Rothamsted Experimental Station Report for 1965

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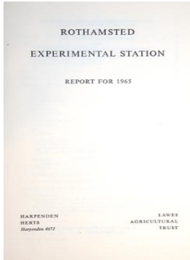
ROTHAMSTED

EXPERIMENTAL STATION

REPORT FOR 1965

Plant Pathology

P. H. Gregory

P. H. Gregory (1966) *Plant Pathology ;* Rothamsted Experimental Station Report For 1965, pp 115 - 136 **- DOI: https://doi.org/10.23637/ERADOC-1-60**

PLANT PATHOLOGY DEPARTMENT

P. H. GREGORY

H. L. Nixon, who has been electron microscopist since 1948, and E. P. Serjeant left, and R. L. Griffith was appointed to the staff.

E. P. Serjeant gained the Ph.D. and Mrs. B. Okusanya the M.Sc. degree of London University.

Visiting workers included: Mr. Anupam Varma and Mrs. Prabhati Varma (Allahabad), Dr. R. R. Frost (Manchester), Dr. D. A. McCarthy (Queen Mary College, London), Mr. D. L. Ebbels (Reading), Dr. S. T. Tilak (Aurangabad), Mr. F. O. Akinbadewa and Mrs. Bolajoko A. M. Okusanya (Nigeria).

The virus work has been done under great difficulties, first because of the reconstruction of the upper floor of North Building to house the mycologists, and later when the laboratories on the ground floor were renovated.

B. D. Harrison and J. M. Hirst attended a Plant Pathology Conference at Belfast in September, J. M. Hirst, D. H. Lapwood and G. A. Hide a meeting of the European Potato Association at Wageningen, in June, and A. J. Gibbs, B. D. Harrison, B. Kassanis, A. Kleczkowski and Marion A. Watson the Plant Virus Conference at Wageningen, in July.

Properties of Plant Viruses

Interaction between "univalent" fragments of antibodies and viruses. There is much evidence that antibodies are "bivalent", i.e., have two sites that combine specifically with homologous antigens. This supports the hypothesis that bridging between molecules or particles of antigen is the basis of the mechanism of precipitation or agglutination, and the hypothesis was further strengthened by the discovery that the "univalent" fragments of antibody, produced by digestion of antibody with papain, still combine with antigens, but the combination does not lead to precipitation. However, all the antigens so far used in such work and found not to be precipitated by the univalent fragments of antibodies are small proteins, which can easily be prevented from precipitation in other ways, such as, for example, by complexing of antibody with albumin. Previous work showed that although such antibody–albumin complexes cannot precipitate antigens, they can still combine with them and thus inhibit their precipitation by unchanged antibody, but the degree of inhibition depends on properties of antigen. Such antigens as small proteins, or spherical viruses, are easily inhibited, whereas rod-shaped viruses, such as tobacco mosaic virus (TMV) are not.

Papain-treated antibodies also gave different results with different plant viruses, and with TMV protein in different states of aggregation. Thus, univalent fragments of antibodies to tomato bushy stunt virus (a spherical virus) combined with but did not precipitate the virus. Nor did fragments

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of TMV antibodies precipitate disaggregated TMV protein. However, they did give some precipitation with intact TMV particles. Unless the papain-treated TMV antibody still contained a small residue of bivalent antibody, it seems that bivalence is not a necessary condition for precipitation. The work is to continue to investigate the possibility of residual bivalent antibody causing precipitation. (Kleczkowski)

Strains of the satellite virus. Satellite virus is the name given to the smallest known plant virus, which is also unusual in apparently being able to multiply only in plants simultaneously infected with a tobacco necrosis virus. Like other viruses, it has now been found to exist in strains that differ considerably in their antigenic composition, indicating that they have different protein constitutions. The form that is now common in the roots of tobacco plants grown in unsterilised soil in the Rothamsted glasshouses differs so much from the one previously described that after antiserum to either is fully absorbed by the other strain the precipitation end point with the homologous strain is not detectably changed. Both these strains of the satellite virus can be activated by strains of tobacco necrosis that also differ greatly from each other antigenically. The antigenicity of the individual strains of the satellite virus seems the same regardless of the identity of the activating strain of tobacco necrosis virus, indicating that the protein of the satellite virus is determined entirely by its own nucleic acid and is uninfluenced by that of the activating virus. (Kassanis)

Carrot Motley Dwarf Virus (CMDV)

In experiments at Rothamsted, Woburn, Broom's Barn and Fernhurst carrots (Clucas New Model) were treated with: (1) Menazon granules applied with the seed; and (except at Rothamsted) (2) four "Saphicol" sprays at 10–14-day intervals during late June and July.

At Rothamsted and Fernhurst many migrant *Cavariella aegopodii* (the aphid vector of CMDV) were caught on sticky traps before the end of May; at Woburn and Broom's Barn they did not become numerous until June. Where migrants were numerous in May Menazon granules increased yield by about 5 tons/acre; where they were few, by about 1 ton/acre.

Regression coefficients of yield (tons/acre) on per cent plants infected at harvest were larger at Fernhurst ($B = -0{\cdot}114 \pm 0{\cdot}016$) and Rothamsted ($B = -0{\cdot}140 \pm 0{\cdot}019$), where early migrants were numerous, than at Woburn ($B = -0{\cdot}100 \pm 0{\cdot}23$) or Broom's Barn ($B = -0{\cdot}065 \pm 0{\cdot}19$), where they were few, suggesting that losses per infected plant were greater when plants became infected near to the time of aphid invasion than later. In contrast to the effect of the granular seed dressing, spraying, although it controlled the aphids adequately, gave only a small increase of yield (averaging 1 ton/acre) at all sites where it was used. The peak number of aphids on untreated plots at Woburn exceeded those elsewhere by more than 10 times, and Fernhurst, where the yield response to all treatments was greatest, had next to the smallest infestation, which again suggests that the level of infestation of the plants is not the dominant factor in determining loss. (Watson, Lack, Dunning and Heathcote, in collaboration

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with Mrs. Hilary Davey, Imperial Chemical Industries Ltd., Jealott's Hill Research Station)

Extracting infected leaves in phosphate buffer and 8% butanol was the best method of preserving the infectivity of carrot motley dwarf virus and the 30-mμ-diameter particles associated with infectivity. However, the particles survived chloroform extraction, whereas infectivity did not, and their identity is uncertain, because similar ones were sometimes seen in non-infective preparations made from healthy carrots. When semi-purified virus preparations were centrifuged in a sucrose density-gradient most of the infectivity was associated with particles of 30 mμ diameter and sedimented slowly. (Watson and Lack)

Virus Diseases of Legumes

Purification of pea enation mosaic virus. Pea enation mosaic virus was purified from leaves of bean (*Vicia faba* L.) by extracting the virus in phosphate buffer containing ethylenediaminetetra-acetate plus chloroform and subjecting the aqueous extract to differential high- and low-speed centrifugation, followed by centrifugation in sucrose density-gradients. The virus preparations contained two components with sedimentation coefficients of about 97 S and 116 S respectively. Both components were particles about 30 mμ in diameter, but they could be distinguished in the electron microscope by their general appearance. Purified preparations also contained two antigens not found in healthy plants, which may correspond to the two components distinguished in the analytical ultra-centrifuge and electron microscope. Bean plants manually inoculated with the purified preparations developed symptoms typical of pea enation mosaic virus, and the virus was transmitted in the persistent manner by aphids from such plants. Preparations of the Rothamsted culture of pea enation mosaic virus reacted with antiserum to a United States culture of the virus prepared at Davis, California, by Dr. R. J. Shepherd. (Gibbs and Harrison)

Groundnut rosette virus. Further work with isolates of groundnut rosette virus from different parts of Africa showed the two types distinguished by Hayes: (*a*) "Green rosette" typified by unusual darkening of leaflets without chlorosis; (*b*) the "chlorosis rosette", typified by stunting of the plant and chlorosis of the leaves. Isolates causing each type of symptom were transmitted by *Aphis craccivora*. The chlorosis rosette isolates were transmitted by all races of aphids tested. The green rosette isolate from Nigeria was easily transmitted by a race of aphids from Nigeria but not by one from Uganda. These two races differ enough in measurements and host preferences to be considered as biotypes (Mrs. M. Jones, Entomology Department). A race of aphids from Kenya transmitted the chlorosis rosette isolate very readily, but the green rosette isolates only occasionally.

