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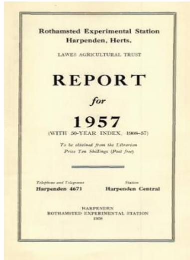
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EYESPOT OF WHEAT AND BARLEY

By

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Introduction

A severe disease of wheat, characterized by eye-shaped lesions, was recognized for many years in France (Föex, 1914, 1919; Föex and Rosella, 1930) and in North America (Heald, 1924; McKinney, 1925), but the causative fungus could not be identified because it failed to produce spores in pure culture. Then Sprague (1931) succeeded in obtaining spores of *Cercosporella herpotrichoides* Fron. in pure cultures of the fungus causing eyespot lesions of wheat in Oregon, and Sprague and Fellows (1934) showed that this was also the fungus isolated by Föex in France. Once its identity was established the fungus was recognized in other countries: in Germany (Schaffnit, 1933) and in Denmark (Nielson, 1934); and in 1934 it became severe in Holland, where the wheat acreage had been doubled after the introduction of a wheat subsidy in 1932 (Oört, 1936). These workers, notably Sprague and Fellows (1934) and Oört (1936), found that the disease was influenced by weather and cultural treatments and was worst where cereals were grown frequently. Eyespot is now known to occur in most European countries, in parts of America, Canada, Africa, Australia and New Zealand.

In Britain eyespot was first recognized in the spring of 1935, when wheat on Broadbalk and other fields at Rothamsted were found to have eyespot lesions bearing spores of *C. herpotrichoides* (Glynne, 1936). In the wet summer of 1937 severely lodged wheat on Broadbalk had eyespot lesions on 90 per cent of the straws, whereas equally heavy standing crops on neighbouring fields were almost free from the disease; eyespot was also found associated with lodging in other districts. It then seemed likely that eyespot might increase lodging and reduce yields in this country. A systematic study of the disease was therefore begun to test this possibility, to assess the extent and importance of eyespot in Britain and to obtain quantitative data on the effects of previous cropping and cultural treatments on the disease and on yields of wheat.

Regional surveys were made in many parts of the British Isles, and at Rothamsted the long-term experiments provided a unique opportunity for studying how the disease varies from year to year under similar treatments, and how manurial and other cultural treatments affect its incidence. Problems revealed by field surveys were studied in laboratory, pot and eventually field experiments (these are briefly summarized under the heading " Eyespot " in *Results of Field Experiments* (*R.F.E.*) issued each year by Rothamsted Experimental Station; the Rothamsted Annual Reports also include brief summaries of some of our results, but will not be referred to here). We now summarize our present knowledge of eyespot as it occurs at Rothamsted and in other parts of the British Isles.

Distribution

Eyespot, first reported only in the southern half of England (Glynne, 1942), is now known to occur all over Britain (Glynne, 1944; Storey, 1947; Glynne and Moore, 1949). It is most often severe on the heavier, better wheat land, least on light, well-drained soil. The order of difference is well illustrated by parallel experiments; on the heavy soil at Rothamsted 64 per cent of the wheat straws were infected at harvest, whereas on the light, sandy soil at Woburn the same variety of wheat treated in the same way had only 2 per cent infected. This advantage, however, was offset by the higher incidence at Woburn of take-all, *Ophiobolus graminis* (which is most severe on light sandy soils) (*R.F.E.* 1956).

History

Eyespot had probably been common on Broadbalk for more than 80 years before the disease was recognized there in 1935; lodging had been recorded on the more fertile plots since 1852, straggling on the less fertile since 1854,* and, on this field, both are chiefly caused by eyespot. The disease had evidently been prevalent in this country for an even longer time. Dialect words such as straggling, brackley, scrawly and scrailing, dating back some 200 years, are still used by farmers to describe wheat crops in which straws fall in all directions as the wheat ripens, and we now know that this condition is mostly a consequence of the eyespot disease. It was probably already prevalent in mediaeval times, when winter wheat alternated with fallow or was followed by spring wheat and then fallow, so that when Richard II says, " We'll make foul weather with despised tears; Our sighs and they shall lodge the summer corn, And make a dearth in this revolting land ", Shakespeare (1597) is more likely to have had in mind the untidy sort of lodging caused by eyespot than that associated with over-manuring.

Spore production

The long delay in identifying the fungus is attributable partly to the sterility of its cultures and partly to its slow growth; when attempts were made to isolate it from straw the eyespot fungus was often obscured by more rapidly growing fungi, such as *Fusaria*, which were sometimes mistaken for the causal organism. Sprague (1931) and Sprague and Fellows (1934) obtained spores by growing the fungus in flasks containing cornmeal agar and keeping them outdoors at temperatures fluctuating between —4° and 16° C. The development at Rothamsted of much simpler and more rapid methods for inducing spore formation within a fortnight on small (3-mm.) discs cut from cultures grown in petri dishes, and on infected straws (Glynne, 1953a), have greatly helped both in diagnosis and in studying the disease.

* *Broadbalk White Book* 1843–1912.

Survival and spread

The fungus survives from one crop to another on pieces of stubble which, lying on the surface of the soil, produce immense numbers of spores in cool, damp weather. On Broadbalk these infected straw bases produce spores abundantly from September to April and in some years later. Spores were produced on infected straws in the laboratory at temperatures below 15° C. They were not detached by blowing dry air at them, but were readily freed and carried in mist droplets. Water falling on infected straws carried the spores in splash droplets up 2 feet or more. They germinate readily in water, and may bud off fresh crops of spores from the mycelium in about a week, but they die if kept dry at room temperature for a few hours. Splashed by rain on to young plants, the spores often infect them at about soil level or in the angle made between the first leaves and shoot or at the edge of the coleoptile enveloping the shoot, where moisture is retained long enough for them to germinate and where they are relatively free from competing micro-organisms. A fresh crop of spores is produced on the infected plants in a few weeks, and these are splashed on to surrounding plants. At Rothamsted spores are seldom found on the growing plants after April. The distance travelled by living spores, depending on that travelled by the splash droplets, must vary widely with rain, wind and temperature. Adjacent plots at Rothamsted retain differences in degree of infection from spring to harvest, suggesting that relatively little spread usually occurs from plot to plot, but in one experiment (*R.F.E.* 1955), in a wet, windy season, the fungus was probably carried some 10 yards from heavily to lightly infected plots, in which the straws developed an unusually high proportion of slight lesions, the result of late infections. The distance eyespot spreads from centres of infection in a healthy crop has been roughly estimated (Oört, 1936), but critical trapping work to determine the distance travelled by spores in the field has not yet been done.

Although very dependent on weather, eyespot incidence also depends on the amount of infective material left by preceding crops. This was measured in an experiment (*R.F.E.* 1957) in which wheat in 1957 followed potatoes preceded by wheat (1955) in one block, and in another block wheat followed wheat preceded by beans. In January and February 1957 the pieces of stubble left on the surface of the soil by the preceding wheat crops were collected from sample areas of land and subjected to sporing tests. The wheat grown in 1955 and 1956 respectively had 60 and 78 per cent straws infected at harvest, and in 1957 the stubble from the first crop provided one and from the second twenty-seven pieces of infected stubble per square yard; from these initial sources, 2 and 47 per cent of the plants growing in the 1957 crop were infected in February and 33 and 79 per cent at harvest. Spread of the disease is favoured by cool, damp weather, which prolongs spore production, gives frequent opportunities for dispersal and infection, especially in luxuriant crops which retain moisture; dry, warm, spring weather not only inhibits spore production, but provides fewer opportunities for dispersal and infection, and infected plants may recover from the disease if the outer leaves die before the fungus has penetrated deeply. Brown-bordered lesions, shaped like an eye, appear within a few weeks of infection, usually on the leaf sheaths. The fungus grows slowly, penetrating successive sheaths, and may kill many tillers and sometimes whole plants in early spring. The fungus eventually penetrates and weakens the central ear-bearing straws; whiteheads with small underdeveloped grain may then result, resembling those caused by take-all, or the straws may fall over, causing straggling in light, lodging in heavy crops.

Increase of eyespot in successive cereal crops

The spread and development of the disease depends so much on the weather that its rate of increase varies greatly in different years and in different parts of the country. Measures that effectively control the disease in most years at Rothamsted and in other parts of southern England are therefore often ineffective in the north and west of the British Isles, where it is cooler and damper in spring and summer. The behaviour of the disease at Rothamsted and in South-east England will first be described and then contrasted with that farther north and west.

