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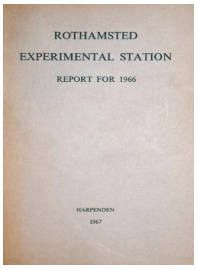
Rothamsted Report for 1966

Full Table of Content



Plant Pathology Department

P. H. Gregory

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PLANT PATHOLOGY DEPARTMENT

P. H. GREGORY

Early in the year the mycologists moved from West Building into the reconditioned upper floor of North Building and so, after 11 years, the Department is now reunited under one roof.

B. D. Harrison and Anthea J. Lack (Mrs. Hickman) left, A. J. Gibbs was seconded to the John Curtin School of Medical Research, Canberra, and J. A'Brook, J. M. Carpenter, D. R. Henden, R. G. Milne, Margaret E. Pullen and J. M. Waller were appointed to the scientific staff.

Visiting workers included: Mr. D. L. Ebbels (Reading), Dr. R. R. Frost (Manchester), Dr. Shirley M. Nash (University of California, Berkeley), Dr. D. A. McCarthy (Queen Mary College, London), Prof. A. D. McLaren (University of California, Berkeley), Mr. G. D. McLaren (Stanford University), Mrs. Bolajoko A. M. Okusanya (Nigeria), Prof. W. C. Snyder (University of California, Berkeley), Mr. Anupam Varma and Mrs. Prabhati Varma (Allahabad).

A. J. Gibbs and B. D. Harrison attended the Ninth International Congress for Microbiology in Moscow. J. M. Hirst attended the First International Biometeorological Conference of the Middle and Far East at Laklouk, Lebanon. At the invitation of C.S.I.R.O. he also attended the Australian Plant Pathology Conference at Toowoomba, Queensland, and visited plant pathologists in other parts of Australia and in the Territory of Papua and New Guinea. B. Kassanis was invited to the Société Francaise de Physiologie Végétale, meeting in Strasbourg, and also to give a seminar in the Biochemical School of the University of Brussels. D. H. Lapwood attended the Third Triennial Conference of the European Potato Association, Zürich. I. Macfarlane was invited to a symposium on "Lower Fungi in the Shallow Sea" at the Institute for Marine Research, Bremerhaven, and visited laboratories in Baarn and Wageningen.

Properties and serology of viruses

A cryptic virus of sugar beet. Electron microscopy showed rounded virus-like particles about 28 mμ diameter in sap of sugar beet taken from the field, and of plants infected in the glasshouse with beet yellows (BYV), yellow net mild yellows (YNMYV) or beet mild yellowing viruses (BMYV).

The particles are fairly numerous (estimated at about 5×10^{10} per ml) in preparations made from sugar beet infected with BYV, BMYV or YNMYV. Of these, preparations from plants with BYV contain most such particles, which are in addition to many of the long particles of BYV. Some cultures of BMYV and YMYV seem free from the cryptic virus, and extracts of plants infected with these cultures do not contain any specific particles identifiable by electron microscopy.

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The 28-mμ particles can be separated from BYV by extraction in 8% butanol followed by high-speed centrifugation. Sugar-beet plants manually inoculated with such preparations apparently became infected but remained symptomless, and sap from them contained only a tenth as many particles as from plants also infected with BYV. The particles were not detected until some weeks after plants were infected.

When sugar beet were infected by manual inoculation with BYV and the cryptic virus only BYV particles were detected for a week or more after the plants developed yellows, but eventually both kinds of particle became numerous. *Myzus persicae* appeared to transmit both from plants infected with both, but this is now uncertain, because particles have been found in old sugar beet on which aphids are believed not to have fed. It may be that the particles invade in some other way, but multiply up in the presence of aphid transmitted virus.

The particles described from Wageningen by Dr. D. Peters (*Virology* (1965), **26**, 159) in fractionated extracts of various aphids, apparently free from plant viruses, were found in our cultures of *M. persicae* (even in one from eggs found on peach in spring 1966). These particles do not resemble the cryptic particles from sugar beet, but are smaller (20 and 24 mμ) and appear "softer" when examined in the electron microscope.

The failure of the cryptic virus to cause symptoms in sugar beet makes it difficult to study, and its status as a plant pathogenic virus remains obscure. It does not need another virus to support its multiplication, but does much better in mixed infections. (Watson, Pullen and Woods)

Properties of virus affected by ambient temperature. Dolichos enation mosaic virus (DEMV), which is serologically related to tobacco mosaic virus (TMV), infects leguminous plants in which it produces a large proportion of particles shorter than 300 mμ, the accepted length of infective TMV. A strain isolated from a single local lesion causes local and systemic necrosis in French-bean plants and has some unusual properties.

Plants infected with this strain and kept at 20° C produce the same amount of virus protein as plants infected with the parent strain, but it has only about $\frac{1}{10}$–$\frac{1}{100}$ the infectivity, seemingly because most of the particles are labile and disintegrate during extraction. The particles were preserved and could be purified when leaves were extracted in phosphate buffer pH 5·2; bringing the purified preparation to pH 8, made most of the virus particles break down to rings (consisting of one to three spirals of the virus-protein helix) and smaller protein units. During the pH change nucleic acid was released but was not infective, probably because the particles were already partly disrupted before or during extraction. When sedimented at pH 8 in the analytical centrifuge the preparation showed three peaks; the smallest had a sedimentation constant of 187S and consisted of complete virus particles, and the two others consisted respectively of the rings (16–28S) and the virus protein, together with the nucleic acid (6–8S). The three components were separated in agar and in sucrose gradient columns, and characterised.

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In the same conditions the parent strain of DEMV did not break down into rings, protein and nucleic acid. Although the necrotic strain behaves in this abnormal manner when produced in plants grown at 20° C, it does not when produced in plants kept at above 30° C. At such high temperatures the two strains differ little in either the quality or quantity of virus produced. (Kassanis and McCarthy)

Interaction between "univalent" fragments of antibodies and viruses. Continuation of this study (*Rothamsted Report* for 1965, p. 116) showed that preparations of papain-digested antibodies to TMV precipitated the virus only because they contained a small proportion of residual undigested "bivalent" antibody. This was found by separating the "univalent" fragments from the residual "bivalent" antibody on a "Sephadex G 200" column.

Kinetics of the interaction between "univalent" antibody fragments and their homologous antigens was studied. The constant of the rate of combination of the "univalent" fragments with TMV is about $\frac{1}{40}$ of that of the original antibody, and about $\frac{1}{3}$ when the antigen is bovine serum albumin. Thus the mode of combination with antigen of the "univalent" fragments differs from that of the original antibody. This, and not necessarily their "univalence", may explain the inability of the fragments to precipitate the antigens.

The extent to which the "univalent" fragments inhibit the precipitation of antigen by the original "bivalent" antibody differs with different antigens. Only small amounts of fragmented antibody are needed to inhibit the precipitation of tomato bushy stunt virus and bovine serum albumin completely, whereas much more is needed with TMV. (Kleczkowski)

Inactivation of infectivity of TMV–RNA during UV-irradiation of the whole virus at two different wavelengths. Inactivation of infectivity of TMV by UV is entirely a result of alterations of the structure of RNA. Any possible alterations in virus protein do not seem to contribute to loss of infectivity. Whereas free RNA is equally susceptible to inactivation by UV at any wavelength (i.e. the extent of inactivation depends entirely on the amount of absorbed radiation energy, irrespective of the wavelength), the RNA inside the virus appears to be about 24 times more sensitive to inactivation at 230 mμ than at 280 mμ. The RNA inside the virus appears to be protected to a considerable extent by protein from damage by UV at 280 mμ and 254 mμ, but not at 230 mμ, when it seems to be even slightly more sensitive than free RNA. (Kleczkowski and A. D. McLaren)

Infectivity changes in cell-free preparations from plants infected with tobacco mosaic virus. Several workers have claimed that the nucleic acid of TMV replicates in cell-free preparations from plants. The evidence for this claim is largely based on increases in infectivity when preparations are incubated a few hours at room temperature, as measured by inoculating test plants with either the preparations themselves or phenol extracts of

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them. It was confirmed that the infectivity of phenol extracts of cell-free preparations obtained from leaves recently infected with TMV often increases when the preparations are incubated at 20° C. However, comparable increases can also occur when the preparations are incubated at 2° C or simply frozen and thawed, i.e. conditions in which virus nucleic acid could not have multiplied. Increased infectivity therefore does not necessarily demonstrate that virus nucleic acid has multiplied. (G. D. McLaren)

Structure of plant viruses with elongated particles. Some viruses with elongated particles were examined in the electron microscope, using uranyl formate as a negative stain, and optical transforms were made from the electron micrographs. Viruses of four types were examined. *Potato virus X group:* potato X, white clover mosaic, hydrangea ringspot and potato aucuba mosaic. *Potato virus S group:* red clover vein mosaic and carnation latent. *Potato virus Y group:* potato Y, bean yellow mosaic and clover yellow vein. *Sugar-beet yellows virus.* All except viruses of the PVY group showed clear morphological sub-units. Within each group particles showed similar structure and could be distinguished from viruses of other groups. The pitch of the basic helix of all these viruses was between 3·3 and 3·7 mμ, and there was no consistent difference between individual viruses. The surface lattice of the viruses could not be determined from the transforms because their surface structure varied too much, and this variability was not decreased by staining for different times at different pH values. (Varma, Gibbs and Woods, with Dr. J. Finch, Medical Research Council Laboratory of Molecular Biology, Cambridge)

Serology of filamentous viruses by micro-gel-diffusion tests. When purified preparations of potato X, red clover vein mosaic and white clover mosaic viruses were treated with ultrasonic vibration in the cold their particles broke into shorter lengths. Particles of white clover mosaic and potato X viruses broke up more easily than those of red clover vein mosaic virus. Untreated preparations of these viruses did not precipitate in micro-gel-diffusion serological tests with homologous antisera, whereas treated preparations of potato X and white clover mosaic viruses gave sharp and specific bands of precipitate. Red clover vein mosaic virus, however, gave only faint precipitate bands after treatment.