Aphids that moulted after acquiring the virus remained infective, but nymphs transmitted more often than adults.

All GRV isolates caused systemic symptoms in *Trifolium incarnatum*, *T. repens*, *Nicotiana clevelandii* and, probably, *N. rustica*. They formed

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local lesions in *Chenopodium amaranticolor*, *C. quinoa* and *C. album*, and *C. amaranticolor* was used as an assay host. The virus was recovered from *T. repens* by *A. craccivora*, but not from *T. incarnatum*. However, the virus was transmitted from *T. incarnatum* to groundnut by leaf-grafting, and by mechanical inoculation to *C. amaranticolor*, as it was from all other systemically infected hosts. (Okusanya and Watson)

Extracts from infected groundnuts remained infective for 1 week at 18° C, and for up to 4 weeks at −20° C. The virus also survived freezing in leaves. Infectivity was lost when sap was heated above 50° C for 10 minutes, or diluted more than $\frac{1}{10}$.

Near-spherical particles, 25–28 mμ in diameter, were seen in electron micrographs from partly purified virus preparations, but could not with certainty be identified as the virus. No similar particles were seen in comparable preparations from healthy plants. (Okusanya)

White clover virus survey. The previously unidentified virus isolates obtained from white clovers from 18 permanent pastures (*Rothamsted Report* for 1964, p. 129) were found to be clover yellow vein virus, either alone or with other viruses.

White clover plants were collected from a further four permanent pastures on each of three farms (at the Plant Breeding Station, Aberystwyth; Grassland Research Institute, Hurley; and Rothamsted): the incidence of the viruses in different fields of the same farm differed greatly. In all, 683 white clover plants from 26 fields have now been collected and tested. Twenty-three per cent of the plants were infected; in order of decreasing incidence the viruses found were: red clover vein mosaic (in 12% of all the plants tested and in 16 of the fields sampled), clover yellow vein (9% of plants; 18 fields), white clover mosaic (4% of plants; 11 fields), arabis mosaic (4% of plants; 8 fields), strawberry latent ringspot (1% of plants; 2 fields), "red leaf" or stolbur (0·5% of plants; 2 fields), tomato black ring (0·1% of plants; 1 field). Arabis mosaic virus was obtained only from plants from North and West England, strawberry latent ring spot from South-west England and tomato black ring from central Scotland. The three most common viruses were found in clovers from all parts of Britain, and were most prevalent in fields where white clover was abundant. White clover mosaic virus seemed to be most prevalent in fields that were cut as well as grazed. (Gibbs and Varma)

Red clover vein mosaic virus. This virus, common in Europe and North America, was identified in Britain for the first time. All British isolates studied were serologically indistinguishable, but some would infect *Chenopodium amaranticolor* giving chlorotic local lesions, whereas others did not. All British isolates reacted with an antiserum prepared against a German isolate of the virus.

The virus was best purified from infected *Pisum sativum* L. "Onward" by differential centrifugation and agar-gel chromatography; the same method worked well for pea streak and clover yellow vein viruses. Preparations made in this way contained many slightly flexible filamentous particles 630–640 mμ long and no detectable amounts of plant protein. The par-

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ticles had a sedimentation coefficient of 160 S, and were most stable in dilute borate buffer. When they did aggregate, most particles seen in the electron microscope were lying side by side, whereas in similar preparations of pea streak virus the particles were aggregated end to end.

After subjecting preparations of red clover vein mosaic virus to ultrasonics most particles were shorter than 630 mμ. This treatment enabled the negative stain used for electron microscopy to penetrate particles and reveal their axial canals and the subunits on their surfaces, but the shortened particles still failed to give specific lines of precipitate in gel-diffusion serological tests. (Varma and Gibbs)

Sărka Disease of Plums

Sărka (plum pox) is a virus disease of great economic importance to the plum industry of Yugoslavia and other Eastern European countries, and in collaboration with Dr. D. Šutić of the Institute for Phytopathology of Belgrade work was done to determine some properties of the virus. It was transmitted by mechanical inoculation from leaves of plum and apricot trees to *Nicotiana clevelandii*, and found to have a thermal inactivation point between 52° and 55° C, and longevity *in vitro* of about 35 hours. The virus has long flexuous particles, of most frequent length 725 mμ. It was transmitted from plum and *N. clevelandii* to seedlings of *N. clevelandii* by *Myzus persicae*, not previously known to be a vector. (Kassanis)

Soil-borne Viruses

Tobacco rattle virus. Fourteen varieties of potato were grown on land in Norfolk infested with the nematode *Trichodorus pachydermus* carrying tobacco rattle virus. The results confirmed what has long been either known or suspected in the Netherlands and Britain, namely that the virus affects different varieties differently. Tubers of the varieties Arran Pilot and Bintje showed no internal symptoms of the spraing type, and tobacco rattle virus could not be detected in them. Tubers of King Edward were only very slightly and of Majestic slightly affected, whereas six out of nine modern varieties showed moderately severe or severe symptoms in 40–70% of the tubers. Tobacco rattle virus was detected in more than 90% of the tubers with symptoms by inoculating extracts to *Chenopodium amaranticolor*; it was transmitted more readily from freshly dug tubers than from those stored weeks or months before testing. The virus was also detected in the roots of two varieties that show tuber symptoms, but not in the roots of Arran Pilot or Bintje. Thus, the susceptibility of tubers and of roots to vector-borne virus seems correlated. (Harrison)

Potato mop-top virus. Corky arcs in the flesh of the tubers of the potato variety Arran Pilot are associated with an apparently undescribed virus we call potato mop-top virus and which is soil-borne. Haulm symptoms are of three types: short bushy shoots (mop-top); yellow blotching and oak-leaf patterns, mostly on lower leaves (confused in the past with frost damage, and with stem mottle caused by tobacco rattle virus); faint chlorotic

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chevrons on some of the upper leaves. The virus was found in many potato varieties, and occurs in Continental Europe as well as the United Kingdom, but some do not show the mop-top type of haulm symptoms or arcs of corky tissue in the tubers. It causes concentric necrotic rings in manually inoculated leaves of *Chenopodium amaranticolor*. Mop-top and tobacco rattle viruses sometimes cause similar haulm and tuber symptoms, but the two differ in many respects, and particles resembling tobacco rattle virus were not detected in plants infected with potato mop-top virus. The two viruses do not infect the same range of potato varieties, mop-top virus is more readily carried through the tubers to progeny plants, and spread of mop-top virus is neither confined to light soils nor associated with nematodes of the genus *Trichodorus*. (Harrison, in collaboration with Mr. E. L. Calvert, Ministry of Agriculture, N. Ireland)

Virus Diseases of Grasses and Cereals

European wheat striate mosaic virus. Adult *Javesella* (= *Delphacodes*) *pellucida* Fabr. injected with extracts of infective hoppers stored for several weeks at -15° C became infective. Experiments with such extracts centrifuged at 8,000 *g* for 10 minutes and layered on to sucrose density gradient columns and centrifuged showed that the virus sedimented more slowly than tobacco mosaic virus, giving a sedimentation coefficient about 120 S. The survival of injected hoppers was impaired by the anaesthetic used (CO_2), and by solids in the inoculum, but apparently not by the presence of the virus.

