ROTHAMSTED RESEARCH

Report for 1973 - Part1

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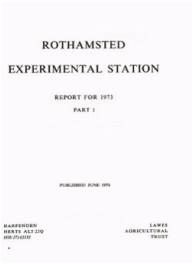

Botany Department

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C. P. Whittingham (1974) *Botany Department ;* Report For 1973 - Part1, pp 88 - 107 **- DOI: https://doi.org/10.23637/ERADOC-1-130**

BOTANY DEPARTMENT

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Introduction

The work of the Botany Department is mainly concerned with crop physiology and in particular the factors limiting yield of cereals, sugar beet and potatoes. There is also work on the biology of weeds.

The long-term aim of crop physiology is to explain how plants grow and to relate quantitatively performance in the field to environmental factors. Ultimately the objective is to identify which plant reactions become rate-limiting under given conditions and to propose desirable changes either in the genetic complement or in agronomic practice. Comparative studies of tropical and temperate grasses have suggested that certain tropical species may be more efficient in utilising solar energy to form plant foodstuffs. Considerable attention has therefore been paid to possible differences in the mechanism of photosynthesis between these two groups of plants with the intention of improving, as far as possible, the productivity of temperate species. However, it has already been established that the high efficiency of some tropical plants is not retained when such plants are grown under temperate conditions. The problem is therefore not likely to be simply agronomic but rather to be one of genetic engineering; thus we need detailed information about the enzyme systems concerned in photosynthesis and related metabolic processes. This long-term objective is being approached through a number of individual studies which may contribute in the short-term to increases in crop yields. Six aspects of the growth of cereals have been investigated in the past year:

1. The relationship between growth, mineral nutrition and irrigation investigated jointly with the Chemistry Department. The aim of these investigations is to explain the differences in growth and yield of wheat and barley at two different sites, namely Rothamsted and Broom's Barn.
2. A study of growth and carbohydrate metabolism in spring wheat to determine why grain yield fails to respond to large amounts of nitrogen which increase leaf area.
3. Determinations of the activity of certain enzyme systems in field-grown wheat with parallel laboratory studies of the isolated enzymes relating to the process of photo-respiration.
4. Investigations of the loss in yield of barley when exposed to moderate levels of air pollution.
5. Studies of the influence of temperature on growth differentiating between the effect of temperature on grain and on the vegetative part of the plant.
6. Investigations of the biochemistry of grains and the influence of growth regulating compounds upon them in an attempt to analyse the factors controlling grain metabolism.

Less extensive investigations are being undertaken with sugar beet and with potatoes. The primary object with both of these crops is to determine which factors control the distribution of dry matter between the vegetative parts and the storage organ and to determine how far this distribution is controlled by growth regulating substances. The effect of irrigation on growth and yield is also being investigated.

In addition to continuing traditional studies on the distribution of weeds in experiments at Rothamsted and Woburn we are also studying the fundamental biology of certain important weed species. In particular we have recently begun to investigate the

https://doi.org/10.23637/ERADOC-1-130

biological characteristics of *Equisetum* that are likely to influence the effectiveness of control by selective herbicides.

Cereal crops

Response of spring wheat to large amounts of nitrogen

Growth. Similar experiments were done in 1972 (*Rothamsted Report for 1972*, Part 1, 92) and 1973 to investigate the physiological factors limiting the yield of Kleiber spring wheat in response to large amounts of nitrogen fertiliser. Extra nitrogen does not increase grain yield of modern stiff-strawed varieties, that do not lodge, as much as expected from studies with older varieties. Although it still increases total dry weight and leaf area, excess nitrogen lowers the efficiency of the leaves in grain production. Treatments were all combinations of nine amounts of nitrogen (0–200 kg N ha^{-1}) and two plant populations (approximately 222 and 412 m^{-2}).

Mean values for most measurements of growth were 8–10% greater in 1973 than in 1972. In 1972 and 1973 mean grain yields were respectively 5·7 and 6·0 t ha^{-1} (467 and 507 g m^{-2} dry matter), mean dry weights of straw plus chaff were 706 and 785 g m^{-2}, and mean values of leaf area index at anthesis were 6·2 and 6·7. Both crops had grain growth periods of about 54 days; anthesis and maturity were two weeks earlier in 1973 than 1972.

In 1972 nitrogen increased the dry weight of grain by 24% from 406 g m^{-2} with no N to 502 g m^{-2} with 125 kg N ha^{-1}. The dry weight of the rest of the top at maturity increased linearly with nitrogen throughout the range from a minimum of 543 g m^{-2} to a maximum of 818 g m^{-2}. Consequently harvest index (grain % of total dry matter) decreased from 42% with no N to 37% with 200 kg N ha^{-1}. Whole plant dry weight and leaf area index at anthesis also increased with nitrogen throughout the range: their respective values with 200 kg N ha^{-1} were 65 and 85% greater than with no N. Nitrogen increased the number of ears from 402 m^{-2} with no N to 560 m^{-2} with 200 kg N ha^{-1} but this was accompanied by a decrease in weight per grain from 41·5 to 35·2 mg; the number of grains per ear was unaffected. In 1973 nitrogen had much smaller effects. Excess nitrogen decreased grain dry weight from 534 g m^{-2}, with amounts of N up to 75 kg ha^{-1} to 480 g m^{-2} with 200 kg N ha^{-1}. The dry weight of the rest of the top increased linearly with nitrogen throughout the range, by 8% between 0 and 200 kg N ha^{-1}. Consequently harvest index decreased with additional nitrogen, as in 1972, from 41% with no N to 36% with 200 kg N ha^{-1}. The addition of 200 kg ha^{-1} increased dry weight by 21% and leaf area index by 16% at anthesis. Although nitrogen increased the number of shoots in May and June, it did not increase final ear number. The 10% decrease in grain yield with nitrogen above 76 kg ha^{-1} was accompanied by a 7% decrease in ear number and a 10% decrease in number of grains per spikelet. Nitrogen increased dry weight per grain slightly.

In neither year did sowing rate affect dry weight of grain or straw at maturity. It increased ear number and decreased the number of grains per ear (fewer grains per spikelet in both years and also fewer spikelets per ear in 1972). Denser sowing increased dry weight and leaf area index until three weeks before anthesis in 1972 and until two weeks after anthesis in 1973. (Thorne)

Measurement of photosynthetic activity in field conditions. Last year two different types of apparatus for supplying $^{14}CO_2$ to leaves in the field were compared. The two methods produced different response curves relating photosynthesis to varying light intensities (*Rothamsted Report for 1972*, Part 1, 99). Further work showed that this was related to the difference in linear air velocity in the two types of leaf chamber, although both were supplied at the same volume flow rate.

Further improvements were made to the field apparatus. Light alloy cylinders, size

https://doi.org/10.23637/ERADOC-1-130

5·5 litres, were filled in the laboratory with gas mixtures containing $^{14}CO_2$ at pressures up to 34·5 bar. The cylinders were fitted with standard pressure regulators and fine needle valves. The leaf chambers were redesigned to give a higher light intensity at the leaf surface. The apparatus is easier and cheaper to build and simpler to use than those previously described and is now used extensively both in the field and in controlled environment conditions. (Kendall and Young)

Photosynthesis of the flag leaf and, in 1973, the leaf below the flag leaf, was estimated by measuring the uptake of $^{14}CO_2$ when the leaves, in their natural positions in the canopy, were exposed for 15 seconds to 300 ppm CO_2 containing 830 mCi mol^{-1} $^{14}CO_2$. Four leaves per plot were treated. Photosynthesis was not saturated in bright sunshine (980 W m^{-2} total radiation) so that photosynthesis and radiation were measured simultaneously and treatment effects calculated after doing an analysis of covariance of photosynthesis on the logarithm of radiation intensity.

In 1972 the rate of photosynthesis per unit area of flag leaves during the grain filling period decreased with increase in nitrogen throughout the range tested. The rate with 200 kg N ha^{-1} was about 75% of that with no N, but nitrogen increased flag leaf photosynthesis per unit ground area because it caused a big increase in the leaf area index contributed by flag leaves. The decrease in photosynthetic rate per unit of leaf area was almost certainly caused by the smaller light intensity in the leafier crops produced by ample nitrogen fertiliser; total radiation at the level of the flag-leaf node was 55% of that above the crop with no N and 30% with 200 kg N ha^{-1}. In 1973 the rate of photosynthesis per unit leaf area was affected negligibly by nitrogen which also had no detectable effect on penetration of radiation into the crop canopy, presumably because effects on leaf area index were so small. Measurements made six and 19 days after anthesis indicated that the intensity of total radiation at the level of the flag leaf was 62% of that above the crop, at the level of the leaf below the flag leaf 35%, and at ground level 15%. Photosynthetic rate per unit leaf area decreased as the leaves aged and the rate for the leaf below the flag leaf was less than one-half that of the flag leaf. Mean values, corrected to an incident radiation of 560 W m^{-2}, were for the flag leaf 18·4, 15·5 and 9·2 mg CO_2 dm^{-2} h^{-1}, 5, 20 and 33 days respectively after anthesis. Values for the leaf below the flag leaf at five and 20 days were respectively 8·2 and 6·5 mg CO_2 dm^{-2} h^{-1}.

