ROTHAMSTED RESEARCH

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Introduction

The work in the Botany Department continues to concentrate on studies of the physiology of the cereal crop with particular reference to wheat. These studies extend from observations of crop development in the field to measurements of activity of isolated enzymes in the laboratory. The experimental work in the field constitutes the contribution of the Botany Department to the work of the Multidisciplinary Group studying the factors which control yield in wheat and is reported by that Group on p. 19. Most of the work concerned with the study of enzymes relates to those enzymes specifically concerned with photosynthesis and especially to ribulose bisphosphate (RuBP) carboxylase/oxygenase. This is part of the contribution by Rothamsted to the nationally agreed priority programme of research on photosynthesis in relation to agricultural production. Other studies in the laboratory are concerned with the development of the wheat plant and of the role of growth hormones in the formation and development of the wheat grain.

Studies continue on the physiology of the sugar-beet crop and here special emphasis has been given to the effect on temperature of leaf expansion and canopy development. This work continues to be undertaken in association with the Crop Productivity Group at Broom's Barn.

Photosynthesis

Work on photosynthesis is concentrated on two main topics, firstly the kinetic properties, structure and function of RuBP carboxylase/oxygenase and secondly, the photosynthetic

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characteristics of wheat plants grown under controlled conditions and of protoplasts and chloroplasts isolated from them.

RuBP carboxylase/oxygenase

Purification and activities. A purification procedure has been developed that consistently provides RuBP carboxylase from wheat with specific activities near 1·2 μmol CO_2 min^{-1} mg^{-1} protein at 25°C. The enzyme obtained from spinach by the same purification procedure, had activities of at least 1·8 μmol min^{-1} mg^{-1}; a value as high as any recorded in the literature. Present efforts are directed towards designing a more rapid procedure. Gel electrophoresis of the purified wheat enzyme after denaturation indicated a protein typical of higher plant carboxylases, with a large subunit $M_r = 56\,000$ and a small subunit $M_r = 14\,500$ in a 1:1 ratio. Electrophoresis on gradient polyacrylamide gels suggests that the intact enzyme is composed of eight large and eight small subunits with $M_r = 560\,000$.

Studies have been made of the activation of the isolated enzyme by CO_2 and Mg^{2+} ions. Progressive exposure to low temperature during purification deactivates the enzyme and the presence of CO_2 and Mg^{2+} at elevated temperatures is necessary to restore the enzyme to full activity. At 40°C a 30 min incubation period is required and at 30°C, 160 min. It is clear that for wheat carboxylase the catalytically active state of the protein is an enzyme/CO_2/Mg^{2+} ternary complex for both carboxylase and oxygenase activities. The enzyme ternary complex is stabilised in the presence of tight binding effector molecules, e.g. fructose-1,6-bisphosphate (FBP). Such effectors decrease the optimal concentrations of CO_2 and Mg^{2+} needed for activation. Activation in the presence of Mg^{2+} and of FBP with $^{14}CO_2$ showed that appreciable amounts of CO_2 were trapped, suggesting a quarternary complex involving enzyme/CO_2/Mg^{2+}/FBP. FBP is a potent competitive inhibitor of RuBP binding and we suggest that activating CO_2 and Mg^{2+} are bound within the RuBP-binding site on the enzyme. (Gutteridge, Cornelius, Parry and Schmidt)

Affinity for carbon dioxide. Measurements made of the relative affinity for CO_2 of ribulose bisphosphate (RuBP) carboxylase/oxygenase from a number of agricultural crop species gave K_m (CO_2) values from 10·5 to 14·4 μM (*Rothamsted Report for 1980*, Part 1, 50) but variations in the measurements on individual enzymes made it difficult to judge the validity of small differences within this range. Further measurements have now been made on RuBP carboxylase/oxygenase isolated from leaves of a wider range of species with the object of detecting enzymes with larger differences in K_m (CO_2). The species examined were Common Horsetail (*Equisetum arvense*), *Ginkgo biloba*, bracken (*Pteris aquilina*), willow (*Salix babylonica*), Lawson's cypress (*Chamaecyparis lawsoniana*), moss (mainly *Atrichum undulatum*) and maize (*Zea mays*). In each instance the RuBP carboxylase/oxygenase was purified and isolated as a freeze-dried powder using the improved isolation procedure referred to in *Rothamsted Report for 1980*, Part 1, 50 (Machler, Keys & Cornelius, *Journal of Experimental Botany* (1980) **31**, 7–14; Bird, Cornelius & Keys, *Journal of Experimental Botany* (1980) **31**, 365–369). Some modifications for particular species especially the addition of 'Tween 80' and insoluble polyvinylpyrrolidone (PVP) to the extraction buffer for recovery of enzyme from willow, bracken and *Gingko* were necessary. Three independent measurements were made on the same occasion of the K_m (CO_2) value for each enzyme and independent duplicate measurements were made on each occasion on RuBP carboxylase/oxygenase isolated from barley which was used as a control. Measurements were made in atmospheres of either N_2 or 21 % O_2. In the absence of O_2 the mean values for K_m (CO_2) ranged from 12·4 μM for the enzyme from willow to 26·7 μM for the maize enzyme with the remainder in the range 15·4–17·9 μM. The barley enzyme gave an overall mean value of 11·4 μM. In the presence of

