ROTHAMSTED RESEARCH

Rothamsted Report for 1967

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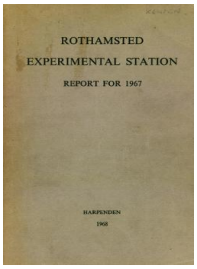

Plant Pathology Department

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J. M. Hirst (1968) *Plant Pathology Department ;* Rothamsted Report For 1967, pp 120 - 140 **- DOI: https://doi.org/10.23637/ERADOC-1-120**

PLANT PATHOLOGY DEPARTMENT

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Viruses and virus diseases

Viruses depend on host-cell systems for their ability to multiply and are more intimately associated with these systems than are most other types of plant pathogen. No way has been found to destroy them chemically without irreparably damaging the host cells. The control of virus diseases rests in using healthy planting material and preventing this from becoming infected. The spread of some diseases can be checked by killing their vectors with pesticides, and heat therapy and meristem culture are now widely used to re-establish virus-free lines of clonal varieties that have become infected. The value of this, even with a virus once thought not to be harmful, is demonstrated by the line of King Edward potatoes that was first freed from paracrinkle virus in 1955 (*Rothamsted Report* for 1964, 282–290). Paracrinkle-free King Edward potatoes occupied 84% of the acreage of that variety entered for seed certification in Scotland in 1967, and in 1966, the national average yield of King Edward (10·8 ton/acre of ware) exceeded that of Majestic (10·2 ton/acre) for the first time, although previously it usually produced about 0·5 ton/acre less than Majestic.

Work on the structure of viruses, their inactivation, multiplication, variation and transmission continued, with results described below.

Properties of plant viruses

Effects of ultraviolet radiation on protein of tobacco mosaic virus. Irradiating tobacco mosaic virus (TMV) with doses of ultraviolet radiation much larger than needed to destroy all detectable infectivity "denatures" protein subunits so that they no longer conform to the structure of virus particles. These subunits fall out and progressively "erode" the walls of the particles until the particles are completely broken into amorphous masses. The quantum yield for denaturation of protein subunits is about 0·0003 at a wavelength of 254 mμ. The infectivity of virus is much more susceptible to inactivation than is denaturation. Thus when 99% of infectivity is inactivated less than 0·5% of virus protein subunits are likely to be denatured. The quantum yield for the destruction of antigenicity of protein subunits is about ten times greater than that for their denaturation. (Kleczkowski and McLaren)

Evidence for "dark-reactivation" of irradiated tobacco necrosis virus in *Chenopodium amaranticolor*. Some of the damage caused in tobacco necrosis virus by ultraviolet radiation can be repaired in darkness in *Chenopodium amaranticolor* (dark reactivation), but not in French bean or

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in tobacco. By contrast, photoreactivation of the irradiated virus was observed in French bean and in tobacco, but not in *C. amaranticolor*. Thus the kind of damage in the irradiated virus repaired by photoreactivation in French bean or tobacco seems to be repaired in the dark in *Chenopodium*. In the conditions in which there is no evidence of any repair, the quantum yield for inactivation of the nucleic acid inside the virus is about $6{\cdot}5 \times 10^{-4}$, and the amount of radiation energy that must be absorbed by the nucleic acid to halve its infectivity is about 0·3 joules/mg. (Kleczkowski)

The effects of heat on viruses. Heat therapy has been important in producing virus-free stocks of clonal varieties of many crops. It is more likely to succeed with viruses that have spherical particles than with those that have elongated ones, but the way it frees plants from infection is not known. To gain information, two spherical viruses that differ greatly in stability and many other properties are being compared, namely tomato bushy stunt virus (TBSV) in young tomato seedlings and brome grass mosaic virus (BGMV) in barley seedlings. Both are easily inactivated by heat *in vitro*, but BGMV particles are much the more fragile, and a proportion contains ribonucleic acid in two pieces which are non-infective. BGMV particles also undergo structural changes as the pH of the medium is changed from 5 to 7 (Incardona, N.L. & Kaesberg, P. *Biophys. J.* (1964), **4**, 11).

Multiplication of BGMV was not hindered when the host was grown at high temperatures, and at 36° C the ratio of infective and non-infective particles was the same as at 20° C. The proportion of non-infective particles increased with time from inoculation when the plants were kept in the glasshouse at temperatures fluctuating around 20° C. Although BGMV particles can withstand 36° C in host plants, they lose shape, and their RNA breaks into small pieces after 1 hour at 36° C in phosphate buffer at pH 6·8. By contrast TBSV particles can be completely inactivated *in vitro* by heat without obvious changes in their appearance or their RNA. TBSV multiplies best at 20° C and as ambient temperature is increased the virus content of plants decreases. At 36° C TBSV soon degrades in plants, first losing infectivity and then serological activity, and no more virus is formed after a few days. The rates of decrease differed from one experiment to another, depending on the season. Also, the degradation was faster in plants previously at 20° C than in those at 26° C. (Kassanis and Lebeurier)

Protein composition of perfect and defective Dolichos enation mosaic virus. Exposure to high temperature is not damaging to all viruses. Last year an isolate of Dolichos enation mosaic virus (NDEMV) was described (Kassanis & McCarthy, *Rothamsted Report* for 1966, p. 111) as having particles that were unstable at pH 8, unless the infected French bean plants were kept above 30° C. Presumably the RNA or coat protein produced at 20° C is defective, for a fault in either might render the nucleoprotein particles unstable.

In seeking an explanation of this unusual behaviour, the coat proteins of virus particles grown at 20° and 35° C were compared. Virus was first

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purified by differential centrifugation at pH 5, and then any slight contamination of perfect with defective virus, or vice versa, was removed by a combination of ultra-centrifugation at pH 8 and gel filtration on agarose columns. Proteins isolated from these preparations by the alkali method had a sedimentation constant of 4 S. When acidified, both proteins aggregated into rods with the helical form of complete virus particles, and quite unlike the stacked disc structure of TMV "A" protein. At pH 5 the protein produced at 35° C formed rods of similar length to virus particles, as would TMV "A" protein. In contrast, the protein produced at 20° C formed much shorter rods, with none longer than 50 mμ; however, at pH 3·5–4·0 it did form rods as long as those formed by 35° C virus protein at pH 5. Both proteins had the same electrophoretic mobilities at pH 7 when run on cellulose acetate membranes.

The soluble tryptic peptides of reduced, carboxymethylated NDEMV proteins were mapped by high-voltage electrophoresis at pH 6·5, and chromatography on paper. The patterns of spots on the two-dimensional maps, located by the usual reagents, were similar with both, but differed considerably from those of common TMV and other TMV strains. NDEMV proteins differ strikingly from those of other strains in their many neutral peptides, and the complexity of this region of the maps necessitated further electrophoretic separation at pH 1·9. The resulting three-dimensional maps reveal that 8 of a total of 15 peptides are in the neutral region. Semi-quantitative comparisons of the amino-acid constituents of each peptide indicate that most of the peptides are probably identical in the two proteins. The possibility that the difference between the virus proteins formed at 20° and 35° C lies in an amino-acid substitution in one of the neutral peptides is being investigated. (Carpenter)

The distribution of viruses within infected cells. Recently the electron microscope has been increasingly in demand from other departments and institutions. During 1967 the Siemens I was temporarily installed in the Nematology Department, where it is being used mainly for examining sectioned material. The Siemens IA, which replaces it, can therefore be used mostly for virus work (Woods). The new instrument allows more attention to be paid to the distribution of viruses within infected cells. Knowing where virus particles are formed and accumulate may suggest ways of interfering with their development; so far five viruses have been studied.