Water consumption. The lack of response to large amounts of nitrogen may be caused by a shortage of water following greater transpiration by the leafier crop produced by ample nitrogen. To test this soil moisture was measured with a neutron probe. Increasing the sowing rate or applying 200 kg N ha^{-1} caused the soil to be drier. In 1972 effects were obvious at most depths down to 120 cm; that of sowing rate was present in early May and persisted until September; that of nitrogen showed from mid-July onwards. During the grain filling period the largest treatment effects were between 30 and 70 cm below the soil surface; the mean effect of additional nitrogen was to decrease the moisture content of the soil from 23·6 to 18·8% (w/v) whereas doubling the sowing rate decreased it from 22·5 to 19·9%. In 1973 nitrogen had a small effect on moisture content of the top 15 cm of soil in early July, decreasing the mean water content from 18·6 to 15·4%. The effect disappeared later as the soil dried further. Denser sowing decreased soil moisture between depths of 30 and 60 cm from the first observation in late May onwards: mean values during July and August for the low and high sowing rates were 17·9 and 21·6% respectively. Calibrations are not yet available to show whether these differences in soil water content represent differences in water potential likely to affect plant growth. However, a comparison between the relative effects of sowing rate and nitrogen on water consumption with their effects on plant growth suggests that shortage of water is probably not an important factor in restricting the response to nitrogen fertiliser. (Thorne)

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Biochemical factors. The activities of certain enzymes associated with photosynthesis, and the initial products of photosynthesis were determined in the flag leaves of wheat grown with different levels of nitrogen. The enzyme activities were measured in crude extracts of flag leaves, as far as possible at optimum conditions to estimate maximum activity. The distribution of carbon between different products of photosynthesis was determined after the application of $^{14}CO_2$. Labelled samples were immediately cut from the leaves and frozen in liquid nitrogen. Compounds were extracted as previously described (*Rothamsted Report for 1972*, Part 1, 100). Measurements were made at intervals from anthesis to senescence of the flag leaves.

The activity of ribulose 1,5-diphosphate carboxylase, the carboxylation enzyme of photosynthesis in wheat, was always greater in the flag leaves from plants grown on plots treated with 200 kg N ha^{-1} than those grown with 100 kg N ha^{-1} or no nitrogen, fixing respectively 66·5, 57·2 and 47·8 mg CO_2 dm^{-2} h^{-1} at anthesis. These activities fell steadily to 23·2, 13·3 and 6·8 mg CO_2 dm^{-2} h^{-1} by the end of the grain filling period. During the period of maximum grain filling, the activity of this enzyme was more than adequate to support the rates of photosynthesis observed in the field. The greater ability of the flag leaves grown with much nitrogen to carboxylate ribulose 1,5-diphosphate did not result in faster rates of photosynthesis.

The enzymic synthesis of serine from glycine, probably responsible for the loss of CO_2 during photorespiration (*Rothamsted Report for 1972*, Part 1, 101) was also measured. There was little difference in activity from leaves grown at different levels of nitrogen (33·9, 29·6 and 31·2 μmol CO_2 evolved h^{-1} g^{-1} fr. wt. at 200, 100 and 0 kg N ha^{-1} respectively measured 11 days after anthesis). This activity remained constant throughout the grain filling period, falling to a lower level only at about 35 days after anthesis. The activity of this enzyme was initially only about 10% of the activity of the carboxylase when compared on a similar basis. As grain filling progressed it became more significant (25% of the carboxylase activity) particularly with little nitrogen.

There was evidence that nitrogen fertilisation affected the distribution of carbon between the initial products of photosynthesis. Five treatments were investigated (0–200 kg N ha^{-1}) and the trends were evident throughout the range. Early in the period of grain growth, most of the label assimilated during 15 seconds was in phosphoglyceric acid and glyceric acid, decreasing steadily from 46% of the total ^{14}C assimilated in leaves from untreated plots to 39% with 200 kg N ha^{-1}. Incorporation into sucrose also decreased from 18 to 13%. Incorporation into malic and aspartic acids increased from 1 to 4%, into alanine from 0 to 3% and into glycine from 1 to 2%. There was no change in the proportion of radioactivity in serine (about 3·5%) and none was detected in glycollate. There was therefore a tendency towards greater incorporation into some of the nitrogen containing compounds in leaves grown at high nitrogen treatments. At a particular nitrogen level, the distribution of ^{14}C in products of photosynthesis in flag, middle and lower leaves was very similar.

Towards the end of the grain filling period (40 days after anthesis) some changes in the distribution of ^{14}C were noted. There were no differences in the percentages incorporated into phosphoglyceric acid and glyceric acid (33%) and into sucrose (10%) between leaves from the different nitrogen treatments. Incorporation into malic and aspartic acids was small, but incorporation into alanine increased from 4% with no nitrogen to 7% with 200 kg N ha^{-1}. Glycollate contained 11·5% of the total radioactivity with no nitrogen and 5% with 200 kg N ha^{-1}, and measurements made 32 days after anthesis showed that this increase occurred earlier in leaves grown on low nitrogen. The radioactivity incorporated into glycine and serine was not greatly affected by nitrogen treatment: the amount in glycine (4%) had increased, whilst that in serine (0·5%) decreased compared with values determined during early grain growth. (Thomas)

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Metabolism of glycine, serine and glycerate in leaves. To explore the possible significance of two carbon compounds in wheat metabolism further experiments were done in the laboratory with detached leaves. In CO_2-free air in the light ^{14}C serine accumulated in detached wheat leaves when [^{14}C] glycollate, glycine, serine, glycerate or glucose were supplied in solution through the vascular system. The production of sucrose from the above substrates was always less in CO_2-free air than in air. More CO_2 was produced in air than in CO_2-free air from serine but less from glycerate and glucose; production of CO_2 from [1-^{14}C] glycine and [1-^{14}C] glycollate was nearly the same in air and CO_2-free air. The dependence of sucrose synthesis upon simultaneous CO_2 assimilation could be partly relieved by adding an α-keto acid to the solution of [^{14}C] substrate. Whilst α-keto acids promoted sucrose synthesis from serine most when the oxygen content of the atmosphere was between 2 and 10%, synthesis from serine in atmospheres with 300 ppm CO_2 was fastest in the presence of 20% oxygen. It follows that some product of CO_2 assimilation or alternatively the added keto acids can interact with serine in the leaf to allow its conversion to a substance, perhaps glycerate, that can be either a substrate for sucrose synthesis or for photorespiration.

In the absence of CO_2, the photorespiratory conversion of serine to sucrose and to CO_2 can continue rapidly only if an α-keto acid is also added to the leaf. For every serine produced from glycollate, two glyoxylate molecules have to be converted to glycine, probably in the peroxisome, so here there should be no shortage of α-keto acid. Therefore the effect of added α-keto acids must be on serine metabolism at some other site in the cells. (Waidyanatha)

The glycollate pathway. Metabolism of products of photosynthesis, by way of the glycollate pathway, is responsible for much of the CO_2 released by leaves in the light by photorespiration. When intermediates of the pathway are applied in solution to illuminated leaf discs or segments, they are converted mainly to sucrose. No mixture of intact chloroplasts, peroxisomes, mitochondria or soluble protein yet prepared has been able to catalyse the complete sequence of reactions from glycollate to sucrose. Therefore the intracellular distribution and properties of the enzymes in leaves that catalyse reaction steps between serine and sucrose were reinvestigated.