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21% O_2 the mean K_m (CO_2) values ranged from 18·6 μM for the willow enzyme to 39·9 μM for the maize enzyme with the remainder in the range 20·4–25·5 μM. The barley enzyme gave an overall mean K_m (CO_2) of 18·5 μM. Some variations existed between replicate measurements on the same enzyme, the cause of which is not known, but the magnitude of these is insufficient to account for the range of K_m (CO_2) values found. It appears that K_m (CO_2) values can differ considerably between RuBP carboxylase/oxygenase isolated from different species. Further data are required before it is possible to say with certainty that the enzymes differ in their sensitivity to O_2.

Yeoh, Badger and Watson have shown differences in the K_m (CO_2) for RuBP carboxylases in various grasses from 13 μM in *Secale cereale* to over 60 in some C_4 species (*Plant Physiology* (1980) **66**, 1110–1112) and a similar range in C_3 and CAM plants with the upper limit in certain aquatic species (*Plant Physiology* (1981) **67**, 1151–1155). Even larger differences were reported by Jordan and Ogren (*Nature, London* (1981), **291**, 513–515) because this survey included some photosynthetic bacteria. Jordan and Ogren concluded from their study that the balance between the carboxylase and oxygenase activities of the enzyme does vary and this is often due to differences in K_m (CO_2). We intend to confirm the differences in kinetic parameters found in different species and to investigate the consequences of these for photosynthesis. (Bird, Cornelius and Keys)

Affinity for oxygen. A method has been developed to measure the K_m for oxygen of the oxygenase activity of RuBP carboxylase/oxygenase. A five- to ten-fold expansion of the scale of oxygen uptake has been used to increase the accuracy of the determinations; the electrode is calibrated with nitrogen, air and water saturated with oxygen. This enables a more precise assessment of the oxygenase activity of the enzyme at low substrate concentrations. The data were analysed by the statistical method of Wilkinson (*Biochemical Journal* (1961), **80**, 324–332). The mean K_m (O_2) value for wheat carboxylase/oxygenase was 0·36 mM which is in close agreement with the K_i (O_2) value for the carboxylase of 0·41 mM. This corresponds to an atmospheric concentration of 30% oxygen. Assays at higher concentrations (above 1 mM) always gave higher rates than anticipated from Michaelis–Menten kinetics and double reciprocal plots showed a downward trend for higher concentrations. When these assays at higher O_2 concentrations were included in the weighted K_m a mean value of 0·496 mM was obtained. The reason for the increased rate at high O_2 concentrations has not been resolved, but addition of superoxide dismutase and catalase, singly or in combination, did not influence the rate observed. The increased rate is dependent upon both RuBP and enzyme being present, but could involve some other reaction, which takes up oxygen, which may be non-specific. The method described above for wheat has been used to determine the K_m (O_2) for a bracken RuBP carboxylase preparation. The mean K_m (O_2) of 0·326 mM (from five experiments) for this enzyme does not agree with the K_i (O_2) value obtained from a set of carboxylase assays in air and nitrogen which gave a value of 0·877 mM O_2. Further experiments on bracken enzyme are continuing. (Hall, Keys and Bird)

Effects of sulphur dioxide on the activities of RuBP carboxylase. Field experiments conducted over the last 8 years in the vicinity of a brickworks in the Marston Vale in Bedfordshire have indicated that aerial pollutants at these sites may reduce crop yields (*Rothamsted Report for 1980*, Part 1, 56). These results and those from other groups have stimulated studies to determine the biochemical mechanisms of plants which are affected by aerial pollutants. Research has been initiated to determine the effect of SO_2 on RuBP carboxylase/oxygenase. Exposure to SO_2 leads to the formation of SO_3^{2-} which is then oxidised to SO_4^{2-}. Both SO_3^{2-} and SO_4^{2-} inhibit RuBP oxygenase catalysis by similar amounts. Preliminary studies suggest that sulphite also accelerates the activa-

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tion of the oxygenase. Future studies will investigate further the general pattern of inhibition by these and other ions and will include comparisons of enzymes extracted from pollutant-resistant and susceptible cultivars of ryegrass and cereals. (Parry and Gutteridge)

Photosynthetic characteristics of wheat. An attempt was made to produce wheat leaf material of contrasting properties to investigate factors limiting photosynthetic rate per unit leaf area. Growth temperature was varied since earlier studies (*Rothamsted Report for 1975*, Part 1, 32) showed an effect on photosynthetic capacity. More recently (*Rothamsted Reports for 1978*, Part 1, 39–40, and *for 1979*, Part 1, 51) it was shown that at lower temperatures, capacity for photosynthesis might be limited by metabolism of hexose phosphates to sucrose. This may account for failure to observe stimulation of photosynthesis at lower temperatures when photorespiration is decreased by lowered oxygen in the atmosphere (e.g. Jolliffe & Tregunna, *Canadian Journal of Botany* (1973), **51**, 841–853). However, it seemed important to find out whether adaptive changes caused by conditions during growth altered the response. Thomas (*Rothamsted Reports for 1975*, Part 1, 33, and *for 1976*, Part 1, 37) showed that in field conditions the relative rate of photorespiration to gross photosynthesis by flag leaves was increased by increased nitrogenous fertiliser applied to the crop. For this reason two levels of N were used.

