ROTHAMSTED RESEARCH

Report for 1977 - Part 1

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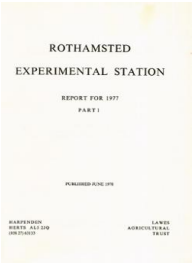

Plant Pathology Department

E. Lester

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PLANT PATHOLOGY DEPARTMENT

E. LESTER

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Introduction

There was a substantial improvement this year, compared with last, in growth and yield of cereals, associated with low levels of most foliage diseases and, in spring barley, late development of mildew, a situation reflected in generally poorer responses to fungicide treatments. The season was, however, favourable to take-all, which caused large yield decreases in some experiments. The summer weather encouraged potato blight which became epidemic on unsprayed King Edward at Rothamsted for the first time in 9 years: fortunately, the drier weather of September and October prevented much tuber infection. Aphids were few and late arriving and a supply of our own seed for the 1978 field experiments is assured, unlike the two previous seasons when stocks of King Edward had to be discarded because of much virus infection (p. 228). The use of serologically distinct strains of potato soft-rot bacteria is helping us to elucidate different aspects of soft-rot and blackleg epidemiology and an active search for bactericides has been started using standard techniques already developed (p. 224). Investigations on potato gangrene have shown that the pathogen is able to move systemically in rooted cuttings and can be

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isolated, though infrequently, from stems of growing plants during the season. The possible role of stem lesions in gangrene epidemiology, revealed by the failure of the stem cutting technique effectively to control the disease, is being investigated in parallel with efforts to develop rapid identification of the pathogen in soils (p. 225). Improvements in the efficacy of fungicidal treatment for the control of tuber diseases in order to maintain the health of seed tubers is one of our main objectives. The possibility of increasing the systemic activity of thiabendazole has been demonstrated and several other fungicides have been assessed (p. 226).

Studies on the overwintering of *Rhynchosporium secalis* in the field have demonstrated unexpected effects of herbicides and fungicides on sporulation and have shown that the disease is able to spread throughout the winter (p. 214). The significance of crop residues and volunteers as a source of infection for a neighbouring winter barley crop has been clearly established (p. 214). The season was favourable for the development of take-all but our tentative prediction of greater damage after a 1-year break than after wheat was not borne out, although the relative percentage plants infected was as predicted. In spite of the effort put into take-all studies over the years, aspects of the disease remain baffling, although some are better understood now that the relationships, both taxonomic and pathogenic, between the take-all pathogen and related fungi are becoming a little clearer (p. 216). The work on cereal diseases in reduced cultivation systems (p. 217) has compared methods of straw disposal with the unexpected result that straw burning has increased eyespot in the following crop in some treatments, reinforcing the need to investigate the effects of cultivations, to which attention was drawn last year. The possibility that oat golden stripe virus may be present commonly in oat crops infected with oat mosaic virus is suggested. Cultivar differences in susceptibility to oat golden stripe virus and differential accumulation of the virus in roots and tillers have been demonstrated (p. 212).

Further tests of ryegrass clones resistant to ryegrass mosaic virus have confirmed their resistance to all but one isolate of the virus so far collected and the few plants found to be infected on first testing have been shown to be free of virus on subsequent tests (p. 218). A new virus record for Britain, brome mosaic virus, is reported (p. 218). Red clover necrotic mosaic virus has been confirmed in farm crops of red clover and substantial differences in cultivar susceptibility observed in the field have been confirmed for some varieties by manual inoculation. It is tentatively suggested that the virus may have a fungal vector (p. 219).

In an attempt to anticipate disease problems that might arise should lupins become a more important farm crop, we have shown that several pathogens of field beans and peas are highly pathogenic to lupin in laboratory tests but these results were not repeated in pot tests using field soil. An interaction between virus and fungus infection of lupins that apparently results in virus infected plants becoming more susceptible to invasion by the fungus is described (p. 220).

In a field experiment on chocolate spot of winter beans to investigate effects of seed-rate, spacing, irrigation and fungicides, the best treatment combination gave a yield almost 70% greater (at 5 t ha^{-1}), than the worst combination (p. 221). The complexity of the virus position in field beans is emphasised by the discovery that a previously-noted bright yellow vein-banding symptom is caused by an agent that is transmitted by aphids only in the presence of a helper virus, usually pea enation mosaic virus (p. 221). The finding of virus-like particles in several field bean and broad bean cultivars showing no symptoms (p. 222), akin to the beet cryptic virus situation, suggests that this may be a phenomenon worthy of further investigation to establish the extent of its occurrence and significance in annual crops.

Seed contamination is shown to provide a ready means of introducing infections of *Pyrenopeziza brassicae* into crops of oilseed rape; fungicidal seed treatment and/or foliar

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sprays offer the possibility of control. Spores of this pathogen and of *Leptosphaeria maculans*, the cause of canker, have been trapped in oilseed rape crops throughout the period January–June and the detrimental effect of dalapon reported previously has been shown to occur also with the canker fungus (p. 222).

From further studies of virus inactivation, a possible role of chelating agents in disruption of protein-RNA bonds is suggested (see below). Comparison of the effects of polyacrylic acid on TMV multiplication in tobacco plants and protoplasts indicates a different action and collaborative work with the Biochemistry Department has demonstrated a lack of effect of b proteins on virus multiplication in protoplasts (see below). Investigation of the distribution of beet cryptic virus has shown it to be present in cultivars of fodder beet, red beet and mangel and that a similar but serologically unrelated particle was present without causing symptoms in one variety of red beet (p. 210).

Properties of viruses and virus diseases

Uncoating of tobacco mosaic virus (TMV) on the cell surface. Last year we described experiments which showed that more than half of the TMV infiltrated into the intercellular spaces of *Nicotiana tabacum* cv. Xanthi-nc or adsorbed on to leaves of *Phaseolus vulgaris* by immersion lost its infectivity in 24 h. Further experiments with *P. vulgaris* have shown that 5 min after immersion in TMV about 3% of the adsorbed virus was inactivated and 50–60% after 1 h. Electron microscopy showed that about 30% of the virus particles were shortened to differing extents and about 10% were lost entirely in the next 24 h. These studies suggested that inactivation possibly proceeds by the uncoating of virus particles from one end rather than by breaking of particles at random. Virus adsorbed to the surface of tobacco protoplasts was not inactivated. TMV treated with EDTA degraded a little more readily than untreated virus and we suggest that inactivation and uncoating possibly take place in a hydrophobic environment by a disruption of bonds between protein subunits and RNA and are facilitated by chelating substances. (Kassanis and Kenten)

Effects of polyacrylic acid and b proteins on TMV multiplication in tobacco protoplasts. Protoplasts incubated at 10°C for 1–3 days with 50–100 μg ml^{-1} of polyacrylic acid (PA) mol. wt. $3{\cdot}5\times10^3$ before inoculation with TMV gave virus yields decreased by 40–60%. The same reduction occurred when PA was added to the protoplasts 7 h after inoculation and some reduction was found even when PA was added 24 h after inoculation. The virus yield of PA-treated protoplasts was reduced to the same extent whether treated protoplasts were kept at 25° or 32°C after inoculation, indicating that the action of PA in protoplasts is of a different nature from that found in plants (Gianinazzi and Kassanis, *Journal of General Virology* (1974) **23,** 1–9). Further, in White Burley protoplasts, multiplication of the mutant Ni 118, which causes necrotic lesions on this cultivar, was also decreased by PA but lesion number on leaves was unaffected.

Protoplasts obtained from leaves sprayed to run off with 20 mg PA ml^{-1} were infectible, although sprayed leaves produced 80–90% fewer lesions than unsprayed leaves. It is possible, however, that any protoplasts made resistant by PA were destroyed during extraction. (Kassanis and White)

Partially purified preparations of b proteins (Gianinazzi *et al. Journal of General Virology* (1977), **34,** 345–351) or well purified preparations (Antoniw, in press) were used to test whether incubation with b proteins induced virus resistance in protoplasts. Protoplasts from Xanthi tobacco were incubated with 60–200 μg b protein ml^{-1} for 1 day at 10°C or for 5 h at 25°C before inoculation with TMV. After inoculation the protoplasts were returned to the medium containing b proteins. We found no difference in virus

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yield when compared with control protoplasts. (Kassanis and R. F. White, with Antoniw, Biochemistry Department)

Beet cryptic virus in some cultivars of *Beta vulgaris*. Kassanis, White and Woods (*Phytopathologische Zeitschrift*, 1978) reported that beet cryptic virus (BCV) was present in 90% of plants of three sugar-beet cultivars without causing symptoms. They also showed that the virus caused a stunting disease of spinach beet and that a virus very similar in some characteristics but not apparently serologically related to BCV was present in cultivars of red beet, again without causing symptoms. Individual plants of fodder beet, red beet and mangel were examined for the presence of a BCV-like virus using the electron microscope. In the two fodder beet cultivars examined, 26 out of 30 plants of cv. Red Otofte and 27 out of 30 plants of cv. Monorosa were infected. Out of 24 plants examined in each case, mangel cv. Red Intermediate had 16 and red beet cvs. Little Ball and Boltardy had respectively 22 and 10 infected plants. In all cultivars except Boltardy, the virus gave a positive reaction against an antiserum prepared to BCV from sugar beet. (R. F. White and Woods)

Viruses affecting arracacha (*Arracacia xanthorhiza*). Further studies of arracacha virus A (*Rothamsted Report for 1976*, Part I, 252) have shown that the 125S component is infective alone while the 92S component is not infective. Like raspberry ringspot virus, addition of the 92S component to the 125S component enhances its infectivity, supporting our suggestion that arracacha virus A is a member of the NEPO virus group. (Kenten, with Dr. R. A. C. Jones, International Potato Centre, Peru)

Biodeterioration

Moulding can greatly decrease the quality of stored agricultural produce, while saprophytic fungi growing on grain before harvest may hasten senescence, decrease yield and germination and give rise to flours of inferior colour. Moulds may decrease food value or produce toxic metabolites while their spores may cause allergy or infection. Our work concerns the identification of the moulds, the sources from which they come, conditions which favour their growth and chemicals to prevent moulding.

