ROTHAMSTED RESEARCH

Report for 1968 - Part 1

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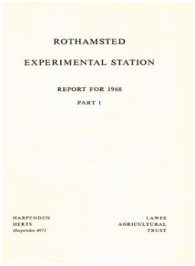

Plant Pathology Department

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PLANT PATHOLOGY DEPARTMENT

J. M. HIRST

Plant pathology began officially at Rothamsted 50 years ago with the establishment in 1918 of an Institute of Plant Pathology with an annual grant of just over £4000 to pay for both entomology and mycology. Since then it has helped to change the face of agriculture although perhaps not quite so much as tractors have, the first of which appeared on the farm at about the same time.

In our Jubilee Year we were glad to welcome many of the delegates to the successful First International Congress of Plant Pathology, held in London in July. We were also pleased that Dr. Mary D. Glynne, a foundation member of the department, temporarily forsook her retirement to contribute a valuable paper on 'Fungus diseases of wheat on Broadbalk' to Part 2 of this Report.

Viruses and virus diseases

Inactivation

Reactivation of irradiated potato virus X in different plants. Some damage caused by ultraviolet radiation to tobacco necrosis virus is repaired in darkness in *Chenopodium amaranticolor* (*Rothamsted Report for 1967*, p. 120) and because of this dark reactivation, photoreactivation is not detectable in this host. To see whether dark reactivation is a general feature of irradiated viruses in this host, the infectivities of irradiated preparations of potato virus X were assayed in it and in two varieties of tobacco (*Nicotiana tabacum* vars. Xanthi-nc and White Burley). When inoculated plants were kept in darkness to prevent photoreactivation, the residual infectivity of the irradiated virus was the same in all three plants. When inoculated plants were exposed to daylight, photoreactivation occurred equally in all three plants. Thus, in contrast to tobacco necrosis virus, potato virus X gave no evidence of dark reactivation in *C. amaranticolor*. (Govier and Kleczkowski)

Inactivation of potato virus X at different wavelengths. Quantum yields for the inactivation of potato virus X by monochromatic ultraviolet radiation of wavelengths ranging from 230 to 290 nm were measured with reference to the energy absorbed by the whole virus and by the virus nucleic acid (RNA). The yields depended on the wavelength, but those with reference to the energy absorbed by the RNA ranged much less (with extreme values of 10^{-3} and $1{\cdot}9 \times 10^{-3}$) than those with reference to absorption by the whole virus. Consequently, the action spectrum for inactivation of a dilute solution of the virus resembled the shape of the absorption spectrum of the RNA. The amount of photoreactivation increased as the wavelength increased and as the year progressed from May

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to July; the extreme values of the photoreactivable sector were 0·43 and 0·86. (Govier and Kleczkowski)

Formation of pyrimidine dimers in the RNA of tobacco mosaic virus. When the RNA isolated from tobacco mosaic virus is irradiated with ultraviolet, part of the infectivity lost can be restored by photoreactivation. By contrast, the RNA in the intact virus is completely protected from photoreactivable inactivation. Therefore, changes in the RNA of virus irradiated intact could not cause inactivation of the photoreactivable kind. Dimerisation of adjacent pyrimidine residues is currently thought to be the photoreactivable damage in irradiated deoxyribonucleic acids. To test if this applies to the RNA of tobacco mosaic virus, tritium-labelled virus and the RNA isolated from it were irradiated so that the two preparations had about the same residual infectivity. The RNA from each preparation was then degraded, fractionated by chromatography, and the radioactive label was used to find the photoproducts. Pyrimidine dimers of cyclobutane type were found in the irradiated free RNA, but not in the irradiated virus. Therefore the possibility was not excluded that the dimers are responsible for the photoreactivable kind of inactivation. (Carpenter and Kleczkowski)

Structure and relationships

The satellite virus of tobacco necrosis virus. The three strains of satellite virus (SV) isolated at Rothamsted share very few antigenic determinants, as shown by serological tests with the homologous and heterologous antisera, but all three strains (serotypes) multiply only in the presence of tobacco necrosis virus (TNV). Of the different TNV strains, strain D did not aid the multiplication of any of the three SV strains, but the others seemed equally able to do so. Strains SV_1 and SV_2, when inoculated mechanically with TNV to tobacco leaves, produced enough virus to be detected serologically (precipitation titre of sap $\frac{1}{8}$ to $\frac{1}{16}$) whereas SV_3 did not. However, SV_3 produced amounts of virus similar to the other two strains in tobacco roots infected with the aid of *Olpidium brassicae*.

Mr. M. Rees of the John Innes Institute, Norwich University, investigated the amino acid composition of the virus protein of SV_1 and SV_2. They were as different in their amino acid composition as they were in their antigenic determinants. The virus protein subunit of SV_1 contains 213 amino acids and has a molecular weight of *c.* 24000, only half the value reported by Reichman (*Proc. natn. Acad. Sci. U.S.A.* (1964) **52**, 1009). The molecular weight of SV is important in interpreting its dependence on TNV. It encloses the smallest nucleic acid (RNA) of any known virus, which is presumably the reason for its inability to multiply on its own. Its protein is serologically unrelated to that of TNV and therefore it is coded for by the SV-RNA. Assuming a triplet code system, about half of the 1200 nucleotides in the SV-RNA will be needed to code for the *c.* 200 amino acids present in the virus protein subunit. Therefore it has enough nucleotides to code for at least another protein (probably an enzyme specific for the multiplication of SV) and not, as Reichman's results suggested, only enough to code for its own protein. (Kassanis)

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Aggregation of tobacco mosaic virus protein. The coat protein of tobacco mosaic virus (TMV) polymerises into rods resembling virus particles when aqueous solutions are acidified. These aggregates are stable between pH 3·0 and 6·5 but outside these limits they dissociate into subunits. This system has been used to study how virus particles are assembled. Two types of protein rod are known. In one, the polypeptide chains are packed in a helix (as in the virus), whereas in the other they are in discs, consisting of 17 polypeptide chains, stacked face to face. The two forms have very similar interchain bonding, so their energies of formation cannot be very different, but the conditions that determine the type of aggregate formed have not yet been defined.

When preparing TMV protein by treating virus with alkali it was noticed that 'stacked-disc' protein aggregation occurred in solutions at pH 10·5 for several days. Further investigation confirms that purified protein (4 S) does polymerise in the range of pH 7 to 11 and that the rods produced always have a stacked-disc structure indistinguishable from similar rods formed in acid solutions but are stable over the wider range pH 3 to 11.

The slow polymerisation at 5° C allowed aggregation to be studied with the electron microscope, the analytical ultra-centrifuge and polyacrylamide gel electrophoresis. At first, intermediate-sized structures form, of which the most important are double discs, that stack to give dimers, trimers and, ultimately, very long rods. Because RNA is not present, there is no sharp limit to the length of the rods, as illustrated by the broad, rapidly diminishing peak (150 S) observed in the analytical centrifuge and rods up to 1 μ long seen in the microscope. The process is little affected by pH between pH 7 and 11 or by the alkali used, but is much affected by ionic strength and is faster at 0·1M than at 0·02M. When formed, the rods were little affected by changes of pH, ionic strength or protein concentration.

Acidifying a freshly prepared protein solution (4 S) invariably leads to the formation of helical aggregates, whereas identical treatment of old, partially aggregated, suspensions may produce large stacked-disc aggregates. Both types of polymerisation do occur simultaneously.

The stacked-disc structures are not only stable over a wide pH range but can form in alkaline solutions, so it is improbable that subunits are linked by carboxyl–carboxylate hydrogen bonds, as suggested for the virus and polymerised protein, and postulated as the reason for their pH sensitivity. Aggregation probably depends entirely on the formation of hydrophobic inter-subunit bonds (hence the dependence on ionic strength), although once formed the structure may be stabilised by polar bonding. (Carpenter)

Tomato spotted wilt virus. This virus has a structure and behaviour unique among plant viruses. Superficially the virus particle resembles that of influenza virus but the electron microscope shows that, in tomato, it develops by budding from complex cytoplasmic membranes. The process of budding differs from that occurring in the myxoviruses (e.g. influenza) and is reminiscent of that in mouse leukemia and mammary tumour viruses.

Potato virus X. The electron microscope shows that 'inclusion bodies',

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long known from light microscopy in leaf cells infected with potato virus X, are unusual and complex structures that may prove diagnostic for strains of potato virus X, as 'pin wheels' have for the potato virus Y group of viruses. (Milne)

Virus-like particles in sugar beet. The search for a host free from these spherical, virus-like particles (*Rothamsted Report for 1967*, p. 124) has failed and they have now been found in *Beta patula*, previously thought to contain none. Their concentration decreased below the limit for detection when plants were grown at 36° C for 5 weeks, but many particles reappeared ten weeks later in bud cuttings taken from the crowns. Attempts are now being made to obtain plants free from the particles by culturing apical meristems. (Pullen)

Filamentous virus-like particles, about 12 mμ thick, occurred in thin sections of the cytoplasm of sugar beet infected with the water mottle strain of beet mosaic virus. In addition to these, which were expected, numerous round particles, about 57 mμ in diameter, occurred in the nuclei of diseased leaves, often close to the nucleolus. Whether these are associated with this strain of beet mosaic virus or with the spherical particles referred to in the previous paragraph, is uncertain. (Ammar)

Variability

Naturally occurring defective strains of TMV. Yellow lesions occur in tobacco plants (type White Burley, var. Judy's Pride) infected with the type strain of TMV. From these, strains were isolated after repeated passage through single lesions that differed strikingly from the type strain. One strain caused yellow local lesions only; another, yellow local lesions followed by a systemic bright-yellow mottle, but the systemically infected plants later produce leaves without symptoms. From a single lesion caused by the 'yellow systemic' strain, a mutant was isolated that produced local lesions consisting of several concentric green and yellow rings, symptoms resembling those produced by viruses of the ring-spot type. From this 'ring-spot' variant, another was isolated that produced concentric rings and oak leaf patterns in some of the uninoculated leaves.