Two enzymes are known which may be responsible for sucrose synthesis in higher plants. One catalyses the transfer of glucose from UDPglucose to fructose to form sucrose directly; the other transfers glucose from the same donor to fructose-6-phosphate to form sucrose phosphate which is subsequently hydrolysed to sucrose. The second of these enzymes, probably the one predominantly used *in vivo* for sucrose synthesis, is generally thought (on the basis of assays made on fractions separated from leaves in non-aqueous media) to be present only in chloroplasts. However, very low activities of the enzyme were recovered and it has been shown that chloroplasts isolated in non-aqueous media are contaminated with cytoplasm (*Rothamsted Report for 1972*, Part 1, 337). We have now studied the distribution of UDPglucose-fructosephosphate glucosyltransferase between fractions of leaves separated in aqueous solutions. The fractions were extracted and assayed by the methods described by Hawker (*Biochemical Journal* (1967), **105**, 943–946) and Lyne and ApRees (*Phytochemistry* (1972), **11**, 2171–2176) and pea leaves proved more suitable than wheat. Leaf fractions containing mostly intact chloroplasts (Cockburn *et al.*, *Plant Physiology* (1968), **43**, 1415–1416) contained only insignificant amounts of activity; most of the activity was in the soluble fraction. The enzyme could not be demonstrated in the chloroplasts even after treatment with dimethyl sulphoxide (Delmer & Albersheim, *Plant Physiology* (1970), **45**, 782–786) or a detergent (Triton X100). However, the activity in the soluble fraction was still insufficient to account for rates of sucrose synthesis commonly observed *in vivo*. Some improvement was

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achieved when the extraction medium was changed to 0·1*M* Tris buffer pH 6·8 containing 0·1% bovine serum albumin. By decreasing the concentration of buffer, omitting both sodium fluoride and EDTA and including 10 m*M* magnesium chloride in the reaction mixture the activity measured was increased by a factor of four. The reaction measured was the transfer of glucose from UDPG to fructose-6-phosphate and not the alternative reaction involving free fructose. Rates of sucrose synthesis measured from leaves of pea, spinach, wheat and bean were 17·9, 25·0, 9·2 and 27·7 μmol h^{-1} g^{-1} fr. wt. respectively. Even if all the carbon assimilated in photosynthesis was converted directly to sucrose these rates would be more than adequate. UDPglucose pyrophosphorylase, another enzyme essential for sucrose synthesis, was also mainly in the soluble fraction. It was confirmed that ribulose diphosphate carboxylase which is known to be present only in the chloroplasts, was retained in them after isolation by our techniques. It follows that neither the enzyme UDPglucose pyrophosphorylase nor UDPglucose-fructosephosphate glucosyltransferase are located in the chloroplasts.

The conversion of serine to glycerate probably occurs in the peroxisomes, but glycerate kinase, the enzyme thought to be essential for the first step between glycerate and sucrose has been found only in the chloroplasts. Thus it appears that in the C_2 pathway either carbon has to re-enter the chloroplast as glycerate before conversion to sucrose or there must be a route which does not involve any enzyme system in the chloroplasts but depends on availability of carbon compounds and energy in the cytoplasm derived indirectly from the chloroplasts. (Keys and Bird)

Effect of temperature on grain growth. Previous experiments showed that warming complete plants of wheat or the ears alone increased early grain growth but not final grain yield largely because warmed ears matured sooner than cool ones (*Rothamsted Report for 1971*, Part 1, 104 and *for 1972*, Part 1, 93). The air used to control ear temperature was warmed but not humidified, so that the vapour pressure deficit (VPD) was 9·3 mbar at 20°C and 18·5 mbar at 25°C compared with 5·1 mbar at 15°C. To discover whether the earlier senescence was caused by the warmer temperature or the dryness of the air an experiment was done in which ears were treated with cool wet (15°C 60% RH = 6·0 mbar VPD), hot wet (20°C, 70% RH = 7·2 mbar VPD) or hot dry (20°C, 49% RH = 11·9 mbar VPD) air from ten days after anthesis until maturity. The 5°C difference in temperature was the largest obtainable while maintaining similar vapour pressure deficits in the cool and hot wet treatments. The air round the rest of the plant was similar to that of the cool treatment. Otherwise the experiment was similar to that done in 1972.

There was no difference between the hot wet and hot dry treatments; both increased ear growth and decreased growth of the rest of the shoot during the first 21 days of treatment, and caused the ears to mature faster than in the cool treatment, as previously. Ear dry weight for the hot wet and hot dry treatments, expressed as % of that of the cool treatment were 117 and 119% after ten days of treatment and 86 and 89% at maturity. So the effects observed in previous experiments can be attributed to the difference in temperature rather than to the accompanying differences in moisture content of the air. (Pearman and Thorne)

In other experiments determinations were made of the concentration of endogenous growth substances in wheat ears grown at different temperatures. In 1971 it was shown that the gibberellic acid (GA) content of the grain increased more rapidly with ears at 20° than at 15°C but the temperature of the rest of the plant was less important (*Rothamsted Report for 1971*, Part 1, 110). Plants from the 1972 experiment have now been analysed, and confirm the earlier result. All the plants were grown at 15°C. In ears maintained at 25°C the GA concentration of the grain increased more rapidly than at

https://doi.org/10.23637/ERADOC-1-130

20°C; this may be correlated with the faster accumulation of starch and quicker maturation. The GA content of the flag leaves and the top internodes of the stems was not affected by ear temperature.

The ears of some plants were sprayed with 5000 ppm chlormequat-chloride (CCC) at the start of the temperature treatment. This had little effect on the accumulation of GA in the ears, but the concentration of GA in the top internode of the stems was higher than in the untreated plants at all three temperatures both at 17 and 24 days after anthesis. The reason for this is not known. The CCC treatment had no effect on final dry weight.

A cytokinin found at R_F 0–0·4 on chromatograms run in isopropanol : ammonia : water (8 : 1 : 1, by vol.) was also present in the ears. The highest concentration was found at the first sampling, seven days after anthesis, when the temperature treatment started. The cytokinin content decreased with time in all treatments, but the decrease was less in ears kept at 20° than at 15° or 25°C. Flag leaf extracts and stem extracts obtained by centrifugation contained two active substances, one with R_F 0–0·4 and the other with R_F 0·5–0·9, similar to zeatin. These also decreased with time but more was retained in flag leaves and stems from plants with ears warmed to 20°C than in the other treatments. (Radley and Wheeler)

Carbohydrate metabolism of detached ears. Other experiments have continued to study the effects of growth substances on the accumulation of starch in developing wheat grains of detached ears. The technique described previously (*Rothamsted Report for 1972*, Part 1, 97) has been modified with a shorter incubation time. Detached ears were incubated for 21 hours with their stalks in ^{14}C-sucrose solution. The amount of radioactivity accumulated in both the starch and the total sugars of the grain was less after application to each individual grain of 15 μg CCC or of 10 μg GA_3, although neither substance had a significant effect when applied through the cut stalk. But as described in the last Report, the GA precursor (—)-Kaurene can cause significant increase in the radioactivity in the sugar and starch of the grain. These results suggest that it is only GA which is synthesised within the grain and not that applied externally which stimulates carbohydrate formation. (Radley)

Growth and yield on different sites. Experiments to analyse the factors that result in smaller yields of winter wheat and barley at Broom's Barn as compared with Rothamsted were continued for a third year.

Similar experiments sown with winter wheat (var. Cappelle-Desprez) and barley (var. Julia) were set up at Rothamsted and Broom's Barn and the effects of irrigation factorially combined with six rates of nitrogen fertiliser (increasing by equal steps from 31 to 188 kg ha^{-1}) investigated. Winter wheat was sown on 20 October at Rothamsted and 10 October at Broom's Barn with 25 kg N ha^{-1} combine-drilled. Differential N was applied on 13 April at Rothamsted and 12 April at Broom's Barn. Barley was sown on 9 March at Rothamsted and 12 March at Broom's Barn and nitrogen treatments applied at sowing. All four experiments were fertilised with sufficient phosphorus and potassium in the seedbed. Irrigation was applied to bring the soil to within 5 mm of field capacity at the beginning of June and thereafter whenever the water deficit exceeded 25 mm until mid-July.

Samples of shoots were cut at ground level at intervals throughout growth to determine total dry weight, shoot numbers and leaf area indices; final yields of grain and straw were determined both from similar sample areas and by combine-harvesting.

Soil core samples for estimating the size of root systems were taken from plots of winter wheat given 125 kg N ha^{-1} following the shoot sampling in mid-June, using methods described in *Rothamsted Report for 1973*, Part 2, 28.

https://doi.org/10.23637/ERADOC-1-130

The main growth data obtained and so far analysed are summarised in the following paragraphs. Further information including grain yield is given in the reports of the Chemistry Department (p. 49) and of Broom's Barn Experimental Station (p. 278). (Sampling dates are given with the Rothamsted date first.)

Winter wheat. At the end of the winter (23/22 March) there was no significant difference between shoot numbers or dry matter yields on the two farms, although Rothamsted wheat had a smaller leaf area. From 29/30 May onwards unirrigated crops at Broom's Barn generally produced less dry matter than at Rothamsted, although the difference was not always consistent at higher rates of N supply.