Tobacco mosaic virus. It has long been recognised that TMV forms three-dimensional crystals within infected cells, but such arrangements have only recently been seen in extracted and purified virus *in vitro*. Small crystals do form from purified virus preparations in the presence of a basic protein, such as cytochrome c or histone, when the concentration of salts is not greater than 0·05 molar and the pH is adjusted so that the net charges on the virus particles and protein molecules are opposite.

Tomato spotted wilt virus. This is an unstable virus, unlike most others infecting plants. Sections examined in the electron microscope showed that

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superficially it resembles the myxoviruses (influenza, measles and rinderpest). The early stages of assembling the virus are being examined to see how close the similarity is. Of three strains of the virus used, two produced particles readily seen in sections of infected plants. The third, a strain obtained from Wye College, causes similar symptoms but does not produce specific particles resolved by the electron microscope, perhaps because it is defective and produces mainly or only infective RNA. (Milne)

Viruses in insects. The distribution of acute and chronic bee paralysis viruses in the mid-brains and mushroom bodies of honeybees has been studied in collaboration with the Bee Department (see p. 216). (Milne). An attempt to determine the size, shape and distribution of particles of wheat striate mosaic virus within viruliferous leaf hoppers (*Javasella pellucida*) and their eggs failed, because no particles of the virus were found. (Milne and Atherton)

Variability of insect-transmitted viruses. Viruses commonly change in plants, and variants often differ in their pathogenicity and modes of transmission.

Plants infected with henbane mosaic virus (HMV) may contain several strains having similar particles and serological properties but with different pathological abilities and that change in relative abundance with time after infection.

Strain B of HMV causes necrotic local lesions in inoculated leaves of *Nicotiana* spp. and systemic spread at first causes necrosis with no mottling of the younger leaves. However, after several weeks the initial strain is replaced by less-virulent variants, some of which protect plants against infection with typical HMV. The necrotic strain alone never does this, even when HMV is inoculated at dilutions near the infection end point.

Strain B is difficult to isolate except by aphids allowed to make only single probes into systemically infected plants or by using local lesions as inocula. Partially purified preparations of HMV mixed with Strain B in ratios of 1 : 10 000 infect plants as easily with HMV as when HMV is diluted in buffer. It seems that, if the necrotic lesions produced by strain B initially contain HMV or mild strains of HMV, the contaminating viruses are unable to multiply at once. Yet they always occur in plants that are inoculated, however carefully the lesions are isolated.

Strain B is much less easily transmitted by aphids than HMV or the mild strains, and it is more easily transmitted from plants that have already developed mild strain infections, but inoculated plants receive both viruses, and Strain B cannot then be isolated alone from lesions except by single aphid probes. (Watson)

Two strains of cucumber mosaic virus (CMV), one from lettuce (LCMV) and one (YCMV) derived from Prices No. 6 strain, differ in their ability to be transmitted from plants or through parafilm membranes by *Myzus persicae*. Changes with time in the concentration of each strain in infected *Nicotiana glutinosa* plants were estimated from the number of local lesions produced in *C. amaranticolor* inoculated with extracts from infected leaf fragments of equal weight. The concentration of each increased up to

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30 days and then decreased, but whereas aphids transmitted LCMV readily up to almost 3 weeks after inoculation and then only slightly less readily, YCMV was transmitted by only about 7% of aphids at 10–14 days after inoculation and not at all thereafter, although the concentration of virus in the plant continued to increase until 30 days. (Watson and Pullen)

Similarly, the failure of aphids to transmit groundnut rosette virus (GRV) from plants infected by manual inoculation seems not to be because virus is less concentrated in them than in plants infected by aphids. Some groundnuts were inoculated manually and others infected by aphids, and sap from each group was then assayed in *C. amaranticolor*. More local lesions were produced by sap taken 3 weeks after inoculation than 6 weeks and more by a strain from Kenya than one from Nigeria, but sap from manually inoculated and aphid-infected groundnuts produced similar numbers of lesions. (Watson)

The incidence and spread of viruses

Virus diseases in root crops

Potatoes. In recent years seed potatoes once-grown at Rothamsted have been used for most experiments, and the results have been satisfactory, usually with fewer than 1% of plants infected with potato virus Y or leaf roll. In 1967 winged aphids were unusually common between mid-June and mid-July, but a severe infestation did not develop because they left few progeny. However, the winged aphids proved unusually active in spreading potato virus Y, so that by late summer some crops had at least 3% of plants infected. As many as 60 plants showing leaf-drop streak, the primary symptom, were found around a single secondarily infected source plant showing severe mosaic. Such spread has not occurred at Rothamsted for at least 20 years, and as once-grown seed is now widely used for English potato crops, it will be interesting to follow its consequences. (Gregory)

Sugar beet. Beet yellows and beet mild yellows viruses together infected about half the plants on the ley–arable experiments by 30 August. Beet mosaic virus also infected between 8 and 30% of plants in different parts of the same experiment. This disease, usually seen only close to seed crops, has been rare at Rothamsted since 1950, and the reason for its occurrence is not known, but aphids were very active this year. (Watson)

Spherical virus-like particles occur in all sugar-beet plants examined, and also in beet seed. The prevalence of these particles hinders study of their possible transmission and effects on the growth of sugar beet. The search for particle-free hosts has proved difficult. None of the standard plants used for virus assays became infected either by aphids or mechanical transmission. Of 7 *Beta* spp. tested, only *B. patula* seems free from particles, but a further collection of 17 species has yet to be examined. Serological and other tests do not suggest the particles are of any of the spherical viruses known to infect sugar beet. Yet they are very abundant in plants, in addition to being widespread. Purified preparations made from the commercial varieties Camkilt and Sharpes Klein E, and from material from plant breeders' lines, contained from 10^9 to 10^{10} particles/ml of plant sap.

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Concentration seems to depend solely on the age of the host plant and to be independent of the presence of other viruses. (Pullen)

The spread of turnip yellows and carrot motley dwarf viruses and their effect on yield. The means by which turnip yellows virus (TYV) (turnip mild yellows) is spread in brassica crops is not understood, but it seems likely that *Myzus persicae* is its chief vector. Much more is known about the spread of the two viruses causing carrot motley dwarf (CMDV), one of which is a persistent virus transmitted by *Cavariella aegopodii*. The spread of these viruses and their effect on yield was studied on variously exposed and infected plots of carrot (for CMDV) and swedes and turnips (for TYV).