Grass viruses. Cocksfoot mottle virus (CFMV) was transmitted by both the adults and the larvae of the cereal leaf beetle *Lema melanopa* L. All 80 adults caught at Rothamsted on wheat, barley and timothy during May and June were virus-free. Two experiments were made in which adults were fed for 5 and 6 days on infected wheat and then transferred every 3 days to healthy wheat; $\frac{6}{10}$ beetles transmitted in the first, and $\frac{8}{10}$ in the second. Several beetles were still infective 9 days after the infection feed, and one infected wheat after 15 days. The larvae were less efficient vectors than adults, and only three larvae of about 100 tested transmitted the virus.

The virus particles of phleum mottle virus (PMV), isolated at the Welsh Plant Breeding Station, are almost spherical, about 30 mμ in diameter, with sedimentation coefficient of 112 S. Extracts of infected plants were infective when heated at 60° C for 10 minutes, but not at 65° C. PMV did not precipitate with CFMV antiserum or with antisera to Weidelgrasmosaikvirus or brome mosaic virus. CFMV did not precipitate with PMV antiserum. (Serjeant)

Virus transmission by artificial feeding. The aphid *Sitobium avenae* three times transmitted one isolate of barley yellow dwarf virus (BYDV) after feeding on infective plant extracts through a "Parafilm M" membrane, but failed to do so in many other attempts with this and other isolates. Without suitable conditions we cannot tell whether this failure is because of faulty technique or because our isolates of BYDV differ from those in

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the U.S.A. Attempts to transmit purified preparations by artificial feeding using *S. fragariae* in controlled environmental conditions have all succeeded, giving up to 30% transmission. Similar feeding of aphid vectors on extracts of infected plants twice transmitted pea enation mosaic, but failed to transmit beet yellow net and carrot motley dwarf viruses. (Lack)

Viruses in Horse-radish

Field trials were started in 1963 by Messrs. J. & J. Colman of Norwich with 47 clones of horse-radish (*Armoracia rusticana* Gaertn.) obtained from 11 different countries. Several of these showed symptoms suggesting virus diseases, and in 1965 all were tested for virus by inoculating sap from them to *Nicotiana tabacum* var. White Burley, *Brassica chinensis* L. var. Pte sai, *Brassica rapa* L. and *Chenopodium amaranticolor*, and using *M. persicae* to transmit to *N. tabacum*, *B. chinensis* and *B. rapa*. Three viruses were obtained and identified by symptoms, electron microscopy and serology. No virus was detected in 16 clones; 25 were infected with arabis mosaic virus (AMV); 17 with cabbage black ringspot virus (CBRSV); and 6 with cauliflower mosaic (ClMV). CBRSV has previously been reported infecting horse-radish in several countries.

In the field a few of the plants infected with AMV had necrotic lesions on the oldest leaves, but most looked healthy. Plants with CBRSV had chlorotic ringspots on the oldest leaves and mosaic. All those infected with ClMV also contained CBRSV; they were more stunted and showed a more severe mosaic than plants with CBRSV alone. As now grown, most horse-radish crops are probably infected, yielding less than healthy ones and providing a potential source of virus for other crops. However, as some clones seem virus-free, healthy horse-radish could be propagated from these. (Lack)

Culturing the apical meristems of horse-radish infected with ClMV and CBRSV produced a virus-free clone. (Varma and Lack)

"Docking Disorder"

The type of "Docking Disorder" (stunting of sugar beet on sandy alkaline soils) in which affected beet plants have shallow spreading roots and occur in sharply defined patches resembling "kite-shaped fairy rings" (*Rothamsted Report* for 1962, 113–114) was studied at three sites: Heacham and Barney in Norfolk, and Knodishall in Suffolk. The disorder was found at these sites in sugar beet in 1962 and in the same areas of subsequent cereal crops. Plots were treated in autumn 1964 with soil fumigants; dichloropropane-dichloropropene (DD) and chloropicrin (CP) both injected into the soil at 400 lb/acre, and 20% pentachloronitrobenzene powder (PCNB) rotavated into the soil at 5·5 cwt/acre. In 1965 spring barley was grown at Heacham and Knodishall, and sugar beet at Barney.

All chemicals, except PCNB at Barney, increased the growth of plants, CP most and PCNB least. Barley plants at Knodishall in July were about 24 in. high in untreated plots, and 30, 37 and 40 in. high in plots treated with PCNB, DD and CP respectively, but storms preferentially lodged the

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barley growing on fumigated plots at both sites, so that the direct effect of the fumigants on yield could not be measured. The sugar beet, which at Barney this year looked almost normal, yielded 7% less on PCNB plots, 7% more on DD plots and 17% more on CP plots than on untreated plots.

No pathogen seemed specifically correlated with the stunting of growth of any of the crops. Plants from all three sites were tested for viruses by inoculating their sap to indicator plants, but none was found. At Knodishall roots of barley from different plots contained similar amounts of *Gloeosporium*, *Fusarium*, *Cylindrocarpon*, *Pythium* and *Phoma*. Resting spores of *Olpidium* were common in roots of plants, especially from CP-treated plots, and resting spores of a chytrid (similar to *Cladochytrium caespitis* Griff. and Maubl.) occurred in local dense masses in roots, especially from untreated plots and PCNB-treated plots. This chytrid was not found in roots from Barney and Heacham, but in 1962 a Plasmodiophoralean fungus, *Polymyxa betae*, in addition to *Olpidium*, was found in roots of stunted and normal plants from Barney, and in 1962 *Cylindrocarpon* was obtained more often from the roots of stunted beet than from unstunted at all three sites.

At Knodishall in 1965 the total number of nematodes was similar on all plots, but whereas in untreated soil growing normal barley 65% of these were plant parasites, in untreated soil growing stunted barley 40% were parasites, and in PCNB-, DD- and CP-treated soils 31, 12 and 11% respectively were parasites; saprophytic Rhabditids had increased to make the total number similar. Fumigants did not affect different genera of the plant-parasitic nematodes selectively, and all decreased equally. *Pratylenchus* was the only plant parasite more prevalent in untreated soils growing stunted plants than in untreated soils growing normal barley (7,000 and 4,000 *Pratylenchus*/litre respectively). In samples taken at different depths in untreated soil the three commonest plant parasitic genera were most common at different depths: *Tylenchorhynchus* 0–3 in., *Paratylenchus* 6–12 in., whereas *Pratylenchus* was most common in the deepest samples collected (15–18 in.). (Gibbs, Lacey and Salt, with Greet and Hooper, Nematology Department)