Wheat (*Triticum aestivum*, cv. Kolibri) was grown in constant environment rooms illuminated with a mixture of fluorescent tubes (total 15 kW) and incandescent lamps (total 2·4 kW) giving 600 μE m^{-2} s^{-1} at plant level for 16 h photoperiods. Two temperature regimes (day/night) were used, 23°/18° and 13°/10°, with corresponding relative humidities of 50%/80% and 74%/88%. Plants were grown in a mixture of sand and peat and fed with Hoagland solution or with Hoagland solution supplemented with sodium nitrate to increase the nitrate content by a factor of four. Subsequently, pots were given 50 ml of the same solutions twice a week for 4 weeks. The unsupplemented level of N supplied was inadequate to keep the plants fully green in the warm room. Thus plants were grown under four different conditions, warm or cool with high or low N fertiliser.

The length and breadth of the third leaf was studied to determine equivalent stages of development for plants grown in each condition. Full expansion occurred after 17 days in the warm room and 29 days in the cool room. The final length of the third leaves in the cool room was only 73% of that in the warm room. There was little effect of N status on leaf size at either temperature. Dry weight per unit leaf area increased with leaf age especially in leaves from the cool room from plants of the lower N status. Other measurements were made in the middle of a photoperiod at each of five stages in the development of the third leaf, two before, one at full leaf expansion and two after. This ensured that effects of leaf age were included in the study. Chlorophyll and soluble protein per unit leaf area were increased by the cool condition and by increased N status.

Growth and chemical composition. Growth of the whole plant under the experimental conditions was studied by sampling 20 plants per treatment weekly for 8 weeks (warm room) and 10 weeks (cold room). Little effect of N on total dry weights was observed, lower N always yielding a little less than the higher N, except in the later harvests in the cool room. Here lower N yielded much lower dry weights. More tillering occurred at high than low N and more in the cooler conditions. Root/shoot ratios were higher in the cool treatment, especially with lower N.

Measurements of chemical composition of the third leaf were made; for each treatment samples of 0·5–1·5 g fresh weight were extracted and separated by Sephadex ion-exchange chromatography into fractions containing sugars, amino acids, organic acids and

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phosphate esters (Redgewell, *Analytical Biochemistry* (1980), **107**, 44–50). Other samples were extracted so that polysaccharides could be measured.

Sugars were estimated by gas chromatography (GC), by enzymic methods and by high pressure liquid chromatography (HPLC). Highest sucrose levels were found in the cooler, lower N treatment before maximum leaf expansion, then fell off rapidly. Little difference was observed in the other treatments. Levels of fructose and glucose relative to sucrose were low, but older leaves showed significant amounts of another sugar, possibly raffinose, when analysed by GC and HPLC.

Amino acids were estimated by GC. Increased N status and cooler conditions increased amounts of amino acids, especially glutamate and serine.

Levels of RuBP in the phosphate ester fraction were measured by a specific enzyme method. Amounts were highest in the cool lower N treatment but decreased rapidly after maximum leaf expansion. We are attempting to develop a rapid method for phosphate ester analysis by HPLC.

Starch levels between 6 and 13% dry matter (DM) were found in the samples with highest levels in the warmer lower N treatment and in the older leaves. (Kendall)

Photosynthesis and photorespiration. Detailed gas exchange measurements were made to determine rates of photosynthesis and photorespiration in relation to other measurements including stomatal resistance.

A leaf chamber was used in which the atmosphere was stirred and the temperature controlled. Leaf temperature, the temperature of the gas mixture in the chamber, and the CO_2 content and relative humidity of gas leaving the chamber, were measured and recorded on punch tape. An ionisation chamber was used to measure $^{14}CO_2$ in gas mixtures. Illumination of the leaves, by high-pressure mercury vapour fluorescent lamps, was varied by altering the distance from the lamp and by inserting screens between the lamps and the leaf chamber.

Recorded information was processed by a small computer to give stomatal resistances, CO_2 concentrations within leaves, photosynthetic rates and respiratory rates. Photosynthesis was most rapid at, or near, full leaf expansion. At 20°C the leaves grown in the warmer conditions and given the higher level of N showed the fastest rates. Leaves from plants grown in the cooler conditions, irrespective of N levels, tended to show decreased rates of photosynthesis as the light was increased to more than 1600 $\mu E\ m^{-2}\ s^{-1}$. This was partly explained by increased stomatal resistances but might be partly the result of photoinhibition.