The greater dry matter production at Rothamsted was associated with a markedly greater leaf area index (L) from the end of May onwards. With the lowest N rate, L reached a maximum of about 8·5 at ear emergence at Rothamsted compared with 7·4 at Broom's Barn. With 94 kg N ha^{-1} L at Rothamsted was 10·2 at ear emergence and at Broom's Barn 7·0, while with 157 kg N ha^{-1} it reached 11·4 at Rothamsted and 7·6 at Broom's Barn. Yields of total dry matter (and grain) of wheat at Broom's Barn were not smaller in proportion to the smaller L, perhaps because leaf area in excess of about $L = 7$ did not significantly enhance the overall rate of dry matter production. A value of L in the range 6–8 is likely to be optimal for grain production (Thorne, *Rothamsted Report for 1973*, Part 2, 5–25). More important, therefore, may be the difference in L between the two sites during the later stages when L had declined below about seven. By mid-July with the lowest rate of N L had declined to 4·3 at Broom's Barn, whilst still being 7·5 at Rothamsted and by the end of July was 0·1 and 1·5 respectively. Throughout this period the greater leaf area of wheat at Rothamsted would have been contributing to a greater grain yield. Although similar or greater differences in L occurred with greater rates of N, their effects on grain yield were confused by the severe lodging that occurred on all the better fertilised wheat plots.

The reasons for the smaller leaf areas at Broom's Barn are not yet clear. Shoot density was closely similar on both sites throughout most of the season. Irrigation had little effect on L at Rothamsted, except perhaps at the end of July when little leaf area remained, but it greatly delayed leaf senescence at Broom's Barn and thus diminished the L difference between Broom's Barn and Rothamsted later in the season.

Wheat on well fertilised (125 kg N ha^{-1}) plots at Rothamsted had a total of 115 g m^{-2} dry weight of roots to a depth of 1 m in mid-June, compared with 124 g m^{-2} at Broom's Barn. However, the extra roots at Broom's Barn were confined to the top 50 cm of soil and, between 50 and 100 cm deep, wheat at Broom's Barn had less roots than at Rothamsted. In the deepest layer examined, 87·5–100 cm, wheat at Broom's Barn had only 57% of the amount of roots at Rothamsted: 2·5 cf. 4·4 g m^{-2}.

Barley. Without irrigation and with the lowest rate of nitrogen fertiliser (31 kg N ha^{-1}), barley at Broom's Barn had a greater L than at Rothamsted (except at the 28/26 June sampling) and a greater rate of dry weight growth. With increasing rates of nitrogen both L and rate of dry matter production became greater at Rothamsted, especially after the end of June. The differences could not readily be ascribed to differences in shoot numbers, which were similar at both sites.

Irrigation had little effect on L at Rothamsted, but increased the L of barley at Broom's Barn at the 26 June and 9 July samplings. It appears to have decreased the number of shoots that died, especially with higher nitrogen rates. As a result, irrigated barley at Broom's Barn with 157 or 188 kg N ha^{-1} had L and rates of dry matter production as great or greater than at Rothamsted. It therefore seems probable that any deficiency in productivity of well fertilised but unirrigated barley at Broom's Barn compared with

https://doi.org/10.23637/ERADOC-1-130

Rothamsted can be ascribed to shortage of soil moisture during the later stages of growth. (Welbank, Taylor, P. J. and Widdowson, Chemistry Department)

Soil moisture to a depth of 120 cm was estimated weekly during the growing season on wheat plots receiving 125 kg N ha^{-1} and on barley plots receiving 94 kg N ha^{-1}, using a neutron probe. The rates of N chosen were those expected to give maximum growth and water use. Plant moisture stress was also measured on these plots using a pressure bomb on several occasions, between early July and when the crops ripened. The water potentials of ears of wheat and barley were less negative than the flag leaves at all samplings and on both sites. The ear and leaf water potentials of wheat were more negative at Broom's Barn than at Rothamsted on unirrigated plots (Table 1). The values for irrigated plots at Rothamsted were not significantly different from those for unirrigated plots, but at Broom's Barn irrigation decreased the water stress. The rate of leaf senescence was also greater at Broom's Barn than at Rothamsted, but was decreased by irrigation.

TABLE 1

Plant water potentials (bar)

Date		Rothamsted		Broom's Barn	
		Unirrigated	Irrigated	Unirrigated	Irrigated
		Wheat			
11 July	Ear	−5·9	−6·0	−7·9	−6·5
	Leaf	−10·2	−6·8	−14·0	−9·1
27 July	Ear	−4·7	−4·4	−10·7	−10·6
	Leaf	−7·1	−8·0	−16·3	−16·0
3 August	Ear	−5·1	−4·9	−14·7	−10·7
	Leaf	−12·5	−11·1	>−20·2	−20·2
		Barley			
18 July	Ear	−10·1	−8·8	−11·0	−11·3
	Leaf	−15·2	−11·6	−17·5	−15·0

The leaf water potentials of barley were more negative at Broom's Barn than at Rothamsted but were less negative on irrigated than unirrigated plots. The ear water potentials differed less between sites and were little different on irrigated and unirrigated plots. Only the lowest mean potential (unirrigated barley at Broom's Barn) was reflected in a reduction in grain yield. (Taylor, P. J.)

Effect of aerial pollution on cereal growth. During spring and summer 1973, Woburn Experimental Farm suffered no serious episode of pollution from the near-by Bedfordshire brickfields. No visual damage was observed on barley or on certain selected indicator plants, except gladioli which are particularly sensitive to fluorides. Fortnightly samples of hawthorn leaves taken at Woburn throughout 1972 and 1973 showed steady increase during the summer both in sulphur and fluoride content. During late October 1972, and early August 1973, hawthorn leaves collected at progressive distances from a particular brickworks showed a decrease in leaf fluoride and sulphur with distance from the works. Obvious leaf damage was only observed at distances less than 0·2 km (Table 2).

Spring barley (var. Julia) was grown in three plastic greenhouses through which was blown ambient field air, ambient air filtered through a dust filter, or ambient air filtered through a dust filter followed by an activated charcoal and calcium carbonate filter. Temperatures, relative humidity and light intensity were similar in the three houses, but

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TABLE 2

Sulphur and fluoride content of washed and dried hawthorn leaves (October 1972)

Distance from works (km)	Total sulphur (%)	Fluoride (ppm)
0–1	0·93	388
1–2	0·78	276
2–3	0·64	148
3–4	0·63	135
4–5	0·60	124
5–7	0·52	81

temperatures averaged 6–7°C above those outside in the middle of hot days, and light intensity was approximately 20% less than outside. A plot of barley was also grown outside.

Between April and August, the mean daily ambient sulphur dioxide level was 75 μg m^{-3}, with a highest daily figure of 216 and with 29 days over 100 (although half these days occurred in April). The mean weekly ambient fluoride level was 0·59 μg m^{-3} with a highest weekly figure of 1·33 μg m^{-3} and with 12 weeks over 1·0 μg m^{-3}. Levels of sulphur dioxide and fluoride were satisfactorily reduced by the complete filtration system to means of 13 μg m^{-3} and 0·07 μg m^{-3} respectively. Sulphur dioxide was reduced by 20% in both of the other houses, and fluorides by 39% in the unfiltered and by 64% in the dust-filtered house. Clearly, some sulphur dioxide and especially fluorine compounds were absorbed by the damp plastic surfaces of the houses and the dust filter alone removed a substantial amount of fluoride.

The growth and yield of barley was similar in all three houses. The outside plot was approximately 20 days behind, but the pattern of growth was essentially the same, and the grain yield was similar to that from the houses. Some growth and yield measurements are given in Table 3.

TABLE 3

Growth and yield measurements of barley

	Maximum leaf area index	Maximum leaf number (per m²)	Shoot no. at harvest	Total dry wt. (g)	Ear dry wt. (g)	Grain dry wt. (g)	1000–grain wt. (g)
Complete filter	10·24	3641	672	967	509	423	39·9
Dust filter	10·00	3665	693	998	528	441	41·3
Unfiltered	10·57	3198	666	983	504	419	40·4
Outside plot	9·26	3432	871	1051	518	429	38·5
	±1·18	±630	±132	±109	±51	±38	±2·2

There was considerable uptake of sulphur by the barley plants in all three houses but differences became apparent as the season progressed. By 2 August, the % total sulphur in the washed leaf dry matter was 0·70%, 0·90% and 1·13% for the charcoal, dust and unfiltered houses; plants from the outside plot had only 0·34% total sulphur. The levels of sulphur in the stems, roots, ears and grain were similar for all four treatments, and the final sulphur content of the grain was 0·14%.

The fluoride concentration in washed leaves was highest for plants from outside or in the unfiltered house, although high values (above 20 ppm) were only seen late in July, by which stage over 90% of the leaf was dead. On 2 August, leaf fluoride levels were 13, 15, 36 and 75 ppm for plants from the charcoal, dust, unfiltered and outside plot respectively. The fluoride levels in the stems, roots, ears and grain were similar for all treatments, and did not exceed 10 ppm.