At Rothamsted the first wheat crop after several years under grass is usually free from eyespot or very lightly infected; the second may be lightly or moderately infected, but the disease usually becomes severe in the third, or in the fourth successive crop of winter wheat; this also happens in other parts of South-east England, but the second wheat crops after grass are occasionally moderately or severely infected in wetter years (Glynne and Moore, 1949); since 1938 this has occurred once at Rothamsted (*R.F.E.* 1944), when a second crop had 63 per cent straws infected at harvest, but the unusually high proportion, 38 per cent, with slight lesions suggested late infection. On old arable land the disease increases in successive wheat crops in the same way as it does after grass, but sometimes seems to do so rather more rapidly; at Rothamsted the second winter-wheat crop (after 2 years under non-susceptible crops) on old arable land has once been very severely infected (*R.F.E.* 1955), and in a long-term experiment, in which the same crop sequences are followed on both types of land, eyespot is usually more severe on the old arable land (Fosters) than on land which was relatively recently under grass (Highfield).

Once a high level of infection has been reached, eyespot incidence fluctuates from year to year and is influenced both by weather and cultural treatments. Thus on Broadbalk, plots on which wheat follows wheat, the mean percentage of straws infected at harvest on eight typical plots has varied from 40 to 90 per cent since 1938.

Early sowing increases the chances of severe infection more in some years than in others; thus wheat sown in October had significantly more straws infected than that sown in November in 2 years and about the same in two other years (*R.F.E.* 1943–46).

Spring-sown crops have not been more than lightly infected at Rothamsted and, though they occasionally suffer moderate to severe infection in other parts of southern England, they are much less severely infected than autumn-sown crops. Barley, though about as susceptible as wheat, is mostly sown in spring, and even when grown continuously at Rothamsted is only lightly infected.

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The infection seems to do little harm to the barley crop, but the infected stubble may produce enough spores to cause severe loss in succeeding wheat crops.

Oats and rye, grown in pot experiments under optimum conditions for infection, developed eyespot lesions more slowly than wheat or barley, and are much less susceptible in the field. One oat crop was moderately infected at Rothamsted, and occasional instances of severe infection of oats have occurred in other south-eastern counties (Glynne and Moore, 1949). However, this happens rather rarely, and at Rothamsted (*R.F.E.* 1953) oats in the rotation were as effective as non-cereal crops in reducing eyespot incidence, but were rather less effective at Sprowston (Batts and Fiddian, 1955). Autumn-sown rye in the six-course rotation at Rothamsted becomes lightly infected, and so, like spring-sown barley, helps the fungus to survive.

In Scotland and Ireland, where cooler, damper weather in late spring and summer favour the spread of infection late in the growing season, eyespot develops more rapidly, so that treatments that rarely lead to severe infection in the south and east often do so in the north and west. Then the second successive crop of winter wheat is often severely infected, spring-sown wheat and barley are often moderately infected in Scotland, severely infected in Ireland; and infected oat crops occur comparatively often (McKay *et al.* 1956).

Although climatic differences account for most of these differences, it is also possible that specialized types of the fungus occur in different regions. In Denmark, wheat and rye grown in a six-course rotation experiment were more severely infected than at Rothamsted. Isolates of the fungus from Danish and English wheat were similar, but that from Danish rye differed from them in cultural characters and infected rye much more severely than did isolates from any other cereal.

Decrease of eyespot under different crops

Land, which had become severely infested by growing successive wheat crops, was used to compare the influence of different crops on eyespot, take-all and weeds appearing in test crops of winter wheat. Crops other than wheat or barley strikingly decreased the infection of subsequent wheat. A single year's break from wheat or barley reduced eyespot; in some years the subsequent wheat crop was lightly, in other years severely, infected. Two-year breaks reduced the disease to a harmless level, and were about as effective as 3-year breaks. Potatoes, beans, fallow and even the slightly susceptible oats and ryegrass all effectively decreased the incidence of eyespot; there were probably differences between them which could be determined in more detailed experiments. These breaks also reduced take-all and weeds and had startling effects on the quantity and quality of grain, which for Squarehead's Master ranged from 15½ cwt./acre in infested plots following wheat to 36 cwt. in plots where eyespot, take-all and weeds had been controlled by previous cropping. Both diseases increased the amount of tailcorn, so that the dressed grain showed even greater differences ranging from 10 in diseased to 33 cwt./acre in healthy crops (*R.F.E.* 1953).

Crop rotation

Regional surveys and field experiments show that, although eyespot incidence varies greatly in the same crop sequence in different years and in different places, the frequency of severe infection is so closely related to previous cropping that this can be used to predict the likelihood of loss from eyespot. Thus at Rothamsted in wheat grown in the four-course rotation experiment (in which wheat and then barley alternate with other crops) the proportion of straws infected at harvest has varied in different years from 3 to 86 per cent; more than half the straws have been infected in 9 out of 20 years. On Broadbalk, where wheat follows wheat, this level has been exceeded in 16 years and in the six-course rotation experiment in only 5 years. Similar effects of previous wheat or barley crops on the frequency of severe infection were evident in regional surveys.

As 2-year breaks (free from self-sown susceptible carriers) have almost always reduced eyespot to a harmless level, it is likely that in some regions the sequence of crops in the four-course rotation experiment might be better if the two non-susceptible crops were grown in successive instead of alternate years, but experiments are needed to discover the conditions under which this would be an improvement. Winter wheat following a 2-year break would normally be free from serious infection; and spring sowing would usually protect the following barley crop from appreciable loss in the south and east, but would fail to do so in the wetter, colder regions. It is, of course, unwise to reverse the order and grow winter wheat after spring barley; the small proportion of plants which become infected in the spring-sown barley after a 2-year break sometimes suffices to affect seriously winter wheat grown the following year, even in the south. This occurred at Rothamsted when, on old arable land, winter wheat following spring barley after a 2-year break was seriously affected by eyespot in 2 out of 4 years (*R.F.E.* 1954, 1955). Three-year breaks, such as occur under grass or lucerne in the Rothamsted ley–arable experiments have consistently given negligible eyespot and correspondingly high yields of wheat.

Lodging

Twenty years ago excess of nitrogen and wet, windy weather were regarded as the chief causes of lodging; but now we know that eyespot is equally important. Very heavy crops may lodge because the straws are not strong enough to withstand the buffeting of wind and rain; then they bend over and lie mostly in one direction. When eyespot has weakened the straw bases, not only do much lighter crops lodge, but the straws, which bend abruptly in the middle of the lesions, fall in all directions and lie flat on the ground; loss from lodging is then added to the direct loss of grain caused by the fungus. Measurements made in many years on hundreds of plots at Rothamsted have shown the highly significant effects on lodging, both of weight of straw and of the proportion with severe eyespot lesions (Glynne, 1951; Salt, 1955). Some idea of their relative effects is shown by mean values obtained on Broadbalk where an increase

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in straw weight from 40 to 60 cwt./acre had about as much effect increasing lodging as an increase from 35 to 85 per cent straws with severe eyespot. Wheat following fallow yielded more straw and a smaller proportion with severe eyespot lesions than wheat following wheat; in some years the heavier straw resulted in more lodging, in other years the lower incidence of eyespot resulted in less lodging than after wheat so that long-term averages of the percentage area lodged after wheat and after fallow were about the same (Glynne, 1954).

Because lodging from eyespot causes more loss than non-parasitic lodging, measures to prevent it are most important where cereals are grown frequently on the same land. These measures depend on reducing the severity of eyespot, reducing weight of straw and increasing straw strength; the last two are important whether eyespot is present or not. The methods likely to affect both eyespot and lodging investigated at Rothamsted include varying fertilizers, rate and date of sowing, the use of short, stiff-strawed varieties and spraying with sulphuric acid.

Fertilizers

Fertilizers influence the incidence of eyespot by their effects on plant growth, and they influence lodging by their effects on the disease and on weight of straw.

Increased tillering produces more layers through which the slow-growing fungus must penetrate before it reaches the central ear-bearing straws, thus reducing the severity of eyespot at harvest; but the greater humidity prevailing within a well-tillered crop favours spread of the fungus and development of the disease; the two effects work in opposite directions.