As part of a more general study to obtain CO_2 response curves, the release of CO_2 into CO_2-free air was measured and used to calculate photorespiration. An alternative method involving measurement of gross photosynthesis using $^{14}CO_2$ (Ludwig & Canvin, *Plant Physiology* (1971), **48**, 712–719) gave similar results. Photorespiration ranged from 10 to 17% of net photosynthesis but no evidence was obtained of either an effect of N status or of growth temperature.

When measured at 20°C, net photosynthesis was increased when the O_2 concentration in the atmosphere was decreased but at 13–15°C the effects were small. Quantum efficiency at low light intensities, calculated from incident light, was increased at 20°C by decreasing the O_2 from 21 to 2%. At 13–15°C the value in both 2 and 21% O_2 was similar to the value in 21% O_2 at 20°C. For the C_3 species *Encelia californica*, Ehleringer and Björkman (*Plant Physiology* (1977), **59**, 86–90) found that quantum yield in terms of absorbed quanta increased in 21% O_2 as the temperature was decreased.

Data are being collected concerning the responses of photosynthesis and respiration to CO_2 and light for leaves from the various treatments. Effects of treatment will be

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deduced from effects on the various parameters required to fit the data to current models of leaf photosynthesis. (Lawlor and Young)

Enzyme activities and photoinhibition. Activities per unit area of the third leaf of RuBP carboxylase, serine-glyoxylate aminotransferase, glutamate-glyoxylate aminotransferase, glycollate oxidase and glutamine synthetase were increased by increased N status but little affected by temperature. Maximum activities were reached just before or at full leaf expansion followed by decreases that were more rapid in leaves from plants given less N. The activities of fructose bisphosphatase, nitrate reductase and carbonic anhydrase were highest in leaves grown in the cooler temperature at both N levels but increased N status also increased the activities of these enzymes. Thus, the latter three enzymes responded to the growth conditions in the same way as chlorophyll and soluble protein contents. Even in the cool room in leaves from plants given more nitrate, the activity of nitrate reductase at its maximum was scarcely a fifth of the activity of RuBP carboxylase, fructose bisphosphatase or glutamine synthetase. Also the activity of nitrate reductase declined rapidly with leaf age whilst glutamine synthetase remained high. It seems that nitrate reductase may have a comparatively minor role even in young leaves. Ammonia assimilation, however, remains necessary even in older leaves, because of photorespiration and turnover or degradation of proteins, and there is much glutamine synthetase present.

In spite of the marked differences in appearance and composition produced by the different growth conditions, detached third leaves showed very similar losses of photosynthetic capacity upon high illumination (photoinhibition) (Powles & Osmond, *Australian Journal of Plant Physiology* (1978), **5**, 619–629). These were by 50–70%, whether the photoinhibitory treatment was applied at 23 or 13°C. When the gas mixture was changed back to air the leaves recovered their capacity for photosynthesis to varying degrees. Older leaves from plants grown at the cooler temperature recovered to achieve faster rates of photosynthesis than they exhibited before the treatment causing photoinhibition.

A further investigation was made of the loss of photosynthetic capacity due to photoinhibition. It has been reported that photoinhibition arises from loss of photosystem II activity (Powles, Osmond & Thorne, *Plant Physiology* (1980), **64**, 982–988) but we have found that carboxylase activity in freshly prepared extracts declines and recovers in parallel with capacity for photosynthesis. This is not because of failure to extract the protein following photoinhibition. It is not yet clear whether the decreased activity of RuBP carboxylase is brought about simply by removal of CO_2 as might be expected from the properties of purified RuBP carboxylase.

A preliminary study was made of photosynthetic metabolism at 23 and 13°C using $^{14}CO_2$ during 5 min steady-state photosynthesis. This showed the increased formation of hexose monophosphates at 13°C in leaves grown at 23°/18°, but not in leaves grown at 13°/10°C. There is probably an adaptation involving changes in enzyme activities responsible for metabolism of hexose phosphates. (Boyle and Keys)

Photosynthesis by isolated protoplasts and chloroplasts. The relative concentrations of CO_2 and O_2 affect photosynthesis by protoplasts and chloroplasts in the same way qualitatively as with intact leaves. There are some quantitative differences between effects on chloroplasts and protoplasts both on steady-state photosynthesis and on the lag period (induction) preceding steady-state photosynthesis. With protoplasts, increased CO_2 (HCO'_3) increased the lag period whereas with chloroplasts the lag period was decreased. At high concentrations of CO_2 (10mM-HCO'_3), with both chloroplasts and protoplasts, the lag period was the same in 0% O_2 as in 21% O_2. At lower HCO'_3

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concentrations (< 5 mM), O_2 extended the lag period in chloroplasts but decreased it in protoplasts. Since O_2 stimulates and CO_2 inhibits photorespiration, this process may serve to shorten the lag in protoplasts. Oxygen caused a greater inhibition of steady-state photosynthesis in chloroplasts than in protoplasts. The metabolic pathway responsible for photorespiration is cyclic and involves steps in peroxisomes and mitochondria as well as the chloroplasts. Thus in isolated chloroplasts the rate of photosynthesis may be decreased because glycollate produced by oxygenation of RuBP cannot be metabolised and its accumulation depletes the Calvin cycle of intermediates. In protoplasts, as in intact leaves, 75% of the carbon used to make glycollate is eventually returned to the chloroplast as glycerate and re-enters the Calvin cycle as phosphoglycerate.