All these defective strains were difficult to transmit by inoculating with sap except with the aid of carborundum. Even with carborundum, susceptible plants of Xanthi rarely produced more than 50 necrotic lesions per half leaf, as many as produced by inocula containing 0·01–0·1 μg/ml of type TMV, or about 1/10000 the amount in sap from plants infected with the type strain. In contrast to the type strain, phenol extracts from leaves infected with these strains are as infective as sap which suggests either that leaves contain some free virus RNA or the virus particles are as unstable as the RNA. Phenol extracts of equivalent infectivity, from the TMV type strain ground with healthy leaves produced only an occasional lesion. Rod-shaped particles were rarely seen when extracted sap was examined in the electron microscope, and those seen after concentrating the virus were broken and had many abnormalities along their length.

Sap lost all its infectivity after ten minutes between 70° and 80° C, and most of it in a few days at 20°–25° C. This contrasts greatly with the

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behaviour of type TMV in sap. However, when the type strain was mixed with sap from healthy leaves, at a concentration producing similar numbers of lesions as sap from plants infected with systemic chlorotic strains, it also lost most of its infectivity in 2–3 days. The reason is being sought. (Kassanis and Woods)

Henbane mosaic virus. The necrotic B strain of this virus, described in last year's *Report* (p. 123), varied much less in plants grown at 20°–25° C than at 25°–30° C. These variants isolated from infected plants grown at 25°–30° C retained their characteristic differences whether plants infected with them were at 20°–25° C or at 25°–30° C. (Watson)

Transmission

Potato mop top virus and* Spongospora subterranea *(Wahl.) Lager. Tobacco and tomato plants grown in soil infested with spore balls of *S. subterranea* scraped from powdery scabs on potato tubers infected with potato mop top virus (PMTV) became infected with the virus (*Rothamsted Report for 1966*, p. 115). In additional tests with *S. subterranea* from three other samples of potatoes, virus was transmitted in two but not in the third. Both isolates of *S. subterranea* associated with the virus have been maintained in the roots of tomato plants grown in sand culture and are still associated with the virus. One, kept in this way for several years, has been transferred to 70 successive tomato plants all of which have also become infected with PMTV. It is impossible to be certain that the isolates of *S. subterranea* are not contaminated with another organism that transmits the virus. However, the long series of transmissions, together with the fact that virus has been found associated with *S. subterranea* spore balls, taken directly from the scabs that contain predominantly this fungus, from different places, varieties and at different times, is strong circumstantial evidence that the fungus is the vector.

To see whether the fungus could be dissociated from the virus a host for *S. subterranea* was sought that was unlikely to sustain the virus. *Lolium perenne* supported the fungus and after two passages in this host the culture was returned to and infected tomato but no longer seemed to carry the virus, for inoculation from the tomato roots did not cause lesions in leaves of *Chenopodium amaranticolor*. The virus-free *S. subterranea* was inoculated to the roots of *Nicotiana debneyi* and tobacco and PMTV to their leaves. Some time after the virus had become systemic, zoospores were collected from the roots and transferred to tomato plants, which were later tested for virus but contained none. Further attempts to re-associate the fungus and virus are being made.

Cool, dull weather favours necrotic symptoms caused by PMTV in leaves of tobacco and *C. amaranticolor*. In warm, dull conditions chlorotic rings and spots form in infected tobacco leaves. French bean was also found to be a host. Some shoots from tubers infected with *S. subterranea* and PMTV were virus-free. Electron microscopy showed short, stiff rod-shaped virus particles in dip preparations from tobacco and *N. debneyi* leaves with symptoms and from tomato roots infected with *S. subterranea* and PMTV; they were not numerous and often occurred in bundles.

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The fact that *S. subterranea* can form zoosporangia in *Lolium perenne* is interesting for the epidemiology of powdery scab. The fungus can persist in this stage indefinitely by repeated cycles of infection in solanaceous hosts. Under the conditions we use, tumours form on infected tomato plants after several months but spore balls are not produced, though they have been recorded on tomato elsewhere. However, when zoospores from tomato were inoculated to potato, tumours containing spore balls developed on the roots. Presumably, *S. subterranea* could also survive in the zoosporangial stage in its grass host but the agricultural importance of this would depend on whether the grass roots survived long enough after ploughing to maintain the fungus until potatoes could be infected. (Macfarlane)

Strains of cucumber mosaic virus. The lettuce strain (LCMV) and yellow strain (YCMV) differ greatly in their ability to be transmitted by aphids (*Rothamsted Report for 1967*, p. 123), but this seems not to be explained by the fact that LCMV is less concentrated in infected leaves. Concentrated preparations of each strain were made and aphids were fed on them through Parafilm 'M' membranes, before they were put on healthy tobacco leaves. Only LCMV was transmitted and this not consistently. Transmission may be impeded by the obstruction the film offers to probing, because only 30% of aphids transmitted LCMV from infected leaves covered with Parafilm 'M', whereas 100% transmitted from uncovered infected leaves. (Pullen)

Aphids and viruses

Sugar beet. The prediction from winter weather (Watson, *Pl. Path.* (1966) **15**, 145–149) that aphids would not be prevalent early or spread viruses early in 1968 was fulfilled. Most aphids, including those of sugar beet, were few and arrived late. *Myzus persicae* increased late in July enough to spread yellowing viruses (mainly mild yellowing) and for 17% of plants in Highfield to have symptoms by 31 August. More developed symptoms during September, but this was too late to affect yield. At Woburn, only 10% of plants showed symptoms at the end of August; these were recently infected and none were dwarfed. (Watson)

Potato. In contrast to the considerable spread of potato viruses around infected plants in 1967 (*Rothamsted Report for 1967*, p. 124) there was very little in 1968 when winged aphids were few. Thus in July the crops grown to supply seed for 1969 had no more than 0·09% of plants infected, compared with more than 5% in seed produced in 1967. (Govier)

Cereals. Although most aphid species were few and the summer weather was cold and dull, grass and cereal aphids became exceptionally prevalent. The weekly catches in the suction traps operated by the Entomology Department between Kent and Scotland provided valuable information. The source of these aphids is uncertain, and although some may have overwintered locally on the roots of grasses (Mr. R. Prior, Ministry of Agriculture Plant Pathology Laboratory, Harpenden) most

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may have come from a distance. The vectors of barley yellow dwarf virus (BYDV) most commonly caught early (June–July) were *Sitobium avenae*, *Metopolophium dirhodum* and late (July–August) *Rophalosiphum padi*. The first aphids were found at the end of May, but during warm spells in early June and late July they multiplied rapidly and briefly reached populations of approximately 50–60 per ear on spring wheat.

Virulent BYDV was isolated from stunted and reddened winter oat plants sent from Evesham, Worcs. in late April. Similar virus attacks were seen by National Agricultural Advisory Service officers in winter crops in other Midland and Western areas. In the past such virus strains isolated from winter crops have all been transmitted best by *R. padi*, as were those tested in 1968. The first infected barley plants at Rothamsted were found on 19 June, when they already had advanced symptoms. These almost certainly became infected in May, before aphids were detected in trap catches. By late June and early July, 25–50% of oats showed symptoms, as did 30% of barley and 5–10% of wheat plants. Many more plants had probably just been infected because most *S. avenae* and *M. dirhodum* collected from plants then transmitted BYDV to test plants in the glasshouse. In contrast to the strains isolated early in the season, most of these were avirulent and not readily transmitted by *R. padi*. Present information suggests that plants infected by avirulent strains after mid-June would not have their yields greatly decreased, but nothing is known about how virus inoculated directly into the glumes of developing seeds can affect yields. (Watson)

Aphid multiplication in the presence of fungicides. Cereal plants on which aphids are caged for multiplication usually become severely infected with mildew. For some years we have used griseofulvin as a fungicide, but it has been suggested that this decreased the fecundity of aphids. Wheat and oat plants were sprayed 14 days after emergence (4 plant/pot each with 3 leaves and 6–10 in. tall) with water, griseofulvin (0·1%, 15 ml/pot) or BASF ('BASF—Melthau mittel', cyclododecyl-morpholinacetate, by Badische

TABLE 1

Effect of infestation on plants and aphids (fresh weight g/pot)

Infestation	*R. padi*	*S. avenae*	None	S.E.
Plant weight				
Wheat	4·9	7·1	20·9	± 0·93
Oats	4·7	7·2	27·2	± 0·93
Aphid weight				
Wheat	0·21	0·19	—	± 0·022
Oats	0·27	0·24	—	± 0·022

— = no data available.

TABLE 2

Effect of fungicides on plants and aphids (fresh weight, g/pot)

	Griseofulvin	BASF	Water	S.E.
Yield of plants	14·0	9·3	12·6	± 0·66
Yield of aphids	0·27	0·19	0·22	± 0·027

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Aniline und Soda Fabrik AG. Ludwigshafen am Rhein, at 15 ml/pot of 0·25%).