In pot experiments where all plants are infected as seedlings and kept under humid conditions the effect of tillering predominates and well-nourished plants are less severely infected at harvest than starved ones. Applications of nitrogen (Glynne, Dion and Weil, 1945; Salt, 1953) and of phosphate and potash to soil lacking these nutrients greatly decreased the severity of eyespot. In the field the effect of humidity usually predominates, so that fertilizers tend to increase the severity of eyespot at harvest. Because nitrogen is more often deficient than phosphate or potash, it has received most attention. Annual surveys of wheat on Broadbalk show the mean number of severe eyespot lesions in 16 consecutive years was 50 per cent on plot 7 receiving minerals and nitrogen, and 38 per cent on plot 3 receiving no manure. Application of fertilizers appreciably increased incidence of the disease in 10 years and reduced it in only one year. Nitrogenous fertilizers have increased the proportion of straws infected in most of our field experiments; they also increase weight of straw; both effects increase the tendency to lodge and so reduce response to fertilizers. Restricting the fertilizer, though it reduces lodging, also limits yields, so that other methods of control are preferable.

Soil fertility and disease incidence affect the optimum date for applying nitrogen. Early applications increase the weight of straw and the risk of lodging on the better soils. Thus at Rothamsted, on fertile land heavily infested by eyespot, nitrogen applied to Squarehead's Master in March, April and May had little effect on eyespot; that applied in March increased straw and lodging most, and yielded less grain than that applied in May. Where eyespot was controlled and lodging decreased by spraying with sulphuric acid, grain yields were not significantly affected by the date when nitrogen was applied (Salt, 1955).

By contrast, at Woburn on light, nitrogen-deficient soil, there was much less eyespot and no lodging. Plots of Holdfast which received nitrogen in March had more eyespot and yielded 3 cwt./acre less grain than those which received nitrogen in April. Where nitrogen was withheld until May deficiency symptoms became apparent, and although eyespot incidence was the same as after the April application, yields were 6 cwt./acre less (*R.F.E.* 1955).

Seed rate

Lowering seed rate below normal encourages tillering and promotes drying within the crop, and so helps to reduce the incidence and severity of eyespot at harvest. It also reduces competition between shoots for light and nutrients, and so promotes growth of stronger straws, and this helps healthy (Glynne and Slope, 1957) as well as diseased (Glynne, 1951; Salt, 1955) cereals to resist lodging. In an experiment on eyespot-infested land at Rothamsted (Salt, 1955) reducing seed rate of Squarehead's Master wheat from 3 to 1½ bushels/acre decreased severe eyespot from 74 to 56 per cent straws infected, the area lodged from 72 to 40 per cent and increased grain yield from 20 to 25 cwt./acre. In several experiments plots, sown at the normal seed rate and given nitrogenous fertilizer, suffered so much loss from disease that they yielded less than plots sown at half the seed rate and given no nitrogen; thinly sown plots dressed with nitrogen yielded most grain. Thin sowing has the additional advantage that it reduces the effects of take-all by encouraging plants to produce more roots. Its main disadvantage is that it encourages the growth of weeds, and these must be controlled for thin sowing to be advantageous. Failure to control weeds accounts for inconsistent effects of seed rate on infested land at Rothamsted and Woburn (*R.F.E.* 1955, 1956).

Optimum sowing rates therefore vary, not only with soil, variety, nutrition and weather, but also with disease incidence; they are higher on land free from eyespot and take-all than where either of these diseases is severe.

Varietal susceptibility

Many types of wheat tested in pot experiments all proved susceptible; they included the wild type *Triticum monococcum* and representatives of groups with fourteen, twenty-eight and forty-two chromosomes. Differences in severity of infection of different varieties were evident in field experiments; Deprez 80 had consistently fewer straws infected at harvest than three other varieties (*R.F.E.* 1943–46) and Cappelle (1952) had 31 per cent severely infected straws when Squarehead's Master had 88 per cent. Similar differences were found in a pot experiment (1953) when inoculated Cappelle and Scandia all developed lesions but had only half as many straws severely infected at harvest as four other varieties, and mean loss in grain from infection was 9 per cent in Cappelle, 12 per cent in Scandia, as compared with 20–28 per cent in other varieties. Lower field susceptibility of Cappelle was found in France (Vincent *et al.*, 1952), in Cambridge (Lupton and Macer, 1955) and was evident in experiments at Rothamsted and Woburn (*R.F.E.* 1953–56). Cappelle has the further advantage of a short, strong straw which seldom lodges. Unfortunately it is just as severely attacked by take-all as other varieties, and the serious loss caused by take-all and eyespot are less easily noticed because it yields more than many other varieties; but the serious loss suffered by Cappelle was dramatically apparent in 1955, when, sown in early October on fertile land supplied with ample nutrients, it yielded only 25 cwt./acre on plots severely infested by eyespot and take-all, and 61 cwt./acre where these diseases (and weeds) had been controlled by previous cropping.

Spraying with sulphuric acid

In South-east England cereals were sometimes sprayed with sulphuric acid in spring to control weeds; in France attempts to use it to control foot and root rots gave variable results. At Rothamsted effects of the acid on spore production were studied in the laboratory and its effects on crops studied in field experiments (Dion, 1943). Application of 12½ per cent by volume commercial sulphuric acid at 100 gallons/acre, after sowing and before emergence, stopped spore production on exposed pieces of stubble and prevented early infection of the crop. But the disease spread in spring, possibly from pieces of freshly exposed stubble. The best results followed two sprayings, one applied to the soil in autumn after sowing, the other applied to the crop in early spring. A single spraying was most effective when applied at the five-leaf stage, before the fungus had penetrated beyond the second leaf sheath, which, with outer leaves, was killed by the spray. At Rothamsted this stage is usually reached in the first half of March. By April the fungus had penetrated too deeply to be controlled by spraying, although, like the earlier treatment, it controlled weeds. The acid had little effect on straw yield, but, by reducing eyespot, greatly reduced lodging; it was most successful on well-nourished crops, giving in some years yield increases of 10 cwt./acre (Glynne, 1951; Salt, 1955); but it has sometimes failed to control eyespot or to increase yields (*R.F.E.* 1956; Salt, 1957).

Acid spraying has proved valuable in experiments in which effects of treatments are compared on lightly and heavily infected crops, but its use in farming is likely to be limited. The land is sometimes so wet in early March that the machinery needed for high-volume spraying damages the crop excessively, and reports of low-volume spraying have not been encouraging. But the impressive effects of successful spraying with sulphuric acid suggest that the use of other chemical sprays might usefully be investigated.

Yield of grain

The loss caused by eyespot varies with cultural and manurial treatments; in pot experiments inoculation reduced yields by 19 per cent in well-nourished, by 86 per cent in starved plants; by 22 per cent in thinly and 45 per cent in thickly sown plants. In a series of pot experiments in which all inoculated plants were infected and some straws straggled there was no general lodging, and loss caused by eyespot averaged 33 per cent. Losses of the same magnitude occur in the field, where, although some plants escape infection, loss from lodging must be added to that caused directly by the fungus. Straws with severe lesions, taken from field experiments, yielded only about half as much grain as uninfected straws or those with slight lesions (Glynne, 1944, 1953b).

Take-all and weeds increase with eyespot in field experiments, so that it is often difficult to assess their separate effects. Where eyespot and weeds, but not take-all, were controlled by spraying with sulphuric acid, yields were increased by 46 per cent in 1948, by 39 per cent in 1951. Where take-all was also controlled by previous cropping yield increases have exceeded 80 per cent (*R.F.E.* 1955–56). In experiments which measured effects of previous cropping on a final test crop of winter wheat, take-all and weeds increased and yields decreased in successive wheat crops. Typical figures for plots which carried the first, second and third crops of winter wheat were respectively 37, 28 and 19 cwt./acre for Squarehead's Master in 1953, and in 1956, 37, 24 and 19 cwt./acre for Holdfast, 49, 36 and 24 cwt./acre for Cappelle, and the heaviest crops included least tail corn, so that dressed grain showed even bigger contrasts.

The development of high-yielding varieties which resist lodging, generous application of fertilizers and improved cultural and harvesting methods have greatly increased yields of wheat in recent years. But they fail to give high yields if eyespot and its associated troubles are not controlled. Recognition of the severity and frequency of losses caused by soil-borne diseases have shown that yields of wheat previously regarded as satisfactory are really too low. This has helped to set a higher standard for wheat yields at Rothamsted. Here, yields of 40–45 cwt./acre were regarded as exceptionally high less than a decade ago, but, by deliberately combining favourable treatments on uninfested land, yields of 50–60 cwt./acre have been obtained in each of the last 3 years. Further knowledge and wider application of that already acquired can do much to increase yields of wheat in Britain.