Gladioli did not exhibit serious fluoride damage during the spring barley growing

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season (April–mid-August), and tip burn only became noticeable during the third week of August. By the end of August, total length of tip injury per plant was 7·3, 13·3, 29·4 and 46·8 cm for the charcoal, dust, unfiltered and outside plot respectively. Analysis of 10-cm leaf tips harvested on 20 September 1973 gave 6, 11, 15 and 57 ppm fluoride for the charcoal, dust, unfiltered and outside plot respectively.

To examine the effect of a short episode of relatively serious pollution, a plot of barley at the milky grain stage was fumigated with sulphur dioxide for five days at levels similar to those in a previous period of severe air pollution (i.e. August 1968). The mean daily sulphur dioxide levels for the five-day period were 83 μg m^{-3} for the unfumigated plot and 451 μg m^{-3} for the fumigated plot. Immediately following fumigation, there was a 30% increase in the sulphur content of the green parts of the fumigated barley. Short-term measurements of the rate of photosynthesis, made during the last day of fumigation, showed an inhibition of 25%. One week after fumigation ear weights were 24% lower, but the final yield showed a smaller difference, with a decrease of 17% in both ear and grain weights at harvest. (Hoskin and Dawson)

Sugar beet

Water relationships. Sugar beets often wilt in the field during periods of low rainfall and high evapotranspiration. Little is known of the effect of this on the photosynthetic activity of the leaves or on metabolism and growth of the plant. The flow of water through a plant is related to the difference in water potential at the root surface and in the leaf, and the conductance of the connecting pathway. In a steady state this flow must equal the evaporation from the leaves which is a function of the water vapour deficit of the air. The relative importance of air moisture in determining the growth of sugar beet was examined by keeping pot-grown plants for two weeks in two controlled-environment rooms, one maintained at a constant water vapour deficit of 8·5 mbar during the day and 6·6 mbar at night and the other at 2·5 mbar during the day and 1·9 mbar at night. Other conditions in the two rooms were similar (day temperature 15°C; night temperature 11°C; total visible radiation 550 J cm^{-2} during a 16-h photoperiod). To examine the effect of soil moisture half the pots in each room were kept at, or below, a water content of 13% (equivalent to a soil water potential of −2·5 bar or less) and the rest at pot capacity (25% soil water content, −0·2 bar) by twice-daily weighing and watering. The treatments were applied to young plants early in June and to mature plants toward the end of August.

There were no interactions between the effects of air moisture and soil moisture on the growth of the plants so the effects of each factor are considered separately. When put into growth rooms the young plants weighed 10 g dry and had 13 leaves with an area of 10 dm^2. After two weeks the average dry weight of the plants grown in the humid air was 8% more than those grown in dry air (48 g cf. 44 g) and although the plants bore more leaves (19 cf. 17) their leaf areas were almost similar (20 dm^2). Dry weights of plants grown in wet soil were 15% heavier than those of plants grown in dry soil (50 g cf. 43 g) and although the plants bore the same number of leaves (18) these were larger and the leaf area of the plant greater (22 dm^2 cf. 18 dm^2). The greater size of the young plants grown in humid air and wet soil was only partly accounted for by the changes in leaf area, because net assimilation rates were also increased, from 126 to 133 g m^{-2} wk^{-1} by increasing the humidity, and from 124 to 135 g m^{-2} wk^{-1} by increasing the moisture content of the soil.

When the mature plants were treated in August, they weighed 190 g dry, with 127 g root, and had 40 living leaves with an area of 52 dm^2. Compared with plants grown in dry air, plants grown for two weeks in the more humid conditions had greater dry weights

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(312 cf. 290 g) and larger storage roots (210 cf. 190 g). Plants grown in wetter soil also had greater dry weights (316 cf. 287 g) and larger roots (208 cf. 193 g) than plants grown in the drier soil. Humid air increased the number of living leaves (45 cf. 39) and produced a greater leaf area (52 cf. 46 dm^2) than dry air but increasing the soil moisture did not affect the number of leaves (42), only their area (46 cf. 41 dm^2). Most of the changes in leaf area and number of the mature plants were brought about by changes in the rates of senescence of the older, larger, leaves. The change in leaf area accounts for only part of the difference in growth; humid air also increased the net assimilation rate from 105 to 115 g m^{-2} wk^{-1} and wetter soil increased it from 106 to 113 g m^{-2} wk^{-1}.

Young and mature plants transpired at similar rates in comparable conditions; in humid air they transpired 12 g water dm^{-2} of leaf surface d^{-1} compared with 17 g dm^{-2} d^{-1} in dry air; in wet soil they transpired 16 g of water dm^{-2} d^{-1} compared with 14 g water dm^{-2} d^{-1} when soil water was restricted.

Leaf water potentials (ψ_1) were little affected by varying the water vapour deficit of the air; the potentials averaged −12 and −13 bar for plants in humid and dry air respectively. ψ_1 of leaves of both young and mature plants averaged −11 bar when grown in wet soil. Restricting the soil water decreased ψ_1 to −13·5 bar in young plants and to −16 bar in mature plants. Although increasing the atmospheric humidity did not affect ψ_1 it did increase the stomatal conductance of the leaves, from 0·18 to 0·37 cm s^{-1} in young plants and from 0·30 to 0·42 cm s^{-1} in mature plants. Increasing the soil water content also increased the stomatal conductance of the leaves from 0·19 to 0·36 cm s^{-1} in young plants and from 0·28 to 0·45 cm s^{-1} in mature plants, but in this case, solely because it increased ψ_1. There were effects on the photosynthetic fixation of $^{14}CO_2$ by the leaves correlated with these differences in stomatal resistance.

Mature leaves wilt more readily than young leaves. It has been suggested that young leaves, when water-stressed, close their stomata and consequently conserve their water more than mature leaves but our data showed, to the contrary, that the stomata of young leaves were less responsive to changes in leaf water potential than mature leaves. A mature leaf wilted when ψ_1 approached −15 bar and closed its stomata whereas the stomata of a young leaf on the same plant at the same ψ_1 remained open, allowing more rapid photosynthesis and transpiration. The reasons for the difference in behaviour of young and old leaves are being investigated.

To investigate a wider range of soil water potentials young and mature plants were allowed to dry out the soil. A comparison was made of the effects of plant age and air humidity on the inter-relations between leaf and soil water potentials, on stomatal behaviour, and on the flux of water through the plant and changes in plant resistance. In wet soil ψ_1 in both young and old plants was similar (−5 to −8 bar) and decreased to the same extent as the soil dried to −4 bar, whether they were in humid or in dry air. The calculated stomatal conductance of the plant canopy was greater in young than in mature plants and much greater in humid air than in dry air. These effects of humidity on canopy conductance were much greater at high than at low ψ_1. Canopy conductances were hardly affected as ψ_1 changed between −5 to −13 bar but decreased markedly at potentials below this. There was evidence that the total resistance to the movement of water from the soil to the leaf increased as the soil water potential decreased and as the plant aged. (Milford and Lawlor)

The effects of short-term water stress on the $^{14}CO_2$ fixation and metabolism of sugar beet was investigated in other experiments using plants grown in solution culture.

Plants were grown in a controlled environment to the 12th leaf stage (total fresh weight 65 g) in nutrient solution and the leaf water potential (ψ_1) changed by addition of polyethylene glycol solutions of −0·4 (control), −3·0 and −8·0 bar osmotic potential. Previous work (*Rothamsted Report for 1972*, Part 1, 109) showed considerable growth

https://doi.org/10.23637/ERADOC-1-130

inhibition at these osmotic potentials. $^{14}CO_2$ was fed, for 15 seconds, to mature leaves, six and 24 hours after application of the osmotic solutions. Leaf samples were frozen in liquid nitrogen, extracted in ethanol and the products separated by chromatography. ψ_1 was measured by a pressure bomb and stomatal resistance by gas flow porometry.

As ψ_1 decreased stomatal conductance decreased from about 0·15 cm s^{-1} for the control plants ($\psi_1 = -9{\cdot}4$ bar) to 0·01 cm s^{-1} in stressed plants ($\psi_1 = -18$ bar) but these values showed large variations. Photosynthesis decreased with stress from about 12 mg dm^{-2} h^{-1} maximum in the control plants to about 2 mg dm^{-2} h^{-1} at $\psi_1 = -18$ bar.

Phosphoglyceric acid contained, after 15 seconds, 44 and 36% of the radioactivity (in the control plants) at $\psi_1 = -9{\cdot}4$ bar and $\psi_1 = -18$ bar respectively; sucrose 3·6 and 2·5%, free hexoses 3·5 and 1·5%, aspartate 2·5 and 0·0%, and malate 5·0 and 1·3%. About 16% of the label in control plants was in a compound tentatively identified as UDPglucose but only 8% at −18 bar. Radioactivity in an unidentified group of compounds, possibly also sugar nucleotides, remained unchanged at 7% by the stress treatment.

