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Report for 1970 - Part1

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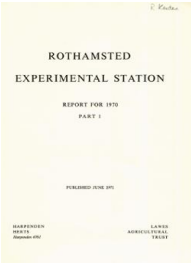
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EXPERIMENTAL STATION
REPORT FOR 1970
PART 1

Plant Pathology Department

J. M. Hirst

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PLANT PATHOLOGY DEPARTMENT

J. M. HIRST

The weather played an unusually important part in our experimental programme. A wet spring, which delayed sowing, was followed by a second unusually dry summer. This restricted the activity of many fungi, and much fertiliser nitrogen seems not to have been utilised, so some experiments were unrewarding and must be repeated. There were unaccountable differences between localities, with potatoes on light soils at Woburn surprisingly yielding more than at Rothamsted, although the reverse was true of winter wheat. Work on foliage diseases has been especially difficult recently; for example, the last severe potato blight epidemic was in 1965 and for the last two years even unsprayed King Edward has failed to produce any blighted tubers, a circumstance unprecedented during the past 20 years. Conversely, the dry summers have allowed us to demonstrate how common scab of potatoes can be prevented by judiciously timed irrigation and favoured work, with the Nematology Department, to analyse the joint effects of *Verticillium dahliae* and *Heterodera rostochiensis* on potatoes.

Properties of viruses and virus diseases

The effect of temperature on the multiplication of strains of tobacco mosaic virus (TMV). The concentrations of complete, infective virus particles (buffer extracts from inoculated leaves), total infective nucleic acid (phenol extracts), and of soluble and insoluble virus protein were estimated in plants infected separately with four strains of TMV and kept constantly at 35°C or in the glasshouse at temperatures varying from 20° to 25°C. At 20°–25°C all four strains produced about ten times as much nucleic acid (RNA) as at 35°C; whether this was stable in plants depended on whether the protein of the strain combined with it. Plants infected with the type strain contained no free virus RNA, whereas the infectivity of PM_2 was all in this form because its protein is non-functional at either temperature. The infectivity of PM_2 increased to a maximum one week after inoculation and then decreased, faster at 35°C than at 20°–25°C. N-118 formed mainly complete virus particles at 20°–25°C but mainly free virus RNA at 35°C, when most of its protein is insoluble.

Strain TC seemed more infective from plants at 35°C than at 20°–25°C but this was because the RNA was badly coated at 20°–25°C and degraded quickly, and not because more RNA formed at 35°C. Moreover, a direct comparison showed that TC and the type strain formed equal amounts of virus RNA at 35°C.

The infectivity of buffer extracts increased when plants infected with either the type strain or N-118 were moved from 35°C to 20°C, but much more with N-118 (500 times in 24 hours), probably because N-118 exists mostly as infective RNA at 35°C. (Kassanis and Bastow)

Phenotypic mixing between strains of tobacco mosaic virus. The RNA and the protein coat of viruses can be separated *in vitro* and the RNA of one virus strain can be recombined with the protein of another. Using strains of TMV where protein rarely or never coats the RNA, we could show that they can combine with protein of a different strain in plants infected with both. Two such defective mutants, PM_2 and N-118, each produced from TMV treated with nitrous acid, did this in plants also infected with Nigerian cowpea virus (CV), a strain of TMV only distantly related serologically to the other strains. The

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infectivity of PM_2 or N-118 was much greater in buffer extracts from plants also infected with CV than from plants infected with PM_2 or N-118 alone. That the RNA of PM_2 and N-118 had been coated with the protein of CV, and so became stable, was shown by the fact that antiserum to CV neutralised their infectivity, because this serological action depends on the protein coat. (Kassanis and Bastow)

Potato virus X (PVX)

Inactivation of the free RNA by ultraviolet radiation of different wavelengths. The action spectra and quantum yields for photoreactivable and non-photoreactivable damage caused by u.v. in the RNA isolated from potato virus X differed from those for similar types of damage in the whole virus. The differences result from the virus protein partly protecting the RNA from damage, and the degree of protection depended on the wavelength of u.v. and on the salt concentration of the irradiated solution.

The photoreactivable sectors of the free RNA and of the whole virus were greater at 290 nm than at 230 nm. At wavelengths longer than 240 nm, the photoreactivable sector of the virus exceeded that of the RNA because, at these wavelengths, the virus protein protects the RNA more against non-photoreactivable than against photoreactivable damage. The behaviour of PVX is intermediate between the behaviour of TMV in which the protein gives much protection to its RNA, and tobacco necrosis virus in which the protein gives no protection. (Govier and Kleczkowski)

Properties of the virus and its degradation products. Some strains of PVX were more readily degraded than others into protein and RNA by incubating at pH 10·4. The electrophoretic mobility of the protein in 0·067 *M* phosphate buffer at pH 7 exceeded that of the virus. The molecular weight of protein separated from freshly purified virus was about 30 000 daltons for all strains tested. However, after storage for a few weeks at 4°C, virus preparations yielded proteins with molecular weights of 27 000 and 24 000 daltons. This may have resulted from protease activity originating either from the host plant or bacterial contamination. Similar degradation occurred during brief incubation of the virus with trypsin; longer incubation decomposed the protein into small peptides. The free protein seemed only slightly related serologically to the intact virus, as antisera against the virus contained very little antibody reacting with free protein. Immunodiffusion tests with free protein often gave two or more precipitation bands, suggesting more than one kind of virus protein. (Carpenter, Govier and Kleczkowski)

Aphid transmission of viruses

The role of 'helper' viruses in aphid transmission. It has long been known that potato aucuba mosaic virus (PAMV) and potato virus C (PVC), which are not transmitted by aphids from plants in which they are present alone, can be transmitted from plants also infected with another ('helper') virus that is aphid-transmitted. PAMV can be helped by potato virus Y (PVY) or potato virus A (PVA), and PVC by PVY. We have found that dual infections are not necessary, and that PAMV or PVC can be transmitted from plants infected with them alone, provided that the aphids feed first on a leaf infected with the helper virus. They are not transmitted by aphids that feed in the reverse order. The helper virus need not be infective, for transmission occurred equally whether or not the helper virus in the first leaf to be fed on was inactivated by exposing the leaf to ultraviolet radiation. This, and the fact that aphids can acquire PAMV or PVC and the helper virus from different plants, shows that the helping process reflects changes within the aphid and not in either the source or test plants. A possible explanation is that particles of the

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helper virus adhere to the surface of the aphid's stylets and provide points of attachment for the PAMV or PVC particles, which cannot adhere directly to the stylet surface. The mechanism may become easier to study by feeding aphids on infective leaf extracts instead of leaves, for we have recently found that aphids carrying PVY can acquire PAMV by feeding on such extracts through artificial membranes.

PVY and PVA are not the only helper viruses for PAMV, which we have also transmitted by aphids carrying henbane mosaic, tobacco severe etch or beet mosaic virus. PVC, also, was transmitted by the aid of PVA or beet mosaic virus. These results have obvious practical implications in the field spread of such viruses as PVC and PAMV. (Kassanis and Govier)

Infection by aphids of leaves sprayed with TMV. TMV is so readily transmitted mechanically, and so concentrated in infected plants, that almost any insect that feeds first on an infected plant and then on tobacco might be expected to transmit it. Yet aphids that readily transmit much less infective viruses fail to transmit it, even from plants also infected with viruses such as henbane mosaic virus (HMV), which are aphid transmitted. Indeed, from plants infected first with HMV and then with TMV, it became increasingly difficult to transmit HMV as time went on and sap from these plants failed to precipitate with antiserum to HMV. Further evidence that TMV suppresses HMV, and that the effect may be specific, came from work with plants infected with HMV and cucumber mosaic virus (CMV), from which aphids readily transmit both viruses. Infecting such plants with TMV greatly stunted them and three weeks later electron microscopy of leaf-dip preparations showed many TMV particles and a few of CMV but none of HMV. Fasted aphids always transmitted CMV from these plants, but only once transmitted HMV. (Watson)

Aphids apparently fail to transmit TMV from plants because they fail to acquire it rather than because they cannot infect healthy plants, for we confirmed (Teakle & Sylvester, *Virology* (1962), **16**, 363–365) that aphids infected leaves that were sprayed with suspensions of TMV before they fed on them. Sprayed leaves of *Nicotiana tabacum* cv xanthi-nc and *N. glutinosa* developed few lesions unless aphids were fed on them, but from three to 25 times as many when 30 fasted *Myzus persicae* were kept on them for 3 hours. We could not confirm the claim (Lojek & Orlob, *Science* (1969), **164**, 1407) that such aphids infected unsprayed leaves to which they were transferred later, but our failure may have been caused by using a clone of aphids not adapted to *Nicotiana* sp.

Aphids fed on leaves sprayed with purified preparations of HMV did not cause infection, perhaps because this relatively unstable virus soon became inactivated. Leaves rubbed before the sprayed suspension dried, developed an average of 90 lesions/leaf, but only ten when rubbed 10 minutes later and usually none after 1 hour. (Watson and Cook)

Molecular weights of plant virus proteins. The molecular weights of protein units of several plant viruses were estimated by the SDS/polyacrylamide gel electrophoresis method. The estimate of 23 000 daltons for protein of the satellite virus of tobacco necrosis virus agrees with the 22 800 daltons estimated chemically (Rees, Short & Kassanis, *Virology* (1970), **40**, 448). Estimates of 27 000 daltons for the proteins of tobacco rattle and of alfalfa mosaic viruses also agree well with unpublished estimates by others but did not agree with the size of alfalfa mosaic protein estimated by Hull, Rees and Short (*Virology* (1969), **37**, 404). Tropaeolum ringspot virus protein has an estimated molecular weight of 40 000 daltons.

Our estimate for the major protein in tomato bushy stunt virus (41 000 daltons)

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contrasts with the 55 000 daltons estimated by Hersch and Schachman (*Virology* (1968), **6**, 234). However, our estimate and the 41 000 daltons estimated for turnip crinkle virus protein agree with recent estimates by others. The suggestion that particles of these viruses are composed of a core of 60 protein subunits and an outer shell of 180 subunits separated by the nucleic acid seems impossible, as others have concluded recently, because 180 subunits of 41 000 daltons would alone account for the total estimated weight of protein in the particles.