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BRACKEN THIAMINASE

By

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Studies of enzymes *in vitro* led to the discovery, some thirty or forty years ago, that on occasion, compounds of similar structure to the substrate were capable of competitively inhibiting the enzyme activity. Later, it was demonstrated that the bacteriostatic action of sulphanilamide was antagonized by the essential metabolite *p*-aminobenzoic acid. This led to the Woods–Fildes hypothesis, which contends that because of the structural similarity of sulphanilamide and *p*-aminobenzoic acid, sulphanilamide blocks the enzyme system concerned with the metabolism of *p*-aminobenzoic acid and interferes so much with an essential metabolic function of the bacterium that growth is inhibited. Sulphanilamide and *p*-aminobenzoic acid compete for the enzyme system, and bacteriostasis results when the inhibitor is successful in the competition. It was then recognized that chemotherapeutic substances might be designed by the synthesis of substances of analogous structures to essential metabolites, thereby destroying the organism by a competitive antagonism of the essential metabolite. However, because of the present limited state of our knowledge of the multitude of co-ordinated reactions which are an essential and indispensable condition of the existence of organisms, attempts to design chemotherapeutic substances in this way have met with little success. It is certain, however, that as biochemical knowledge accumulates, living processes will become more and more susceptible to control and that an increasing number of drugs, weedkillers and insecticides will be deliberately designed and synthesized. It is for the purpose of obtaining such knowledge that a study of the enzymes of bracken (*Pteridium aquilinum* (L.) Kühn) has been undertaken; for none of the weedkillers which have been tested have proved to be practically useful for eradicating bracken specifically. The investigations so far have only occupied part of the time over the past three years and although a number of the enzymes common among other organisms have been identified in bracken, as yet our knowledge is fragmentary and unco-ordinated and only the work done on bracken thiaminase will be discussed here.

Introduction

Thiaminases were first recognized in certain species of fishes. Their discovery followed the demonstration that the disease in animal populations known as "Chastek paralysis" was in fact a thiamine (vitamin B1) deficiency and developed when certain species of raw fish were included in the diet of foxes, although thiamine was present in adequate amounts. The substance in fish responsible for the destruction of the dietary thiamine was shown to be an enzyme by Sealock, Livermore and Evans (1943). The relevant literature has been reviewed by Yudkin (1949) and Harris (1951).

The thiaminases are enzymes which catalyse the fission of the methylene–quaternary-nitrogen bond of thiamine (Fig. 1). This usually occurs only in the presence of certain amines, the pyrimidine moiety being transferred to the amine according to the general equation,

$$P\text{–}CH_2\text{–}T^+ + RNH_2 \longrightarrow P\text{–}CH_2\text{–}NHR + T + H^+ \quad . \quad (1)$$

where P and T stand for the pyrimidine and thiazole components of thiamine respectively (Woolley, 1953; Fujita *et al.*, 1952; Sealock and Davis, 1949). Thiaminases catalysing the transfer reaction have been found in fishes, shellfish, bacteria and ferns (Harris, 1951; Fujita, 1954), but only with the bacterium *Bacillus aneurinolyticus* Kimura et Aoyama is there good evidence of the production of a thiaminase capable of catalysing the hydrolytic fission of thiamine (Fujita, Nose and Kuratani, 1954)

$$P\text{–}CH_2\text{–}T^+ + H_2O \longrightarrow P\text{–}CH_2OH + T + H^+ \quad . \quad . \quad (2)$$

Studies of plant thiaminase stem from the observation of Weswig, Freed and Haag (1946) that rats fed on a ration containing 40 per cent of air-dried bracken and adequate thiamine developed acute thiamine deficiency. Thomas and Walker (1949) confirmed this work and showed that bracken contained a thermolabile system capable of destroying thiamine. The active system was extracted from the dried leaf by Evans, Jones and Evans (1950), who concluded that it contained an enzyme and established that the thiazole component of thiamine was one of the products of the reaction. The situation was, however, complicated by the reports of Somogyi (1949) and Somogyi and Muralt (1949) that the factor responsible for the inactivation of thiamine in fern and bracken extracts was thermostable and passed a dialysing membrane. Subsequently, Evans and Jones (1952) obtained evidence that aqueous extracts of bracken contained an enzyme capable of catalysing the transfer reaction of equation (1), but the work of Fujita, Okamoto and Nose (1955) with the variety of bracken *var. japonicum* suggested that an enzyme was present which was capable of catalysing both the transfer and the hydrolytic fission reactions (equations 1 and 2).

It was known that horses suffer from thiamine deficiency when the proportion of bracken in their diet is high (Roberts, Evans and Evans, 1949) and it seemed likely that the deficiency was brought about by the destruction of the dietary thiamine by factors in the bracken. It follows therefore that such factors are unlikely to be present in the common plants of pastures. Also, Fujita (1954) examined large numbers of higher plants for the presence of thiaminase and found activity only in the herb *Celosia crista*.

Apart from the need to clarify the conflicting reports of the nature of the thiamine-destroying factors which are present in bracken, it was of particular importance to establish whether an enzyme system which attacked thiamine was present in bracken but absent from most of the higher plants. Such a difference might be exploited for the development of a specific bracken-killing agent.

Preliminary experiments

Examination of aqueous extracts of dried and fresh bracken showed that the destruction of added thiamine was small and that it could be greatly increased by the addition of certain amines. Further, over 90 per cent of the activity was abolished by heating the extracts for 15 minutes at 100°. These results, in agreement with those of Evans and co-workers, suggested that the bracken extracts contained a thiaminase capable of catalysing the transfer reaction according to equation (1). Previously, thiaminase activity had been followed by measuring the disappearance of thiamine, although evidence for the transfer reaction had been got by the isolation of the pyrimidine–amine product, or by fluorimetric or chromatographic techniques. As a result of the preliminary experiments, it became clear that it would be desirable to develop a method by which the formation of the pyrimidine–amine product could be measured. The availability of such a method would make it possible to determine the precise nature of the reaction by which thiamine was destroyed. For the activating effect of amines might have been due either to their stimulating the hydrolytic fission of thiamine or to their entering into the transfer reaction.

Spectrophotometric method of estimating thiaminase transfer activity

Tests with a variety of amines suggested that bracken thiaminase destroyed thiamine most readily in the presence of pyridine. If the transfer reaction was involved it would lead to the production of 1 mol. *N*-(4-amino-2-methylpyrimidin-5-yl)-methylpyridine heteropyrithiamine, called HPT hereafter, Fig. 1) per mol. of thiamine destroyed and could be followed by measuring the rate of formation of HPT. A method of estimating small amounts of HPT in the presence of thiamine was therefore worked out (Kenten 1957). The method depends on the destruction of thiamine by incubation with strong alkali and subsequent oxidation of the HPT to 2-methyl-pyrichromine by alkaline ferricyanide. The 2-methyl-pyrichromine is estimated spectrophotometrically at 386 mμ. Using this method, studies were made of the effect of pH, thiamine concentration and pyridine concentration on the rate of formation of HPT by extracts and partially purified thiaminase preparations from bracken.

These studies helped to establish standard conditions for the estimation of thiaminase activity under which the rate of formation of HPT was directly proportional to the amount of extract or thiaminase preparation present. This new method was of value in following the thiaminase activity in work on the purification of bracken thiaminase. Kenten (1957) defined one unit of thiaminase activity as that amount of enzyme which catalyses the formation of 1 μmole of HPT in 1 hour under the standard conditions, and the specific activity as the number of enzyme units/mg. of N of the enzyme preparation.

The course of the reaction

Using either water extracts of dried bracken leaf or partially purified thiaminase preparations, it was shown that in the presence of pyridine 90–95 per cent of the thiamine added was converted to HPT. The activity was virtually completely destroyed by heating the extracts or thiaminase preparations for 15 minutes at 100° before testing. With the small amounts of extract used no destruction of thiamine in the absence of pyridine could be detected. These results suggested that destruction of thiamine with both the extracts and the partially purified thiaminase preparations took place through the transfer reaction and that side reactions were small.

By fractionation with ammonium sulphate and calcium phosphate gel, concentrated, partially purified thiaminase preparations were made from water extracts of dried bracken leaves. The best preparation represented a purification of about sixty-fold. It catalysed the formation of 3,150 μmole of HPT/hour/mg. of N of the preparation under the standard conditions. Using such preparations, no evidence was obtained that they were capable of catalysing the destruction of thiamine in the absence of amines.