Occurrence of *Polymyxa betae* Keskin in sugar beet from an area with one form of "Docking Disorder". In December 1962 spore balls of a Plasmodiophoralean, resembling *Polymyxa* or *Ligniera*, were found in roots of diseased sugar beet brought by A. J. Gibbs (see p. 121) from a kite-shaped patch at Barney, Norfolk. Soon afterwards a very similar fungus, also associated with diseased sugar beet, was described from Germany and since named *Polymyxa betae*. Further observations of the Norfolk fungus this year on plants grown in field soil and in sand showed that it has zoosporangia as well as spore balls of the *Polymyxa* type, corresponding closely with the German material. Infection is often very dense, and the roots seem severely damaged by the time the spore balls mature. (Macfarlane)

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Fungus Diseases of Cereals

Intensive cereal growing experiments

Spring barley. The mean grain yields and incidence of take-all (*Ophiobolus graminis*) and eyespot (*Cercosporella herpotrichoides*) in continuous barley (4th, 5th and 6th successive crops) and in barley after an oats–beans break for the 3 years 1963–65 on Little Knott field were:

	Nitrogen cwt/acre			
	0	0·3	0·6	0·9
Grain yield, cwt/acre				
Continuous barley	24·0	32·6	35·2	37·2
Barley after oats–beans	39·8	44·5	43·0	39·0
% Take-all (early July)				
Continuous barley	60	46	46	41
Barley after oats–beans	3	1	2	1
% Eyespot (early July)				
Continuous barley	9	17	18	16
Barley after oats–beans	1	3	2	1

It is not surprising that continuous barley responded to more nitrogen fertiliser than barley after oats–beans, but even with 0·9 cwt N/acre, it yielded less than the barley after oats–beans with no nitrogen. Larger yields might have been obtained from continuous barley had more nitrogen been used, but the response curve indicates that further increases in yield would have been small. The response to each increment of nitrogen above 0·3 cwt/acre was only marginally economic, and larger amounts would certainly be uneconomic had they needed an additional field operation to apply them. Assuming that 0·9 cwt N/acre is economic, the loss of yield incurred by growing barley continuously was only 7 cwt/acre, or 16%. However, the yield of the Proctor barley after oats–beans was limited because the crop lodged when given more than 0·3 cwt N/acre. When stronger-strawed varieties are bred the benefits of rotation will probably be much greater. (Slope and Cox)

Spring wheat. The mean grain yield and incidence of take-all and eyespot in Jufy spring wheat grown continuously (4th, 5th and 6th successive crops) and after a beans–oats break in the 3 years 1963–65 on Little Knott were:

	Yield (cwt/acre)	% Take-all (early July)	% Eyespot (early July)
Continuous spring wheat	33·6	40	15
Spring wheat after beans–oats	37·8	5	3

Because the preceding break crop was oats, the comparison with continuous wheat is not greatly influenced by nitrogen residues. All the wheat crops were given 0·6 cwt N/acre; in the same experiment other continuous wheat plots were given 0·9 cwt N/acre, and these yielded slightly less than the plots given 0·6 cwt. Lodging was not serious in any of the crops, and the smaller yield (4 cwt/acre, 11%) of the continuous spring wheat is reasonably attributable to the effects of soil-borne diseases. In these experiments only moderate loss of yield was incurred by growing spring

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barley or spring wheat continuously, despite the prevalence of take-all. This is because infection was mostly on seminal roots only and, consequently, its effect on yield was small. However, the results of previous experiments show that many crown roots sometimes become infected in spring crops (especially wheat), and when they do yields are much decreased. Unfortunately we do not know what conditions favour crown-root infection. It must be emphasised that, in each year of our experiments, there was a *risk* that take-all would cause severe loss of yield. Farmers who grow spring wheat or barley continuously should realise this and should be prepared for small yields in some years. (Slope and Cox)

Winter barley. Farmers who have grown successive crops of spring barley on the same land without serious losses from soil-borne diseases may be tempted to sow some winter barley to distribute work more evenly through the year. However, on land previously cropped with wheat or barley the risk of take-all and eyespot causing serious loss is much greater with winter-sown than spring-sown barley. All barley varieties are susceptible to eyespot, and winter-sown crops are exposed to infection that spring-sown ones usually escape in eastern and southern England. Take-all is more often severe in winter than in spring wheat, and winter barley can also be expected to be more often attacked than spring. To test this we started an experiment to measure how previous cropping affects the incidence of eyespot and take-all, and the yield of Pioneer winter barley on a site in Hoos field where previously two spring-barley crops had been grown. In the first year of this experiment there were no differences in previous crops to compare, but the prevalence of eyespot and take-all and the small yield of the winter barley show the dangers of growing this crop on infested land:

	Nitrogen (cwt/acre)		
	0·25	0·50	0·75
Grain yield, cwt/acre	14·4	21·8	24·8
% plants with take-all	94	93	86
% straws with eyespot	54	45	43

Contrary to expectation, damage by birds was negligible. Barley leaf blotch (*Rhynchosporium secalis*) was prevalent in the winter barley, but not in neighbouring plots of spring barley. Leaf blotch has not yet been severe in spring barley at Rothamsted, but a nearby source of many spores on winter barley might increase the risk of spring barley suffering in years favourable to the fungus. (Slope and Cox)

Continuous wheat growing and the decline of take-all. Take-all was prevalent and severe on all plots in the Decline of Take-all experiment on Great Field I, but the 5th and 7th successive wheat crops had fewer plants infected and yielded more than the 2nd and 4th crops, although the yield differences were not statistically significant:

	Number of successive wheat crops			
	2	4	5	7
% plants with severe infection	75	86	66	62
Grain yield, cwt/acre	29·6	29·3	31·8	32·4

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Thus for the 6th successive year take-all "decline" has been evident on this site.

On Broadbalk, where results first led us to postulate that *O. graminis* is partially inhibited when wheat is grown continuously, take-all was much more severe on plot 7 than in any year since records began in 1938:

	Number of successive crops after fallow				
	2	3	4	7	14
% plants infected, March	4	9	20	25	25
% plants infected, July	40	57	94	98	81

Take-all on winter oats. In June 32% of plants in a winter oat crop (var. Padarn) had typical take-all lesions on their roots, although few plants had more than two crown roots infected. *Ophiobolus graminis* was isolated from these lesions, but whether it is the oat strain *O. graminis* var. *avenae*, not previously recorded at Rothamsted, has not yet been determined. (Cox)

Effect of paraquat on take-all. In June 1964 the incidence of take-all was measured in the 3rd successive winter wheat crop after old pasture at Jealott's Hill Research Station, where traditional ploughing and seedbed cultivation are being compared with direct seeding after spraying with paraquat. Ploughed, unsprayed plots had 21% of straws infected, and un-unploughed, sprayed plots 4%. Paraquat is thought to be quickly inactivated on contact with the soil, so it is unlikely to affect take-all by killing *O. graminis* in the soil. At Rothamsted, to test this possibility, 7-in.-diameter pots were filled with naturally infested soil, and the soil surface in some of the pots was sprayed with amounts of paraquat equivalent to 2 pints/acre (R1) or 6 pints/acre (R2). Cappelle winter wheat was sown on 1 December, 12 days after spraying, and the pots kept in an unheated glasshouse. On 2 June plants from unsprayed soil had 95% seminal and 97% crown roots infected by *O. graminis*, compared with sprayed soil (R1) 98% seminal, 98% crown and (R2) 93% seminal, 93% crown. At Jealott's Hill, therefore, take-all was probably less prevalent in the sprayed than in the unsprayed crops because of the different cultivations, not because paraquat directly affected the fungus. (Slope)