After spraying, some pots were infested with 25 apterae/pot of *R. padi* or *S. avenae*. Aphids were counted after 17 to 24 days and weighed 44 days after emergence. Aphid infestation prevented tillering but uninfested plants had at least four tillers. Both fungicides slightly decreased aphid number at 17 days, the decrease had disappeared at 44 days and griseofulvin may even have slightly increased multiplication. Differences in plant yield could not be attributed to effects of the fungicides on mildew because, unusually, mildew appeared only on a few oat control plants. (Tables 1 and 2.)

At the end of the experiment there were 50–100 mg of aphids per g of plant and several plants had been killed. Clearly aphids do not damage plants in crops anything like so severely, presumably because they do not usually arrive until well after tillering, are exposed to lower temperatures and restricted by various factors such as rain, parasites and predators. (Watson and Allen)

Spore dispersal

Spore deposition within crops. The deposition of sugar-beet pollen in wheat crops (*Rothamsted Report for 1967*, p. 127) was again studied as a guide to the behaviour of fungus spores. The spore trap array was extended through the source crop and to 100 m downwind of its leeward edge in wheat, and with two additional trapping poles within the first 10 m. Unfortunately, northerly or easterly winds predominated during the flowering period and few tests were possible.

With the collaboration of Mr. A. C. Chamberlain (Atomic Energy Research Establishment, Harwell) laboratory and field tests began on the deposition of *Lycopodium* spores on plants and spore traps, using spores labelled with radioactive iodine. Preliminary results indicate that making surfaces sticky may greatly increase spore capture, particularly in strong winds.

Splash dispersal. The effects of drop size and height of fall on splash patterns were tested in still air. The volume of liquid deposited was assessed by colorimetric assay of dissolved Napthol Green B. Scarcely any splash droplets were caught beyond 10 cm from collisions of 1 mm diameter drops. Drops of 2–5 mm diameter splashed from 40 to 70% of their volume further than 10 cm after falling at least 1 m. The distance splash droplets travelled was increased as drop size and height of fall increased. Consequently, small drops often deposited more splash droplets per unit area within 30 cm of the impact than large drops. When total deposition was measured on consecutive 5 cm annuli outwards from the impact point, there was a maximum 20–40 cm out from fast collisions.

A new dropping mechanism is being constructed for tests in crops but a few tests made from a target 40 cm above ground in a crop of field beans (*Vicia faba*) showed that no fluorescein was detectable further than 4 m from the source. There was some evidence of preferential movement in the space between rows when winds were light. (Hirst and Stedman)

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Moulds and actinomycetes from stored crops

It has been known for 250 years that dust from mouldy grain can cause respiratory diseases in man, but the hazards to health of workers and livestock have only recently been appreciated. Recognition of the problem, and probably much of its cause, stems from the increased use of machines. Biologists have done little to inform engineers of the kinds, quantities and sizes of harmful spores in these dusts. Fungus spores are usually the largest particles, and most on fodders range from 40 μ to 4 μ diameter; some cause aspergillosis, ulceration and abortion in cattle, others that produce poisonous metabolites, are being increasingly implicated as causes of mycotoxicoses. Actinomycetes, most of which have spores less than 2 μ diameter, can penetrate into the alveoli of the lungs, and they cause diseases such as fog fever in cattle and farmer's lung disease and bagassosis in man.

Spore concentration on farms. Average spore concentrations in the open air reach 10^3 to $10^4/m^3$ air in summer, exceptionally and briefly $10^6/m^3$ spores have been recorded among crops. Numbers may be much larger during some farm operations when the material handled has moulded or heated spontaneously. Recently air sampled in an open shed when large hay bales were being opened contained $1{\cdot}7 \times 10^8$ spores/m^3, and this is by no means a record (Lacey & Lacey, *Trans. Br. mycol. Soc.* (1964), **47**, 547; *Rothamsted Report for 1964*, p. 142). In 1968, weather was poor for haymaking, and some hay that lay wet for 10 days before baling had $2{\cdot}3 \times 10^7$ spores/g (dry weight), of which approximately half were *Cladosporium*, a quarter actinomycetes and bacteria, and a tenth of 'Aspergillus type'. Downwind of the baler working in this crop there were $1{\cdot}1 \times 10^8$ spores/m^3 (111 in every cubic centimetre) in approximately the same proportions but with about $10^6/m^3$ of each of *Helminthosporium*, *Epicoccum*, *Mucor* and *Aspergillus*. When an Andersen sampler, operated by a compressed air injector, was towed across the field on a low trolley after baling, many of the thermotolerant and thermophilic organisms common in mouldy hay were isolated, including *Thermomonospora viridis*, *Streptomyces griseus* and *Thermoactinomyces vulgaris* frequently, but *Micropolyspora faeni*, an important cause of farmer's lung, only once.

Control of moulding in damp hay. In the laboratory, additives tested to prevent moulding of hay included 'Hay Savor' (a proprietary material marketed by Agil Ltd., Maidenhead); 2 phenyl phenol; sodium 2 phenyl phenate and oxyquinoleine (supplied by S.D.C. Chemicals Ltd., Regent Street, London W.1); also propionic and formic acids. Adding 'Hay Savor' at 0·1–2·0% by weight to hay containing 25–40% water had no effect on heating or moulding. Hay with 40% water did not mould after adding 0·5% of 2 phenyl phenol, its sodium salt or oxyquinoleine. Further tests confirmed that 0·5 and 1·0% of propionic acid prevented moulding of hay with 30 or 50% water; 1% of formic acid did not prevent moulding in hays with 30 or 40% water.

Field experiments were handicapped by wet weather and difficulties of

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applying materials during baling. It was possible to treat hay baled at 34% water only with 'Hay Savor' (2 lb/ton) or a 1:1, propionic:formic acid mixture (at 2% by fresh weight). Rain soon afterwards wetted bales to a depth of about 1 cm. After storage the outsides of untreated and 'Hay Savor'-treated bales were visibly mouldy and the hay inside was classified 'very mouldy' with abundant thermophilic actinomycetes, *Aspergillus fumigatus* and *Absidia* spp. but the acid-treated hay was only slightly mouldy with few thermophiles. Untreated, 'Hay-Savor' and propionic: formic acid treated hays had respectively 70, 41 and 3 ($\times 10^6$) spores/g dry weight, and the hay with 'Hay Savor' yielded 75% as many colonies as untreated hay and the other treatment only 9%.

Bagassosis. The squashed and chopped fibre remaining after sugar is extracted from cane is called bagasse. When self-heated and mouldy it carries dust that causes bagassosis, a respiratory disease clinically similar to farmer's lung, and frequent in factories where stored bagasse is compressed into particle board. Samples of bagasse associated with a recent occurrence of bagassosis (Hargreaves *et al.*, *Lancet* (1968) pt 1, 619) were examined and air was sampled during experimental production of particle board. *Thermoactinomyces vulgaris*, to which the patient was sensitive, was always present although not abundant unless bagasse was being disturbed. The microflora of the 60 samples examined differed greatly, with up to $1{\cdot}06 \times 10^8$ actinomycetes and bacteria and $1{\cdot}13 \times 10^8$ fungus spores/g dry weight. The commonest fungi included *Allescheria terrestris*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *Cladosporium* spp., *Humicola lanuginosa*, *Paecilomyces varioti*, *Penicillium* spp., and yeasts (including *Kluyveromyces marxianus*, *K. fragilis*, *Candida guillermondii*, *C. tropicalis* and *C. krusei*). Actinomycetes isolated frequently include *Streptomyces albus*, *S. griseus* and grey *Streptomyces* spp. (including *S. antibioticus*, *S. griseoflavus*, *S. thermoviolaceus* and *S. violaceoniger*), *Thermoactinomyces vulgaris*, *Thermomonospora viridis*, and unidentified species related to Nocardia, Pseudonocardia and Thermoactinomyces. *M. faeni* was only occasionally isolated. (Lacey)

Actinomycetes from stored crops. Some samples of hay treated with propionic and formic acids contained many white thermophilic actinomycetes with sporophores, dichotomously branched to various degrees and carrying single oval spores, on both substrate and aerial mycelium. Similar types were isolated from straw and bagasse. They show an almost continuous range of morphology, from those resembling *Thermomonospora viridis* and *T. curvata* to those resembling Actinobifida, but the many intermediates make division into species difficult. (Lacey, with Mr. T. Cross, Bradford University)

Foot and root diseases of cereals

Bioassay of *Ophiobolus graminis* in soils. We give below results of field experiments in which the occurrence of take-all (*Ophiobolus graminis*) has been related to the cropping history, manuring and yield of winter wheat and spring and winter barley. Full understanding of such results requires

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better ways of measuring the distribution and persistence of *O. graminis* in soil than we have now, and more knowledge of the way its development is affected by the physical and biological environment. At present there is no way of estimating *O. graminis* populations by direct isolation from soil, so bio-assays with host indicators must be used. Previous experiments suggested much variability in the infectivity of replicate soil samples and in the results obtained in different environmental conditions. Rooms with controlled lighting and temperature now provide reproducible environments for assays of two kinds. The first (a modification of the assay used by Ogilvie & Thorpe, unpublished) measures the intensity of attack on plants in terms of an 'infection index'. The second seeks to measure the number of 'infective units'/unit volume of soil.

Preliminary experiments showed that discrete take-all lesions could be counted only during the first 3 weeks of the growth of wheat seedlings at 13° C, later than this multiple lesions developed, probably from a single source of inoculum. A standard test was designed in which 16 germinated wheat seeds were sown in approximately 300 cm^3 of test soil. After 35 days the roots were washed, trimmed to 6 cm below the seed and scored for the percentage of the five first-produced seminal roots that had take-all lesions, the 'Infection index'.