Several plant viruses have isometric particles 25–30 nm in diameter, containing about 20% RNA and sedimenting as a single component when centrifuged. These viruses can be placed in two or three groups on the basis of molecular weight or other characters. One group, including bromegrass mosaic, broad bean mottle and cowpea chlorotic mottle viruses have sedimentation coefficients smaller than 100S, are unstable in molar calcium chloride solution and contain protein with a molecular weight near 20 000 daltons. The others are stable in calcium chloride and divide into two groups. One includes tomato bushy stunt and turnip crinkle viruses, with sedimentation coefficients of 130–140S and proteins of approx. 40 000 daltons. The other group has particles with sedimentation coefficients between 110 and 125S and proteins of 30 000 daltons and includes cocksfoot mottle, turnip rosette, southern bean mosaic and tobacco necrosis viruses.

Electrophoretic estimates of molecular weight of proteins of ononis yellow mosaic and dulcamara mottle viruses were about 20 000 daltons, which is close to that estimated for the related viruses turnip yellow mosaic, wild cucumber mosaic and belladonna mottle. (Carpenter, Cook and Gibbs)

Nucleotide nearest neighbours in nucleic acids, and the 'genetic code'. The amino acid composition of the proteins in various culture lines of animal cells was estimated. These and published data on proteins and nucleic acids from other organisms and viruses were used to test whether the nearest neighbour base content of the nucleic acids was correlated with the amino acid composition of the proteins they specified, and to see whether the correlations obtained were related to those predicted from the bacterial 'genetic code'. Statistically significant correlations were found for several amino acid/doublet combinations, but these were not always what would be expected from the bacterial 'genetic code', nor was it possible to suggest explanations for such anomalies as the shortage of CG doublets in all mammalian cell DNAs and the small viruses of mammals. (Gibbs with Marjorie Byers, Biochemistry Department)

Sites of virus multiplication in plant cells. Previous attempts to identify the sites in infected cells where viruses multiply have been made by estimating the virus content of different cell fractions, or finding the distribution of virus particles or the regions where radioactively labelled compounds, such as uridine, accumulate. However, these methods may merely show where the virus accumulates or is assembled, rather than where it is synthesised.

Zaitlin, Spencer and Whitfeld (*Rep. Div. Pl. Ind., C.S.I.R.O. Aust.* (1968) 114–115) showed that cycloheximide but not chloramphenicol inhibits the incorporation of labelled amino acids in TMV particles in infected leaves. This suggests that the gene specifying the virus protein is translated by ribosomes in the cytoplasm, which are inhibited by cycloheximide, rather than by those in the chloroplasts or mitochondria, which are inhibited by chloramphenicol. We confirmed this and showed that broad bean mottle (a bromovirus), eggplant mosaic (a tymovirus), and the U5 strain of TMV, the particles of which frequently occur in the chloroplasts, all respond similarly to these inhibitors. (Gibbs)

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Henbane mosaic virus (HMV). This virus has been maintained in tobacco and henbane plants in our glasshouses for many years without symptoms altering. Our early electron micrographs showed flexuous rods about 730 nm long, as described by Brandes (*Phytopath. Z.* (1959), **60**, 132–142). However, a modification of the purification method used by Damirdagh and Shepherd (*Phytopathology* (1970), **60**, 132–142) yielded long, stiff filamentous particles 834 ± 51·5 nm long from infected White Burley tobacco. Leaf-dip preparations stained with phosphotungstic acid, or shadowed with platinum iridium, also gave particles of this greater length.

To try to explain this difference we are examining the lengths of particles of various isolates of HMV and the variant HMBV (*Rothamsted Report for 1967*, 123; *Rothamsted Report for 1969*, Part 1, 143). (Watson, Plumb and Woods)

Tropaeolum ringspot virus. This virus is difficult to purify because it becomes aggregated and insoluble. Best preparations have come from homogenising infected *N. clevelandii* leaves in 0·1*M* phosphate buffer, pH 8, containing a reducing agent. The homogenate is passed through muslin, 8% butanol added and the mixture clarified at 10 000 rpm for 20 minutes, and the supernatant fluid centrifuged at 35 000 rpm for 2½ hours. The pelleted virus can be resuspended in 0·02*M* borate buffer, pH 8, containing a reducing agent, and further purified by repeated centrifugation. (Cook)

Gel diffusion serological tests using sap from the youngest leaves of systemically infected plants of *Chenopodium amaranticolor* have shown that this virus is closely related serologically to broad bean vascular wilt virus and to PO virus (Kima & Hagedom, *Phytopathology* (1959), **49**, 656). (Cook and Gibbs)

Cocoa necrosis virus. This virus, at present known only from infected cocoa in Ghana and Nigeria, causes veinal necrosis and translucent lesions in leaves of Amelonada cocoa. It was readily transmitted mechanically to 11 out of 25 herbaceous species inoculated. Inoculated primary leaves of *Phaseolus vulgaris* (var. 'Prince') developed chlorotic rings that were useful for assaying virus suspensions. The virus infected *Gomphrena globosa* and tomato systemically, causing leaves to curl and twist.

In French bean sap the virus lost infectivity when heated for 10 minutes at 65°C or kept for 3 or 4 days at 20°C. Virus preparations mixed with neutral phosphotungstate contain isometric particles, 24–26 nm in diameter, some of which are penetrated by phosphotungstate. Some of the particles contain about 40% nucleic acid, some about 30%, and some none. The vector is not known, but these properties suggest that it may be a nematode. However, it did not precipitate with antisera to the known common NEPO viruses. (Kenten)

Pepper veinal mottle virus. A disease, common in all pepper (*Capsicum*) cultivars grown in the Eastern Region of Ghana, was found to be caused by a non-persistent aphid-transmitted virus, with flexuous particles 750 nm long. It did not precipitate with antisera to two strains of potato virus Y, or to tobacco severe etch, beet mosaic, clover yellow vein and lettuce mosaic viruses, so seems to be a new member of the potato virus Y group, and we have called it pepper veinal mottle virus. It could not be purified by methods suitable for potato virus Y and other members of this group. Yields (5–10 mg virus/kg infected petunia leaf) of largely mono-dispersed virus with few contaminants, as judged by examination in the electron microscope and analytical ultracentrifuge, were obtained by a method that may be useful for other viruses of the potato virus Y group. The method depends on maintaining a large concentration of borate (0·3–0·5 *M*), a small concentration of Mg^{2+} (0·01 *M*) and pH 7·5–8 throughout the purification, which

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is achieved by precipitating with polyethylene glycol from extracts of infected petunia leaf clarified with carbon tetrachloride-ether. A further two precipitations of the virus with polyethylene glycol, followed by two or three cycles of differential centrifugation give colourless preparations that suspend in 0·03 *M* borate–0·01 *M* Mg^{2+} pH 7·8 and remain largely mono-dispersed for at least a week at 0°C. (Kenten with Dr. A. A. Brunt, Glasshouse Crops Research Institute)

Electron microscopy of virus infected tissues

Barley yellow striate mosaic virus. This virus occurs in Italy (Conti, *Phytopath. Z.* (1969), **66**, 275) but it is not known among crops in Britain. Transmitted by *Laodelphax striatella*, the virus has bacilliform particles and causes symptoms resembling those of European wheat striate mosaic.

Viruliferous *L. striatella* were dissected and the salivary glands, fat bodies, intestine, ovaries and testes fixed and embedded separately for electron microscopy. Thin sections showed virus particles, locally in large concentrations, only in the salivary glands. Longitudinal sections of particles showed a densely stained central area 315 × 32 nm surrounded by a halo-like structure. Transverse sections showed the central area comprised a dark-staining central core 15–20 nm diameter, surrounded by an unstained area and a dark-staining 'coat' 30–32 nm diameter. The particles were often clustered within a membrane, many had uncoated central cores and some were partially coated. (Conti, Turner, Plumb and Cook).

Tissues infected with viruses of the potato virus Y group. It has often proved difficult to demonstrate virus particles in tissues infected with viruses resembling potato virus Y, although sections often show 'pinwheels'. This may be because double fixation with glutaraldehyde and osmic acid, was usual but was found to be detrimental to virus particles. Henbane mosaic virus (HMV, purified by Plumb see page 124) particles withstood one or other of these fixatives, but double fixation caused them to expand and become flexuous. Analytical centrifugation suggests that the expanded particles are not broken.

Sections of *Nicotiana sylvestris* leaves infected with HMV and fixed only in osmic acid, showed virus particles lying appressed to the pinwheel lamellae and parallel to their longitudinal axes. Sugar-beet leaves infected with sugar beet mosaic virus and fixed only in osmic acid also show abundant virus particles, but these seem to lie in uniseriate rows within invaginations of the tonoplast. (Vince)

The isolation of leaf protoplasts and their infection by viruses. Cultured animal cells become infected when immersed in solutions of viruses to which they are susceptible. By contrast, plant cells are infected only via wounds or by vectors, and the cell wall is usually regarded as the principal barrier to infection. Japanese workers found that tobacco leaf cell protoplasts, released enzymatically, can be infected in virus solutions and we tested this potentially valuable technique.

To prepare protoplasts, the lower epidermis was peeled from slightly wilted leaves and the enzyme solutions were infiltrated *in vacuo*. The source of enzymes is important; one fungal pectinase was ineffective whereas another separated the cells. Of two cellulases from *Aspergillus*, only one digested cell walls but not enough to release many protoplasts. The Japanese enzymes (pectinase from *Rhizopus* and cellulase from *Trichoderma*) worked well and released protoplasts faster when the two enzymes were mixed. However, sequential treatment in pectinase, to separate cells, and then in cellulase, to remove cell walls, allowed protoplasts to be obtained from different fractions of leaf tissue.

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Protoplasts were prepared from five tobacco cultivars (White Burley, Samsun, Java Bright Yellow (Japanese) and Xanthi-nc, and, less readily, from French bean and Chinese cabbage, but we could not remove the walls of cells from *Chenopodium amaranticolor*. The factors affecting protoplast survival are still obscure but protoplasts from leaves not fully expanded, although the most easily isolated, survived only a short time as did most protoplasts from leaves with even mild nitrogen deficiency. Leaves that had just become fully expanded were therefore used.

We confirmed that poly-L-ornithine (a polycation that enhances uptake of proteins by mammalian cells) is required for TMV to infect tobacco leaf protoplasts. After exposure to TMV (1 μg/ml), protoplasts were washed in fresh mannitol by centrifuging gently and incubated in light at 25°C. The Japanese workers report virus to be most abundant 12 hours after infection, but in our experiments multiplication was slower; it was first detected after 18 hours and virus content increased 20-fold during the next 42 hours. We also infected tobacco leaf protoplasts with potato virus X.

