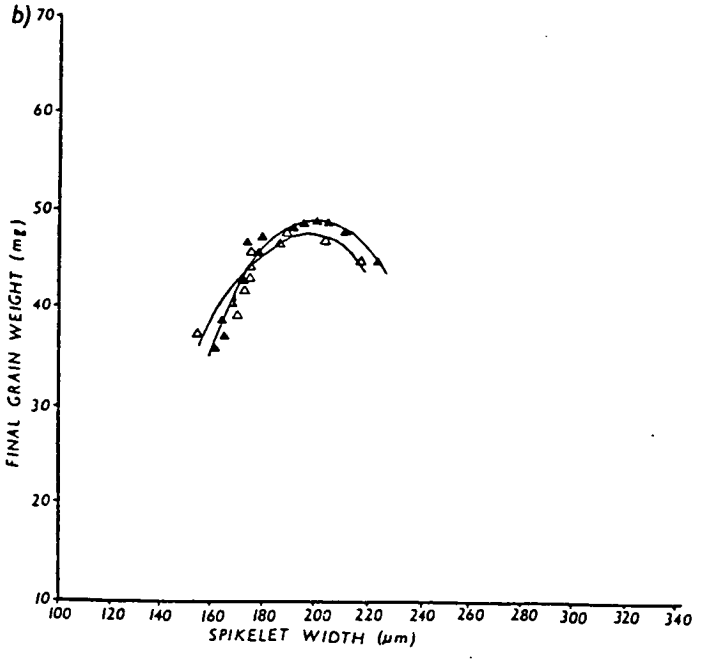

b)
FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
100
120
140
160
180
200
220
240
260
280
300
320
340
SPIKELET WIDTH (μm)

Table 30. The 95% confidence limits (cl) for final grain weight at different positions along the spike in Maris Mink and Proctor plants at different nitrogen treatments

Final grain weight and confidence limits in different treatments

	ML/H		MH		PL/H		PH	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
3.5	39.0	3.7	35.0	4.3	32.0	8.6	39.7	3.3
5.5	45.5	2.9	39.1	4.9	39.3	5.1	44.3	3.7
7.5	48.0	2.9	41.8	4.7	45.3	2.7	47.6	3.7
9.5	47.4	2.9	41.9	3.9	46.6	3.7	49.1	2.2
11.5	45.5	3.1	38.0	14.7	47.2	2.5	48.6	2.2
13.5	44.3	2.9	37.8	3.9	46.4	1.3	47.5	2.4
15.5	43.1	2.7	38.5	3.7	45.5	2.2	47.3	2.4
17.5	41.5	2.7	35.8	3.7	45.1	2.4	46.6	1.9
19.5	40.0	2.7	31.5	4.1	43.7	2.9	45.3	2.2
21.5	37.8	2.5	28.9	4.3	41.6	2.5	42.7	2.7
23.5	3.7	2.4	28.3	9.8	39.3	2.5	41.4	2.7

Table 31. The position of the largest and smallest set grain and the mean weight (mg) of this grain in L/H and H treatments.

	Grain set:	largest grain:		smallest grain:	
		position	weight	position	weight
ML/H	3.5-27.5	7.5	48.0	27.5	25.0
PL/H	3.5-31.5	9.5	46.2	31.5	27.8
MH40	3.5-21.5	9.5	41.9	21.5	24.6
PH40	3.5-31.5	9.5	49.1	31.5	31.0

Numbers of grain were highest on the mainstem in both the ML/H and MH sets. ML/H plants had a higher value of $\bar{x}N$ on all ears when compared with the corresponding ears on MH plants (Table 32). There was little difference between the $\bar{x}N$ overall between PL/H and PH treatments.

ML/H plants had larger ΣG than the MH plants and this increase was attributable partly to an increase in the $\bar{x}G$ and partly to an increase in $\bar{x}N$ relative to the MH plants (Table 32). There was a significant difference between ears so that in both Maris Mink treatments ΣG was highest in the MS, second highest in T1 and smallest in T5 (Table 33).

There was no significant difference in ΣG between PL/H and PH treatments (Table 33).

Table 32. Total grain weight per ear (mg), mean individual grain weight per ear (mg), mean grain number per ear for mainstem and tiller ears and total grain weight per plant under different nitrogen regimes

		Treatment:			
		ML/H	PL/H	MH	PH
MS	x̄G	40.2	39.3	36.5	43.5
	x̄N	27.0	23.4	19.9	17.6
	ΣG	1085.4	919.6	726.4	765.6
Tc	x̄G	28.1	-	27.4	-
	x̄N	15.9	-	14.9	-
	ΣG	445.6	-	408.3	-
T1	x̄G	37.5	32.0	29.6	42.5
	x̄N	22.9	16.4	19.7	27.1
	ΣG	857.8	524.0	583.1	1151.8
T2	x̄G	35.0	32.6	25.2	41.3
	x̄N	18.3	14.8	15.6	24.2
	ΣG	639.7	482.8	393.1	995.3
T3	x̄G	35.3	30.4	26.5	38.7
	x̄N	16.1	16.3	12.6	17.6
	ΣG	566.7	495.5	333.9	681.1
T4	x̄G	35.3	34.1	25.1	37.2
	x̄N	15.7	17.2	13.6	16.3
	ΣG	554.7	585.4	341.4	606.4
T5	x̄G	25.5	33.5	24.6	-
	x̄N	16.7	15.0	11.6	-
	ΣG	425.0	502.5	285.4	-
	TGW	5331.3	4288.9	3324.7	4200.2

Table 33. Results from two way analysis of variance for total grain weight per ear (ΣG) in the different treatments and different ear positions within Maris Mink and Proctor plants, including least significant difference values (LSD)

Source	d.f.	variance ratio (F)	signif.	LSD
ML/H.MH				
Nitrogen treatment	1,7	28.1	***	213.3
Ear position	7,7	7.0	**	148.9
PL/H.PH				
Nitrogen treatment	1,4	1.6	ns	
Ear position	4,4	0.4	ns	

5.2. DISCUSSION

The hypothesis for these experiments was that by supplying plants with low concentrations of nitrogen, nitrogen deficiency would result and give rise to plants producing small primordia.

Varying the amount of nitrogen given to plants had different effects according to duration of application. Giving low amounts of nitrogen up to d40 resulted in smaller primordia at double ridge stage than giving larger amounts of nitrogen upto d40. The difference in size was maintained throughout subsequent stages.

Giving low amounts of nitrogen upto d20 gave results complicated by the fact that on transfer to nitrogen rich compost on d20 plants had not completed initiation of primordia and since widths were only measured up to d20 there are no data for sizes of subsequently produced primordia, although as expected for the other plants, low nitrogen supply led to smaller primordia at stage 4 than those given high nitrogen.

Reducing the nitrogen supply during the vegetative stage, before spikelet primordia production, did not to alter spikelet primordia size at stage 4.

Dale (1972) showed that a delay in supplying nitrogen to plants soon after germination caused a reduction in plant size and reduced the photosynthetic activity of the first leaf. The result of delaying the supply of nitrogen so that it is given 8 days rather than 2 days after germination had a long term influence on leaf emergence rates and final leaf size such that the mainstem leaves up to the fifth leaf may be 20% smaller and emerge 5 days later than normal (Dale, 1972). Although, in ~~the~~ present experiment, nitrogen was not totally withheld, its supply was substantially reduced to the extent that visual symptoms of the deficiency were seen 10 days after sowing (Dale, 1976). The reduction of nitrogen

supply during the vegetative phase would be expected to reduce the plant's capacity for assimilate production, by delaying leaf emergence, producing smaller leaves and reducing the photosynthetic activity of the leaves. It might be expected therefore, that low nitrogen given over the vegetative period would cause a reduction in plant growth and spikelet primordia size at stage 4 but this was not the case.

Plant growth was reduced over the period when low nitrogen was supplied, but recovered rapidly after the nitrogen supply was increased. The size of spikelet primordia at double ridge stage was not altered and was similar between LN/HN and HN treated plants, for both cultivars. This suggests that conditions prior to spikelet initiation have little effect on spikelet primordium size at stage 4. However, low nitrogen must influence the size of spikelet primordia in that plants treated with low nitrogen for more than 14 days had smaller primordia than those treated with high nitrogen. Hence, it must be conditions during the period when the apex becomes reproductive, that are important in determining the size of spikelet primordia at stage 4. It would appear that as long as favourable conditions are restored by the time spikelet primordia production starts, the size of the spikelets will be unnaffected by the previous poor conditions; in other words there is no memory effect, at least in the short-term.

Lowered nitrogen supply for forty days causes an irreversible reduction in spikelet primordia size. Plants given low nitrogen for the first 40 days after germination showed classic symptoms of nitrogen deficiency with small dry weight, light green chlorotic leaves, lower leaves turning yellow and short and thin stems. Leaf and tiller emergence were retarded and fewer leaves and tillers produced. Nitrogen deficiency probably limited the size of spikelets by inhibiting cell growth, as it does in leaves (Dale, 1972). The assimilate requirement of spikelet primordia at stage 4 must be very small and so it is unlikely that small

primordia are due to limited assimilate supply. Small cells in spikelets may determine the size grain ultimately achieve.

Varying nitrogen supply during the reproductive stage of apical development clearly altered spikelet primordia size but to what extent does spikelet primordia size determine grain size? Was grain size different in plants where spikelet size at stage 4 was unaltered? According to the Cottrell and Dale hypothesis small primordia would invariably give rise to small grain. Similarly cultivars and treatments which had the same primordia sizes at stage 4 would give rise to plants with the same size grain. On the removal of specific nitrogen regimes and transfer to nitrogen rich compost, nitrogen deficiency should have been alleviated. According to the hypothesis, alleviation of the nitrogen deficit would not result in large grain because the small primordia produced during the nitrogen deficient period had already limited grain size. What was not known was whether the alleviation of the nitrogen deficiency might actually result in the production of larger grains than predicted.

In fact, where the different nitrogen treatments were maintained until primordia initiation ceased, primordia size at stage 4 appeared to determine grain size, as predicted. However, grain size varied where conditions were only maintained for a short period, as in the plants given low nitrogen before the onset of spikelet primordia production and those given low and high nitrogen for only 20 days. Cultivar response to conditions varied affecting the spikelet/ grain size relationship and the reasons for this are discussed below.

The size of spikelet primordia at stage 4 suggested that plants given low nitrogen over the vegetative period of apex development would develop similar size grain to those given high nitrogen from sowing until d40. Maris Mink, however, went on to produce larger grain following the reduced nitrogen treatment. In Maris Mink

plants, a growth spurt occurred following the removal of the low nitrogen and the introduction of the high nitrogen supply. In Proctor the plants appeared to be slower in utilising the increased nitrogen supply. Perhaps the ability to utilise the increased nitrogen supply rapidly conferred an advantage on Maris Mink plants, thus allowing increased grain production. At this stage no firm ideas can be put forward as to the form of the advantage. LN/HN treated Maris Mink does have faster tiller emergence and may be related to the increases in grain weight.

Although plants transferred on day 40 subsequently grew in nitrogen rich compost, grain production was smaller in plants given low nitrogen prior to this time. This means that the spikelets producing the grain were still suffering as a result of low nitrogen supply prior to grain growth even though the plants were growing in a nitrogen rich compost over this crucial period.

Grain nitrogen reserves have been shown to be sufficient to allow maximum development of seedlings for 4 to 5 days after sowing (Dale, 1972) but after this period, an external supply of nitrogen is needed if developing leaves are to expand fully and achieve maximum rates of photosynthesis (Metivier and Dale, 1977). If the nitrogen supply was inadequate, one might expect a cumulative effect on successive leaves which would become smaller as carbohydrate supply from previously formed small leaves, necessary for development of later leaves, is limited (Dale, 1976). The supply of low nitrogen for 40 days may mean that not only are smaller primordia produced due to the direct result of nitrogen shortage but also that the smaller plants produced may be less able to supply carbohydrate for the developing grain.

At this point it is difficult to see whether small primordia or small plants or a combination of both are determining grain size. If small primordia at the double ridge stage limit grain size then one would expect that if low nitrogen conditions were only

maintained upto and during double ridge stage and then alleviated, small grain would still be produced.

Plants that were transferred at d20 were only subjected to a shortage of nitrogen for a brief period, upto and during double ridge stage (stage 4). In Maris Mink small primordia on d20 did appear to give rise to small grain. In Proctor, however, this was not the case and plants given low nitrogen produced larger grain than would be predicted by spikelet size at stage 4.

Why is there this difference between the two cultivars? The timing of tiller production in the plants transferred at day 40 was found to vary between cultivars so that tillers in Maris Mink plants given high nitrogen took longer to emerge than those in Proctor. Thus Maris Mink had more unemerged, non-autonomous, tillers present over the period when the mainstem was initiating spikelet primordia. This may affect spikelet primordia size and ultimately grain size, through a competition mechanism.

Proctor transferred at d40 produced larger grain than Maris Mink plants given the same treatment. There was no size difference in spikelet primordia at double ridge stage between the 2 cultivars, although Proctor did have larger florets at stage 9 than Maris Mink. Later stages in Maris Mink plants were reduced in size and this was associated with smaller grain. Tillers emerged and grew more slowly in Maris Mink with the MH40 plants producing two more primary tillers than Proctor, as well as secondary tillers which were not produced by Proctor. Over the early stages of development both cultivars had tillers that were dependent on the mainstem but by stage 9 only Maris Mink was supporting later formed and young tillers. It could be that while tillers are developing they have a high demand for nitrogen and thus limit the amount available to the developing mainstem spikelets. Certainly it has been shown that higher concentrations of nitrogen are

required for maximum tiller bud growth than for that of the plant (Fletcher and Dale, 1974).

While both cultivars were supporting tillers their spikelet primordia and floret sizes were similar. Proctor then appeared to produce larger florets, commencing at around the time tiller 3 developed its own root system and could support its self.

Perhaps, the longer that plants are grown under low nitrogen conditions, the more long-term the effect of reduced primordia size. Whilst low nitrogen conditions for 40 days affect spikelet primordia size there may also be an effect on the whole plant reducing the possibility of compensation. Small grains may be more a consequence of small plants than small primordia per se.

At day 20, plants given low nitrogen had smaller dry weights than H20 plants. However, following the removal of the nitrogen limiting condition the plants appeared to recover, producing larger, greener leaves and more tillers than the L40 plants. From this it would appear that alleviation of the nitrogen deficit does occur on transfer to the nitrogen rich compost. So, although Maris Mink plants in general recover from the nitrogen deprivation, grain producing spikelets do not. It may be that growth of the rest of the plant, especially that of the tillers, uses up the new supply of nitrogen at the expense of the spikelets. In PL20 where tiller growth was less vigorous the spikelet primordia were probably released from nitrogen deficiency and so were able to produce larger grain. Compensation must have occurred in later stages of plant development, counteracting the effect of small primordia on final grain weight. These results suggest that spikelet primordia size does play a role in determining grain size. If plant size and thus ability to supply assimilate to developing grain is the determining factor of final grain size then small grain should not be produced. Small grain were produced in the L20 batch of plants when compared with the

H20 batch of plants and this corresponded with spikelet primordia size in the two batches.

So, in both cultivars it would appear that altering nitrogen supply to plants during the period of spikelet initiation limits the size grain will ultimately achieve. Altering nitrogen supply to plants with spikelets at the double ridge stage, reduces spikelet size but still allows for later compensation, the extent of this being determined by cultivar.

So far, in considering the relationship between spikelet primordia and grain size nothing has been mentioned about the position of spikelets within the ear and size. Except for the most basal primordia, throughout these experiments the size of each grain in relation to its position on the spike appeared to be set by stage 4 and this was not altered in subsequent stages. The first formed basal primordia were the exception and their size appears to be set later, at stages 7 and 9. These results are of considerable importance because even when spikelet size is increased overall, the relationship between spikelets remains the same thus limiting any obtainable increase in grain weight.

It might be expected that at stage 4, basal spikelet primordia nearer to the collar would be largest and that the further away a primordium was from the collar the smaller it would be since nutrients would have further to diffuse to reach it. Later in spike development, at stage 5, vascular procambium develops in the lower mid-primordia but only later does this connect to the main vascular system. Some time later the rest of the primordia develop procambia, with the most distal ones only becoming connected to the main vascular system around stage 11 (Kirby and Rymer, 1974). It is likely that this sequence of vascular development leads to the hierarchy of floret size, development and grain sizes up the ear.

The lower mid-primordia develop vascular connections first. These spikelet primordia are the most advanced, have the largest florets at stages 7 and 9 and produce the largest grain. Vascularisation then proceeds both acro and basipetally and it appears that the longer the primordia takes to become vascularised the smaller the resulting florets grow. This again is compatible with a nutritional hypothesis.

The size differences between different primordia established in the early stages of development remain the same throughout later stages and are reflected in the weight distribution along the ear of the grains finally produced. The basal grains are smaller than the lower mid grain on the apex because although they are in the most favourable position for nutrients early on they were slow to become vascularised and lost their initial advantage. This may explain why, with the exception of the most basal primordia, there is a good correlation between the size of spikelet primordia at stage 4 and final grain weight.

When the grain weight curves for plants grown under the same nitrogen regimes, but transferred to the greenhouses on different days, are compared the shape seemed to be very similar. That is to say grain size relative to their position along the spike remained the same in plants transferred at d20 and in d40. If the size relationship is set when primordia are at stage 4 then a change in size of primordia produced after d20 in Proctor would have altered the relationship. Thus if the primordia initiated on plants transferred to the greenhouse had been wider at stage 4 than those initiated in the same position on the spike of growthroom-grown plants, the shape of the grain weight curve would have been shallower for the greenhouse grown plants with the grain at the distal end of the ear being closer in weight to those of the heaviest grain at the centre of the ear. Since this was not the case, it is probable that primordia formed after d20 were the same size in both sets of plants.

When the most advanced spikelet is at developmental stage 4, the apex is not vascularised but nutrients reach the spike via a vascular 'disc' at the collar and then are transported to the developing spikelets by diffusion (Kirby and Rymer, 1974; R. Hill unpublished data for Proctor barley). Conditions up to d20 might affect the size of later formed primordia if the size of the vascular 'disc' supplying the apex was smaller in low nitrogen treated plants. A smaller disc may limit the nutrient supply reaching the apex and this may affect the size of later formed primordia. Kirby and Faris (1970) found that barley plants grown at high densities have discs with smaller diameters plants grown at low density, showing that disc size can be influenced by the environment. The smaller disc size was associated with a reduction in spikelet number and, although it was not measured, there may have been a reduction in spikelet primordia size.

Until this fixed size relationship of spikelets within the ear can be broken the increase of grain weight will be limited. The ideal would be to produce an ear with grain of uniform size, all grain being the size of those arising from the mid-basal spikelets which produce the largest grain in current cultivars.

SECTION 6. THE EFFECT OF TILLERS ON MAINSTEM SPIKELET AND FINAL GRAIN SIZE

6.1. INTRODUCTION

The results described previously indicate that under certain conditions environment can have an effect on spikelet and grain size in barley. In addition, it was noted that the degree of tiller production varied with nitrogen supply. This raises the possibility of differences in competition between the mainstem and developing tillers making it impossible to differentiate between the effect of environment *per se* on primordium dimensions and its indirect effect due to tiller interactions. Two approaches were adopted to clarify this conflict.

The first used the mechanical removal of tillers to eliminate the effects of tiller competition. Such an experiment suffers the inevitable flaw that it is not possible to discount completely the effects of any wound response arising from mechanical injury. Similarly it is not possible to estimate the effect of organic material lost through tiller removal although this might be expected to be small. To avoid these problems a second approach was to use a plant which does not produce tillers. Whilst the uniculm cultivar, Compana, is not near-isogenic to either of the other cultivars studied, so that comparisons cannot rule out the effects of the differing genotype on development, it does provide a useful alternative. The aim of the experiments described in this chapter was 1) to determine the extent to which tillers affected spikelet primordia size and final grain weight on the mainstem and, 2) to determine the effect of nitrogen supply on plants when tillering was not a complicating factor.

The cultivars and treatments used are as follows:

Cultivar	Tiller regime	Nitrogen treatment	Code
Maris Mink	Detillered	High	(M)Dt
	Freely tillering	High	(M)C
	(ie. control)	Low	(M)L
Proctor	Detillered	High	(P)Dt
	Freely tillering	High	(P)C
	(ie. control)	Low	(P)L
Uniculm Compana Uniculm		High	(UC)H
		Low	(UC)L

6.2. RESULTS

6.2.1. EFFECT OF TILLER REMOVAL

Tillers were removed as they emerged (see Methods and Materials) but all other cultural conditions were the same as used for the H40 plants in the previous chapter.

Tillers are initiated close to the mainstem apex and when they were removed the apex might have been damaged which would then alter the growth and development of the rest of the plant. A number of extra measurements were taken on a group of detillered plants and freely tillering plants to try determine the extent of the injury suffered by the plant after detillering. Leaf length and emergence rate of leaves were measured in both detillered and freely tillering plants. These measurements were taken from the time of removal of the first tiller until the time the most advanced spikelet primordium on the mainstem was at stage 6.