Effect of heptachlor on take-all. We have reported previously that heptachlor controlled take-all of wheat grown in pots (*Rothamsted Report* for 1962, p. 115; for 1963, p. 103). A field experiment testing the effect of heptachlor sprayed on the soil before ploughing has now been completed. Heptachlor neither decreased take-all nor increased the yield of winter wheat, even where 8 lb. active ingredient/acre was applied in two successive years. (Slope, with R. Bardner, Entomology Department)

Varietal susceptibility to take-all. The four winter wheat varieties compared last year were again grown in naturally infested soil in pots. All

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plants became infected, but Heines 4013 (H) much less severely than Cappelle (C) or Prestige (P); Rothwell Perdix (R) was intermediate:

	Variety			
	H	R	C	P
% crown roots infected, April	7	6	16	15
% plants severely infected, July	20	68	84	80

The same varieties were also compared in a microplot experiment on land naturally infested by *O. graminis*. In April Heines 4013 and Rothwell Perdix had slightly fewer infected plants than Cappelle or Prestige (H 45, R 42, C 56, P 57), but in July take-all was equally prevalent and severe on all four varieties. Eight varieties of winter wheat (including Rothwell Perdix and Cappelle) were also compared in a trial on naturally infested land at Boxworth Experimental Husbandry Farm, Cambridge. Although the heavy boulder clay at Boxworth is reputed not to favour take-all, all the varieties were severely and equally affected. We cannot explain why varieties that differ in their susceptibility to *O. graminis* in pots are equally infected in the field. (Cox)

Eyespot on Broadbalk. Routine sampling on plot 7 showed that the 1st, 2nd, 3rd, 4th, 7th and 14th consecutive wheat crops after fallow had respectively 40, 56, 39, 52, 45 and 54% of straws with eyespot, of which 15, 27, 16, 25, 19 and 25% had severe lesions. (Cox)

Effect of CCC on eyespot of wheat. Chlorocholine chloride (CCC) shortens wheat stems, can prevent lodging and has also increased grain yields in crops that did not lodge. To see whether it affects the incidence of eyespot, Squareheads Master winter wheat was sown in John Innes compost in 7-in.-diameter pots on 24 November 1964 and was inoculated with *Cercosporella herpotrichoides* on 15 December and again on 1 February. On 4 May one-third of the pots were watered with 100 ml of a $10^{-3}M$ solution of CCC (R 1), one-third with 100 ml of a $10^{-2}M$ solution (R 2) and one-third left unsprayed. On 30 June the percentage of straws with severe eyespot lesions was 30 in the unsprayed plots, 21 in R 1 treatments and 6 in R 2 treatments. When used in the field to dwarf stems less CCC is used than in our experiment and it is sprayed on to the crop, not watered on to the soil. An experiment has been started to see whether under these conditions CCC affects eyespot in field crops. (Slope, with E. C. Humphries, Botany Department)

***Cephalosporium* stripe of wheat.** Cappelle winter wheat was sown in John Innes compost in 7-in.-diameter pots in an unheated glasshouse, and the soil in half the pots was inoculated with *Cephalosporium gramineum* grown on autoclaved sugar-beet seed. Five wireworms were buried shallowly in each of a quarter of the pots on three occasions: 18 January, before plant emergence (treatment A); 22 March, when the wheat was at the three-leaf stage (treatment B); and 20 April, when the wheat plants had two tillers (treatment C); the remaining pots had no wireworms (treatment D). Plants showing symptoms of *Cephalosporium* stripe were counted on

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11 June, and all plants were grown to maturity and the grain yield of each pot determined.

	Treatment A	Treatment B	Treatment C	Treatment D
% plants surviving	65	72	83	81
% of surviving plants infected,				
not inoculated	0	0	0	0
inoculated	50	49	7	3
Grain yield, g/pot				
Not inoculated	20·7	21·8	21·4	20·2
Inoculated	15·6	16·7	20·9	21·4

Although fewer plants survived in pots where wireworms were buried in the soil before plant emergence or at the three-leaf stage than in other pots, the loss of plant did not affect grain yield per pot, presumably because surviving plants compensated for those that died. Thus wireworms decreased yield only by increasing the prevalence of *Cephalosporium* stripe. (Slope, with R. Bardner, Entomology Department)

Cereal Experiments with Soil Fumigants

Effect of formalin on take-all (1964 and 1965). Spring wheat sometimes yields poorly and fails to respond to nitrogen fertilisers on the light sandy soil at Woburn. In an attempt to find the reason an experiment was started in 1964 in collaboration with Widdowson (Chemistry Department) on Butt Close, in which soil was treated with formalin. The experiment failed in its main purpose because the spring wheat was severely attacked by take-all and cereal cyst-nematode, but the effects of formalin were striking. In June 1964 the mean % straws with take-all was 56 on untreated plots (—), only 1 on plots treated with formalin (F). In 1965 the residual effect of formalin was compared with new formalin; in May the mean % plants with take-all was:

Treatment 1964	Treatment 1965	% take-all
—	—	67
F	—	45
—	F	5
F	F	1

Formalin again controlled take-all well in the year it was applied, but its residual effect was small. We have previously shown that, although there is usually little take-all in wheat grown immediately after one or two non-susceptible crops, the disease can become as prevalent in the second wheat crop after the break as it is where wheat has been grown continuously. Thus in the formalin experiment, as in experiments in which crops previous to wheat were varied, the prevalence of take-all in wheat seems not to be proportional to the amount of infected plant residues left in the soil. This is surprising, because wheat roots are thought to be infected by *O. graminis* only when they grow close to infected residues or infected living roots. We assess take-all in crops by examining roots from only the top 3 or 4 in. of soil. These assessments would not be proportional to the total amount of

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infected residues if deeper roots can be infected when shallow roots are not. This seems unlikely. More probably, a crop with as few as 1% plants infected may leave enough fungus, *when evenly distributed*, to cause severe take-all in the following crop; much more than this may be superfluous. If this idea is correct its practical implications for the chemical control of take-all are important. A control measure aimed at killing the fungus in the soil must be either cheap enough to use every year or it must be so effective that the amount of *O. graminis* left alive is very much less than is needed to infect only 1% of the crop. Neither of these conditions will be easy to satisfy. Formalin is a powerful biocide, and in 1964 we twice applied 200 gal of a 40% solution/acre, but even this had little effect on the incidence of take-all in the second wheat crop after treatment. These results reinforce our conclusion that chemical control of take-all is most likely to be achieved by preventing crown-root infection in early summer, not by killing the fungus in the soil. Moreover, it seems probable that the wheat plant itself will need to be used to distribute the chemical to the roots. (Slope)

Soil fumigants and spring wheat, 1965. At Rothamsted in Pastures field, where the incidence of soil-borne diseases was very small, formalin had no consistent effect on grain yield, which averaged 27·2 and 27·8 cwt/acre in untreated and formalin-treated soils respectively. By contrast, on Little Knott, which had been intensively cropped with cereals for the past 20 years, formalin increased the average yield from 24·5 to 31·7 cwt/acre. The increase was associated with decreases in the incidence of take-all, eyespot and root damage by cereal-root eelworm. Thus, in June, formalin treatment decreased the percentage of plants infected by take-all from 44 to 6%, and those damaged by eelworm from 44 to 20%. In July the percentage of straws infected by eyespot was decreased from 31 to 12%.

At Woburn, in an experiment started by the Nematology Department (see p. 150), several different soil sterilants were compared on a site known to be infested by take-all and cereal-root eelworm. Both diseases were controlled by all treatments except mercuric chloride, which was broadcast and rotavated in (Table 1).