In contrast to the decreased proportion of label in sugars, etc., there was an increase in the intermediates of the glycollate pathway. Glycine increased from 4·5 to 12·5%, serine from 3·2 to 6·5% and glycerate from 4·8 to 13·8% at $\psi_1 = -9{\cdot}4$ and $\psi_1 = -18$ bar respectively. The small amount of ^{14}C in glycollate (less than 1%) was not appreciably affected; alanine increased with stress from 2·5 to 4·2% of the total label.

Comparison of sugar beet with wheat (*Rothamsted Report for 1972*, Part 1, 100) showed that a much smaller proportion of ^{14}C in sugar beet accumulated in sucrose, and the effects of water stress were less. However, sugar beet, like wheat, contained more radioactivity in serine and glycine as a result of stress. This would be consistent with the synthesis of sucrose at least in part from intermediates of the glycollate pathway (as discussed in a previous section). (Lawlor and Lenton)

Growth regulators. Tests to evaluate the activity of new plant growth regulating chemicals which may increase sugar yield in beet were made with rooted sugar-beet leaves or on young whole plants grown in glasshouses and controlled environment rooms. One of the substances tested, PRB-8 (α-chloro-β-(3-chloro-*o*-tolyl)proprionitrile) increased root size of whole plants by 25% within five weeks of application. As a result a field trial was set up in collaboration with the British Sugar Corporation in Norfolk. At the beginning of June when plants had an average of ten leaves, PRB-8 at rates of 350, 700 and 2800 g a.i. ha^{-1} was sprayed on to the leaves. After two weeks all rates of application had reduced growth but subsequent harvests suggested that application at 700 g ha^{-1} promoted shoot growth and to a lesser extent root growth. The concentration of sugar in the root was also slightly increased in earlier harvests so that total sugar per root was higher than in control plants but the difference had disappeared by final harvest.

Further experiments on the effects of externally applied growth substances have been made with young seedlings. The effect of benzyladenine(BA), gibberellic acid(GA_3), indol-3-ylacetic acid(IAA) and abscisic acid(ABA) on lateral shoot development of young plants was examined in two experiments. Axillary buds were treated by application of the growth substances in 70% alcohol to the base of petioles of fully expanded leaves. BA released buds from apical dominance and after 3–4 weeks considerable growth of the lateral buds had taken place. This effect was promoted by the addition of GA_3 but reduced by IAA. ABA also promoted lateral shoot development once growth was initiated by BA. GA_3, IAA and ABA had no effect when applied alone or in combination. BA treatment decreased root dry weight compared with untreated plants, presumably because the developing lateral shoots were competing with the root for the supply of photosynthetic assimilates. GA_3 alone promoted root growth confirming previous

https://doi.org/10.23637/ERADOC-1-130

results (*Rothamsted Report for 1972*, Part 1, 99). The combined treatment of BA and GA_3 also decreased the concentration of sugar in the storage root although separately they had no effect.

In an experiment where GA_3 was applied in aqueous solution to the root and shoot of young plants the rate of leaf production was reduced without affecting the total leaf area per plant. A further experiment was designed to examine the interaction of GA_3 with BA and ABA on leaf expansion. All factorial combinations of the three growth substances were applied in 70% alcohol to the midrib of young unexpanded leaves. Area and dry weight of the treated leaves, measured after three weeks, was increased by GA_3 and BA but not by ABA, which completely suppressed the stimulatory effect of GA_3 and partly that of BA. An additional and somewhat unexpected effect of ABA was an elongation of petioles of the treated leaves and of the immediately older and younger leaves. GA_3 promoted petiole growth only in the treated leaf, and to a lesser extent than ABA. Thus ABA acted in a similar manner to GA_3 in promoting petiole growth, while acting in opposition to GA_3 in its effect on lamina expansion. (Garrod)

Endogeneous growth substances. The naturally occurring growth substances present in beet were also analysed and their variation during plant development studied.

The ethyl acetate soluble gibberellin-like substances obtained from methanol extracts of leaves and roots of young plants were partially purified as their sodium salts on columns of Polyclar AT, and were then chromatographed on silica gel C using ethyl acetate : chloroform : acetic acid (15 : 5 : 1, by vol.) as developing solvent, and on Kieselguhr G using benzene : acetic acid : water (8 : 3 : 5, by vol.). Bioassay with the barley endosperm test suggests that there is a substance present in leaves with an R_F similar to GA_1 (17·4 pg GA_3 equiv g^{-1} fr. wt.), whereas roots contain a compound having an R_F similar to GA_3 (87·1 pg GA_3 equiv g^{-1} fr. wt.).

Two GA-like substances have been detected in root xylem exudate and agar diffusates of shoot apices, which were examined as possible sites of GA synthesis in sugar beet.

Young sugar-beet seed clusters harvested two weeks after anthesis were found to contain a GA_1/GA_3-like compound (31·6 pg GA_3 equiv g^{-1} fr. wt.) together with smaller quantities of a compound more polar than GA_8.

The effect of temperature on GA concentration was studied by transferring two weeks prior to harvest two clonal lines of Sharpe's Klein Monobeet to growth rooms giving 12-hour days at four temperature regimes combining 18·5° or 12·5°C days with nights at 14·0° or 8·0°C. There were marked differences between the GA concentrations of the two clones in their response to the four temperature regimes. In clone 1 the concentration of the more polar GA in leaves was increased by warm days or nights compared with cooler conditions. The concentration in the leaves of clone 2 and the roots of both clones varied little with temperature. The less polar GA was present in very low concentration in clone 1, but clone 2 (both tops and roots) contained much more when grown in cool nights, regardless of day temperature. (Garrod, Lenton and Radley)

The suitability of Polyclar AT and Sephadex LH20 column chromatography for purification of sugar beet cytokinins is being investigated. A basic and neutral ethyl acetate fraction of young sugar-beet plants was purified on columns of Polyclar AT and eluted with 0·1*M* phosphate buffer pH 8. Aliquots from these fractions were further purified on silica gel thin layer plates using butan-1-ol : H_2O : 20*N* NH_4OH (86 : 14 : 5, by vol.) or water-saturated $CHCl_3$: MeOH (5 : 1, v/v) as developing solvents. Soybean callus assay has revealed the presence of several biologically active compounds in both roots and leaves.

Several cytokinin-like substances were found in bleeding sap from root stumps of sugar beet and in centrifuged extracts of petioles, using the *Amaranthus* and radish bio-

https://doi.org/10.23637/ERADOC-1-130

assays. Extracts of leaves, purified on Amberlite IR-120(H) ion-exchange column, were assayed by the oat leaf senescence test and by a similar assay using sugar-beet leaf discs. Both assays indicated more biologically active material in extracts of mature sugar-beet plants than in extracts of seedlings. (Garrod, Lenton and Wheeler)

Glycine betaine is a well established constituent of sugar beet; leaves contain 2–6 g kg^{-1} fresh weight. Glycine betaine (5 g $litre^{-1}$) caused chlorophyll retention in the oat leaf senescence assay. Therefore, some of the cytokinin-like activity found in sugar-beet extracts may be attributed to glycine betaine. Glycine betaine was active in several other growth tests. At 1 g $litre^{-1}$ it diminished adventitious root formation on petioles of detached leaves and hypocotyls of detached shoots of dwarf French bean. These leaves and shoots survived several weeks whereas, without root formation, they died: this is again reminiscent of cytokinin activity. Unlike cytokinins, glycine betaine failed to promote the elongation of coleoptiles excised from wheat seeds 24–30 hours after germination.

Auxins, present in the acidic and non-acidic ethyl acetate-soluble fractions of plant extracts, were separated on paper chromatograms developed with propan-2-ol : ION ammonia (4 : 1, v/v) and assayed using the wheat coleoptile extension test. Two active substances were found. One in the acidic fraction had an R_F similar to IAA. The other substance was found in both acidic and non-acidic fractions and had an R_F similar to indole-3-ylacetonitrile (IAN); it was also found in root stump exudates and excised petiole exudates. A sample was hydrolysed with 1*N* NaOH at 100°C for 1 hour to yield a compound with chromatographic properties and biological activity similar to IAA; it was inactive in the pea epicotyl section test, consistent with its identity as IAN. (Wheeler)

Potatoes

Physiology of the potato in relation to tuber yield. The introduction of a night break was shown to regulate tuber initiation in King Edward potatoes grown in constant environments (*Rothamsted Report for 1972*, Part 1, 94). Thus plants of comparable total leaf area could be produced which differed only in the presence or absence of tubers; even so, no differences in net assimilation rate or photosynthetic activity of the leaves could be detected between such plants. The absence of tubers produced a compensatory increase in stem and root growth but the reduction in stem and root growth after tuber initiation was not great enough to account wholly for tuber growth. By feeding radioactive carbon dioxide to the leaves it has been possible to examine the distribution of carbon compounds within the stem before and after tuber initiation. Whereas after tuber initiation the majority of assimilate is translocated directly to the tubers, prior to tuber formation a greater fraction is metabolised in the stem. Present experiments are investigating the level of various metabolites in the stem, stolons and tubers and the activity of certain hormones which probably influence the activity of relevant enzymes. (Frier and Taylor, C. J.)