In our experience conditions for growing plants that consistently yield viable protoplasts especially need to be better defined. (Macfarlane and Carpenter)

Mycoplasma-like organisms (MLO)

Attempts were made to grow, in artificial media, the MLO in clover plants suffering phyllody diseases. The media tested, included those in which animal mycoplasmata will grow, media other workers have claimed to support growth of MLO from plants and a medium with a composition resembling that of phloem sap.

The only medium in which there was any suggestion of growth was based on a plant tissue-culture medium containing coconut milk extract, yeastolate, sucrose (8%), two plant hormones and cholesterol. The pH was 6·8 and the cultures were kept under aerobic conditions. In the liquid medium, structures resembling mycoplasmata appeared between four days and two weeks after setting up the cultures but did not continue growing or form colonies.

Attempts were also made to grow the plant MLO in cells of the leafhopper, *Agallia constricta*, that had been cultured for a year and, the moth, *Antheraea eucalypti*, which had been cultured for ten years. There was no evidence that MLO from the clover grew in the cells. (Grace)

Diseases of cereals

Epidemiology of cereal aphids and barley yellow dwarf virus (BYDV), 1969–70

Overwintering of cereal aphids. Only a very small proportion of cereal aphids (adults or nymphs) survived the winter. None was found on turves incubated in the glasshouse during the winter, and cereal crops had no natural infestations after mid-November. Also, of aphids placed on cereals on 20 November, *Rhopalosiphum padi* disappeared after a few days, and although *Sitobion avenae* survived longer, the last two were found on 12 January. Some may have survived below ground, but results with the turves suggested that these were very few.

R. padi and *S. avenae* on Powys oat seedlings were exposed outside at intervals between October and April and examined periodically. A few aphids exposed from November to January survived to 18 February, but none survived to 13 March, probably because there were two weeks of frost and snow in early March. Aphids exposed from 13 March survived and began multiplying during April. In these tests *R. padi* survived longer than *S. avenae*, whereas the reverse has more often been true during late spring. (Plumb)

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Phenology of BYDV and its aphid vectors. During the warm dry autumn of 1969 many *R. padi* were caught in suction traps, and as many winter cereals were drilled early, severe BYDV infection seemed possible. However, lack of rain delayed the germination of many crops until after cold weather had greatly decreased aphid numbers.

The spring aphid migration was later than usual and *R. padi* (300/week) was much commoner than *S. avenae* (1 or 2/week) in late May. Thereafter aphids increased much as in 1969, but *Metopolophium dirhodum* (2000/week) was abundant in mid-July. Numbers decreased greatly during August but *R. padi* increased during September and October, as in previous years. The first cereal aphid caught in a trap at 1·3 m above ground level that transmitted BYDV was caught on 27 May, but the next not until 22 June. The proportion of aphids that were infective was smaller than in 1969; of 779 aphids tested 4·2% were infective; 2% of *R. padi*, 7·3% of *S. avenae* and 4·1% of *M. dirhodum*. (Plumb and Cook)

Effects of BYDV and aphids. Small central areas of plots of Zephyr barley were artificially infested on 18 May with *R. padi* and *S. avenae* with or without BYDV. *R. padi* transmitted virus to the infested plants and decreased their yield by 60% but, as in 1969, they failed to survive or spread virus outside the infested areas. *S. avenae* also transmitted virus to infested plants, decreasing their yield by 80%, but also multiplied and spread virus during the next six weeks, which were warm and dry. However, plots around the infested area did not show significant decreases in yield attributable to infection. *S. avenae* and *M. dirhodum* multiplied on infested plots *not* treated with insecticide, until in July there were, respectively 38 and 29 per plant. This infection was probably enough to affect yield directly; whole plots, without introduced virus, that were sprayed with menazon on 28 May yielded, on average, 11·5% more than comparable unsprayed plots. Other evidence suggests that this difference was attributable to aphid feeding and not to late natural infection with avirulent strains of BYDV. (Plumb and Cook)

European wheat striate mosaic (EWSM). Before 1970 the greatest recorded incidence of this disease, for which no causal agent is known, was 5% of plants in Norfolk in 1956. Several fields at Rothamsted and in Lincolnshire, Yorkshire and Lancashire had more in 1970. The worst, a field of 11·2 ha near Grantham, Lincs., had areas during June with 25–50% plants infected, many of which died, decreasing yield by at least 10%. *Javesella pellucida* (Fabr.), the plant hopper vector of EWSM were numerous and infective at Grantham. *J. pellucida* collected at Rothamsted were not infective but became so when fed on diseased plants.

Symptoms of EWSM were first seen at Rothamsted early in May (Table 1), when the number of infected plants varied inversely with distance from a nearby hedgerow. By July, 6·5% of all plants showed symptoms in the area nearest the hedgerow, and those

TABLE 1

Appearance of EWSM symptoms and yield of marked, infected plants

Dates when fresh symptoms were recorded and plants marked	No. of plants marked with fresh symptoms	No. of marked plants that produced ears	Yield of marked plants as % of symptomless controls
6 May	298	22	2·4
20 May	223	56	1·4
4 June	246	132	2·9
18 June	77	77	12·5

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infected produced little grain, which was much shrivelled but able to germinate. There may have been many more late infections but ripening prevented recognition of symptoms.

The distribution patterns and occurrence of most infection in winter wheat suggest that EWSM was spread mostly during autumn or early spring and came from reservoirs of infection or vectors in hedgerows. The mild autumn may have allowed nymphs and possibly adults of *J. pellucida* to remain active unusually late in 1969, although the records show that fresh symptoms appeared until the end of June suggesting further spread during summer. It is therefore puzzling that, at Rothamsted adjacent spring wheat and barley had fewer than 0·1% plants infected. (Plumb)

Methods used to isolate viruses causing similar symptoms and with similar vectors produced no particles from EWSM infected plants. Injecting the preparations into *J. pellucida* did not make them infective or produce virus particles. An Italian line of a related plant hopper *Laodelphax striatella* (Fallen) *Homoptera*, *Delphacidae* readily transmitted EWSM but the causal agent seems to have a longer latent period in this vector than in *J. pellucida*. In Britain, *L. striatella* occurs only on damp grassland and at present seems unlikely to be an important vector. (Watson, Conti and Plumb)

Cereal powdery mildew (*Erysiphe graminis*)

Epidemiology. Cappelle winter wheat was again sown at intervals between mid-August and late-March in two series of unreplicated plots where consecutive sowings were contiguous or isolated from one another (*Rothamsted Report for 1969*, Part 1, 151). One contiguous series was sown with seed treated with ethirimol. There was no evidence that the fungicide decreased the establishment of mildew during autumn, but it is known to be less effective against mildew on wheat than on barley.

Table 2 shows there was much more mildew on plots sown early in the autumn of 1969 than of 1968 but this seems to have had little effect on the incidence of disease during summer 1970. In 1970 mildew was not found on contiguous August or September sowings before June. During late June, mildew was worst on isolated September or October sowings. Hence, severe mildew on wheat during autumn does not necessarily result in an attack the following summer. Possibly local differences in survival may explain the erratic distribution of serious mildew infection during 1970. (Jenkyn)

TABLE 2

Effect of sowing date and proximity to inoculum on mildew of winter wheat

	% area of 2nd youngest leaf infected with mildew					
	1968–69			1969–70		
	29 October	14 May	27 June	29 October	18 May	30 June
Contiguous plots[1] sown:						
Mid-August	0·4	0·1	2·1	18·1	0	0
Mid-September	0·9	0·1	1·4	10·1	0	0·1
Mid-October	—	—	1·9	—	0	0
Mid-November	—	—	1·1	—	0	0
Late March	—	—	0·1	—	—	0
Isolated plots sown:						
Mid-September	0	0	1·4	5·3	0·1	2·5
Mid-October	—	—	0·1	—	0	2·1
Mid-November	—	—	0·2	—	0	0·8
Late March	—	—	0	—	—	0

[1] The series without ethirimol

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The apparatus in which cereal seedlings can be grown isolated in spore-free air was further developed and tested. It succeeded both in producing healthy seedlings and simultaneously in incubating infected seedlings in isolation. Plants were exposed each week just after inoculation to estimate changes in the time needed for symptoms to appear. In late April this 'incubation period' was about 14 days, but by mid-May it had shortened to 8 days, and from the end of May to mid-October never exceeded 7 days and was often only 5 days. Pots of healthy seedlings were exposed (at first weekly but later for 1 day per week) both on the edge of a barley crop and among potatoes and then incubated in isolation to estimate changes in viable airborne spores of barley mildew. Plants exposed close to the barley developed few infections before 28 May, and were most severely infected in mid-June (70 pustules/leaf/24 hours). Infection decreased erratically until there was little a week before harvest, on 14 August and for a week afterwards. Thereafter changes reflected the growth, killing and regrowth of volunteers, and infection continued until ploughing early in November. (Jenkyn, Hirst and King)

Experiments with ethirimol. The dry summer probably limited not only the yield of cereals but also the incidence of mildew in our experiments, although rather less so at Woburn than at Rothamsted. In the spring barley variety trials, mildew (per cent area of 2nd youngest leaf infected at Growth Stage 11.1) was, at Woburn, respectively 0·4 and 2·2% with and without ethirimol (1 lb/acre 80% a.i. wettable powder as seed dressing) and 0·1 and 1·0% at Rothamsted. Yields were not significantly affected by the fungicide and further information could not be extracted on the interactions between fungicide, nitrogen fertiliser and yield noted last year. Sultan succumbed to mildew as much as the susceptible variety Zephyr at both sites.