C. aegopodii appeared at the end of May and was common during June (200–400 aphids/ft² of sticky trap/week). By comparison *M. persicae* was scarce, although it was much more prevalent than in recent years. Natural infections by both viruses appeared early in exposed plots (i.e. those not protected from aphids by cages for 6 weeks from early June). By the end of July, on sprayed and unsprayed plots respectively, TYV had infected between 30 and 40% of swede plants and 70 and 80% of turnips.

Deliberate infection decreased the yield of turnips considerably (7·5 ton/acre) and of swedes less (3 ton/acre, not significant), but yield was not significantly increased in either crop by spraying plots exposed to natural aphid infestation (Table 1).

TABLE 1

Yield of roots (ton/acre)

	Caged plots, all sprayed		Exposed plots		
	Deliberately infected	Uninfected	Unsprayed	Sprayed	
	A	B	C	D	
Swede	15·9	18·8	19·3	20·9	±1·45
Turnip	14·4	21·9	20·8	23·0	±2·12
Carrot (1)*	11·4	15·3	8·4	7·3	±1·12
Carrot (2)*	9·5	15·2	7·2	10·0	±0·85

* See text

The naturally occurring CMDV in the exposed carrot plots caused more severe symptoms than the stock culture used to infect the caged plants artificially. The yields reflect this difference, but are complicated by a probable effect of shading by the cages and by differences in plant populations between treatments (Treatment A had more and Treatment D fewer plants than the average). In Table 1 Carrot (1) gives the actual average yield and Carrot (2) the yields adjusted for plant population. (Watson and Pullen)

Virus diseases of grasses and cereals

Ryegrass mosaic virus. The incidence of ryegrass mosaic virus (RMV) was measured in seed crops of S 22 Italian ryegrass sown during 1966 in Hampshire. Crops sown under barley in spring contained more infected plants than those sown direct during the autumn. No relation was found

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between virus incidence and amounts of fertiliser applied or systems of management, either in this survey or in a perennial ryegrass trial at the Grassland Research Institute, Hurley. Thus, these observations do not support the suggestion that an interaction between RMV and much nitrogen causes intensively managed perennial ryegrass swards to degenerate. Field trials will test whether under-sowing in spring does increase infection and whether the virus can be transmitted by crushing together healthy and infected leaves, as glasshouse experiments suggest.

The effect of RMV on yield of ryegrass was measured in pots in which healthy plants and others infected in November were both cut in spring, for hay in summer, and during autumn. Yields of S 22 were unaffected by infection in spring, but diminished in later cuts by 20 and 30% respectively; those of S 24 perennial ryegrass were decreased by 23 and 19% in spring and summer respectively, but unaffected in autumn.

Cocksfoot mottle virus. Thirty per cent of test plants placed in a cocksfoot–lucerne sward and exposed only during cutting on two occasions, became infected with cocksfoot mottle virus (CFMV), confirming a previous observation (*Rothamsted Report* for 1964, p. 132). *Lema melanopa*, the vector of CFMV, occurred from July to September and was most often caught, with a portable suction trap, during August, but catches were never large. Test plants were exposed for 14-day intervals throughout the period July to September, but only 9% became infected. The relative importance of vector and mechanical transmission of CFMV therefore needs further study. (A'Brook)

Soil-borne virus diseases of oats. Patches of stunted plants showing a mosaic have been noted in crops of winter oats (Powys) in Devon. Powys oats were sown in soil from such patches in pots which were kept wet in the glasshouse during winter. Two months later pale green flecks and chlorotic eyespots developed on the leaves of about a third of the plants. Seedlings, manually inoculated with sap from such chlorotic lesions, developed similar systemic symptoms, but only a small and variable proportion of plants became infected. Cool, dull weather favours symptom expression. Electron microscopy showed slightly flexuous rods, about 14 mμ wide and of many different lengths, but most often 600–700 mμ, in cut-leaf preparations from lesions on plants infected either from soil or by manual inoculation. Field occurrence, soil transmission, symptoms and particles indicate a disease very like North American soil-borne oat mosaic.

Lower fungi identified in the roots of oat plants grown in soil from an affected field were *Lagena radicicola*, *Olpidium brassicae* and *Polymyxa graminis*. The *Olpidium* was isolated, but has not yet transmitted the virus.

Systemic infection by tobacco rattle virus was found in another sample of stunted Powys oats with brilliant yellow streaks on the leaves. Virus rods, of two lengths, about 140 and 300 mμ, were plentiful. Oat mosaic virus was also present in the same crop, again in Devon. (Macfarlane, with Messrs J. E. E. Jenkins and S. C. Melville, National Agricultural Advisory Service)

Barley yellow dwarf virus was common in samples of winter oats and

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wheat from Southern counties during spring, and during summer from other parts of the United Kingdom, including Scotland. To measure incidence at Rothamsted more accurately, samples of oats were taken at intervals across experimental plots. On Fosters ley–arable experiment there were 70% of infected plants in outer and 25% in inner rows. On the Garden Plots the comparable figures were 50 and 25%. The numbers of "blasted spikelets" (empty basal glumes, showing silvery white in oats) were proportional to the percentage of plants infected, but it could not be proven that this symptom provided a reliable diagnosis. (Watson)

Fungi and actinomycetes

Much of our work on these organisms is ecological, concerned either with how attacks start or how changes in microbial populations can be manipulated to benefit crops. Experiments on these subjects often last for several years, and results are best described on a crop basis, whereas work on spore dispersal, which is described first, has more general application.

Spore dispersal

Spore deposition within crops. Much is known about concentrations of spores in the air within and above crops, how they are influenced by weather and how spores are dispersed over long distances (see p. 373). Much less is known of their transport to and deposition in crops, whether or not these contain sources of spores. To study these important processes a sugar-beet steckling crop was used as a convenient, potent and identifiable source of day-liberated pollen grains. These grains are about 20 μ diameter and similar enough in size to the spores of many foliage pathogens to represent them aerodynamically. At Broom's Barn two adjacent 50 m square plots of sugar-beet stecklings produced pollen during July, and when wind direction was suitable pollen was collected down wind in plots of spring wheat or beans (50 m downwind and 25 m wide), separated from the sugar beet by a 5 m path. Pollen concentration and "area dose" (the number of spores passing through unit area perpendicular to the wind in unit time) were estimated by rotorod traps. Deposition was measured on the sticky surfaces of horizontal microscope slides and 0·5 cm diameter sticky vertical cylinders, crudely simulating leaves and stems respectively. Traps were arranged on poles sited at the upwind edge of the wheat or beans and at 10 m intervals downwind, at 5–55 m from the sugar beet. At each distance there were four traps at heights up to 1 m, and other traps above the crops at 2, 4 and 6 m.