Potato X and white clover mosaic viruses were proved to be serologically related by microprecipitin tests, but no relationship was detected by micro-gel-diffusion serological tests. (Varma and Gibbs)

Replication of tobacco rattle virus. Following earlier work (7.17) on the differing roles of long and short particles of tobacco rattle viruses, the effect of temperature was studied in relation to the accumulation in inoculated leaves of long and short particles of two isolates, one from Brazil and the other from Scotland. When the leaves were sampled from 1 to 6 days after inoculation the optimum temperature for virus accumulation decreased with increasing time. For instance, with the Scottish isolate it was 22° C after 1 day and 14° C after 6 days; with the Brazilian isolate it was

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about 4° C higher after each period. In general, accumulation of short particles was well synchronised with that of long ones, but the specific infectivity of the long particles decreased with increasing time at the higher temperatures. (Frost and Harrison)

Chronic bee-paralysis virus. Preparations of this virus (see Report of the Bee Department, p. 213) contained particles of three main sizes, all mostly about 21 mμ wide, but of three different average lengths, 42, 52 and 61 mμ, with sedimentation coefficients of $S_{20,w}$ 97, 110 and 125 respectively. They were serologically indistinguishable. Unfractionated preparations contained nucleic acid of the ribose type only, with a molar base composition of guanine 20%, adenine 24%, cytosine 28% and uracil 28%. The slowest sedimenting (97S) particles seemed to contain about 10% nucleic acid and the fastest 20–25%.

The virus was not precipitated in cold 1*N*-HCl or 1*N*-KOH, but both reagents partially degraded the particles, so that, although they were of similar shape as before, they were smaller (20–40 mμ long and 20–30 mμ wide), were penetrated by negative stain and contained no nucleic acid. They produced two broad boundaries in the analytical centrifuge with sedimentation coefficients of 65 and 72S. An antiserum prepared against untreated preparations of the virus reacted specifically with alkali-treated preparations, but its titre with it was only one-quarter as great as with untreated virus. (Gibbs)

Virus classification and nomenclature. The merits and demerits of the VAC (Vernacular name and cryptogram) system of naming viruses proposed in collaboration with Dr. D. H. Watson and Prof. P. Wildy (7.19) was much discussed. Referenda were organised to sound opinions in Britain by the Society for General Microbiology and by the Federation of British Plant Pathologists. Most of those who answered preferred the VAC system to any alternative, though some felt that the time was not yet ripe for any new system. Further discussions at the International Congress for Microbiology and in the International Committee for Nomenclature of Viruses led to the establishment of a subcommittee (Chairman: A. J. Gibbs) to evaluate the VAC system. It is now proposed that the names should take the form: Turnip yellow mosaic virus R/1:2/35:S/S:S/Cl:TYM-group. (Gibbs and Harrison)

Virus diseases of potatoes

Production of virus-free potato clones. Excising apical meristems (100–200 μ across) from potato sprouts is one method of producing healthy clones from varieties that are totally virus-infected. The meristems are grown aseptically in artificial media into plantlets which, when big enough to handle, are transferred to soil in pots. Using this method, virus-free clones of the following varieties were produced: Golden Wonder was freed from potato virus A; Arran Comet from potato virus X; Sharpe's Express, Orion and Epicure from potato virus S. Of 196 excised meristems, 20 grew into plants, of which 19 were virus-free. The health of the plants was

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verified by serological and infectivity tests. Golden Wonder was also tested by grafting to Up-to-Date and *Solanum demissum*, which are indicator plants for potato virus A. It is of interest that potato virus X was eliminated from Arran Comet by this method, because it was generally assumed that, with this virus, the method works only when meristems are excised from plants that have previously been treated to diminish their virus content, either by keeping them at 37° C or spraying with thiouracil. (Kassanis and Varma)

Tobacco rattle virus in potatoes. Potato varieties differed in the frequency with which tobacco rattle virus was transmitted from mother tubers to progeny plants and tubers. In varieties such as Pentland Dell, whose tubers showed severe tuber symptoms (spraing) in the first year of infection, transmission was rare, but in others, such as King Edward, which show very slight tuber symptoms, transmission was much commoner. The symptoms shown by haulms of plants differed much between varieties, but characteristically did not involve all shoots on a plant. A proportion of the tubers produced by plants showing these stem-mottle symptoms were of poor shape and had internal corky markings whose severity was related to that of primary symptoms in the same variety. (Harrison)

Potato mop-top virus. Tobacco seedlings became infected when grown in soil infested with the virus, even when the soil was previously air-dried, suggesting that the vector is not a nematode. Preliminary tests indicate that the powdery scab fungus (*Spongospora subterranea*) transmits the virus, which is retained by its resting spores. Tobacco was found to be a host of *S. subterranea*. The virus, which seems to occur in plants in only small amounts, is not very stable *in vitro*, and had elongated particles. (Harrison)

Virus diseases of legumes

Pea early-browning virus (PEBV). Major serological differences were previously reported between isolates of PEBV from Britain and the Netherlands, but only small differences were found between different isolates from Britain. However, pea varieties that are field resistant in the Netherlands are also resistant when grown in soil from some fields in Britain, but not from others. The difference between the British sites seems to lie in the prevailing virus strain. A virus isolate from a site where some varieties were resistant caused only necrotic local lesions in the inoculated leaves of the resistant ones, whereas isolates from sites of the other type caused diffuse local lesions followed by systemic infection. Isolates of both types were transmitted by a new vector, the nematode *Trichodorus anemones*, which did not transmit a Dutch isolate. Both types of British isolate were found together in one field, but not in another. (Harrison)

True broad-bean mosaic and broad-bean stain viruses. Plants showing symptoms characteristic of infection with true broad-bean mosaic virus

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(TBBMV) were found in crops of broad beans (*Vicia faba* L.) in southern England in 1965 and 1966, but none of about 100 isolates from these plants reacted with an antiserum to TBBMV. The virus from these plants is serologically unrelated to TBBMV, and can be differentiated from it on other host plants. It is associated with an economically important staining of the pod and beans ("Evesham stain"), and has been called broad-bean stain virus (BBSV).

BBSV and TBBMV have similar isometric particles 28 mμ in diameter, with a polygonal outline and no obvious morphological subunits. They have nucleic acids with similar molar base ratios: guanine 23%, adenine 27%, cytosine 18%, uracil 32%. Preparations of BBSV, but not of TBBMV, contain nucleic acid-free particles, in addition to particles containing nucleic acid.

BBSV and TBBMV have particles with the same morphology, and nucleic acid of the same base composition as viruses of the cowpea mosaic group of viruses. BBSV, but not TBBMV, is serologically related to cowpea mosaic, red clover mottle (guanine 20%, adenine 29%, cytosine 20%, uracil 30%), squash mosaic and Fl (*Acta virol.* (1966), **10**, 182) viruses. None of these viruses is serologically related to broad-bean mottle virus. (Gibbs)

Cowpea mottle virus. This virus from Nigeria has isometric particles, which are about 25–30 mμ in diameter, rounded and show no obvious morphological sub-units. These particles sediment as a single component with a sedimentation coefficient of 137, and contain nucleic acid with a molar base ratio of: guanine 25%, adenine 25%, cytosine 26%, uracil 25%. In serological tests the virus was not precipitated by antisera to turnip crinkle, turnip rosette, tomato bushy stunt, *Pelargonium* leaf curl, broad-bean mottle, carnation ringspot, carnation mottle, cocksfoot mottle, brome mosaic, *Phleum* mottle, cowpea chlorotic mottle and cowpea mosaic viruses. (Mrs. Prabhati Varma and Gibbs)

Red clover vein mosaic virus. Irradiation with ultra-violet (2,537 Å), even for a short time, decreased the infectivity of the virus considerably. More infectivity was lost in plants kept in the dark than those kept in the light, showing that the virus is photoreactivable: the amount of reactivation increased as the time of irradiation increased.

Purified preparations (*Rothamsted Report* for 1965, p. 118) of this virus gave absorption spectra characteristic of nucleoproteins with a small amount of nucleic acid. The ratio of optical densities at 2,800 and 2,600 Å suggested that RCVMV has 6·25% nucleic acid. Its nucleic acid molar base ratio (guanine 31·5%, adenine 24·2%, cytosine 22·7%, uracil 21·6%) differs from those of all other reported virus nucleic acids.