With spring wheat varieties at Rothamsted, ethirimol (as above) was less effective and mildew more prevalent than on spring barley. Mildew occupied 6·9% of 2nd leaves on treated plots and 9·2% on untreated plots. On average Kolibri had least mildew, respectively 1·0 and 1·9% and yielded 25·9 and 26·1 cwt/acre. This differed strikingly from the imported dwarf wheat variety Inia which had 27·9 and 30·0% mildew and yielded only 15·3 and 13·9 cwt/acre. (Jenkyn with Moffatt, Farm)

The experiment intended to measure how infection at different stages of growth affects spring barley was again hampered by little mildew. By early June, 2nd youngest leaves of plants then without fungicide had 0·5 or 0·6% area infected, compared with none on plants from seed dressed with ethirimol ($\frac{1}{4}$ or 2 lb/acre, wettable powder 80% a.i.). Although seedling emergence was scarcely affected, the treated plots produced four or nine more fertile tillers/m of row (mean of 80/m). Yields from treatments where fungicides were applied did not differ significantly from the untreated control (28·5 cwt/acre). Applying fungicide to prevent mildew early or late in growth slightly increased yields, respectively 29·5 and 29·8 cwt/acre, and controlling mildew throughout the season, increased yield to 31·1 cwt/acre.

After harvest, volunteer seedlings growing where 2 lb/acre of ethirimol had been applied as two sprays, with or without 2 lb/acre as a seed dressing, were less infected than seedlings where none or only a seed dressing of $\frac{1}{4}$ lb/acre was used. This was from active residues in the soil and not in the shed grain. (Jenkyn)

Brown rust (*Puccinia hordei*) of spring barley. Akka, a short-season spring barley, is potentially useful where early harvesting or late drilling are necessary. It was included in the variety trials and some plots were drilled with the other varieties on 2 April and others in late May. Akka remained free from mildew but had much more brown rust than other varieties; so much, when sown late, that this disease may seriously limit its useful-

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ness. At Growth Stage 11.1 (11.2 for 'normal' sown Akka) the area of the second youngest leaf infected with brown rust at Rothamsted and Woburn, respectively, averaged 0·2 and 0·6% for other varieties, 0·6 and 2·9% for Akka sown on 2 April and 11·9 and 17·6% for Akka sown in May. (Jenkyn with Moffatt, Farm)

Systemic fungicides on spring barley and spring wheat. Several systemic fungicides were tested for their activity against various diseases of wheat and barley. All were applied as seed dressings and two sprays; wherever possible, diseases were assessed between applications in the hope of separating the effects of different applications. The weather was so unfavourable to root diseases that their incidence was negligible. Ethirimol, benomyl, EL 273 and thiophanate methyl all greatly decreased mildew (Table 3). EL 273 and thiophanate methyl decreased loose smut of barley but not as much as benomyl.

Fungicides have been said to increase yields of cereals free from known pathogens and the pathogenicity of the epiphytic flora has been questioned. Several we tested apparently affected *Sporobolomyces* but as senescence and leaf damage also affect it, we are not certain that the decrease is directly attributable to the fungicides. (Jenkyn and Prew)

TABLE 3

Effect of fungicides on diseases and yield of spring barley (Zephyr) and spring wheat (Rothwell Sprite)

	Spring barley				Spring wheat		
Treatment	Mildew[1]	Brown rust[1]	Loose smut[2]	Yield (tonnes/ha) ±0·224	Mildew[1]	Sporobolomyces[3]	Yield (tonnes/ha) ±0·189
None	2·0	0·5	94	4·62	16·7	12·5	2·76
Benomyl	0·0	0·2	6	5·12	2·4	1·4	3·16
Ethirimol	0·0	0·7	95	4·68	1·2	4·7	3·27
Furidazole	1·6	0·6	95	4·62	14·0	16·0	2·95
EL 273 (Eli Lilly)	0·2	0·3	17	4·35	3·9	10·0	3·13
Thiophanate methyl	0·0	0·4	45	4·79	4·0	6·1	2·90
W524 (Cela)	0·9	0·6	125	4·59	11·9	7·8	3·45

[1] % area of 2nd youngest leaf infected at growth stage (G.S.) 11.1/11.2
[2] Mean number of smutted ears per plot (total ears/plot *c.* 7000)
[3] Mean number of colonies per segment of leaf exposed (*c.* 7·5 cm²)

The effects of frequent cropping with wheat and barley on take-all and yield. Yields of winter wheat in the 'Wheat after intensive barley experiment, Little Knott' (Table 4), contrasted sharply with those of 1969. The best yield after fallow was only 1 cwt/acre less, but the best yields after wheat were 15 cwt/acre less than in 1969. Take-all incidence has not yet been assessed for samples taken during summer, but observations suggest that infection was less in 1970. Rain during spring delayed farm work and nitrogen fertiliser was not applied to the wheat until 13 May, and only 0·4 in. of rain fell during the next 5 weeks before the ears emerged. These unusual circumstances could have helped cause the differences in yield. If fertiliser nitrogen did not become available to the wheat more soil nitrogen could be expected to be absorbed by wheat after fallow than after wheat.

Assays of *Ophiobolus graminis* in soils in October 1969, and estimates of take-all incidence in May, again showed less of the fungus and disease after growing many successive susceptible crops than after a few; crops with most take-all yielded least (Table 5). (Slope, Henden and Broom)

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TABLE 4

Grain yields (cwt/acre) of winter wheat (Joss Cambier) on Little Knott, Rothamsted, 1970

Previous crops			Crop in	Nitrogen, cwt/acre				
1967	1968	1969	1970	0·6	1·0	1·4	1·8	Mean
Po	sB.1	Fa	wW.1	47·5	49·4	48·8	53·8	49·9
wW.7	Fa	wW.1	wW.2	32·4	34·9	40·6	38·9	36·7
Be	sB.1	wW.2	wW.3	22·0	25·7	29·2	31·7	27·8
sB.1	sB.2	wW.3	wW.4	24·7	32·5	32·8	33·8	30·9
sB.2	sB.3	wW.4	wW.5	29·0	33·0	31·6	26·9	30·1
sB.3	sB.4	wW.5	wW.6	32·3	30·8	41·9	33·4	34·6
sB.4	sB.5	wW.6	wW.7	30·0	28·5	30·2	36·1	31·2
sB.7	sB.8	wW.9	wW.10	32·9	34·2	35·4	39·2	35·4
sW.7	sW.8	wW.9	wW.10	29·2	32·0	39·2	34·8	33·8

Abbreviations used in Tables 4 to 8:

Po = potatoes
Be = field beans
Fa = fallow
M = mixed crops
sB = spring barley
sW = spring wheat
wW = winter wheat

Numbers after crop symbols indicate successive crops susceptible to *O. graminis*.

TABLE 5

Assays of O. graminis *in soil and incidence of take-all on winter wheat (Joss Cambier); Little Knott, Rothamsted*[1]

Previous crops			Crop in	λ/150 cm³ soil[2]	% assay roots infected[2]	Infection[3] index	% plants infected
1967	1968	1969	1970	Oct. 1969	Oct. 1969	Oct. 1969	May 1970
Po	sB.1	Fa	wW.1	0·5	1·8	3·7	1
wW.7	Fa	wW.1	wW.2	4·5	10·0	14·5	11
Be	sB.1	wW.2	wW.3	13·2	11·1	34·0	30
sB.1	sB.2	wW.3	wW.4	10·2	16·2	32·5	31
sB.2	sB.3	wW.4	wW.5	8·8	10·4	33·1	29
sB.3	sB.4	wW.5	wW.6	15·1	9·9	29·5	31
sB.4	sB.5	wW.6	wW.7	5·4	9·7	22·1	20
sB.5	sB.6	wW.7	Fa	9·1	13·9	25·3	—
sB.7	sB.8	wW.9	wW.10	6·0	13·0	22·0	11
sW.7	sW.8	wW.9	wW.10	3·4	5·5	28·0	19

[1] Information only from plots subsequently given 1·0 cwt N/acre
[2] Data from D. Hornby
[3] *See Rothamsted Report for 1968*, Part 1, 134

TABLE 6

Grain yields (cwt/acre) of winter wheat (Cappelle), Woburn, 1970

Previous crops				Crop in	Nitrogen, cwt/acre				
1966	1967	1968	1969	1970	0·5	1·0	1·5	2·0	Mean
wW.1	wW.2	Ley	Po	wW.1	21·2	23·1	25·1	24·8	23·6
wW.1	Ley	Po	wW.1	wW.2	21·6	24·3	26·8	23·6	24·1
Ley	Po	wW.1	wW.2	wW.3	17·2	18·7	17·9	17·9	17·9
wW.1	wW.2	wW.3	wW.4	wW.5	17·4	18·5	20·5	16·8	18·3

In the 'Woburn intensive wheat experiment', even wheat grown after two 'break' crops (ley, potatoes) yielded disastrously little (Table 6). During May and the first two weeks of June, rainfall (0·26 in.) was only about one-tenth of average and the wheat

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suffered too much from drought to benefit from large amounts of nitrogen fertiliser or freedom from take-all. In May, wW.1, wW.2, wW.3 and wW.5 had respectively 0, 2, 15 and 12% plants infected.

The 'Saxmundham intensive wheat experiment' begun in 1965 ended this year. Grain yields, as last year were very disappointing, especially those from wheat following ley or beans, which were only 30 cwt/acre, no more than the best from the 6th successive wheat crops (Table 7). Although nitrogen fertiliser was broadcast in late March, well before the weather became dry, even wheat after wheat responded little to more than 0·6 cwt N/acre.

TABLE 7

Grain yields (cwt/acre) of winter wheat (Cappelle), Saxmundham, 1970

Previous crops				Crop in	Nitrogen, cwt/acre			
1966	1967	1968	1969	1970	0·6	1·2	1·8	Mean
wW.2	wW.3	Ley	Be	wW.1	28·0	29·8	28·9	28·9
wW.2	Ley	Be	wW.1	wW.2	26·2	29·8	30·8	28·9
Ley	Be	wW.1	wW.2	wW.3	24·1	28·2	28·3	26·9
Ley	wW.1	wW.2	wW.3	wW.4	24·5	28·0	28·1	26·9
wW.2	wW.3	wW.4	wW.5	wW.6	26·0	28·6	30·4	28·3

Mildew was severe on crops given most nitrogen, but known foot and root-rot diseases seem not responsible for the small yields. An average of 14% of straws had brown foot rot and 12% had eyespot. Take-all was negligible in first wheat crops and more common in third successive wheat crops (20% of plants infected in June) than in sixth wheat crops (6%), but infections were mostly confined to seminal roots.