Preliminary analysis of the results suggests that the first 10 m of both crops removed many pollen grains from the air and greatly modified vertical profiles of spore concentration. At the upwind edge of the trap crops much the greatest pollen concentration was in the lowest metre, but within 10 m the vertical pollen concentration profiles became small-scale replicas of the "eroded profiles" occurring in spore clouds that have travelled several hundred miles over the sea (p. 373). Thus, over most of the length of the trap crops the greatest pollen concentration was at two or

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three times crop height, and the greatest area dose occurred somewhat higher. Beyond 15 m from the downwind edge of the source most of the pollen deposited in trap crops probably came not from air that had travelled through these crops but by turbulent transfer from the denser cloud above. (Hirst, Stedman and Carter)

Splash dispersal. The spores of plant pathogens can be spread through the air, in flowing water and in splash droplets. Little is known about the efficiency of splash dispersal in crops or of the gradients it produces, so deposition of splash droplets containing fluorescein was measured in dry weather and over short grass, around an "ideal" collision surface at ground level and at ½ and 1 m above. The volume of droplets deposited per unit area at ground level and up to 1 m, was considerably modified by the height of the impact and the wind velocity. In one test, droplets were detected at 16 m downwind of an impact point 1 m above ground. In future we hope to make more realistic tests by operating among crops and during rain.

Lycopodium spores were liberated simultaneously from the same position, both by splashing spores that were stained in crystal violet and then suspended in the fluorescein solution and also releasing dry, unstained spores into air. Preliminary examination of deposition measured on a downwind axis suggests that the two methods of dispersal gave different gradients. (Hirst, Stedman and Carter)

Accurate knowledge about splash dispersal will depend on developing efficient quantitative spore traps. The difficulties of sampling the steep gradients of splashed spores are considerable, because many are featureless and stick even to wet surfaces. Of several prototype traps tested none seems acceptable. (Hirst and Gilmour)

Dispersal gradients of potato blight. With the intention of eventually producing better designs for experiments with air-dispersed pathogens, dispersal gradients of *Phytophthora infestans* were again measured, but in an experiment designed differently from last year (*Rothamsted Report* for 1966, p. 135). The design was altered so that spore deposition and lesion gradients could be measured from a central inoculated strip source (north to south) in replicated strips of potatoes running east and west and sprayed with fungicide 0, 5 or 8 times. The results have been quite different in the two years. Blight was slower to develop in 1967 than in 1966. Unsprayed and early sprayed strips developed fewer lesions as distance from the source increased, i.e. there was a pronounced gradient. In 1966 there was only a slight gradient and some evidence that a substantial proportion of the initial inoculum arrived from outside the experiment. The strips that received the full spray programme had not produced any blight lesions by 22 August, when the haulm was burnt off. (Gregory, Henden and Lapwood)

Moulds and actinomycetes from stored crops

Moist barley. Barley grain with a moisture content of 30% was stored in 45-gal drums under 6 in. of dry straw (*Rothamsted Report* for 1966,

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p. 133). The drums were sealed with butyl rubber sheet after 1, 6 or 11 days, when the temperature of the grain 1 ft deep was, respectively, 23°, 41° and 45° C. After sealing, the temperature of the heated grain fell rapidly to near the ambient temperature and remained there until the drums were unsealed 6 months later. There were few mould or actinomycete spores in drums when they were sealed at 1 or 6 days. In those sealed after 11 days spores of *Aspergillus fumigatus*, *Absidia corymbifera*, *Streptomyces fradiae* and *Micropolyspora faeni* (a cause of farmer's lung disease) were abundant. During 6 months these species became slightly less common and yeasts increased, especially in the drums where the grain had not heated. During the 3 months after unsealing, self-heating raised the temperature to 39–43° C and *M. faeni* became abundant in grain from all drums, but most in those that had been allowed to heat for only 6 days before sealing.

Although little or no growth of actinomycetes or fungi other than yeasts occurred while the drums were sealed, because conditions were almost anaerobic, potentially pathogenic moulds and actinomycetes survived for at least 6 months, probably both as spores and mycelium. The fact that, after unsealing, *M. faeni* became most abundant in the drums sealed after 6 days may mean that mycelium was formed from spores germinated during this initial period. To check these pathogens in commercial stores, self-heating must be minimised both by covering the exposed grain surface with plastic sheet at night and during other delays in filling and by removing at least 3 in. of grain daily when grain is being taken from unsealed silos that are unloaded from the top.

Control of moulding in damp hay. Moulding of hay, stored in 4 l. Dewar flasks (Festenstein *et al.*, *J. gen. Microbiol.* (1965), **41**, 389) was prevented at water contents up to 50% by mixing with 1–2% of propionic or formic acid. More acid was needed to prevent moulding at the larger moisture contents, and more formic acid was needed than propionic acid. Field experiments gave inconsistent results, although moulding was less in acid-treated bales. (Lacey)

Ecology of fungi and actinomycetes in stored fodder and sugar-cane bagasse. Few samples of fodder or bagasse are free from spores of *Aspergillus* spp., and the abundance of some species often indicates the initial moisture content of the material. Table 2 groups the *Aspergillus* spp. isolated from hay (H), barley (G) and sugar-cane bagasse (B) according to their frequency and the moisture content of the sample. The species listed in the fourth group were obtained too infrequently to draw definite conclusions about the optimum initial water content of the substrate. (Lacey and Channer)

Yeasts are characteristic components of the microflora of well-stored moist barley grain. The most common isolates resembled *Endomycopsis chodati* and *Hansenula anomala*. Other species isolated occasionally included *Pichia membranaefaciens*, *P. farinosa*, *Debaromyces phaffii*, *D. hansenii*, *Candida krusei*, *C. tropicalis*, *C. guillermondii* and one of the *C. parapsilopsis* group. (Lacey)

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The actinomycete previously called *Thermopolyspora polyspora*, an important cause of farmer's lung disease, is now considered to be incorrectly identified. It is being redescribed as a new species, *Micropolyspora faeni*. (Lacey, with Mr. T. Cross and Miss A. M. Maciver, Bradford University)

TABLE 2

Occurrence of Aspergillus *spp. in stored products*

Name	Source (see text)	Relative abundance
Mostly from substrates containing little water (e.g. hay at <25%)		
A. amstelodami	H,G	Common
A. repens	H,G	Frequent
A. chevalieri	H	Occasional
A. chevalieri var. *intermedius*	H	Occasional
A. ruber	H	Occasional
Mostly from substrates stored with medium water contents (e.g. hay at 25–35%)		
A. versicolor	H,G,B	Common
A. nidulans	H,G,B	Common
A. terreus	H,G,B	Common
A. candidus	H,G	Frequent
A. oryzae	H,G,B	Frequent
A. sydowi	H	Occasional
Mostly from substrates containing much water (e.g. hay at >35%)		
A. fumigatus	H,G,B	Common
A. niger	H,G,B	Frequent
Other species isolated		
A. carneus	B	Occasional
A. clavatus	G	Occasional
A. fischeri	G	Occasional
A. niveus	H,B	Occasional
A. parasiticus	B	Occasional
A. ochraceus	H	Occasional
A. tamarii	B	Occasional
A. ustus	H	Occasional

Potato diseases

With other tuber diseases continuing to displace potato blight as the main topic of research, the types of experiment and observation made on fungi have changed greatly, and surveys have increased.