TABLE 1

Effect of soil treatments at Woburn

Treatments	Take-all: % Plants in June	Take-all: Disease rating in July	Cereal-root eelworm: % Plants damaged* in June
Nil	43	52	88
Rotavated (R)	46	45	88
Mercuric chloride (R)	41	39	89
Dazomet (R)	2	2	1
Methyl bromide injected (I)	27	18	3
Chloropicrin (I)	15	30	1
DD (I)	9	13	2
Formalin drench	4	12	14

* Deformed roots.

Dazomet, applied by the same method, controlled both diseases best, and DD (usually used as a nematicide) very effectively controlled take-all

https://doi.org/10.23637/ERADOC-1-60

as well. But brown foot rot (*Fusarium* spp.) increased in treated soils from 4·1% of straws infected in untreated plots to 11·4% in plots treated with DD. Grain yields after treatment with chloropicrin, DD and formalin were similar, and averaged 30·8 cwt/acre, compared with 22·4 cwt/acre in untreated controls. (Salt)

Potato Diseases

Potato blight spraying. Tests of the stage of potato blight (*Phytophthora infestans*) epidemics at which fungicidal spraying or haulm destruction give the maximum yield of uninfected tubers were continued at Rothamsted, at Woburn and on black fen soil at Mepal, Cambs. With more than average rain in May, crops grew fast and blight appeared early. In late June infected plants were common on cull piles and clamp sites in the Fens, and blight was found on the experiments on 21, 2 and 6 July respectively. Haulm grew unusually lush, and when it straggled in mid-July the lower leaves died (about 40% of leaf area). The first spray preceded disease outbreak and official disease warning (13 July) except at Woburn, where a focus developed early in an adjoining experiment. Table 2 shows the yields

TABLE 2

Tuber yield (tons/acre) and blighted tubers (tons/acre, and %)

		Rothamsted	Mepal	Woburn
No fungicide	Total	16·04	12·87	13·13
No acid	Blighted	3·40 (21·2%)	0·71 (5·5%)	0·96 (7·5%)
4 Fungicide sprays	Total	16·55	13·80	17·67
Acid 1–5%	Blighted	1·16 (7·0%)	0·14 (1·0%)	1·25 (7·1%)
4 Fungicide sprays	Total	17·42	16·07	—
Acid 10–20%	Blighted	2·02 (11·6%)	0·71 (4·4%)	—
4 Fungicide sprays	Total	18·51	16·86	—
Acid 50%	Blighted	3·37 (18·2%)	1·36 (8·1%)	—
4 Fungicide sprays	Total	19·57	16·15	19·06*
No acid	Blighted	5·23 (26·7%)	2·42 (15·0%)	2·19 (11·5%)

* Estimated from samples.

on the unsprayed plots, plots sprayed four times with fungicide but not burnt-off with sulphuric acid and other plots sprayed with sulphuric acid when 1–5, 10–20 and 50% of the foliage still alive at disease outbreak was blighted. The earlier haulm was destroyed, the smaller the percentage of tubers blighted. At Rothamsted, a fungicide spray before any forecast or outbreak delayed foliage infection by about a week, but the difference disappeared by the time a quarter of the foliage was destroyed and there were no significant differences in total yield. Even such small differences could be profitable by allowing a further week of growth before the need to destroy haulm at the early stages necessary to decrease tuber infection. (Hirst, Stedman and Hide)

Tuber susceptibility to potato blight. Field studies designed to analyse the process of tuber infection were continued with two tuber-susceptible varieties Up-to-Date (UD) and King Edward (KE), and two tuber-resistant varieties Majestic (MJ) and Arran Viking (AV).

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Phytophthora infestans was introduced on 29 June and released, by removing inoculation sleeves, on 5 July; by 14 July first lesions were seen around the inoculated plants and by 24 July the fungus had spread to all plots, affecting 0·5% UD, KE and MJ, and 1% AV foliage. Wet weather favoured spread, and UD and KE were 50% defoliated by 4 August and had few leaves alive after mid-August. MJ and AV were more resistant, and were half defoliated by 7 and 11 August respectively; little foliage remained by 2 September.

First infected tubers were found on 26 July on plants dug from rows adjacent to the infectors, when 21% UD, 19% KE, 4% MJ and 6% AV tubers were blighted; similar amounts were shown on 29 July from sample areas. By 31 August 57% UD, 63% KE, 5% MJ and 7% AV tubers showed active rots.

The epidemic developed so rapidly that the apparatus could not be installed in time to study the early phases of the blight attack in detail. However, the main occasions of spread to tubers were traced. The first was on 14 July, when 0·49 in. rain fell. The most important infection period was between 19 and 25 July, when 2·06 in. rain fell (0·89 in. on 23 July alone). Further tubers were infected after 0·28 in., rain on 28 July and 0·71 in. on 2 August. Little or no rain fell from 3 to 18 August, the period during which the destruction of foliage was almost completed, but with 0·5 in. on 19 and 20 August a few surface tubers were infected; 0·3 in. on 24 August led to further infection, but was less important in this experiment than in areas where more of the foliage was still alive.

Soil samples were taken daily and the moisture content determined at the surface and the tuberising zone 5 in. below the ridge surface. Most tuber infection occurred when soil moisture at 5 in. depth reached 20% (on a dry wt basis), when the clay soil, although "sticky", could be readily removed from around tubers because of a water film between the soil and tuber surface. After 4 August, during the drier weather, soil moisture at 5 in. depth decreased to about 15% and reached 20% again only on 25 August and was maintained for 3 days, during which period tuber infections further increased.

Catches in spore traps on stems showed that spores were washed down from stem lesions on all rainy days. Throughout the epidemic spores were detected in the surface soil under Majestic foliage, but fewer after 10 August, when over 50% foliage was destroyed, than earlier. At 5 in. depth spore numbers declined after 5 August. Spores in the soil do not necessarily come directly from foliage, because the fungus was often found sporulating from the eyes and lenticels of blighted tubers underground, particularly tubers of UD and KE. (Lapwood)

Common scab (*Streptomyces scabies*). Field trials, started in 1964 to test whether fungus on seed tubers is important in determining the incidence of common scab, were repeated. Tubers of the variety Majestic were separated into classes called "severe", "moderate" or "slight" scab, and "clean" tubers. Some "clean" tubers were also treated with formalin, and the five lots planted in an experiment duplicated on Highfield and Fosters (Highfield usually produces more scabbed tubers than Fosters). At harvest the

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mean percentage surface area of tubers affected on progeny in this wet year was less than 1% for all treatments on both fields. A small quantity of "very severely affected" seed with over 65% surface area affected (as compared with 45% for "severe") also showed about 1% on the progeny on the two fields.

Experiments were started to investigate the frequency of irrigation required to decrease the incidence of common scab on Majestic. A small trial at Rothamsted with 4 blocks of 4 treatments compared: (1) plots receiving rain only; (2) plots where rain was supplemented by overhead irrigation to bring soil at 4 cm depth to field capacity only when soil moisture tension reached 50 cm mercury in porous pot tensiometers; (3) irrigation to field capacity when soil moisture tension reached 30 cm; and (4) irrigation to maintain soil at field capacity.