Leaf length after emergence (Table 34)

In both cultivars and treatments, the older the leaf the smaller the final length. There was no significant difference in any of the leaf lengths between detillered and freely tillering plants of either cultivars.

Leaf emergence (Table 35)

There was no significant difference in the number of leaves that emerged between treatments. In Maris Mink over the period from d18 to d20, 0.2-0.3 leaves emerged per day, in both treatments. In Proctor, over the period from d18 to d28, 0.3 leaves emerged per day in both treatments.

Table 34. Time course of mean ($\bar{x}$) leaf length (cm) after emergence and the 95% confidence limits (cl) for leaves 1-4, in Maris Mink and Proctor

The length of leaf at different days from sowing

			6	7	8	9	10	12	13	14	15
L1	MC	$\bar{x}$	-	5.7	7.6	10.1	-	12.1	-	12.1	12.2
		cl	-	4.9	4.5	5.7	-	7.8	-	6.9	7.4
	MDt	$\bar{x}$	-	5.4	7.6	10.0	-	12.7	-	13.0	13.1
		cl	-	2.5	3.7	5.3	-	7.3	-	15.3	15.9
	PC	$\bar{x}$	5.5	7.3	9.0	-	12.2	12.3	12.3	12.3	12.3
		cl	9.2	9.8	10.4	-	13.5	13.7	14.9	10.6	13.9
	PDt	$\bar{x}$	5.9	7.5	9.2	-	11.1	11.3	11.4	11.4	11.4
		cl	7.3	14.7	11.0	-	18.4	16.1	12.7	9.9	13.2

			11	12	13	14	15	16	18	19
L2	MC	$\bar{x}$	-	10.1	14.0	16.6	16.9	17.2	-	17.3
		cl	-	7.1	6.5	10.0	12.7	10.6	-	10.2
	MDt	$\bar{x}$	-	10.4	14.3	16.7	17.2	18.1	-	18.1
		cl	-	15.7	13.0	13.5	11.2	7.8	-	7.8
	PC	$\bar{x}$	5.3	7.0	9.2	12.0	15.5	20.1	20.1	20.1
		cl	21.6	27.4	35.3	37.2	35.3	23.5	18.6	15.4
	PDt	$\bar{x}$	5.3	7.4	10.1	12.9	16.5	18.1	18.2	18.2
		cl	16.1	15.5	16.7	23.5	18.8	17.6	12.0	9.7

		13	14	15	16	18	19	20	21	22	23
L3 MC	x̄	4.4	7.3	9.7	13.4	-	21.5	-	21.6	21.7	21.7
	cl	9.0	5.3	7.3	9.8	-	8.6	-	6.7	6.9	4.3
MDt	x̄	4.2	7.0	9.9	13.1	-	11.1	-	21.4	21.6	21.6
	cl	25.5	15.5	19.0	21.6	-	8.6	-	11.2	10.4	5.4
PC	x̄	-	0.9	1.2	-	9.2	13.3	17.8	21.0	22.1	22.1
	cl	-	18.4	23.5	-	4.3	3.7	5.5	14.0	13.3	10.6
PDt	x̄	-	0.4	1.8	-	13.1	15.2	20.2	21.3	22.1	22.2
	cl	-	5.9	5.9	-	19.4	15.9	17.1	9.8	11.6	5.3

		18	19	20	21	22	25	26	27	28	29
L4 MC	x̄	-	9.9	-	18.9	21.2	-	29.2	29.6	30.1	30.2
	cl	-	6.5	-	12.2	10.2	-	13.4	12.3	12.3	12.0
MDt	x̄	-	10.7	-	17.1	20.9	-	29.1	29.4	32.1	32.1
	cl	-	14.0	-	18.1	13.3	-	16.4	16.8	15.5	17.6
PC	x̄	0.4	4.5	-	8.1	15.9	26.2	27.0	27.0	27.0	27.0
	cl	8.2	8.2	-	7.8	14.3	13.1	10.8	11.2	10.9	9.5
PDt	x̄	1.0	2.8	7.9	-	14.4	25.1	-	25.9	25.9	25.9
	cl	19.0	15.4	12.6	-	16.9	18.2	-	16.3	10.3	7.6

Table 35. Time course for leaf emergence in Maris Mink and Proctor, including the 95% confidence values (cl)

	Number of leaves emerged at different days from sowing										
	18	19	20	21	22	23	24	25	26	27	28
MDt	-	3.8	-	4.0	-	4.3	-	-	5.0	-	5.2
(cl)	-	5.4	-	4.2	-	4.4	-	-	1.9	-	2.6
MC	-	3.7	-	4.4	-	4.8	-	-	5.0	-	6.0
(cl)	-	2.5	-	3.3	-	2.1	-	-	3.1	-	1.2
PDt	3.0	-	4.0	-	4.0	-	4.2	-	5.0	-	6.0
(cl)	2.0	-	1.9	-	3.0	-	3.2	-	4.1	-	1.4
PC	3.0	-	4.0	-	4.2	-	4.4	-	5.3	-	5.8
(cl)	1.8	-	2.2	-	3.5		1.6	-	3.3	-	1.8

Mainstem spikelet primordia initiation and development (Tables 36, 37)

Initiation of spikelet primordia in detillered plants was slowed. Between d20-d30 MDt initiated 14.6 spikelets whereas MC plants initiated 17.2. PDt between d20-d28 initiated 12.3 spikelets whereas PC initiated 13.4 over the same period. Between d20 and d30 MDt initiated more but had actually produced fewer spikelet primordia overall than PDt.

Collectively the data from Tables 34-37 suggest that there were no major effects of injury.

Table 36. The number of mainstem primordia initiated between day 20 and day 34 in detillered and freely tillering plants of both cultivars

	Number of primordia present at different days from sowing				
	20	24	26	28	30
MDt	5.5	7.5	12.0	13.5	20.1
range	4-7	5-8	9-14	11-15	17-22
MC	7.0	11.5	12.4	17.0	24.2
range	6-8	7-14	11-14	16-18	21-29
PDt	9.5	10.8	14.8	16.7	21.8
range	7-11	9-12	12-16	13-18	18-24
PC	8.6	13.5	21.5	22.0	-
range	7-11	10-16	18-24	16-28	-

Treatment did not appear to alter primordium development (Table 37).

Table 37. Development of the most advanced spikelet primordia on the mainstem from day 20 until day 36 for both detillered and freely tillering plants in both cultivars

	Stage of most advanced primordia at different days from sowing								
	20	22	24	26	28	30	32	34	36
MDt	3.0	3.0	3.0	4.0	4.0	4.0	5.4	7.0	8.2
MC	4.0	4.0	4.0	4.0	4.0	7.0	-	7.2	8.2
PDt	4.0	4.0	4.0	4.0	4.0	5.3	5.6	6.0	7.0
PC	4.0	4.0	5.7	6.3	6.5	-	7.0	-	9.0

Mainstem spikelet size (Figure 18a, 18b, 19a, 19b; Tables 38, 39)

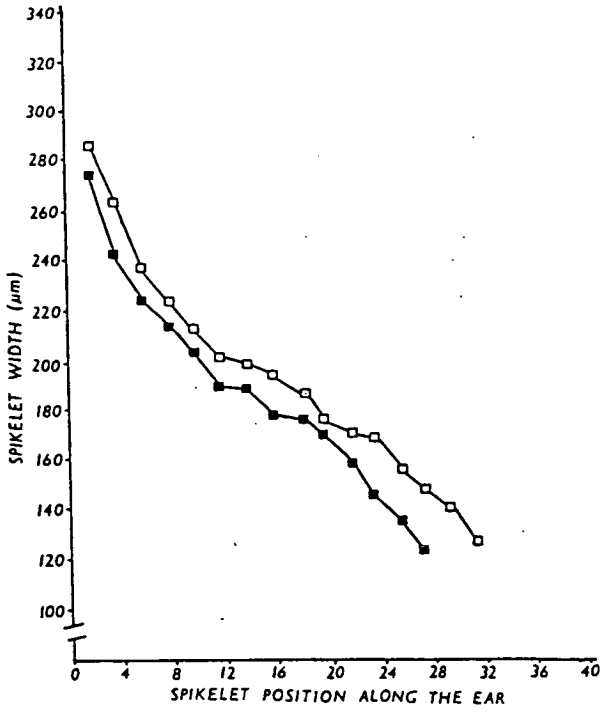
The width of spikelet primordia at double ridge stage became progressively less from base to tip of the developing spike in both freely tillering plants and detillered plants and there was no significant difference between treatments at this stage (Figure 18a, 19a, Table 38).

The widths of central florets at stage 7 were larger than the controls in both MDt and PDt (Figure 18b, 19b, Table 39). There is the suggestion that the presence of tillers appear to affect the size of spikelet primordia later in development.

Figure 18a. The mean width of spikelet primordia at the double ridge stage of development, at various positions along the spike, for MDt plants (□) and for MC plants (■).

Figure 18b. The mean width of spikelet primordia at the double ridge stage of development, at various positions along the spike, for PC plants (▲) and for PDt plants (△).

a)


SPIKELET WIDTH (µm)
340
320
300
280
260
240
220
200
180
160
140
120
100
0
4
8
12
16
20
24
28
32
36
40
SPIKELET POSITION ALONG THE EAR

b)

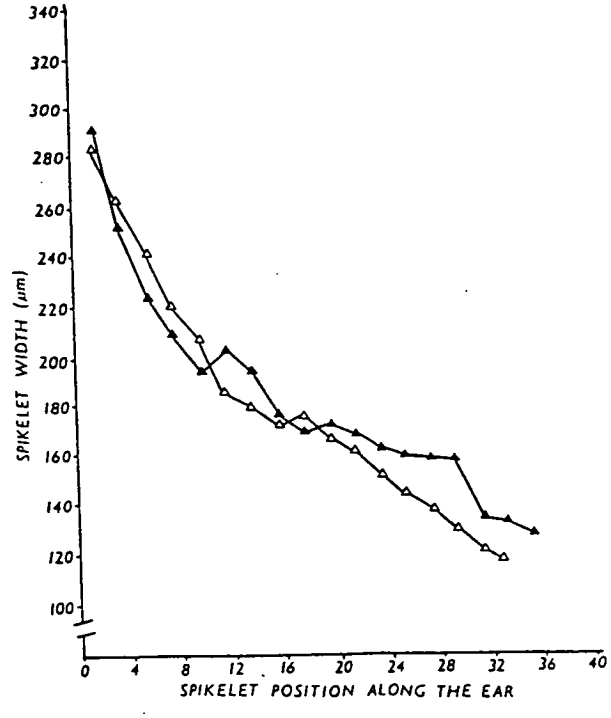
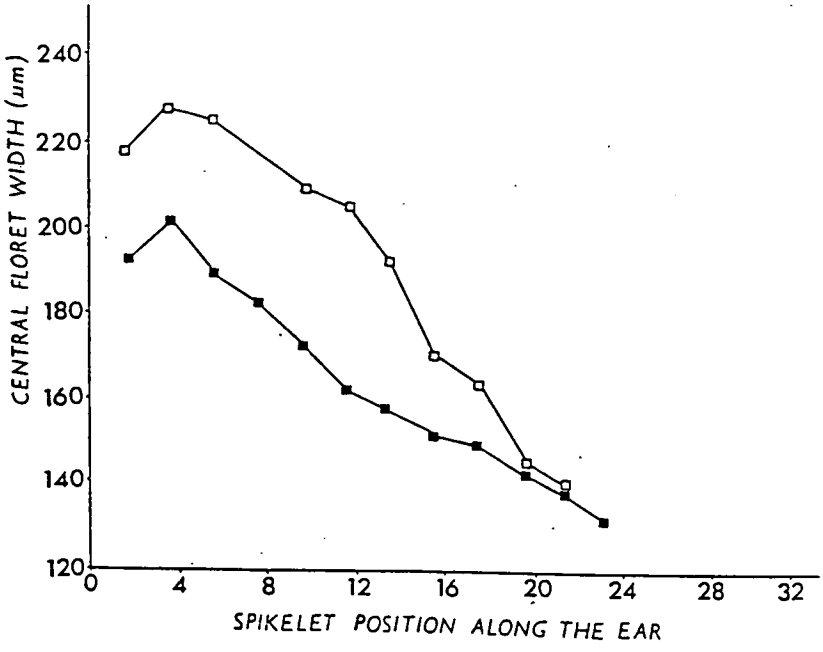

SPIKELET WIDTH (µm)
340
320
300
280
260
240
220
200
180
160
140
120
100
0
4
8
12
16
20
24
28
32
36
40
SPIKELET POSITION ALONG THE EAR

Figure 19a. The mean width of the central floret at stage 7 (lemma initials), at various positions along the spike, for MDt plants (□) and for MC treated plants (■).

Figure 19b. The mean width of the central floret at stage 7 (lemma initials), at various positions along the spike, for PC plants (▲) and for PDt plants (△)

a)


CENTRAL FLORET WIDTH (µm)
240
220
200
180
160
140
120
0
4
8
12
16
20
24
28
32
SPIKELET POSITION ALONG THE EAR

b)

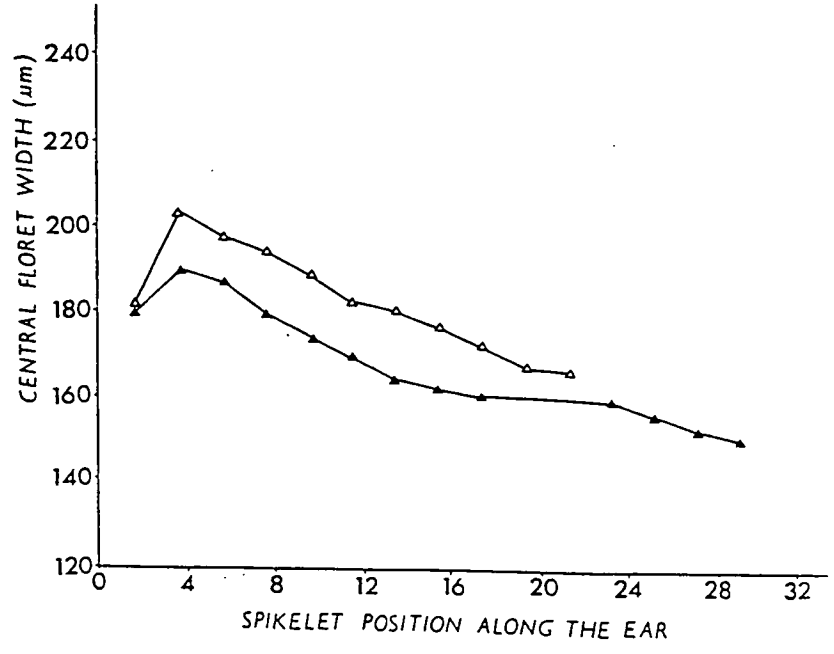

CENTRAL FLORET WIDTH (µm)
240
220
200
180
160
140
120
0
4
8
12
16
20
24
28
32
SPIKELET POSITION ALONG THE EAR

Table 38. The 95% confidence limits (cl) for spikelet primordia width along the spike at stage 4 in detillered and freely tillering plants in both cultivars

	Spikelet primordia size along spike at stage 4							
	MDt		MC		PDt		PC	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	290.0	17.7	277.6	14.1	287.7	10.9	295.1	17.3
3.5	267.3	20.4	244.2	12.4	265.5	12.5	255.3	14.7
5.5	239.4	17.4	231.0	10.4	243.3	10.9	225.2	12.5
7.5	225.9	15.9	213.2	9.6	222.4	12.6	210.5	11.5
9.5	214.6	14.9	206.0	8.0	208.6	15.1	195.0	10.9
11.5	203.7	18.2	192.5	10.6	187.8	10.9	205.6	9.5
13.5	200.9	17.4	191.5	11.3	181.3	11.2	198.7	9.2
15.5	195.1	17.0	178.3	9.9	174.5	12.7	179.6	10.1
17.5	185.5	12.5	178.7	11.8	177.4	11.6	172.7	12.7
19.5	177.7	18.4	170.6	8.6	169.3	10.4	174.9	13.4
21.5	173.5	20.7	159.6	9.6	162.4	16.7	170.4	8.8
23.5	171.6	20.4	142.9	7.6	152.6	16.9	167.5	9.8
25.5	157.8	15.3	136.6	8.4	146.3	17.1	163.4	7.1
27.5	149.1	10.6	122.4	10.8	139.8	10.7	163.7	18.2
29.5	142.7	7.6	-	-	130.7	31.6	161.8	18.2
31.5	127.2	4.3			122.8	18.7	138.7	10.6

Table 39. The 95% confidence limits (cl) for floret widths at stage 7 in freely tillering and detillered plants in both cultivars

	Floret size along the spike at stage 7							
	MDt		MC		PDt		PC	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	195.9	8.4	194.1	8.0	182.4	12.8	180.1	21.9
3.5	213.4	9.1	203.8	8.9	205.3	12.0	191.1	13.0
5.5	208.9	13.1	191.1	7.0	198.2	10.2	187.9	9.8
7.5	202.4	8.7	183.8	7.3	195.0	9.1	180.0	7.7
9.5	200.2	12.1	173.7	6.2	189.5	12.6	174.3	10.2
11.5	189.3	10.8	163.2	8.3	181.8	6.7	170.1	7.6
13.5	181.0	11.6	159.8	7.7	177.0	5.5	164.2	10.9
15.5	171.5	10.4	152.8	5.4	173.8	9.6	163.3	8.1
17.5	164.2	12.3	150.9	10.5	168.2	5.4	160.6	6.2
19.5	157.9	7.1	143.5	7.7				
21.5	137.8	4.5	139.2	8.0				

Mainstem final grain weight (Figures 20a, 20b, 20c, 21a, 21b, 21c; Tables 40, 41)

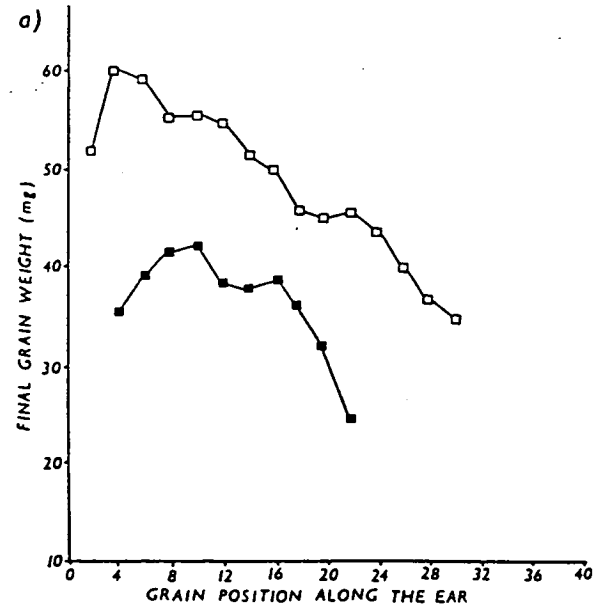
Detillered plants in both cultivars produced heavier grain than for those of the freely tillering plants (Figure 20a, 21a, Table 41) In Dt plants the largest grain was set in a lower position 3.5 than in C (7.5). MDt set 8 more grains than MC and PDt set 9 more than in any of the other treatments (Table 40).

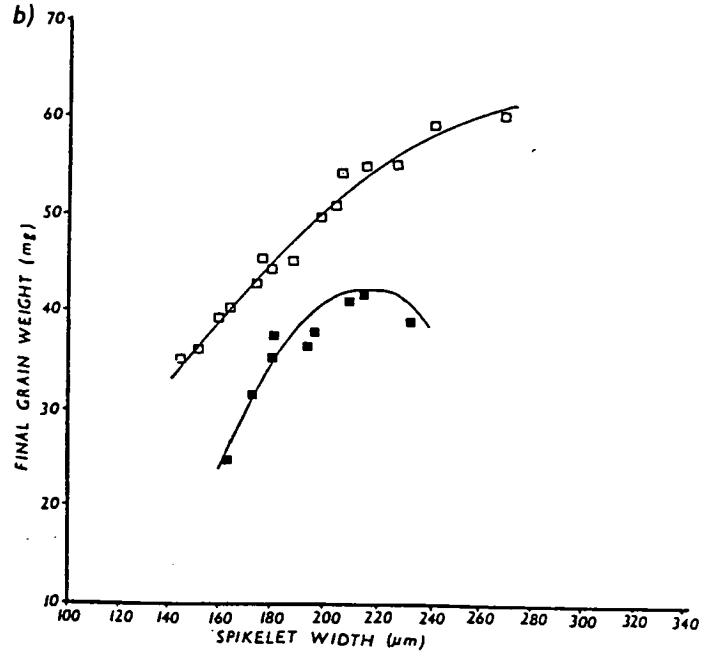
In Maris Mink, the shape of the curve relating final grain weight and spikelet primordia width at double ridge stage was similar for both treatments, although in MDt the curve was displaced up the y axis, because larger grain were set than in MC (Figure 20b, 20c).

Figure 20a. The mean weight of grain set at various positions along the ear for MDt plants (□) and for MC plants (■).

Figure 20b. The relationship between final grain weight and the width of spikelets at the double ridge stage at various positions along the ear for MC treated plants (■) ($y = -230.38 + 2.58x - 6.12x^2$; $r^2 = 0.927$, $n = 9$) and for MDt treated plants ($y = -41.46 + 0.70x - 1.20x^2$; $r^2 = 0.978$, $n = 15$) (□).