In serological tests this virus proved distantly related to cactus 2, carnation latent, chrysanthemum B, passiflora latent and potato S viruses, but not related to potato virus M. (Varma and Gibbs)

Clover yellow vein virus. This virus, which is common in Britain in white

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clover, was also isolated from naturally infected *Medicago lupulina* showing bright vein yellow mosaic. *Nicotiana clevelandii* infected with it had long flexuous particles ranging from 700 to 800 mμ long. The modal length of particles differed from plant to plant, but was commonly 767 mμ, suggesting some relationship with viruses of the potato Y group. This was confirmed by serological tests. Serologically it was distantly related to bean yellow mosaic, lettuce mosaic and pea mosaic viruses. However, it failed to react with antisera prepared against potato Y, cocksfoot streak and henbane mosaic viruses. (Varma and Gibbs)

Viruses in crops of red clover (*Trifolium pratense* L.). Red clover plants from nine permanent pastures in England and Wales were tested for sap-transmissible viruses, and the viruses obtained were identified by the symptoms they caused in test plants, by electron microscopy and serology. Of the 265 plants tested, 14% were infected. Pea mosaic was common and widespread, occurring in 8% of all plants and in seven fields. Other viruses isolated were: arabis mosaic, bean yellow mosaic, red clover mottle and red clover vein mosaic. No virus was isolated from seedlings grown from seed from 89 commercial seed crops in England. Red clover mottle virus was not transmitted by *Sminthurus viridis* L. (Mrs. Prabhati Varma and Gibbs)

White clover viruses. Random samples of 60–66 plants of nine varieties on replicated plots at Bristol N.A.A.S. were grown in separate pots in the insect-free glasshouse. Symptoms were recorded, and sap of 40–44 plants of each variety was inoculated to *Chenopodium amaranticolor*, *Nicotiana clevelandii* and pea. Viruses were identified by symptoms, electron microscopy and serology. The remaining 20–22 plants of each variety were tested for clover yellow vein, red clover vein mosaic and white clover mosaic viruses by slide agglutination tests.

No significant varietal differences were observed in the incidence of red clover vein mosaic and white clover mosaic viruses, whereas clover yellow vein virus infected the variety N.Z. Mother significantly less than Kent Wild White and Pajbjerg Milka.

C.B. Pasture was the variety most susceptible to infection and Kersey was the most resistant. Of the 560 plants tested, 64% were infected. Red clover vein mosaic virus was found in 36% of the plants, white clover mosaic virus in 29% and clover yellow vein virus in 20%. The following were also found: potato stolbur (2%), witches' broom (2%), clover phyllody (1%) and bean yellow mosaic (0·2%). Another 2% of the plants were infected with viruses not yet identified. (Varma, Gibbs and Woods)

Effect of viruses on clovers. Subterranean clover and white clover strains were grown on Jensen's medium in tubes and mechanically inoculated with viruses under aseptic conditions. After nearly 3 months, with all the following combinations virus infection resulted in slight (but non-significant) decreases in number of nodules, leaves and dry weight:

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Virus	*Legume*	*Rhizobium*	*Other effects of virus infection*
Red clover vein mosaic virus	Subterranean clover		
	(*a*) abundantly nodulating strain	Effective strain	Plants produced bigger inflorescences than healthy control
	(*b*) sparsely nodulating strain	Effective strain	Flower formation inhibited
	White clover (Aberystwyth S 100)	Effective strain	—
		Ineffective strain	—
White clover mosaic virus	White clover (Aberystwyth S 100)	Effective strain	—

(Varma, with P.S. Nutman, Soil Microbiology Department)

Effect of red clover vein mosaic on yield of peas. Six representative varieties of peas were sown on replicated plots on 23 April 1965. Plots were divided into two sets, one set was manually inoculated with the virus, and in the other natural infection was recorded on four dates. Varieties Big Ben and Kelvedon Wonder had significantly fewer natural infections than Gregory Surprise and Lincoln. Natural infection diminished yield by 50% or more, the effect being greater the earlier plants were infected (Table 1).

TABLE 1

Loss of yield from pea plants infected with red clover vein mosaic virus

	Date symptoms first observed (1965)				
Naturally infected	29 June	16 July	26 July	6 Aug.	Mean
Mean yield (g) of all varieties	4·1	6·3	6·3	7·1	5·9
% decrease	72	58	58	52	60

(Mean yield of uninfected control plants—14·8 g)

	Date plants inoculated		
Artificially infected	11 June	1 July	Mean
Mean yield (g) of all varieties	4·5	6·7	5·6
% decrease	64	46	55

(Mean yield of uninoculated control plants—12·5 g)

The variety Dark Skinned Perfection suffered most. Infection decreased the number of pods per plant, peas per pod and pea weight, but the fewer pods per plant was the main factor in loss of yield. Some other pathogens may also have affected yield: all varieties were infected with *Ascochyta pisi*, and Kelvedon Wonder was severely damaged. (Varma and Gibbs)

Alfalfa mosaic in lucerne crops. In field experiments the incidence (number infected plants per unit area of crop) of alfalfa mosaic virus (AMV) in plots sown with lucerne alone was three times that when rows of lucerne and cocksfoot alternated. The incidence of AMV in plots cut five times each year was one-sixth that in plots cut the usual three times. AMV-infected plants were not more sensitive to frequent cutting or winter damage than comparable uninfected plants. Neither spring top-dressing with "Nitro-Chalk" nor paraquat spray affected the incidence of AMV.

Samples of commercial lucerne seed were tested for AMV. Three out of 16 seed lots produced 1–3% AMV-infected seedlings; these seed lots

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were from England, France and Portugal, and the AMV isolates obtained were indistinguishable. (Gibbs)

Virus diseases of other crops

Carrot motley dwarf and plant number. In an experiment at Woburn the yield of marketable roots was increased by 3 tons/acre when menazon granules were applied to the soil at time of sowing and 5 tons by "Saphicol" sprayed three times in June and July.

Cavariella aegopodii, the only known vector, appeared on sticky traps in mid-May (about the time the carrots were germinating), and alatae colonised most plants during the first week in June when the sticky trap count was 140 per ft^2 and increased to 400 in the following week. Aphids did not increase on the plants because of heavy rain in June, and because some infested leaves were eaten by rabbits, but some aphids persisted through June and July.

Untreated plots became 50% infected with motley dwarf virus, and sprayed plots averaged 14%. The small yield on untreated plots was not wholly because of infection. Plant number ranged from 60,000 to 300,000/acre, as a result of poor seeding, rabbit damage and injury from herbicides. Partial regression coefficients on plant number and percentage virus infection were significant and correlated, indicating greater virus incidence on plots with fewer plants. Correcting yields for differences in plant number showed a loss of about $\frac{1}{2}$ ton/acre for each additional 10% of infection. This is less than indicated by the effect of treatments (e.g. 5 tons/acre from spraying), partly because the sprayed plots became infected later than the untreated and partly because eliminating plant number also eliminated some of the effect of infection. Possibly CMDV spreads more in plots with fewer plants, but this would be independent of treatment.

In experiments in 1964 and 1965, with 500,000–850,000 plants/acre, but otherwise comparable with this year's, plant number did not affect yield, but virus infection affected both yield and plant number. In 1964, when there was an average of 50% infection compared with 28% in 1965 and 1966, plant numbers averaged 680,000/acre on treated plots and only 500,000 on untreated. When plants were fewer than 500,000/acre yield was proportional to the square root of plant number. (Watson and Pullen)

Turnip and swede mild yellows host range. Turnip mild yellows virus closely resembles other viruses described causing mild yellows of brassicas, potatoes, sugar beet and *Physalis floridana* in continental Europe, Canada and California in its transmission by *Myzus persicae* and failure to be manually transmitted.

Turnip mild yellows has no common host with beet mild yellowing virus. It does not infect *Claytonia perfoliata* or sugar beet. It differs from Western mild yellows (U.S.A.), Malva yellows and flax yellow "disease" in failure to infect sugar beet, flax, radish, cucumber or lettuce. However, Western mild yellows is almost certainly a name embracing several viruses, because different ones are said to infect various of these hosts.

Turnip mild yellows resembles Western mild yellows, the Canadian

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turnip latent virus and Physalis yellow-net complex, and also potato leaf roll virus, in its ability to infect *Physalis floridana*. (Watson)

Virus transmission

"Artificial feeding"; transmission of non-persistent viruses. Aphids will probe through stretched "Parafilm M" membranes into solutions containing sucrose. When removed immediately after probing partially purified preparations and placed on host plants they can transmit some non-persistent viruses. Viruses so transmitted have short particles. Pirone (*Virology* (1964), **23**, 107) transmitted alfalfa mosaic by this method. We have repeated this, and also transmitted a strain of cucumber mosaic isolated from lettuces in the field (LCMV). Once only we also transmitted a yellow strain of cucumber mosaic virus (YCMV), isolated from Price's No. 6 strain, but this strain is also difficult to transmit by aphids from plants.