Tuber samples showed that the incidence of scab decreased with increasing frequency of irrigation, and on 20 July 3·0, 2·0, 0·5 and 0·2% surface area of tubers was already affected for treatments (1) to (4) respectively. Earlier than this the only "dry" weather necessitating irrigation treatments (2) and (3) was from 26 June to 7 July (when only 0·13 in. rain fell), which suggests that this was the "infection period".

To test at what stage of development Majestic tubers were most susceptible to scab infection, seed tubers were either: (1) chitted for a few months; (2) chitted for a few weeks; or (3) not chitted (seed stored at 5° C) before planting, in an attempt to delay the time when tubers formed and developed. Plots arranged in three blocks were sampled regularly from early June, and the times the tubers formed and the rates they developed were assessed by riddling tubers into size grades differing by 0·5-cm units. Tubers were scored for the surface area affected, and the distribution of scab lesions was also described by dividing the length of the tuber into six regions from stolon attachment (heel) to apical (rose) end. First lesions were obvious on 12 July, when the distribution of lesions depended on the size of the tuber. Progeny from the chitted seed (1) and (2) had most lesions at the rose end of tubers 4·0 cm or more in diameter; lesions were most abundant in the middle (body) region of tubers 2·5–4·0 cm, and were distributed more variably on smaller tubers. Few non-chitted (3) progeny graded over 3·0 cm, but, like treatments (1) and (2), most lesions were in the "body" region; smaller tubers showed mainly "heel" end infections. Assuming that the larger tubers were formed before smaller ones and that infection occurred during late June or early July, then on 28 June for treatments (1) and (2) tubers grading over 4·0 cm were then 2·0 cm and those 2·5–4·0 cm were 1·0–2·0 cm; for treatment (3) tubers less than 3·0 cm were then 0·5–1·0 cm. This suggests that, when conditions favoured infection, tubers larger than 1·0 cm were already resistant to infection at the "heel" end, whereas smaller tubers were not. (Lapwood)

Other tuber diseases. The survey of fungus diseases of King Edward seed tubers showed some unusually large differences in stocks from different localities. The percentages of tubers (macroscopically) infected in Scottish A, Irish (predominantly Northern Ireland) and English stocks were, respectively, 60, 39 and 11 with skin spot (*Oospora pustulans*); 9·8, 3·7 and

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5·2 with gangrene (*Phoma* spp.); 4·9, 15·4 and 1·7 with powdery scab (*Spongospora subterranea*); 34·3, 12·0 and 35·9 with common scab (*Streptomyces scabies*). Averaging all sources, there was less blight (0·7% tubers infected) and dry rot (1·9%) than in previous years, but the incidence of *Verticillium* spp., silver scurf (*Helminthosporium atrovirens*) and black scurf (*Rhizoctonia solani*) were within the range of variation in the previous two years.

Our microscopic test of the ability of fungi to spore on excised eyes does not discriminate between species of *Verticillium*. Dr. Q. D. Macgarvie (East Craigs) and G. A. Hide therefore collaborated in identifying species isolated from a sub-sample of stocks. Neither *Verticillium albo-atrum* nor *V. dahliae* was found among over 400 isolates from seed stocks, although *V. dahliae* was isolated from English ware tubers. More than three-quarters of the isolates from seed tubers were *V. tricorpus*. Of the remainder, *V. nubilum*, claimed to be the cause of "coiled sprout", and *V. nigrescens* were almost equally common.

Information on the prevalence of diseases needs matching with knowledge of the damage they do. Repetitions of experiments made in 1964 to compare yield from clean, moderately or severely infected tubers gave very different results in 1965, perhaps because it was a wet instead of a dry summer. Severely skin-spotted tubers of King Edward and Majestic yielded 30·4 and 18·5% respectively less than clean tubers (7% less in 1964). Severe black scurf did not affect the yield of Majestic tubers, but significantly decreased King Edward yield by 7·4% (33% on Majestic and 40% on King Edward in 1964). Infection of progeny tubers increased with severity of seed tuber infection; averaging the two varieties, the progeny of clean, moderately and severely infected seed had, respectively, 18·5, 29·3 and 37·0% buds infected with *O. pustulans* at harvest; from seed graded for black scurf 1, 11 and 18% of plants developed the "*Corticium*" stage and 6·5, 33·0 and 41·1% of their tubers had sclerotia at lifting. (Hirst, Hide and Stedman)

Olpidium brassicae (Wor.) Dang., as a Pathogen

Olpidium is common in the roots of many species of plants, but there has been little evidence that it causes damage. That it can be pathogenic is indicated by work with cabbage plants grown in sand culture. Plants infected with *Olpidium* usually had severely chlorotic leaves and shorter, thicker roots than uninoculated plants, which were not only greener but larger. In water culture, chlorosis of inoculated plants depended on the concentration of Fe (supplied as tartrate) in the culture solution and was also alleviated by painting the leaves with $FeSO_4$. Roots of infected chlorotic plants in water culture were also shorter and thicker than those of green plants, but tended to become normal when Fe was supplied to the leaves. Thus, in suitable conditions, *Olpidium* infection of cabbage caused Fe deficiency, resulting in chlorosis and stunted roots and loss of yield. Whether uptake of Fe is specially affected by *Olpidium* is not known, but it seems more likely that infection decreases the amount of effective root and that iron supply (often near-limiting in sand cultures) is severely restricted

https://doi.org/10.23637/ERADOC-1-60

giving immediate and conspicuous symptoms. In the field *Olpidium* infection might be a contributory factor in iron chlorosis, particularly where chlorosis is enhanced by cool, wet weather. (Macfarlane)

Diseases of Sitka Spruce Seedlings in Forest Nurseries

Collaboration with the Research Staff of the Forestry Commission continued with experiments at Ringwood, Wareham and Old Kennington nurseries. The persistent effect on seedling survival and growth of soil sterilants applied at Ringwood was particularly striking. Chemical treatments last applied in December 1962 substantially increased survival and growth of Sitka spruce sown in March 1963, 1964 and 1965. This year 1,380 seedlings survived and grew to a mean height of 1·11 in. in untreated soil, compared with 1,476, 1,569 and 1,574 measuring 1·70, 2·35 and 1·82 in. in plots previously treated with formalin, metham-sodium and dazomet respectively. In contrast, soil treatments with quintozene and nabam in March 1963 failed to increase growth, and increased numbers only in 1963 and 1964. Fallowing half the plots in 1964 had no effect on numbers of seedlings in 1965, but increased growth by 23% in untreated soil and by 19 and 16% respectively in those treated with sterilants and fungicides.

To see whether growth responses could be related to root damage by soil-borne pathogens, root segments were plated after surface sterilising in sodium hypochlorite solution, and nematodes were extracted from soil washed off roots. The parasitic nematode *Hoplolaimus uniformis* abundant in untreated soil and in soil treated with nabam, was still rare in soils treated three years previously with sterilants. The evidence that this nematode alone accounted for most of the stunted growth at Ringwood is convincing, except for the fact that the fungicide quintozene controlled the nematode as effectively as metham-sodium, and yet failed to increase growth. Species of *Cylindrocarpon*, *Pythium* and *Fusarium* were the most common fungi isolated. They were rare or absent from roots in the first year after treatment, but *Cylindrocarpon* and *Pythium* had returned to treated soils by the third year, and similar numbers were isolated from roots grown in soil given any treatment. They occurred on 64 and 26% respectively of root segments plated. Only 8% of roots yielded *Fusarium* spp. in plots treated with metham-sodium, compared with a mean of 20% in all other treatments. The increase in growth after fallowing may have been a nutritional effect, as fallowing had no effect on numbers or types of fungi or parasitic nematodes isolated from roots.