To study these effects further, the products of photosynthesis from $^{14}CO_2$ ($H\ ^{14}CO'_3$) were studied. Reactions were conducted in the vessel of an O_2 electrode and small samples of chloroplast or protoplast suspensions were removed at intervals, extracted and fractionated into neutral compounds, organic acids, phosphate esters and amino acids (Redgwell, *Analytical Biochemistry* (1980), **107**, 44–50). Subsequently the radioactivity in each fraction was measured and, after thin-layer chromatography (Arrabaca, *Ph.D. Thesis, University of London* (1981)) the radioactivity in individual compounds was measured. With protoplasts, intermediates of the glycollate pathway were relatively more radioactive when the concentration of $^{14}CO_2$ was low and O_2 was high. In chloroplasts under these conditions, glycollic acid was the main product. Chloroplasts incorporated more radioactivity into sucrose than expected.

The flag leaves of some wheat species, especially the more primitive diploids, have been reported to show higher rates of photosynthesis per unit leaf area than modern varieties of *Triticum aestivum* (Dunstone, Gifford & Evans, *Australian Journal of Plant Physiology* (1973), **26**, 295–307). Photosynthetic metabolism of the flag leaves of some of these species of *Triticum*, *T. dicoccoides*, *T. dicoccum*, *T. monococcum* and *T. urartu* was investigated using the pulse-chase $^{14}CO_2$ method described by Thomas and Long (*Planta* (1978), **142**, 171–174). There was no evidence for operation of the C_4 photosynthetic pathway. Differing extents of labelling of glycollate pathway intermediates were observed and will be investigated further. (Holbrook and Keys)

Growth of winter wheat

Tillering in winter wheat. Ear number in winter wheat can be modified by altering the time and rate of N application although there are unpredictable differences between crops grown in different seasons and sown on different dates. These are due mainly to differences in survival of tillers. In the multidisciplinary experiments (see p. 20) tiller survival ranged from 30 to 51% over three seasons. To study the causes of tiller death in more detail plants were grown in the glasshouse so that plant density, tillering and growth were comparable with the field. In a preliminary experiment in an open-sided glass-roofed cage, plants were grown at a density of 300 m^{-2} either in rows in beds or in three sizes of pots (19 or 13 cm diameter round or 9 cm square). Seed of cv. Hustler was sown on 23 September and 6 November, close to the dates used in the field. All plants were supplied with ample water and nutrients.

The early-sown plants tillered from October to December and the maximum shoot number was maintained until tillers began to die in early March. In contrast, shoot numbers of the later sowing increased from January to late March and then declined rapidly. For both sowings tillering ceased close to the double-ridge stage of apical development and tiller death started when the stems began to elongate. The youngest tillers (secondary tillers and the fourth on the main stem) died first, followed by the third tillers, but together these constituted only a small part of the total DM produced. Some

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of the first and second tillers died before anthesis even though they had formed ears and stems, resulting in a more serious loss of DM and potential grain sites. With the slightly warmer temperatures in the cage than in the field, all developmental stages occurred earlier and more tillers were produced (maximum shoot numbers for early and later sowing: 2000 and 1800 m^{-2} in the cage as compared with 1300 and 1000 m^{-2} in the field), but the general pattern of tiller production and death and the final ear numbers (500–600 m^{-2}) were similar. The tillering pattern, ear numbers and dry weights were similar for the beds and the 19 and 9 cm pots. The small square pots are being used in further experiments in which effects of the environment on tillering pattern and growth are being studied. This system permits standard plant densities to be maintained while allowing plants to be moved for treatment and sampling. (Thorne and Wood)

Grain set and growth in wheat. Two possible causes of limited grain set in wheat are a suboptimal supply of assimilate and correlative inhibition due to hormonal control. Recent results suggest there may be an interaction between assimilate supply and hormone activity.

It was previously reported (*Rothamsted Report for 1979*, Part 1, 45) that abscisic acid (ABA) applied to wheat ears at anthesis prevented grain set in the third and fourth florets of each spikelet, although semi-dwarf cultivars were largely insensitive. It has now been shown that detached wheat ears cultured in nutrient medium (*Rothamsted Report for 1980*, Part 1, 55) respond to ABA when cultured in 1% but not in 4% sucrose.