Viruses with long particles, such as henbane mosaic, potato virus Y and cabbage black ringspot, were all tested, but although these were all readily transmitted by aphids from plants, and the partially purified preparations used were at least as infective as those of LCMV, none was transmitted. Aphids fed on a concentrated preparation of red clover vein mosaic virus also failed to transmit. (Watson, Lack and Pullen)

Transmission of cucumber mosaic virus from plants infected with two strains. The yellow strain of cucumber mosaic (YCMV) was not transmitted more readily by aphids from *Nicotiana glutinosa* or tobacco plants simultaneously infected with the lettuce strain (LCMV) than from plants infected with it alone. When saps from separately infected *N. glutinosa* plants were mixed and inoculated in proportions of 1 : 1, 10 : 1 or 40 : 1 YCMV to LCMV, the first symptoms to appear were those caused by LCMV, which could be transmitted by aphids, although less readily than from plants infected with LCMV alone. Later, when symptoms caused by YCMV developed, neither virus was usually transmissible. YCMV did not usually infect plants in which LCMV was already established, but seemed to compete successfully when both strains infected simultaneously. (Watson)

Frequency of aphid flight from virus-infected plants. Experiments were done to find whether flight-mature alate aphids from virus-infected plants flew at different air temperatures from those on uninfected plants. Some *Aphis fabae* Scop. colonies were grown on virus-infected broad beans (*Vicia faba* L.) and sugar beet (*Beta vulgaris* L.), and others on uninfected plants. Alate aphids were collected from these colonies up to 1–2 hours after their final moult, and put for their teneral period on virus-infected or healthy plants for 18 hours in the dark at about 20° C. The plants were then put into brightly lit cabinets in a glasshouse at about 10° C, and the temperature increased at about 3–4° C/h to about 20° C. The aphids that flew from the plants at different temperatures were counted. *A. fabae* flew from broad beans infected with pea enation mosaic or pea mosaic

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viruses at the same rate as they left comparable healthy plants, and rearing the aphids on virus-infected plants also had no effect. (Mrs. Prabhati Varma and Gibbs)

Transmission of strawberry latent ringspot virus (SLRV). The ability of the nematode *Xiphinema diversicaudatum* to transmit SLRV was confirmed, using specimens from a glasshouse where roses were infected. The nematodes transmitted SLRV to cucumber but not to pea, which is readily infected by arabis mosaic virus transmitted by *X. diversicaudatum*. SLRV was transmitted by adult male, adult female and juvenile *X. diversicaudatum*, and by virus-carrying adults kept without plants for 32 days; it was also transmitted up to three times by nematodes transferred to fresh plants every 2–3 days, though not necessarily to consecutive plants. (Harrison)

Arabis mosaic virus and *Xiphinema diversicaudatum*. At two sites in south-west England, where arabis mosaic and its vector *X. diversicaudatum* were common, the virus was isolated only from plants or seeds of *Trifolium repens*, *Bellis perennis* and *Sagina procumbens*, but not from 11 other dicotyledonous species or from grasses. The virus was not isolated from pollen collected in the same neighbourhood by bees. In a field experiment at one site the nematicide "D-D" (400 lb/acre) controlled the nematode, and hence checked the spread of the virus, but after one year nematodes surviving in the rocky subsoil started to reinfest the treated soil.

All nine varieties of *T. repens* grown in the experiment became infected with arabis mosaic virus. The population of *X. diversicaudatum* seemed unaffected by *T. repens* growing in the soil, but the population was smaller on plots also sown with *Phleum pratense*. In pots in the glasshouse, with soil containing 750 *X. diversicaudatum*/litre, the nematode population was unaltered after one year in pots sown with either wild white clover infected with arabis mosaic virus or *Lolium perenne*; the population decreased to one-fifth in pots sown with *Phleum pratense* or kept fallow, and it increased three-fold in pots sown with S. 100 *T. repens*. (Gibbs)

Transmission of satellite virus by *Olpidium brassicae*. Transmission of tobacco necrosis virus (TNV) by zoospores of the fungus *Olpidium brassicae* depends on having the right combination of TNV strain, species of test plant and isolate of the fungus (see *Rothamsted Report* for 1964, p. 132). Three strains of satellite virus (SV_1, SV_2, SV_3) have now given somewhat similar results. In the roots of naturally infected plants satellite virus occurs only together with TNV, whose help it needs to multiply. When these three satellite viruses were tested in tobacco in combinations with strains A and B of TNV and the three isolates of *O. brassicae* previously reported only SV_3 was transmitted. However, tobacco plants grown in unsterilised loam were recently found infected with SV_2 and a new isolate of *O. brassicae* obtained that transmits SV_2.

Plants used in the transmission experiments were raised in sand and soon transferred to water culture. Roots of these plants (usually with three expanded leaves) were then exposed for 12 hours to a suspension of

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zoospores containing appropriate amounts of TNV and SV. The roots were then washed and grown on in water culture for 3 weeks by which time virus in infected roots had reached amounts that could be assayed serologically and electron-microscopically. The type of virus can be identified serologically, using the individual antisera diluted so that they react only with homologous virus. (Kassanis and Macfarlane)

Fungus diseases of cereals

Take-all and the yields of successive barley crops. The mean grain yields and the incidence of take-all (*Ophiobolus graminis*) in May and early June in successive crops of Proctor barley after an oats–beans break in the 2 years 1965–66 in Little Knott field were:

	Nitrogen (cwt/acre)			
	0	0·3	0·6	0·9
Grain yield (cwt/acre)				
1st crop after oats–beans	37·3	46·0	43·8	42·9
2nd crop after oats–beans	31·0	37·2	43·4	44·2
3rd crop after oats–beans	20·9	32·6	36·8	41·4
% plants with take-all (May)				
1st crop after oats–beans	2	2	2	2
2nd crop after oats–beans	12	12	8	6
3rd crop after oats–beans	28	24	26	18
% plants with moderate or severe take-all (July)				
1st crop after oats–beans	5	0	0	0
2nd crop after oats–beans	27	20	12	8
3rd crop after oats–beans	52	32	25	14

These results illustrate some of the difficulties of measuring the effect of take-all on yield and of interpreting the response of barley to nitrogen. Beans are generally thought to enrich the soil with nitrogen. If this is so, a 2nd barley crop after beans should need more fertiliser nitrogen than a 1st crop. In our experiment the 2nd barley crop needed 0·3 cwt nitrogen/acre more than the 1st. However, the 3rd barley also needed 0·3 cwt nitrogen/acre more than the 2nd, which suggests that either the beans left the equivalent of 0·6 nitrogen/acre available for the 1st barley and the equivalent of 0·3 cwt/acre for the 2nd or that the 2nd barley crop depleted the soil of nitrogen as much as the beans enriched it. To ascribe the differences in yield between the 1st, 2nd and 3rd crops after beans entirely to differences in the amount of nitrogen available in the soil means ignoring the effect of previous crops on the incidence of take-all. Take-all was unimportant in the 1st barley after oats–beans, but was twice as prevalent in the 3rd barley crop as in the 2nd. A regression of plot yields on the incidence of moderate plus severe take-all in July in all barley crops grown after barley in this experiment from 1962 to 1965 indicates that each 1% of plants with take-all causes a loss of 0·9% grain yield. Estimates derived from correlations must be accepted with caution, but using this estimate to allow for the effect of take-all in the years 1965–66 the predicted yields in the absence of take-all are:

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	Nitrogen (cwt/acre)			
	0	0·3	0·6	0·9
Grain yields (cwt/acre) adjusted for loss caused by take-all				
1st crop after oats–beans	39	46	44	43
2nd crop after oats–beans	41	46	49	48
3rd crop after oats–beans	40	46	48	48

Thus the differences in yield between the 1st, 2nd and 3rd crops can be explained by the difference in the incidence of take-all. (The predicted yields for the 2nd and 3rd crops given 0·6 and 0·9 N/acre are potential yields; the 1st crops after beans did not yield this potential because they lodged.) However, the presence of take-all was partly determined by fertiliser nitrogen; if nitrogen from beans acted similarly it may have caused the differences in incidence of take-all between the 1st, 2nd and 3rd crops after oats–beans and thus, indirectly, the differences in yield. Our information is too little to show which interpretation is correct. (Slope and Etheridge)

Continuous wheat growing and the decline of take-all. The decline of take-all experiment in Great Field I ended this year. The soil was too wet to sow the wheat until 3 January; soil conditions at sowing were very bad, and crop establishment, growth and yield were poor. The mean yield from all treatments was only 29·5 cwt/acre. As in previous years, take-all was more prevalent in the 2nd and 3rd successive wheat crops after oats than in the continuous wheat (8th successive crop), but yields did not differ significantly. The mean grain yields and incidence of take-all in continuous wheat (5th, 6th, 7th and 8th successive crops) and in the 2nd and 3rd crops after a crop not susceptible to take-all were, in the 4 years 1963–66:

	Continuous wheat	2nd and 3rd crops
Grain yield (cwt/acre)	32·2	29·6
% straws with take-all (July)	64	75