There has been much less take-all and eyespot in this than in comparable experiments at Rothamsted and Woburn. This may explain why previous cropping has affected grain yields so little at Saxmundham, but certainly not why the best yields have been so much less than those at Rothamsted. (Slope and Broom)

Development of take-all in soils from different Rothamsted fields. On most of our fields take-all has become common in the second or third successive wheat or barley crops, but recently barley has been grown successively with little take-all on three (Claycroft, West Barnfield and Foster's Corner). To test whether these soils suppressed the development of take-all, wheat seedlings were grown in them, with and without added *O. graminis*, and infection compared with that of similar seedlings grown in sand or soil from Stackyard and Little Knott where take-all is severe. In early May, six replicate pots were filled with

TABLE 8

Percentage of seminal roots with take-all after 35 days

Soil	Cropping					No. of infected root-pieces added/pot			
	1966	1967	1968	1969	1970	0	4 (±4·74)	8	Mean (±2·73)
Claycroft	sB	sB	sB	Fa	wW	2·9	25·3	39·4	22·5
Foster's Corner	M	sB	sB	sB	Fa	1·0	15·8	46·8	21·2
West Barnfield	Ley	wW	sB	sB	sB	6·6	25·4	61·3	31·1
Stackyard	wW	Fa	wW	Fa	wW	1·0	19·9	39·7	20·2
Little Knott	wW	Po	sB	Fa	wW	1·4	22·8	46·0	23·4
Sand	—	—	—	—	—	0·0	25·8	57·7	27·8
				Mean (±1·93)		2·2	22·5	48·5	24·4

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approximately 300 cm^3 of the soils and 0, 4 or 8, 1 cm pieces of take-all infected wheat root were added about 1 cm below the 16 wheat seeds planted in each pot. After 35 days growth in a controlled environment (15°C day/10°C night) the proportion of seminal roots infected was estimated. None of the soils prevented take-all developing and West Barnfield soil favoured it as much as sand (Table 8). As eight pieces of diseased root infected approximately twice as many roots as four pieces these results support the view that the 'Infection Index' (*Rothamsted Report for 1968*, Part 1, 134) is approximately proportional to the number of infective units in soils. (Broom)

Effects of P and K manuring on yield and severity of take-all. See report of the Chemistry Department, p. 46 (Slope and Broom with Margaret Chater and G. E. G. Mattingley, Chemistry Department)

Estimating soilborne *Ophiobolus graminis* by host infection tests. To make our assay methods (*Rothamsted Report for 1968*, Part 1, 135) both sensitive and reproducible, it has been necessary to study the effect of environmental variables and the distribution of the few infective units present in most tests.

Moisture. To define and reproduce a moisture stress in soils during host infection tests, we measured the moisture characteristic curve of soil from Butt Furlong, Woburn, using the suction plate method, and the moisture content at permanent wilting point by the sunflower method. From these an average moisture stress can be reproduced by changing the frequency of irrigation (Couch, Purdy & Henderson, *Bull. Res. Va. polytech. Inst.* (1967), **4**, 23 pp).

Temperature. The number of infective units of *O. graminis* per 150 cm^3 (λ) was estimated in soils where spring wheat or barley had been grown often, using temperatures between 11° and 27°C, in Wisconsin tanks. Attempts were made to minimise differences between tanks in light, air temperature and soil moisture but even so the response of λ to temperature differed in each experiment and seemed to be related to the date when the soil was sampled. In June, the response was linear and positive but later there were fewer infective units and λ became maximal at a temperature that decreased progressively until it reached 18°C in November. Natural inoculum was postulated to consist of colonised host fragments differing in size and capacity to survive and infect; also that it changes in amount because of annual additions and continual loss of viability, and that the smaller or less vigorous units are infective only at the greater temperatures. The fractions of a soil sampled in August, that passed through a U.S. Standard 25 mesh sieve contained more infective units than the fraction retained, and its λ was greatest at 22°C whereas λ for the retained fraction was greatest at 19°C. At each temperature the sum of λ from the two fractions was nearly equal to λ for the whole soil. Only whole soil and the retained fraction were infective when soil collected in May–June was sampled and λ was greatest at 19°C. The average weight and relative area of assay plant roots were measured in experiments where little infection occurred. Weight increased less than area, indicating a change in root morphology and the number of root axes per plant increased with temperature to a maximum at 23°C. Differences in the completeness with which roots searched the soil samples seem not to account for the differences in λ between temperatures.

Sensitivity. Sand containing short lengths of infested wheat straw was used to investigate how the distribution and number of infective units affected estimates of λ.

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The results suggest that the variability we reported last year should not have been attributed to poor mixing. A possible explanation is that the estimate of infective straws by Garrett's seedling test overestimated the proportion of straws that were infective in dilution series and so should not have been used to calculate the expected values of λ. (Hornby)

Seasonal fluctuations of *O. graminis* inoculum. Routine estimates of λ and disease severity (DS) in assay seedlings were continued on soil from Butt Furlong while carrying its fourth successive crop of spring barley. After harvest in August 1969, the steady decreases in λ and DS were temporarily reversed after September ploughing but were then resumed to give, by 24 November, the smallest λ (0·8 infective units/150 cm^3 of soil) yet recorded. DS and λ usually varied similarly but were occasionally up to a few weeks out of phase with one another. Both DS and λ increased until 22 December to a subsidiary maximum ($\lambda = 5{\cdot}4$) before declining somewhat erratically, even after resowing, to 13 July ($\lambda = 2{\cdot}1$). Other evidence confirms that take-all developed unusually little in 1970 and although λ increased to 6·7 by 27 July, DS remained erratic. In Harwood's Piece at Rothamsted, the changes were similar but occurred later. The increases after ploughing (on 27 October 1969, $\lambda = 4{\cdot}3$) were larger and continued until 22 December ($\lambda = 14{\cdot}8$). The winter minimum occurred on 19 January 1970 ($\lambda = 2{\cdot}1$) and the subsidiary peak was on 16 March ($\lambda = 4{\cdot}2$).

These results are difficult to explain. The temporary increase after ploughing could be attributed to infected stubble being incorporated and decomposing, so that although infective units were added they soon lost infectivity. The subsidiary peaks, during December at Woburn and March at Rothamsted, remain unexplained because the increase in λ was not accompanied by the expected decrease in DS if the cause were simply the fragmentation and decomposition of infected host residues. (Hornby, Henden and Parkes)

The effects of aureofungin and benzoxazolone on take-all. Winter wheat seedlings grown in a glasshouse in naturally infested soil were used to test these materials applied as sprays, as seed steeps and to soil. After 38 days growth, no treatment showed significantly fewer roots with take-all lesions so the work was discontinued. (Hornby)

Serology of *O. graminis*. Studies of the conditions affecting the amount of protein in mycelial extracts, in the hope of preparing better antigens, showed that 2-day-old cultures (23°C) yielded most, amounting to about 30% of the total N of the mycelium. Seven antigens were detected in these extracts by immunodiffusion tests in agar against the antiserum prepared in 1968. The amount of the different antigens depended on the buffer used for extraction; 0·01 *M* phosphate-buffered saline pH 7·5 containing 0·01 *M* EDTA was the best tested. One of the three major antigens was unstable and was not detected in extracts stored at 4°C for 3 weeks. The amounts of protein extracted were only slightly increased by adding sodium diethyl-dithiocarbamate or sodium ascorbate to the extracting buffer, or by several buffer extractions of acetone powders made from mycelium, which suggests that extraction was not limited by a polyphenoloxidase system. (Hornby and Govier, with Olsson, Biochemistry Department)

Other root diseases of cereals. Often the incidence of important pathogens such as *Ophiobolus graminis* and *Cercosporella herpotrichoides* does not explain differences in yield of cereals similarly manured and in similar rotations. Even where these pathogens are not present, the yield of successive cereal crops may decrease progressively. There

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are many possible explanations other than pathogens but it is necessary to know whether unrecognised pathogens contribute to these differences. We therefore began a survey of fungi in roots of winter wheat, initially adopting methods best suited to the visual recognition of lower fungi including chytrids, and resumed work on *Pythium* root rot, briefly studied in 1967. Both problems are difficult because most infections will probably be of fine roots which are so difficult to sample, especially when plants are old and the ground dry, and because only very small samples can be examined microscopically.

Infection by chytrids and other pathogens. Samples of 1 cm long root segments, cut 5 cm below the seeds, were cleared and stained (Phillips & Hayman, *Trans. Br. mycol. Soc.* (1970), **55**, 158) to reveal infections on primary and secondary seminal and crown roots. Of 617 root pieces of winter wheat examined during March and April, from selected plots at Saxmundham, Woburn and Rothamsted, 40% were uninfected. Unidentified fungi were present as mycelium in 22% and as thick walled resting spores in 12%, sporangia or resting spores of *Olpidium* in 15%, *Pythium* in 12%, *Endogone* in 9%, eggs or larvae of nematodes, probably *Pratylenchus*, in 7%, and *Lagena* sp., *Ligniera* sp. and *Polymyxa* sp., respectively, in 3, 1 and 1%. Younger roots or branches seemed least infected and crown roots contained more mycelium (64%) and *Pythium* (28%) than seminal roots but had fewer chytrids and nematodes.

Plants from plots continuously cropped with winter wheat had fewer clean roots (29%) than from wheat after other crops (42%) because they contained more mycelium, *Pythium* and *Endogone*. The greatest differences in kinds of fungi infecting roots were between sites with similar crop sequences. Chytrids were more abundant at Saxmundham (*Olpidium* 40%, *Lagena* 15%) than on Stackyard Field at Woburn (8 and 4%) or Pennel's Piece at Rothamsted (10 and 1%), *Pythium* was most abundant at Rothamsted (32%), Woburn (18%) and least at Saxmundham (1%). Unidentified mycelium was commoner at Woburn (38%) than at Rothamsted (18%), and again least at Saxmundham (4%); some of it probably belonged to *Ophiobolus*, *Rhizoctonia* and *Fusarium*. *Endogone* became more common and chytrids less so as the season advanced but the dry weather prevented continued sampling. (Salt)

Pythium ***root rot.*** Infection of spring cereals was less common in 1970 than in 1967, probably because the summer was drier. Late in May, samples of Cappelle wheat from Pennel's Piece, had 33% plants with symptoms, whereas Kolibri spring wheat had only 5%. Between June and August, 19% of spring barley plants (Julia) from Hoosfield were infected on plots without phosphatic fertiliser and only 10% on the other plots sampled.