Chemical preservation of damp hay. The spread of fungi from untreated hay into hay treated with propionic acid and ammonium propionate is described in the Report of Chemical Liaison Unit (Lacey, with Lord and Cayley)

Retention of harvest dust by filter units on combine harvester cabs and a ventilated helmet. Combine harvesters operating in oilseed rape and cereal crops can generate much dust, composed largely of fungus spores at least half of which may be *Cladosporium*. On one occasion the load in the air close to a combine harvester driver was 2 g dust m^{-3} containing $7{\cdot}5 \times 10^9$ spores. Sponge rubber or paper filters fitted to combine harvester cabs retained most of this dust, so that the atmosphere inside usually contained less than 5% and sometimes less than 1% of the dust in the air outside whether measured by spore number or weight of dust. Failure to clean cabs and the common practice of leaving cab doors open, substantially reduced the benefit conferred by filters. During combining without a cab, an 'Airstream' helmet (Racal Amplivox Ltd.) supplied air to the wearer containing 95% fewer spores than outside. (Lacey, with K. A. McLean, ADAS, Eastern Region)

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Microflora and grain quality

During ripening. Before ear emergence the embryonic grain was sterile or colonised by very small numbers of bacteria and fungi ($<0{\cdot}6\times10^6$ propagules g^{-1}). After emergence from the leaf sheaths, numbers of propagules on the grain increased rapidly to maxima at harvest of 2000×10^6 g^{-1} (bacteria) and 18×10^6 g^{-1} (fungi). By contrast, in 1976, colonisation was less and maximum numbers occurred earlier in the season. Fungicides applied to winter-sown (cv. Cama) and spring-sown (cv. Sappo) wheats, once, twice and three times between heading and harvest modified the grain surface microflora and affected yield, grain weight and germination, seedling height and shoot dry weight (Table 1). However, whereas captafol was very beneficial on Cama, it was much less effective on Sappo, although it decreased *Alternaria* more than any other fungicide on both. 'Delsene M' (carbendazim+maneb) was best on Sappo. Causing spring wheat to lodge greatly increased the incidence of *Fusarium* on the grain, decreased *Alternaria* and other fungi, decreased the yield (to less than one-third of that from supported wheat) and germination of grain (by 14%) but had little effect on seedling shoot dry weight. Irrigation, in this wet season (unlike 1976) and increased application of nitrogen fertiliser to the crop had little effect on the microflora or on yield, germination and shoot dry weight. Chlormequat slightly increased *Alternaria* and decreased *Fusarium* on the grain and improved yield and germination.

During storage. The colonisation of stored wheat by micro-organisms was dependent on the initial grain water content, the temperature and aeration. Germination was related inversely to numbers of fungi.

TABLE 1

Effect of fungicidal sprays between heading and harvest on yield of wheat harvested in 1977 (% change from unsprayed controls)

	Fungicide	Yield	1000 grain weight	Germination	Shoot dry weight	Shoot height
Winter wheat (Cama)	Benomyl	−5·2	−0·03	−2·5	+29·4	+4·7
	Captafol	+12·6	+4·7	+4·2	+38·7	+8·2
Spring wheat (Sappo)	Benomyl	+1·7	+1·1	+1·3	+0·4	—
	Captafol	+1·0	+3·3	−1·7	+7·7	—
	Carbendazim +maneb ('Delsene M')	+2·3	+1·2	+4·6	+6·2	—

Milling and baking tests. The results of tests on grain harvested in 1976, carried out by the Flour Milling and Baking Research Association are shown in Table 2. The flour yield from both cultivars was lower than expected, probably because grains were small following the drought. All flours had good colour but the benomyl-treated winter wheat was worse than other winter wheat flours, perhaps because of greater colonisation by *Alternaria*. Alpha amylase in cv. Cama was increased significantly by fungicidal treatment, more by captafol than benomyl. Loaf scores were best for control and benomyl-treated Cama flours, exceeding the average score (24) for bread from English wheat flour and significantly better than treatments containing captafol. By contrast, the benomyl-treated Sappo flour gave a loaf score significantly worse than all other treatments.

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Micro-organisms were few in all flours but numbers of bacteria in the fungicide-treated Cama flours were less than one-fifth of those in the control flour. (Hill)

TABLE 2

Effect of fungicidal sprays applied to wheat during ripening in 1976 on flour, milling and baking properties

Cultivar	Treatment	Flour yield %	Protein % (at 14% H_2O)	Flour colour grade*	Alpha-amylase (Farrand units)	Loaf score (max 50)	Bacteria (No. of propagules $g^{-1} \times 10^{-3}$)	Fungi
Cama (winter)	Control	70·9	11·6	−0·4	9·4	33	12·1	0·4
	Captafol	71·7	11·4	−0·3	21·5	23	3·1	0·4
	Benomyl	73·5	11·5	0·2	15·9	35	2·1	0·5
	Captafol+ Benomyl	72·1	11·8	−0·5	24·9	17	1·3	0·4
Sappo (spring)	Control	69·8	13·2	0·2	1·3	24	3·9	0·3
	Captafol	71·0	13·4	0·3	1·1	28	3·6	0·5
	Benomyl	70·2	13·8	0·2	1·3	18	3·0	0·3
	Triadimefon	70·5	13·7	0·1	1·3	25	3·3	0·2

Note: Results based on single samples
* scale from −2 to +12, lowest score best

Cereal diseases

Oat mosaic virus (OMV). The effect of this virus on yield was tested in a small-pot experiment. Winter oats, cvs. Peniarth and Powys, were inoculated with OMV by spray-gun in November and the plants kept in a cool glasshouse without artificial illumination throughout the winter. During this time infected plants had pronounced mosaic symptoms and grew markedly less well than the water-sprayed controls. In spring, the pots were placed outside and additional fertilisers given. At harvest in July, panicle number per pot was little changed by infection but grain number was decreased by 25% and grain weight by 33%. (Macfarlane and Plumb)

Oat golden stripe virus (OGSV). This virus, which was reported last year (*Rothamsted Report for 1976*, Part 1, 256–257) but not named, is soil-borne and has tubular particles of two lengths, 152±1 and 305±2 nm. An apparently identical virus has also been reported from Aberystwyth (*Report of the Welsh Plant Breeding Station for 1976*, 141–3) where the name oat tubular virus (OTV) is preferred. Both names have disadvantages as the golden stripe symptoms are distinct only late in plant growth and when temperatures are greater than 20°C, whereas particle structure can be seen only by electron microscopy.

In addition to the known sites of infection of OGSV in Devon and Kent, one or two areas about 10 m diameter were found in an 8-ha crop of Maris Osprey winter oats in Warwickshire in March this year. Almost all the crop was infected with OMV and the OGSV infected plants were found only after detailed visual examination and electron microscopy. It is possible that OGSV is commonly present in crops infected with OMV. Winter oats cvs. Peniarth, Pendrwm, Maris Osprey and Maris Quest were sown in boxes of infected Kent soil in October 1976 and within 6 weeks, 60% of the roots but none of the leaves of Peniarth contained OGSV; OMV was present in only half as many roots. The fungus *Lagenocystis radicicola* occurred in all roots and *Pythium* spp. and *Polymyxa graminis* were present in 40% but there was no apparent association of a particular fungus with virus infection. Bait oat seedlings placed in water for 1 or 3 days along with roots of infected plants and subsequently grown in sand culture, did not become infected by virus although some seedling roots became heavily infected by *Lagenocystis radicicola* or *Olpidium brassicae*, neither of which thus seems to be the vector. Although OGSV was

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first found in roots, OMV was the first virus detected in leaves. All leaves of Maris Osprey contained OMV alone on 25 February, even though 60% of the roots contained OGSV particles.

Most plants sown in infected soil showed symptoms of OMV in spring; these disappeared in June and July when symptoms of OGSV became apparent. Maris Quest and Peniarth showed symptoms of OGSV on 16·9 and 16·7% tillers respectively, whereas Pendrwm (11·3%) and Maris Osprey (6·4%) had significantly ($P = 0·05$) fewer. However, the effect of OGSV on yield was greatest for Maris Osprey, a decrease of 55% over OMV infection alone, whereas yields of Peniarth and Pendrwm were decreased by 45 and 35% respectively and Maris Quest was unaffected. Wheat cv. Cappelle-Desprez and barley cv. Maris Otter showed no symptoms of OGSV infection and virus particles were not found in roots or leaves. Successive transfers of OGSV were made in *Nicotiana clevelandii* kept at 15°C day/10°C night after inoculation. (Plumb and Macfarlane)

Powdery mildew (*Erysiphe graminis*) on winter barley

Epidemiology of mildew. Early sown winter barley, despite being infected with powdery mildew in the autumn and winter, is often very much less affected in the following spring and summer than later sown crops. In 1976, we began a research project aimed at explaining the differences in disease susceptibility shown by winter barley sown at different times. We have measured disease development in the field on crops sown at different times and shown the effects of vernalisation on crop growth and disease susceptibility in the glasshouse.