Survey of fungal diseases of seed tubers. For the fifth season seed tubers, obtained with the help of the Potato Marketing Board, from representative farms in England and Wales were examined for pathogens. The results illustrate well the type of differences in varietal susceptibility that are now becoming evident (Table 3). The survey is also providing valuable information on the prevalence of the pathogens in different regions. (Hide and Griffith)

Gangrene (Phoma spp). This disease is very erratically distributed between stocks of seed tubers. For example, during the last five years only 6% of King Edward stocks had more than a quarter of their tubers showing gangrene lesions at planting time, but on average 7·6% of tubers in all stocks bore lesions. There is evidence that many more seed tubers carry the

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TABLE 3

Survey of fungal diseases of seed tubers 1966–67

(Per cent tubers infected/per cent stocks with infected tubers)

Examination*	Disease	King Edward	Majestic	Pentland Dell	Arran Pilot	Record
P	Skin spot (*Oospora pustulans*)	36/96	41/96	37/97	20/71	55/100
P	Gangrene (*Phoma spp.*)	9/78	8/62	7/85	5/60	6/70
P	Dry rot (*Fusarium caeruleum*)	1/20	2/43	1/42	10/83	2/38
R	Blight (*Phytophthora infestans*)	3/60	1/23	0/3	2/23	1/34
R	Black scurf (*Rhizoctonia solani*)	20/93	20/97	24/97	24/91	14/90
R	Powdery scab (*Spongospora subterranea*)	13/73	13/76	14/79	10/63	5/72
R	Common scab (*Streptomyces scabies*)	24/91	40/99	22/88	29/89	23/96
No. of stocks examined		153	146	33	35	50

* R = at receipt. P = at planting-time.

fungus than show lesions. Wounding sub-samples of the stocks surveyed and storing them at 5–7° C increased the incidence of lesions about four-fold in 1966–67.

Soil, brushed from lesion-free seed tubers and stored air dry, remained infective to test tubers throughout the storage season. The infectivity of such soils was related to the incidence of gangrene in the stocks from which they came, as also was the infectivity of shallow parings of peel from lesion-free tubers.

Inoculating uniformly wounded tubers and storing them cool provides a sensitive assay for gangrene inoculum in soil, tubers or dead haulm. However, it is difficult to discriminate visually between *Phoma foveata* and *P. solanicola* in lesions on test tubers. In 1966 the yield from gangrene-infected seed tubers averaged about 10% less than from clean tubers (*Rothamsted Report* for 1966, p. 129), and infected seed produced fewer ware tubers. After 12 weeks' storage the proportion of infected ware tubers depended both on the health of the seed and the treatment of the ware after harvest (Table 4). The effects were larger with King Edward than with Majestic. Dipping the tubers in organo-mercurial fungicide immediately before wounding them prevented almost all wound infection, but dipping after the tubers were damaged was less effective. (Griffith)

TABLE 4

Percentage of King Edward tubers with gangrene after storage

	Gangrene infection of seed tubers		
	Lesion-free	Moderate[1]	Severe[2]
"As dug"	0	0·3	0
"Twice riddled"	0·8	8·6	4·9
"As dug and damaged"	0·7	17·6	16·0

[1] Lesions occupying about $\frac{1}{10}$ area of tuber.
[2] Lesions occupying about $\frac{1}{2}$ area of tuber.

Verticillium spp. on potatoes. Wilting, unilateral chlorosis of leaves and premature death of potato haulm has been common at Woburn in recent years. Nutritional deficiencies and damage by eelworm are probably complications, but the symptoms are associated with sporulation of

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Verticillium dahliae within the pith cavity of moribund stems, and later, with the abundant development of microsclerotia. Studying the role of *Verticillium* spp. has been hampered by a failure to find stocks free from all species, by doubts about their individual pathogenicities and by failure to induce different amounts of disease in the field by inoculating soil or by introducing seed tubers from affected crops.

Some of these difficulties are now becoming better understood. The most pathogenic species present seems to be *V. dahliae*, but this has not so far been isolated from seed tubers. There is also evidence that the expression of symptoms can be delayed by large dressings of fertiliser. In one field at Woburn a patch where affected plants appeared early in 1962 bore them again in 1965 when the next potato crop was planted and when it was evident that the patch was infested with *Heterodera rostochiensis*. Collaboration with D. C. M. Corbett (see the Report of the Nematology Department, p. 152) provided evidence that both *H. rostochiensis* and *Pratylenchus neglectus* may aid invasion of potatoes by *V. dahliae*. (Hide and Hirst)

The incidence of coiled sprout, a disorder of unexplained etiology, was studied in a field experiment at Rothamsted, which confirmed that chitting of seed, deep planting and soil consolidation increased the number of coiled sprouts produced by both King Edward and Majestic tubers (see p. 317). Most coils were on chitted tubers and always occurred between 1 and 2 in. from the tuber surface. This was unaffected by depth of planting, suggesting that chitted sprouts coiled soon after they began growing in soil. King Edward had more coils than Majestic.

At Falmouth, Cornwall, long-sprouted, short-sprouted and recently desprouted Arran Pilot seed tubers were grown in rolled and in pointed ridges, both sprayed with pre-emergence herbicides, or under ridges worked down and rebuilt by cultivations. On 12 July most coils were from seed with short sprouts (25% of stems) and fewest (0·2%) from desprouted tubers; tubers with long sprouts had 17% coiled stems. Worked-down ridges had fewest coils, and rolled-sprayed ridges the most fasciated (swollen) coils. The long-sprouted seed under pointed–sprayed ridges yielded most.

Verticillium nubilum, which has been claimed to be a cause of coiled sprout, was isolated from 8/94 Majestic and 0/124 King Edward coils at Rothamsted and *V. tricorpus* from 22/94 and 24/124 coils respectively. However, normal stems also yielded these fungi in similar proportions, *V. nubilum* on 4/184 Majestic and 1/178 King Edward stems, and *V. tricorpus* on 21/184 and 36/178 stems. At Falmouth *V. nubilum* was isolated from 39% of coils and *V. tricorpus* from 46%, but a third of the coils yielded neither species. (Lapwood, Hide and Hirst)

Skin spot and other blemishing diseases. The proportion of eyes infected by *Oospora pustulans* increases while tubers are stored. Before "healthy" tubers were obtained by propagation from stem cuttings, it was not possible to conclude whether this increase resulted from new infections or long-delayed incubation of old ones. To find out, "healthy" tubers of three varieties were inserted among bulk-stored King Edward potatoes and

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examined 3 months later, together with similar control tubers stored for the same time in damp peat. The eyes of the control tubers were free from *O. pustulans* and *Helminthosporium atrovirens*, but of those exposed in the bulk store, the proportion with *O. pustulans* ranged from 4% on Pentland Dell to 17% on King Edward, and from 16% of Pentland Dell to 35% of Majestic eyes showed *H. atrovirens*. From 18 to 78% of eyes from the bulk-stored samples had *Rhizoctonia* runner hyphae, but so had up to 10% of control tubers. The results are inconclusive with *Rhizoctonia*, but suggest that both *Oospora* and *Helminthosporium* caused new infections during storage. The test needs repeating, but it may be significant that spores of the last two fungi were detected in air sampled from within the potatoes using a modified Hirst spore trap. While these fungi are as prevalent on potatoes as they now are, their ability to spread within stores is only of academic interest, but should attempts to improve seed health succeed, it could be an important method of reinfection. (Hide and Stedman)