Difference between bracken and higher plants and its bearing on bracken eradication

It was therefore clearly established that bracken contained a thiaminase capable of catalysing the transfer reaction (equation 1), but without significant hydrolytic activity (equation 2). Tests with a number of higher plants failed to provide any evidence for the presence of an enzyme system capable of destroying thiamine. This difference suggested two possible lines of approach towards the development of a specific bracken-killing agent. In the first place, it might be possible to synthesize a thiamine analogue which would not be attacked by thiaminase but which would combine with it and destroy its catalytic activity. If the thiaminase system is essential, then introduction of such a compound into the bracken plant might inactivate this system and so disturb the metabolism of the plant that its death would follow. Secondly, it might be possible to introduce a substance into the bracken plant on which the thiaminase would act to release a toxic compound. That this latter type of approach can be successful has been demonstrated by Wain and co-workers (Wain, 1956). With certain homologues of the substituted phenoxyacetic acids, selectivity was achieved by exploiting differences in the β-oxidation systems of plants; only certain plants having the capacity to convert the homologues into the phytotoxic substituted phenoxyacetic acids.

To facilitate studies of the action of bracken thiaminase on thiamine analogues it was necessary to develop a general method for following thiaminase action. The spectrophotometric method described previously can be used only when pyridine is the acceptor amine and heteropyrithiamine is one of the products of the reaction.

Manometric studies of bracken thiaminase

Since both the reactants and the products of the thiaminase transfer reaction are bases and the extent of ionization of the products, at about neutral pH, may differ from that of the reactants, acid may be released. If the reaction takes place in bicarbonate–CO_2 buffer, the amount of acid released, and hence the extent of thiaminase action, can be followed manometrically by measuring the evolution of CO_2.

The following examples of systems in bicarbonate–CO_2 at pH 7·5 make this clear. If the acceptor amine is a strong, or moderately strong base (pKa 10 or greater), then the two extreme possibilities may be written

$$\text{P–CH}_2\text{–T}^+ + \text{RNH}_3^+ \longrightarrow \text{P–CH}_2\text{–NH}_2^+\text{R} + \text{T} + \text{H}^+ \quad (3)$$

$$\text{P–CH}_2\text{–T}^+ + \text{RNH}_3^+ \longrightarrow \text{P–CH}_2\text{–NHR} + \text{T} + 2\text{H}^+ \quad (4)$$

and between 1 and 2 mol. of acid would be released according to the extent of ionization of the pyrimidylmethyl–amine product. If the acceptor amine is a very weak base (pKa 5 or less) and the pyrimidylmethyl–amine product is a strong base, then the reaction can be formulated

$$\text{P–CH}_2\text{–T}^+ + \text{RNH}_2 \longrightarrow \text{P–CH}_2\text{–NH}_2^+\text{R} + \text{T} \quad . \quad (5)$$

and no release of acid, and hence no evolution of CO_2, would take place. For studying such systems (equation 5) therefore, the manometric method is not suitable, but they can be avoided by the choice of an amine acceptor of suitable pKa.

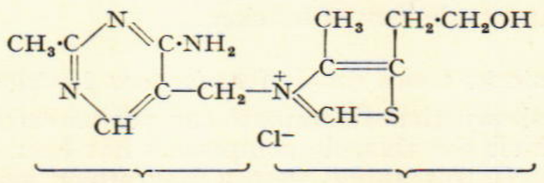
Quantitative studies using aniline, pyridine, piperidine and trimethylamine showed that, although a small part (5–10 per cent) of the thiamine was lost by side reactions under certain conditions, there was a reasonable agreement (within 10 per cent) between the calculated and experimental outputs of CO_2 (Kenten, 1958). This suggested that the method was suitable for studying the action of thiaminase, and it was accordingly used to test the activity of thiaminase towards structural analogues of thiamine.

Behaviour of thiaminase with structural analogues of thiamine

Thiamine analogues were synthesized which differed in structure from that of thiamine in that only the thiazole portion was replaced by different amines.

The results of manometric tests with these compounds showed that some of them were attacked by the bracken thiaminase and that none of them, when present in relatively small amounts ($5 \times 10^{-3}M$), was capable of appreciably inhibiting the action of bracken thiaminase on thiamine. In particular, heteropyrithiamine and quinilinothiamine (Fig. 1), in which pyridine and quinoline replace the thiazole heterocycle, were readily attacked by thiaminase at about half the rate of that with thiamine. When the primary amino group of thiamine, which is carried on the pyrimidine portion of the molecule, was replaced by hydroxyl the resultant compound, oxythiamine, was attacked very slowly. Oxythiamine at concentrations of $5 \times 10^{-3}M$ was, however, without appreciable effect on the rate of the thiaminase reaction with thiamine. These results suggested that in searching for a structural inhibitor of thiaminase a more profitable approach would be to vary the nature of the pyrimidine component of thiamine. Accordingly, several analogues were synthesized in which the pyrimidine heterocycle was replaced by a substituted benzene ring and in which the thiazole component was the same, or very nearly the same, as that of thiamine. Tests showed that none of these compounds was attacked by thiaminase. One of them, 3-(*o*-aminobenzyl)-4-methylthiazole (ABMT, Fig. 1), was found to be a very powerful inhibitor of thiaminase action. Concentrations of $2 \times 10^{-6}M$ ABMT inhibited bracken thiaminase by 15–20 per cent and at $5 \times 10^{-5}M$ inhibition was virtually complete (Kenten, 1958). The structural requirements for inhibition appear to be very exacting, for if the amino-group of ABMT is replaced by a nitro-group the capacity to inhibit thiaminase is lost; $5 \times 10^{-3}M$ concentrations of the nitro-compound being without

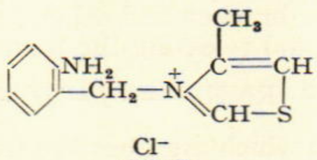
FIG. 1.



Pyrimidine moiety (P) Thiazole moiety (T)

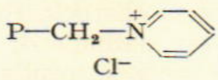
Thiamine

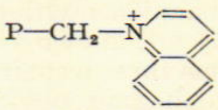
N-(4-amino-2-methylpyrimidin-5-yl)methyl-5-(β-hydroxyethyl)-4-methyl thiazolium chloride



ABMT

3-(*o*-aminobenzyl)-4-methylthiazolium chloride





Heteropyrithiamine

N-(4-amino-2-methylpyrimidin-5-yl) methylpyridinium chloride

Quinilinothiamine

N-(4-amino-2-methylpyrimidin-5-yl) methylquinolium chloride

effect. Inhibition of thiaminase by ABMT would be expected to be competitive because of its structural resemblance to thiamine, and in fact Sealock and Goodland (1944) have obtained evidence that fish thiaminase is inhibited competitively by ABMT. With bracken thiaminase, however, the inhibition is not competitive because increasing the concentration of thiamine fails to decrease the amount of inhibition. Also, the amount of inhibition increases with increase in time of exposure of the thiaminase to the ABMT. It is possible that the ABMT initially combines reversibly with the enzyme at the same site as thiamine but that a second irreversible reaction follows which gives an inactive ABMT–enzyme complex.

Injection of ABMT into bracken

Single injections of up to 5 mg. of ABMT into the stems of bracken fronds caused no obvious damage to develop in a period of 3–4 weeks. These injections were made into pot-grown bracken in early August, when growth was almost complete. The size of the fronds was such that, assuming even distribution, the concentration of ABMT in the frond would be at least $3 \times 10^{-4}M$; *in vitro* $5 \times 10^{-5}M$ ABMT is sufficient to bring about nearly complete inhibition of the thiaminase system.

There are several reasons why the ABMT should have failed to affect the bracken. In the first place, it may not have been translocated to the site of thiaminase action. Secondly, it may have been attacked by another enzyme system in the bracken and its capacity to inhibit thiaminase destroyed. Thirdly, it may be that the thiaminase system is not important, or only important at certain stages of growth, to the metabolism of bracken.

Thiaminase substrates which could give rise to a phytotoxic substance

It has been shown that thiaminase can act on certain thiamine analogues in which the thiazole component has been replaced by another amine. If this amine were a phytotoxic substance (X), then it is possible that combination with the pyrimidylmethyl component of thiamine might mask its toxic properties. Thus, if the pyrimidylmethyl-X compound were administered to a mixed population of plants it might be expected that only those containing the thiaminase system (i.e., bracken and other Pteridophytes) would be capable of splitting it and releasing the toxic substance X.

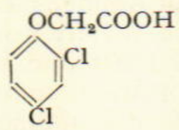
$$\text{P–CH}_2\text{–X} + \text{RNH}_2 \longrightarrow \text{P–CH}_2\text{–NHR} + \text{X}$$

Few amines are known which are toxic to plants when administered in very small quantities. The amino-compounds, 1-*iso*propyl-2-nonyl-4 : 4-dimethyl-2-imidazoline (Allen and Skoog, 1951) and (5-chloro-2-benzimidazolylthio)-acetic acid (Rebstock, Ball, Hamner and Sell, 1957) are known to cause severe damage or kill certain plants, but when these were injected into bracken fronds in amounts of up to 5 mg. the only obvious effect was a slight chlorosis at the tip of the frond with the higher amounts of the latter compound.