Although the differences are small, they are important because the smaller yields of the 2nd and 3rd wheat crops cancel out much of the benefit gained in the 1st after one not susceptible to take-all. In this experiment spring oats were grown as nonsusceptible crop. As expected, take-all was not prevalent in the 1st wheat crop after oats (on average 13% straws were infected in the years 1964–66), but the mean yield was only 3 cwt/acre (9%) more than the continuous wheat (65% infected). We cannot explain the small yield of the wheat after oats. In previous experiments on Great Field and on several other fields at Rothamsted, Cappelle wheat has consistently yielded 50 cwt/acre or more when grown after crops other than cereals and given amounts of fertiliser similar to those used in the take-all decline experiment. Moreover, a regression of plot yield on the percentage straws with take-all in Cappelle wheat *grown after wheat or barley* in experiments on Great Field (including the take-all decline experiment) during 9 successive years indicates that each 1% take-all decreased yield by 0·6%. From this estimate the expected yield of wheat after oats in the take-all decline experiment would be 30% more than the yield of continuous wheat. Either our estimate of the loss caused by take-all is invalid or some other, unrecognised, factor limited the yield of wheat after oats. (Etheridge and Slope)

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Eyespot and take-all on Broadbalk. Routine sampling on plot 7 showed that the 1st, 2nd, 3rd, 4th, 8th and 15th consecutive wheat crops after fallow had respectively, 8, 38, 59, 46, 32 and 36% straws with eyespot, of which 1, 12, 26, 17, 12 and 12% had severe lesions.

Take-all was, as in 1965, more severe than usual on all sections, including VA (3rd after fallow), which is not sprayed with herbicide.

	Number of successive crops after fallow					
Take-all:	1	2	3	4	8	15
% plants infected—						
May	4	10	58	20	30	32
July	8	32	86	64	57	66
% plants severely infected, July	2	19	65	40	32	28

(Etheridge)

Soil fumigants for cereals

Formalin fumigation for spring wheat. At Woburn formalin applied as a drench to soil during winter controlled take-all in the subsequent spring-sown wheat crop (*Rothamsted Report* for 1965, p. 127). At Rothamsted in 1966 the cumulative and residual effects of formalin were compared on two adjacent fields with contrasting cropping history:

Site	Formalin applied		Take-all in July (% straws)		Eelworm damage (% straws)	Grain yield (cwt/acre)
	1965	1966	1965	1966	June 1966	1966
Little Knott	—	—	92	71	19	29·4
	—	F	95	9	1	37·0
	F	—	34	92	27	24·0
	F	F	32	28	5	35·9
Pastures	—	—}	2	{56	0	35·7
	—	F}		{22	0	36·8
	F	—}	2	{51	0	35·7
	F	F}		{22	0	37·6

On Little Knott, where cereals have been grown frequently for more than 20 years, take-all was more severe and yields smaller in 1966 on soil treated with formalin in 1965 than on untreated soil, despite a large decrease in take-all in 1965. On Pastures, formalin applied in 1965 before a crop almost free from take-all had no effect on the development of disease in the 1966 crop. On both these fields root damage by cereal cyst nematode was less severe than on Butt Close at Woburn: on Little Knott, but not on Pastures, it was affected by formalin similarly to take-all. Experiments with different fumigants should show whether the rapid increase of pathogens in the second year after fumigation is a general phenomenon or can be delayed by materials with greater biocidal properties than formalin (see Report of Nematology Department, p. 143).

The incidence of severe take-all in both fields decreased with increasing amounts of nitrogenous fertiliser applied:

Nitrogen (cwt/acre)	% straws with severe take-all: Little Knott —	Little Knott Formalin	Pastures —	Pastures Formalin
0	67	18	48	12
0·5	58	8	36	19
1·0	36	2	27	20
1·5	44	8	18	3

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Details of grain yields are given in the Report of the Chemistry Department (p. 59), which is collaborating in the experiment. Formalin increased yields most where root diseases were most prevalent. Without formalin grain yields on Little Knott were substantially less than on Pastures with all dressings of nitrogen, whereas with formalin and nitrogen similar maximum yields of 40 cwt/acre were obtained on both fields. (Salt)

Dazomet and "D-D" fumigation for spring wheat. The effects of fumigants on disease incidence and yield of April-sown Kloka spring wheat were tested at Rothamsted (Hoosfield) and Woburn (Lansome), in collaboration with the Nematology Department.

Dazomet, rotavated in at 100, 200 and 400 lb/acre, decreased the incidence of take-all in June and July samples by up to 32% on Hoosfield and by up to 17% on Lansome, where the disease was less prevalent. This was associated with increases in mean yield of up to 1·9 cwt/acre and 7·4 cwt/acre respectively. On Hoosfield, where nematode populations were very small, injection of "D-D" at 200, 400 and 800 lb/acre was associated with an increase of take-all in July and a progressive decrease in yield. On Lansome, where nematodes were more numerous, there was a similar increase in take-all, but mean yields on "D-D"-treated plots were increased by up to 5 cwt/acre. Incidence of eyespot was generally small, and showed an irregular pattern of association with the treatments.

The effects of the fumigants on the soil microflora were assessed by a direct plating method, using the Andersen air sampler, developed by Buxton & Kendrick (*Ann. appl. Biol.* (1963), **51**, 215) to assess *Pythium* and *Fusarium* in soils. The method was modified, principally by decreasing the weight of soil used. Bacteria were counted by the soil-dilution plate method.

In the 1-in. and 5-in. horizons of untreated plots fungal populations were greatest during November, with $1{\cdot}9 \times 10^5$ and $1{\cdot}5 \times 10^5$ propagules/g respectively. The December and February samplings showed a progressive decline to the smallest count of $5{\cdot}8 \times 10^4$ and $6{\cdot}8 \times 10^4$ propagules/g in March. After sowing in April, the counts were higher in May and August. Bacterial populations at the 1-in. and 5-in. horizons fluctuated irregularly from $5{\cdot}7 \times 10^6$ to $7{\cdot}1 \times 10^6$/g, and from $3{\cdot}3 \times 10^6$ to $8{\cdot}8 \times 10^6$/g respectively. Three weeks after applying dazomet at 400 lb/acre in March, the numbers of fungi and bacteria in the 1-in. horizon drastically decreased. Fungal populations recovered only slowly, whereas bacteria soon increased to numbers greater than in untreated plots. A similar but smaller effect on fungi and bacteria was observed at the 5-in. horizon in plots given 800 lb/acre of "D-D" in November. The qualitative effects of treatments on the soil microflora were small. Of 1,248 isolations from all plots sampled, the fungi isolated most frequently were: *Trichoderma* spp. (13·2%), *Penicillium* spp. (10·6%), sterile forms (9·1%), *Phoma* spp. (8·1%) and *Microdiplodia* sp. (7·5%). (Ebbels)

Population of *Fusarium* in Rothamsted soils

During May, June and July soil samples were collected from plots on Broadbalk, Hoosfield and Barnfield, and from Broadbalk Wilderness, and

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their *Fusarium* content estimated. Weighed samples of these soils were plated directly on the Nash medium, selective for *Fusarium*, and the resulting colonies counted and identified. Parallel isolations were made from diseased wheat and barley plants where present in the plots.

The total counts of propagules of the *Fusarium* species ranged from 1,000 to 5,000/g of soil. In Broadbalk plots, continuously sown to wheat, one of these, *F. roseum* "Culmorum", had reached populations between 2,000 and 3,000/g. The same fungus was recovered from brown lesions on culms of wheat growing in these plots. The most "Culmorum" occurred where complete fertilisation, including much nitrogen, had been applied, and the fewest where fertiliser had not been used. Similar but less-striking differences were obtained in barley plantings on Hoosfield. On Barnfield, where only broad-leaved crops have been grown for more than 100 years, and in Broadbalk Wilderness, "Culmorum" was very rare.

Although *F. nivale* and its perithecial stage, *Calonectria nivalis*, was commonly isolated from *Fusarium*-infected wheat and barley plants at Rothamsted, in addition to *F. roseum* "Culmorum", the snow mould *Fusarium* was not recovered from the soil. This agrees with the fact that *F. nivale* does not normally form chlamydospores, whereas "Culmorum" produces them abundantly and therefore is ideally soil-borne.

In addition to these two *Fusarium* pathogens of cereals, *F. tricinctum* and *F. roseum* "Avenaceum" were also isolated from infected plants, and very rarely from the soil also, *F. roseum* "Graminearum" was not encountered in these tests either on cereals or in the soil. (Nash and Snyder)

Fungus diseases of potatoes

Potato blight phenology, forecasting and control. In the series of experiments begun in 1952 and ended in 1966 much has been learnt about the origin and early development of blight attacks, the effect of defoliation on yield, the occurrence of tuber infection and the weather with which it is associated. In later years the susceptible variety King Edward was used to test whether a routine first spray before the issue of a disease forecast was beneficial, and to find the optimum stage of the attack at which to destroy the haulm to prevent infection of tubers. Precautionary sprays delayed the outbreaks and early development of blight, but the differences did not persist or significantly increase yield. In rapid epidemics, with frequent heavy rain, the yield of healthy ware was increased by burning-off the haulm when less than 5% of it was blighted. However, in drier years fewer tubers were infected, and differences in yield of uninfected tubers were negligible or slightly less when haulm was destroyed early.