In experiments with Sitka spruce seed poor quality seed with a germination of 18% and good seed with 81% were each sown at a rate to give 1,800 viable seeds/square yard. At Ringwood the good seed yielded 1,408 seedlings, and dressing the seed with 50% thiram dust had no beneficial effect, whereas the poor seed yielded only 602 seedlings, and treatment with thiram increased emergence by 69% to give 1,019 seedlings/sq yd. A similar result was recorded at Old Kennington, where thiram increased the emergence of poor seed by 31%. The increases in emergence were similar in untreated soils and in soils partially sterilised with formalin or

https://doi.org/10.23637/ERADOC-1-60

dazomet, which suggests that the emergence losses were caused by seed-borne and not soil-borne pathogens. If so, the damage done by seed-borne pathogens depends very much on seed bed conditions, because in the same experiments in 1963 and 1964 poor and good seed both yielded similar numbers of seedlings, and thiram had no beneficial effect on either.

Seed that failed to germinate on sterilised quartz grit in Petri dishes yielded several pathogenic fungi not yet isolated from dead or dying seedlings in nursery soils. One of these, *Rhizoctonia solani*, although found on less than 1% of seed plated, killed every seedling when tested as a pure culture. An unidentified fungus, isolated from many excised endosperms of ungerminated seed incubated at 5° or 10° C, grew very slowly on natural media; it varied in culture from a floccose pale brown mycelium to a compact orange colony with blue-green edges, and produced branching heads of conidia resembling a *Botrytis*. (Salt)

Aerobiology

In the orchard at Wisbech where the concentration of *Venturia inaequalis* ascospores in the air has been measured since 1953, apple scab was well controlled by spraying, although more leaves than usual survived through the winter and weather favoured the disease. This suggests that methods of spray timing, proposed from work done during a series of dry springs, are also effective in the more stringent conditions of wet spring months.

In collaboration with the Meteorological Office and the Meteorological Research Flight of the Royal Air Force we have extended earlier studies (Hirst, J. M., *Trans. Br. mycol. Soc.* (1961) **44**, 138–139) of the vertical distribution of spores and the interception of immigrant uredospores of *Puccinia graminis*. The earlier work suggested that, in unstable air, the concentration of airborne spores decreased approximately logarithmically with height but that spores from distant sources might be most common at heights between 3,000 and 5,000 ft, because of preferential deposition of spores from the base of the cloud. Later flights were aimed to study how the spore cloud produced by England is depleted as it travels downwind over the North Sea. Pollens and *Cladosporium* conidia were used as examples of large and small spores usually liberated by day, and a composite group of "damp-air spore types" (*Sporobolomyces*, *Tilletiopsis* and ascospores) as an example of spores probably liberated chiefly at night. All flights were during summer, and samples were collected in the middle of the day at heights between 100 and 10,000 ft above sea-level, during a "saw-tooth" pattern of continuous alternating ascent or descent (at 1,000 ft/min and 180 knots). Analysis of the catch is laborious, so although four flights were made in the period 1962–64, results are only just complete.

In the first flight on 15 June 1962, 200 miles N.E. from Orfordness, Suffolk, spores of all three indicator groups were least common about 75 miles out and concentrations (*Cladosporium* 1,510/m^3 at 3,500 ft, pollens 430/m^3 at 15,000 ft and damp-air types 80/m^3 at 1,500 ft) were greatest 175 miles from the coast. Meteorological analysis suggests that spores caught near the coast were released either from Brittany, France, on 14 June or Essex, England, on the morning of the flight. The region with fewest

https://doi.org/10.23637/ERADOC-1-60

spores represented day travel over the English Channel or night travel over England, and the dense cloud north of the Netherlands was liberated from S.W. and C. England during 14 June. On the second flight, on 17 July 1963, these observations were repeated, but spores were fewer because of cooler weather and cloud- and rain-enforced modifications to the flight plan. Nevertheless, spores were caught all the way across the North Sea on a line roughly from Lincolnshire to Esbjerg in Denmark. It was hoped that a longer flight might pass through the daytime spore cloud produced over England the previous day and into that of the day before. Stronger winds prevented this, but we think we distinguished spore clouds that came from England on the morning of the flight and others that came from England and Ireland the previous day.

The third flight, because of a sudden change to easterly winds, could not test depletion of spore clouds along the track from King's Lynn north for 350 miles to a position midway between the Orkney Is. and Stavanger, Norway, but instead provided an interesting cross-wind section of air over the region Estonia–Poland 36 hours earlier. Particularly interesting was the discovery of widespread but small concentrations of *Puccinia graminis* uredospores, showing that Mediterranean regions of Western Europe are not the only possible source for their introduction to the British Isles.

The fourth flight, on 16 July 1964, was again of "saw-tooth" pattern along the line 515 miles E.N.E. from Middlesborough into the Skagerrak. Spore concentrations were large, with *Cladosporium* and pollen densest about 300 miles from the coast, coincident with fewest damp-air liberated spores, which were most abundant at 100 and 375 miles. The heights of maximum concentrations were below 2,500 ft for pollens and for damp-air types (except for a rise to about 3,500 ft at the extremity of the flight), but for *Cladosporium* spores rose from 200 ft near the coast to 4,000 ft when 400 miles out. The spore concentration at 300 miles from the coast was such that had the spores been instantaneously washed-out by rain, at least 10 spores/sq cm would have been deposited. (Hirst and Stedman)

Airborne Moulds and Actinomycetes from Crops in Storage

Moist storage of barley grain in concrete staved silos. The spore content of the air in silos over high-moisture barley, and of the grain stored in this way, was examined for the remainder of the 1964–65 storage season (see *Rothamsted Report* for 1964, p. 142). Before any grain was extracted, moulding occurred in the top of the stored grain to a depth of as much as 1 ft, the depth varying with the method of capping. A layer of silage covered by a polyethylene sheet, used in one silo, allowed the least moulding. In another silo a layer of straw, used to absorb condensed water under a polyethylene sheet, moulded with much *Thermopolyspora polyspora*, so forming a potential farmer's lung disease hazard. *Aspergillus fumigatus*, *A. flavus*, *A. nidulans*, *A. terreus*, *A. candidus*, *A. glaucus*, *Mucor pusillus*, *Absidia corymbifera*, *Humicola lanuginosa*, *Thermoactinomyces vulgaris* and *Thermopolyspora polyspora*, all potential human and animal pathogens, were isolated from the surface layer of mouldy grain; numbers

https://doi.org/10.23637/ERADOC-1-60

of each differed in different silos, possibly because the water content of the grain differed.

While grain was being drawn from the silos, all tended towards the same microflora, with *Penicillium* spp. and yeasts predominant, but the rate the grain was withdrawn had a large effect. When removed too slowly the grain may self-heat to 40° C or more, and then *Mucor pusillus*, *Absidia corymbifera* or *Aspergillus* spp. may predominate. Removing at least 3 in. of grain each day seems enough to prevent self-heating, and to check the growth of potentially pathogenic fungi.

Extracted and rolled grain had fewer spores than grain in the silo, because many spores are deposited on the silo walls. Other spores may escape as clouds of dust at the bottom of the extraction chute or around the rolling equipment. Efficient dust respirators should therefore be used wherever dust is liberated in or around the silo, and the grain should be handled, wherever possible, in well-ventilated premises. (Lacey)

https://doi.org/10.23637/ERADOC-1-60