A computer simulation of a mildew epidemic has also been prepared, which indicates that the smaller amount of mildew on early sown crops may be due to disease escape. In the spring, growth of early-sown crops is rapid while mildew development is slow due to the prevailing low temperatures. (N. White, Jenkyn, Finney and Turner)

Deposition of* Erysiphe graminis *spores. Theoretical predictions of the rate of deposition of particles on obstructions in flowing air may be tested in a wind tunnel or in the field. Horizontal slides, vertical cylinders and barley plants were exposed at various heights in a plot of mildew-infected spring barley. Suction traps were used to measure the concentration of spores in the air at each exposure height. Deposition on vertical surfaces often exceeded the number expected from the measured concentration by more than ten times. Much of this extra deposition was probably due to the presence of many large clumps of spores giving enhanced impaction. However, the frequent occurrence of clumps does not afford a complete explanation, as the deposit on horizontal surfaces, which might also be expected to increase as a result of clumping, was less than expected. Analysis of the deposition of *E. graminis* spores within crops will be complicated by the presence of a range of particle sizes; dispersion by clumps as well as by single spores could, however, be epidemiologically important. (Bainbridge and Stedman)

Carbon assimilation and translocation. In 1976 radioactive ^{14}C was used in a field experiment with spring barley to investigate the effect of mildew infection on the movement of material assimilated by the second and third youngest leaves. However, powdery mildew infection was slight in this experiment. Perhaps for this reason no effect on assimilation or translocation was detected at any feeding. Of the ^{14}C supplied at G.S. 45 (Zadoks *et al. Weed Research* (1974) **14**, 415–421) to the second and third leaves, 49 and 30% respectively was in the ear at harvest but over half was in the awns and structural parts of the ear. The majority of the ^{14}C assimilated by the second and third leaves at G.S. 58 moved rapidly down the plant but some was subsequently remobilised to appear

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in the endosperm of the grain at harvest, with very little in the structural parts of the ear. The ^{14}C assimilated by the second leaf at G.S. 75 was immediately moved to the grain. Almost 90% of it was present in the ear at harvest, with less than 1% in the structural components. (Finney and Bainbridge)

Observations on *Rhynchosporium secalis*

Effect of herbicides and fungicides on the number of spores on barley stubble and volunteers. The reported effect of paraquat on the number of spores of *R. secalis* on barley stubble and volunteers (Stedman, *Plant Pathology* (1977) **26**, 3) was confirmed in experiments in 1976 and 1977. In both years a single spray of captafol was applied to plots unsprayed or sprayed 7 days previously with paraquat. In 1976, shed grain germinated in July but volunteers did not become infected until October by which time they were well tillered. Plots were sprayed with paraquat in late October but plants on these plots retained some green tissue until late December. Captafol was ineffective in decreasing spore numbers per plant. Paraquat substantially increased spores per plant 1 week after treatment and substantially decreased them at all sampling dates after mid-November. At the end of February, $1{\cdot}5\times10^6$ spores per plant were washed from control plants compared with $3{\cdot}2\times10^4$ spores from plants sprayed with paraquat.

Two experiments in 1977 with volunteer plants at a much earlier growth stage again showed that captafol tended to increase the number of spores per plant for 2–4 weeks after treatment. Single sprays of benomyl, carbendazim, triadimefon and thiophanate tested in one experiment, decreased the number of spores per plant 7 days after spraying; at 14 days all had decreased the number by over 95% (to $3{\cdot}5\times10^4$ spores per plant) compared with unsprayed plants.

Glyphosate applied in early October or 3 weeks later had no effect on the number of spores on stubble and did not cause the large increase in spore number produced on volunteers that followed paraquat treatment. Five weeks after treatment, spore number on volunteers was decreased by ca. 80% compared with the number recovered on the day of spraying and by 97% compared with untreated on the same sample date. More spores were found on plants sprayed with paraquat than on those sprayed with glyphosate in the 3 weeks after spraying but thereafter, more were found on the latter. (Stedman)

Viability of spores. Spores washed weekly from the remains of volunteers sprayed with paraquat in late October 1976 were inoculated on to pot-grown barley plants (cv. Maris Otter) up to the end of February. Viable spores were found to be present throughout the period. A series of healthy pot-grown plants which were exposed in a plot of barley infected with *R. secalis* and removed to isolation after each rain occasion throughout January and February confirmed that viable spores were present and that spread of infection took place throughout the winter. (Stedman and Parkins)

Spread. In late October 1976, a field was drilled with winter barley cv. Maris Otter, (previous crop wheat). This field was on the down-prevailing-wind side of a source of *R. secalis* spores on barley stubble and volunteers. In early February and subsequently every 3 weeks, 25 plant samples were taken every 10 m on a down-prevailing-wind line across the field. Spores of *R. secalis* from the samples were estimated by washing the plants and counting the spores on a haemocytometer slide. On the first samples, spore number declined from $3{\cdot}3\times10^5$ spores per plant at 10 m to zero at 60 m. By early March, spores were found on all samples across the field to the further side at 220 m but a steep gradient persisted within the first 60 m. By late March this gradient had almost disappeared and by mid-April, spore numbers were approximately uniform across the field. (Stedman)

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Take-all disease. In these reports the following abbreviations are used for simplicity:

Gaeumannomyces graminis var. *tritici* = Ggt;
G. graminis var. *graminis* = Ggg;
Phialophora radicicola var. *radicicola* = Prr;
P. radicicola var. *graminicola* = Prg.

Epidemiology of take-all. From the results of soil assays for take-all infectivity we predicted last year (without much confidence) that winter wheat in 1977 might be more at risk from take-all after a 1-year break than after previous wheat (*Rothamsted Report for 1976*, Part 1, 261). This prediction was partly correct for the fields we are studying: the percentage of plants with take-all in July was 24, 4 and 5 in the first, second and third wheats after oats respectively. However, our caution was justified because the infection on the first wheat was mostly slight and the crop damaged little. In other experiments at Rothamsted, take-all developed extensively and seriously decreased yields (up to 50%), so the season cannot be described as unfavourable to take-all. Thus it seems that though the inoculum surviving the oat break in 1976 was widespread (74% of soil cores with infected seedlings in September 1976), it was insufficient to cause serious take-all. However, soil assays during June to September this year showed a large increase in infectivity during the first wheat crop, so that assay seedlings grown in soils from stubble on September 6 had four times as many roots infected as those in the assay of oat stubble soils the previous September, though the proportion of soil cores infected was similar (78%). This increase was not entirely a consequence of the widespread inoculum surviving the oat break, because infectivity also greatly increased in soils from the second and third wheats where infectivity had been small in 1976. It seems that the inoculum of the take-all fungus has recovered from the exceptionally dry summers of 1975 and 1976, and we will be surprised if take-all is not common in the winter wheat on our sites in 1978, at least in the early spring. (Slope and Gutteridge)

Preservation of fungi of the* Gaeumannomyces-Phialophora *complex. The ability to maintain cultures of the take-all fungus (Ggt) without introducing undesirable changes is often an important requirement for studies of the pathogen. The successful reconstitution and growth of two isolates shortly after vacuum-drying in skimmed milk was reported in *Rothamsted Report for 1971*, Part 1, 147. Six years and 2 months later, vacuum-dried phialospores contaminated with mycelial fragments no longer produced new growth when reconstituted with tap water but macerated mycelium treated similarly produce normal growth. The parent material of the macerate, Og12, had been sub-cultured in 1971 and maintained on agar by twice-yearly transfers and storage at 5°C. In wheat-seedling pathogenicity tests, the revived culture did not cause disease, whereas the agar-maintained culture infected 12 out of 20 plants and 20·8% of roots.