Potato blight remains an important disease, and it seems that the greatest improvements now to be made are to improve fungicides and their application (see report of Insecticides Department), and to destroy more of the overwintering sources of *Phytophthora infestans* in seed tubers, cull piles and clamp sites—mainly an advisory problem. (Hirst, Stedman and Hide)

Blight infection of potato tubers. In the final trial of the series designed to analyse some of the processes of tuber infection, started in 1960, the two

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tuber susceptible varieties Up-to-Date (UD) and King Edward (KE), and the tuber-resistant Majestic (MJ) were used as before, but the tuber-resistant Arran Viking (AV) was replaced by Pentland Dell (PD).

Phytophthora infestans was introduced on 6 July and released by removing inoculation sleeves on 11 July. As in 1965, wet weather favoured rapid development of the epidemic, and by 4 August most of the middle and lower canopy leaves of UD, KE and MJ showed multiple infections "peppering" leaflets, although few leaves were dead. By 14 August 50% UD and KE, and 35% MJ were defoliated by blight. Despite 2 weeks of dry weather from mid-August, foliage continued to be killed, and by early September about 90% were dead. Pentland Dell, a variety hypersensitive to Race 4, the race of the fungus that was introduced, remained unaffected and showed no lesions on foliage or tubers throughout the epidemic.

First tuber infections were found on 8 August on UD, and on 11 August on KE, but not until 15 August on MJ, by when 14% UD and 5% KE tubers had active rots. On 8 September 29% UD, 8% KE and 6% MJ tubers were affected.

There were two main periods during which tubers became infected, the first from 31 July to 9 August, when 1·65 in. of rain fell, and the second from 29 August to 4 September, when 2·06 in. fell. Soil samples taken daily, to measure moisture and to detect the fungus, showed that these were periods when soil moisture in the tuberising zone reached 20% or more (on a dry-weight basis), confirming the 1965 observations. These periods coincided with the occasions when *P. infestans* could be most readily detected on and in the soil. The frequency with which the fungus was recovered, both from surface soil and at 5 in. depth, decreased gradually during the dry period of August, but with the early September rains it increased again, though not to the previous amount. *P. infestans* was not detected at 5 in. depth (in the tuberising zone) after 13 September, or in surface soil after 15 September. (Lapwood)

Survey of fungal diseases of seed tubers. In collaboration with the Potato Marketing Board, samples of 50 tubers were examined: (R) soon after receipt on farms, and (P) at planting time after chitting. This year 150 stocks each of King Edward and Majestic, and 30 stocks each of Arran

TABLE 2

Survey of fungal diseases of seed tubers 1965–66

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

Examination	Disease	King Edward	Majestic	Pentland Dell	Arran Pilot
P	Skin spot (*Oospora pustulans*)	56/98	55/96	68/100	49/97
P	Gangrene (*Phoma* spp.)	10/76	5/60	10/68	6/62
P	Dry rot (*Fusarium caeruleum*)	1/17	3/47	1/32	8/69
R	Blight (*Phytophthora infestans*)	3/51	1/24	0/6*	1/22
R	Black scurf (*Rhizoctonia solani*)	17/92	18/92	27/97	26/100
R	Powdery scab (*Spongospora subterranea*)	10/77	12/86	9/65	15/81
R	Common scab (*Streptomyces scabies*)	16/88	27/93	13/87	17/94

* One blighted tuber found in each of two stocks (0·1% of total tubers). No attempt was made to identify the race or to confirm that the infected tubers were not rogues.

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Pilot and Pentland Dell were sampled. The table shows the percentage of tubers infected, and of stocks with some infected tubers. On King Edward skin spot was almost as prevalent as in 1962–63. More than half of the tubers that eventually showed gangrene already had lesions when they arrived—an abnormally large proportion. Powdery scab occurred in more than three-quarters of the stocks examined, a fact that acquired greater importance from the knowledge that this fungus can transmit the mop-top virus (see p. 115). Verticillate fungi were again isolated from a sub-sample of tubers, and confirmed the distribution of species reported by MacGarvie and Hide (*Pl. Path.* (1966), **5**, 72).

A further sample of 50 tubers from each stock was cooled, damaged and stored cold for a week before being placed on chitting trays alongside similar undamaged samples. This treatment only slightly increased dry rot and gangrene at planting time. Soil adhering to seed tubers of each stock was brushed off and inserted into jab wounds in tubers of a stock of King Edward tubers free from gangrene. After 8 weeks some test tubers showed lesions caused by *Phoma* spp., *Fusarium* spp. or both pathogens; the proportion of test tubers infected by soils was correlated with the incidence of gangrene in the corresponding seed-tuber samples in May. (Hide and Griffith)

Field experiments on the effects of fungal diseases of seed tubers

Skin spot. Yields from plots planted with clean (C), moderately (M) or severely (S) diseased seed tubers were again compared. In the first of two experiments on skin spot (*Oospora pustulans*) tubers were selected according to the area of skin spotted. Plots planted with S tubers of King Edward yielded 29% less than those planted with C tubers and of Majestic 6% less. Plots planted with S tubers had fewer plants and stems/plant, and large tubers accounted for more of the yield than with C. Infection of the buds of progeny tubers increased with the severity of skin spotting on the seed; averaging the two varieties, progeny from C, M and S had, respectively, 35, 52 and 65% of buds infected. A second experiment was made with a King Edward stock in which skin spots were almost restricted to the eyes; grades were selected according to the number of live eyes/tuber in March, as follows: C = >3, M = 1 or 2, and S = 0. Eventually some S tubers produced enough secondary buds to give a 58% stand of plants and 53% of the yield of C plots; M plots gave 98% of the plants and 95% of the yield of C plots. (Hirst, Hide, Griffith and Stedman)

Black scurf. King Edward and Majestic tubers, approximately one-tenth covered by *Rhizoctonia* sclerotia (S), yielded respectively 8% and 20% less than C tubers. Plant stands were similar, but increased prevalence of *Rhizoctonia* affected stem number and tuber size, as did increased skin spot. Infections of growing plants by the *Corticium* stage, and of progeny tubers by sclerotia, were increased more in King Edward than in Majestic by increasing the amount on seed tubers, but the progeny of M tubers (only about 2% sclerotial cover) with both varieties had nearly as much infection as progeny of S tubers (10%). (Hirst, Hide, Griffith and Stedman)

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Gangrene. King Edward and Majestic tubers with about half their area (S) occupied by gangrene lesions produced crops that yielded, respectively, only 7% and 13% less than those from C tubers. Gangrene affected plant growth oppositely from skin spot and black scurf, because it increased stem number and decreased mean tuber size. (Hirst, Hide, Griffith and Stedman)

Common scab. Tubers of the variety Majestic were separated into: "severe", "moderate" and "slight" scab, and "clean" tubers; some "clean" tubers were also treated with formalin. Half of each lot was chitted before planting in randomised blocks (with plots split for chitted and unchitted seed) on two fields, Highfield and Fosters, to test the relative importance of seed tubers or soil as a source of infection. At harvest the mean percentage surface area of progeny tubers affected about 1% on Highfield, and less on Fosters for all treatments. (Lapwood)

Attempts to produce pathogen-free stocks. Potato plants free from *Oospora pustulans* and other tuber-borne fungi can be produced by rooting stem cuttings using mist propagation. Such plants are valuable for experimental work in assaying the infectivity of soils and for measuring the losses caused by infection by individual fungi. If they can be retained free they could also be useful in commercial seed production (*Rothamsted Report* for 1962, p. 120). They have now been grown in two seed-producing areas in Scotland and one in Northern Ireland. After the first year in field soil samples were examined microscopically and only one infected eye was found on 280 tubers examined, but after the second year the stocks at two centres had considerable numbers of tubers infected with *O. pustulans*. We do not know the source of reinfection, or whether additional precautions would have prevented it. However, tubers on one farm in Scotland have remained free from *O. pustulans*, and these stocks are being multiplied. All stocks were recolonised to various extents by *Streptomyces scabies*, *Spongospora subterranea*, *Helminthosporium atrovirens* and *Rhizoctonia solani*. (Hirst and Hide)

Potato coiled sprout. In early June it was noticed that plants from unchitted Majestic seed tubers planted in an experiment at Rothamsted had only 0·3% of stems coiled, whereas chitted tubers of the same stock had more than 25% coiled. The remaining chitted and unchitted seed were then planted at four different depths (1, 4, 8 and 12 in.) and half of the plots were rolled to compact the soil. When sprouts began to emerge the plants were examined for the incidence of coiled sprout. It was increased by chitting, by deep planting and by compacting the soil after planting with a ring roller. Few plants from unchitted seed produced coiled sprouts, and these only in rolled soil. Coils were most abundant with chitted seed planted in rolled soil. The incidence of coiling and fasciation of stem bases was increased more by rolling than by chitting. Chitted seed planted 1 in. deep produced very few coils, even in rolled soil. Not all coils showed lesions, but *Verticillium nubilum* Pethybr. was frequently isolated from lesions. (Lapwood, Hide and Hirst)

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Effect of irrigation on incidence of common scab. The 1966 trial at Rothamsted compared four treatments replicated in four blocks: (1) rainfall only (DRY); (2) rain supplemented by overhead irrigation to bring soil at 4 in. depth to field capacity, but only when soil moisture tension reached 50 cm mercury on porous pot tensiometers ("50"); (3) irrigation to field capacity when soil moisture tension reached 30 cm ("30"); (4) irrigation to maintain soil at field capacity ("10"). The plots were split for three seed treatments: (1) chitted for a few weeks (CHE); (2) not chitted before planting (—E); and (3) planting unchitted seed 4 weeks late (—L) intended to delay the date when tubers formed and developed.

