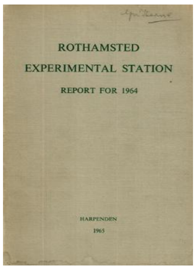
ROTHAMSTED RESEARCH

Report for 1964



Plant Pathology Department

P. H. Gregory

P. H. Gregory (1965) *Plant Pathology Department ;* Report For 1964, pp 126 - 142 **- DOI: https://doi.org/10.23637/ERADOC-1-57**

PLANT PATHOLOGY DEPARTMENT

P. H. GREGORY

The department suffered a grievous blow by the death of E. W. Buxton at the age of 38 years.

A. J. Gibbs spent three weeks as Visiting Professor at the Instituto Nacional de Tecnologia Agropecuria, Buenos Aires, and returned after visiting Moor Plantation, Ibadan. P. H. Gregory visited the Plant Research Institute, Ottawa, and afterwards took part in the Atmospheric Biology Conference, sponsored by the U.S. National Aeronautical and Space Administration, at the University of Minnesota, Minneapolis. He was a member of the U.K. delegation to the Seventh Commonwealth Conference of Plant Pathology, Kew. B. D. Harrison was seconded for six months to the Plant Pathology Department, Waite Agricultural Research Institute, Adelaide. Marion A. Watson took part in the Aphid Symposium at Berkeley, by invitation of the University of California.

Members of the department participated in the Tenth International Botanical Congress, Edinburgh; the Twelfth International Entomological Congress, London; and the Photobiology Congress, Oxford.

Mr. O. Khalifa was awarded the Ph.D. degree of London University.

Visiting workers included Mr. E. Debrot (Venezuela) and Mrs. Eva G. I. Poinar (Berkeley), Dr. Giselda Giussani (Milan), Mrs. B. Okusanya (Nigeria) and Mr. A. Varma (India).

Properties of Plant Viruses

Inactivation by radiation of different wavelengths. The action spectrum for inactivation of nucleic acid from both tobacco necrosis (TNV) and tobacco mosaic (TMV) viruses by ultra-violet radiation follows closely the shape of the absorption spectrum, but the nucleic acid from TNV is more resistant to inactivation.

When the viruses are irradiated intact the two show a striking difference. Intact TNV is as susceptible to inactivation as its free nucleic acid, and infectivity of irradiated preparations is equally restored by exposing inoculated plants to visible light. By contrast the nucleic acid in intact particles of TMV is so protected from radiation that, to be inactivated to the same extent as free nucleic acid, at wavelength 254 mμ, it must absorb about five times more radiation energy; also, it is completely protected from the kind of damage that is reversible by visible light. Consequently the action spectrum for inactivation of intact TNV is the same as that of free nucleic acid (i.e., it follows closely the shape of the absorption spectrum of the RNA), whereas the action spectrum for inactivation of intact TMV differs from the absorption spectrum of either its nucleic acid or protein. (Kleczkowski and Kassanis)

https://doi.org/10.23637/ERADOC-1-57

Mutual precipitation by two viruses. TMV and bromegrass mosaic virus (BMV) are isoelectric at pH values below 4·0 and about 8 respectively. There is thus a wide range of pH values at which particles of these two viruses are oppositely charged, and they precipitate each other quantitatively when equal amounts are mixed in salt-free solutions. Adding NaCl to a concentration of 0·4% or more immediately dissolves the precipitate, and electrophoretic evidence shows that the particles of the two viruses do not interact in these conditions.

Precipitating TMV in salt-free suspensions with BMV did not appreciably diminish its infectivity.