The search for simple, reliable methods of long-term preservation has continued with tests of Boesewinkel's method (*Transactions of the British Mycological Society* (1976) **66,** 183–185). A culture of Ggt isolated from maize in September 1975 and shown to be pathogenic on wheat was preserved at room temperature as small pieces of colonised agar in sterile distilled water in May 1976. One year later, it was sub-cultured and its pathogenicity tested on wheat seedlings. It infected all ten seedlings exposed and 51·8% of roots. Both methods seem worthy of more extensive testing. (Hornby)

Cultures of five isolates of Ggt and three each of Ggg, Prr and Prg grown on 10 g expanded, unmilled aluminium silicate (grade EUP 6X, Johns-Manville (G.B.) Ltd., Richmond, Surrey) with 2% malt extract (2ml g^{-1} silicate) in conical flasks plugged with polyurethane foam and capped with aluminium foil, survived at least 18 months at room temperature. Monthly tests of viability were made by plating individual, colonised

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granules (*c.* 200 g^{-1} silicate) on potato dextrose agar. Only one isolate each of Ggt and Ggg and two of Prg failed to grow after 2 years, but there was no association between survival and the presence or absence of virus in the isolates. This method of preservation seems useful for routine laboratory storage up to 18 months, perhaps longer if the flasks are opened less frequently than in these tests thereby conserving moisture. (Rawlinson and Muthyalu)

Gaeumannomyces-Phialophora *complex: an early isolate of Prr.* In July 1967, a fungus identified as *Ophiobolus graminis* was isolated in a bioassay of spring wheat soil from Road Piece, Woburn and maintained in stock as isolate 4. Its pathogenicity to wheat and growth on wheat coleoptiles were tested in 1977 and revealed that the fungus is Prr. Prr has been isolated previously by us from roots of barley in Hoosfield (*Rothamsted Report for 1974*, Part 1, 227) and seen on the roots of bioassay plants grown in soil from Woburn (see below) but 4 may be the earliest recognised isolate of the fungus in Britain. (Hornby)

The effect of breaks on take-all experiment at Woburn. An experiment of 18 plots designed to develop a number of cropping sequences suitable for studying the take-all decline (TAD) phenomenon in continuous spring barley was laid down in two blocks in 1972 on a part of Butt Furlong that had grown cereals each year since 1967. A knowledge of the populations of the take-all fungus in this soil had accumulated in the previous 4 years and was judged to off-set the obvious disadvantages of using the sloping site for a rotation experiment. Take-all had declined in the field following a peak incidence of the disease in 1969 (in a third cereal crop) and the intention was to eliminate and then redevelop TAD by the phased introduction of 2-year breaks (fallow then beans) so that by 1978, 1st–6th and 12th barley crops would be available for comparison. In each year of the experiment the following assessments and measurements were made:

February	Soil bioassays for take-all Chemical analyses of the soil*	} all plots
1st July	Take-all assessments and height measurements on the barley crop	
August	Yield	
Post-harvest	As February	

*Total N, NH_4, NO_3 and pH

Take-all has remained at a remarkably low level (<4% roots infected in the field) throughout the experiment and can have had very little effect on yield. Although yields have been slightly increased by breaks, disease in the subsequent crops has never built up much above those levels in the continuous barley plots. It is not known whether this represents the persistence of TAD throughout the breaks or reflects unfavourable weather that also produced 2 very low-yielding years (1975, 1·73 t ha^{-1}; 1976, 1·95 t ha^{-1} compared with an average 4 t ha^{-1} for the previous 3 years). The very noticeable occurrence of Prr on many of the plants in the autumn 1977 bioassay, opens up the possibility that this fungus may be associated with the generally low levels of take-all in the experiment. However, it was most abundant in soil from the western block, whereas take-all was similar in both blocks; and in soil that had grown five barley crops, the greatest incidence of take-all coincided with the greatest occurrence of Prr.

Yields and chemical data have confirmed the expected north–south trend caused by the sloping site but an east–west trend also exists and has resulted in as much as 40% more grain from the eastern block in dry seasons. Initial studies indicate that this is

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associated with the texture of the subsoil. (Hornby and Henden, with Catt, Soils and Plant Nutrition Department)

Soil N and take-all. Work on soil N and take-all is described in the Report of the Soils and Plant Nutrition Department p. 279. (Hornby, with Ashworth and Margaret Brown)

The effect of Poly(2-vinylpyridine 1-oxide) (PVO) on silicon content and infection of wheat roots. It is known that silicon accumulates in the endodermis of rice roots (Parry D. W. and Soni S. L. *Annals of Botany* (1972) **36,** 781–783) and also that the uptake of silicon into rice leaves is increased in the presence of PVO (Parry D. W. *Annals of Botany* (1975) **39,** 815–818). Experiments were done to determine whether PVO increased the deposition of silicon in the endodermis of wheat and if so whether this deposition would affect hyphal penetration by Ggt. Wheat seedlings were grown in liquid culture for 4 weeks using a full nutrient solution with the addition of 0.025% PVO and/or sodium metasilicate equivalent to 100 ppm SiO_2.

The % silicon contents of the roots (0·60%) and tops (5·25%), as measured by X-ray fluorescence spectrometry, were not increased by the presence of PVO.

A further pot test was done in which wheat was grown for 10 weeks in one of the following ways:

(a) Wheat was sown directly into a sandy loam soil, naturally infested with Ggt, with or without the addition of PVO and/or sodium metasilicate at the same rates as the liquid culture tests.

(b) Single wheat plants were grown in sterilised soil in 10 mm diameter bore transparent PVC tubing and watered with 0·025% PVO and/or sodium metasilicate (100 ppm SiO_2). After 3 weeks, the tubes were split open and the cores transplanted into pots containing the same soil as (a), thus allowing the undisturbed pretreated root systems to come into contact with Ggt infested soil

In the presence of PVO, there was a slight decrease in % silicon content of the roots (SiO_2+PVO 1·44%, SiO_2 1·84%) and of the tops (SiO_2+PVO 1·68%, SiO_2 2·05%), though the dry weight of the tops was increased (SiO_2+PVO 9·05 g, SiO_2 6·28 g). The proportion of roots with take-all was unaffected by the addition of PVO and/or SiO_2, but was halved by the transplant method (b) Energy dispersion X-ray analysis showed that silicon in the roots is concentrated in the walls of the endodermis. (Prew, Read and Carpenter)

Diseases in reduced cultivation systems

Eyespot* (Pseudocercosporella herpotrichoides) *and straw burning. The joint National Institute of Agricultural Engineering (NIAE)/Rothamsted winter wheat cultivation experiments at Boxworth Experimental Husbandry Farm and Rothamsted have for the last 2 years included a comparison of straw burning versus straw baling preceding cultivation with the NIAE rotary digger or a tine system. In 1976, there was more eyespot on the burnt than the baled rotary-dug plots on both sites but there was no difference between the tine cultivated plots. In 1977, burning increased the incidence of eyespot on both cultivation treatments

% Straws with eyespot—July 1977

	Rothamsted		Boxworth EHF	
	Flexitine	Rotary digger	Chisel plough	Rotary digger
Straw baled	26	46	15	16
Straw burnt	60	62	26	31

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Although on the tine cultivated plots, particularly at Rothamsted, the plant population was less on the unburnt plots, which could account for some of this difference, the plant and tiller counts on the rotary dug plots showed little difference between treatments. (Prew and Read)

Diseases of grass and forage crops

Virus diseases of grasses

Clones of perennial ryegrass resistant to ryegrass mosaic virus (RMV). The selection from within S.23 of two clones of perennial ryegrass resistant to a Rothamsted isolate of RMV (RMV-R) was described in the *Rothamsted Report for 1975*, Part 1, 258. In the *Rothamsted Report for 1976*, Part 1, 266, we reported occasional infection of these two clones following manual inoculation with isolates collected throughout Britain. However, subsequent tests on the inoculated plants have failed to reveal infection and it is apparent that if infection did indeed occur initially, the virus has subsequently been lost. Tests on the resistance of these clones were extended in spring 1976 to nine field trials, planted throughout Britain. At all sites, a proportion of the S.23 plants included as controls became infected (25/40 at Cambridge). In contrast, infection has been found in only one plot of a resistant clone at Saxmundham; glasshouse tests have confirmed that this isolate can infect both resistant clones and their seedling progeny though with greater difficulty than S.23 plants.

In pot experiments, five out of 80 seedlings obtained by crossing the resistant clones became infected after inoculating with RMV-R, in comparison with all 80 of S.23. The resistant progeny yielded about 30% more dry matter than infected S.23 and about 15% more than healthy S.23. (Gibson, with Dr. A. J. Heard, Grassland Research Institute)

Effects of ryegrass mosaic virus on grass yields. During 1976, plots of Italian (cv. S.22) and perennial (cv. S.24) ryegrass, initially infected in spring 1976 by manual inoculation with RMV from various sources and protected from infestation by the mite vector of ryegrass mosaic with a plastic house, yielded 11% less than healthy plots. Between June 1976 and June 1977, the proportion of infected tillers of S.22 decreased from 60 to 10% and of S.24 from 21 to 12%. Although infected plots again yielded less, the differences were not significant. Infected plants may have been eliminated by competition from healthy plants or have been 'cured' of virus by the very high temperatures inside the house during the summer of 1976. Inoculation with a mixture of RMV and ryegrass spherical virus (RSV) decreased yield only as much as RMV alone. (Gibson and Plumb)

The occurrence of brome mosaic virus in Britain. Brome mosaic virus, not previously reported from Britain despite being known in Continental Europe, has now been found at Rothamsted in *Phleum bertolonii*. Physically and serologically, the isolate was indistinguishable from an American strain and the two could be clearly differentiated only by the symptoms they caused in *Chenopodium murale*. (Gibson and Kenten)

Virus diseases of clovers

Red clover necrotic mosaic virus (RCNMV). This virus which stunts and distorts red clover, decreased the yield of cv. Hungaropoly over three cuts by an average of 60% in glasshouse tests.

RCNMV, previously reported only at research institutes and trials centres, has now been identified in two farm crops of cv. Hungaropoly in South Wales. In one of these, 75% of plants were infected. At the Cockle Park trials centre of the National Institute of Agricultural Botany (NIAB), where RCNMV was widespread, cvs. Hungaropoly,

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Teroba and Robina were 35–45%; Granta, Kuhn, Otet and Tetri 10–20%; and all other cultivars 10% or less infected. No RCNMV was found in cvs. Bombi, Hermes III, Merkur, S123 and Temara.