Because of the readiness with which heteropyrithiamine and quinilinothiamine were attacked by bracken thiaminase, it seemed likely that a thiamine analogue containing a substituted pyridine ring in place of the thiazole would also be attacked. It was decided therefore to synthesize some pyridine analogues of the phytotoxic phenoxyacetic acids. Since quite small modification of a molecule can abolish phytotoxicity, the chances that the pyridine compounds would have toxic properties were small. However, the structural requirements for phytotoxicity are not clearly understood, and there were no other grounds on which to base the choice of structures. Two compounds were synthesized (Fig. 2), 6-chloro-2-methyl-pyridin-3-oxyacetic acid and 5-bromopyridin-3-oxyacetic acid. These compounds had no obvious effect on bracken when injected into the fronds as described above; they were also without significant activity as compared with 2 : 4-D or MCPA in inhibiting the germination of cress seeds.

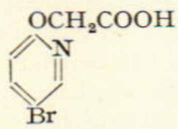
Because of the lack of toxicity of these imidazole and pyridine derivatives, no attempt was made to synthesize and test the pyrimidylmethyl-substituted compounds. It may be that other amines, for example, pyridine compounds with halogen or halogen and methyl substituents in other positions, will prove to be toxic to bracken, and there is scope here for much further work.

FIG. 2.

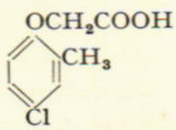


2 : 4D

2 : 4-dichlorophenoxyacetic acid

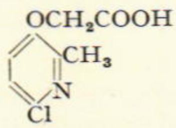


5-bromopyridin-2-oxyacetic acid



MCPA

4-chloro-2-methylphenoxyacetic acid



6-chloro-2-methylpyridin-3-oxyacetic acid

The role of thiaminase

The physiological role of thiaminase is not clear. Thiamine apparently has a limited and erratic distribution (Harris, 1951; Fujita, 1954). Among the fishes, for example, it occurs only in some fresh-water and salt-water species. It could be that thiaminase has some special role in those organisms in which it occurs. Alternatively, *in vivo* it may catalyse a reaction common to most organisms and only show activity with thiamine *in vitro* in certain cases. If thiamine is the substrate of thiaminase *in vivo*, then thiaminase would catalyse the synthesis of thiamine analogues having an amine other than the thiazole moiety of thiamine attached to the methylene bridge. Such compounds are as yet unknown in biological material. From the activity of thiaminase in bracken extracts it can be calculated that the rate of metabolism of thiamine by thiaminase in the fresh leaf could be of the order of 4,000 μg./g. of leaf/hour at 17° C. Watanabe (1952) finds that bracken contains 0·66 μg. of thiamine/g. fresh weight.

With certain amines the transfer reaction is reversible. Therefore the possibility exists that thiaminase takes part in the synthesis of thiamine by catalysing the exchange of 5-β-hydroxyethyl-4-methyl thiazole with a pyrimidinmethylamine precursor. Such a role for thiaminase would appear to presuppose different pathways of thiamine synthesis in, for example, different but closely related fishes.

The present work and that of Fujita (1954) show that thiaminase will act on certain thiamine analogues. Also Woolley (1953) has found that carp thiaminase catalyses the transfer reaction between the pteridine analogue of thiamine and *p*-aminobenzoic acid or *p*-aminobenzoylglutamic acid, with the formation of pteroic acid or pteroylglutamic acid, although the yields were extremely small.

Woolley does not consider that this is the mode of biosynthesis of these compounds. The results do, however, indicate the potentialities of thiaminase as a synthesizing enzyme.

The results of the present work with bracken thiaminase and that with other thiaminases suggests that for the transfer reaction to take place readily the substrate must have a pyrimidine ring attached by a methylene bridge to an amine. The nature of the amine may be varied widely, but little variation in the pyrimidine ring is permissible. It is therefore of particular interest that preliminary results suggest that certain purines and pyrimidines are active as acceptor amines in the bracken thiaminase transfer reaction. It may be that the physiological function of thiaminase lies in the metabolism of these compounds or in helping in such actions as the nucleic acids may be concerned in, and that is not restricted to reactions involving thiamine.

Conclusion

Although the investigations have as yet produced no results of immediate practical value for the eradication of bracken, a start has been made on the synthesis of compounds which may be of value, and a good deal of information about the thiaminase system has been obtained. New techniques for studying thiaminase action have been developed, and the information obtained by these methods will help to elucidate the physiological role of thiaminase. In particular, the investigations have shown that ABMT is a powerful inhibitor of thiaminase *in vitro*, although it is apparently without effect on the bracken plant. Further studies of compounds having a close structural resemblance to ABMT would appear to be worthwhile, as the availability of compounds which would inhibit thiaminase *in vivo* might help to throw light on the role of this enzyme and might also be useful as bracken-killing agents.

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THE RESIDUAL EFFECTS OF THE MANURIAL AND CROPPING TREATMENTS IN THE AGDELL ROTATION EXPERIMENT

By

R. G. Warren

The Agdell Rotation Experiment was started by Lawes and Gilbert in 1848 and tested two crop rotations and three manurial treatments.

The two cropping schemes were both four-course rotations, one of which was the Norfolk four-course with swedes, barley, clover (or beans) and wheat; the other rotation had the same root and cereal crops, but a fallow replaced the leguminous crop of the Norfolk four-course rotation. The two rotations, side by side, occupied 2½ acres in Agdell Field, on a soil which is one of the heaviest on the Rothamsted farm. The clover, undersown in the barley, failed in some winters, and beans were then sown as a substitute. Although in the early years of the experiment there were more bean than clover crops, the ratio for the whole period was two crops of clover to one of beans.

The manurial treatments were O, P (which was changed to PK in 1884) and NPK; these were put down as three strips across the two rotations. The manures were given once in 4 years and applied to the swede crop only. Part of the nitrogen was supplied as ammonium salts and the remainder as rape cake. Until 1912 the inorganic nitrogen fertilizer consisted of equal parts of ammonium chloride and ammonium sulphate, but afterwards it was given entirely as sulphate. Rape cake was replaced by castor meal in 1940 and in later years. Phosphorus and potassium were supplied as

TABLE 1

Agdell rotation experiment 1848–1951

Average dressings of manures, cwt./acre, applied every fourth year

Manure	Treatment PK	NPK
Ammonium sulphate	—	2
Rape cake (or castor meal)	—	18
Superphosphate	4	4
Potassium sulphate	3	3½

Supplementary minerals, sodium sulphate at 100 lb./acre and magnesium sulphate at 200 lb./acre, were given whenever potassium sulphate was applied.

superphosphate and potassium sulphate. Throughout the experiment the amount of nitrogen given in each form remained unchanged, but the dressings of superphosphate were increased in 1904 from $3\frac{1}{2}$ to $4\frac{1}{2}$ cwt./acre and those for potassium sulphate from $2\frac{3}{4}$ to $4\frac{1}{2}$ cwt./acre in 1896. The average dressings of the manures are given in Table 1, in which all the inorganic nitrogen has been expressed as ammonium sulphate.

In addition to the comparisons of three manurial treatments on two rotations, Lawes and Gilbert included a test of management when roots were grown. On half of each plot the roots and leaves were carted off; on the other half the produce was eaten on the land by sheep, or, if the weather was unsuitable, the leaves and the roots (after slicing) were spread over the ground. This test was continued until 1900, but in later years the crop was removed from the whole of each of the six plots.

Summary of the Results of the Agdell Experiment

The experiment was continued until 1951, but by then the crops on the NPK plots were affected by soil acidity. The swede crops were generally ruined by clubroot, which first appeared on the acid plots but spread to the other plots. Table 2 gives the average yields of the crops for the first eighteen courses (1848–1919) before the disturbance from soil acidity became serious.

Table 2

Average yields, Agdell Field, 1848–1919

	No manure		PK		NPK	
	Fallow rotation	Clover rotation	Fallow rotation	Clover rotation	Fallow rotation	Clover rotation
Swedes, tons ...	1·7	0·6	8·8	9·6	18·0	15·9
Barley, cwt. ...	11·4	10·8	12·0	12·0	16·4	18·4
Beans, cwt. ...	—	7·7	—	10·7	—	13·1
Clover hay, cwt.	—	30·7	—	58·6	—	60·2
Wheat, cwt. ...	13·8	12·8	16·3	17·7	16·9	17·8

The average results for the eighteen courses show that the two rotations gave similar yields of roots and cereals and that the main advantage of the clover rotation was the extra produce as beans or clover.