Satellite virus is isoelectric near pH 7, and so there is a considerable range of pH values at which its particles and those of TMV are oppositely charged. However, when salt-free solutions of the two were mixed in this range no precipitate formed and no interaction between them was observed in the ultracentrifuge. The small charge density of particles of the satellite virus probably explains the failure to interact. (Kleczkowski and Kassanis)

Effects of salt and pH on precipitation of antigen–antibody compounds. Further knowledge of the mechanism of precipitation of antigen–antibody compounds was sought, using as antigens tobacco mosaic virus (TMV) and human serum albumin (HSA). At pH 7 neither precipitated with its antiserum from salt-free solutions, but precipitates formed rapidly when salt was added. The precipitates formed in the presence of salt dissolved rapidly when suspended in distilled water. TMV combined with antibody to the same extent whether salt was present or not. Ultracentrifugation also showed that without salt TMV particles that have combined with antibody at the ratio of equivalence do not aggregate.

The compound formed at equivalence between HSA and its antibody contains 6 times more antibody than antigen and precipitates at pH 6·0 (which is near the isoelectric point of antibody) even without salt, but it dissolves when the pH is raised to 7·0.

All these facts argue against the widely accepted "lattice hypothesis" of precipitation and favour the idea that antigen–antibody compounds are hydrophobic and, as such, precipitate when sufficiently discharged by salt or by suitably adjusting the pH. (Kleczkowski)

Electron Microscopy of Plant Viruses

Limits of resolution. Fine structure of plant virus particles is best seen by direct electron microscopy in negatively stained preparations. The limit to resolution is set by the maximum difference in weight density that can be arranged between the biological material and the negative stain around it, which effectively limits resolution to about 2–3 mμ (about 3 times the limit of our electron microscope). Contrast in electron microscopy of negatively stained particles apparently comes from changes in density and thickness through the whole specimen, including the supporting film, and the layer of carbon that builds up on the surface of the specimen during microscopy. The best evidence for this is that it explains some images

https://doi.org/10.23637/ERADOC-1-57

difficult to explain in other ways. Fine structure can be seen easily in stacked disc structures of TMV protein, whereas detail in the helical structure of the intact virus is seen very imperfectly. The images given by aggregates of 12 satellite virus particles are difficult to explain in any other way (Kassanis and Nixon (1961) *J. gen. Microbiol.* **25**, 450–471), as also is the limited success obtained in resolving the subunits in all except one group of small polyhedral plant viruses. High-contrast pictures of subunit arrangement in these small particles will be possible only when images in the upper and lower halves of a particle coincide and reinforce each other. Suggested protein shells with more than 32 subunits are not symmetrical or pseudo-symmetrical about planes through their centres, so that coincidence is not to be expected.

A new generation of electron microscopes with resolutions approaching atomic dimensions may even increase the difficulties because improved resolution is obtained by operating the objective lenses at wider apertures, leading to lower image contrast at focus, and bigger phase contrast effects just off focus. Using lower voltages to get images of greater inherent contrast is not at present possible, as the images are not bright enough at the high magnifications needed, but in time equipment for image intensification, by what is essentially a closed-circuit television link between the microscope and an external viewing screen, may help to close the gap. (Nixon and Woods)

Cucumber mosaic viruses. The electron microscope and the analytical ultracentrifuge were used to examine isolates of cucumber mosaic virus, including chrysanthemum aspermy virus in purified preparations made at the Glasshouse Crops Research Institute. All require to be fixed with formaldehyde before mixing with phosphotungstate for negative staining, and then all preparations look alike, with polyhedral particles of very small contrast showing no internal structure. All have sedimentation rates in the range 97–102 S, not much affected by concentration, and spectrophotometry suggests a nucleic acid content of about 30%. (Nixon and Woods, with Mr. M. Hollings and Dr. O. M. Stone, Glasshouse Crops Research Institute, Rustington)

Other co-operative projects involving electron microscopy are in progress with: Central Coconut Research Station, India; Cocoa Research Institute, Ghana; East Malling Research Station; National Vegetable Research Station, Wellesbourne; and Rubber Research Institute of Ceylon. (Nixon and Woods)