A range of response to ABA, both on intact plants and in detached ears was found in a comparison of five tall and six semi-dwarf cultivars. Bract size was inversely related to sensitivity to ABA, and the sucrose and the reducing sugar content of the bracts were greater in insensitive cultivars. The ratio of reducing sugar to sucrose was greater in sensitive cultivars. ABA increased this ratio in both types indicating an effect of ABA on sugar metabolism rather than uptake.

Detached ears of the wheat cv. Highbury, cultured in nutrient solution contained grains which became greater in volume than grains on intact plants (*Rothamsted Report for 1980*, Part 1, 55). Removal of the two lowest grains of each spikelet also caused an increase in volume of the third grain compared with complete ears. Increases due to the two treatments were additive. Cell size was measured in the aleurone layer surrounding the endosperm, and total cell number was estimated. The increased area of the aleurone layer caused by detached ear culture was due to increased cell division rather than increased cell size. Partially de-graining ears on intact plants also caused an increase in cell number, but de-graining cultured ears did not give a further increase in cell number, instead there was an increase in cell size. This provides a method of investigating some of the factors limiting grain volume. (Radley)

Auxin in developing wheat grain. Previous bioassay results have shown differences in free and bound auxin of sprouting susceptible and resistant wheat cultivars during grain growth (*Rothamsted Report for 1980*, Part 1, 55). A highly sensitive physical assay for indoleacetic acid (IAA) based on HPLC and natural fluorescence (Crozier *et al.*, *Planta* (1980), **150**, 366–370) was evaluated as a method for measuring IAA in grains of cv. Hobbit (resistant) and cv. Hustler (susceptible) harvested from the field in 1980.

Several different extraction and purification methods were examined to minimise release of IAA from conjugates and to optimise recovery and clean-up prior to analysis by HPLC. Radiolabelled IAA was used as an internal standard to correct for recovery losses. Fractions from HPLC containing IAA were collected, methylated and rechromatographed to constant specific activity.

There was a rapid decline in the free IAA content and a gradual increase in the bound

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IAA content of grain for both cultivars during the later stages of ripening. Simultaneous estimates of the free IAA fraction by bioassay were in broad agreement with the HPLC-fluorimetric method but this was not so with the bound IAA fractions. There was agreement with the samples from cv. Hustler but the apparent decline in the biological activity of bound IAA from cv. Hobbit could be explained by the presence of ultraviolet light absorbing compounds interfering in the bioassay. (Lenton)

Gibberellins in developing wheat grain. The proposed structures of several novel 1β-OH gibberellins (GA) isolated from developing wheat grain (*Rothamsted Report for 1979*, Part 1, 46) have now been confirmed by partial synthesis and have been assigned the following numbers: GA_{54} (1β-OH GA_4), GA_{55} (1β-OH GA_1), GA_{60} (1β-OH GA_{20}), GA_{61} (1β-OH GA_9). In addition, re-examination of the 1978 wheat endosperm sample by gas chromatography–mass spectroscopy (GC–MS) using wall-coated open tubular (WCOT) capillary columns of fused silica confirmed the presence of GA_{62} (1β-OH 2,3 dehydro GA_9), an isomer of GA_7.

Samples of wheat grains, cv. Huntsman, were harvested in 1979 at 1, 3 and 4 weeks post-anthesis and the GAs purified and analysed by GC–MS using standard packed glass and WCOT capillary columns. The major component present at 3 weeks was GA_{54} with much smaller amounts of GA_{39} and GA_{44} and traces of GA_{60} and GA_{61}. There was no evidence of an accumulation of the less polar compounds at the earliest harvest but there was a large increase during grain growth of a polar compound with a mass spectral fragmentation pattern consistent with the structure 2β-OH GA_{54}. Confirmation of the proposed structure by partial synthesis and the necessary feeding experiments may confirm that it is a biologically inactive end-product of GA metabolism in developing wheat grain. Support for this idea comes from the observation that no bound GAs were released from enzymic digests of the acidic butanol fractions.

Further prepurification of plant extracts is required before full advantage can be taken of the high resolving capacity of capillary columns. Recent work has shown that major impurities of developing wheat grains can be resolved from the GA_3/ABA region by HPLC using Spherisorb-50DS columns and an acetic acid–methanol gradient system. The elution patterns of the new GAs on HPLC and their biological activity is currently under investigation. (Lenton, with Professor J. MacMillan, University of Bristol)

'PP333.' A new plant-growth regulator, 'PP333' produced by ICI, was compared with the growth retardant chlormequat chloride on barley, cv. Proctor, and wheat, cv. Maris Dove, grown in pots in a greenhouse. 'PP333', 0·5–4·0 mg a.i. per pot, applied to cereals at growth-stage 13, shortened barley less than wheat, and the effect persisted to shorten all main stem internodes. A larger amount of chlormequat chloride, 16–316 mg per pot, was needed to produce similar shortening. 'PP333' induced more tillers per plant, particularly in barley where 2–4 mg per pot more than doubled their number, but the number of ears per barley plant was affected less, and in wheat not at all. Fresh weight of ripe grain per plant decreased with increasing doses of 'PP333' especially in wheat; the number of grains per plant decreased without much effect on weight of 1000 grains. 'PP333' delayed ripening of ears, and senescence of leaves, particularly of the late forming tillers.