This test was used to compare two naturally infested soils (Woburn, much; Rothamsted, little) that were kept in the open in large pots after collection in autumn 1967 and then sown with field beans in spring 1968. On three occasions a core of soil 5 cm × 15 cm deep was taken from each of the 24 replicate pots for each soil; each core filled one assay pot. Although the soils had been thoroughly mixed when taken from the field, the infection of test plants varied erratically between replicate assay pots. This could have been caused by different soil conditions but more probably indicated that surviving inoculum sources were few and of variable strength. Despite this variability, there were large differences in mean infection indices between the soils and the sampling dates. (Table 3)

TABLE 3

Bio-assay of persistence of Ophiobolus graminis *in soil*

	Infection index (%)	
Soils sampled	Woburn soil	Rothamsted soil
30 November 1967	22·9	3·7
17 April 1968	10·1	1·0
3 September 1968	1·9	0·05

This assay was also used to compare soils from the Cereal Disease Reference Plots with different cropping histories. From one plot of each treatment, 24 random soil cores were taken before sowing in spring and again after harvest. The occurrence of take-all was also estimated from plant samples of the previous and intervening spring wheat crops. The infection in individual assay pots again ranged widely, but the mean infection indices usually agreed with the estimate of crop infection, even where this was much less than expected from the previous cropping. (Table 4.)

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TABLE 4

Crop infection and infection index for Ophiobolus graminis *in soils with different cropping histories*

Previous crops	1963	W2	Be	W2	W2	W2	0
	1964	W3	0	Be	W3	W3	W1
	1965	W4	W1	0	Be	W4	W2
	1966	W5	W2	W1	0	Be	W3
	1967	W6	W3	W2	W1	0	Be
Crop 1967							
% plants infected, June		46	59	2	2	—	—
% roots infected, June		4·5	8·6	0·1	1·1	—	—
Soil assay, March 1968							
Infection index %		8·7	17·4	1·7	1·3	1·1*	0·7
No. of cores infective		24	23	13	6	5	13
Crop 1968		W7	Be	W3	W2	W1	0
% plants infected, June		41	—	4	41	9	—
% roots infected, June		4·0	—	0·2	12·3	2·1	—
Soil assay October 1968							
Infection index %		59·1	3·1	24·1	74·1	35·3	2·4†
No. of cores infective		24	8	17	22	22	4

* Mean weighted by 1 core with index 20·8; if excluded Index = 0·2
† Mean weighted by 1 core with index 53·8; if excluded Index = 0·2
W1, W2, etc. = 1st, 2nd, successive wheat crops; Be = field beans; O = oats.

Possibly the most important result is the fact that several cores were still infective 2 years after a wheat crop had been grown. The 24 replicate cores represent only an area of 470 cm² (approx. 1½ ft²) from each plot, so it is not surprising that take-all appears so readily among susceptible crops. Indeed, if field conditions were as favourable to *O. graminis* as this assay, a two-year break would be much less effective than it usually is. (Slope, Henden and Etheridge)

A quantitative technique for estimating the pathogenic population of *O. graminis* in soil was also developed from the preliminary method described in last year's Report (p. 138). A series of volumetric dilutions of the medium to be tested is made with sand, several replicate pots prepared at each dilution and a single wheat seedling is grown in each pot. At the end of the test, the presence or absence of root infection is recorded and the results used to obtain the 'maximum likelihood' estimate of density, using the assumptions given by Maloy and Alexander (*Phytopathology* (1958) **48**, 126–128). The estimate is of 'infective units', which are envisaged as pieces of plant debris colonised by *O. graminis*. Last year's work suggests that these are most likely to occur in the coarser debris in soil and that they will not be uniform in size or shape.

Populations were estimated either directly with whole soil or indirectly with debris extracted quantitatively from soil. But for the difficulties of extraction, the latter is the easier and the more sensitive method. Estimates are expressed as the number of infective units/150 cm³ of soil merely because the pots used hold that volume. The factors affecting the density estimate were investigated, using whole soil, because the automatic extraction of debris has not yet been perfected. The estimate depended on the temperature (largest estimates at 20–25° C) and increased with time (in

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a 6-week experiment sampled weekly). Two or four seedlings grown in 150 cm^3 of test medium and scored collectively gave slightly larger estimates than one seedling/pot. Eight seedlings/pot gave smaller estimates, probably because of difficulty in examining the tangled root systems efficiently. The confirmatory test, of rotting wet roots in the light until perithecia form, occasionally increased the score of plants infected, but increased the estimate only slightly. Air-dried soil gave an estimate similar to tests made on samples of soil straight from the field; this is convenient because whole soil can be diluted more easily when dry. However, disease severity (measured as the proportion of seminal roots infected, cf 'infection index') was considerably decreased by air drying. An experiment with parallel dilution series of the same soil revealed no large variation in replicate estimates of density. The effect of soil moisture and the time and temperature for optimum estimates have yet to be decided.

Preliminary estimates were made of the seasonal fluctuations in the density of infective units in the top 15 cm of cereal soils at Woburn. They are based on experiments with whole soil, made arbitrarily at 20° C for 3 weeks with one plant/pot, so are comparative rather than maximal for 20° C. During the growth of a barley crop on Butt Furlong the maximum estimate rose to 11·5 infective units/150 cm^3 of soil in late June. In early May the figure was 0·1/150 cm^3 and at harvest it was 0·5/150 cm^3. While the rows remained undisturbed, the estimate for soil between rows was always less than that from soil within rows. In the fallow period after a wheatcrop on Road Piece, the population of infective units declined from 2·7/150 cm^3 on 21 September 1967 to 0·8/150 cm^3 on 28 February 1968.

Measuring the density of inoculum suggests that the variability common in assessments based on counts of lesions or infected roots results from few large infective units occurring sporadically among pots. The density estimates should also make it possible to infer the spatial distribution of infective units, especially in the fallow period after ploughing. (Hornby)

The prevalence of take-all has usually been assessed by examining crop samples. This may be best for measuring the effect on yield but is not necessarily the best way to estimate the hazard to future crops or to define how interactions between soil, climate and the soil microflora affect the development of the disease. Bioassays will find a place in research and advisory work only if they provide extra or more accurate information reasonably easily. Our two approaches have different starting points. The first developed from field experiments, so the variable infectivity of individual soil cores provides important information and also, with much replication, what seem sensible assessments. Measurement of the infection index already adds considerably to the results from field experiments. The second approach, designed to study the form and distribution of surviving inoculum, provides information comparable with the first, but should be better suited to measuring the number and distribution of sources. When debris extraction is improved, the estimates of density may be easier to convert to a simple assessment technique. Although, for some purposes, such as studying 'decline' and microbial interactions, it may be essential to retain whole soil. Only extensive use and comparison between assay

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methods and plant samples on the same experiment can establish confidence in estimates of *O. graminis* in soil.

***Serological identification of* O. graminis.** An antiserum prepared against components of the mycelium reacted with mycelial extracts in gel diffusion tests. The technique should provide a rapid means of checking the identity of isolates and it is hoped to develop a fluorescent antibody technique to aid the identification of the fungus associated with roots or soil debris. (Hornby and Govier)

***Microbiology of* O. graminis.** Other work in collaboration with the Soil Microbiology Department is described on p. 84. (Hornby and Brown)

The effects of frequent cropping with wheat or barley

Spring barley. The Intensive Spring Barley Experiment, begun in 1961 in Little Knott field, ended. In each of the last 3 years continuous barley (6th–8th crops) was compared, at 4 amounts of nitrogen fertiliser, with 1st, 2nd, 3rd and 4th barley crops grown after two successive crops (oats, beans) not susceptible to *O. graminis*. Table 5 shows the mean grain yields and incidence of take-all in early July. Eyespot (*Cercosporella herpotrichoides*) was not prevalent (only 11% straws infected in the continuous barley). Most broad-leaved weeds were controlled by herbicide sprays, but knot grass (*Polygonum aviculare* agg.) and mayweed (*Tripleurospermum maritimum* ssp. *inodorum*) were prevalent in continuous barley not given nitrogen. Perennial grass weeds (*Agropyrum repens* and *Agrostis gigantea*) were only partially controlled by autumn sprays of amitrole or paraquat; wild oats were controlled by hand-weeding. Some crops lodged each year, most affected was the 1st barley given 0·6 or 0·9 cwt nitrogen/acre. Take-all, prevalent in all barley crops except the first, was most common in the 3rd and 4th crops, but became less prevalent in continuous barley. Thus, take-all declines in continuous barley as in winter wheat (*Rothamsted*

TABLE 5

Average grain yields and incidence of take-all in spring barley (Proctor), 1966–68

	Nitrogen (cwt/acre)			
	0	0·3	0·6	0·9
Grain yield, cwt/acre				
1st crop after oats, beans	34·3	40·4	44·6	44·6
2nd crop after oats, beans	23·3	34·1	40·5	43·8
3rd crop after oats, beans	16·4	26·8	34·4	41·3
4th crop after oats, beans	18·1	27·2	34·1	39·9
Continuous barley since 1961	17·1	29·1	38·2	40·6
% plants with moderate or severe take-all, early July				
1st crop after oats, beans	6	1	2	1
2nd crop after oats, beans	26	22	12	7
3rd crop after oats, beans	55	34	26	17
4th crop after oats, beans	47	39	27	15
Continuous barley since 1961	28	27	13	12

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Report for 1963, p. 108). Although less attacked by take-all, continuous barley did not consistently yield more than the 3rd or 4th crops, possibly because other factors, such as weeds, counteracted the benefit from decreased take-all. Nitrogen fertiliser decreased the proportion of plants moderately or severely attacked by take-all, but there is not enough evidence to decide whether barley after barley responded to much nitrogen because this decreased take-all or because successive barley crops depleted available soil nitrogen.