Biology of perennial weeds

Agropyron repens and *Agrostis gigantea*

Germination and persistence of seeds in cultivated soil. The field experiment investigating the germination and longevity of seeds of *Agropyron* and *Agrostis* in relation to different cultivation regimes was continued (*Rothamsted Report for 1972*, Part 1, 106–107). Plots were given the same treatment as in 1971–72 except that paraquat was not applied and rotavation replaced chisel-ploughing.

In all treatments, most seeds that germinated did so during the autumn of the first year (1971) (Table 4). In treatment 1 many ungerminated but viable *Agropyron* seeds were

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TABLE 4

Percentage of Agropyron(*AR*) *and* Agrostis(*AG*) *seeds giving emerged seedlings under different cultivation regimes*

Cultivation regime	1971/72	In crop (1972)	Remainder estimated from core samples	1972/73	In crop (1973)
AR1	10·3	0·12	5·2	0·59	0·20
AR1 S	6·5	0·10	16·5	0·55	0·18
AG1	34·8	0·13	18·8	0·19	0·05
AG1 S	27·7	0·16	16·2	0·17	0·07
AR2	34·9	0·26	11·3	0·46	0·35
AR2 S	8·3	0·44	4·3	0·47	0·32
AG2	5·2	0·13	24·2	0·64	0·41
AG2 S	5·9	0·18	25·6	0·49	0·14
AR3	26·8	5·43	2·6	0·23	0·21
AR3 S	11·1	4·64	3·5	0·13	0·12
AG3	3·2	1·08	44·4	1·09	0·88
AG3 S	3·1	1·42	21·5	0·90	0·65

Treatment (1) Ploughed late autumn, then spring-tine cultivation simulating no control preceding a spring cereal; (2) Autumn cultivation followed by spring-tine cultivation simulating some control preceding a spring cereal; (3) Intensive autumn cultivation, simulating control preceding winter wheat.
S = paraquat spray in 1971. 'In crop' indicates the percentage of seeds giving seedlings during the time a cereal crop would be growing.
1% = 30 seedlings m^{-2} for *Agropyron* and 20 seedlings m^{-2} for *Agrostis*.

buried by ploughing and only three seedlings m^{-2} appeared after tine cultivation during the first year. Ploughing during the second autumn brought some of these near the soil surface so that twice as many seedlings would appear in the crop during the second year. In contrast to *Agropyron*, *Agrostis* germinated more readily on the soil surface in the first autumn and fewer seedlings emerged in the second year. In treatment 2, as many seedlings of both species would be expected to appear in the crop in the second as in the first year. In treatment 3, about 150 *Agropyron* seedlings m^{-2} would germinate in the crop in autumn 1971; very few seeds germinated during the second year but most of these did so after the last autumn cultivation. Because even shallow burial enforces dormancy on *Agrostis*, fewer seeds of this species than of *Agropyron* germinated in treatments 2 and 3 during the first year. In treatment 3 the absence of spring cultivations prolonged the enforcement of dormancy so that more *Agrostis* persisted until the second year in this than in the other two treatments.

Germination tests in the glasshouse of soil from cores taken from one block of the experiment showed that 25% of the *Agrostis* but only 7% of the *Agropyron* seed remained viable in the soil after a year. During the second year 3% of the remaining viable *Agrostis* and 6% of the *Agropyron* seeds gave emerged seedlings. A parallel investigation in pans of shallow soil in the glasshouse showed that germination of *Agropyron* was complete within a year with all cultivation regimes, whereas that of *Agrostis* was incomplete after two years.

The results of these experiments suggest that seeds of both species are not innately dormant but that dormancy is more readily enforced on *Agrostis* than on *Agropyron*. They also indicate that as many weed seedlings may appear in a second as in a first crop because dormancy may be enforced as a consequence of the cultivations.

Growth of seedlings in winter wheat. Winter wheat (var. Cappelle) was drilled on 13 October 1972 and, to simulate different times of emergence, seedlings of *Agropyron* and *Agrostis* were transplanted at the one leaf stage about 22 cm apart into the cereal

https://doi.org/10.23637/ERADOC-1-130

rows during early November when the wheat plants also had one leaf or during mid-March when they had four to five leaves and three to four tillers. Half the plots received 63 kg ha^{-1} of nitrogen as 'Nitro-Chalk' on 26 April and the other half none. Crop and weed seedlings were sampled on 26 May, 4 July and 8 August (when the wheat was ripe) and a further sample of weeds from the stubble on 12 September.

About 336 wheat plants established per square metre and the crop grew quickly during a mild winter. Nitrogen slightly but not significantly increased the number and weight of shoots m^{-2} before ear emergence but not afterwards. There was no difference in the growth of the wheat on plots planted early or late with either species. The mean grain yield of the plots was 5·23 t ha^{-1} (41·7 cwt ac^{-1}).

A similar percentage of weed seedlings survived until the end of May in all treatments but during June about half the *Agrostis* seedlings and during July half the *Agropyron* seedlings planted late into plots given nitrogen died; more *Agropyron* seedlings died in this treatment during the next two months so that only about one-quarter ultimately survived. Late planting of the weed seedlings resulted in decreased growth. There was an interaction on the growth of the weed seedlings of the time of their planting out and the nitrogen treatment of the plots. Nitrogen increased the dry weight of seedlings planted early from 0·97 to 1·38 and from 1·62 to 2·30 g m^{-2} in early July and at harvest time respectively and decreased that of the late planted ones from 0·34 to 0·19 and from 0·73 to 0·33 g m^{-2}. The decreases with nitrogen of the late planted seedlings were due both to poorer survival and less growth per survived seedling whereas the increases of the early planted seedlings were due solely to increases in the growth of individual seedlings. *Agrostis* grew more rapidly than *Agropyron* throughout the experiment; by the beginning of August the early sown seedlings of *Agrostis* were 70% heavier than the *Agropyron*. Also, the weight of late planted *Agrostis* seedlings without nitrogen increased in the stubble from 0·86 to 4·83 g compared to an increase from 0·60 to 1·72 g m^{-2} for *Agropyron*. Since many *Agrostis* but few *Agropyron* seedlings died, the differences in growth per survived seedling were even greater.

Very little rhizome was formed by the weed seedlings before crop harvest, *Agropyron* producing only 8 cm weighing 0·013 g and *Agrostis* 33 cm weighing 0·080 g m^{-2}. In the stubble, despite dry weather, growth was rapid and many rhizomes were formed. The influence of planting time persisted (Table 5). (Williams)

TABLE 5

The effect of planting time and nitrogen on the number, length and dry weights of rhizomes m^{-2} of Agropyron *and* Agrostis *seedlings*

	Agropyron				*Agrostis*			
	Early		Late		Early		Late	
	—	N	—	N	—	N	—	N
Number	41	33	26	3	115	90	28	11
Length (cm)	121	131	47	7	383	258	115	32
Dry weight (g)	0·54	0·62	0·16	0·03	1·70	1·07	0·39	0·08

***Equisetum arvense* (Field horsetail).** Work, begun in 1972 (*Rothamsted Report for 1972*, Part 1, 105–106) on the biology and control of *Equisetum* continued in 1973. An experimental area was sown with strips of spring barley, kale, potatoes, or beans with one strip left uncropped to test for residual effects of the herbicides used.

The plot treated with chlorthiamid remained free of *Equisetum* in the following year (1973) but sufficient residues persisted to severely inhibit the establishment of all crops tested. On the aminotriazole- and *N*-phosphonomethylglycine-treated plots crop establishment and growth was unaffected.

https://doi.org/10.23637/ERADOC-1-130

Another trial was started in spring 1973 on a lightly-infested site at Rothamsted. Treatments were 4·6 or 9·2 kg a.i. ha^{-1} of chlorthiamid applied to the soil surface and lightly raked in on 27 March (before the *Equisetum* emerged) *or* 4·0 or 6·0 kg a.i. ha^{-1} of *N*-phosphonomethylglycine as a foliar spray on 3 July *or* rotary cultivation every three or six weeks between mid-June and early September.