Figure 20c. The relationship between final grain weight and the width of florets at stage 7 (lemma initials) at various positions along the ear for MC plants (■) ($y = -286.87 + 3.71x - 0.01x^2$; $r^2 = 0.912$, $n = 11$) and for MDt plants (□) ($y = 88.37 - 0.65x + 2.41x^2$; $r^2 = 0.937$, $n = 10$).


a)
FINAL GRAIN WEIGHT (mg)
GRAIN POSITION ALONG THE EAR


b)
FINAL GRAIN WEIGHT (mg)
SPIKELET WIDTH (μm)

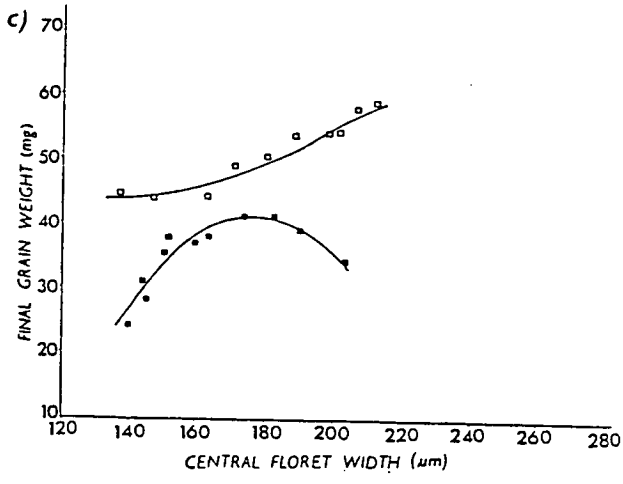
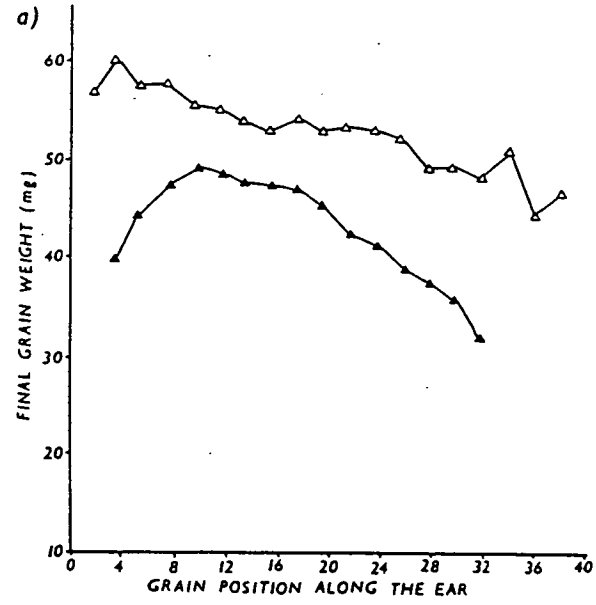
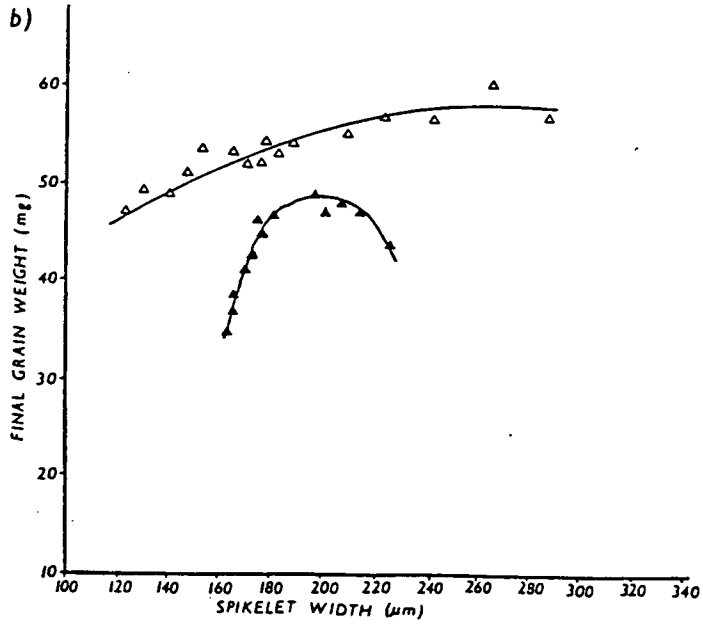

c)
FINAL GRAIN WEIGHT (mg)
CENTRAL FLORET WIDTH (μm)

Figure 21a. The mean weight of grain set at various positions along the ear for PC (▲) and for PDt treated plants (△).

Figure 21b. The relationship between final grain weight and the width of spikelets at the double ridge stage at various positions along the ear for PC plants (▲) (y= -301.95 + 3.54 - 8.92 x^2; r^2= 0.904, n= 13) and for PDt plants (△) (y= 22.07 + 0.27 - 4.99x^2; r^2= 0.918, n= 16).

Figure 21c. The relationship between final grain weight and the width of florets at stage 7 (lemma initials) at various positions along the ear for PC plants (▲) (y= -694.03 + 8.60x - 0.02x^2; r^2= 0.949, n= 11) and for PDt plants (△) (y= 137.55 - 1.07x + 3.40x^2; r^2= 0.854, n= 10).


a)
FINAL GRAIN WEIGHT (mg)
60
50
40
30
20
10
0
4
8
12
16
20
24
28
32
36
40
GRAIN POSITION ALONG THE EAR


b)
FINAL GRAIN WEIGHT (mg)
60
50
40
30
20
10
100
120
140
160
180
200
220
240
260
280
300
320
340
SPIKELET WIDTH (μm)

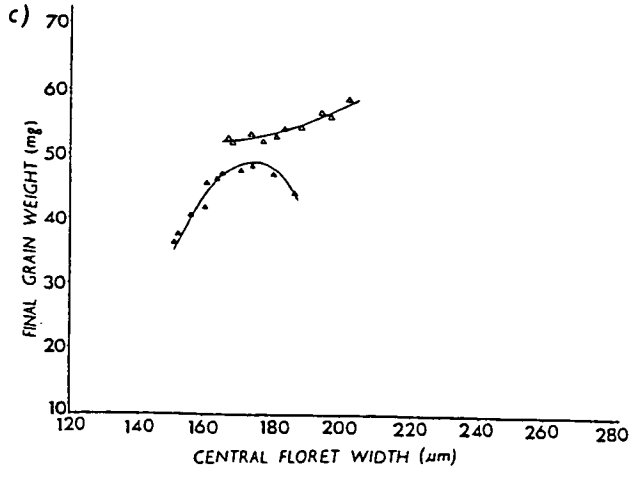

c)
FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
120
140
160
180
200
220
240
260
280
CENTRAL FLORET WIDTH (μm)

Table 40. The position of mainstem grains set and the position and weight (mg) of the largest and smallest mainstem grains set in detillered and freely tillering plants of both cultivars

	Grain set:	largest grain:		smallest grain:	
		position	weight	position	weight
MC	3.5-21.5	9.5	41.9	21.5	24.6
MDt	1.5-29.5	3.5	60.3	29.5	35.0
PC	3.5-31.5	9.5	49.1	31.5	31.0
PDt	1.5-37.5	3.5	60.4	35.5	44.5

Table 41. The 95% confidence limits (cl) for final grain weight at different positions along the spike in freely and detillered plants of both cultivars

Final grain weight and confidence limits in different treatments

	MDt		MC		PDt		PC	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	51.7	9.0	-	-	56.8	5.5	-	-
3.5	60.3	3.9	35.0	4.3	60.4	5.5	39.7	3.3
5.5	59.2	3.9	39.1	4.9	57.3	5.5	44.3	3.7
7.5	55.0	5.1	41.8	4.7	57.9	3.3	47.6	3.7
9.5	55.3	4.1	41.9	3.9	55.3	3.3	49.1	2.2
11.5	54.5	5.9	38.0	14.7	54.8	3.3	48.6	2.2
13.5	51.0	4.5	37.8	3.9	53.8	3.5	47.5	2.4
15.5	49.5	4.5	38.5	3.7	52.9	3.9	47.3	2.4
17.5	45.4	3.5	35.8	3.7	54.3	3.5	46.6	1.9
19.5	44.8	3.9	31.5	4.1	52.5	3.7	45.3	2.2
21.5	45.5	5.3	28.9	4.3	53.2	2.5	42.7	2.7
23.5	43.4	9.6	28.3	9.8	53.0	3.1	41.4	2.7
25.5	39.7	2.7	-	-	51.6	2.7	38.9	2.3
27.5	36.7	10.4	-	-	48.8	3.7	37.2	3.5
29.5	-	-	-	-	49.0	4.5	35.5	1.4

In PDt the curve for grain weight and primordia width was disimilar in shape to that for PC. This is because in PDt differences in grain weight at different positions along the ear were smaller than in PC (Figure 21b, 21c). The basal grain in PDt plants did not show a marked reduction in size when compared with other positions.

6.2.2. EFFECT OF VARYING THE NITROGEN SUPPLY ON UNICULM COMPANA

Initiation and development of spikelet primordia (Tables 42, 43)

Initiation in UCH from d20 to d32 was slower than in MC and PC. However UCH had already initiated 4.4 more primordia than MC and 2.8 more than PC by d20. UCL plants had a faster spikelet primordia initiation rate than either ML or PL, and by d22 UCL had initiated 7.4 more spikelet primordia than ML plants and 3.4 more than PL plants. Initially UCH plants initiated more spikelet primordia than UCL but between d20 and d30 the UCL plants initiated 4.9 more spikelet primordia than the UCH plants (Table 42).

The rate of development of the most advanced primordia was similar in UCH and PC plants But slightly faster than in MC plants. The rate appears fastest of all in UCL, plants in this treatment having already reached stage 4 before d20 and reaching stage 8 faster than in UCH, ML and PL plants (Table 43). However it should be noted that this higher developmental rate is only marginal being only 0.1 units more than UCH at d20 (ie. 4.1 *cf* 4.0) and at d26 it had a lower score (ie. 4.5 *cf* 5.2).

Table 42. The number of mainstem primordia initiated between day 20 and day 34 for Uniculm Compana, Maris Mink and Proctor grown in different nitrogen regimes

	Number of primordia present at different days from sowing							
	20	22	24	26	28	30	32	34
UCH	11.4	11.4	12.2	15.8	16.5	17.3	21.0	-
range	9-13	9-13	10-14	13-18	15-17	15-19	20-22	-
MC	7.0	11.0	11.5	12.4	17.0	24.2	-	25.4
range	6-8	10-12	7-14	11-14	16-18	21-29	-	23-26
PC	8.6	10.0	13.5	21.5	22.0	-	-	29.3
range	7-11	9-13	10-16	18-24	16-28	-	25-31	28-32
UCL	9.5	12.4	-	13.7	19.5	20.5	-	-
range	8-11	10-13	-	12-15	18-20	18-22	-	-
ML	-	5.0	7.0	10.3	11.3	14.7	16.0	16.8
range	-	4-6	5-8	8-12	10-14	14-15	15-18	15-17
PL	-	9.0	10.0	15.6	22.3	-	-	24.8
range	-	6-11	9-11	10-21	20-25	-	-	21-27

Table 43. Development of the most advanced spikelet primordia on the mainstem from day 20 until day 36 for Uniculm Compana, Maris Mink and Proctor grown in different nitrogen regimes

	Stage of most advanced primordia at different days from sowing								
	20	22	24	26	28	30	32	34	36
UCH	4.0	4.2	5.0	5.2	6.9	7.1	7.8	-	9.0
MC	4.0	4.0	4.0	4.0	4.0	7.0	-	7.2	8.2
PC	4.0	4.0	5.7	6.3	6.5	-	7.0	-	9.0
UCL	4.1	4.3	-	4.5	7.0	8.0	-	-	-
ML	-	3.0	3.0	4.0	4.0	4.0	4.6	4.8	6.5
PL	3.0	3.0	4.0	4.0	4.0	4.0	4.4	4.4	-

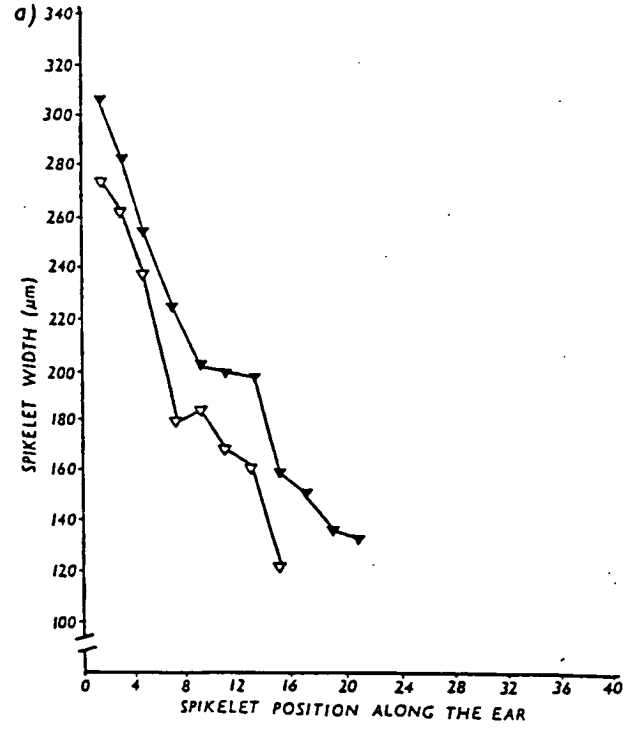
Mainstem spikelet size (Figure 22a, 22b; Table 44)

At the double ridge stage the widths of primordia of UCH were marginally larger than those of MC and PC from primordia 1.5 to 15.5 after which the widths were smaller (Figures 22a, 11a, 13a; Tables 8, 18, 44). UCL plants had significantly smaller primordia at double ridge stage than the UCH plants (Figure 22a; Table 44).

At stage 7 the widths of the central florets were significantly larger in UCH than UCL (Figure 22b; Table 44). UCH also had significantly larger central florets than MC or PC. UCL had slightly larger central florets than either ML or PL plants, but the difference was not significant.

Figure 22a. The mean width of spikelet primordia at the double ridge stage of development, at various positions along the spike, for UCH plants (▼) and for UCL plants (▽).

Figure 22b. The mean width of the central floret at stage 7 (lemma initials), at various positions along the spike, for UCH plants (▼) and for UCL plants (▽).


a)
SPIKELET WIDTH (μm)
340
320
300
280
260
240
220
200
180
160
140
120
100
0
4
8
12
16
20
24
28
32
36
40
SPIKELET POSITION ALONG THE EAR

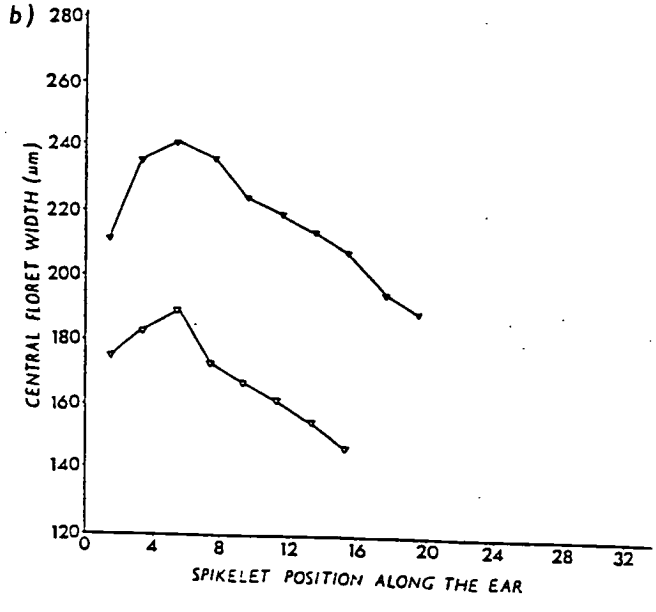

b)
CENTRAL FLORET WIDTH (μm)
280
260
240
220
200
180
160
140
120
0
4
8
12
16
20
24
28
32
SPIKELET POSITION ALONG THE EAR

Table 44. The 95% confidence limits (cl) for the width of spikelet primordia along the spike, at stage 4 in Uniculm Compana at different nitrogen treatments

	Spikelet size and cl at stage 4				Floret size and cl at stage 7			
	UCH		UCL		UCH		UCL	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	311.4	13.2	278.5	17.8	212.3	12.9	176.5	11.5
3.5	288.4	16.5	267.2	17.4	237.2	14.1	184.7	13.6
5.5	258.8	16.9	223.6	12.5	242.2	12.4	190.9	8.5
7.5	227.6	9.9	195.3	8.7	236.8	10.5	173.8	9.2
9.5	203.1	11.3	180.2	15.6	228.7	11.8	168.3	9.1
11.5	202.2	13.7	173.8	10.1	220.8	9.2	162.6	9.8
13.5	203.7	13.7	162.0	11.0	214.5	10.1	155.0	8.7
15.5	161.0	12.4	149.4	12.5	208.7	15.6	147.8	8.3
17.5	154.3	12.5	124.8	12.0				

Mainstem final grain weight (Table 45, 46 and Figure 23a, 23b, 23c)

Grain was significantly heavier in UCH than in UCL plants and larger than was suggested by central floret size (Figure 23a; Table 46). UCH also had significantly larger grain than that set by either MC or PC and the largest grain set were at position 7.5 in all cultivars.

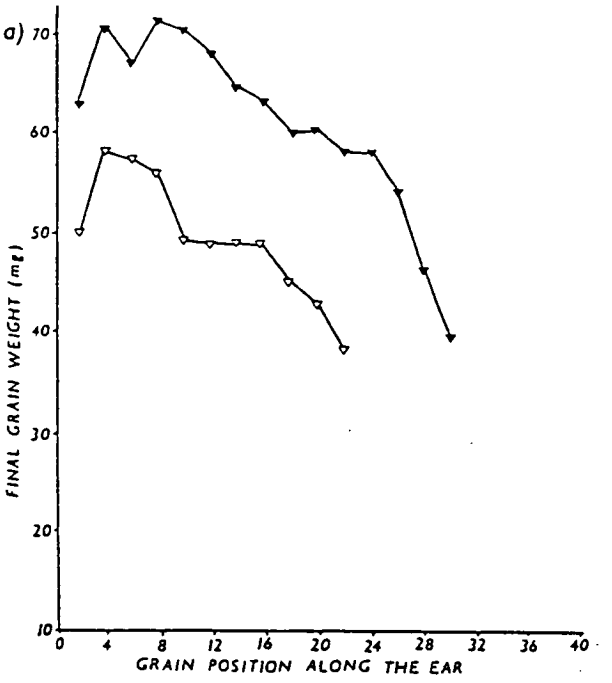
UCL produced heavier grains than either ML or PL. In UCL, as in UCH, the largest grain was set at position closer to the base of the ear than in ML or PL (Table 45, 11, 21).

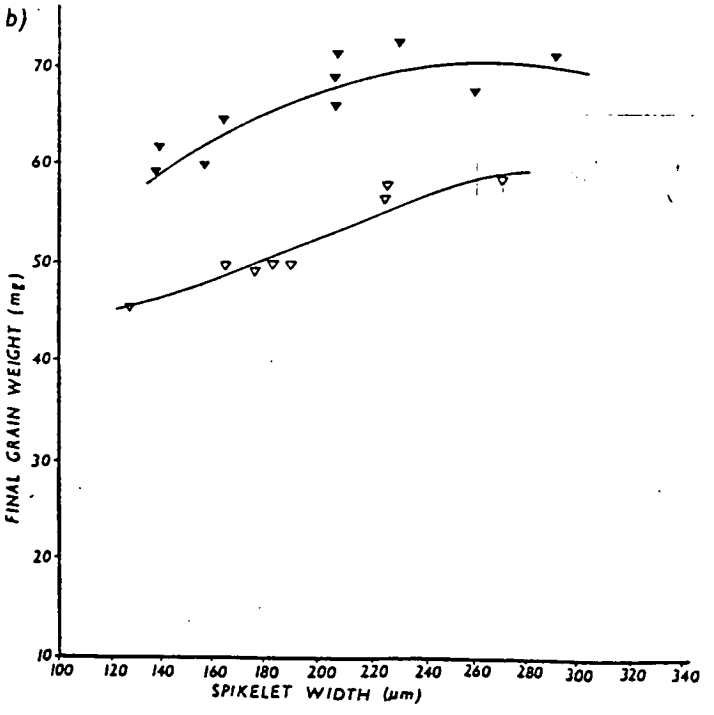
There was a good correlation between the width of spikelet primordia at double ridge stage and final grain weight. The shape of the UCH curve was shallower than the curves for MC and PC.