Plants of the red clover cultivars recommended by the NIAB in 1977 were placed in the dark for 18 h and then manually inoculated with a phosphate-buffered extract of RCNMV using carborundum as an abrasive. The infection of cultivars was similar to that at Cockle Park; cvs. Hungaropoly and Teroba were 35–40%, Granta, Kuhn, Redhead and Tetri 13–27%; and Merkur 7% infected. Only 1% of cv. Norseman was infected and S123 remained healthy. Of the five most susceptible cultivars four were tetraploid. Some evidence from field crops suggests that the virus may be seed-borne but results of further tests of seed, used to sow crops which subsequently showed RCNMV symptoms, have been negative.

Weevils, especially *Apion aestivum*, and the aphid *Acyrthosiphon pisum* were the most common insects collected from infected crops; however, these insects and a few other beetles all failed to transmit RCNMV. In soil from infected areas a few nematodes were found that belong to the genus *Longidorus*, which includes species known to transmit viruses with a similar morphology to RCNMV but red clover grown in this soil remained healthy. However, when healthy seedlings were grown together with plants and soil from infected sites, one seedling became infected with RCNMV. As a barrier prevented foliage contact, it is assumed that the virus entered the seedling root via the soil. A fungal vector may be responsible. (Bowen and Plumb)

White clover mosaic virus (WCMV). At Cockle Park, WCMV was more widespread in red clover than RCNMV and many plants were infected with both viruses. Red clover infected with only WCMV shows a faint mottle, whereas in plants infected with both, the more severe symptoms of RCNMV predominate. WCMV is readily manually transmitted and in glasshouse tests with cv. Hungaropoly, infected plants yielded 26, 21 and 16% less than healthy plants at the first, second and third cuts, respectively. Plants infected with both WCMV and RCNMV were no more severely affected than plants infected by RCNMV alone. (Bowen and Plumb)

Cucumber mosaic virus from white clover. All attempts to transmit this isolate to white clover by aphids (*Myzus persicae* and *Acyrthosiphon pisum*) and by sap failed, whether the source plant was tobacco cv. Xanthi-nc or white clover. The virus caused mottle symptoms in subterranean clover, induced reddish ring lesions on inoculated leaves but no systemic infection in field bean cv. Minden and, in contrast to results reported last year, infected tomato systemically.

The clover isolate and a cucumber mosaic virus isolate from swede were purified by slightly modified published methods and some of their properties compared. The two isolates had similar absorption spectra with A_{260}/A_{280} ratios of 1·65 and were indistinguishable in reciprocal immunodiffusion tests. When purified by extracting in 0·5M–KH_2PO_4, 0·001M–*EDTA*, 0·02M–*DIECA*, 0·1% thioglycollic acid, pH 7·5 and resuspending ultracentrifuge pellets in 0·005M–$Na_2B_4O_7$, 0·005M–*EDTA* pH 9, both isolates sedimented as a single boundary with a sedimentation coefficient of 92*S*. When extracted in 0·5M–sodium citrate, 0·1% thioglycollic acid, pH 6·5, precipitated with 10% polyethyleneglycol and resuspended in 0·05M–sodium citrate pH 7, followed by ultracentrifuging and resuspending the pellets in distilled water, both isolates sedimented with a major boundary at 96*S* but with two additional faster-sedimenting boundaries suggesting the presence in these preparations of aggregates of two and more particles.

During isopycnic centrifugation, both isolates degraded in CsCl but sufficient of the clover isolate remained after 16 h for the buoyant density to be determined (1·360)

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although this value probably represented partially degraded particles. After treating with formaldehyde, both isolates were stable in CsCl; the clover isolate precipitated and produced a hyper-sharp band during isopycnic centrifugation but the swede isolate did not. Precipitation seemed not to affect the buoyant density as both isolates gave values of about 1·386. Both isolates were stable in Cs_2SO_4 solutions without formaldehyde treatment and had buoyant densities of about 1·312. The two methods of purifying virus gave particles with the same buoyant density but the method of preparing solutions for isopycnic centrifugation was important. When small amounts of concentrated virus were added to incompletely mixed buffer and caesium salt solution, the virus particles completely or partially degraded and often gave several bands of low and inconsistent densities. Consistent values were obtained when caesium salt solution (60% w/w) was added to virus diluted in buffer and immediately mixed. (Govier)

Diseases of grain legumes

Susceptibility of lupins to *Fusarium* increased by virus infection. During the unusually dry summer of 1976, it was difficult to distinguish wilt symptoms caused by clover yellow vein virus (CYVV), *Fusarium oxysporum* and drought. Plants containing CYVV were usually infected by *F. oxysporum* also (*Rothamsted Report for 1976*, Part 1, 269). When white lupins (cv. Kievsky) were grown during winter in pots of compost in the glasshouse, inoculation at flowering time with bean yellow mosaic virus (BYMV) or CYVV decreased plant growth and pod numbers; CYVV also caused wilting and early death. Inoculation of the compost with *F. oxysporum* when the young seedlings were transplanted decreased growth slightly but had no effect on pod numbers and caused no root-rot or wilt. *F. oxysporum* was isolated from the cortex of roots and hypocotyls of almost all plants inoculated at transplanting but from the steles, in the hypocotyl and stem base, only of plants that had been inoculated with both virus and fungus. *F. oxysporum* was isolated less frequently from plants inoculated with the fungus at flowering than at transplanting but again the fungus was isolated more frequently from plants inoculated with both virus and fungus. (Salt and Cockbain)

Fungus diseases of lupins. No foliar diseases were noted on April-sown white lupins (cv. Kievsky) in field experiments on heavy land at Rothamsted and on light land at Woburn but root-rot and wilt affected small numbers at both sites. At Rothamsted, wilting first appeared on 17 June after cool wet weather, reached a maximum a week later and declined with the onset of warmer drier conditions. Wilted plants had brown rotted roots from which several species of *Pythium* were consistently isolated. *F. oxysporum* was also isolated frequently from roots but no symptoms of vascular wilt disease were found in stems. Wilted plants often produced new lateral roots from the hypocotyl region and resumed growth although stunted. Wilted plants per plot ranged from 0 to 7% (mean 2%) irrespective of the wide range of plant populations tested. Benomyl applied to the soil at sowing increased wilt from 1·7 to 2·2% possibly because it is ineffective against *Pythium* and may have removed some antagonists.

During July and August, a few well-grown plants suddenly wilted and died having been girdled at the cotyledonary node with a stem lesion caused by *Botrytis cinerea*. (Salt and Ingleby)

Further details of these experiments are given in Field Experiments Section Report, p. 128.

Pathogenicity tests. Most isolates of *Pythium* spp. *Botrytis cinerea*, *Rhizoctonia solani*, *Fusarium avenaceum*, *F. solani*, *F. oxysporum* and *F. oxysporum* var. *redolens* from lupin,

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pea or field bean were highly pathogenic on young lupin seedlings grown axenically in boiling-tubes of agar containing Hoagland's nutrient solution. *F. avenaceum* isolated from field beans was the most damaging fungus on all three legumes. Pathogens from lupin were usually more pathogenic on lupin than those from pea or field bean. *Pythium vexans*, *P. ultimum* and *P. arrhenomanes* from cereals were non-pathogenic or only slightly so on all three legumes. *R. solani* from potato was only weakly pathogenic on lupin and isolates from field beans sugar beet and wheat caused moderate damage.

This method for selecting possible pathogens from among a large number of isolates is not a reliable indication of pathogenicity under field conditions. For example, isolates of *Pythium* and *Fusarium* selected as most pathogenic by this method, gave only sporadic infections when used as inoculum in pots of sterilised sand or unsterile field soil in the glasshouse. The conditions that predispose lupins to root rotting in the field need further investigation. (Salt and Ingleby)

Chocolate spot (*Botrytis fabae*) of winter beans. In a factorial experiment, seed (cv. Throws MS) was sown at 126 or 377 kg ha^{-1}, at 18 or 53 cm row spacing, irrigated twice with 25 mm of water or unirrigated, and sprayed once with benomyl at 1·12 kg a.i. ha^{-1} (30 May), sprayed twice (30 May and 23 June) or left unsprayed. By the beginning of May, two chocolate spot foci had formed in the surround of the experiment and by mid-May, when flowering was beginning, chocolate spotting was present on lower leaves on all plots. By early June first pods were forming. The disease continued to develop through the season but only slowly, so that by the end of July when flowering was complete, the disease on unirrigated, unsprayed plots occupied 8·1% of leaf area on lower leaves (node 7) and 5·2% on upper leaves (node 15). On unsprayed plots irrigation almost doubled the amount of disease. With spraying, disease amount was similar on irrigated and unirrigated plots. One benomyl spray almost halved the amount of disease while two sprays decreased it by almost two thirds. Disease was not affected by seed rate or row spacing. Yield was increased 13% by the wider row spacing and 19·4% by the higher seed rate. Although both these treatments decreased tillering and the number of pods per tiller, increased pod weight more than compensated. Pod weight was further increased 15·2% by the benomyl spray applied on 30 May; the second spray gave little further increase. Irrigation decreased yield in all treatments, even though it did not affect pod set and it increased disease only in unsprayed plots. The yield of the treatment comprising irrigated, unsprayed, low seed rate and close spaced was 2·96 t ha^{-1} compared with 5·01 t ha^{-1} an increase of 69·3%, for the converse treatment. (Bainbridge and Finney)