Seed was saved from treated plants to compare their germination and growth with seed from untreated plants. There was no obvious effect on germination or early growth of seed from 'PP333'-treated plants but some evidence that wheat plants were shorter at maturity. (Wheeler, with Lord, CLU)

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Sugar beet

Leaf development. The times of appearance and rates of expansion of individual leaves were measured in eight irrigated crops grown on the crop productivity areas at Broom's Barn between 1978 and 1981 with different sowing dates (24 April, 18 May), N application (0 and 125 kg N ha^{-1}) and spacing (43, 66 and 120 thousand plants ha^{-1}). The aim was to determine the factors governing how fast and large the leaf canopy grew and to analyse those factors regulating leaf development independent of seasonal fluctuations.

The times when these crops reached leaf area indices (L) of 2·5–3·0, sufficient to intercept most of the incident radiation, ranged from late June to mid-August and maximal L ranged from 1·5 to 5·0. These differences in L and the quantities of radiation intercepted by the crop were reflected in different yields of total DM and sugar (*Rothamsted Report for 1978*, Part 1, 64; *Rothamsted Report for 1980*, Part 1, 71). Leaf area index depends on the rates at which leaves appear and expand, the duration of expansion and on how long they are retained by the plant. Controlled-environment experiments have shown that all these aspects of leaf growth vary linearly with temperature over the range experienced by field crops (Milford & Riley, *Annals of Applied Biology* (1980), **94**, 431–443). Hence leaf growth and development was measured against accumulated thermal times (°C days) rather than days from sowing.

Linear regressions on thermal time above 0°C accounted for most of the variation in leaf appearance within a season provided separate regressions were fitted for leaves produced early and late in the season. Each leaf up to leaf 20 required about 30°C d to be accumulated before it unfolded from the apex. This requirement varied between seasons (from 27 to 37°C d) but not with different husbandry treatments. More thermal time was required before each subsequent leaf appeared. The amounts varied with season from 40 to 55°C d and more was required when crops were deprived of N or grown at high density.

Leaves produced first grew successively larger and later ones successively smaller. This sequence was controlled by developmental processes within the plant and influenced by season and agronomy. Leaf 10 was the largest in most crops but the eighth leaf was largest in the low-N crop and the 12th in that grown at low density. Leaves of crops grown in 1980 and 1981 were, on average, 12% larger than in the other 2 years. Early leaves of the late-sown crop were 35% larger and leaves 8–16 in the low-density crops 18% larger than their counterparts in standard crops grown alongside. Leaves from the sixth onwards were 30 and 60% smaller, respectively, in the high-density and low-N crops. These changes in leaf size were generally caused more by changes in the mean thermal rate of expansion (cm^2 °C d^{-1}) than in the duration of leaf expansion. Size was only increased by a longer duration of expansion in the early leaves of the late-sown crop.

Further analysis of the growth curves revealed that, after unfolding, individual leaves initially expanded linearly with accumulated temperature above 3°C at thermal rates that varied systematically with leaf position but were the same irrespective of season, sowing date, spacing or nutrition. The overall mean rates of thermal expansion for the whole of growth were determined by how long leaves were able to continue expanding linearly at rates dictated by their temperature environment. This was because, although the overall thermal duration of expansion from unfolding to maturity was not greatly altered by husbandry, the thermal duration of the linear phase was extended by sowing the crops late, at low density or with more N and shortened by growing them at high density or with less N.

In sugar-beet crops expansion of the first 20 leaves contributes virtually all the leaf surface up to values of L of 3·5–4·0. These leaves appear and initially expand linearly with accumulated temperature; so, therefore, do leaf-area indices up to values of 3·5–4·0. The thermal rate of increase in L thus also provides a basis for analysing canopy growth

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in crops grown on different sites in different seasons. Changes in temperature alter leaf growth without changing the intrinsic thermal rates of the processes involved so leaves merely complete growth sooner without change in size. Therefore, although time to reach full leaf cover changes, the final size of the canopy does not. On the other hand, change in the intrinsic thermal rates of expansion changes leaf size, and changes in maximal L accompany changes in the rate of canopy expansion. There were significant differences in the thermal rates of expansion of L in the crops grown at standard density that we studied. These ranged from ΔL values of 0·36 100°C d^{-1} in the low N crop, 0·52 100°C d^{-1} in the three standard crops grown between 1978 and 1980, 0·67 100°C d^{-1} in the late-sown crop, to 0·79 100°C d^{-1} in the 1981 standard crop. These differences in rate were closely associated with differences in the percentage of N in the lamina DM. (Milford, with Broom's Barn)

Effect of light quality on bolting. Endogenous gibberellins are involved in the vernalisation stimulus (*Rothamsted Report for 1975*, Part 1, 44) and the GA antagonist ancymidol had some effect in delaying the onset of bolting (*Rothamsted Report for 1976*, Part 1, 43). A means of synchronising and modifying the bolting response is required to establish the role of endogenous growth regulators at all stages of apical transition and stem elongation. The phytochrome system is known to be involved in the flowering response and therefore the effect of red and far-red light on bolting was used as a tool for further work on the involvement of growth substances.