Previous tests on mature tubers from commercial stocks showed a uniform distribution of infection on excised eye-plugs from different parts of tubers. In 1967 plots were planted with King Edward tubers free from *O. pustulans* (F) (*Rothamsted Report* for 1966, p. 129), other plots were planted with similar tubers inoculated with *O. pustulans* (I) and a third group of plots with tubers from the original commercial stock (S). *O. pustulans* infections were found on all treatments. Plants from F seed were infected most often when adjoining naturally infected Arran Victory marker plants. In I and S plots there was more infection on plots mechanically cultivated than in plots that remained undisturbed after spraying with pre-emergence herbicides. Infection was greatest at the rose-end eyes of progeny tubers. By contrast, *H. atrovirens* most frequently infected skin around the heel-end eyes and stolon scar of progeny tubers. (Hide)

Control of tuber-borne pathogens. Effective and safe ways of killing pathogens on tubers are needed to replace the organo-mercurial fungicides. Tests since 1965 have not so far revealed superior fungicides, and *O. pustulans* has been decreased most by heating tubers. Water at 50° C for 10 or 20 min was slightly more effective than air at 45° C for 8 hr. Higher temperatures caused considerable damage, so careful control of temperature would be required in commercial treatment. As with organo-mercurial fungicides, the best heat treatments almost prevented sporulation of *Oospora* and *Helminthosporium* until the tubers were planted, but by the time the progeny crop was lifted these pathogens had re-established themselves to, respectively, a fifth and a half of their prevalence on the crop from untreated seed. The best hot-water treatment decreased *Rhizoctonia* sclerotia to less than a half of their prevalence on controls, slightly better than attained by fungicide. (Hide)

Common scab, *Streptomyces scabies*. The fourth and final year of testing the relative importance of seed and soil-borne inoculum of common scab confirmed the conclusion that soil infestation is the more important. On either the Highfield or Fosters sites the progeny crop of Majestic tubers was similarly infected by common scab irrespective of whether the seed was

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chitted or not, whether it was severely, moderately or slightly infected, whether it was free from scabs and surface sterilised in formalin. The average of the surface area of tubers scabbed was 6% on Highfield and 2·5% on Fosters. The difference was much greater when expressed, more commercially, as the proportion, by weight, of severely infected tubers ($\frac{1}{8}$ of surface scabbed) which averaged 30% on Highfield and 5% on Fosters.

In previous reports (*Rothamsted Report* for 1964, p. 137, and 1966, p. 131) we have noted the incidence of common scab differing with the amount of nitrogen fertiliser applied. The effect is probably an indirect one, reflecting different dates of tuber formation, some coinciding with wet and others with dry soil. In 1967 both June and July were mainly dry, so that all tubers were formed in dry soil, and the amount of nitrogen had no effect on the amount of scab. With 0, 1 and 2 cwtN/acre (as "Nitro-Chalk"). 2·8, 3·2 and 2·8% of tuber surfaces were scabbed on the susceptible variety Majestic, 1·1, 0·8 and 1·5% on King Edward and 3·2, 3·0 and 3·1% on Pentland Dell, which was the least affected of the three varieties in 1966.

It is thought that *Streptomyces scabies* can enter tubers through stomata but that lesions develop only as the tuber swells. The period when any internode of a tuber is susceptible is brief, because stomata are soon converted into lenticels, and it occurs in sequence from the heel end as the tuber grows. Infection is prevented when the soil is wet, so irrigation can be used to control common scab. A field trial at Rothamsted in 1967 aimed to test the effect of different frequencies of irrigation over a 4-week period. It included four irrigation treatments: control, rain but no irrigation; rain supplemented by overhead irrigation to bring soil at 4 in. depth back to field capacity when soil-moisture tension reached 10, 30 or 50 cm of mercury, measured by porous-pot tensiometers (respectively "10", "30" and "50"). Plots were split for three treatments of Majestic seed tubers: chitted for several weeks before early planting (CHE) and unchitted seed planted early (—E) or four weeks later (—L) to delay the date when tubers formed. Irrigation began only when —E plants began to form tubers and ended 4 weeks later (14 June to 12 July).

TABLE 5

Mean % surface area of tubers affected by scab at lifting

Treatments	Seed		
Irrigation*	CHE	—E	—L
"10"	1·1	1·3	0·7
"30"	1·7	2·0	1·6
"50"	2·9	2·2	1·9
Control (Dry)	8·3	6·5	9·9

* For treatments see text

June and July were dry except for a brief spell in late June and one after 22 July. Scab infection was prevented by "10" during the irrigation period. At lifting (Table 5) lesions occurred only at the heel end of tubers on CHE sub-plots, the result of infection before irrigation began; they occurred only at the rose end of —E and —L tubers, because they were still swelling when irrigation stopped. Because the weather was mainly fine the

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"50" plots usually needed irrigation only 1 day later than the "30" plots. This probably explained why scab incidence differed little between these two treatments. Irrigation for the correct 4-week period (—E) greatly decreased scab, especially "10", but this required 11 waterings, against 7 for "30" and 5 for "50", so was probably the least profitable.

An impressive demonstration of the way wet soil can prevent scab occurred naturally at Woburn, where Majestic seed tubers were planted on a light sandy soil infested with *Streptomyces scabies*. At lifting an average of 60% of the surface area of tubers was scabbed, but many had a white belt of uninfected skin between the severely scabbed rose and heel ends. This was the result of almost 1 in. of rain on 25 June decreasing soil-moisture tension below 30 cm Hg. for 5 days. (Lapwood)

Controlling soil moisture by irrigation can be used briefly to permit as well as to prevent scab infection. Plots were planted with CHE and —E tubers under a mobile Dutch Light glasshouse on scab-infested soil at the School of Agriculture of Nottingham University, Sutton Bonington. During the early part of growth all plots were trickle irrigated to maintain moisture tensions less than 15 cm Hg. When —E plants began to tuberise, and there were already many tubers swelling on the CHE plants, irrigation was stopped on different plots for 0, 5, 10 or 15 days, when it was resumed.

At lifting the tubers exposed to these treatments had, respectively, 1, 20, 34 and 37% of their surface area scabbed on —E plots, and 1, 12, 27 and 22% on CHE plots. Only the heel end of tubers from —E plots was scabbed, whereas tubers from CHE plots had a belt of lesions around their middles.

Plotting the phyllotaxis of the shoot on tubers, by following the sequence of eyes, showed that scab affected 4, 5 and 6 internodes respectively on tubers from plots where irrigation was interrupted for 5, 10 and 15 days, with both CHE and —E plants. Plotting the phyllotaxis also emphasises how, on long oval tubers, such as Majestic, the first-formed internodes became much longer than those at the rose end, so when scab lesions develop they disfigure the tuber disproportionately. It is therefore particularly necessary to begin protective irrigation in time to check the infection of the first-formed internodes. (Lapwood and Dr. T. F. Hering, University of Nottingham School of Agriculture)

Foot and root diseases of cereals

One of plant pathology's greatest needs and opportunities is to develop systemic fungicides effective against foot and root diseases. Until they are found, decreasing the damage caused by diseases such as take-all (*Ophiobolus graminis*) and eyespot (*Cercosporella herpotrichoides*) must depend on other kinds of fungicides and methods, of which several possible ones were investigated.