Winter barley. The Intensive Winter Barley Experiment, begun in 1965 in Hoosfield, also ended. Because this crop ripens earlier than other cereals it often suffers serious damage from birds. This happened on the experiment in 1968, so yields are not reported. Table 6 shows the incidence of take-all and eyespot as means from 3 nitrogen treatments (0·3, 0·6, 0·9 cwt N/acre) that had only small effects on eyespot and, surprisingly, on take-all. One year free from barley greatly decreased take-all, but had less effect on eyespot. Unexpectedly, eyespot was less prevalent in barley after oats than in barley after beans, although 8% of straws had been infected in the winter oat crop. This also happened in 1965, the only other year oats and beans were compared, but we cannot explain it.

TABLE 6

Incidence of take-all and eyespot in winter barley (Maris Otter), 1968

Previous crops	% plants with take-all		% straws with eyespot	
	Total	Severe	Total	Severe
sB.wO.wB.Be	5	2	38	21
sB.Be.wB.wO	6	3	21	11
sB.wB.Be.wB	36	18	45	24
sB.wB.wB.wB	26	9	40	19

(sB = spring barley, wB = winter barley, wO = winter oats, Be = winter beans)

Winter wheat. Table 7 shows the grain yields and incidence of take-all and eyespot in winter wheat in the Saxmundham Intensive Wheat Experiment. As expected, there was little take-all or eyespot in wheat grown after ley-beans, but surprisingly there was little take-all, and large yields, in the 2nd wheat crop after the 1-year ley. Possibly soil conditions did not favour the take-all fungus, because, although take-all was prevalent in the 3rd successive wheat crop, few crown roots were attacked and the disease caused little loss of yield. Thus, where given optimum nitrogen, the 3rd wheat crop yielded only 6 cwt/acre less than the best yield of wheat after ley-beans.

In contrast, take-all was prevalent and very severe in the 3rd wheat crop in the Intensive Cereal Experiment at Woburn. In this crop many crown roots were attacked, even where much nitrogen was given, and the yields were much decreased. The best yield of the 3rd wheat crop was 14 cwt/acre less than the best yield of wheat after ley-potatoes, which was itself unaccountably small (Table 8). Many crops in south-east England yielded poorly in 1968. On this one, although mildew was prevalent, it was not unusually severe on upper leaves or ears in early July and less than 10% of

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TABLE 7

Grain yields and incidence of take-all and eyespot in winter wheat (Cappelle) at Saxmundham, 1968

	Nitrogen (cwt/acre)		
	0·6	1·2	1·8
Grain yield, cwt/acre, after:			
Barley, ley, beans	46·3	44·1	40·0
Barley, ley, wheat	38·9	45·5	38·8
Barley, wheat, wheat	34·1	40·1	34·1
% plants with take-all, 19 June, after:			
Barley, ley, beans	2	5	2
Barley, ley, wheat	16	14	11
Barley, wheat, wheat	41	29	34
% straws with eyespot, 19 June, after:			
Barley, ley, beans	7	5	5
Barley, ley, wheat	37	20	9
Barley, wheat, wheat	58	41	40

TABLE 8

Grain yields and incidence of take-all in winter wheat (Cappelle) at Woburn, 1968

Previous crops	Nitrogen (cwt/acre)			
	0·5	1·0	1·5	2·0
Grain yield, cwt/acre				
Fallow, ley, potatoes	28·2	34·5	32·6	29·0
Fallow, potatoes, wheat	21·8	27·2	28·4	28·9
Fallow, wheat, wheat	17·2	17·7	20·3	20·7
% plants with take-all, July				
Fallow, ley, potatoes	5	7	2	2
Fallow, potatoes, wheat	37	20	17	23
Fallow, wheat, wheat	93	96	95	97

plants had eyespot. In 1967 there were, in this experiment, patches of wheat with both severe magnesium deficiency and severe take-all. We thought the deficiency might have made the wheat more susceptible to take-all, but applying magnesium fertiliser to half-plots in autumn 1967 did not decrease take-all in 1968, and only slightly increased grain yields. It now seems probable that take-all enhances magnesium deficiency, rather than the converse. (See Chemistry Department report, p. 54.) (Slope, Etheridge and Palmer)

Soil fumigation for wheat crops

The effects of repeated fumigation. Continued tests of the residual and cumulative effects on take-all of fumigating soil with formalin gave rather different results from previous years (*Rothamsted Report for 1967*, p. 136). As before, samples taken in May from Little Knott showed some control of the disease in the year of fumigation (Table 9) and more disease in the second crop after fumigation than in unfumigated plots. On Pastures, differences were smaller and reversed, with most take-all where formalin was applied and least after fumigation in 1967 (throughout, the crop year

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TABLE 9

Formalin fumigation and take-all of wheat (Cappelle)

	% plants infected			Yield (cwt/acre)	
	May	August			
Little Knott	Total	Total	Severe	Grain	Straw
No formalin	20	33	27	33·3	37·0
Formalin in 1967	36	76	27	28·9	28·9
Formalin in 1968	12	78	19	31·1	36·2
Formalin in both years	8	55	22	33·7	35·0
Pastures					
No formalin	17	36	18	26·3	39·5
Formalin in 1967	14	50	21	27·8	36·7
Formalin in 1968	26	45	24	23·0	39·0
Formalin in both years	26	49	24	22·1	36·5

is referred to, although fumigant may have been applied the previous autumn).

During the summer the pathogen infected many more plants in fumigated than in unfumigated soil but did not damage them severely. Therefore in August there was more take-all in both fields after fumigation in 1967 or 1968 than on untreated plots. There were only small differences in proportions of severe infections. Consequently, the only effect on yield was a decrease after fumigation in 1967 on Little Knott, but not on Pastures where severe lodging depressed yield erratically. (Salt with Widdowson, Chemistry Department)

The comparison of fumigation with 'D-D', dazomet and formalin for winter wheat on Claycroft field, Rothamsted ended (see *Rothamsted Report for 1967*, p. 138).

No fumigant affected root-browning caused by *Fusarium* sp. Again, the use of 'D-D' led to many ears being deformed and much smaller yields in the year when it was applied (Table 10). (Ebbels)

TABLE 10

Disease incidence and yield of winter wheat (Cappelle) grown after soil fumigation

1967	1968	'Take-all rating'*	Eyespot (% straws infected)	Browning root-rot (% plants infected)	Grain yield (cwt/acre)
—	—	10·6	31·9	22·0	32·8
F	—	16·2	29·6	28·8	31·5
—	F	6·4	15·2	38·7	33·6
F	F	5·2	20·8	37·9	30·8
D	—	8·3	45·1	27·2	32·7
D	D	7·1	28·6	38·7	18·7
R	R	6·2	22·0	37·9	33·1
Z	R	14·5	32·0	20·2	30·7
R	Z	2·5	30·4	37·8	33·8
Z	Z	10·2	37·7	41·7	32·8
S.E.		±1·94	±4·44	±4·22	±0·96

F = formalin drench at 350 ml/yd²
D = 'D-D' injected at 800 lb/acre
R = rotavated; no fumigant
Z = dazomet broadcast and rotavated in at 400 lb/acre
— = no fumigation

* (See Ebbels, *Ann. appl. Biol.* (1969), **63**, 81–93)

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Methyl bromide on Broadbalk. In April, winter wheat growing on soil treated with methyl bromide in the previous October was taller and had more shoots per plant than wheat growing in untreated soil. Fumigation decreased the percentage of plants with take-all lesions from 17 to 6% and of roots knotted by *Heterodera avenae* from 10 to 3%, but had no effect on eyespot infection. However, during summer *O. graminis* spread faster among plants in fumigated soil, and by August 46% were infected there compared with 27% on untreated soil. (Salt with Corbett, Nematology Department) (see also Jenkinson, Pedology Department, p. 71)

Wilting of field beans (*Vicia faba* L)

During the last two years, scattered plants in field bean crops at Rothamsted, Woburn and Saxmundham have been observed to wilt and die prematurely. The tap roots were rotted and dry, had only a few late-produced lateral roots, and the remains of the epidermis and cortex formed a black necrotic sheath. No parasite could be seen in microscope sections but some roots resembled those with boron deficiency described by Warington (*Ann. Bot.* (1926) **40**, 27–42). Fungi isolated from affected roots and known wilt-causing Fusaria, obtained from the Commonwealth Mycological Institute, did not produce the same symptoms on beans in pots. The cause of the condition remains unknown to us.