In contrast with the effect of crop rotation on yields, there were large responses to the manurial treatments. Nitrogen increased the yields of swedes by 6–9 tons/acre, and on the following crop of barley the residues of the nitrogen manures gave 4–6 cwt./acre more grain; there was, however, no residual effect on wheat, which was the last crop after the application of the nitrogen manures. The PK fertilizer treatment also gave large increases in yields; the increases per acre were for swedes 7–9 tons, clover 30 cwt., beans 3 cwt. and wheat 2·5–5 cwt.

Except for the period of the fed and carted treatments of the swede crop, the whole of each crop in the Agdell Rotation Experiment was removed from the land. While there was no evidence from the crop yields of any accumulation of residues of the nitrogen manures, the wheat crops on the unmanured and PK plots showed that there were residues of the PK fertilizers at the end of the manuring cycle. The response of the wheat crops to residues of these fertilizers provided no estimates of the amounts of phosphorus and potassium that had accumulated over the whole period of the experiment, nor of the ability of the residues to supply adequate amounts of these nutrients to crops which have higher requirements than wheat.

Effects of the Accumulated Residues of PK Fertilizers

After the conclusion of the Rotation Experiment in 1951 the field was fallowed for 1 year and was then cropped with cereals till 1955. The cereals received only a uniform dressing of nitrogen. During this period a pH survey of the experimental site was made. Serious soil acidity (i.e., pH 5·0–5·5) was found only on the NPK plots, but small areas at the ends of the PK plots which adjoined the NPK plots were either slightly acid or devoid of calcium carbonate. The soils of the remainder of the PK plots and the whole of the unmanured plots contained 2–3 per cent $CaCO_3$. To correct the soil acidity, differential chalk dressings (with a maximum of 5 tons $CaCO_3$/acre for the most acid areas) were applied in the winter of 1953–54. After an interval of 2 years beans and potatoes were grown in successive years to measure the combined effects of the residues of the PK fertilizers applied during the years of the Rotation Experiment. The potatoes received a uniform dressing of nitrogen, but none was given to the beans. The yields were:

Table 3

Effects of the accumulated residues of fertilizers applied in the Agdell rotation experiment

Fertilizer treatment (1848–1951) ...	None		PK		NPK	
Rotation	Fallow	Clover	Fallow	Clover	Fallow	Clover
1956 Beans, grain cwt./acre	8·5	5·2	26·2	20·0	18·2	19·2
1957 Potatoes, total tubers tons/acre ...	4·4	3·0	14·4	8·6	15·5	14·1
Percentage ware (1½-inch riddle) ...	91	70	95	92	94	92

Good crops of both beans (with yields up to 26 cwt. grain/acre in 1956) and potatoes (up to 15·5 tons/acre in 1957) were grown on the residues of previous manuring. The highest yields were equal to those obtained on other fields of the Rothamsted Farm where 0·6 cwt. P_2O_5 and 1·2 cwt. K_2O/acre was applied to beans and 1·0 cwt. N, 1·0 cwt. P_2O_5 and 1·2 cwt. K_2O together with 12 tons farmyard manure/acre was given to potatoes. Both crops on Agdell showed large increases in yield due to the residues of phosphorus and potassium in the soils of the PK and NPK plots. For beans the average increase was 14 cwt. of grain/acre, and for potatoes 8·5 tons of tubers/acre. These increases are the combined effects of phosphorus and potassium residues, but the design of the original experiment does not make it possible to separate the effects of the two nutrients. In seven of the eight comparisons between the rotation with fallow and the rotation with clover the latter gave lower yields in the residual years. Even on the unmanured plot, the extra crops (taken as clover or beans) on the clover section have further exhausted the levels of phosphorus and potassium in the soil, which were already very low. In addition to lowering the yields of beans and potatoes by 3 cwt. and 1 ton/acre respectively, the further soil exhaustion caused by the clover crops reduced the proportion of ware potatoes from 90 to 70 per cent. An indication of the cause of the large increase in the proportion of small tubers is given by the phosphorus and potassium composition of the tubers from the fallow and clover sections. The percentages of potassium in the potatoes from the two sections were almost identical, but the crop from the clover section had a much lower phosphorus content. The values were:

	Percentage in dry matter	
	P	K
Unmanured plot:		
Fallow rotation	0·149	1·26
Clover rotation	0·114	1·30

It seems probable, therefore, that although the available phosphorus and potassium in the soil were both very low as a result of cropping for a hundred years without manure, the phosphorus shortage was more acute than the potassium shortage on the clover section, and was mainly responsible for the lower yield and the large reduction in percentage of ware potatoes.

The PK residues in the soil have increased both the percentages of phosphorus and potassium in the crops and also the yields, and the two effects have accentuated the differences in the uptakes of both nutrients on the unmanured and the fertilizer plots. The results are given in Table 4.

TABLE 4

Phosphorus and Potassium Contents of Potatoes, Agdell Field 1957

	Fertilizer treatment 1848–1951 every 4 years					
	O		PK		NPK	
Rotation	Percentage in dry matter					
	P	K	P	K	P	K
Fallow ...	0·149	1·26	0·206	1·75	0·197	1·87
Clover... ...	0·114	1·30	0·180	1·41	0·186	1·50
	Total nutrients in crops, lb./acre					
Fallow ...	3	24	13	110	13	119
Clover... ...	2	18	7	54	12	96
	Gain from fertilizer residues, lb./acre					
Fallow ...	—	—	10	86	10	95
Clover... ...	—	—	5	36	10	78

The percentages of the two nutrients in the potatoes illustrate the high demand made by this crop for potassium relative to phosphorus. The residues of the PK fertilizers have increased the concentration of phosphorus by one-half and potassium by one-quarter of the values in the crops of the unmanured plots. These increases, together with those in yields of tubers, amount to 5–10 lb. extra P and 36–95 lb. extra K/acre in the crops grown on the residues, and provide evidence additional to that obtained from the Exhaustion Land Experiment on the availability of both phosphorus and potassium accumulated in soils from applications of fertilizers over a long period.

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In the Exhaustion Land Experiment the value of the residues was established only for barley, but even for this crop the experiment supplied no information, by direct measurement, on the adequacy of the PK residues for growing maximum crops, nor on the separate effects of the two nutrients. From the yields of barley and the crop composition it was possible to deduce that the yields were governed mainly by the phosphorus supplies in the soil and that, for these amounts, the potassium residues were more than sufficient. A 2-year scheme of modified cropping and manuring was started in 1957 on part of the Exhaustion Land to give a more complete assessment of the residues. Six crops, barley, wheat, potatoes, sugar beet, swedes and kale are being grown side-by-side, and new dressings of P and K fertilizers have been applied to establish phosphorus and potassium response curves by which to measure the individual nutrient effects of the residues. During the period 1856–1900 wheat was grown for the first 20 years on the site now known as the Exhaustion Land, and potatoes in the remaining years. The average annual dressings of P and K fertilizers were 3 cwt. of superphosphate and 2·5 cwt. of potassium sulphate/acre. The accumulation of fertilizer residues in the soil has therefore occurred under conditions which were abnormal but were favourable to the build-up, for wheat is one of the less-exhausting crops, and the rate of manuring was high. The potatoes, although they have a much higher nutrient requirement, did not yield well.

The results from the Agdell Experiment have a greater interest, since the conditions were nearer normal farming practice. The cropping system followed was a Norfolk four-course rotation, and the manuring was not excessive. PK fertilizers were applied to the roots every fourth year, and were equal to 1 cwt. of superphosphate and 1 cwt. of potassium sulphate/acre for each of the hundred years of the experiment. The uptakes of phosphorus and potassium by the crops of the first eight courses were determined by Lawes and Gilbert. The average yields for the eighteen courses up to 1919 differed little from those for the first eight courses, but by 1930 the crops on the NPK plot were affected by soil acidity. Up to 1919 therefore the estimated average excesses of added fertilizer phosphorus and potassium over the amounts of the two nutrients withdrawn by the crops on the fallow and clover sections of the PK and NPK plots were per year:

Fertilizer treatment	PK		NPK	
	P	K	P	K
	lb./acre			
Rotation with fallow	5	15	8	20
Rotation with clover	3	7	5	10

The higher annual excess of phosphorus on the NPK plot arises from the phosphorus contained in the rape cake applied to this plot, while the higher potassium is due in part to the same cause and in part to the absence of K fertilizer applications on the PK plot for the first 32 years of the experiment. If these excesses have accumulated in the soil in an available form during the past hundred years there would be sufficient phosphorus and potassium for a further ten courses of the rotation.