The effect of CCC on eyespot of wheat. In 1965 eyespot of winter wheat in pots was decreased by watering unusually large amounts of CCC on the soil (*Rothamsted Report* for 1965, p. 126). However, the incidence of

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eyespot was not significantly affected in the field by applying 2½ or 5 lb/acre of CCC to winter wheat.

In 1967 Champlein winter wheat was sprayed with 2½ lb CCC/acre at the three-leaf stage on 7 February (E), six-leaf stage on 21 March (M) and at the early "shooting" stage on 26 April (L); control plots (C) were not sprayed, and plot yields were unaffected by the tractor during spraying. The results are shown in Table 6. Although Champlein is susceptible to *C. herpotrichoides*, it has very strong straw and does not easily lodge. In our experiment 4% of the area lodged on unsprayed plots and none on sprayed plots. In Germany spraying with CCC has increased yield of infected crops more than it did in our experiments, perhaps the varieties grown had weaker straw. (Slope and Etheridge with E. C. Humphries, Botany Department)

TABLE 6

Effect of CCC on eyespot and winter wheat

Treatment (see text)	C	E	M	L
Straw with eyespot (% in June)	37	35	39	32
Crop height (in.)	39·7	37·0	35·9	35·1
Grain yield, (cwt/acre)	54·9	60·4	58·0	58·6

Flame cultivation and eyespot. J. E. Callwood of the National Agricultural Advisory Service and technical staff of Shell-Mex and B.P. Ltd. collaborated in two experiments testing how flame-burning barley stubbles affected the prevalence of cereal foot and root rots. Plots were flame cultivated on 28 September before and after ploughing, then sown with Champlein wheat on 21 October 1966 and scored in July 1967. Table 7 shows that the incidence of each pathogen was almost unaffected by the treatments. (Slope and Etheridge)

TABLE 7

Effect of flame cultivation on cereal diseases

	Flamed before ploughing	Flamed after ploughing
Eyespot (% straws)		
Flame cultivated	56	54
Not flamed	51	51
Sharp eyespot (% straws)		
Flame cultivated	29	26
Not flamed	23	26
Take-all (% plants)		
Flame cultivated	70	75
Not flamed	78	68

Chemical control of take-all. Collaborative work is described in the report of the Insecticides and Fungicides Department (see p. 186) (Waller and Slope with A. H. McIntosh, Insecticides Department)

Soil fumigation with formalin. The residual and cumulative effects of treating soil with formalin were again tested on Little Knott and Pastures. In 1967 "Cappelle" winter wheat replaced spring wheat, and formalin was applied both to stubble and after cultivation. The winter wheat in 1967 suffered less than the two preceding spring wheat crops from *Heterodera avenae*, but eyespot increased to an average of 36% on Little Knott, where

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formalin had no effect. On Pastures eyespot incidence was 15% where formalin was applied to the stubble, 28% where applied after cultivation and 37% without formalin.

The effects on take-all were consistent and striking but also disappointing (Table 8). By July, on Pastures, take-all had severely attacked 28, 39 and 42% respectively of plants on plots treated after cultivation, on stubble or not at all. Comparable figures on Little Knott were 30, 26 and 35%. In 1965 when these experiments began, Pastures had not grown cereals frequently. Table 8 below shows that formalin has not prevented take-all

TABLE 8

Formalin fumigation and take-all of wheat

	Severe take-all (%)		
	1965	1966	1967
Pastures			
No formalin	0	30	33
Formalin in 1965 only	0	35	26
Formalin in 1966 only	0	13	56
Formalin in 1967 only	0	30	34
Formalin every year	0	14	40
Little Knott			
No formalin	54	34	7
Formalin in 1965 only	9	68	1
Formalin in 1966 only	54	5	67
Formalin in 1967 only	54	34	26
Formalin every year	9	13	35

becoming progressively more prevalent. In contrast, Little Knott had been frequently cropped with wheat and barley. Here the untreated plots seem to have experienced a natural "decline of take-all" (*Rothamsted Report* for 1966, p. 213) from 54 to 7%. Although in this field formalin much decreased take-all, it seems, in the following year, to have interfered with its natural decline, and this crop was the most infected. Repeated applications of formalin seem to be becoming progressively less effective on both fields (see also p. 56). (Salt with Widdowson, Chemistry Department)

Other soil fumigants. Measurements were made at Rothamsted (Hoosfield) and Woburn (Lansome) of the effects of applying "D-D" and 85% dazomet dust in 1966, or in 1966 and 1967 on spring wheat (*Rothamsted Report* for 1966, p. 125).

Dazomet had little effect on eyespot, but had both immediate and residual effects on take-all, decreasing its incidence in the first crop but increasing it in the second. Both effects were larger at Woburn than at Rothamsted; at Woburn plots given most dazomet in both years had 3% plants with take-all compared with 26% in the untreated plots. "D-D" also had more effect at Woburn than Rothamsted, though it behaved differently from dazomet and affected eyespot more than take-all, diminishing the incidence of eyespot where it was applied both years and increasing it where it was applied in 1966 only. The greatest increase in yield, 5·0 and 7·3 cwt/acre respectively at Rothamsted and Woburn, came from applying 400 lb/acre of dazomet each year. Applying large amounts of "D-D" each year decreased yield at Rothamsted by 6·7 and at Woburn

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by 2·8 cwt/acre. Some of this loss may result from toxic effects on the wheat, which had deformed and partially sterile ears (see also Report of the Nematology Department, p. 156). On Lansome the prevalence of such ears increased with both the amount of "D-D" and of nitrogen used; the extremes were 10% of affected ears with 50 units of N and 200 lb/acre of "D-D", and 45% with 150 units of N and 800 lb/acre of "D-D".

At Rothamsted 800 lb/acre of "D-D" also diminished yields of winter wheat (Cappelle), and 85% of ears were deformed. Dazomet (85% dust) rotovated in at 400 lb/acre decreased take-all but neither it nor "D-D" affected eyespot. Formalin applied as a drench at 370 gal/acre, decreased eyespot, but no fumigant increased yield significantly.

All the fumigants increased the amount of ammonium-N in the first 6 weeks after treatment, probably because they impeded nitrification. Dazomet also increased nitrate-N, presumably because it contains 17·3% N in the molecule. The only differences persisting until 22 weeks after fumigation were more ammonium-N and less nitrate-N in soil treated with "D-D".