On 25 July, 10·0, 5·0, 5·0 and 0·1% surface area of tubers was affected by scab in treatments DRY, "50", "30" and "10" respectively for CHE plots, whereas on 1 August 4·0, 3·5, 1·0 and 0·1% were affected on —E plots. Failure to obtain better scab control with the "30" CHE treatment was attributed to an underestimate of moisture stress because soil tensiometers were placed only in —L plots, where the crop was much less advanced.

From the irrigation frequency, scab incidence and lesion distribution on tubers, the main infection periods were judged to have occurred during dry weather from 1 to 19 July and 19 to 29 August. Irrigation was stopped on 25 July, and at final harvest on 15 September —L tubers showed 3·0, 1·5, 2·0 and 0·3% scab for treatments DRY, "50", "30" and "10" respectively. Tubers from treatment "10", plots previously kept at field capacity, now showed infections confined to the rose end of tubers, which corresponded to the August infection period.

In another experiment to test at what stage of development Majestic tubers are most susceptible to scab infection, the above three seed treatments CHE, —E and —L were also set up as randomised block experiments on: (1) heavy clay-with-flints, scab-infested, land (Highfield) at Rothamsted; (2) on a light sandy scab-infested soil (Schoolfield) at Woburn. Tensiometers measured soil moisture. The main dry periods, and presumably the main scab-infection periods, were similar at both sites (early to mid-July and late August). Plots were sampled regularly, scored for scab and lesion distribution described by dividing the length of the tuber into six regions from stolon attachment (heel) to apical end (rose).

At Woburn CHE plots, planted 11 May, had formed all their tubers by late June (i.e. before the July infection period), and scab infection was confined to the middle (body) and rose end. Tubers were just forming in the —E plots in late June and early July and scab lesions covered the whole tuber surface from the "heel" end. The —L plots began to form tubers in late July, and some were infected during the July, some during the August and some during both infection periods. Infection during both periods was shown by some tubers that had a few lesions at the heel end, the body clean and an infected rose end. At Rothamsted there was much less scab than at Woburn, but the lesions were distributed similarly on the tubers. These results confirm observations in 1965 that the larger the tuber at the time conditions favour infection, the greater the area of tissue at the heel end free from lesions. (Lapwood)

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Effect of nitrogen on incidence of scab. Observations in 1964 indicated that increasing the amount of nitrogen fertiliser increased the incidence of scab on King Edward tubers (*Rothamsted Report* for 1964, p. 137). Three nitrogen treatments, NIL, 1 cwt and 2 cwt N/acre as "Nitro-Chalk", were tested in a small field trial with three varieties that differ in scab resistance (Pentland Dell, resistant; King Edward, moderately susceptible; and Majestic, susceptible). Varietal plots were split for N treatment in four randomised blocks. At harvest Pentland Dell showed 0·4, 0·7 and 0·7%, King Edward 0·7, 1·4 and 2·7%, and Majestic 2·7, 3·8 and 3·1% surface area affected by scab lesions for increasing N respectively. Although King Edward again showed a trend with N similar to that in 1964, the other two varieties did not. (Lapwood)

Lower fungi parasitic in roots

Plasmodiophorales. The organism that causes powdery scab of potatoes, *Spongospora subterranea*, has been maintained now for more than a year in the zoosporangial stage in tomato roots growing in sand irrigated with nutrient solution. Zoospores were transferred to new host plants every 2 weeks. This amply confirms observations in Holland that the zoospores from zoosporangia gave rise to further zoosporangia. Zoospores encysted on root hairs and passed into the host cell, leaving the empty envelope outside, much as do zoospores of *Polymyxa betae*. Yields of *S. subterranea* zoospores vary more than those of *Olpidium* and are usually much smaller, but as many as 3×10^7 zoospores have been obtained from 2 g (fresh weight) of roots. Zoospore suspensions may be valuable as inoculum in the study of some aspects of powdery scab.

A *Polymyxa* in sugar beet, provisionally identified with *P. betae* Keskin, was reported last year. A similar fungus, probably *P. graminis* Ledingham, was found in 1966 in roots of barley and wheat grown in soil from Bagthorpe, Norfolk. Zoosporangia were found both in surface cells of the roots and in the inner cortex, as originally described by Ledingham. A *Polymyxa* was also found in roots of diseased Italian ryegrass from an infested site in Suffolk. These fungi have not previously been recorded in Britain.

Chytridiales. Another new record is of a fungus closely similar to *Rhizophydium graminis* Ledingham, which was found in the roots of barley grown in sand containing roots of diseased cereals from Saxmundham. It parasitises root hairs and other surface cells, forming zoosporangia and resting sporangia on the outside and sending a fine mycelium within. A similar fungus was seen on sugar beet and *Chenopodium* spp. grown in soil from Barney, Norfolk, and on tobacco grown in Kettering loam. Tobacco plants were also infected by zoospores of the original isolate on barley. These Rhizophydia seem to be very common.

Lagenidiales. A fungus, provisionally identified as *Lagena radicicola* Vanterpool and Ledingham, was first observed in roots of barley grown in Bagthorpe soil, but has since been found in wheat roots from Rothamsted farm. Our fungus resembles more the form described by Truscott

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(*Mycologia* (1933), **25**, 263) than that originally found by Vanterpool and Ledingham when studying "browning root rot" of wheat in Canada. Infected plants growing in sand have many fewer fine roots than uninfected plants. It is likely that this fungus, although not previously reported in Britain, is widespread, and is probably important in cereal-root disease. (Macfarlane)

Diseases of Sitka spruce seedlings in forest nurseries

Soil partial sterilants last applied at Ringwood in December 1962 have had remarkably persistent effects in improving survival and growth of Sitka spruce sown in 1963, 1964 and 1965. The effects are now wearing off; in 1966 there was no residual effect on seedling survival, and only metham-sodium increased growth, and this by a mean of only 8%. Improved growth was associated with control of the ectoparasitic nematode, *Hoplolaimus uniformis*, and to its very slow population increase in treated soils (precautions were taken to minimise contamination of treated soil by cultivating each yard square plot individually).

By contrast, at Old Kennington nursery, where *H. uniformis* is rare, growth responses after sterilants were confined to the first crop after treatment, and then they seemed to be associated with nutritional factors, especially soil acidity and the supply of nitrogen as ammonium rather than nitrate. For example, where nitrogen was supplied as "Nitro-Chalk" seedlings grew to 1·38 and 2·25 in. in untreated and formalin-treated soils respectively, whereas with ammonium sulphate they grew to 2·39 and 3·14 in. Annual dressings of ammonium sulphate since 1962 acidified the soil to pH 4·2 (in 0·01*M*-$CaCl_2$), which is near the optimum for growth of Sitka spruce, whereas in plots supplied with "Nitro-Chalk" the pH was 5·2.

Inoculation experiments. The nematode, *H. uniformis*, and three fungi commonly isolated from roots were tested singly and together for their effects on growth of seedlings in pots containing Ringwood soil that had been sterilised in steam–air mixtures. The soil came from part of the nursery that had been bare fallow for at least 6 years and contained very few parasitic nematodes. Steaming for 30 minutes at 125°, 155° and 210° F decreased growth from a mean of 4·8 in. in unsteamed soil to 4·3, 3·4 and 3·8 in. respectively, despite a lapse of about 8 weeks between steaming and sowing. Adding 50 *H. uniformis*/500 g soil after seedlings had become established shortened plants from a mean of 4·5 to 3·7 in. The nematode retarded growth by 25% in soils steamed at 155° and 210° F, and by 11% in unsteamed soil and soil steamed at 125° F. Sterilised sugar-beet seed was inoculated with *Pythium ultimum*, *P. irregulare*, *Cylindrocarpon* spp. and *Fusarium oxysporum* and mixed with soil in which Sitka seed was then sown. This had no effect on the growth of the Sitka seedlings whether or not the soil also contained nematodes. However, *Pythium* killed many seedlings, especially in soil steamed at 125° F, where the natural population of *Pythium* survived.

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The "psychrophilic seed fungus". This fungus endophyte in Sitka seed was mentioned in last year's Report (p. 134) and in the *Report on Forest Research* for 1966 (H.M.S.O.) Provisionally it was called the "psychrophilic seed fungus" because it was isolated more readily at below 10° C, when it was not over-run by faster-growing fungi, than at 20° C. However, in pure culture it grows well at 20° C, and so is not truly psychrophilic. Incubating damp seed contaminated with the fungus at 10° C for 32 days decreased germination at 20° C from 82 to 40%. During the 32 days the proportion of ungerminated seed that yielded the fungus increased from 5 to 50%. After dusting with 50% thiram the germination of similarly treated seed decreased only to 72%, and the fungus was recovered from 30% of the seed.