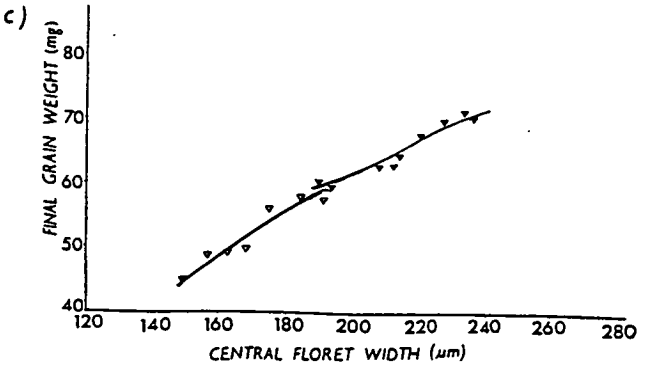
Figure 23a. The mean weight of grain set at various positions along the ear for UCH plants (▼) and for UCL plants (▽).

Figure 23b. The relationship between final grain weight and the width of spikelets at the double ridge stage at various positions along the ear for UCH plants (▼) ($y= 11.75 + 0.48x - 9.85x^2$; $r^2= 0.773$, $n= 10$) and for UCL plants (▽) ($y= 31.46 + 0.11x - 4.15x^2$; $r^2= 0.900$, $n= 8$).

Figure 23c. The relationship between final grain weight and the width of florets at stage 7 (lemma initials) at various positions along the ear for UCH plants (▼) ($y= 92.97 -0.51x + 1.78x^2$; $r^2=0.947$, $n=9$) and for UCL plants (▽) ($y= -116.57 +1.70x - 4.12x^2$; $r^2= 0.934$, $n= 7$).


a)
FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
0
4
8
12
16
20
24
28
32
36
40
GRAIN POSITION ALONG THE EAR


b)
FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
100
120
140
160
180
200
220
240
260
280
300
320
340
SPIKELET WIDTH (μm)


c)
FINAL GRAIN WEIGHT (mg)
80
70
60
50
40
120
140
160
180
200
220
240
260
280
CENTRAL FLORET WIDTH (μm)

There was a good correlation between the widths of spikelet primordia at double ridge stage and grain weight for UCL (Figure 23b), the correlation still held for floret size (Figure 23c).

Table 45. The position of grains set and the position and weight (mg) of the largest and smallest grains set in Uniculm Compana, Maris Mink and Proctor grown in different nitrogen regimes

	Grain set:	largest position	grain weight	smallest position	grain weight
UCH	1.5-29.5	7.5	71.1	29.5	40.5
MC	3.5-21.5	9.5	41.9	21.5	24.6
PC	3.5-31.5	9.5	49.1	31.5	31.0
UCL	1.5-21.5	3.5	58.3	21.5	38.5
ML	3.5-21.5	9.5	34.9	21.5	24.0
PL	3.5-31.5	9.5	42.7	31.5	27.3

Table 46. The 95% confidence limits (cl) for grain weight along the ear in Uniculm Compana treated with high and low nitrogen

Final grain weight and confidence limits

	UCH		UCL	
	$\bar{x}$	cl	$\bar{x}$	cl
1.5	62.9	8.2	50.4	4.1
3.5	70.8	3.5	58.3	2.7
5.5	66.9	4.3	57.5	2.4
7.5	71.1	3.1	56.2	2.4
9.5	70.2	3.7	49.6	5.5
11.5	67.9	3.5	49.0	4.3
13.5	64.9	4.9	49.3	2.7
15.5	63.3	4.3	49.0	5.3
17.5	59.5	3.7	45.3	4.5
19.5	60.8	4.7	43.0	1.0
21.5	58.0	6.1	38.5	6.9

6.3. DISCUSSION

6.3.1. EFFECT OF TILLER REMOVAL

There was no significant difference between treatments in spikelet primordia size at double ridge stage although detillered plants did produced larger florets.

Tiller removal only occurred after the plants had reached the double ridge stage so any competition would not have been removed until the later stages of mainstem apex development. However, it seems unlikely that tillers reduce the size of spikelets at stage 4. The size of the whole apex when the first primordia reach stage 4 is tiny in comparison to the rest of the plant. The nutrient and assimilate requirement of the mainstem apex must be small and it seems unlikely that a decrease in nutrient and assimilate supply from the mainstem as a whole, due to the presence of tillers, could affect spikelet primordia growth at this stage. Removal of tillers at emergence probably benefits the rest of mainstem growth. Detillering has been shown to enhances mainstem leaf growth (Gu and Marshall, 1988). When plants were detillered leaves grew larger and thicker thus causing net photosynthesis per unit area to be increased (Gu and Marshall, 1988). It would appear that following tiller removal, assimilate and nutrients that would have gone to supporting tiller growth are utilised by mainstem leaf growth. The immediate, direct result of increasing assimilate availability due to tiller removal is to increase the leaf size so that assimilate output is further increased. It is suggested that some of the additional assimilate goes to the rapidly developing spikelets, causing the increase in floret size observed at stage 7.

Another possibility is that removing tillers leads to a change in growth substances complement or sensitivity within the mainstem. Perhaps when the tillers are present they reduce

spikelet size at stage 4 by a cumulative inhibition mechanism involving growth substances. Complex hormone interactions certainly do occur between the mainstem and tillers. Cytokinin supply has been implicated in limiting early growth of tiller buds while GA_3 and IAA probably limit their subsequent growth (Sharif and Dale, 1980b). It has been suggested that nitrogen supply may alter tiller bud growth via a growth substance mechanism (Dale, 1979); the evidence for this being that tiller bud growth does not occur when exogenous nitrogen is withheld but does occur under the same circumstances when cytokinin is added (Sharif and Dale, 1980a). Tiller removal was only performed when the tillers had emerged and until that time there would have been a 'communication network' between the tillers and the mainstem. On removal of the tillers not only would the flow of photoassimilate from the mainstem to the tillers stop but any flow of growth substances to and from tillers would stop as well. An alteration in growth substance concentration within the mainstem after removal of tillers could allow the production of larger florets. If a growth substance mechanism was operating while tillers were present and limiting mainstem spikelet size at stage 4 then tiller removal before they developed would be expected to allow for an increase in primordia size at stage 4.

In both cultivars, detillered plants produced significantly larger grain, a result similar to that found in other detillering experiments (Gu and Marshall 1988; Kemp and Whingwiri, 1980; Kirby and Jones 1977). Mainstems are continually exporting assimilate to tillers. So, tiller removal must not only increases the availability of assimilate and nutrients to the developing mainstem spikelets but also the whole of mainstem development. Also the quicker tillers emerge and so reduce their reliance on the mainstem the better the chance is that the mainstem will be able to utilise assimilate production for its own development.

The extent to which Maris Mink was out-yielded by Proctor probably reflected the fact that tiller production started earlier and was faster in Proctor than in Maris Mink. As a consequence, the mainstem of Maris Mink was probably supporting at least one tiller, if not more, throughout mainstem spikelet primordia development. There was a reduction in leaf size, floret size and hence final grain weight because Maris Mink plants were supporting tillers throughout the period of leaf expansion and primordia initiation.

Grain size appears to be the product of a combination of conditions experienced throughout the life of the plant. Gu and Marshall (1988) suggest that detillering influences grain growth principally by enhancing cell division in the endosperm and back up this suggestion by showing that in detillered plants grain shape changed becoming 25% longer than grain of control plants. It would appear however, that if detillering increases leaf size and thus the photosynthetic capacity of the plant, which in turn allows for an increase in floret size then the growth of grain is most probably determined by events prior to anthesis as well. This is borne out by the fact that the earlier detillering occurs, the greater is the increase in mainstem grain yield (Gu and Marshall, 1988). Tillers appear to limit mainstem grain size by reducing the size and photosynthetic capacity of mainstem leaves thus reducing their assimilate production and export.

6.3.2. COMPARISON BETWEEN MARIS MINK, PROCTOR AND UNICULM COMPANA TREATED WITH HIGH AND LOW NITROGEN

As with Proctor and Maris Mink there was a difference in spikelet size at stage 4 between the uniculm plants given high nitrogen and those given low nitrogen and by stage 7 the former had significantly larger florets than the latter. It therefore appears that the difference between high and low nitrogen treatments in the size of spikelet primordia at stage 4 must, initially at least, be due directly to nitrogen supply and not the presence of tillers.

Initially low nitrogen treated uniculm plants would have had sufficient nitrogen in the seed for normal growth and development but around d10 exogenous nitrogen is required if this growth and development is to be sustained (Dale 1976). Reduced nitrogen supply will progressively limit plant growth mainly because assimilate supply from previously formed leaves will be reduced (Dale 1986). However, as previously mentioned, assimilate supply to the spikelet primordia at stage 4 is very small and is probably not the limiting factor. It is more likely that the nitrogen required for developing spikelet cells at stage 4 is limited, so spikelet sizes are smaller. Assimilate supply might be more of a limiting factor as the spike becomes larger and requires more assimilate for the development of florets .

The effect of low nitrogen was to reduce the size of the central florets and substantially reduce final grain weight. The floret sizes in UCL plants were similar to those of PL and ML plants with the prediction that grain size would be similar in this set of plants. However, the final grain weight of the uniculm plants treated with low nitrogen, was higher than that of the corresponding Maris Mink and Proctor plants. Whether this is due to the absence of tillers or to genetic differences is not certain.

On transfer to the greenhouse, Proctor and Maris Mink plants previously grown under low nitrogen conditions entered a more favourable environment and thus were able to support tiller outgrowth. Perhaps by supporting tiller outgrowth Proctor and Maris Mink mainstem grain size was unable to reach its potential. Proctor and Maris Mink plants treated with low nitrogen produced three primary tillers which set grain. These tillers had not emerged when the plants were transferred to John Innes potting compost no. 1 in the greenhouse at day 40. Normally these tillers would have died and it has been suggested that photoassimilate is diverted away from late developing tillers to support mainstem extension (Ong *et al*, 1978, in Lolium perenne). It has also been suggested that young tillers developing late on in the mainstem development normally die due to an increase in competition for light among shoots (Kirby and Faris, 1972; Simmons and Lauer, 1986) Additionally, there is internal competition between younger tillers and the mainstem for nutrients in such a way that even when the tillers survive they often produce no ears or are totally suppressed such that they never develop further than buds (Aspinall, 1961, 1963). However, in other grasses where the environment is not limiting, mainstems have been known to support defoliated (Gifford and Marshall, 1973; Rogan and Smith, 1974; Rawson *et al*, 1976) and shaded tillers (Ong and Marshall, 1979). In such plants the mainstem produces smaller grain but makes a large contribution to the recovery of the tillers thereby increasing seed production per plant (Gu and Marshall, 1988).

Uniculm plants, and plants with their tillers removed, out-yielded mainstems of freely tillering plants. The extent to which this occurred may depend upon the time at which tillers develop. However, for total grain production it may not be advantageous to remove tillers altogether as they are an important yield determining component.

SECTION 7. THE EFFECT OF CCC ON SPIKELET PRIMORDIA AND GRAIN SIZE

7.1. INTRODUCTION

The correlation between the size of primordia and grain weight is not absolute because events after primordium initiation can have an effect on subsequent growth and on the extent of grain filling that will occur. It would appear that a potential maximum for the size of each individual grain is set during the period of spikelet initiation. The extent to which grains will attain their potential varies, but any reduction from the maximum occurs all the way along the spike in such a way that the relative sizes of the grains are maintained. With all the experimental treatments described so far, grains near the tip of the ear have been smaller than grains produced at the centre of the ear, mirroring the patterns of primordium sizes obtained. There has been no substantial change in the sizes of grains set relative to each other, on the same ear.

The average size of distal spikelets at the double ridge stage of development is much smaller than the basal ones at the same stage. This results from the fact that distal spikelets develop, and hence pass from one stage to the next, more rapidly than basal ones. The increase in the rate of development is marked and paralleled by an increase in the rate of growth, however distal spikelets are smaller than proximal ones from stage 4 onwards so that the rate of growth can be said not to keep up with the rate of development (Cottrell & Dale, 1984). GA_3 is known to accelerate the rate of development of spikelet primordia and it has been suggested that endogenous gibberellin accumulation in the later stages of apex development is responsible for the increase in the rate of development seen (Cottrell *et al.*, 1984). It follows that a compound such as CCC which affects gibberellin metabolism and status , might be expected to cause a reduction in the rate of

development of spikelet primordia. Application of such a compound at an appropriate time could delay the rate of development in the distal spikelets which might then be able to reach similar sizes to those of the basal ones, by virtue of their higher growth rates. A consequence of this could be a more uniform and larger spikelet size along the ear.

The growth retardant chlormequat (= [2-chloroethyl]-trimethylammonium chloride), CCC (Tolbert, 1960) reduces lodging in field crops of wheat when applied after the start of internode elongation and treatment increases harvestable yields of grain. When applied at this late stage CCC is found to have little or no effect on the gross morphology of wheat plants in the field. This is presumably a consequence of the fact that maximum tiller number, leaf number, spikelet number and even potential floret number have already been determined by this stage (Hutley-Bull & Schwabe, 1982). The use of CCC in barley and wheat showed the surprising result that small yield increases are seen even in the absence of lodging (Koranteng & Matthews, 1982; Hofner & Kuhn, 1982).

CCC applied to wheat plants early in development, soon after the beginning of spikelet initiation, has a pronounced effect on plant morphology. Early application of the compound to barley causes changes in development in the first three weeks following application bringing about permanent morphological changes in the plants, including early tiller bud growth resulting in an increased number of tillers producing ears (Jaddoa, 1986; Koranteng & Matthews, 1982). This effect appears to result from a retardation of the growth of the main shoot and the first two primary tillers allowing more assimilate to be available for the late primary and early secondary tillers. In consequence, the plant has more sinks (tillers) of more uniform strength during the later stages of growth and this allows a greater proportion of the late tillers to produce ears (Koranteng & Matthews, 1982).

CCC inhibits the cyclization of trans-geranyl-geranyl-pyrophosphate and hence prevents the accumulation of kaurene, a GA_3 precursor (Hofner & Kuhn, 1982). Cartwright & Waddington (1982) have used mepiquat chloride (1,1-dimethyl-piperidinium chloride), a compound with similar biological properties to CCC, on barley. Both CCC and mepiquat chloride slow down the rate of spikelet primordium production when applied at double ridge stage (Jaddoa, 1986; Cartwright & Waddington, 1982). It is likely that the earliest developing spikelet primordia on a plant will be slightly retarded by treatment allowing the later ones to 'catch up' (Cartwright & Waddington, 1982). The effect of early application of mepiquat chloride on grain sizes is quite pronounced (Cartwright & Waddington, 1982) and grains along the ear showed much more uniform sizes, remaining similar until closer to the tip.

The aim of the experiment now described was to use CCC to see whether it was possible to obtain the predicted distribution of primordium sizes, and to see if any uniformity in primordium sizes was reflected in more uniform grain sizes along the mainstem ear. In addition to studying the effect of CCC application on the mainstem ear, the effect of early application on the survival and yield of tiller ears was investigated since an increase in the synchrony of primordium development between tillers has been reported (Waddington, 1983; Jaddoa 1986; Cartwright & Waddington, 1982). If grain size increases in the tiller ears as a result of CCC treatment, then we have a powerful tool for bringing about increases in all of the components of yield.

Both Maris Mink and Proctor were treated with 175 ppm CCC in solution applied as a root drench. The plants were grown in the growthrooms for 40d after sowing and then transferred to the greenhouse to set grain. CCC was given once to 4 batches of plants of both cultivars, on 4 separated dates, all the other conditions being the same as those of the H40 treated plants

(Section 5). The treatments are set out in Table 47. The results of spikelet primordia widths were compared with those of the H40 treated plants. A separate batch of control plants was grown for grain and their tiller and leaf emergence recorded at the same time as the CCC treated plants. There was no significant difference in final grain weight, leaf and tiller emergence between H40 plants and the controls of this experiment. Too few data for primordia width in Maris Mink plants, given CCC at d28, were available and are not therefore presented.

Although both Maris Mink and Proctor where given the same treatments the response of plants following treatment were completely different, so for the sake of clarity the results for the two cultivars have been presented seperately.

Table 47. Table of treatments for Maris Mink and Proctor

CCC treatment	Code
No CCC	Control
Day 16	CCC 16
Day 20	CCC 20
Day 24	CCC 24
Day 28	CCC 28

7.2. RESULTS

Maris Mink

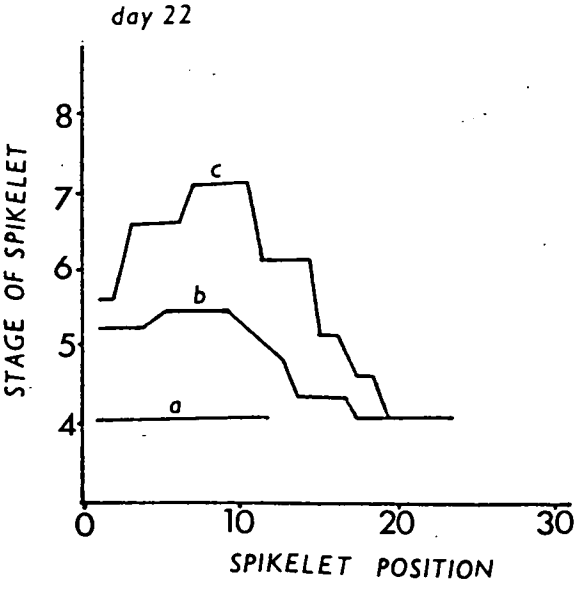
Mainstem develoment and initiation of spikelets (Tables 48, 49 & Figure 24)

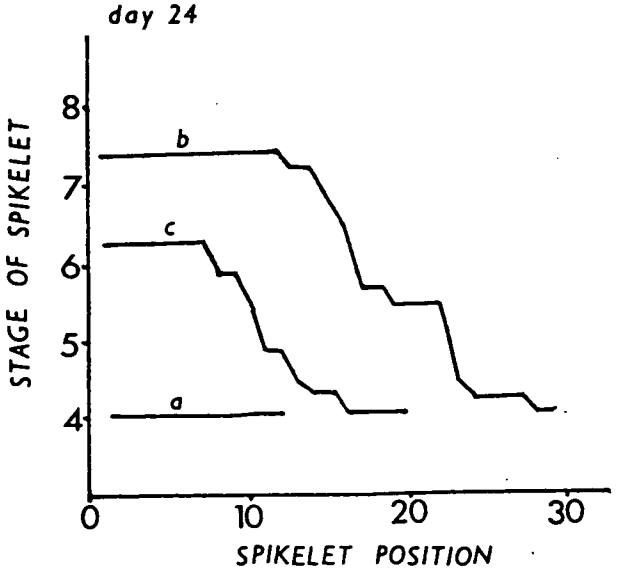
Information on the rates of initiation and development of spikelets in control and CCC-treated plants can be obtained from Tables 48, 49 & Figure 24. Upto d26 treated plants initiated primordia faster than the controls but after d26 initiation ceased in CCC-treated plants but continued in the controls. By day 26, the most advanced primordia on the CCC-treated plants had reached stage 9 but the control plants were still at stage 4. Controls only reached stage 9 at d40. This agrees with the finding of Cottrell (1982) who showed that primordium initiation ceased when the most advanced primordia on the apex reached stage 9, and also shows the effect of treatment in shortening primordial development.

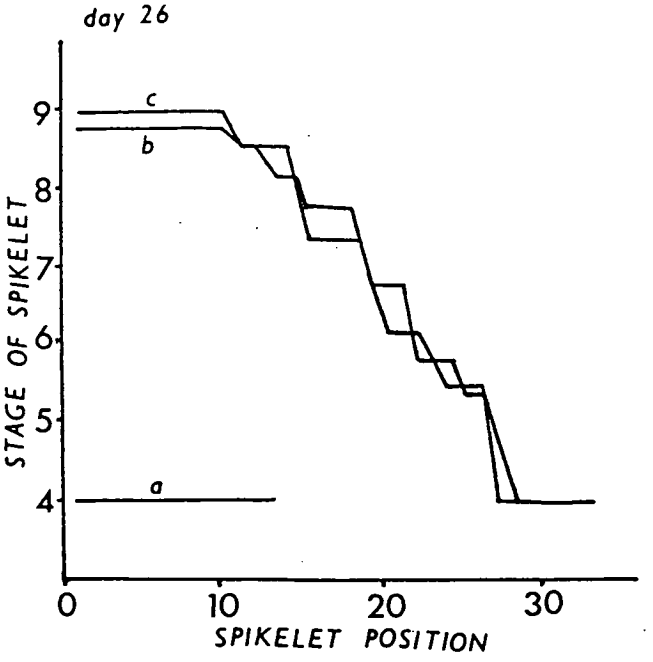
At day 22 the most advanced primordia on CCC 16 plants were at stage 7 whilst in control plant primordia were at stage 4. The most advanced primordia on CCC 20 plants were already past stage 5 even though CCC had only been applied two days previously, illustrating the immediate effect of this compound on the rate of primordium development. By day 26, the most advanced primordia on both CCC 16 and CCC 20 plants were at stage 9 indicating that the there was no decrease in the rate of development of primordia despite the fact that the compound had been applied 6 or 10 days previously. Control plants showed rapid development between days 26 and 30 with developmental rates similar to those shown by earlier CCC-treated plants.

It is possible therefore that CCC-treatment might act by bringing forward the timing of this burst of developmental activity .