These materials and chloropicrin were also tested for their effects on pea wilt (*Fusarium oxysporum f. pisi*). Chloropicrin and dazomet controlled the disease (see p. 372). (Ebbels)

Soil fumigants would offer substitutes for break crops in controlling soil-borne diseases if they were cheaper and easier to apply. However, those effective against take-all seem also to prevent the natural process of its decline, so they may resemble most break crops in benefiting only a single crop, and may even increase the disease in subsequent untreated crops. There is also some evidence suggesting that, when fumigants are used regularly, they may become ineffective. The results of these experiments support the idea that the natural "decline of take-all" is an effect of biological activity. (Salt and Ebbels)

Methods of assessing *Ophiobolus graminis*. Research on take-all is hampered by lack of satisfactory methods for estimating amounts of soil-borne inoculum. The possibility of isolating the fungus directly from soil was re-examined, but met with little success, and estimating the ability of extracted organic debris to infect indicator plants seems more rewarding. Wet sieving shows that most of the active fungus is in the organic fraction retained on sieves larger than 40 mesh (U.S. Standard), with apertures of 420 μ. Test seedlings grown in the fractions containing larger debris were infected faster and more severely than those grown in smaller fractions. The efficiency of the test can be increased by packing debris in 2·5 cm lengths of 1 cm dia. P.V.C. tubing and placing a seed in each, so that it resembles Garrett's wheat-seedling test (*Ann. appl. Biol.* (1938), **25**, 742). *O. graminis* infecting the seedling roots is initially identified 3 weeks later by the presence of runner hyphae or lesions. It can be identified more reliably by cutting off the roots and keeping them wet for 4 weeks in daylight and at room temperatures, when they rot and perithecia develop. The perithecial test also seems the more sensitive; it has not only confirmed all diagnoses from runner hyphae or lesions but also showed *O. graminis* in some roots without these symptoms, including some grown in debris that passed a

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50-mesh sieve (297-μ apertures) but was retained by a 100-mesh sieve (149-μ aperture). (Hornby)

Pythium root rot. Browning of roots is frequently seen when examining cereal plants for take-all. One common type agrees closely with "Pythium root rot" (Vanterpool & Truscott *Canad. J. Res.* (1932), **6**, 68–93), in which the roots are rotted and contain abundant smooth thick-walled oospores. Its incidence was recorded during May and June on wheat and barley from fields at Rothamsted, Woburn and Saxmundham. An average of 20% of winter wheat plants had one or two infected roots, irrespective of the locality or the treatment given to the plot. The condition was more prevalent and severe on spring barley. At Woburn from 60 to 80% of plants were affected, a third so severely that on average a fifth of their roots were infected. At Rothamsted it was less prevalent, particularly where much phosphate had been applied.

Segments of washed or surface-sterilised root were plated-out, and the fungi that grew from them were identified. Table 9 shows the percentage of

TABLE 9

Fungi isolated from seemingly healthy and diseased root segments

	Wheat		Barley	
	Healthy	Diseased	Healthy	Diseased
Aereobasidium bolleyi	31	43	34	67
Cylindrocarpon spp.	5	24	9	24
Fusarium spp.	7	12	6	12
Pythium spp.	5	24	2	9

517 seemingly diseased and of 369 seemingly healthy root segments that yielded the fungi most often isolated. *Pythium* spp., which are very sensitive to surface sterilants, were probably under-estimated in these assays, and later isolations using a selective medium (Eckert & Tsao *Phytopath* (1962), **52**, 771–777) yielded them more often. Ten of 24 *Pythium* isolates were pathogenic to Cappelle wheat and Maris Badger barley grown in pots. *Aureobasidium bolleyi* was only slightly pathogenic and did not make *Pythium* spp. more damaging. The isolates of *Fusarium* and *Cylindrocarpon* were not pathogenic. (Waller)

Diseases of conifer seedlings in forest nurseries

Experiments at Ringwood, Hants, which ended in 1966, showed that stunting of seedling trees there is mainly caused by ectoparasitic nematodes and was controlled only by soil fumigants, one application of which was beneficial for two or sometimes three seasons.

New experiments were started at Kennington (Oxford) and Wareham (Dorset) nurseries to find how to improve seedling emergence and survival rather than growth. *Phytophthora cactorum* was isolated consistently from dying seedlings of Noble Fir with severe root rot in one seed-bed at Old Kennington. Seedlings and transplants of Sitka Spruce and Lodgepole Pine with less-severe root rot in nearby seed-beds yielded mainly *Cylindrocarpon* and *Pythium*. However, a pure culture of *P. cactorum* was highly

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pathogenic to Sitka Spruce seedlings in dishes of distilled water and caused brown root rot within 4 days at 20–25° C. *Rhizoctonia solani* was often isolated from diseased seed and seedlings that failed to emerge at Wareham, and occasionally as a seed-borne contaminant.

At Old Kennington damping-off of Sitka Spruce seedlings was mainly caused by *Pythium* spp. and was prevented by formalin applied to the soil during winter. A nabam drench applied to seed-beds before sowing and after emergence prevented damping-off but greatly damaged the seedlings. Thiram (80% wettable powder) similarly applied was ineffective. (Salt)

The "Psychrophilic seed fungus". The spread and effect of this unnamed endophytic fungus of Sitka Spruce (*Rothamsted Report* for 1966, p. 133) was studied in seed-beds. Inoculum was prepared by incubating small pieces of agar cultures with autoclaved Sitka Spruce seed at 15° C for 6–8 weeks. One yard square plots were inoculated, just before the live seed was broadcast, by placing a single row of inoculated seeds 1 in. apart across the middle of each plot. Emerged seedlings were counted in a 2-in.-wide strip along the row of inoculum and in 2 or 4 similar strips across uninoculated parts of the plots. At Wareham inoculation decreased emergence by 57% where seed was sown early (8 March), by 16% where sown at the usual date (22 March) and had no effect where sown late (5 or 19 April). In other experiments 21% of seed sown on 22 March failed to emerge in inoculated strips, irrespective of pH ranging from 4·5 to 6·5; Norway Spruce suffered more than Sitka Spruce. The experiments at Old Kennington were sown on 20 March, and inoculation decreased emergence by from 24 to 30%. Fumigation with formalin before sowing had no effect, but treating the seed with 50% thiram controlled the attack completely. (Salt)

Staff and visiting workers

P. H. Gregory retired after 9 years as head of the department, but, happily, will continue to work in the department. J. A'Brook left and D. A. Govier and D. Hornby were appointed. R. H. Kenten was seconded to the West African Cocoa Research Institute, Tafo, Ghana, and J. M. Waller to the Coffee Research Foundation, Ruiru, Kenya. Mr. A. Varma was awarded the Ph.D degree of London University.

Visiting workers included: Miss Susan Allitt (Department of Botany, University of Cambridge); Dr. J. G. Atherton (Department of Microbiology, University of Brisbane); Dr. M. V. Carter (Waite Agricultural Research Institute, Adelaide); Mr. D. L. Ebbels (A.R.C. Scholar), Mr. J. Gilmour (Forestry Research Institute, Rotorua, New Zealand); Dr. Genevieve Lebeurier (Laboratoire des Virus de Plantes, Strasbourg); Mr. I. I. Kondratyev (Moscow Agricultural Academy).

G. A. Hide attended a meeting of the Pathology Section of the European Potato Association at Vollebekk, Norway, in August. J. M. Hirst attended an F.A.O. Symposium on Crop Losses in Rome during October, and D. H. Lapwood visited the Institute of Phytopathological Research, Wageningen, Netherlands, in June to discuss work on common scab (*Streptomyces scabies*).

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