In another test moist seed from 15 different sources was incubated for 24 days at 10° C and its germination then tested at 20° C. Germination was not impaired in any of the 11 samples in which the fungus was not found, whereas it decreased from 46 to 9% in two samples in which 55% of the ungerminated seed were infected. Of the two other samples, one had 10% infected seed, and its germination was unaffected by incubation, and the other had 30%, but only few seeds were viable even before incubating at 10° C. When or how seed becomes infected is unknown, and the behaviour of the fungus in nursery seed-beds is obscure. It was isolated from seed buried for several months in the seed-bed, but not from seed sown the previous season. (Salt)

Airborne moulds and actinomycetes from grain in storage

Moist storage of barley in concrete staved silos. Further measurement of the spore content of air in silos over moist barley, and of the grain itself, gave results resembling those reported last year (*Rothamsted Report* for 1965, p. 135), except that there were smaller numbers of potential human and animal pathogens. In most silos the topmost grain was mouldy after the storage period before grain was used, but less was mouldy when covered with silage than under straw and polyethylene or butyl rubber sheet. During extraction of the grain spores were few and consisted mostly of *Penicillium* spp. and yeasts. They increased when grain was used slowly, and to prevent moulding about 3 in. of grain should be removed daily, particularly at the end of the season when the ambient temperature is increasing.

Small-scale storage. Grain of 20, 30 and 45% water content was stored in 45-gal drums provided with a rigid inner polyethylene liner and cased outside with glass fibre to decrease heat loss. Each drum held about 3 cwt grain, which was covered with 6 in. of chopped straw, and sealed as airtight as possible with a polyethylene or butyl rubber sheet.

There was little spontaneous heating during storage for 6 months, when the appearance of the grain resembled well-stored commercial grain at similar water contents. Grain stored at 20% water content was free-running, and showed slight yeast growth, which increased slightly with increasing depth. After storage at 30% water content there was a white

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yeast layer 3–4 in. deep over the surface of the grain, but the grain was still free running; few fungi were found below this layer. After storage at 45% water content grain at the top of the drums was compacted into a solid, yeast-rich layer, immediately below which were colonies of *Penicillium* sp. Grain below the mouldy layer was dark brown and acid. The absence of the yeasty crust from grain stored at 45% under the butyl rubber sheet was the only difference from grain stored under a polyethylene sheet (a thicker grade of polyethylene was used than is usual in commercial silos).

After opening the drums cold prevented spontaneous heating of the grain for about 2 weeks, but then the temperature of the drum containing grain of 30% water content increased to about 33° C, with abundant growth of *Absidia corymbifera* and *Streptomyces fradiae*. Heating of the 20% and 45% drums was negligible, and little moulding occurred. (Lacey)

Aerobiology

Improved volumetric spore trap. In collaboration with the Burkard Manufacturing Co. Ltd., Rickmansworth, Herts, a version of the suction spore trap was developed that can operate unattended for 7 days. The new trap was thoroughly tested in the field. A battery-powered pump is also being developed so the trap can be used in places remote from electricity supplies. (Hirst)

Seasonal occurrence of airborne *Ophiobolus graminis* ascospores. A ½-acre plot on Great Field I, infested with take-all, was sown with wheat in November 1964. The crop was harvested in the following September, and in November 1965, after disc-harrowing, wheat was drilled directly into the stubble to leave as much perithecial material as possible on the surface. From July 1965 a Hirst spore trap was operated continuously (except for short breaks for cultivations or during continuous frost) near the middle of the plot.

Each day's spore-catch was examined, and the number of *O. graminis* ascospores estimated on an area of the dust deposit corresponding approximately to ⅓ m³ of air. In 1965 the first ascospore appeared on 20 August and the last of the season on 29 December. In 1966, when the wheat matured somewhat earlier, the first was recorded on 6 August. In 1965 over 75% of the season's catch came between mid-September and late October, but in 1966, with less than 10% of the previous year's catch, there was no peak period.

	Aug.		Sept.		Oct.		Nov.		Dec.		Total	
	1965	1966	1965	1966	1965	1966	1965	1966	1965	1966	1965	1966
Rainfall (in.)	2·1	3·2	4·0	1·6	0·9	3·5	2·8	2·0	4·7	3·6	14·5	13·7
No. of rain days	13	12	19	9	13	22	19	22	23	24	87	89
No. of days spores seen	6	6	12	6	7	9	11	5	3	2	39	28
Total spores for month (in ⅓ m³ per day)	12	2	86	4	49	3	6	3	1	1	154	13

Young wheat seedlings with partly exposed roots, growing in plastic seed-trays, have been exposed in the field close to the trap weekly since

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December 1965 to test possible direct infection by ascospores from the air (see Brooks, *Trans. Br. mycol. Soc.* (1965), **48**, 237). Although in the laboratory many young roots became infected when ascospores fell on them from wetted stubble in a humid chamber, no infection has yet been observed in these field tests. (Gregory and Henden)

Effect of fungicide spraying on potato-blight gradients. Experimenters using small plots for fungicide trials commonly observe sharp boundaries to infected areas corresponding to boundaries between sprayed and unsprayed control plots. But published records demonstrate strong infection gradients when blight spreads from a focus over an unsprayed crop, and some interference between sprayed and unsprayed plots would therefore be expected. This apparent contradiction was investigated in a preliminary experiment on West Barnfield, isolated more than 200 yards from other potatoes.

An area of six rows of King Edward potatoes, 200 yards long, was left unsprayed to become naturally infected with *Phytophthora infestans*. On each side were two sprayed guard rows, then two sprayed rows followed by a 60-yard gap of fallow land and then two more sprayed-test test rows. The whole was surrounded by fallow land. Copper oxychloride ("Coppesan") was applied at 0·25% copper from a pneumatic knapsack sprayer at about 80 gal/acre on 15 and 24 June, 6, 15 and 27 July, and 5 August 1966. Blight was assessed on two dates as follows:

	15 August			25 August		
Arrangement of rows	% leaves infected	Lesions per 100 leaves Observed	Lesions per 100 leaves Expected	% shoots infected	Lesions per 100 shoots Observed	Lesions per 100 shoots Expected
Fallow to end of field	—	—	—	—	—	—
Sprayed test row	1	1	1	16	16	17·4
Sprayed test row	6	6	6	12	14	12·8
60 yards fallow	—	—	—	—	—	—
Sprayed test row	6	6	6	50*	66*	69·3
Sprayed test row	2	2	2	20	28	22·3
2 sprayed guard rows	—	—	—	—	—	—
Infector rows (unsprayed) 2 rows sampled	82	<473	197	—	—	—
	86	<565	172	—	—	—
2 sprayed guard rows	—	—	—	—	—	—
Sprayed test row	8	9	9·4	19	21	21·1
Sprayed test row	7	7	7·2	16	20	17·4
60 yards fallow	—	—	—	—	—	—
Sprayed test row	3	4	4	18	20	19·8
Sprayed test row	3	4	4	18	20	19·8
Fallow to end of field	—	—	—	—	—	—

* A discordantly large value, attributable to some factor operating in one half the row only (possibly rain soon after spraying).

(1) The unsprayed rows became infected naturally, and secondary lesions spread locally around the primary foci. This is clearly shown by the excess of observed lesions/100 leaves over the number expected from the percentage leaves infected, assuming random distribution of lesions (estimated from the multiple infection transformation).

(2) Spraying achieved its immediate object of protecting the foliage while blight was developing on the unsprayed strip. By abolishing sites for infection, spraying also abolished conditions for establishing an infection

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gradient, which requires that a spore concentration gradient can establish infections freely on susceptible tissue.

(3) During the 20 days after the last spray new susceptible sites evidently arose at random in the sprayed rows (by new growth of the plants, or by erosion of protective deposit). Some or perhaps all of these sites became infected, but whether by fungus from the local infectors or from more distant sources, or a mixture of both, is unknown.

(4) That these infections on the sprayed rows did *not* become centres of local spread is shown by the close agreement between the observed number of lesions/100 shoots and the number expected from the percentage leaves infected were the lesions randomly distributed in the row.

The randomly distributed new sites on the sprayed rows may not have arisen until the infector strip was producing saturating amounts of airborne inoculum, i.e. enough to infect all sites whether adjacent to the infector strip or 60 yards distant, and if this happened an infection gradient would be lacking, although the usual gradient of spore concentration would have existed.

Ignoring the aberrant row, there is evidence of only slightly fewer infections in the distant test rows than in the rows close to the infected area, but more experiments, necessarily costly in land, are needed to measure interference between plots. Because spraying destroys the conditions for setting up an infection gradient, the lack of such gradients in conventional small-plot experiments with epidemic foliage diseases cannot be interpreted as proving there is no interference between sprayed and unsprayed plots. (Gregory and Henden)

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