Occasional plants from Barnfield had a different, black spongy rot at the stem base. *Pythium* sp. and *Phytophthora* sp. were isolated from the necrotic tissue which was full of oospores. The pathogenicity of these isolates has yet to be tested. (Hornby and Salt)

Potato tuber diseases

Survey of fungal diseases of seed tubers. Seed produced in 1967 and sampled by Potato Marketing Board officers from farms in England and

TABLE 11

Survey of fungal diseases of seed tubers, 1967–68

(% tubers infected/% stocks with infected tubers)

Examination	Disease	King Edward	Majestic	Pentland Dell	Pentland Crown	Record
R	Skin spot (*O. pustulans*)	65 /100	52 /96	73 /100	43 /100	70 /100
P	Gangrene (*Phoma* spp.)	6 /62	4 /53	8 /68	13 /73	4 /59
P	Dry rot (*Fusarium caeruleum*)	2 /43	3 /36	2 /43	4 /60	3 /69
R	Blight (*Phytophthora infestans*)	<1 /11	<1 /4	0 /0	<1 /7	<1 /14
R	Black scurf (*Rhizoctonia solani*)	20 /98	22 /96	27 /96	27 /96	17 /90
R	Powdery scab (*Spongospora subterranea*)	22 /85	16 /87	6 /71	3 /47	5 /62
R	Common scab (*Streptomyces scabies*)	32 /94	50 /100	34 /100	30 /100	27 /97
Number of stocks examined		53	53	28	30	29

R = at receipt P = at planting time

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Wales had more skin spot and common scab but less blight than seed produced in 1966. Pentland Crown, examined for the first time, had less skin spot and powdery scab and more gangrene and dry rot than other varieties (Table 11) (Hide and Griffith)

The prevalence of gangrene. Soil and skin parings from lesion-free seed tubers are often infective when inoculated to test tubers (*Rothamsted Report for 1967*, p. 130). Inoculum that is not usually expressed as lesions must be very common, for although an average of only 7% of tubers in the survey stocks showed gangrene when stored on chitting trays until planting-time, the proportion was increased to 38% when sub-samples were uniformly wounded and stored at 5° C, and only 23 out of 193 of these damaged samples had fewer than 10% of tubers infected.

The presence of lesions on seed tubers gave little indication of the potential inoculum on them or on their produce. In 1967 grades of visible infection of seed tubers (see below) did not consistently affect the incidence of disease whether the seed was stored 'as dug', riddled or riddled and dipped in an organo mercurial fungicide. Hence the produce of the same King Edward stock, uniformly damaged and stored cool (5° C) from crops at Rothamsted or Terrington gave 93–98% of gangrene-infected tubers, irrespective of the grade of seed selected. At Mepal (Black Fen Soil) the stock developed much less (28–35%) gangrene. At Mepal and Terrington King Edward seed stocks, with from 11 to 90% of tubers showing gangrene, were planted, both unselected and after removing all tubers with lesions. After 6 months storage, infection of the produce showed little relation to infection visible on the seed, and the produce from lesion-free tubers averaged as much infection as produce from unselected seed, respectively 6·8 and 7·2% at Mepal and 39·2 and 39·6% at Terrington.

Such results suggest that gangrene lesions probably indicate the previous maltreatment of a stock more than the amount of fungus it carries. They also help to explain the prevalence of pathogenic *Phoma* spp. on the haulm of plants from seed with or without lesions. (Griffith)

Estimating the effects of diseases. Between 1964 and 1968 we have done several series of experiments to measure the effects of skin spot (*O. pustulans*) *Rhizoctonia solani* and gangrene (*Phoma* spp.). Initially there were no healthy stocks, so we could only select a fairly heavily diseased seed consignment (stock) and compare the yield and diseases of its produce with those of plots planted entirely with tubers selected into grades of disease severity. In addition to stock, the grades were severe, moderate and 'clean' (without macroscopic symptoms but often known to be infected microscopically). Seed stocks usually contain a gradation of symptoms, so farm practice was represented only by the comparison between 'clean' and stock because plants on severe or moderate plots would not exhibit the usual compensatory growth of plants for diseased or absent neighbours. Yield compensation was measured in artificial gaps experiments, where various proportions of randomly selected plants were removed at emergence or flowering time. The effect of diseases on seed tubers are of such concern to farmers that a summary of the results should be given. How-

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ever, the results are now too numerous to report comprehensively here, and it is with some misgivings that the main effects are quoted because we must omit many necessary provisos. For example, different fertiliser dressings have not been tested and we shall not quote accurate descriptions of the disease categories, nor say which stocks were chitted. The effects diseases have on growth seem consistent but the yields in Table 12 refer to specific experiments and are not yet considered reliable generalisations.

Planting tubers selected for severe skin-spotting by *O. pustulans* delayed the emergence of shoots and decreased the final plant population, but less than selecting grades of tubers with more than 2, 1 or 2, and no 'live eyes' at planting, although sprouts eventually emerged from half the tubers that seemed blind. Infection decreased the number of stems/plant and, especially with Majestic, increased the proportion of oversize ware. In some years *Rhizoctonia* had similar effects to *Oospora*, but the differences were not consistent between years or between sites. The proportion of eyes on progeny tubers that became infected with *Oospora* increased with the severity of skin-spotting grades but not when tubers were selected according to the number of 'live eyes'. Increasing severity of seed infection by *Rhizoctonia* sclerotia increased the incidence of the *Corticium* stage on stems, of *Rhizoctonia* hyphae on eye-plugs and sclerotia on the progeny tubers. There was strong evidence that *Oospora* and *Rhizoctonia* interact because selecting for increased severity of one, decreased the occurrence of the other disease on progeny tubers. Gangrene (*Phoma exigua var. foveata* syn. *P. foveata*) increases sprouting and sprout branching, so infected seed tubers often produced more stems/plant and more tubers smaller than $2\frac{1}{4}$ in. than did lesion-free seed. Seed with lesions sometimes but not always produced more infected tubers. In the first experiment, at Rothamsted in 1966, gangrene had much less effect on yield than in 1967 and 1968; on average it seemed the most damaging of the diseases studied, especially to King Edward.

Table 12 shows that, when severe, each disease can cause considerable or severe loss but, except for gangrene, 'clean' tubers did not yield as much as 5% more than the unselected diseased stocks. Much of this difference is attributable to compensatory growth, the extent of which we measured in experiments with artificial gapping of uniform crops (Table 13). Field

TABLE 12

Effects of seed tuber diseases on total yield 1964–68

(Mean % difference from yield of 'clean' seed)

Disease	Selected for:	Variety	No. of tests	Seed tuber health category: Moderate	Severe	Unselected stock
Skin spot	Extent of skin spotting	King Edward	8	−13	−25	(−4)
		Majestic	4	+2	−13	(−4)
Skin spot	Proportion of live eyes	King Edward	2	−6	−48	−3
Gangrene	Extent of lesions	King Edward	6	−10	−27	−16
		Majestic	3	−5	−21	−7
Rhizoctonia	Extent of sclerotia	King Edward	4	−5	−7	(−3)
		Majestic	4	−4	−6	(−3)

( ) Figures in brackets based on 1 or 2 tests fewer than stated.

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TABLE 13

Effect of compensatory growth on yield of artificially gapped plots

(Average % of full crop)

	% plants removed				
Removed at:	4	8	12	16	24
Emergence[1]	98·9	96·6	95·5	95·0	(91·0)[4]
Flowering[2]	98·5	95·3	91·6	88·5	(83·0)[4]
Harvest[3]	95·4	91·7	86·1	82·3	—
Theoretical	96·0	92·0	88·0	84·0	76·0

No. of tests incorporated in average (1) = 4, (2) = 5, (3) = 3, (4) = 2

experiments with potatoes are seldom claimed to indicate significant differences smaller than 5% of yield. The results of random gapping immediately before harvest were very close to the theoretical estimates of yield, suggesting that these experiments distinguished differences smaller than 5%. By contrast, there was much compensation for gapping at emergence and flowering when, respectively 16 or 8% of plants could be removed before yield was decreased by more than 5%. The small differences between 'clean' and stock tubers in Table 12 should not yet lead to the conclusion that tuber diseases are unimportant because not all causes of yield decrease were tested; there are many other ways in which diseases cost money. While most stocks are diseased, small losses will be widespread and, if ubiquitous, even a very small proportional loss may be very costly (for example a 1% loss on the 6 million ton maincrop in Great Britain would represent slightly less than £1 million per annum). (Hide, Hirst, Griffith and Stedman)

Control

By fungicides. Dipping seed in organo mercurial fungicides decreases tuber-borne inoculum of *Oospora*, *Phoma* and *Rhizoctonia*, but does not eliminate it. Disadvantages of these materials include ineffectiveness against infection encountered during growth, against the black leg bacterium and toxicity to operators. Field trials to find safer and more effective substitutes have been in progress for two years and twelve materials were tested in 1968.

The chemicals, applied to seed tubers (King Edward once grown at Rothamsted) as dusts or dips, included organo mercurials, chlorinated phenol derivatives, dithiocarbamates, oxathiins and benzimidazoles. Treating seed immediately after lifting with chlorinated phenols ('Aardisol' and 'Aretanol') and one organo mercurial ('Agallol') decreased yield by 1–2 tons/acre, but one benzimidazole (dipped in 0·1% 'Thiabendazole lactate "S4" ', for 5 minutes) significantly increased yield by 2 tons/acre. Most treatments (except dipping in 'Plantvax' and 'Vitavax', 0·2% a.i. for 5 minutes) increased the proportion of seed-sized tubers. Dusting well chitted seed immediately before planting delayed emergence and, on average, decreased yield by 2 tons/acre, although seed treated with another benzimidazole (Du Pont's E F 1991) yielded as much as untreated seed despite the damage to sprouts.