Figure 24. The development of spikelet primordia at various positions along the spike on days 22, 24 (page 153), 26 and 30 (page 154) from sowing, for Maris Mink plants treated with CCC at d16 (b), d20 (c), d24 (d) and control plants (a).


day 22
STAGE OF SPIKELET
8
7
6
5
4
c
b
a
0
10
20
30
SPIKELET POSITION


day 24
STAGE OF SPIKELET
8
7
6
5
4
b
c
a
0
10
20
30
SPIKELET POSITION


day 26
c
b
a
9
8
7
6
5
4
STAGE OF SPIKELET
0
10
20
30
SPIKELET POSITION

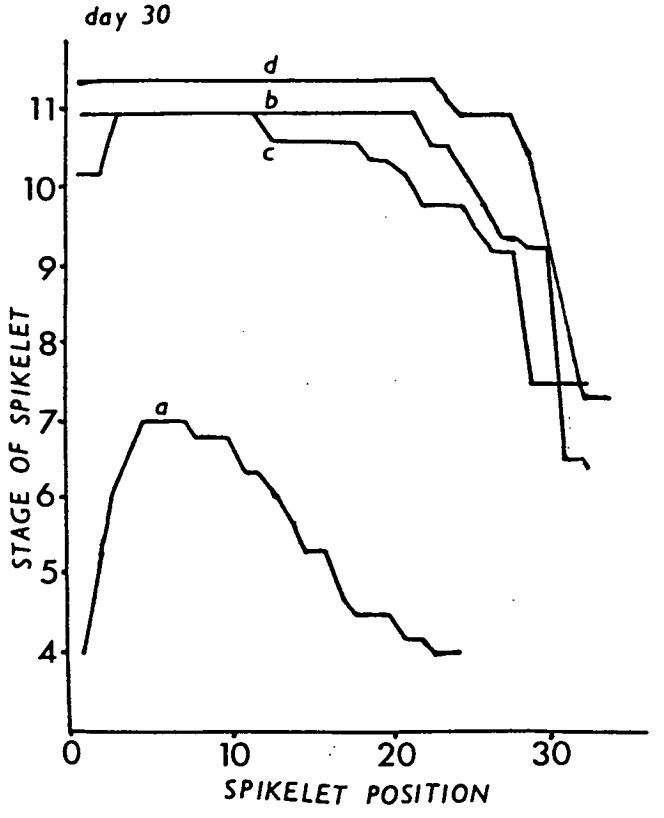

day 30
d
b
c
a
11
10
9
8
7
6
5
4
STAGE OF SPIKELET
0
10
20
30
SPIKELET POSITION

Table 48. Time course for the number of mainstem primordia initiated for Maris Mink plants grown under different CCC treatments

	Number of primordia present on apex on different days from sowing						
	22	24	26	28	30	32	34
Control	11.0	11.5	12.4	17.0	24.2	-	25.4
range	10-12	7-14	9-14	16-18	21-29	-	23-27
CCC 16	24.0	29.2	32.0	-	32.1	-	-
range	22-26	24-32	29-34	-	39-34	-	-
CCC 20	18.4	19.1	28.0	-	32.0	-	-
range	15-20	18-20	25-30	-	30-33	-	-
CCC 24	-	-	-	-	34.2	-	-
range	-	-	-	-	30-37	-	-

Table 49. Time course for the development of the most advanced mainstem spikelet primordia for Maris Mink plants grown under different CCC treatments

	Stage of most advanced primordia on different days from sowing							
	22	24	26	28	30	32	34	36
Control	4.0	4.0	4.0	4.0	7.0	-	7.2	8.2
CCC 16	7.0	7.4	8.8	-	11.0	-	-	-
CCC 20	5.4	6.2	9.0	-	11.0	-	-	-
CCC 24	-	-	-	-	11.5	-	-	-

Plant dry weight, leaf and tiller emergence (Tables 50, 51)

CCC application appears to cause a reduction in total plant dry weight. The rate of response to treatment varies according to the date of treatment - the earlier the treatment the slower the response (Table 50).

Table 50. Time course in days from sowing of plant dry weight of Maris Mink plants treated with CCC and control plants starting from the second day after each treatment. The day of CCC treatments is marked thus: <-> and 95% confidence limits for each harvest date and treatment is shown in brackets ()

	plant treatment:				
Time	CCC16	CCC20	CCC24	CCC28	control
20	238.0	<->			252.8
	(6.4)				(29.8)
22	257.1	234.1			255.9
	(27.3)	(12.5)			(66.2)
24	289.8	238.8	<->		332.2
	(14.8)	(5.2)			(58.0)
26	353.0	286.6	779.0		410.6
	(20.9)	(31.2)	(63.4)		(23.5)
28	642.2	379.0	894.4	<->	580.4
	(29.4)	(49.1)	(54.7)		(79.4)
30	851.0	459.0	957.4	704.2	990.7
	(50.6)	(33.2)	(60.4)	(28.2)	(60.1)
32	748.8	642.7	1001.0	1352.8	1454.9
	(20.3)	(29.5)	(33.9)	(51.2)	(88.7)
34	891.4	654.4	1081.0	1161.7	1474.4
	(13.8)	(82.4)	(85.1)	(28.1)	(43.8)
36	-	911.7	-	-	1800.5
	-	(79.3)	-	-	(90.3)

Leaf and tiller emergence was slightly accelerated after treatment with CCC. All the plants produced a maximum of 9 leaves on the mainstem. Ear bearing stems on the CCC treated plants also grew substantially taller than the coresponding control plant ears.

Table 51. Time course in days from sowing for leaf and tiller emergence in CCC treatment and control plants starting from the second day of each treatment. The day of CCC treatment is marked thus: <->

	days from sowing:								
	20	22	24	26	28	30	32	34	36
CCC16	L4		------L5		--L6		------L7		----------
	T2		----------		T3		----------		T4--------
CCC20	<->		-L4		--L5		------L6		--L7----------
	<->		-T2		------T3		----------		----T4---
CCC24				<->			-----L6		--L7----------
				<->			-----T3		----------T4
CCC28						<->			-L6--L7--------
						<->			-T3----------
control			--L4		------L5		------L6		--L7--------
			------T2				----------T3		--------

Mainstem spikelet size (Figures 25a, b, c & Tables 52, 53, 54)

At stage 4, the widths of spikelet primordia were found to become progressively less from the base to the tip of the spike in the control as well as in CCC 20 and CCC 16 treatments (Figure 25a). Values could not be obtained for CCC 24 and CCC 28 since the primordia had passed this stage of development when harvested on days 26 and 30 respectively. In fact, CCC 28 plants developed so rapidly after treatment that there were not enough data for collection at the stages, 4 ,7 and 9.

There was no significant difference in whole spikelet primordium sizes at stage 4 for any of the treatments (Table 52).

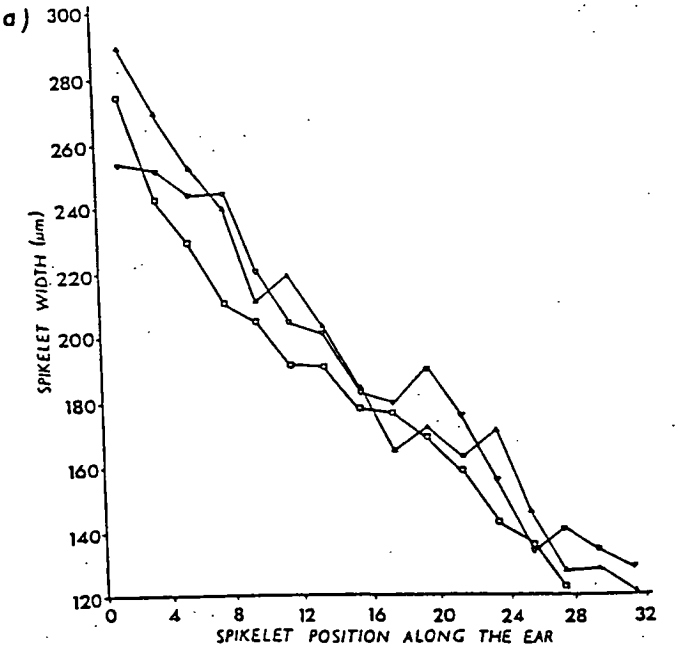
At stage 7, the width of the central floret was largest between spikelets 4 and 7 and decreased progressively toward the tip of the spike. The central florets of the most basal primordia increased progressively in size from the base of the spike to position 4 and the size at a given position along the spike was significantly smaller on control than on CCC treated plants (Figure 25b, Table 53). The width differences between central florets along the spike that were established by stage 7 were found to be similar at stage 9 (Figure 25c). At this stage the control plants had significantly smaller central florets, at a given position on the spike, than the CCC treated plants (Table 54).

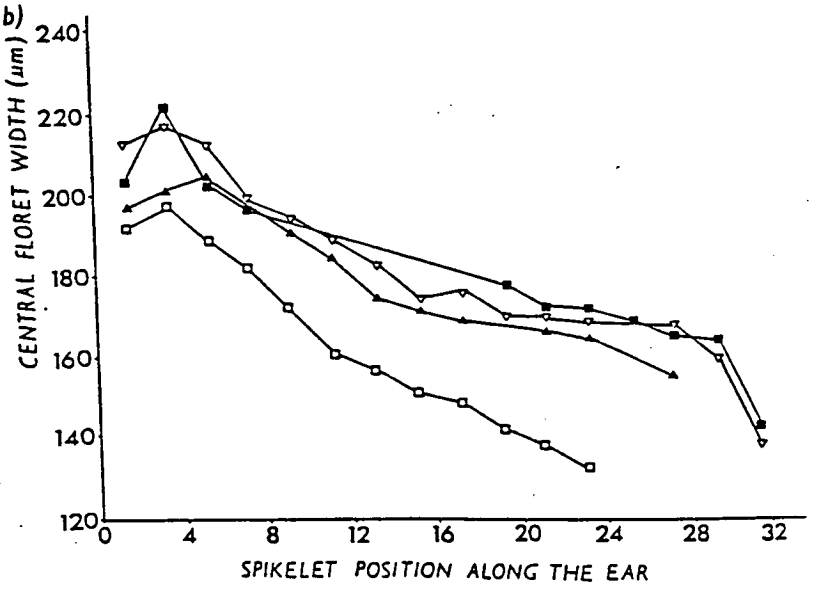
The data for floret size showed that for all treatments there was a progression in such a way that the more distal the spikelet position the more that spikelet had grown by stage 9 in relation to its size at stage 7. This trend resulted in there being a reduction in the absolute size differences between spikelets on the apex during development from stage 7 to stage 9.

Figure 25a. The mean width of spikelet primordia at the double ridge stage of development, at various positions along the spike, for Maris Mink plants treated with CCC at d16 (▽), d20 (▲) and for control plants (□).

Figure 25b. The mean width of the central floret at stage 7 (lemma initials), at various positions along the spike, for Maris Mink plants treated with CCC at d16 (▽), d20 (▲), d24 (■) and for control plants (□).

Figure 25c. The mean width of the central floret at stage 9 (stamen initials visible as 3 distinct mounds), at various positions along the spike, for Maris Mink plants treated with CCC at d16 (▽), d20 (▲), d24 (■) and for control plants (□).


a)
SPIKELET WIDTH (µm)
300
280
260
240
220
200
180
160
140
120
0
4
8
12
16
20
24
28
32
SPIKELET POSITION ALONG THE EAR


b)
CENTRAL FLORET WIDTH (µm)
240
220
200
180
160
140
120
0
4
8
12
16
20
24
28
32
SPIKELET POSITION ALONG THE EAR

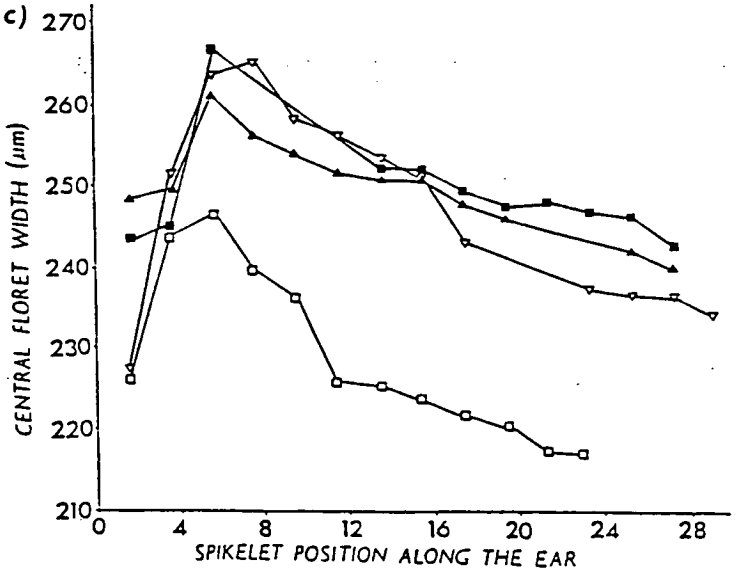

c)
CENTRAL FLORET WIDTH (µm)
270
260
250
240
230
220
210
0
4
8
12
16
20
24
28
SPIKELET POSITION ALONG THE EAR

Table 52. The 95% confidence limits (cl) for spikelet primordia sizes at different positions along the spike, at stage 4, for different CCC treatments in Maris Mink plants

	Spikelet primordia width and confidence limits					
	CCC 16		CCC 20		control	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	254.4	38.8	291.6	30.5	277.6	14.1
3.5	253.0	37.0	271.5	37.8	244.2	12.4
5.5	245.5	24.6	254.3	21.6	231.0	10.4
7.5	246.3	27.2	241.4	32.1	213.2	9.6
9.5	221.1	33.3	212.5	22.1	206.0	8.0
11.5	205.3	24.1	220.3	28.4	192.5	10.6
13.5	203.7	32.5	204.3	18.2	191.5	11.3
15.5	184.7	27.6	185.0	17.2	178.3	9.9
17.5	181.6	21.9	165.7	21.4	178.7	11.3
19.5	155.8	12.0	172.6	28.8	170.6	8.6
21.5	133.4	14.3	163.7	10.2	159.6	9.6
23.5	140.6	21.4	172.1	22.1	142.9	7.6
25.5	133.7	22.7	146.8	14.7	136.6	8.4
27.5	128.8	20.2	127.8	11.0	122.4	5.5

Table 53. The 95% confidence limits (cl) for floret sizes at different positions along the spike , at stage 7, for different CCC treatments in Maris Mink plants

	Floret size and confidence limits along spike							
	CCC 16		CCC 20		CCC 24		control	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	212.5	17.0	201.3	10.9	207.4	12.9	194.1	8.0
3.5	220.9	6.0	204.7	9.7	226.1	14.2	203.8	8.9
5.5	210.2	10.2	208.7	6.4	206.2	3.9	191.1	7.0
7.5	203.7	10.9	-	-	200.6	6.0	183.9	7.3
9.5	197.9	8.2	193.9	12.0	-	-	173.7	6.2
11.5	192.6	8.2	186.7	7.2	-	-	163.2	8.3
13.5	185.4	9.7	176.3	10.3	-	-	159.1	7.7
15.5	177.8	6.9	174.2	13.2	-	-	152.8	5.4
17.5	178.7	9.7	171.5	9.9	-	-	150.9	4.7
19.5	172.3	12.7	172.1	6.7	179.5	22.7	143.5	7.7
21.5	171.2	13.6	172.8	29.5	173.3	24.2	139.2	8.0
23.5	171.2	13.6	168.1	21.4	173.1	8.9	133.7	4.1
25.5	-	-	165.0	15.0	170.0	6.0	-	-
27.5	169.6	17.4	-	-	168.9	10.2	-	-
29.5	160.6	12.6	156.7	13.2	165.2	7.7	-	-
31.5	139.9	11.1	-	-	144.6	22.2	-	-

Table 54. The 95% confidence limits (cl) for floret sizes at different positions along the spike , at stage 9, for different CCC treatments in Maris Mink plants

	Floret size and confidence limits along the spike							
	CCC 16		CCC 20		CCC 20		control	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	227.8	9.7	248.9	13.1	243.9	27.6	226.7	14.2
3.5	251.9	7.1	249.5	7.4	245.1	16.7	244.4	10.5
5.5	264.2	9.3	261.6	12.1	267.9	17.5	247.2	10.5
7.5	265.6	10.3	256.5	11.5	-	-	240.0	9.8
9.5	258.9	9.8	254.5	10.1	-	-	236.9	9.8
11.5	256.7	14.4	252.3	7.8	-	-	226.1	13.3
13.5	254.1	7.7	251.7	1.7	252.4	12.2	234.0	5.9
15.5	253.7	14.4	251.8	8.5	252.1	6.5	234.0	5.9
17.5	243.5	9.0	248.8	8.4	250.3	10.8	221.1	9.4
19.5	-	-	247.2	10.9	248.2	15.9	220.7	7.9
21.5	-	-	-	-	249.0	14.2	217.9	29.5
23.5	238.1	10.8	-	-	248.2	14.2	217.9	23.5
25.5	237.1	6.6	242.6	11.1	247.8	11.1	-	-
27.5	237.1	9.5	240.6	28.4	243.8	14.9	-	-

Final grain weight (Tables 55, 56, 57, 58; Figures 26, 27a, b, c)

At all positions along the ear, the grains set in CCC-treated plants were larger than those at the same position in control plants, although no differences could be detected between the different CCC treatments (Figure 26; Table 56). The total number of grain set was higher on the CCC-treated plants because of enhanced grain set at both the base and tip of the ear (Table 55). Later applications of CCC caused the largest grain to be set nearer the collar. Grain weights at the tip of the ear were significantly smaller than those at the base, the decrease in grain size towards the tip being much more pronounced in the controls than in CCC-treated plants.

When final grain weight was plotted against spikelet primordia width at double ridge stage there was a correlation between the two, the shape of the curve being the same in all treatments but the positioning on the y axis showing the CCC treatment curves to be shifted upwards relative to the control curve (Figure 27a). There was no shift along the x axis as the widths of the primordia at this stage were similar.

Plotting widths of the central florets at stage 7 and 9 against final grain weight gave a curve more truncated on the x axis. This is due to the changes occurring in the central floret width / position curves where basal florets decrease in size relative to the other florets and where the difference between the largest floret and the smallest decreases as the apex develops. The CCC curves were higher on the y axis than the control curve because the CCC treatments had the larger grain. The curves also shifted along the x axis relative to the control curve and due to the CCC treatments having larger florets than the controls (Figure 27b).