Of the roots that appeared diseased only about half contained *Pythium* oospores and rather fewer yielded *Pythium* spp. when cultured. At Woburn 27% of volunteer barley seedlings were infected in October and *Pythium* spp. were isolated from 90% of their diseased roots. Of three morphological groups of *Pythium* isolated, one resembling *P. arrhenomanes* was most damaging to sterile barley seedlings. (Waller)

Chemical control of root diseases of cereals

The time of applying formalin. Applying formalin to soil before sowing spring wheat controlled take-all better than applying it in autumn before winter wheat. This suggested that mycelium not killed by the formalin had more time to multiply and cause damage after fumigation during autumn than in spring. Take-all failed to develop in experiments done to test this suggestion, but the results strikingly illustrate the differences in yields that made 1970 such a difficult year for experimenters.

At Rothamsted, formalin applied in autumn decreased take-all incidence in winter

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wheat from only 9 to 1% of plants infected in April, and from 5 to 2% of straws infected in July, yet it increased grain yield from 31·1 to 38·8 cwt/acre. Only 3% of straws of spring-sown wheat were infected in July and yields, which averaged 24·4 cwt/acre, were unaffected by formalin whether applied in spring or autumn. On light land at Woburn wheat germinated well, but it almost failed later and winter and spring crops, respectively, yielded only 3·1 and 4·0 cwt/acre without formalin, 6·1 and 6·2 cwt/acre after formalin applied in autumn, and spring wheat 10·0 cwt/acre after applying it in spring. Yields were not increased by nitrogen fertiliser in amounts up to 1·8 cwt/acre. Foot- and root-rotting fungi were not prevalent but cereal cyst nematodes were and, in May, severely deformed, respectively, 40 and 60% of roots on plots with and without formalin. Nematodes and summer drought seemed the main factors restricting yield. (Salt)

Seed dressings and soil borne diseases. Seed of Cappelle winter wheat dressed with benomyl (50% wettable powder), oxycarboxin (75% w.p.) or thiabendazole (60% w.p.) (each at 1 oz and 4 oz w.p./63 lb of seed) was sown in October 1969. The heavier dressings of benomyl and thiabendazole halved the incidence of eyespot in April, but by July eyespot and take-all were equally prevalent in all plots; benomyl and thiabendazole more than doubled the incidence of sharp eyespot. No treatment affected yield. (Prew with A. H. McIntosh, Insecticides Department)

Root rot and wilt of field beans (*Vicia faba* L.)

Root rot and wilt. Field beans grown in pots of unsterilised Barnfield soil, or in steamed soil with chopped roots of wilted plants added, became affected by black root rot. The roots contained stem eelworm (*Ditylenchus dipsaci*) species of *Pythium*, *Fusarium*, *Rhizoctonia* and *Chaetomium*, arbuscles of a mycorrhizal fungus, and oospores of *Olpidium brassicae* and of a fungus provisionally identified as *Phytophthora megasperma* Drechsler. (Hornby)

A pure culture of *P. megasperma* was obtained from infected roots placed in water, and a zoospore suspension from this was used to infect radicles emerging from surface-sterilised bean seeds. Eight days later, when the radicles had blackened and were producing zoosporangia, small pieces were plated on water agar, where they produced a little mycelium and sporangia. The mycelium grew well and produced oospores on V.8 juice agar. Zoospores produced in sterile water from discs of this culture were inoculated to beans just emerging from steamed soil; 14 days later these plants began wilting, their tap roots and hypocotyls were blackened and contained typical *P. megasperma* oospores and produced zoosporangia when placed in water. Surviving plants were stunted and had a few fibrous roots, whereas uninoculated plants grew well. *P. megasperma* did this also when inoculated together with *Pythium*, *Rhizoctonia* or *Fusarium* isolates from beans. The other fungi did not cause wilting or affect growth when inoculated alone, although all plants eventually developed some cortical blackening from which *Fusarium* spp. were isolated. (Salt)

On Barnfield, beans with black roots infected by *P. megasperma* wilted and died during May and June; treating plots before sowing with 'Temik' (10% at 11 kg a.i./ha), benomyl (50% at 22 kg a.i./ha) or gamma BHC (50% at 2·5 kg a.i./ha) did not prevent the rot. During dry weather in July wilting increased greatly on all plots except those treated with 'Temik', but *P. megasperma* was rare. 'Temik' prevented damage to leaves by *Sitona* beetles and decreased the numbers of stem eelworm, and increased yield of beans from 12 cwt (1·56 t/ha) to 20 cwt/acre (2·49 t/ha). It did not greatly affect the proportion of blackened roots, but decreased the proportion of blackened root nodules.

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During July and August most bean crops at Rothamsted and Woburn were stunted and yellow with many plants dead; the first bean crop on a part of Broadbalk was also affected. Root systems generally were black and dusted with white patches of *Fusarium* spores. It seems that *P. megasperma* is only one of several causes of unhealthy bean roots, the relative importance of which has still to be found. (*See also* Bardner, Cockbain and Fletcher, Entomology Department). (Salt and Hornby)

Potato diseases

Bacterial soft rots. Field experiments assessed the effect of inoculating seed tubers with *Erwinia carotovora* var. *atroseptica* on the appearance of blackleg symptoms, and of this bacterium and *E.c.* var. *carotovora* on the date seed tubers rotted and contaminated progeny tubers. Both experiments were planted with chitted seed tubers of Majestic and Pentland Crown grown at Rothamsted from Scottish V.T. tubers derived from stem cuttings four years previously.

In the experiments to assess seed tuber contamination, plots were planted with seed tubers stab-inoculated, on the day before planting, at the stolon scar, with one or other of the bacteria or none, or with tubers dipped in soil slurries with or without these bacteria on the day of planting (30 April). Few uninoculated seed tubers rotted before late August. At lifting on 29 September, 33% of dipped and 50% of stab-inoculated seed tubers had rotted, the remainder were still firm, many hollow and with the tissues 'glassy' or slightly brown. Few seed tubers stab-inoculated with *E.c.* var. *carotovora* rotted early in the season but by late August more had rotted than of the uninoculated seed tubers. Rotting was earlier and more common in seed tubers inoculated with *E.c.* var. *atroseptica* than with *E.c.* var. *carotovora*. Seed tubers dipped in slurries containing bacteria were less rotted than when stabbed but again *E.c.* var. *atroseptica* was the more damaging. The soil was dry and often hot while the crop was growing and this together with the time many seed tubers took to rot probably limited the spread and survival of bacteria in soil. Of 360 progeny tubers, taken at lifting equally from all 12 treatments, only 53 rotted when placed in environments favouring the bacteria. These were distributed almost uniformly between treatments and only from seven were varieties of *E. carotovora* isolated.

In the other experiment *E.c.* var. *atroseptica* was stab-inoculated, on 30 April, to the middle and rose or heel ends of seed tubers planted on 1 May. Inoculating the rose end or middle of seed tubers allowed bacteria to enter more sprouts. Among 252 plants of each variety inspected on 13 August, only five Majestic stems showed blackleg above ground, and of these some that were infected in June seemed healthy in August because the invaded stems had withered. Seed tubers inoculated at the heel end also took longer to rot. Examining plants lifted at intervals showed that many more stems were invaded below ground than showed symptoms above. Infection was seen in Pentland Crown only when the stems were cut to expose brown vascular tissue. Few stolons or tubers showed symptoms, the first stolons on 29 July and the first progeny tubers on 11 August. After lifting 60 tubers per variety only 13 Majestic and 14 Pentland Crown could be induced to rot and only five and three respectively gave typical *E.c.* variety isolates. (Lapwood and Martin)

Irrigation practice for scab control. A further experiment at the Ministry of Agriculture's Gleadthorpe Experimental Husbandry Farm, near Mansfield, Nottinghamshire, tested how different irrigation regimes affected common scab and yield of four varieties. Regimes A and B (Table 9) were designed to control scab, regimes D and E to increase

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TABLE 9

Effects of irrigation on scab and yield of potatoes, Gleadthorpe E.H.F., 1970

Irrigation regimes	A	B	C	D	E	F
No. of irrigations	8	7	10	5	3	0
			Mean % tuber surface scabbed			
Variety						
King Edward	1·5	4·2	2·3	5·3	20·6	19·4
Majestic	2·8	7·1	2·3	9·0	20·6	32·3
Record	2·4	2·5	1·4	4·2	9·1	7·7
Pentland Crown	0·1	0·8	0·1	0·6	2·2	2·1
			Ware yields ($1\frac{1}{2}$ to $3\frac{1}{4}$ in.) tons/acre			
King Edward	19·8	18·6	18·4	20·1	18·3	12·1
Majestic	18·5	18·7	18·0	17·9	17·4	11·2
Record	19·8	18·4	20·6	18·7	18·6	7·3
Pentland Crown	21·2	20·3	19·9	19·6	19·1	13·1

S.E. between irrigation regimes—(a) scab ±2·25; (b) yield ±0·74
S.E. between varieties (a) scab ±2·03; (b) yield ±0·69

A 0·6 in. (15 mm) soil moisture deficit before irrigation allowed for 4 weeks after tuber initiation of KE, and then 1·5 in. (38 mm) deficit.
B 0·6 in. (15 mm) deficit for 2 weeks and then 1·5 in. deficit.
C Irrigation to field capacity at tuber initiation and then at 0·8 in. (20 mm) deficit.
D Irrigation when 1·5 in. (38 mm) deficit.
E Irrigation when 2·25 in. (57 mm) deficit.
F No irrigation to supplement rain.

yield. Regime C was a compromise aimed to achieve both objectives and regime F was unirrigated.

Irrigation regimes began when tubers first began to swell on King Edward plants, not, as in 1969, when this happened, rather earlier, on Majestic. Plants began emerging during the third week of May when the soil moisture deficit already exceeded 0·6 in. (15 mm). The first irrigations to prevent scab were on 8 June (A and B) and 9 June (C). Except for a few days in August there was little rain throughout the growing period, so irrigations to increase yield were required until the end of August.