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Nitrogen

The manuring and cropping treatments have produced marked differences in the nitrogen, phosphorus and potassium contents of the soils. In Table 5 the total nitrogen contents are given of soil samples taken on three occasions, two during the course of the experiment and the third in 1953, 2 years after it had ended.

TABLE 5

Total nitrogen, as percentage of surface soil (0–9 inches)

	Treatment					
	O		PK		NPK	
Year	Fallow rotation	Clover rotation	Fallow rotation	Clover rotation	Fallow rotation	Clover rotation
1867	0·127	0·130	0·123	0·135	0·129	0·130
1913	0·118	0·141	0·122	0·148	0·127	0·147
1953	0·119	0·152	0·121	0·152	0·118	0·144

On the soils of the fallow rotation the effects of time and manuring are in accordance with those shown by the Broadbalk plots with similar fertilizer treatments (Warren, R. G. 1956: *Proc. Fert. Soc.* 37). The levels of nitrogen on both the unmanured and the PK plots had fallen by 1913 to the same value, 0·12 per cent N (which is a little higher than on Broadbalk) and had not changed during the next 40 years. For the NPK plot there was a small increase over the other two plots, as on Broadbalk, due to the extra nitrogen in the greater plant residues. Unlike the Broadbalk soil, however, the increase in nitrogen of the Agdell NPK plot was not maintained, and by 1953 the level had fallen to that of the unmanured plot. This decrease may possibly be due to the development of soil acidity on the NPK plot, which affected yields after 1930. The explanation must remain in doubt, since weed growth in the cereal crops increased rapidly as the soil became acid and the crop yields fell. No figures are available to show whether the amount of weed growth below the cutter-bar of the binder was less than the amount of plant residues that would be ploughed in after harvesting a good clean crop.

The greatest effect shown by the figures in Table 5 arose from the replacement of fallow by clover, which increased the nitrogen content of the soil by 0·03 per cent. This increase occurred on both manured and unmanured plots, and it is surprising that the soil of the unmanured plot of the clover rotation had increased in nitrogen as much as the soil of the PK and NPK plots in the same rotation. With lower yields on the unmanured plot, and especially of clover, the smaller amounts of crop residues would return less nitrogen to the soil than residues on the manured plots. The records of the Agdell experiment mention frequently the abundance of weeds on the unmanured plot, but, though the weeds would undoubtedly help to conserve soil nitrogen, the source of the additional nitrogen to compensate for the smaller residues of the clover crops is unknown, since the weeds were predominantly non-leguminous.

The extra nitrogen in the soils of the clover rotation towards the end of the experiment as compared with those of the fallow rotation,

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was about 600 lb./acre, but its rate of mineralization to produce inorganic nitrogen for subsequent crops was small. This is illustrated by the yields of the plots which received phosphorus and potassium but no nitrogen fertilizers. Such data provide the only estimate in this experiment of the availability of the accumulated soil nitrogen. The average yields of barley and the mean total nitrogen contents of the soils for the later years of the experiment were:

	PK plots	
	Fallow rotation	Clover rotation
Barley, grain cwt./acre, mean of 10 seasons, 1913–51	10·5	11·6
N per cent in soil, mean of 1913 and 1953 samples ...	0·12	0·15

The results support the conclusion drawn from the Hoosfield Barley Experiment that, at a level of 0·15 per cent N in the soil, the extra amount of old organic matter residues above the amount in a starved soil provides but little nitrogen for barley. However, further tests of the value of the extra organic nitrogen are needed with other crops, especially with those which would benefit from the nitrogen that is mineralized in the autumn.

Phosphorus and potassium

The phosphorus and potassium analyses of the soil samples taken in 1913 and 1953 reflect the manurial treatments, type of rotation, the carting of roots versus the " feeding " of roots on the plots and also the effect on yield of the more acute soil acidity of the NPK plot of the clover rotation.

Although the comparison of carting versus feeding of roots ceased after 1900, the effect (as measured by soil analysis) on the potassium returned to the land on the " fed " portions of the PK and NPK plots was still detectable in the soil samples taken in 1913. The feeding, compared with carting, gave an average increase of 3 mg. readily soluble K/100 g. soil, the amounts being greater on the fallow rotation and less on the clover rotation. This extra potassium had been reduced to negligible quantities by 1953. The two rotation sections of the unmanured plot showed no differences in soluble potassium on either sampling date, nor were there any differences in the effects of the carting and feeding treatments on the nitrogen and phosphorus contents of any of the plots on these occasions.

The changes in phosphorus and potassium contents of the soils due to manuring and crop rotation are set out in Table 6 for the 1913 and 1953 samples, after averaging the results for the carting and feeding sections of the plots.

The moderate dressings of PK fertilizers that were applied in this experiment have increased the readily soluble phosphorus and potassium in the soils, especially of the fallow rotation. By taking a crop of clover or beans once in 4 years instead of a fallow the supplies of these two nutrients were greatly reduced. On all the fertilizer plots, except the PK plot in the clover rotation, the differences over the unmanured plot which were built up in the earlier years continued to increase, and by 1953 the highest values for soluble P and K attained (and especially for potassium) would be regarded as adequate for crops other than those having a very high requirement, such as potatoes and sugar beet. On the PK clover rotation plot, unlike the other fertilizer plots, the levels of soluble phosphorus and potassium have remained unchanged during the last 40 years. This difference in behaviour, especially as compared with the NPK clover rotation plot, would appear to indicate some abnormality in the PK plot. It is more probable, however, that the NPK clover plot is the discordant one, owing to the onset of soil acidity during the years 1913–53, which led to lower crop yields and uptakes of nutrients, and consequently to greater phosphorus and potassium residues in the soil.

TABLE 6

Phosphorus and potassium in the Agdell soils 1913 and 1953

Fertilizer Treatment...	O		PK		NPK	
Rotation: fallow (f), clover (c)	f	c	f	c	f	c
	P soluble in $0{\cdot}5M$-$NaHCO_3$, mg./100 g.					
1913	0·25	0·20	1·05	0·55	1·00	0·55
1953	0·30	0·20	1·35	0·55	1·15	0·75
	K soluble in $0{\cdot}5N$-HAc, mg./100 g.					
1913	8·5	8·5	17·0	13·0	15·5	11·5
1953	9·0	9·0	20·5	14·0	20·0	17·0

The acidity of the clover and fallow parts of the NPK plot, though an unfortunate development for the main objects of the Agdell experiment, has provided some information on the availability of phosphorus in the soil derived from applications of superphosphate given before and during the period of acidity. The clover section was the more acid at the conclusion of the experiment, with about half the area at pH 4·8–5·0 and the remainder at pH 5·2, while the soil of the fallow part was almost entirely within the range pH 5·4–5·6. The chalking carried out in the winter of 1953–54 raised the values to a little above pH 7. In 1956 and 1957, when beans and potatoes were grown to measure the residual values of the PK fertilizers, the yields were good and similar to those on the PK plot, which had not developed acidity. The uptakes of phosphorus given in Table 4 were almost identical for the fallow sections of the two plots, while for the clover sections the amount of phosphorus in the potato crop of the NPK plot which had been acid was greater than that of the PK plot. Although no comment can be made on the state of the phosphorus during the period of soil acidity, the results of the potato crop show that after bringing the soil to pH 7 the availability of the phosphorus residues was at least equal to that of the residues in the soil which had not been acid.

The yields and nutrient contents of the potato crop in 1957 and the soil analyses have established two facts: first, that residues of PK fertilizers have accumulated, and secondly that these residues are available. However, the three types of figures, taken singly or collectively, do not give quantitative estimates of the reserves built up in the soil by the fertilizers. To obtain this information half of each plot will be sown with a crop able to exhaust the soil rapidly, such as grass cut for silage, to which adequate nitrogen fertilizer will be given. The value of PK fertilizer residues for a wider variety of crops will continue to be tested in the Exhaustion Land Experiment. As the manuring and cropping treatments of the Agdell experiment have produced a set of soils with different phosphorus and potassium contents, the halves of the plots not put down to grass will be used for a more precise standardization of methods of soil analysis. The Agdell series is much more suitable for this purpose than the series on the Exhaustion Land, where the levels of nutrients are generally either "low" or "medium". With the additional information that would be obtained from this extension of work suggested for Agdell Field, soil analysis could profitably be used more frequently for avoiding the accumulation of fertilizer residues to an unnecessary degree and (of equal importance) to prevent a gradual exhaustion of the soil.