In July the 'disease rating' for stem base browning, caused by *O.*

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pustulans was unaffected by oxathiins (70%) (untreated 71%), but slightly decreased by organo mercurials (50%), chlorinated phenols (56%) and dithiocarbamates (50%). 'Thiabendazole' and E.F. 1991 decreased the disease rating, respectively to 21% and 8% and were also most effective in decreasing infection of tuber eyes at a month after lifting (35% and 14% eyes infected, untreated 77%). *Rhizoctonia* infection of eyes was controlled best by Uniroyal F 849 (10% dust applied before planting) and decreased by the benzimidazoles, oxathiins and chlorinated phenols. *Helminthosporium atrovirens* (silver scurf) was decreased by benzimidazoles, organo mercurials and dithiocarbamates ('Trametan' as 50% dust and 'Polyram' as 7% dust). (Hide and Hirst)

Progenies of stem-cuttings. In the *Report for 1966*, p. 129, we referred to the production of pathogen-free tubers from rooted cuttings of potato stems. The stocks have now been multiplied sufficiently in Scotland for experiments at Rothamsted and Woburn. When multiplied to a field scale and planted and harvested mechanically, diseases reappear increasingly after the third year of multiplication. This is presumably from inoculum introduced by machinery or surviving from previous potato crops, because small stocks of these tubers cultivated by hand and grown where potatoes have not previously been grown remained uninfected. Therefore fungicides will probably be required to maintain the health of these stocks in commerce. Some varieties, especially King Edward, also produce many small tubers when healthy, so it may also be desirable to plant at wider spaces within the rows.

At Rothamsted in 1967, 'healthy' stocks of King Edward produced in total 8% more than the stock inoculated with *Oospora* and 4% more than imported Stock Seed, but these differences disappeared in terms of saleable yield. In 1968, there were two similar trials with King Edward, Majestic and Pentland Dell, results for which are averaged here. At Rothamsted, inoculating 'healthy' stocks with *Oospora* at planting did not decrease yield but inoculating with *Rhizoctonia* decreased saleable yield by 9%. However, 'healthy' stocks produced 10% more saleable ware than either once-grown or new Stock Seed. At Woburn, where other factors affected growth (see below) King Edward and Pentland Dell yielded poorly. Inoculation with *Oospora* or *Rhizoctonia* had no effect and, although the total yield from 'healthy' was slightly greater than from once grown or Stock Seed, there were no differences in saleable ware. (Hide, Hirst and Stedman)

Heat treatment of seed tubers. Attempts to free tubers from fungal pathogens by hot water and hot air have been made for some years (*Rothamsted Report for 1967*, p. 133). Hot water seems most effective and the best treatments greatly decreased *O. pustulans* and *H. atrovirens* up to planting, but the fungi became re-established by lifting time. The treatment most effective against fungi (50° C for 30 minutes) was harmful to tubers and more than halved the yield. If satisfactory fungicides can be found, heat treatment probably has little future. (Hide)

Gangrene. The fungi that cause such diseases as gangrene and skin

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spot become damaging only when large populations develop on plants or in soil. Their rapid multiplication makes it difficult to stabilise small populations, and our present aim is to eliminate them by using clean stocks and fungicides. However, this may not be possible, so we are also testing alternative measures. Results now reported are from field experiments made in 1967.

Removing soil-borne inoculum from seed tubers of 4 varieties by pressure washing scarcely decreased their chances of contracting gangrene when damaged and stored cool, washing and dipping in hypochlorite only halved the chances, but dipping in 'Agallol' (an organo mercurial fungicide) decreased it to 5%. Tests of substitutes for mercurials are not yet complete.

In mid-July only about 10% of the progeny tubers set by plants from diseased seed tubers of 4 varieties developed gangrene when wounded and stored at 5° C for 16 weeks. Whereas more than 95% did when they were wounded in October. King Edward and Red Craigs Royal became affected (50% in August) earlier than Pentland Crown and Majestic (50% in September). Much of the inoculum for this increase probably came from the dying haulm. Removing haulms before they began dying decreased gangrene to about a third in the produce of untreated or fungicide dipped seed, but not quite to a half where the seed tuber was artificially inoculated with *P. exigua var. foveata*. Seed growers might benefit by removing haulm and lifting earlier than they now do.

A relationship between damage, temperature and infection has long been recognised but many details need clarifying and have important practical implications, as in the design of machines. For example, a rubber-spool type grader caused 25% less gangrene than a reciprocating riddle. 'Heat curing' is widely recommended and is certainly valuable although it should be repeated after every exposure to damage. Tests at the Potato Marketing Board Experimental Station at Sutton Bridge showed that warm storage was ineffective against inoculated deep crush wounds and less effective against inoculated shallow crushes than clean cuts. *P. exigua var. foveata* was more able to penetrate slight wounds than *P. exigua var. exigua*. (Griffith)

Coiled sprout. The incidence of coiled sprout of potatoes has been reported to be increased by conditions in which seed tubers are stored, by infection with *Verticillium nubilum* and by soil compaction. To test these factors experiments were designed in collaboration with Dr. J. Moorby, University of Nottingham, Sutton Bonington and Dr. J. H. Lennard, Edinburgh School of Agriculture.

Arran Pilot seed tubers were stored in three ways at Sutton Bonington; on chitting trays at 15° C from mid-November; at 4° C from mid-November until the end of February then transferred to chitting trays at 15° C; and throughout at 4° C, unchitted. Before planting, seed of each treatment was immersed in soil slurries half with and half without *V. nubilum*. Experiments were planted at Falmouth, Cornwall; Rothamsted; Sutton Bonington and near Edinburgh, but only the results of the first two are discussed here. Half the plots were sprayed with a pre-emergence herbicide and on the remainder the ridges were worked-down after plant-

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ing, inter-row cultivated and then gradually rebuilt as the plants grew. Coiling and yield were estimated at Rothamsted at two weekly intervals from the time plants emerged.

Of the storage treatments, seed chitted throughout at 15° C had most coils, 36 and 31% of stems respectively at Rothamsted and Falmouth. Chitting seed at 15° C only from February caused fewer coils (18 and 12%) and unchitted seed produced fewest (0·1 and 0·6%). At Rothamsted these three treatments yielded respectively 8·3, 8·5 and 6·5 tons/acre on July 29 and 17·3, 19·3 and 15·0 tons/acre on August 12. Similarly, at Falmouth, when lifted on July 8, the treatments yielded 7·0, 8·9 and 6·0 tons/acre.

Inter-row cultivation halved the proportion of coiled stems (17–8%) at Rothamsted without affecting yield, but at Falmouth cultivated plots had more coils (12%) and yielded less (6·2 tons/acre) than herbicide-sprayed plots (8% of stems and 8·4 tons/acre). The same seed planted on unridged plots at Rothamsted confirmed previous results that excessive soil compaction increases coiling (5% of stems in unrolled and 19% in rolled soils).

Inoculating seed tubers with *V. nubilum* had no effect on coiling at Rothamsted and at Falmouth increased it from 7·8% to 12·4% of stems. Inoculation did not affect yields and *V. nubilum* was isolated from 24% of stems both from inoculated and uninoculated seed. At Rothamsted chlamydospores, morphologically indistinguishable from those produced in culture by *V. nubilum*, occurred in superficial lesions on coiled and straight stems and later they were abundant throughout the moribund tissue of the underground stem bases. (Lapwood, Hide and Hirst)

Common scab (*Streptomyces scabies*). The experiments to test effects on common scab of different amounts of nitrogen fertiliser ended with two experiments on scab-infested soil at Rothamsted and Woburn. The weather was similar at both farms and soil was dry (i.e. more than 60 cm Hg tension as measured by porous pot tensiometers set in the ridge) from about 14 to 24 June when most infections occurred and again between 6 and 10 July. With 0, 1 and 2 cwt N/acre (as 'Nitro-Chalk') at Rothamsted, 7·0, 8·0 and 8·0% of tuber surfaces were scabbed on the susceptible variety Majestic, 0·7, 0·8 and 0·6% on King Edward and 0·8, 1·2 and 0·8% on the supposedly more resistant Pentland Dell. The equivalent figures at Woburn were 17·5, 25·3 and 23·6% on Majestic, 11·6, 21·5 and 16·3% on King Edward and 10·6, 17·1 and 17·0% on Pentland Dell. Except that tubers from plots without nitrogen at Woburn were least affected, the fertiliser treatment had little or no effect on scab incidence. This might have been expected, if as earlier results suggested, nitrogen mainly influences scab by altering the date when tubers start to form, because in 1968 *Rhizoctonia solani* attacked and severed many of the first-formed stolons of all varieties. Plotting the growth of tubers by following the phyllotaxis of eyes (*Rothamsted Report for 1967*, p. 135) showed that during the 10 days in June when the soil was dry five internodes were scabbed on Majestic, but during the following 10 days when the soil was wet, the next three internodes formed remained free from lesions.

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Now that the examination of tubers from earlier experiments is complete, a clearer account can be given of the relationship between tuber growth, soil moisture and scab distribution. In 1967, tubers from covered plots at Sutton Bonington (*Rothamsted Report for 1967*, p. 135) where irrigation was interrupted for 5, 10 and 15 days, had 4, 5 and 6 internodes scabbed respectively, and the reason for the differences was shown by relating eye numbers formed at different dates before, during and after the period when the soil was allowed to dry. On average only one eye separated from the apex and only one internode formed on tubers during each of the 5-day periods; the fact that four internodes were infected during the first 5-day period suggested that the last two or three internodes formed before irrigation stopped were susceptible to infection when the soil was allowed to dry. A similar analysis of how distribution was affected by a rain storm at Woburn in June 1967 (*Rothamsted Report for 1967*, p. 135) showed that, when soil was wet for 5 days, only 1·0–1·5 internodes remained uninfected, that the blemish-free internodes were those formed just *before* the rain, and that the three internodes formed during and immediately after, when the soil was wet, were subsequently scabbed when the soil dried, confirming the result at Sutton Bonington.