Bean yellow vein-banding virus. Several times in recent years field beans showing a bright yellow vein-banding symptom, together with symptoms characteristic of pea enation mosaic virus (PEMV), have been observed in crops at Rothamsted. At first it was thought that these plants were infected with an aberrant strain of PEMV but recent studies have shown that the vein-banding symptom is caused by another agent, provisionally named bean yellow vein-banding virus (BYVBV), which is transmitted by aphids (*Acyrthosiphon pisum*) in a persistent manner only in the presence of helper viruses. Thus when BYVBV was separated from PEMV by passage through French bean (resistant to PEMV) it ceased to be aphid-transmissible and could be transmitted only by mechanical inoculation. However, the virus became aphid-transmissible again when plants infected with it were inoculated with PEMV. It seems that aphids need to acquire BYVBV and PEMV from the same plant in order to transmit the dependent virus, because aphids that were fed the viruses sequentially, irrespective of sequence, transmitted PEMV only. Although PEMV has been associated with all isolates of BYVBV that have so far been collected from the field, which suggests that PEMV is the usual helper virus, recent tests

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have shown that another persistent aphid-borne virus, bean leaf roll, can also serve as a helper virus.

Except in broad bean, field bean and French bean, no symptoms developed in standard test plants that were mechanically inoculated with BYVBV. However, the virus was recovered from several legumes, including groundnut (*Arachis hypogaea*), subterranean clover (*Trifolium subterraneum*) and white lupin (*Lupinus albus*) that had been inoculated by aphids with BYVBV + PEMV but which showed symptoms only of PEMV. In pot tests, field beans infected with BYVBV + PEMV yielded 77% fewer pods and 89% less seed than uninfected plants, whereas field beans infected with PEMV alone yielded a similar number of pods to uninfected plants and only 9% less seed. (Cockbain)

Vicia cryptic virus. While attempting to purify bean leaf roll virus from field bean plants cv. Minden, spherical virus-like particles *c.* 30 nm in diameter were found at low concentration in the sap of apparently normal uninoculated plants. Similar particles, provisionally named vicia cryptic virus (VCV), were found subsequently in field bean cultivars Herz Freya, Maris Bead, Minor and Throws MS and in broad bean cultivars Masterpiece Green Longpod, Midget, Primo and Threefold White, but not in broad bean cultivars Beryl and Minica, nor in pea cultivars Freezer 69 and Jade. About 50% of Minden plants grown from one seed lot were found to contain VCV and in a few plants the particles were sufficiently concentrated to be detected by electron microscopic examination of crude sap (*c.* 1 particle per field at a magnification of 40 000). Attempts to transmit the particles by mechanical inoculation and by aphids were unsuccessful; transmission by seed, dodder and grafting is under investigation. (Kenten, Cockbain and Woods)

Other observations on fungus and virus diseases in field beans are given in Field Experiments Section Report, p. 127.

Diseases of brassica crops

Studies on *Pyrenopeziza brassicae*. The possibility that this fungus may be seed-borne in winter oilseed rape was investigated. Naturally infected tissue from plants grown in field experiments and commercial crops was cleared in lactophenol and alcohol and examined microscopically. By early May in cvs. Eurora, Rapora and Primor, infection on some plants extended to the inflorescence where rachides, pedicels, sepals, petals, filaments and ovary walls were ramified by hyphae and often carried sporing acervuli. By mid-July in the more susceptible cv. Eurora, infection was extensive on pods and hyphae penetrated placentae, repla, funicula and immature seeds lacking a testa. All these tissues could also carry sporing acervuli, even within undehisced pods. Acervuli were seen on funicula immediately adjacent to seeds with a mature testa, but no spores or hypae were seen on testas or in embryos of 200 mature seeds from severely infected pods.

These observations link with a record of *P. brassicae*-like spores seen in centrifuged washings from a commercial sample of Eurora seed used in 1974–75, when this variety and others were first severely attacked by *P. brassicae*. Rape seed, immersed in a spore suspension held under momentary vacuum, readily produces infected seedlings. True seed transmission has yet to be demonstrated but these observations suggest spores loosely adhering to seed coats, or trash-borne in seed samples, could provide a ready means of transmission and cause initial foci within crops grown from untreated seed.

In a field experiment, benomyl seed treatment (0·5 g kg^{-1}) of cv. Eurora, followed by foliar sprays (1·12 kg ha^{-1}) in January and April, gave good control of natural infection, even on plots treated with dalapon (*Rothamsted Report for 1976*, Part 1, 270). When assessed in late May, plots treated with dalapon and benomyl showed 1·7% infection

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compared with 31·2% for dalapon alone but this control did not increase yield. A single spray of either benomyl (1·12 kg ha^{-1}) or triadimefon (0·25 kg ha^{-1}) but not pyroxychlor (0·34 kg ha^{-1}), applied in late April at the start of maximum stem extension in a severely infected self-sown crop of cv. Victor (fourth successive crop), decreased incidence and severity of infection on uppermost foliage, inflorescences and stems by approximately 50%.

Five types of spore trap, including rotorod samplers, pre-impinger, splash-traps and bait plants were used in continuing studies on the epidemiology of *P. brassicae* and *Leptosphaeria maculans*. Conidia of both fungi and ascospores of *L. maculans* were detected during periods of rainfall throughout the period January to June. In a field experiment, most infection by both fungi occurred on plots treated with dalapon, which decreased leaf surface wax, and least on plots treated with propyzamide.

Most spores of *P. brassicae* were dispersed by run-off water from infected leaves and by larger splash droplets distributed over fairly steep gradients near soil level. Although dispersal was positively correlated with wind direction during periods of rainfall, it was not directly proportional to rainfall, presumably because continuous, excessive rain washed spores from leaf surfaces. Dispersal of fewer spores over greater distances by wind-blown rain, smaller droplets or other means was possible but unlikely to be as effective in causing secondary infections because few spores germinate readily on normal leaf surfaces. (Rawlinson and Muthyalu)

Clubroot (*Plasmodiophora brassicae*). There are two distinct stages in the life cycle of *P. brassicae*. The first, initiated in a root hair by a zoospore that has emerged from a resting spore, culminates in the formation of thin-walled zoosporangia and (secondary) zoospores. The second is a plasmodium associated with the clubroot gall and eventually forms resting spores. The role of the secondary zoospores is obscure. They probably link the two stages but may also be a subsidiary means of multiplication. Root hairs of cabbage plants, grown at 19°C in a steamed mixture of Woburn soil and sand infested with resting spores, contained abundant zoosporangia after 2 weeks. The infected roots, thoroughly washed and placed in water, released zoospores and small seedlings placed in these preparations sustained many root-hair infections with zoosporangia. Some of these infections could have resulted from resting spores of the original inoculum adhering to the infected roots but root washings made by shaking infected roots in water immediately after freeing from soil were much less infective than water in which the same roots were later left to release zoospores. This evidence suggests that secondary (or zoosporangial) zoospores of *P. brassicae* like those of the related fungus *Spongospora subterranea* (*Rothamsted Report for 1968*, Part 1, 128) can repeat the zoosporangial cycle, but we have yet to maintain *P. brassicae* in the zoosporangial stage through successive transfers of zoospores.

When roots of small cabbage plants with two unfolded leaves were placed in a zoospore suspension for 1 day, some shoots were inadvertently exposed to the spores. The plants were transferred to solution culture and, within 1 week, conspicuous galls developed on leaf bases and stems. The galls contained the second stage of *P. brassicae* which later formed resting spores. The rapid appearance of these stem tumours suggests that, in this situation, the secondary zoospores behaved differently and directly initiated the gall-forming stage. (Macfarlane)

Work on systemic fungicidal and growth regulating sprays is described in the Report of Insecticides and Fungicides Department, p. 151. (Macfarlane, with McIntosh, Insecticides and Fungicides Department)

Potato diseases

Planting conditions this year were not ideal and good seed beds were difficult to achieve

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on heavier land. Soils in May were cool and wet which delayed emergence and *Rhizoctonia* damaged emerging shoots and early-forming stolons. After average June rain, common scab was severe on Maris Piper at Woburn and wilting blackleg stems were common in the Pentland Crown seed crop at Rothamsted. July was dry and warm and towards the end of the month unirrigated crops, especially of King Edward, were showing signs of stress. By contrast, August was wet, late-blight was found on Pentland Crown at the end of the month and, for the first time since 1968, became epidemic especially on unsprayed King Edward. Drier weather in September and October prevented much blight infection of tubers and allowed most crops to be lifted before the onset of November rains. Yields of Pentland Crown were reasonably good, averaging 42 t ha^{-1}.