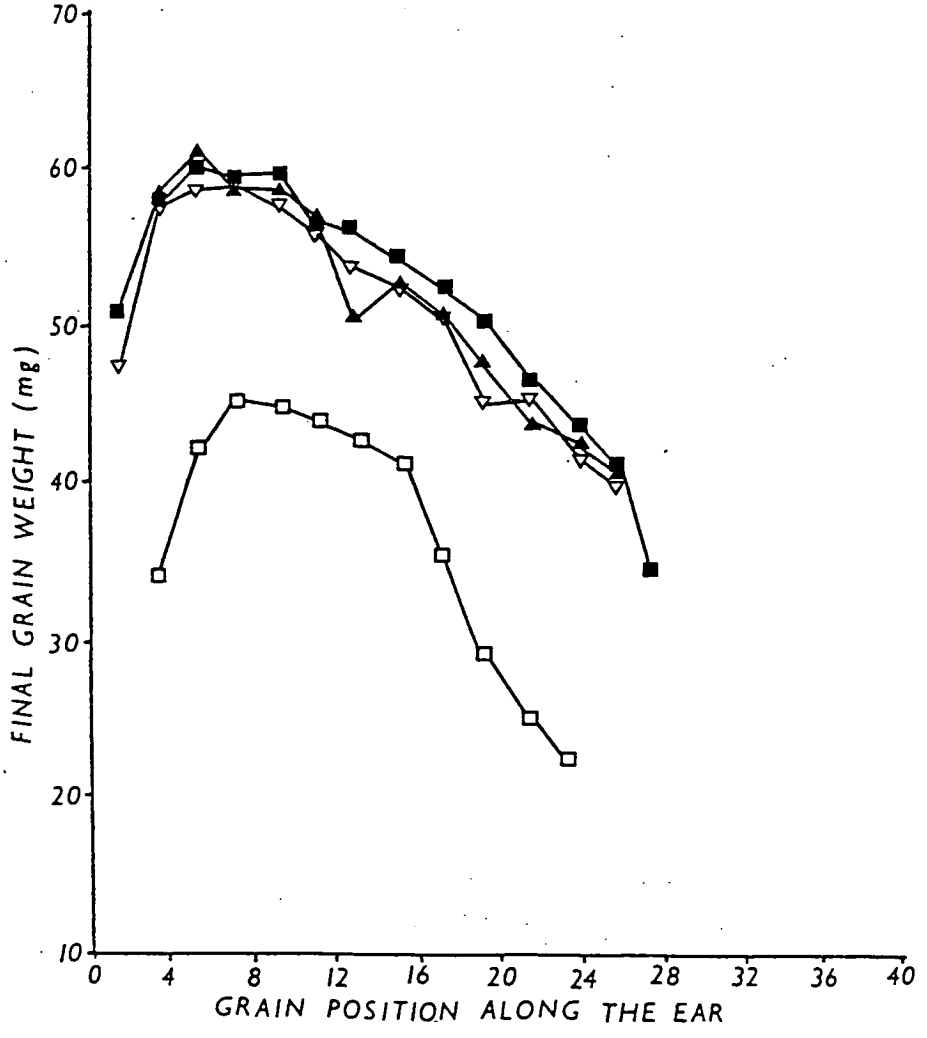
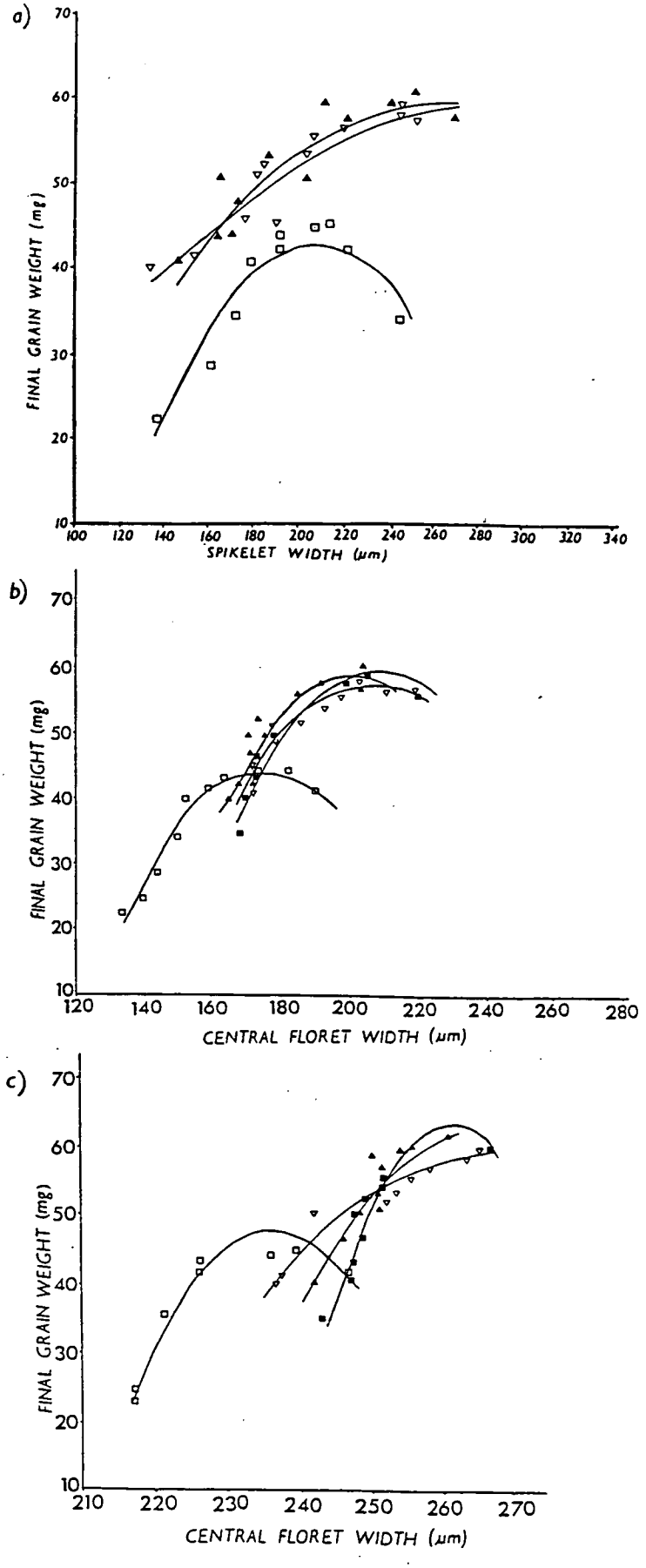

70
60
50
40
30
20
10
FINAL GRAIN WEIGHT (mg)
0
4
8
12
16
20
24
28
32
36
40
GRAIN POSITION ALONG THE EAR

Figure 27a. The relationship between final grain weight and the width of spikelets at the double ridge stage at various positions along the ear for Maris Mink plants treated with CCC at d16 (▽) (y= -4.41 + 0.40x - 5.79x²; r²= 0.873, n= 12), d20 (▲) (y= -40.43 + 0.76x - 1.45x²; r²= 0.857, n= 12) and for control plants (□)(y= -154.64 + 1.93x - 4.69 x²; r²= 0.928, n= 10).

Figure 27b. The relationship between final grain weight and the width of florets at stage 7 (lemma initials) at various positions along the ear for Maris Mink plants treated with CCC at d16 (▽) (y= -419.96 + 4.53x - 0.01x²; r²= 0.942, n= 11), d20 (▲) (y= -508.06 + 5.63x - 0.14x²; r²= 0.892, n= 9), d24 (■) (y= -537.60 + 5.74x - 0.01x²; r²= 0.937, n= 8) and for control plants (□) (y= -386.15 + 4.92x - 0.01x²; r²= 0.965, n= 10).

Figure 27c. The relationship between final grain weight and the width of florets at stage 9 (stamen initials visible as 3 distinct mounds) at various positions along the ear for Maris Mink plants treated with CCC at d16 ( ) (y= -1265.39 + 9.88x - 0.02x²; r²= 0.955, n= 9), d20 ( ) (y= -3485.87 + 27.03x - 0.05x²; r²= 0.822, n= 9), d24 ( ) (y= -6324.78 + 48.81x - 0.09x²; r²= 0.909, n= 9) and for control plants ( ) (y= -3704.03 + 31.77x - 0.07x²; r²=0.0915, n= 8).


a)
FINAL GRAIN WEIGHT (mg)
SPIKELET WIDTH (μm)
b)
FINAL GRAIN WEIGHT (mg)
CENTRAL FLORET WIDTH (μm)
c)
FINAL GRAIN WEIGHT (mg)
CENTRAL FLORET WIDTH (μm)

The correlations between the sizes of primordia / florets and final grain are correlations between the relative sizes of these one to another rather than absolute sizes.

Table 55. The position of MS final grain and the mean weight (mg) of the largest and smallest set in Maris Mink plants

	Grain set:	largest grain:		smallest grain:	
		position	weight	position	weight
Control	3.5-23.5	7.5	45.0	23.5	23.4
CCC 16	1.5-25.5	7.5	59.5	25.5	40.4
CCC 20	3.5-25.5	5.5	61.1	25.5	40.9
CCC 24	1.5-27.5	5.5	60.0	27.5	35.0

Table 56. The 95% confidence limits for final grain weight at different positions along the spike in different CCC treatments

	Grain size and confidence limits along spike									
	CCC 16		CCC 20		CCC 24		CCC 28		control	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	47.3	5.7	-	-	51.0	9.2	-	-	-	-
3.5	57.5	3.3	58.4	5.3	57.7	1.8	48.5	10.9	34.0	3.1
5.5	58.4	2.4	61.1	3.9	60.3	1.8	54.8	10.5	42.1	3.1
7.5	59.7	2.0	59.1	5.5	59.1	1.4	54.7	6.9	45.2	2.9
9.5	57.2	2.2	59.1	3.3	59.9	1.2	54.2	6.5	44.6	2.9
11.5	55.6	0.4	57.1	3.1	57.5	1.4	52.8	6.3	43.9	2.7
13.5	53.3	1.4	50.5	7.1	56.8	1.2	53.0	3.7	42.2	2.7
15.5	52.0	1.4	53.3	2.5	54.8	1.2	50.3	4.9	40.5	3.1
17.5	50.6	1.4	50.5	2.7	52.9	1.2	49.8	3.5	35.3	2.7
19.5	45.1	4.7	47.7	2.5	50.9	1.0	46.8	3.1	29.1	4.9
21.5	45.9	1.8	43.7	2.9	47.4	1.6	43.5	3.9	25.1	3.9
23.5	41.5	2.0	43.6	3.9	44.3	2.0	37.8	7.7	23.3	4.1
25.5	40.2	4.5	40.7	2.4	41.4	2.4	34.6	4.7	-	-

Table 57. Total grain weight per ear (mg), mean individual grain weight per ear (mg) and mean grain number per ear for mainstem and tiller ears in Maris Mink for different CCC treatments

		Treatment:			
		Control	CCC 16	CCC 20	CCC 24
MS	$\bar{x}G$	36.0	51.1	52.1	52.1
	$\bar{x}N$	18.5	21.9	20.4	22.2
	ΣG	666.0	1119.1	1062.8	1156.6
Tc	$\bar{x}G$	24.0	48.9	49.4	48.0
	$\bar{x}N$	14.9	16.7	16.4	17.3
	ΣG	357.6	816.6	810.2	830.4
T1	$\bar{x}G$	26.7	53.4	61.6	59.2
	$\bar{x}N$	20.5	26.2	18.6	23.8
	ΣG	547.5	1399.1	1145.8	1409.0
T2	$\bar{x}G$	27.9	55.7	54.7	57.2
	$\bar{x}N$	14.9	20.0	19.6	21.6
	ΣG	547.4	1114.0	1072.1	1235.5
T3	$\bar{x}G$	26.6	54.6	51.6	54.5
	$\bar{x}N$	12.6	17.2	18.5	21.3
	ΣG	335.2	939.1	954.6	1160.9
T4	$\bar{x}G$	28.0	51.5	52.2	52.2
	$\bar{x}N$	11.4	18.2	17.1	20.1
	ΣG	319.2	937.3	892.6	1049.2
T5	$\bar{x}G$	27.4	45.3	48.1	53.4
	$\bar{x}N$	11.7	18.1	16.6	17.5
	ΣG	320.6	819.9	798.5	934.5
	TGW	3417.8	7567.2	7107.0	8268.1

Table 58. Results from two way analysis of variance for total grain weight per ear (ΣG) in the different treatments and different ear positions within Maris Mink plants, including the least significant difference values (LSD)

Source	d.f.	variance ratio (F)	signif.	LSD
Treatment(CCC)	3,21	33.2	***	187.5
Ear position	7,21	11.5	***	123.8

In CCC-treated plants the mean grain weight, $\bar{x}G$ on tillers 1-3 was greater than that for the mainstem ears whereas in control plants, $\bar{x}G$ on the mainstem ears was larger than that on any of the tillers. In all cases, values of $\bar{x}G$ on CCC-treated plants were larger than those on any of the ears set on control plants (Table 57).

The values for total grain weight, ΣG for mainstem and primary tillers in the CCC-treated plants was significantly larger than for the controls . This increase was attributable to an increase in the $\bar{x}G$ and an increase in the mean grain number, $\bar{x}N$ relative to the control plants. There was no significant difference in ΣG between the different CCC-treatments (Table 58). In both control and CCC-treated plants the values of ΣG was highest in T1 and decreased progressively for T2-T5 (Table 57). The values of ΣG was lower for Tc than for the T1 in all CCC treatments and the control but the ΣG on tiller Tc was always higher than that on secondary and tertiary tillers. The difference in ΣG was significantly different between the tillers in all treaments (Table 58) .

Proctor

Mainstem initiation and development of spikelets (Tables 59, 60 and Figure 28).

CCC-treated plants initiated primordia more slowly than the control. Between d24 and d28 no primordia were initiated on CCC-treated plants and little change in the stage of development of the apex was seen. This contrasted with the control plants where primordium initiation could be observed and where there was a increase in the developmental stage of all but the most distal primordia. At d24 the number of primordia initiated on, and the developmental stage of, the apex on CCC 16 plants lagged behind that of CCC 20 plants, and this trend was maintained until at least d36 (Table 59).

Between d28 and d32 there was an increase in the rate of primordium initiation and development in both CCC-treated and control plants. CCC 16 plants had the fewest primordia and these were also the least advanced (Table 60). The control plants and CCC 28 treated plants had the most and furthest advanced primordia.

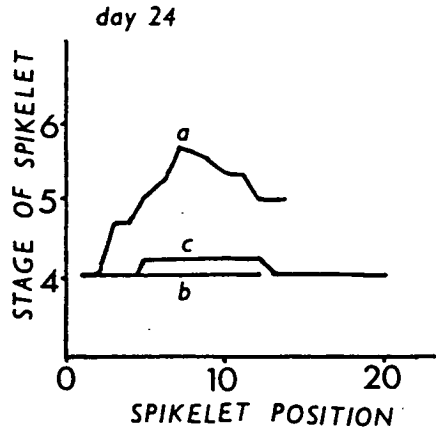
Table 59. Time course for the number of mainstem primordia initiated for Proctor plants grown under different CCC treatments

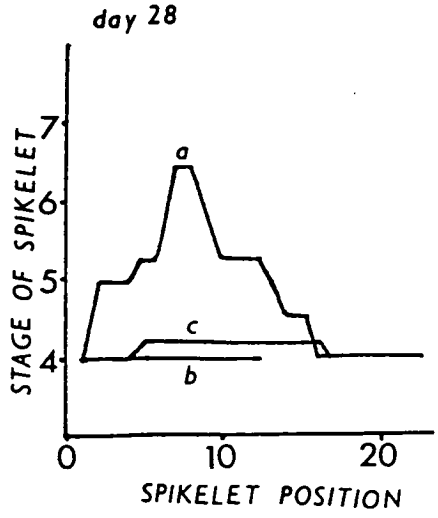
	Number of primordia present on apex on different days from sowing							
	22	24	26	28	30	32	34	36
Control	10.0	13.5	21.5	22.0	-	29.0	29.3	40.0
range	8-11	10-16	18-24	16-28	-	25-32	27-32	39-42
CCC 16	-	12.0	-	12.3	-	21.0	-	24.4
range	-	10-13	-	11-13	-	19-23	-	23-25
CCC 20	-	20.1	-	20.2	-	27.3	-	32.0
range	-	19-22	-	19-23	-	25-29	-	29-34
CCC 24	-	-	-	-	-	20.0	-	27.2
range	-	-	-	-	-	18-23	-	25-29
CCC 28	-	-	-	-	-	27.0	-	36.1
range	-	-	-	-	-	25-28	-	35-37

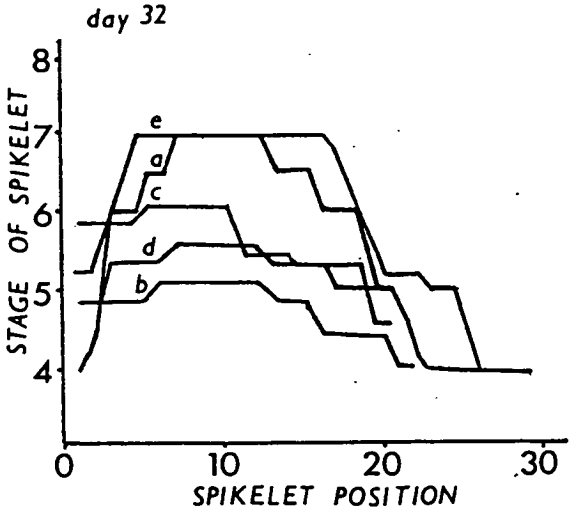
Table 60. Time course for the development of the most advanced mainstem spikelet primordia for Proctor plants grown under different CCC treatments

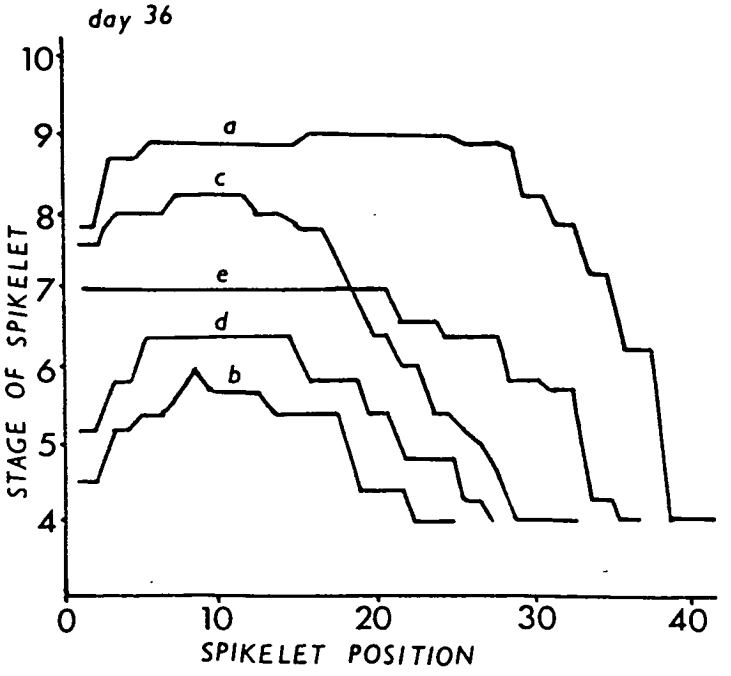
	Stage of most advanced primordia on different days from sowing							
	22	24	26	28	30	32	34	36
Control	4.0	5.7	6.3	6.5	-	7.0	-	9.0
CCC 16	-	4.0	-	4.0	-	5.0	-	6.0
CCC 20	-	4.2	-	4.2	-	6.0	-	8.2
CCC 24	-	-	-	-	-	5.6	-	6.4
CCC 28	-	-	-	-	-	-	-	7.0

Figure 28. The development of spikelet primordia at various positions along the spike on days 24, 28 (page 175), 32 and 36 (page 176) from sowing, for Proctor plants treated with CCC at d16 (b), d20 (c), d24 (d), d28 (e) and control plants (a).


day 24
STAGE OF SPIKELET
6
5
4
a
c
b
0
10
20
SPIKELET POSITION


day 28
STAGE OF SPIKELET
7
6
5
4
a
c
b
0
10
20
SPIKELET POSITION


day 32
STAGE OF SPIKELET
8
7
6
5
4
e
a
c
d
b
0
10
20
30
SPIKELET POSITION


day 36
STAGE OF SPIKELET
10
9
8
7
6
5
4
a
c
e
d
b
0
10
20
30
40
SPIKELET POSITION

At d32 there was no real difference between CCC 28 and control plants suggesting that the compound had not had much effect on apex growth in the first four days after application. However by d36 the effect of CCC application plants could be seen and the treated plants were found to be behind control plants, both in terms of primordium number and stage of primordial development (Figure 28).

CCC 20 plants were affected by the compound, but their apices did not develop as rapidly as control plants and they were not affected to the same extent as CCC 16 plants where the apices were extremely delayed with respect to spikelet initiation and development. Perhaps, in CCC 16 plants the compound was applied at a time when the plants were particularly susceptible. CCC 20 plants did not show such an extreme response to the compound and by d36 had recovered to the extent that they were second only to the controls with respect to primordium development.

For CCC 20 plants, therefore, CCC-treatment appeared to have an initial effect which then was gradually lost. This could also be observed in CCC 24 and CCC 28 plants although the data are not available to show how the response may have declined with time. During the period from 4 to 8 days after its application, i.e. during the early stages of its effect, CCC-treatment appears to have stopped the increase in the developmental stages of CCC 28 primordia. At some time between the application of CCC and d32 the initiation and development of primordia on CCC 24 plants must have been greatly impaired since at d32 the developmental stages of these primordia were found to lag well behind those of the controls and most of the CCC-treated plants, being greater only than those of CCC 16 plants. By d36 these plants were beginning to recover from the effect of CCC-treatment since development and initiation of primordia resumed (Figure 28).

Plant dry weight, leaf and tiller emergence (Tables 61, 62)

CCC treatment appeared to reduce plant dry weight in all treatments when compared with the control values (Table 61). Differences in plant dry weights values between CCC treatment varied, but appeared to have no easily distinguishable pattern.

Table 61. Time course in days from sowing of plant dry weight of Proctor plants treated with CCC and control plants starting from the second day after each treatment. The day of CCC treatments is marked thus: <-> and 95% confidence limits for each treatment on each harvest date is shown in brackets ()

	plant treatment:				
	CCC16	CCC20	CCC24	CCC28	control
20	218.6	<->			300.0
	(16.4)				(24.5)
24	410.6	261.8	<->		549.2
	(29.9)	(15.4)			(40.9)
28	442.0	406.1	670.6	<->	719.1
	(37.8)	(81.2)	(41.4)		(81.2)
32	898.1	606.4	918.6	709.0	1399.0
	(33.9)	(55.1)	(80.7)	(75.7)	(87.6)
36	957.1	1039.9	1094.8	1095.0	1701.1
	(50.7)	(91.5)	(146.5)	(73.4)	(67.5)
40	1401.6	1300.0	1397.8	1879.4	1959.0
	(115.5)	(88.9)	(69.6)	(474.4)	(35.9)

CCC treatment appears to slow leaf and tiller emergence (Table 62). Plants produced a maximum of 10 leaves on the mainstem and the ear bearing stems of the control plants grew taller than those of the CCC treated plants.