All irrigation regimes greatly increased yield (Table 9) and all except E decreased scab. The extra water required to control scab (regimes A, B and C) had little effect on yield. The different incidence of scab on King Edward and Majestic between A and B can be attributed to the omission of a single irrigation to B on 23 June. Regimes A, B and C were all less effective in controlling scab on Majestic than on King Edward because Majestic tubers started to form before irrigation began. The results confirm resistance to scab in Record and even more in Pentland Crown. They also show that even in a very

TABLE 10

Survey of fungal diseases of seed tubers 1969–70

(% tubers infected/% stocks with infected tubers)

Examined	Disease	King Edward	Majestic	Pentland Crown
R	Skinspot (*Oospora pustulans*)	35/90	24/84	26/86
P	Gangrene (*Phoma* spp.)	9/71	8/55	14/75
P	Dry rot (*Fusarium caeruleum*)	1/35	3/56	6/79
R	Blight (*Phytophthora infestans*)	1/33	0/7	0/7
R	Black scurf (*Rhizoctonia solani*)	37/92	35/100	36/96
R	Powdery scab (*Spongospora subterranea*)	16/71	11/78	1/21
R	Common scab (*Streptomyces scabies*)	34/94	63/100	30/100
Number of stocks examined		49	45	28

R = at receipt; P = at planting

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dry season almost all scab infection can be prevented by keeping the soil moist during the first weeks of tuber formation. (Lapwood with Mr. L. W. Wellings, Experimental Husbandry Farm, Gleadthorpe)

Survey of fungal diseases of seed tubers. Seed tubers grown in 1969 (Table 10) had more than average infection with common scab and black scurf but less blight and skin spot. Gangrene on tubers at planting was near average and most on Pentland Crown, but soil carried by seed tubers was less infective than usual. (Hide and Griffith)

Gangrene. Soil around progeny tubers was unusually infective in mid-July 1969 when the first samples could be taken. Later in the season infectivity varied erratically before becoming stabilised just before lifting at amounts less than in previous years. The earlier differences probably reflected the dry summer restricting the establishment of *Phoma exigua* var. *foveata* on below-ground parts of plants, and even later growth on senescent potato haulm was probably small. This may also explain why so little gangrene developed on the progeny tubers in field experiments and why the effects of different treatments were equivocal.

Experiments on stored tubers were more rewarding. Tests in controlled environment cabinets supported growers' ideas that cold storage before, as well as after, wounding increases gangrene. Other tests confirmed that warm storage and clean-cut wounds produce less gangrene than crush wounds and cool (2–6°C) conditions. Except at 2°C spore suspensions caused most infections at 95% R.H. but infective soil was usually more infective at 75% R.H.

Both thiabendazole and benomyl greatly decreased the incidence of gangrene on stored tubers, provided they were applied before or just after wounding; but applied 2 or 4 weeks after wounding they had no effect. Thiabendazole (as a dust 10% a.i. at 5 g/kg of tubers) prevented gangrene even when dusted on tubers 4 weeks before they were wounded and contaminated with infective soil. Thiabendazole was more effective than benomyl in preventing gangrene in stored tubers especially when applied as a dip; 5 minutes in 0·1% a.i. thiabendazole suspension was as effective as dusting with benomyl (10% a.i. at 5 g/kg of tubers). A preliminary test suggested spraying tubers might be less effective than dusting or dipping (with less a.i.). (Griffith)

***Verticillium dahliae* and nematodes.** Further work on the chemical control of these pathogens is described on p. 153. (Hide with D. C. M. Corbett, Nematology Department)

Chemical control of tuber diseases

Effect of benzimidazole fungicides on yield and infection. The succession of dry summers has prevented reliable conclusions about the effect of benomyl and thiabendazole on diseases caused by tuber-borne fungi. Once again, too little gangrene developed on wounded tubers of the 1969 experiment (*Rothamsted Report for 1969*, Part 1, 167) for the results to be conclusive. Since 1968 the incidence of *Oospora pustulans* and *Helminthosporium solani* on untreated tubers has decreased progressively, whereas *Rhizoctonia solani* has become more prevalent, perhaps reflecting their different reaction to the weather. Only 1968, the wettest year, gave evidence that fungicides significantly increased yield. The yield of total and saleable ware tubers was unaffected in 1970 by dusts incorporating 1, 5 or 10% a.i. in kaolin, applied at 10 lb/ton of seed tubers (5 g/kg) (Table 11). The fungicides were least effective against *Rhizoctonia*, which as usual occurred very erratically.

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TABLE 11

Effects of fungicides on tuber yield and infection of King Edward and Pentland Crown: Rothamsted 1970

	Tons/acre		% tuber eyes infected		
	Total	> 1¾ in.	*Oosp.*	*Rhiz.*	*Helm.*
King Edward					
Untreated	13·33	9·93	4·0	25·1	11·6
Kaolin	13·37	10·38	3·3	1·7	6·5
1% benomyl	13·87	10·68	0·6	16·7	1·0
5% benomyl	13·44	10·16	0·9	8·4	0
10% benomyl	13·71	10·45	0·3	0·6	0
1% thiabendazole	14·10	11·07	0·3	0·6	0
5% thiabendazole	13·59	10·65	0·6	20·0	0·3
10% thiabendazole	13·59	10·87	0·3	7·5	0
Mean	13·62	10·51	1·3	10·1	2·4
Pentland Crown					
Untreated	12·69	12·14	1·9	44·5	15·3
Kaolin	12·91	12·37	1·3	72·5	20·6
1% benomyl	13·24	12·69	0	11·0	1·1
5% benomyl	12·86	12·41	0	1·0	0
10% benomyl	12·95	12·43	0	10·8	0
1% thiabendazole	12·42	11·87	0	15·8	1·9
5% thiabendazole	12·43	11·93	0·4	6·7	0
10% thiabendazole	12·82	12·21	1·1	5·8	0·7
Mean	12·79	12·26	0·6	21·0	5·0
S.E.	±0·289	±0·310	±0·72	±7·20	±2·05
			Infection of seed tubers (March 1970)		
King Edward			3·1	0	6·2
Pentland Crown			11·2	28·2	31·0

Because many potatoes are grown on black fen peat soils and some systemic fungicides are said to be less effective in these than in mineral soils, we tested benomyl and thiabendazole at the Arthur Rickwood Experimental Husbandry Farm, Mepal, Camb. The experimental fungicide F849 (Uniroyal) was also included because earlier experiments showed it to be exceptionally effective against *Rhizoctonia*, which is especially troublesome on these soils. Table 12 shows that 5% benzimidazole dusts and 1% F849 (applied as above) were not completely effective and did not significantly affect yield. (Hide and Hirst)

TABLE 12

Effect of fungicides on disease incidence: Mepal, 1970

	Total yields (tons/acre)	% tuber eyes infected		
		Oosp.	*Rhiz.*	*Helm.*
Seed tubers at planting		13·8	80·8	9·6
Progeny tubers after lifting				
Untreated (kaolin)	8·00	13·8	61·1	22·3
Benomyl 1% dust	8·18	4·6	40·9	0·5
Benomyl 5% dust	8·11	1·5	35·8	0·2
Benomyl and F849, 1% dusts	7·72	2·3	16·4	2·6
Thiabendazole 1% dust	9·22	2·3	42·4	9·8
Thiabendazole 5% dust	7·62	2·0	47·3	0·5
Thiabendazole and F849, 1% dusts	7·66	6·0	28·9	6·8
F849	8·76	12·1	43·6	27·9
	±0·423	±2·23	±8·43	±3·04

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Maintaining 'healthier' seed tuber stocks. The use of rooted stem cuttings to produce tubers free from some tuber pathogens (*Rothamsted Reports for 1966*, 129 and *1968*, Part 1, 145) has been adopted by the Department of Agriculture for Scotland. So far they have succeeded but our work suggests that the greatest danger of re-infection occurs as the stocks enter usual crop rotations and are lifted and handled mechanically. Re-infection may be delayed by strict hygiene and by using fungicides, especially those that are active, after planting, against fungi surviving in soil. At Dunning, Perthshire, seed tubers of three varieties with different amounts of infection by *Oospora*, *Rhizoctonia* and *Helminthosporium* were treated with dusts containing benomyl or thiabendazole (1% a.i. in dusts as above) in April 1969. Both fungicides almost eliminated *O. pustulans* and *H. solani* but neither was effective against *R. solani* where this was prevalent.

At the High Mowthorpe Experimental Husbandry Farm we began an experiment in which 5% a.i. benomyl dust was applied to both English seed tubers and the healthier ones from Scotland. Table 13 shows that benomyl much decreased *Oospora* and *Helminthosporium* but not *Rhizoctonia* and did not affect yield significantly. The progeny tubers of treated seed from Scotland and England were almost equally infected.

TABLE 13

Effect of benomyl on total yield and disease incidence: High Mowthorpe, 1970

(tons/acre and % tuber eye-plugs infected)

	English seed				Scottish seed			
	Tons/ acre	*Oosp.*	*Rhiz.*	*Helm.*	Tons/ acre	*Oosp.*	*Rhiz.*	*Helm.*
Majestic								
Seed tubers planted	—	35·7	46·5	67·5	—	1·7	15·6	8·7
Progeny, untreated	12·45	48·3	94·1	47·5	12·88	6·2	81·9	55·0
Progeny, 5% benomyl	12·52	0·5	77·4	0·8	11·86	0·3	59·3	0
Pentland Crown								
Seed tubers planted	—	46·8	9·0	8·5	—	0	12·5	0
Progeny, untreated	12·88	19·5	63·9	56·6	11·46	1·6	3·4	9·7
Progeny, 5% benomyl	12·96	0	52·9	1·1	12·92	0·6	40·3	1·2
Record								
Seed tubers planted	—	32·3	55·9	93·7	—	4·0	16·0	16·0
Progeny, untreated	10·62	30·0	87·5	25·7	10·45	3·6	76·1	46·9
Progeny, 5% benomyl	10·82	1·6	65·1	4·7	11·21	0·4	48·8	0·4
	±0·498	±2·93*	±11·25*	±5·03*	±0·498	±2·93*	±11·25*	±5·03*

* Applies to progeny tubers only, not seed.

Benomyl and thiabendazole are not known to be active against the pathogens causing blight, common and powdery scab, or blackleg. At economic rates they seem unlikely to control *Verticillium dahliae* and *Rhizoctonia solani*. The last may have been increasingly prevalent in the recent dry years or may be able to increase when *Oospora*, to which it seems antipathetic, is scarce. There is at present, no indication of the development of strains with an increased tolerance to these fungicides. However, such strains may appear so we have begun to study the concentrations tolerated by isolates of *Oospora*, *Helminthosporium* and *Rhizoctonia*, from potato stocks treated repeatedly with these fungicides.

Machine dusting of tubers with fungicide. In conjunction with the National Institute of Agricultural Engineering (Scottish Station), prototype machines were developed for dusting small quantities of seed tubers. Recent trials suggest that small units could treat

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30 cwt/hour and it is hoped to develop a machine that will operate unattended at the end of seed dressing lines.