Diseases caused by bacteria

Underground spread of* Erwinia carotovora *varieties. Investigations on the underground spread of a serologically distinct isolate of *Erwinia carotovora* var. *atroseptica* from inoculated seed to progeny tubers (*Rothamsted Report for 1976*, Part 1, 271), were extended to include var. *carotovora* (tuber soft rot). Both organisms behaved similarly and in July, were found to have spread into soil from inoculated tubers placed in the crop in June but neither could be isolated from the induced rots of progeny tubers in early August (following a dry July). However, August was wet and both organisms were isolated from induced rots in early September and in early October from plots in which inoculated tubers had been placed in mid-August and mid-September respectively. Both *E. carotovora* varieties were readily detectable in late October in soil surrounding the remains of inoculated tubers. (Harris and Lapwood)

Spread of* Erwinia carotovora *var.* carotovora *from stem lesions to progeny tubers. In contrast to 'blackleg' (var. *atroseptica*) stem lesions, which originate from underground, stem lesions associated with leaf scars or damage are often caused by var. *carotovora*. To assess whether such lesions are important as a source of inoculum for the infection of progeny tubers, stems were inoculated with var. *carotovora* in June, July or August or not at all. The organism was recovered from soil samples taken near the base of inoculated stems during the season and at final harvest was isolated from induced progeny tuber rots from all inoculated plots but not from controls. Filter paper traps suspended weekly for 7 days in plots from August to September failed to detect any aerial spread. (Harris and Lapwood)

Potential bactericides

'In vitro' *tests.* Several substituted 8-hydroxyquinolines and their complexes with zinc and copper were tested against *E. carotovora* var. *atroseptica* in liquid culture. ID_{50} values ranging from 15 ppm for 2-carboxy-4,8-dihydroxyquinoline to 1·0 ppm for 5-nitro-8-hydroxyquinolinate-zinc were recorded. The finding that other metal chelating agents were effective inhibitors of bacterial growth suggests this may be due solely to their removing iron from the culture medium. (Harris and Lapwood, with Cayley, Chemical Liaison Unit)

'In vivo' *tests.* In developing methods to assess the value of potential bactericides in preventing soft rotting of stored tubers, our standard wound technique for estimating gangrene incidence (*Annals of Applied Biology* (1977), **87**, 7–15) has been combined with the 'bucket' test, in which tubers are induced to rot in an anaerobic atmosphere (*Rothamsted Report for 1973*, Part 1, 142). Wounded tubers in plastic nets were immersed in chemical for 1 min, loaded directly into plastic buckets, the air displaced by nitrogen

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and the lid sealed. After 10 days at 15°C tubers were removed and cut to record the numbers and extent of rotting wounds. Both 8-hydroxyquinoline and SD 740823AX at 1500 ppm significantly decreased the incidence of soft rot compared with controls. (Harris)

Gangrene (*Phoma exigua* var. *foveata*)

Survival in soil. Loam inoculated with pycnidiospores and stored in plastic bags at 5, 10 and 15°C was tested periodically for the presence of the pathogen using the Arran Banner slice technique (*Annals of Applied Biology* (1977), **87**, 7–15). Populations declined gradually and were undetectable in about 2½ years (5°), 2 years (10°) and 8 months (15°) in air dry or moist soil and 1 year, 8 months and 6 months respectively in soil kept at or near field capacity. Soils inoculated in February 1976 and placed in the field (*Rothamsted Report for 1976*, Part 1, 272) had populations in August 1977 as large as those in dry soil kept at 5°C for the same period.

Infection of stems. Pycnidia which form on dying potato stems, usually after burning off, exude spores which can contribute to tuber contamination at harvest. The origin of stem infection is uncertain but it has been suggested that pycnidia in rotting seed tubers release spores into the vascular system of the shoot. In two experiments, shoots were cut from plants growing in the field and their cut ends immersed in a concentrated spore suspension for 24 h and transferred to water for a further 48 h. The stems were then surface disinfected and sections plated on malt agar and *P. exigua* var. *foveata* was occasionally isolated. In a further experiment, stem cuttings about 10 cm long were dipped in spore suspension (3×10^6 spores ml^{-1}) and rinsed in water before rooting. Subsequent sampling and isolation showed that the pathogen survived and was transported within the rooted cutting which remained outwardly healthy.

In a pot experiment, seed tubers with extensive gangrene were planted and plant samples taken periodically for isolation from different parts of the shoot. Over a 3-month period about 1800 stem segments were plated from a total of 147 stems and *P. exigua* var. *foveata* was recovered from only 13 segments (from nine stems), usually near the stem base. In a field experiment more pycnidia of var. *foveata* developed on stems after burning off in plots planted with seed contaminated at planting with a soil slurry containing pycnidiospores than in plots planted with actively rotting seed. Although 1977 proved to be a year when pycnidia developed profusely on stems, it seems unlikely that many of these lesions arose from inoculum released into the vascular system from rotting seed tubers.

Wound type and inoculum. The incidence of gangrene in stored tubers depends *inter alia* on the type and frequency of wounds. Because the importance of different wound types in practice is poorly understood, experiments were done in which stored tubers were washed, dipped in soil slurries containing different known concentrations of pycnidiospores and, after drying, wounded with brass teeth of different shapes. In other experiments, samples of tubers freshly harvested from experimental plots were similarly wounded. Where tissue was crushed and inoculum concentration was in the range of 5×10^2 to 5×10^5 spores g^{-1} adhering soil, over 70% of wounds became infected and variations in inoculum or depth of wound had little effect. By contrast, simple cut wounds rarely became infected unless inoculum concentration exceeded 10^5 spores g^{-1}. 'Crush' wounds of various shapes were frequently infected on field samples but again the incidence on cut wounds was negligible.

Cultural properties. *P. exigua* var. *foveata* is usually distinguished from var. *exigua* by the production of anthraquinone pigment. Var. *exigua* and some isolates of var. *foveata* produce an antibiotic substance ('E') which is detected in agar cultures by its decolouri-

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sation of methylene blue and by a green colour which develops within 5 min of adding sodium hydroxide solution. In tests on filtrates of isolates of both vars. growing in malt extract or Czapek Dox liquids, no green colour developed after addition of sodium hydroxide. However, both filtrates rapidly decolourised methylene blue although only that of var. *exigua* inhibited fungal growth when incorporated into agar media. When vars. *foveata* and *exigua* were grown together on potato dextrose agar, a line of purple pigment (apparently anthraquinone) developed between them. This effect has also been observed on Czapek Dox agar and when liquid culture filtrates of the two vars. were mixed. (Adams)

The use of fungicides against tuber pathogens

Seed treatment. In separate series of experiments, seed derived from stem cuttings (healthier seed) yielded up to 10% more than commercial certified (tuber selected) seed and the progeny tubers of fungicide-treated or healthier seed developed less disease in store. In collaboration with the Potato Marketing Board, samples of four King Edward seed stocks (one healthier and three commercial stocks) were treated with thiabendazole (Storite) before sprouting and planted on two farms in Lincolnshire. Seed tubers carried 133 g thiabendazole t^{-1} compared with the 40 g t^{-1} currently recommended for ware treatment. Total yield was not affected by fungicide treatment but averaged 8% more from healthier seed; effects on saleable yield remain to be determined after storage. In samples taken at lifting, tubers from healthier seed had fewest infections of *Polyscytalum pustulans* and *Rhizoctonia solani* and in all stocks the incidence of these pathogens and *Helminthosporium solani* was greatly decreased by thiabendazole, which also slightly decreased incidence of *Colletotrichum coccodes* (black dot) on skin round tuber eyes. (Hide, Bell and Cayley)

Uptake of fungicides into plants. In 1976, we found that more thiabendazole was taken up by potato shoots from solutions than by potato plants from soil treatments. The effect of soil type on uptake was investigated in 1977. Most was taken up by plants growing in sand followed by clay loam (Rothamsted), sandy loam (Woburn), loam (Kettering), peat/sand compost (EFF) and black fen peat (Mepal) and uptake was inversely related to the distribution coefficients for thiabendazole between these soils and water. In one experiment when 0·24 g per pot was incorporated into growing media, plants in sand and sandy loam were slow to emerge, had small root systems and remained stunted. In loam and peat/sand compost, plants developed necrosis of leaves, beginning at leaf margins 7 and 8 weeks after planting respectively. After 10 weeks stems contained $>$ 10 ppm (sand, sandy loam and loam), 7 ppm (peat-sand compost) and $<$ 1 ppm (fen peat) thiabendazole and toothpicks infested with *P. exigua* var. *foveata*, used to inoculate stems, induced lesions only on plants in fen peat.

Carbendazim, iprodione, triadimefon and thiabendazole were compared by standing potato shoots for 16 days in solutions containing 0·3, 1·0, 3·0 and 10·0 ppm of fungicide before inoculation. All fungicides at 3 and 10 ppm decreased lesion size and treatment with iprodione and triadimefon for 2 days before transfer to water for the remainder of the test was as effective as treatment for 16 days. Plants growing in sand or peat/sand compost were watered with solutions of 'NF 48', iprodione, triadimefon, thiabendazole and a mixture of carbendazim and 8-hydroxyquinoline before inoculation. No lesions developed on treated plants growing in sand but only iprodione prevented formation of lesions on plants in peat/sand compost.

Uptake into tubers. Potato cuttings grown in compost containing thiabendazole produced tubers with the fungicide in internal tissues (*Rothamsted Report for 1975*,

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Part 1, 271). To determine whether thiabendazole can be transported to developing tubers, growing plants were lifted, the mother tuber removed and the stem base inserted in vials containing 10 ppm thiabendazole solution. After 6 weeks, progeny tubers contained at least 1 ppm thiabendazole internally. Attempts to investigate whether the fungicide can penetrate the skin of developing tubers were unsuccessful because treated tubers failed to swell.