Table 62. Time course in days from sowing for leaf and tiller emergence in Proctor plants treated with CCC and control plants starting from the second day of each treatment. The day of CCC treatment is marked thus: <->

	days from sowing:										
	20	22	24	26	28	30	32	34	36	38	40
CCC16	--------L5-------------L6------L7--------										
	--------T2------T3-------------T4--------										
CCC20	<->-------------L5------L6-------------L7										
	<->-------------T2------T3-------------T4										
CCC24			<->-------------L6-------L7-------								
			<->-----------------------------T4								
CCC28					<->-------------L7-----L8						
					<->---------------------T4						
control	L4------L5------L6------L7-------L8-------										
	--------T3-------------T4---------------										

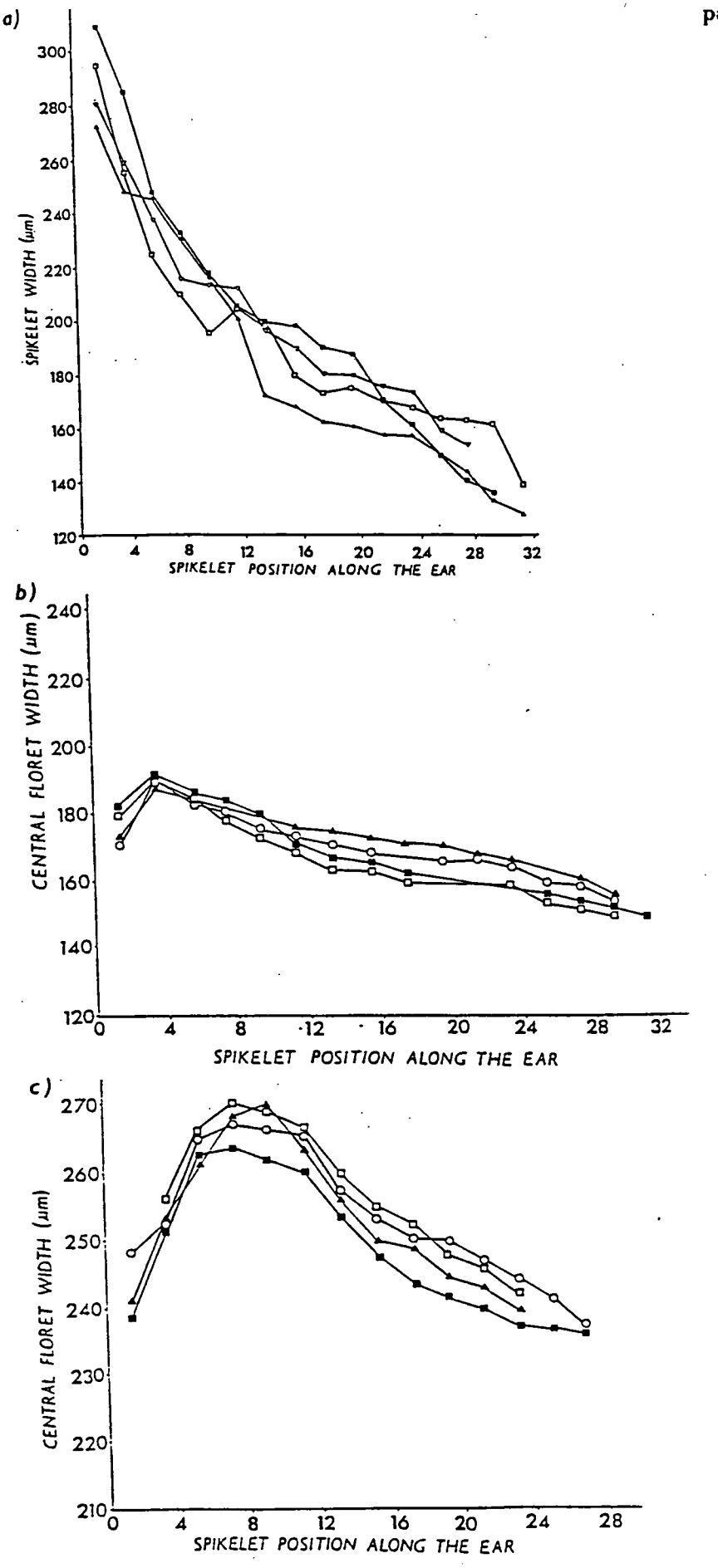
Mainstem spikelet size (Figures 29a, b, c & Tables 63, 64, 65)

At stage 4, the widths of spikelet primordia were progressively less from the base to the tip of the spike in control plants and plants of the CCC 20, CCC 24 and CCC 16 treatments (Figure 29a). Values could not be obtained for CCC 28 plants since the primordia were past this stage of development when harvested. There was found to be no significant difference in spikelet primordium width at stage 4 between control CCC 16 and CCC 24 and CCC 20 plants (Table 63).

Figure 29a. The mean width of spikelet primordia at the double ridge stage of development, at various positions along the spike, for Proctor plants treated with CCC at d16 (▽), d20 (▲), d24 (■) and for control plants (□).

Figure 29b. The mean width of the central floret at stage 7 (lemma initials), at various positions along the spike, for Proctor plants treated with CCC at d20 (▲), d24 (■), d28 (●) and for control plants (□).

Figure 29c. The mean width of the central floret at stage 9 (stamen initials visible as 3 distinct mounds), at various positions along the spike, for Proctor plants treated with CCC at d20 (▲), d24 (■), d28 (●) and for control plants (□).


a)
SPIKELET WIDTH (µm)
300
280
260
240
220
200
180
160
140
120
0
4
8
12
16
20
24
28
32
SPIKELET POSITION ALONG THE EAR
b)
CENTRAL FLORET WIDTH (µm)
240
220
200
180
160
140
120
0
4
8
12
16
20
24
28
32
SPIKELET POSITION ALONG THE EAR
c)
CENTRAL FLORET WIDTH (µm)
270
260
250
240
230
220
210
0
4
8
12
16
20
24
28
SPIKELET POSITION ALONG THE EAR

No data were available for floret size in plants treated with CCC 16 due to the extreme slowness of development of the primordia over the harvesting period. In the other treatments the width of the central floret at stage 7 was largest at spikelets 3.5. There was no significant difference in the size of central florets at a given position along the spike between control plants, CCC 20 and CCC 24 plants and CCC 28 plants (Figure 29b; Table 64).

Table 63. The 95% confidence limits (cl) for spikelet primordia sizes along the spike, at stage 4, for different CCC treatments in Proctor

Spikelet primordia size and confidence limits at stage 4

	CCC 16		CCC 20		CCC 24		control	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	282.4	30.9	272.7	11.8	310.1	22.7	295.1	17.3
3.5	260.7	31.9	248.3	22.1	285.1	32.7	255.3	14.7
5.5	239.1	32.1	245.9	20.9	248.6	30.2	225.2	12.5
7.5	217.6	31.1	230.5	21.4	233.2	36.6	210.5	11.5
9.5	214.2	36.1	216.5	22.5	218.7	37.0	195.0	10.9
11.5	213.3	42.5	200.1	18.2	205.9	38.8	205.6	9.5
13.5	197.9	25.6	190.1	20.8	199.9	27.2	198.7	9.2
15.5	190.7	18.6	172.1	33.1	198.8	20.4	179.6	10.1
17.5	181.3	16.7	168.2	20.7	190.1	16.7	172.7	12.7
19.5	180.0	13.7	161.5	20.7	187.1	10.4	174.9	13.4
21.5	175.7	10.2	160.8	8.0	170.6	12.3	170.4	8.8
23.5	173.4	23.5	156.5	7.1	161.7	12.2	167.5	9.8
25.5	158.6	12.9	156.8	9.0	149.0	11.2	163.4	7.1
27.7	-	-	143.5	9.0	139.0	3.7	163.7	18.2

Table 64. The 95% confidence limits (cl) for floret sizes at different positions along the spike, at stage 7, in different CCC treated Proctor plants

	Floret size and confidence limits along spike							
	CCC 20		CCC 24		CCC 28		control	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	175.5	15.3	185.2	10.0	172.9	5.1	180.1	21.9
3.5	190.0	12.8	193.4	9.5	192.1	10.2	191.1	12.0
5.5	-	-	188.2	10.9	184.0	2.9	187.9	9.8
7.5	-	-	186.0	9.1	181.6	3.4	180.0	7.7
9.5	-	-	182.8	8.7	177.0	7.3	174.3	10.2
11.5	178.2	10.6	172.5	9.7	174.4	5.2	170.1	7.6
13.5	176.7	8.8	168.1	8.7	172.4	12.9	164.2	10.9
15.5	173.6	10.9	167.2	7.9	169.6	3.6	163.3	8.1
17.5	172.3	8.9	163.3	4.5	-	-	160.6	6.2
19.5	171.9	7.5	-	-	167.7	13.2	-	-
21.5	169.4	8.4	-	-	168.9	12.3	-	-
23.5	168.2	7.9	-	-	165.1	9.1	159.7	5.5
25.5	-	-	156.9	5.4	160.2	6.5	155.9	10.3
27.5	161.2	6.1	153.5	8.8	159.8	7.6	152.9	13.6
29.5	156.3	6.4	150.9	6.6	154.7	9.1	150.2	10.9
31.5	-	-	145.2	10.4	-	-	-	-

The width differences between central florets at different positions along the spike were established at stage 7, but there were no significant differences between the sizes of the central florets at a given position in the control plants or on any of the CCC treated plants by stage 9 (Figure 29c; Table 65).

Table 65. The 95% confidence limits for floret sizes at different positions along the spike, at stage 9, in different CCC treated Proctor plants

	Floret size and confidence limits along the spike							
	CCC 20		CCC 24		CCC 28		control	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	242.1	3.7	239.6	22.8	249.6	1.7	148.2	2.6
3.5	254.4	11.6	252.8	16.2	253.7	7.1	257.7	11.1
5.5	262.8	10.6	263.5	12.0	266.5	12.1	266.9	16.3
7.5	269.1	16.7	264.1	8.6	269.0	12.7	272.2	15.1
9.5	271.5	11.9	262.6	5.7	267.0	12.2	271.5	19.9
11.5	264.8	10.9	261.6	10.5	266.3	12.1	265.9	27.2
13.5	257.4	9.9	254.3	6.9	258.1	13.3	261.5	18.8
15.5	250.5	15.1	247.8	8.3	254.1	14.5	255.6	19.8
17.5	249.5	13.0	243.8	20.4	251.3	15.2	253.5	25.5
19.5	245.6	9.2	242.2	23.0	251.0	17.6	249.2	9.4
21.5	243.6	17.6	240.0	22.8	247.1	15.3	247.1	8.5
23.5	240.3	11.7	237.6	17.4	245.8	19.6	242.9	13.2
25.5	-	-	237.4	29.4	241.9	16.3	-	-
27.5	-	-	236.0	7.0	237.6	24.0	-	-

In Proctor, as in Maris Mink the size variation between different primordia on the spike decreased by stage 9 and whilst in the latter cultivar there was an increase in growth rates of central florets on the most distal primordia, in Proctor it was the central florets between positions 5 and 12 which grew the fastest between these stages 7 and 9 resulting in a shift of the position

of the largest primordia distally along the apex. This increase in growth rate by the central florets in the lower middle region of the apex was seen in all P plants regardless of treatment.

Final grain weight (Tables 66, 67, 68, 69 and Figures 30, 31a, b, c)

The sizes grains set at different positions on mainstem ears differed between control and CCC-treatments. For given positions on the ear, grains on control plants were larger than those on CCC-treated plants (Figure 30; Table 67), a complete reversal of the situation obtained with Maris Mink.

Unlike Maris Mink, where the total number of grain set was found to be higher on the CCC-treated plants, no difference could be detected between plants of different treatments in Proctor (Table 66). As usual, grain weights at different positions within individual ears differed. In all Proctor plants treated with CCC the largest grains were set further up the spike than in Maris Mink, mirroring the changed positions of maximum central floret sizes at stage 9.

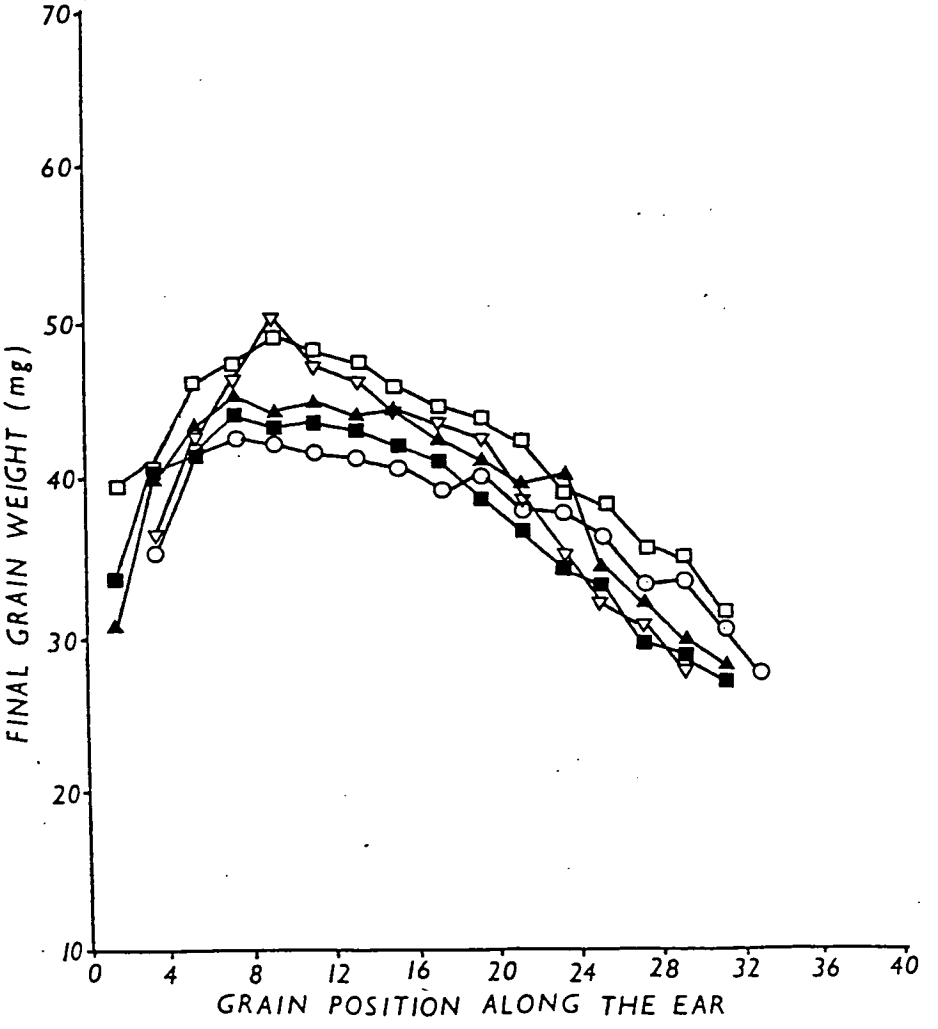

70
60
50
40
30
20
10
FINAL GRAIN WEIGHT (mg)
0
4
8
12
16
20
24
28
32
36
40
GRAIN POSITION ALONG THE EAR

Table 66. The position of MS final grain and the weight in mg of of the largest and smallest set in all treatments

	Grain set:	largest grain:		smallest grain:	
		position	weight	position	weight
Control	1.5-31.5	9.5	49.0	31.5	31.5
CCC 16	3.5-31.5	9.5	50.1	31.5	28.0
CCC 20	1.5-31.5	7.5	45.5	31.5	27.4
CCC 24	1.5-29.5	7.5	43.9	29.5	27.0
CCC 28	1.5-33.5	9.5	42.6	33.5	27.0

Table 67. The 95% confidence limits for final grain weight at different positions along the spike in different CCC treated Proctor plants

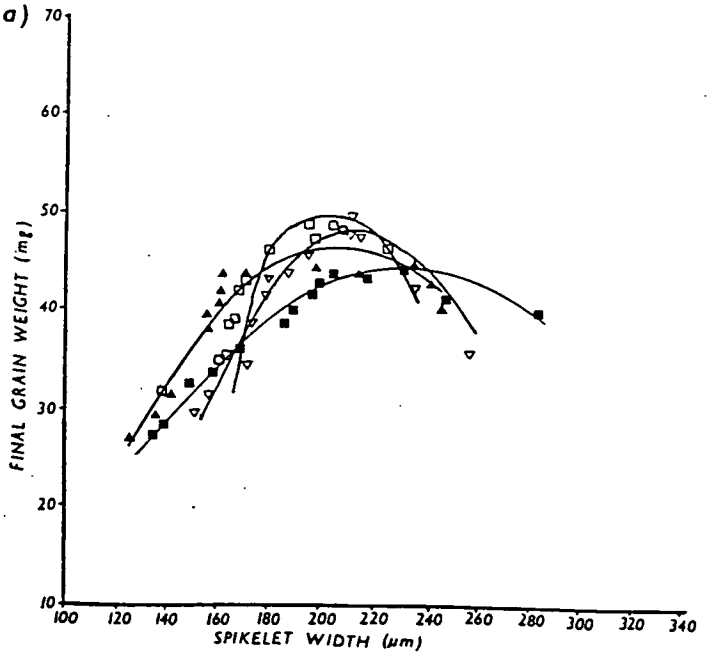
	Final grain weight and confidence limits along spike									
	CCC 16		CCC 20		CCC 24		CCC 28		control	
	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl	$\bar{x}$	cl
1.5	-	-	30.6	8.0	33.6	5.7	-	-	40.0	7.4
3.5	35.8	2.4	40.5	4.5	40.1	3.3	35.8	4.1	40.8	5.5
5.5	42.9	1.6	43.1	2.9	41.3	3.7	41.6	3.1	46.4	4.5
7.5	47.9	1.9	45.2	2.7	44.5	2.5	42.7	2.5	48.1	3.7
9.5	50.3	1.5	44.3	2.4	43.9	2.0	42.6	2.5	49.4	3.3
11.5	48.0	1.3	44.8	2.7	44.1	1.6	41.9	2.5	48.7	2.5
13.5	46.0	1.2	44.0	2.4	43.0	1.4	41.5	2.5	47.9	1.3
15.5	44.5	1.2	44.1	1.5	42.0	1.6	40.9	2.4	46.1	1.7
17.5	42.9	1.5	42.3	1.4	41.3	1.6	39.7	2.4	44.8	1.1
19.5	41.3	2.2	41.6	2.5	39.0	1.6	41.1	2.7	44.2	2.0
21.5	39.7	2.9	40.2	1.2	36.7	1.8	38.1	3.1	42.7	2.0
23.5	35.1	2.9	39.6	3.7	34.5	2.7	38.6	4.5	39.3	2.9
25.5	32.1	2.9	34.6	1.6	33.2	4.1	36.3	1.3	38.6	2.9
27.5	30.0	3.3	31.9	2.2	29.3	5.0	33.0	1.9	35.6	3.3
29.5	28.0	2.0	29.7	2.9	28.3	2.9	33.8	1.4	35.3	3.3
31.5	-	-	27.7	2.4	27.0	5.9	30.6	2.6	31.5	6.9

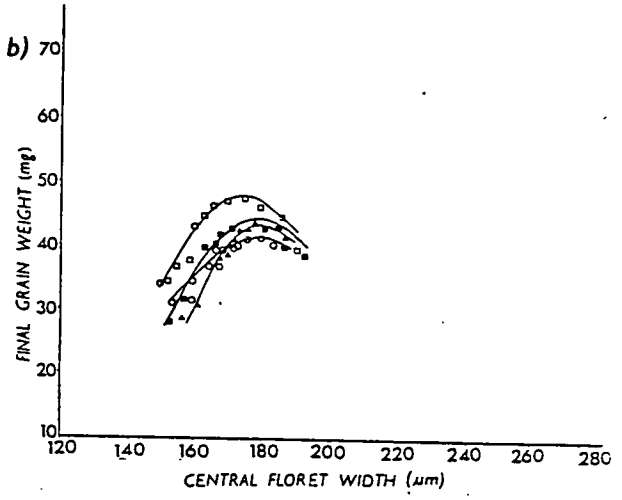
When final grain weight was plotted against spikelet primordia width at double ridge stage (Figure 31a) there was a correlation between the two, with the two most basal primordia being out liers. The shape of the correlation curve in all treatments was the same and only the control curve was shifted up the y axis. This was not the case for Maris Mink where all the CCC treatment curves are shifted up this axis relative to the control curve. In Maris Mink this was because the CCC treatments had larger grain weights than the controls whereas in Proctor the position was reversed. There was no shift along the x axis in either cultivar and widths of the primordia were similar.

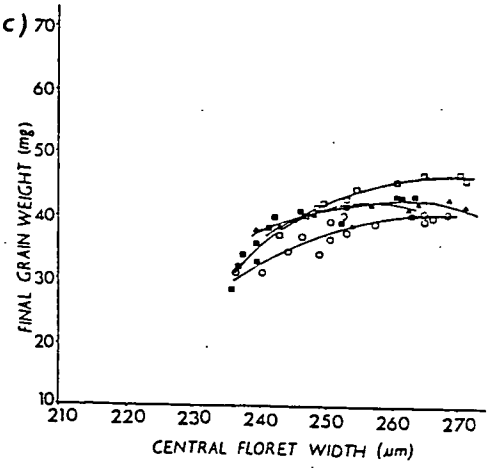
Figure 31a. The relationship between final grain weight and the width of spikelets at the double ridge stage at various positions along the ear for Proctor plants treated with CCC at d16 (▽) (y= -194.08 + 2.27x - 5.31x^2; r^2= 0.936, n= 12), d20 (▲) (y= -89.19 + 1.33x - 3.23x^2; r^2= 0.933, n= 13), d24 (■) (y= -46.46 + 0.78x - 1.67x^2; r^2= 0.968, n= 14) and for control plants (□)(y= -264.60 + 3.10x - 7.64x^2; r^2= 0.926, n= 13).