Agronomic effects of healthier seed potatoes. Chitted and unchitted seed tubers of commercial stocks and stocks multiplied from rooted stem cuttings were again compared with additional treatments testing the effect of benomyl and of reinfecting the healthier seed with *Oospora* and *Rhizoctonia*. At Woburn, where the crops grow well, the stocks from rooted stem cuttings of four varieties yielded, on average, 11% more tubers than the commercial stocks, but there was no difference at Rothamsted. Chitting had the greatest effect, especially on Pentland Crown; benomyl did not affect ware yield. (Hide and Hirst)

When free from such fungi as *O. pustulans*, King Edward often produces more small tubers than when infected. Although this is not usually at the expense of ware-sized tubers, the small tubers are valueless and could increase the number of ground-keepers, so we began to test whether the proportion of ware-size tubers could be increased by altered spacing. All tubers were dusted with benomyl and planted 12, 16, 20 and 24 in. apart in rows either 28 or 36 in. wide. Chitting early or late had little effect. The greatest ware (>1¾ in.) yield came from large (4·0 oz) tubers planted 16 in. apart in 28 in. rows, although small tubers (1·7 oz) produced fewer stems/plant and most tubers over a 2¼ in. riddle. (Hirst, Hide with Widdowson, Chemistry Department and Moffatt, Farm)

Incidence of virus diseases at Rothamsted. The potato experiments planted with home grown seed contained few plants with leaf roll (0·2%) or potato virus Y (0·1%), reflecting the scarcity of winged aphid vectors in the seed crops during 1969. Potato virus X increased from the 23% in the 1969 seed crop to 71% in 1970, but roguing held infection with a severe strain at no more than 0·6%, as it was in the seed crop. (Govier)

Joint work reported elsewhere

Boron deficiency in sugar beet at Woburn. Chemical analyses confirming the importance of boron deficiency on Stackyard Field (*Rothamsted Report for 1969*, Part 1, 151) are described in the report of the Chemistry Department, p. 56. (Watson and Plumb with Chater and Mattingley, Chemistry Department)

Fungal component in sub-crystalline layer of cyst-nematodes. *See* Report of the Nematology Department, p. 147. (Salt and Lacey, with Williams, Green, Shepherd, Nematology Department and Callow, Insecticides Department)

Yields of potatoes on the Woburn Ley–Arable experiment. *See* Report of the Nematology Department, p. 155. (Salt with Evans, Nematology Department)

Nematodes associated with Sitka spruce. *See* Report of the Nematology Department, p. 161. (Salt with Gowen and Hooper, Nematology Department)

Biodeterioration

Moulding of stored crops is important not only because the products are spoiled but because the moulds can cause diseases of man and animals and may also form toxins.

Bagassosis. This allergic alveolitis was reproduced in a sufferer by inhaling spores of a thermophilic actinomycete abundant in mouldy bagasse (the fibre remaining after

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extracting sugar from sugar cane). The actinomycete, which is being described as a new species *Thermoactinomyces sacchari*, grows between 37° and 65°C and has spores resembling bacterial endospores. It differs morphologically and in cultural characteristics from *Thermoactinomyces vulgaris* but may share immunological properties with it because the bagassosis sufferer reacted to both (Hargreaves *et al.*, *Lancet* (1968) i, 619). (Lacey with Professor J. Pepys, Institute of Diseases of the Chest, London S.W.3)

Fine structure of *Thermoactinomyces sacchari*. Autolysis of the hyphae make this a difficult organism to manipulate for electron microscopy. The best results were obtained by growing cultures between two layers of cellophane. This provided thin sections through sporangia, sporangiophores and hyphae and made it easier to cut hyphae longitudinally. Specimens prepared in this way were less contaminated by remains of autolysing hyphae than specimens prepared by pelleting organisms washed off agar medium or by embedding in agar. Unexpectedly, mature spores fixed and embedded better than immature spores and hyphae. Of several methods of fixation tested, acrolein followed by osmium tetroxide was the best.

The mycelium of *Thermoactinomyces sacchari* is septate and forms only one spore per cell. Spores usually begin to form within the cell but the process is completed in a terminal sporangium formed as a side branch. Mature spores resemble those of *T. vulgaris* and bacterial endospores. When spores germinated the cortex disappeared first, the core swelled and the spore coats disintegrated before a hypha formed. (Lacey and Vince)

Control of moulding in damp hay. Of two further chemicals tested for ability to prevent moulding in hay; thiabendazole (2% a.i.) failed and benomyl (2% a.i.) prevented moulding in hay containing 32% but not 40% water.

Propionic acid and sodium 2 phenyl phenate needed to be applied uniformly to prevent moulding. Thus even increasing the concentration applied to part of the hay, for example, the top of the swath during baling, would not improve the protection. (Hill and Lacey)

Identification of *Penicillium* spp. and *Talaromyces* spp. from stored products. *Penicillium* spp. and related genera are common moulds of stored products where they may produce toxic substances or allergens. Of 250 isolates that have been classified into 45 species, several produce ascospores typical of *Talaromyces* and included thermophilic species (*T. thermophilus* and *T. emersonii*) and mesophilic species (*T. spiculisporus*, *T. vermiculatus* and *T. wortmanni*).

Most species, e.g. *Penicillium piceum* occurred in several substrates but others were restricted, e.g. *T. emersonii*, to bagasse and mushroom compost and *T. thermophilus* was most abundant on hay treated with formic acid. Interesting but less frequent species included *P. hordei* (grain and straw), *P. lilacinum* (hay), *P. miczynski* (bagasse and grain) and *P. multicolor* (coffee and cocoa). (Hill and Lacey)

Feeding experiments with mouldy hay. Hay was baled at three water contents to produce: (1) a batch that heated to 60°C and was predominantly colonised by thermophilic actinomycetes but with some fungi; (2) a batch that heated less, with *Aspergillus fumigatus* predominant; (3) a batch that did not heat and contained few spores. These were fed to sheep at the Institute of Animal Physiology, Babraham, Cambs.

Within a month of the experiment starting many sheep fed on the first hay developed antibodies to *Micropolyspora faeni* and *A. fumigatus*, those fed the second hay produced antibodies only to *A. fumigatus*, and sheep fed the third hay developed neither antibody.

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Antibodies were detected in lambs shortly after birth but not before suckling. No ill effects of the feeding were noted. (Lacey with Mr. G. A. Embleton, A.R.C. Institute of Animal Physiology, Babraham and Professor J. Pepys and Miss V. Holford-Strevens, Institute of Diseases of the Chest, London, SW3)

Spore dispersal and deposition patterns in crops

Methods of studying how foliage pathogens are spread by air and water are receiving more interest; first, because the importance of foliage diseases of cereals is being increasingly recognised; secondly because resistant varieties so often prove short-lived; and thirdly because we need to know how best to use new types of fungicide. However the patterns of dispersal and deposition are difficult to define when they involve crops, changeable weather and the viability to obligate parasites that exist in many specialised physiologic races.

Our first attempts depended on microscopical examination of spore catches on artificial traps exposed within crops and down-wind of natural spore sources. Large sources of spores are difficult to maintain, so the work was limited to the few occasions when wind direction was correct and spores were being produced. Also, identifying many spores or other natural particles on plant surfaces is impractical, and tests (in conjunction with Mr. A. C. Chamberlain, Atomic Energy Research Establishment, Harwell) showed that surfaces of wheat plants made wet, or sticky like spore traps, caught up to five times as much as when dry. We found it more convenient to disperse *Lycopodium* spores labelled with fluorescein, which is easily dissolved, measured fluorimetrically and related to the number of spores deposited on plant surfaces.

Fluorescein-labelled *Lycopodium* was liberated within wheat at half the crop height. Close to the source, basal leaves caught many more spores than upper leaves, the deposits became equal about 3 m from the source and the proportions were reversed further away, although few spores were caught.

When the crop reached its full height (*ca.* 1 m) the spore source was raised to ear level. This increased the proportion of spores escaping from the crop into the faster winds above. Ears always caught more spores than equivalent areas of leaves or stems, and spores were detectable (limit approx. 100 spores/cm^2) to 17 m downwind after releasing 20 g of *Lycopodium* (approx. $2{\cdot}6 \times 10^9$ spores).

A new method of supporting the vertical pipe down which 'raindrops' are aimed at targets helped us study the dispersal of splash droplets, of 1% fluorescein solution, without disturbing standing crops. Close to a splash target, 30 cm below the flag leaves of wheat, most was deposited on the stems and more on the leaves than the ears. Few splash droplets penetrated further than 4 m through the upper half of the crop, but more travelled above it and 8 m away the ears caught ten times as much fluorescein per unit area as other plant parts. These differences were accentuated by placing the target on the ground, when, in the lower half of the crop a thousand times more fluorescein reached 0·5 m than reached 1·0 m. By contrast, splashing at the level of the ears dispersed detectable fluorescein further, to 5 m in the lower half of the crop and to 11 m on ears. (Stedman and Hirst)

Staff and visiting workers

The sudden death of A. Kleczkowski in November deprived us of a productive and popular colleague. A. J. Gibbs, R. H. Kenten and J. Waller returned from secondments overseas. Myra Chu Chou, F. Bell and R. H. Turner were appointed and R. G. Milne, Connie L. Bastow and N. F. Martin left.

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Visiting workers included Dr. I. Cacciari (C.N.R., Rome); Dr. M. A. C. Conti (C.N.R., Turin); Dr. T. D. C. Grace (C.S.I.R.O., Canberra); Dr. M. S. Mirza (Queens University, Belfast); Miss T. Nart (Exeter University); Mr. E. A. Pizarro (University College of Wales, Aberystwyth). R. A. Hill worked as a 'sandwich course student', M. J. Adams joined as a scholar of the Potato Marketing Board, and P. H. Gregory continued with us at the invitation of the Lawes Trust Commitee.

A. Kleczkowski attended the '8th International Congress of Biochemistry' in Switzerland, A. J. Gibbs the Xth Microbiological Congress in Mexico and D. H. Lapwood a European plant pathology discussion group on 'The epidemiology of *Phytophthora infestans* on potatoes in relation to forecasting'. J. M. Hirst visited West Germany to inspect fungicide trials on cereal crops at the invitation of B.A.S.F.

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