Harvested tubers were induced to take up thiabendazole by cutting a slice of tissue from the heel end and standing them in fungicide solutions for 1 or 2 weeks. Following wound inoculation with *P. exigua* var. *foveata*, all wounds on control tubers developed lesions. Treatment for 1 week with 50 ppm thiabendazole in water decreased infection to 62% and with 50 ppm in ethanol to 19%. Corresponding results after treatment for 2 weeks were 10% and nil. Analyses of tuber flesh indicated that incidence of infection decreased with increasing concentration, 3·5 ppm thiabendazole (dry weight) completely preventing lesion development. (Cayley and Hide)

***Tests* in vitro.** Effects on mycelial growth in culture were tested by adding different amounts of fungicides to malt extract agar cooled after autoclaving. Results are expressed as ED50 values. Thiabendazole and carbendazim prevented the growth of *P. pustulans* at a lower concentration (0·3 ppm) than *R. solani* (1 ppm) and *P. exigua* var. *foveata* (2 ppm). Iprodione prevented growth of *R. solani* at 1·2 ppm and *P. exigua* var. *foveata* at 0·6 ppm, whereas triadimefon, tricyclazole, 2-methylbenzimidazole, 2-hydroxybenzimidazole, 2-aminobenzimidazole and 5-nitrothiabendazole did not affect the growth of any pathogen at up to 5 ppm. Growth of *P. exigua* var. *foveata* was only slightly affected by thiophanate methyl and 'NF 48' at 5 ppm but both were much more effective in decreasing colony size when autoclaved with the media, probably because the rate of decomposition to carbendazim was increased.

Thiabendazole is very effective in controlling the spread of *Helminthosporium solani* from seed to progeny tubers but none of the materials tested, including thiabendazole, greatly affected its growth on pea extract agar when incorporated at 5 ppm or less. However, in one test with 'NF 48' at up to 10 ppm, numbers of conidia produced by colonies decreased with increasing concentration of fungicide. This suggests that reliance on effects on mycelial growth in culture, though a useful and rapid screening technique, could result in discarding material potentially useful in practice. (Hide, Mayne and Cayley)

Potato groundkeepers and perpetuation of diseases. Potato tubers of three cultivars were returned to plots on which they were grown in 1976 and immediately ploughed-in in early March 1977 in an attempt to establish populations of 150 000 (high density—HD), 15 000 (low density—LD) and nil ha^{-1}. Of seven plots of each population, one was ridged in April and the remainder drilled with wheat. In the ridged HD plots, populations of 43 600 and in LD plots, 10 700 ha^{-1} were recorded in June and after harvest tubers were found to be infected with *Polyscytalum pustulans*, *Rhizoctonia solani* and *Helminthosporium solani*. Inexplicably very few (less than 2 ha^{-1}) groundkeepers were found in wheat plots.

Rooted potato stem cuttings planted 60 cm apart in the ridges in April were harvested in October and root systems and tubers examined for pathogens. In HD plots, 15% of cuttings had root systems infected with *P. pustulans* and 92% with *R. solani* whereas in LD plots, the proportions were respectively 2 and 24%. No *P. pustulans* was found on roots in 'nil' plots but 10% of plants were infected with *R. solani*. Both pathogens were found on progeny tubers in plots where root systems were infected but *H. solani* was found only on tubers from HD plots. In all plots almost all (96%) root systems were infected

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with *Colletotrichum coccodes*. Unexpectedly, the greatest number of stem cutting tubers induced to soft rot was from the 'nil' and least from the HD plots; serological identification showed only *Erwinia carotovora* var. *carotovora* in these rots, although both this and var. *atroseptica* were isolated from groundkeeper tubers induced to rot. (Hide, Bell Lapwood and Harris)

Potato virus diseases at Rothamsted. Our own King Edward seed was rejected in 1976 when the parent crop was found to be extensively virus-infected, so the 1977 experiments were planted with Scottish King Edward seed (2 stocks) and with Pentland Crown grown at Rothamsted in 1976. When counts were made in early July, one of the King Edward stocks (AA1) had 0·5% potato virus Y (PVY) and the Pentland Crown had 1·2% leaf roll but otherwise the crops were healthy. Aphids were few and late arriving and there was little early spread of PVY in the experiments. However, some late spread occurred and by the end of August, a King Edward crop grown in isolation to provide seed for 1978 showed 0·8% current year infection with PVY. This is more than we usually see at this time but well below the high levels reached in the past 2 years. No virus infection was found in the Pentland Crown crop grown to provide seed for 1978. (Govier)

The effectiveness of foliar glandular hairs against potato pests. Previous experiments in England demonstrated that *Solanum berthaultii*, *S. tarijense* and *S. polyadenium* have glandular hairs on their foliage that trap insects and protect against certain potato pests, including some virus vectors. Experiments at the International Potato Centre in Peru have facilitated the screening for pest resistance of the numerous seedlines of these species maintained in the North American Potato Collection because quarantine measures needed in England are unnecessary there. The abundances of the aphids *Myzus persicae* and *Macrosiphum euphorbiae* were negatively correlated with the abundances on different seedlines of the four-lobed glandular hairs. These same hairs also provided resistance against flea beetle (*Epitrix* sp.), vector of Andean potato latent virus and, in the USA, protected against the potato leafhopper *Empoasca fabae* (Gibson, with Dr. W. M. Tingey, Cornell University). An additional type of glandular hair, found especially on *S. berthaultii*, protected against the mite *Polyphagotarsonemus latus*, a pest of potatoes throughout the tropics and against a leaf miner fly (*Liriomyza* sp.), which is a pest in several South American countries. (Gibson)

Staff and visiting workers

March saw the retirement of Basil Kassanis, an acknowledged authority on plant viruses and a prolific contributor to our knowledge of them, often in collaboration with others, including the late Sir Frederick Bawden. Practical application of meristem culture and heat therapy, now widely practised especially in horticulture for freeing nucleus material of vegetatively propagated plants from viruses, originated from his work on potatoes. Among the many advances in virus studies that he made during almost 39 years at Rothamsted was his discovery of the existence of 'satellite' viruses.

A. J. Dabek, P. H. Roberts, A. Strowman and Mrs. Caroline Peck left during the year and O. C. Cronshaw, Miss Hilary Davies (supported by Cadbury Typhoo), Mrs. Sheila Gilmour, J. Payne (supported by the Perry Foundation) and Mrs. Pauline Williamson were appointed. B. D. L. Fitt was supported for 3 months by the Perry Foundation to extend his work on root disease and plant nutrition undertaken as an ARC student. He was awarded the Ph.D. degree of London University in June and was appointed in November to undertake epidemiological studies concentrating on splash-borne cereal diseases. The Potato Marketing Board continued their support of P. T. Gans (post-graduate student) and R. I. Harris (research post). R. A. Hill continued with the support

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of the Home-Grown Cereals Authority and N. White continued with an ARC post-graduate studentship. Dr. P. H. Gregory continued to work at the invitation of the Lawes Agricultural Trust. Miss Beatrÿs de Lisdonk spent 3 months as a voluntary worker, A. Nogay joined us in June for 12 months' training in virology and M. Romanos (SRC CASE Award) worked for 3 months with C. J. Rawlinson on mycoviruses.

D. A. T. Constantine, N. M. Harrison, A. J. Ingleby, Miss J. McWilliams and Miss C. W. Nuttall were sandwich course students.

S. J. Eden-Green continued his work in Jamaica on coconut lethal yellowing disease, for which R. H. Kenten was appointed Technical Consultant to ODM.

M. J. Adams attended the European Association for Potato Research, Pathology Section Meeting at Braunschweig, and subsequently visited laboratories at Wageningen.

R. W. Gibson returned from the International Potato Centre, Peru in May, visiting the Boyce Thompson Institute, New York en route to lecture on potato glandular hairs for protection against pests. He left for Peru again in December, to extend his work and to organise a continuing programme at the Centre by invitation.

J. F. Jenkyn spent August to October inclusive as an FAO Consultant assigned to the University of Agricultural Sciences, Bangalore, India and participated in the All India Workshop on Assessment of Crop Losses due to Pests and Diseases, 19–30 September.

R. H. Kenten visited Jamaica in April and October and Florida in May in connection with his consultancy on the Coconut Lethal Yellowing Research Scheme. He also visited the International Rice Research Institute, Philippines to explore collaboration by the Department on rice virus diseases.

J. Lacey gave an invited paper at a symposium in November on Grain Dust and Health at the University of Saskatchewan, organised by Canada Health and Welfare and visited other centres concerned with allergic respiratory disease, grain storage and the use of preservatives to prevent moulding of hay and grain.

R. T. Plumb gave two papers and chaired a session at the 2nd European Conference on Virus Diseases of Gramineae, Montpellier.

R. D. Prew visited France in May as part of Anglo-French liaison on minimum cultivations and cereal rotations and visited farms in Denmark in June at the invitation of BASF.

C. J. Rawlinson attended the 2nd International Mycology Congress at Tampa, Florida as co-chairman and organiser of a Session on fungal viruses.

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