Figure 31b. The relationship between final grain weight and the width of florets at stage 7 (lemma initials) at various positions along the ear for Proctor plants treated with CCC at d20 (△) (y= -873.74 + 10.20x - 0.03x^2; r^2= 0.955, n= 10), d24 (■) (y= -176.20 +8.55x -0.02x^2; r^2= 0.973, n= 10), d28 (○) (y= -422.92 + 5.18x - 0.01x^2; r^2= 0.972, n= 10) and for control plants (□) (y= -763.75 + 9.32x - 0.03x^2; r^2= 0.953, n= 11).

Figure 31c. The relationship between final grain weight and the width of florets at stage 9 (stamen initials visible as 3 distinct mounds) at various positions along the ear for Proctor plants treated with CCC at d20 (▲) (y= -486.87 + 4.00x - 7.51x^2; r^2=0.855, n= 9), d24 (■) (y= 1712.42 + 13.63x - 0.03x^2; r^2=0.881, n=14), d28 (◎) (y= -166.99 + 4.87x - 8.99x^2; r^2=0.833, n=14) and for control plants (▣) (y= 785.70 - 6.15x + 0.01x^2; r^2=0.0981, n=10).


a)
FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
100 120 140 160 180 200 220 240 260 280 300 320 340
SPIKELET WIDTH (μm)


b)
FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
120 140 160 180 200 220 240 260 280
CENTRAL FLORET WIDTH (μm)


c)
FINAL GRAIN WEIGHT (mg)
70
60
50
40
30
20
10
210 220 230 240 250 260 270
CENTRAL FLORET WIDTH (μm)

In both cultivars, when the widths of the central florets at stage 7 and 9 were plotted against final grain weight (Figures 31b, c) the shape of the correlation curve changed, becoming more truncated on the x axis, due to the changes occurring in the central floret width / position curves where basal florets decrease in size relative to the other florets and where the difference between the largest floret and the smallest decreases as the apex develops. The control curve was higher on the y axis than the curves of CCC treatments because the control treatment had the larger grain. In Maris Mink the CCC treatments were shifted up the y axis compared with the control and there was also a shift of the CCC treatment curves along the x axis as their floret widths were larger than the controls.

In control plants of Proctor, values of $\bar{x}G$ on the MS ears were higher than that on any of the tillers. Conversely, in all the CCC-treated plants, the values of $\bar{x}G$ on T1 were higher than those on either the mainstem ears or the other tiller ears (Table 68).

In control plants, T1 had a higher values for $\bar{x}N$ than either the mainstem or any of the other tillers. This contrasted with the situation in CCC-treated plants where the mainstem ears had a larger $\bar{x}N$ values than any of the tiller ears.

The weight of grains was more variable than the number, thus the weight per grain had a bigger influence on total grain weight per plant in the CCC treatments. Consequently, control plants had the highest values of ΣG on T1 whilst the CCC-treated plants produced the greatest values of ΣG on their mainstem ears. The ΣG of the controls was significantly larger than CCC 16, CCC 24 and CCC 28, there was no difference between CCC treatments (Table 69). This is a complete reversal of the situation found in Maris Mink where CCC-treated plants had larger values of ΣG than the controls.

Table 68. Total grain weight per ear (mg), mean individual grain weight per ear (mg) and mean grain number per ear for mainstem and tiller ears in Proctor for different CCC treatments

		Treatment:				
		Control	CCC 16	CCC 20	CCC 24	CCC28
MS	x̄G	44.1	40.5	39.0	37.6	37.8
	x̄N	23.0	23.2	23.1	23.2	23.1
	ΣG	1014.3	939.6	900.9	872.3	873.2
T1	x̄G	43.3	41.4	43.8	40.4	39.5
	x̄N	26.0	19.5	19.7	20.7	15.7
	ΣG	1125.8	807.3	862.9	836.3	620.2
T2	x̄G	42.5	38.0	40.8	40.5	37.0
	x̄N	22.1	18.8	18.2	22.0	19.9
	ΣG	939.3	714.4	742.6	891.0	736.3
T3	x̄G	40.7	36.0	37.9	33.4	36.0
	x̄N	15.6	16.5	19.9	21.3	14.9
	ΣG	634.9	594.0	754.2	711.4	536.4
T4	x̄G	36.8	35.2	36.3	33.6	36.0
	x̄N	18.9	19.2	15.9	12.1	11.9
	ΣG	695.5	675.8	577.2	406.6	428.4
T5	x̄G	-	34.7	32.7	32.0	32.2
	x̄N	-	9.4	8.6	8.9	6.7
	ΣG	-	326.2	281.2	284.8	215.7
	TWG	4409.8	4057.3	4119.0	4002.4	3410.2

Table 69. Results from two way analysis of variance for total grain weight per ear (ΣG) in the different treatments and different ear positions within Proctor plants, including the least significant difference values (LSD)

Source	d.f.	variance ratio (F)	signif.	LSD
Treatment (CCC)	4, 16	4.24	**	124.5
Ear position	4, 16	12.7	***	124.5

7.3. DISCUSSION

Application of CCC reduced plant dry weight and altered mainstem apex development and leaf and tiller emergence. CCC treatment also altered the size of final grain. Plant size in CCC treated plants must not be the sole determinant of grain size because CCC treatment in Maris Mink led to smaller dry weight but larger grain production than the controls. It therefore seems more probable that grain weight in CCC treated plants is determined by a change in growth rate.

The results obtained for the two cultivars, Proctor and Maris Mink seem completely different and hard to reconcile. In Maris Mink there was no long-term effect on apex development but rather a short-term effect where a period of intense development was brought foward in the CCC-treated plants relative to the control. Floret sizes at stages 7 and 9 were larger in CCC-treated plants than controls, as was final mainstem grain size, tiller grain size and number. The application of CCC reduced plant dry weight increment but leaf and tiller emergence was slightly accelerated in the treated plants. Also, the ear bearing stems of CCC treated plants were taller than the controls. In Proctor the short-term effect of CCC sees the rate of development being slowed. Treated plants also showed reduced rates of leaf and tiller emergence and dry weight increment reduced. CCC treatment did not alter spikelet or floret size, but appeared to cause a reduction in grain weight. Treated plants had shorter ear bearing stems than the controls.

CCC, as mentioned previously, causes a lower concentration of GA precursors and partially inhibits GA synthesis (Höfner and Kühn, 1982). So the effects of CCC application are most likely to be mediated via a plant growth substance mechanism

Barley plants treated with CCC show only limited stem shortening and even this effect is mostly transient, with plants later compensating for any shortening (Humphries, 1968; Waddington, 1983; Stevens and Palmer, 1983; Clark and Fedak, 1977). Occasionally CCC has even had similar effects on plant growth and development as GA . Both CCC and GA , had similar effects on stem extension (Adedipe *et al*, 1968; Wünsche, 1969; Halevy and Witter, 1965) and on increasing grain yield and tillering in barley (Koranteng and Matthews, 1982). Even when CCC caused dwarfing in tomato and pea plants, increased amounts of extractable, gibberellin-like substances were produced (Hill, 1973). It has also been suggested that there is an over-production of GA in the upper internodes after CCC has degraded, causing treated plants to be taller than untreated plants (Linser and Kühn, 1962; Cartwright and Waddington, 1982; Jaddoa, 1986). Jaddoa and Flint (1985) found that winter barley treated with CCC had short lower internodes but longer upper internodes, causing treated plants to be taller overall than the controls. Growth retardants such as CCC must not merely affect the biosynthesis of gibberellins since only some of their effects can be reversed by the application of GA (Cleland, 1969). Auxins, however, were found to counteract the effects that application of GA could not (Khan and Tolbert, 1966) and CCC has been shown to reduce the levels of indoleacetic acid and tryptophan, as IAA precursors (Norris, 1966). In tobacco, CCC causes stunting and inhibits sterol synthesis and these effects cannot be totally reversed by the application of gibberellins alone but can be reversed by a combination of gibberellins with β-sitosterol, stigmasterol and cholesterol (Douglas and Paleg, 1974).

It would therefore appear from these results that CCC alters the level of other key metabolites as well as GA and these in turn affect the growth of the plant as a whole.

The results obtained with CCC treated plants may be explained by the following hypothesis (Summary in diagram A). In Maris Mink it is possible that CCC treatment temporarily raises the level of a key metabolite in the cells of the apex which affects the rate of primordia initiation and development and this same metabolite probably alters the emergence rates of the leaves and tillers; with time this effect may decline. In the normal situation, as seen in control plants of Maris Mink, a peak of this compound may occur between d26 and d30 when the peak rate of development occurs. In the CCC-treated plants of Maris Mink where an increase in the level of this metabolite is brought about earlier, than the normal increase.

In Proctor CCC-treatment may also cause an increase in the level of the proposed control metabolite but in this instance it may elevate the compound to such an extent that it becomes inhibitory to leaf and tiller emergence as well as primordium initiation and development. There is a precedent for this type of inhibitory effect in cell cultures where auxins at low concentrations will promote growth but at high concentrations will impede growth. Hence CCC-treatment might initially depress the rate of growth and development until the level of control metabolite had declined sufficiently to allow growth to recommence at the normal rate.

In Maris Mink, application of CCC brought the burst of developmental activity forward when compared to that of the controls but had no long-term effect. In Proctor however, CCC treatment appears to have a long-term negative effect on primordium initiation and development in CCC 16 plants. In CCC 20 plants, and most probably in CCC 24 and 28 plants, the effect of the compound on apex development appears to be more short-term. There was an initial effect in these plants causing the rates of primordium initiation and development to be depressed but after several days the normal rates of growth and development returned. This situation could arise if CCC treatment has two distinct

effects on the apex, a short-term effect that is manifest regardless of the time of application and a long-term effect that is only manifest if the compound is applied sufficiently early in development when the apex is susceptible to it. It has been suggested that the early application of GA_3 acts as a trigger for the production of a self-sustaining stimulus of spikelet development (Cottrell *et al.*, 1982) and that this results in the long-lived effect of a single application of GA_3. At d16 the apex of Proctor plants may still have the ability to be changed in this long-term manner and thus CCC affects the development of the apex over a prolonged time-span. By d20 spikelet development may no longer be susceptible to the long-term effect of the compound and so only the short-term effect is seen in these and the d24 and d28 CCC-treated plants.

Diagram A. Summary of hypothesis:

Maris Mink

add CCC ---> raise key metabolite ---> promotes:
1) spikelet develoment
2) leaf & tiller emergence
3) stem growth
4) grain number & weight

Proctor

add CCC ---> raise key metablite ---> inhibits:
1) spikelet development --->
a) long term in CCC 16
b) short term in rest
2) leaf & tiller emergence
3) stem growth
4) grain number & weight

In control plants of Proctor there was only a slight burst of developmental activity and it occurred earlier than in Maris Mink. It is possible that different cultivars with different developmental patterns are affected differently by the application of CCC. When CCC was applied to Maris Mink during the vegetative stage, spikelet primordia development was temporarily slowed (Koranteng and Matthews, 1982). The same effect was seen in the cultivar Clipper (Cottrell, 1980). Other barley varieties showed a temporary slowing of mainstem apex development when CCC was applied later, at the lemma initial stage (Jaddoa, 1986; Waddington, 1983). As well as causing a slow period of apex development, when applied at the vegetative stage, CCC application in Clipper (Cottrell, 1980) also resulted in a retardation in whole plant development whereas in Maris Mink (Koranteng and Matthews, 1982) whole plant development was accelerated. The increase in developmental rate in Maris Mink was due mainly to the early production of tillers (Koranteng and Matthews, 1982). The timing of CCC application in relation to the cultivars' normal development could determine whether or not the proposed control metabolite retarted or advanced mainstem apex development and plant growth. This difference in initiation and development rates of spikelets as well as the differences in leaf and tiller emergence rates between cultivars perhaps caused the differences in final grain weight.

In both Maris Mink and Proctor CCC, had a long term effect on plant growth because in the treated plants the height of the ear bearing stems was altered. In Maris Mink the treated plants had taller ear bearing stems than the controls while in Proctor they were smaller. The change in stem length found in CCC treated plants appears to be a result of a change in normal levels of growth substance altering the growth of stem internodes (Linser and Kühn, 1962; Cartwright and Waddington, 1982; Jaddoa, 1986). Again presumably, in Maris Mink the change in growth substance

levels promotes some part of stem growth while in Proctor it is inhibitory.

Total grain weight was affected by the application of CCC in three ways: the number of tillers changed, the number of grain per ear was altered and the weight of individual grain per ear produced varied. Maris Mink plants treated with CCC produced one more primary tiller and a number more secondary tillers. Grain number and grain weight per ear was also increased. In Proctor treated with CCC, the number and weight of grain per ear was decreased.

It has been found that CCC frequently causes an increase in grain yield (Larter *et al*, 1965; Bokhari and Younger, 1971; Matthews *et al*, 1982; Jaddoa, 1986) but the way in which the increase occurs seems to differ. Matthews *et al* (1982) found that, in Maris Mink, CCC caused synchronous, early emergence of tillers. This led to a smaller size difference between primary tiller, secondary tillers and the mainstem and thus a yield increase. Maris Mink produces tillers more slowly than Proctor and it has been suggested above that this late development of tillers reduces spikelet primordia and floret size. This would mean that if CCC causes the tillers of Maris Mink to emerge earlier, increasing within-plant uniformity, then spikelet size should have been increased, which indeed was the case. It has been suggested that within-plant uniformity early on in plant development allows available resources to be used more effectively (Matthews et al, 1982) thus leading to grain yield increase.

CCC must affect some metabolic process which leads to synchronous production of tillers. Koranteng and Matthews (1982) found that following the application of CCC to Maris Mink there was an synchronous increase in cytokinin and tiller bud production, cytokinin being a stimulator of tiller bud growth (Sharif and Dale, 1980). Application of BAP, a synthetic cytokinin, has been shown to increase uniformity between shoots and between spikelets

within the ears of barley and it is suggested that this is due to a lowered gibberellin-cytokinin ratio (Williams and Cartwright, 1980). In untreated Proctor plants, where tiller production is earlier and more compressed in time than Maris Mink, CCC could further increase the concentration of the growth substance responsible for tiller bud growth at a time when it was at its most potent. These elevated levels of growth substances could well be inhibitory to the growth of tillers in Proctor, thus explaining their reduced growth.

There is much evidance to suggest that CCC has a wide ranging effect on plant growth substances, altering the production of gibberellins, cytokinins, auxins and many others within treated plants (Khan and Tolbert, 1966; Norris, 1966; Halvey, 1963; Gaspar and Lacoppe, 1968). There are also many examples that illustrate that growth substances are complementary in their action (Johnston and Jeffcoat, 1977; Phillips, 1975; Ruckenbauer and Kirby, 1973; Cottrell, 1980; Rocha, 1979; Skene 1975; Morris and Winfield, 1972; Woolley and Wareing, 1972; Sachs and Thiaman 1967; Langer *et al.*, 1973; Wareing, 1977; Sheldrake, 1973) and so it is probable that if the concentration of one substance is altered by the application of CCC many others are most probably altered be too. What is not know is if the effect of CCC application acts directly on one of these plant growth substances or indirectly through a number of (unknown?) routes.

The effects of CCC on the size of florets, on apex development and on tiller growth seem to be related to individual plant development patterns and timing of application. In plants which have delayed, slow tillering CCC may well be an effective way to increase yield by freeing the plant from within-plant competition allowing for increased spikelet primordia size and number as well as enhanced tiller production and survival. In plants that already have early tiller production application of CCC probably does not serve any useful purpose in increasing yields. In

conclusion it seems premature to claim that all the effects of CCC are related directly to the inhibition of gibberellin synthesis or that CCC is the answer to increasing yield in all barley cultivars. However in many modern cultivars CCC seems to have allowed yield increases to occur.

SECTION 8. OVERVIEW AND CONCLUSIONS

Using the Scanning Electron Microscope techniques (Chapter 2) it has been possible to investigate the relationship between spikelet primordia and grain weight and to test the suggestion of Cottrell and Dale (1984) that spikelet primordia at double ridge stage (stage 4) determined final grain size so that any change in spikelet primordia size at stage 4 would automatically be reflected in grain size. However it was found that this suggestion was not universally applicable.

Spikelet primordia size at double ridge stage could be altered experimentally. However, from results found in the first and subsequent experiments, the size of spikelet primordia at double ridge stage did not automatically determine the size of grain. Whether or not spikelet primoria at stage 4 appeared to determine grain size depended on what happened to the plants after stage 4. So, it does not follow that in an experiment where two treatments produce similar sized spikelet primordia at stage 4, grain size will be similar too, because conditions experienced later on in the life of the plants may well be different. For example, in repeating as precisely as possible the Cottrell and Dale experiment results showed that although spikelet primordia at stage 4 were similar, grain size differed between the two experiments probably due to change in the amount of sunshine received by plants late on in their development. In the experiments where nitrogen supply was altered during spikelet initiation it appeared that if a low level of nitrogen was maintained until spikelet primordia initiation ceased, spikelet primordia size at stage 4 did determine final grain size. The relationship found here between spikelet primordia and final grain weight was not one of absolute sizes rather it was one of relative size, such that small primordia at stage 4 led to small grain. However, if adverse nitrogen conditions were only maintained for a short

period, upto the time the most advanced primordia was at stage 4, the grain sizes appeared unaffected by primordia sizes at stage 4.

So, it appears that there is no single determinant of grain size but rather grain size is controlled by the size of spikelet primordia and the growth of the plant as a whole. If adverse conditions are followed by favourable conditions while the plant is still initiating spikelets then compensation for small primordia at stage 4 can occur leading to the production of larger florets and grain. When plants were given low nitrogen upto d20 so that small primordia were produced at stage 4 it was predicted that small grain would result but increasing nitrogen supply after d20 caused an increase in plant growth and this appeared to allow the plants to compensate for the small primordia and increase grain size beyond what was expected. However, the degree of compensation varied according to cultivar.

Maris Mink and Proctor responded differently to treatment. Following the removal of low nitrogen conditions after d20, Proctor plants appeared more able to respond to the increased nitrogen and compensate for small primordia at stage 4 than Maris Mink plants. The size of grain produced by a cultivar in response to a given set of conditions seems to be related to the growth and development of that cultivar. The stage and rate at which tiller development takes place appears to affect the growth of the mainstem as well as the degree to which compensation can occur, following the removal of adverse conditions. Under the same high nitrogen conditions, Proctor produced larger florets than Maris Mink.

Tiller development in Proctor was earlier and faster than in Maris Mink and so it is likely that the development of tillers had less impact on the growth of the Proctor mainstem. The indications are that the presence of tillers reduces the size and photosynthetic capacity of mainstem leaves and consequently leads to a reduction

in assimilate availability. In turn, this is reflected in a reduction in floret and grain size along the mainstem ear. Small florets are indicative of small grain and in all experiments where florets were small, small grains were produced (Sections 4, 5, 6, 7). Removal of tillers increased floret size and grain size but the extent of the increase was again dependent on cultivar response.

Conditions prior to stage 4 have no effect on spikelet primordia size; there is no memory effect in the short term. Conditions during the period when the apex becomes reproductive are important in determining the size of spikelet primordia. Where low nitrogen was given to plants only prior to them entering the reproductive phase of spikelet primordia production, spikelet primordia sizes at the double ridge stage were similar to those in plants given high nitrogen from sowing. Low nitrogen supply during spikelet primordia production reduced spikelet primordia size at stage 4. Except for the low nitrogen treatment, treatment during spikelet primordia production appears only to alter floret size rather than spikelet primordia size at stage 4. It is suggested that spikelet primordia at stage 4 are extremely small and have minimal requirement for assimilate, in order to alter their size the plant as a whole has to suffer great stress. This appears to be the case in plants supplied with low nitrogen but not in the other treatments.

A change in grain size does not closely reflect a change in plant size. In Maris Mink plants treated with CCC, the increment in plant dry weight was small but grain size was greatly increased in comparison with the control, non-treated, plants. However the rate of spikelet primordia development, leaf and tiller emergence were all increased in the CCC treated plants, suggesting that increased grain size was correlated with plant growth and development rather than plant size.

In all the experiments carried out grain size in relation to its position on the spike was determined at stage 4 and maintained throughout subsequent developmental stages. This relationship does not appear to be altered by changes in the rate of spikelet primordia development. In the CCC experiments, where spikelet primordial developmental rate was altered, being speeded up in Maris Mink and slowed in Proctor, no modification in the position / size relationship occurred.

It now seems premature to suggest that it will be possible to predict absolute grain size. The sizes of spikelet primordia at stage 4 are rarely indicative of relative grain size let alone absolute grain size. Floret size appears to be a more accurate indicator of relative grain sizes. However, even floret size does not always predict grain size as in Proctor plants treated with CCC were floret sizes were similar to those of the control plants but grain size was smaller. It also seems unlikely that it will be possible to alter the position / size relationship of spikelets within an ear because it appears to be determined by the timing and rate of vascularisation within the ear. The only way to achieve an ear with uniform grain base to apex would be to synchronize or speed up spikelet vascularisation and this may prove unachievable. It seems more sensible to investigate further the effects of plant size and timing of tiller and spikelet primordia development on grain size.

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