Establishing a close relationship between internode formation, soil moisture and scab infection prompted further study of tuber development. At harvest in 1967, Majestic had about five ware tubers (>2 in.) per plant and 14 eyes per tuber. Weekly samples of plants from the 1968 fertiliser experiment showed that, on the five largest tubers per plant, approximately two eyes were formed each week. The rate at which eyes separated slowed greatly at five weeks after tuber initiation when these tubers had ten eyes; King Edward and Pentland Dell showed similar patterns of development.

The most important period to protect tubers was defined in an experiment where a crop of Majestic grown on moderately scab-infested land was uniformly irrigated to maintain soil moisture at less than 15 cm Hg tension on porous pot tensiometers. Polythene covers were placed over different plots during each of the eight weeks from tuber initiation, starting 1 June to 18 July. The plots remained covered for two weeks to promote infection by *S. scabies*. At harvest on 28 August, plots that were covered during the first three weeks immediately after tuber initiation were scabbed, e.g. the plot covered on 14 June had 9·5% surface area scabbed, compared with 1% on plots first covered in the 4th or 5th weeks (21 and 28 June) after tuber initiation.

So far the work on common scab has shown that, during dry weather, tubers in scab-infested land will be infected so long as new internodes are formed, that most internodes of a ware tuber are formed within the first five weeks from tuber initiation, that the area scabbed is greatest when the first-formed internodes are infected, because these expand most, and that 4–5 weeks (perhaps less) irrigation or wet weather after tuber initiation should adequately control the disease. The extent of protection can be predicted, for example, to protect seven internodes, requires soil to be kept moist until a further three internodes form and there are 10 eyes on ware tubers; assuming that dry weather immediately follows irrigation when the last three internodes formed would be scabbed. The minimum soil

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moisture necessary to prevent scab infection in different soils and therefore the frequency of irrigation necessary, has still to be determined. (Lapwood)

Results of an experiment on the effect of chemical soil treatment on occurrence of common scab are described in the report of the Insecticides and Fungicides Department (Lapwood and McIntosh Insecticides Department, p. 198).

Problems of potato growing at Woburn

New problems are increasingly recognised in potato crops at Woburn. The effects in long-term experiments of increases in potato cyst-nematode were ameliorated by growing the resistant variety Maris Piper, but the nematode is now recognised as aggravating *Verticillium* wilt, which is important locally (*Rothamsted Report for 1967*, pp. 131, 152, 321). We also suspect that magnesium deficiency, local air pollution and pests and diseases yet to be identified may be complicating factors.

***Verticillium* wilt and nematodes.** Further glasshouse experiments confirmed that *Heterodera rostochiensis* increases the severity of this disease, but left the role of *Pratylenchus minyus* still uncertain.

At Woburn, small plot experiments were made with King Edward in two sites. One was a part of Stackyard, long free from potatoes and with few *H. rostochiensis*, the other in Great Hill where potatoes died prematurely in 1966 and *H. rostochiensis* is common. Half the plots at each site were fumigated with methyl bromide (2 lb/100 ft²) and sub-plots received different amounts of compound fertiliser and magnesium sulphate (Table 14).

TABLE 14

Effects of fertilisers and methyl bromide on potatoes at Woburn

	NPK (13:13:20) (cwt/acre)	White cysts/ plant in July	% plants with *Verticillium*	Total yield (ton/acre)
Stackyard				
Unfumigated	2	0	0	11·56
	10	0	0	19·95
	10 + Mg*	1	0	20·00
Fumigated	2	0	0	10·31
	10	0	0	18·96
	10 + Mg	0	0	20·94
				±2·57
Great Hill				
Unfumigated	2	153	100	6·15
	10	105	87	11·25
	10 + Mg	145	68	12·14
Fumigated	2	0	4	14·64
	10	6	0	19·17
	10 + Mg	5	0	20·42
				±1·10

* 100 lb/acre Mg as Magnesium sulphate

Plants in unfumigated plots on Stackyard began to mature at the end of July, when those on comparable plots on Great Hill were dead; those with

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least fertiliser died first. Fumigation delayed the emergence of plants by about one week, but later improved both growth and yield on Great Hill, but not on Stackyard. *Verticillium dahliae* and *H. rostochiensis* were common only on unfumigated plots on Great Hill. Symptoms of magnesium deficiency showed on parts of both fields and applying magnesium increased yields slightly. In contrast, fumigation almost doubled yield on Great Hill. (Hide and Corbett, Nematology Department)

Chloropicrin fumigation for potatoes on the Ley-Arable Experiment. The Woburn Ley-Arable Experiment, also in Stackyard Field, provides contrasted cropping histories in different plots. Maris Piper potatoes again grew poorly in the continuous arable series (*Rothamsted Report for 1967*, pp. 321–322). Fumigating the soil with chloropicrin (400 lb/acre) strikingly improved growth, first noticeably in May, increasingly later, and increased yield (Table 15). An unexplained result of fumigation was that stems died and turned brown much sooner than in untreated plots.

TABLE 15

Effect of chloropicrin soil fumigation on yield of potatoes

(Total, tons/acre)

	Untreated	Fumigated
Ley-arable*	17·78	21·02
Sainfoin-arable*	18·12	22·02
Continuous arable (H)†	8·53	19·18
Continuous arable (C)†	9·05	21·33

* Potatoes every 10th year.
† Potatoes every 5th year, and 1 year grass for hay (H) or carrots (C) every 10th year

Release of mineral nutrients after fumigation could hardly account for such a large increase in the yield on some plots but not on the others. A more probable explanation is that fumigation controlled pathogens present in the arable but not the ley-arable series. In last year's Report (p. 322) we described such a distribution for *Verticillium*, but examining plants and fine roots in July, and dead haulms later, did not suggest that any of the fungi present in 1968 were responsible for the difference between different plots.

Fumigating the soil would be expected to decrease soil-borne inoculum, but not the inoculum carried on seed tubers. Only traces of *Verticillium* were found from untreated soil either in root samples in July or in dead haulm in September. In July *Oospora pustulans* was unaffected by fumigation but *Rhizoctonia solani* was more common on plants from fumigated soils. Samples from washed fine-roots from untreated soil were plated on water agar and 22% yielded *Pythium*, 16% *Phoma*, 12% *Cylindrocarpon*, 6% *Cephalosporium*, 4% *Verticillium*, 4% *Oospora* and 3% *Rhizoctonia*. Those from fumigated soil yielded fewer, 11% *Pythium* and 6% *Cylindrocarpon*, but similar or increased numbers of the other genera. *Pythium* and *Cylindrocarpon* were equally common on roots from the arable and ley-arable series, so it is improbable that they contributed significantly to the poor growth of potatoes in the continuous arable series. (Salt)

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Diseases of conifer seedlings

The 'Psychrophilic seed fungus'. The effect this unnamed endophyte has on the emergence of Sitka Spruce and other seedlings was confirmed in 1968 at Wareham and Kennington, using methods previously described (*Rothamsted Report for 1967*, p. 140).

At Wareham, emergence of seedlings from broadcast Sitka Spruce within an inch on either side of a line of inoculated seed was decreased by 80% where sown early or late in February, by 30 and 53% where sown early or late in March and by 28 and 50% where sown in April. The average loss over all six sowing dates was 53%, but only 11% where the seed was treated with thiram. Much of the thiram-treated seed that failed to emerge was sown in early February, so the fungicide was least effective when conditions most favoured infection and when seed lay dormant for 2 to 3 months. At Kennington the width of the 'bare strip' of killed seedlings depended on sowing date. It averaged 8·75, 4·25, 0·5, 1·5, 0·25 and 1·75 in. respectively for the six consecutive sowing dates from early February to late April.

Where other conifer species were inoculated similarly with the same isolate from Sitka Spruce seed, they suffered much less than Sitka Spruce. The fungus was re-isolated from ungerminated seed of Western Hemlock, Lodgepole Pine, Japanese Larch and Douglas Fir but not from Grand Fir, Scots Pine, Norway Spruce or Corsican Pine. (Salt, with Mr. R. Brown, Forest Research Station, Alice Holt)

Staff and visiting workers

R. H. Kenten, A. J. Gibbs and J. Waller were on secondment in 1968. J. F. Jenkyn and R. T. Plumb were appointed. D. J. Ebbels was awarded the Ph.D. degree of the University of Reading and took an appointment in Tanzania.

Dr. P. H. Gregory and Dr. Mary D. Glynne worked in the department by invitation. Visiting workers included Mr. D. Ammar (Cairo University); Mr. D. J. Ebbels (Agricultural Research Council Scholar); A. J. Gibbs (Australian National University); Mr. I. I. Kondratyev (Moscow Agricultural Academy); Mr. M. J. Phillips (Science Research Council Scholar) Mr. M. Wurtz (Laboratoire des Virus de Plantes, Strasbourg).

J. M. Hirst was invited to the 60th Anniversary Meeting of the American Phytopathology Society in Columbus, Ohio, in September. B. Kassanis worked on thermal inactivation of viruses at the Laboratoire des Virus de Plantes, Strasbourg, during August. A. Kleczkowski attended the Fifth International Congress on Photobiology, Dartmouth College, Hanover, New Hampshire, in August, J. Lacey an International Symposium on the Taxonomy of the Actinomycetales at Jena, East Germany, in September, R. G. Milne the Fourth Regional Conference on Electron Microscopy at Rome in September, and M. A. Watson the 13th International Congress of Entomology in Moscow in August.

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