Plants of the sugar beet Line G were sown from seed in late October and over-wintered in a glasshouse at a minimum temperature of 5°C. In March, when the plants had produced 12 leaves, temperatures were increased gradually to 15°C and the light treatments applied. Plants were divided into three batches and the control plants given no additional light. The remaining plants were given supplementary light for 16 h each day from either incandescent lamps (at predominantly 730 nm wavelength, far-red), or a red light source (630 nm). Some plants exposed to far-red light were transferred to red light after 2, 3 or 4 weeks and plants under red light were transferred to far-red light at weeks 5, 6 or 7.

Dissection of the shoot apex showed that the first sign of transition from the vegetative to the floral state (the doming of the apex) occurred in late April in the control plants and this indicated the response of Line G to the natural increase in daylength. Tungsten light advanced this process by 3 weeks to the beginning of April (the third week after treatments were applied). Red light delayed the onset of apical transition by one week to the end of April. Subsequent apical development between doming and the time at which stem extension became visible was also affected by light quality as confirmed by the development of plants transferred at different stages from tungsten to red light. When apical doming had only just started (plants transferred at week 3) red light could not halt apical transition but did slow down subsequent development so that visible stem extension was delayed. Transferring plants from red to tungsten light accelerated apical development and doming occurred within 1 week in plants held previously under red light for 4 weeks. Although the onset of visible stem extension was altered the rate of extension once started was unaffected by the light treatments.

The experiment has shown that supplementary light treatments were able to synchronise and alter the time of transition of the apex but were unable to suppress the bolting response entirely. This may have been due to the overriding effect of the increasing rate of change of daylength during the course of the experiment. (Pocock and Lenton)

Root growth and sugar accumulation. Increasing the total DM production of the sugar-beet crop will only increase harvestable sugar if the partitioning of root DM to sugar is

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not adversely affected. Data from recent crop productivity studies show that although root yields increase linearly over a wide range of root DM (7–20 t ha^{-1}) the sugar as percentage root DM differed widely between site and season from 64 to 76%. However, it was not altered by sowing date, N or density on any one site in 1 year.

The DM partitioning within cells was measured in roots of plants grown at three densities in 1980 in the Crop Productivity Group's study at Broom's Barn and the results confirm the pattern described for the 1979 crop (*Rothamsted Report for 1979*, Part 1, 58). However, the maximum sugar content per unit cell volume was lower than in 1979 (180 compared with 220 gg sucrose μm^{-3}) whereas cell wall material was increased by 20%. This resulted in a partitioning of DM to sugar of 66% from August onwards compared to 76% for 1979 even though total root yields were similar.

The development of the storage root in these two identical crops was studied to determine a possible cause for this marked difference. Although the pattern of cell production did not differ early in the season, roots produced a maximum of 400 million cells per week in mid-July in 1980 whereas in 1979 cell division rates continued to rise until mid-August when weekly cell production was 750 million. Cell production paralleled the pattern of uptake of N into the roots with the time of maximum cell production coinciding with the maximum rate of N uptake in each of the crops. The rate of N uptake not only peaked earlier in 1980 but was also higher relative to the rate of cell division during June and July. This resulted in higher levels of N per cell and it is suggested that when N uptake is rapid early in the season (relative to root development and the supply of carbon assimilate) the partitioning of DM to sugar is adversely affected. High levels of nitrogenous substances within the cell during July and August may contribute to the osmotic potential of the cell and reduce its ability to accumulate sucrose. As assimilate supply rises, that which cannot be accumulated is deposited as wall material. Possible mechanisms are being investigated. (Pocock)

Staff and visiting workers

R. W. Soffe retired on 28 February 1981 after 11 years at Rothamsted following periods of service at two other ARC institutes. His expertise and detailed knowledge of horticulture was of the greatest value to all those concerned with the growth of plants in the Botany Department and in other Departments also.

S. Gutteridge, N. P. Hall and C. N. G. Schmidt joined the Department to complement and extend work on the enzymes of photosynthesis and in particular RuBP carboxylase/oxygenase.

CASE students who worked in the Department during the year were B. Newton (University of Essex) and K. Walker (University of Newcastle upon Tyne).

D. W. Lawlor attended the 13th International Botanical Congress in Sydney, Australia, in August and remained in Australia for a further 3 months working at the Australian National and other universities. En route to the United Kingdom he visited the Indian Agricultural Research Institute in Delhi, India.

A. J. Keys, as the United Kingdom Correspondent and Co-ordinator for Theme 1.7, for the OECD Programme in Photosynthesis, attended a workshop at Ettlingen, West Germany, from 11 to 14 October 1981.

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