Plots treated with 9·2 a.i. kg ha^{-1} of chlorthiamid remained free of *Equisetum* throughout the season but a few shoots appeared during August on plots treated with 4·6 kg a.i. ha^{-1}. Shoot growth of *Equisetum* sprayed with *N*-phosphonomethylglycine was severely checked about three weeks afterwards but not killed. New shoots of *Equisetum* appeared above ground within about three weeks of rotavation. Regeneration was almost entirely from buds on rhizomes beneath the disturbed soil and not from the fragmented rhizomes.

Soil core samples taken at Woburn in March 1973 indicated that living roots and rhizomes did not penetrate deeper than 0·9 m although the distribution of dead roots and rhizomes suggested that it had done so in previous seasons. Tubers also occurred more shallowly in March 1973 than in 1972; in 1972 half of them occurred deeper than 50 cm. This change in the depth distribution of the weed continued in the fallow strip throughout 1973 so that by October 82% of the living rhizome and 90% of the tubers occurred in the uppermost 25 cm. At Rothamsted, after a year's fallow, 82% of the rhizomes occurred in the uppermost 50 cm of soil with very little deeper than 75 cm; tubers occurred mostly in the uppermost 25 cm (Table 6).

TABLE 6

Percentage weight of rhizomes(R) and number of tubers(T) of Equisetum *occurring at different depths*

	Woburn						Rothamsted	
Date:	Jan. 1972		Mar. 1973*		Oct. 1973		Sept. 1973	
Previous crop:	Beans		1-year fallow		2-year fallow		1-year fallow after potatoes	
Depth (cm)	R	T	R	T	R	T	R	T
0–25	50	25	47	42	82	90	59	88
25–50	23	22	22	46	9	6	23	12
50–75	15	39	31	10	6	4	15	0
75–100	12	13	0	2	2	0	2	0

* Depth zones are 0–30, 30–60, 60–90 and 90–120 cm

Studies of the germination and growth of plants from tubers were made in the glasshouse. To study the periodicity of germination tubers collected in November 1972 from Woburn were planted in pots of Woburn soil and were buried either in soil outdoors or in a frost-free or warm glasshouse (15°C). Some of the tubers in the frost-free glasshouse were also treated with gibberellic acid(GA) at 100 ppm or with benzyladenine(BA) at 50 ppm. Germination was quickest in the warm glasshouse and was complete within a month. In the cool glasshouse germination of untreated tubers occurred mostly at the end of January but continued until April; germination of all the GA-treated tubers was delayed until April and that of BA-treated tubers almost completely inhibited. Outdoors, germination started in early June and continued during June and July. Germination tests on tubers from the soil cores taken in 1973 showed that most tubers were viable and that the larger tubers germinated much sooner than the smaller ones.

The growth of *Equisetum* from tubers was investigated in two different soils with different nutrient supply. Plants made more growth in Rothamsted than in Woburn soil,

https://doi.org/10.23637/ERADOC-1-130

regardless of the amount of nitrogen supplied; larger applications of nitrogen (216 kg ha^{-1} N as ammonium nitrate) checked the early growth of the species in both soils. With lower amounts of nitrogen (54 or 135 kg ha^{-1}) the species produced 100% more shoot, 50% more tuber and 27% more rhizome weight in Rothamsted than in Woburn soil. Within each soil type extra nitrogen increased shoot weight but had little effect on rhizome weight. Twice as many tubers were formed in Rothamsted as in Woburn soil (493 compared to 261 per plant) although individual tubers were 21% lighter. Added nitrogen did not affect the number of tubers produced. Plants grown in acid, neutral and basic soil of pH 4·5, 6·6 and 8·2 weighed 39, 61 and 22 g dry respectively. In the neutral soil shoots, rhizomes and tubers contributed equally to the total weight but in the acid and basic soils tubers contributed only 17% of the total weight. Almost as much rhizome was formed in the acid as in the neutral soil, although much lower shoot and tuber weights were recorded. Plants had 155, 385 and 106 tubers in the three soils. It therefore appears that the weed does not prefer acid conditions *per se* but probably benefits from decreased crop competition in such situations. (Williams)

Weeds on Broadbalk. *Polygonum aviculare* was again prevalent in all rotation-sections; *P. convolvulus* was also abundant in the no-K plots 11 and 12, and also on the no-Na plot 14. The most noticeable increase this year was in *Convolvulus arvensis* in all crops, following its vigorous growth in 1972 (*Rothamsted Report for 1972*, Part 1, 102). *Aethusa cynapium* was more abundant than in recent years, despite spraying, and *Equisetum arvense* and *Agrostis gigantea* remained serious problems.

In the first six years of the rotation, *Equisetum* has been most abundant in beans and slightly more so in the second round of the rotation than in the first. Amounts in wheat and potatoes did not increase because hand-pulling in potatoes helped to control it.

Occasional plants of the semi-parasite *Odontites verna* were seen in bean plots, always close to the bean rows. They were vigorous and dark green in late August and their pods had not dehisced, whereas *Odontites* plants in wheat were smaller, with purplish leaves, and their seeds were already shed. (Thurston)

Biology of *Alopecurus myosuroides* (Blackgrass). Two herbicides (terbutryne and chlortoluron) were compared for the control of *Alopecurus* in winter wheat. Terbutryne had given inadequate control on this field in previous years although it had controlled autumn-germinating *Alopecurus* on the adjacent Broadbalk. Chlortoluron can be applied later and is slightly more persistent than terbutryne. To compare the efficiency of the herbicides with the periodicity of germination of *Alopecurus* some plots were hand-weeded on the day of application of chlortoluron only, or fortnightly until the end of December, January, February, March or April, or right through to harvest and compared with plots on which all *Alopecurus* seedlings were allowed to develop in the crop.

Very few *Alopecurus* seeds germinated in spring 1973 on Little Knott I so weeding until the end of March was sufficient to keep the wheat free of them, and even the single hand-weeding on 12 December 1972 when chlortoluron was applied removed most of the seedlings.

Both herbicides effectively controlled *Alopecurus*, and at ear-emergence in early June the wheat was noticeably greener, with wider leaves, and possibly slightly taller, on plots where *Alopecurus* was controlled by herbicides or hand-weeding. Unfortunately these better areas of wheat were the first to lodge and were more flattened by harvest than those where *Alopecurus* formed a dense stand. (Thurston)

Spread of *Avena fatua* and *A. ludoviciana* (Wild oats) in the West Midlands. Wild oat samples from randomly-selected farms with more than 8 ha of cereals in Cheshire,

https://doi.org/10.23637/ERADOC-1-130

Shropshire, Staffordshire, Herefordshire, Worcestershire and Warwickshire were collected by staff of ADAS West Midland Region and sent to Rothamsted together with notes on geographical location, soil type, present and previous cropping of each field. The wild oat species present were identified and their distribution compared with the NAAS/Rothamsted wild oat survey (*Rothamsted Report for 1951*, 69).

As shown by comparing the maps for 1951 and 1972, *A. ludoviciana* is still present in South Worcestershire and South Warwickshire but has not spread. There were three samples in 1972 from around Copston Magna where it was found in 1951, but none from north of Coventry.

In contrast, *A. fatua* had spread to all districts of Cheshire, Staffordshire and Herefordshire (which reported no wild oats in 1951), and it had probably increased in the areas where it was already present. Half of the *A. fatua* found in areas previously free of it was in spring-sown cereals. Only two of these fields had been in cereals for less than half of the years for which records were given (mostly five-year periods). The frequency of barley in the preceding four years and the fact that half of the newly-infested areas in Staffordshire and all in Cheshire were on light soils, suggests that spring cereals predominated there. This would explain why spring-germinating *A. fatua* had invaded them but winter-germinating *A. ludoviciana* had not. Even in Herefordshire where soils were heavier, nearly half of the crops mentioned were barley, so there too spring sowing may have kept out *A. ludoviciana*. (Thurston)

Staff and visiting workers

Susan M. Thomas joined the Department to study carbohydrate metabolism in cereals. U. P. de S. Waidyanatha was awarded the Ph.D. degree of London University and has now returned to the Rubber Research Institute in Sri Lanka. D. C. McIlroy has commenced studies as a student of the Potato Marketing Board. Short-term visitors to the Department included Mrs. Ceu Matos of the Centro de Estudos e Formentao do Fruticultura, Alcobaca, Portugal, who spent three months studying the photosynthesis of crop plants.

C. P. Whittingham attended the 36th Winter Congress of the Institut International de Recherches Betteravieres in Brussels and took the chair at a physiology symposium held in connection with the Congress.

Sandwich students who worked in the Department were Elizabeth Brinsden, Susan Thomas and Colin Hill.

https://doi.org/10.23637/ERADOC-1-130