Carrot motley dwarf in the field in 1964. Sticky traps at Woburn and Broom's Barn Experimental Stations caught the first alate *Cavariella aegopodiae* on 25 May, almost the same date as in previous years. The peak of spring invasion occurred in the last week of May, when 120 were trapped. Aphids were fewer at the end of June than at other times, but alatae became numerous again in the third week of July (the "summer peak"), when 28, 35 and 110 were trapped at different sites at Broom's Barn, and 803 at Woburn. The large catch at Woburn was associated with aphids multiplying there on carrots and producing winged forms, but the aphids

https://doi.org/10.23637/ERADOC-1-57

caught at Broom's Barn must have come from elsewhere. At Woburn in July, *C. aegopodiae* made up 75% of the total aphids trapped, and at Broom's Barn about 30%. (Watson and Heathcote)

Winged aphids were first found on carrots in many places during the last week in May, and colonies were established by the middle of June. The infestation became severe on carrots at Woburn, but not at Broom's Barn. On 27 July half the carrot plants on unsprayed plots at Woburn were virus-infected, and of plants tested about half were infected with red-leaf and mottle viruses (motley dwarf) and half with red-leaf virus alone. At this time less than 1% of plants at Broom's Barn showed symptoms, but about 25% did so later.

At Woburn spraying increased the yield of carrots by 27%. Plots sprayed 5 times with menazon yielded 19, and unsprayed plots 14 tons/acre. Loss of yield per infected plant was greater on unsprayed than on sprayed plots, judged from the infected plants recorded in August. This may have been because aphid feeding directly affected yield on some plots, or because infections occurred earlier in the unsprayed plots than in the sprayed plots.

At Broom's Barn fortnightly sprays with "Metasystox" did not significantly affect yield, although there was a 5% increase with six sprays. Menazon used as a seed dressing decreased yield by 17%, whereas at Woburn in 1963 it increased yield. The difference may be because, at Broom's Barn in 1964, pests and diseases were too rare for their control by menazon to compensate for the damage caused to the seedlings by the insecticide, which also may have been greater in 1963 because of the drier weather. (Watson, Dunning, Serjeant and Lack)

Groundnut viruses. Two samples of groundnuts (A and B) from Nigeria, thought to be infected with groundnut rosette virus, were grafted to healthy seedlings of two varieties. Both caused slight stunting, and light- and dark-green "oak-leaf" patterns on the leaves of both varieties, but neither caused yellowing or rosetting. *Aphis craccivora* from Nigeria, when fed for 48 hours on infected plants and then 3 days on healthy seedlings, transmitted the virus from sample A, but not from sample B. Infective aphids caused symptoms in crimson clover (*Trifolium incarnatum*), and the virus has not yet been recovered from diseased clover plants. Crimson clover has not hitherto been recorded as a host of groundnut rosette virus. Other legumes, including *Vicia faba*, *Phaseolus vulgaris*, *Pisum sativum* and *Trifolium* spp., did not become infected.

A. craccivora from Kenya, which had transmitted groundnut rosette there, did not transmit virus from either of the samples from Nigeria. Further tests are needed, but current results suggest that different viruses or virus strains are included under the name of groundnut rosette, and that *A. craccivora* from different regions may differ in their ability to transmit them. (B. Okusanya)

White clover virus survey. Random samples of 20–25 white clover plants were collected from each of 17 old permanent pastures in Great Britain, planted in pots at Rothamsted in insect-free glasshouses and tested for

https://doi.org/10.23637/ERADOC-1-57

viruses; symptoms were recorded, and sap was inoculated to tobacco, broad bean, French bean, pea and *Chenopodium amaranticolor*. The viruses isolated were identified by symptoms, electron microscopy and serological tests.

Viruses were obtained from 14% of the plants collected; only four of the fields failed to yield virus. Of the 14% infected plants, only those with red-leaf virus (1% of the total) showed clearly recognisable symptoms; clover phyllody virus was not found. Three other viruses causing very slight or no symptoms were identified. Arabis mosaic virus, the commonest, was in 5% of the plants collected and occurred in 5 pastures in north and south-west Britain. About one-half of the plants from south-west Britain with arabis mosaic virus also contained strawberry latent ringspot virus, which did not occur alone in any plant. Red clover vein mosaic virus (2% of the total) was found in one pasture in Scotland and one in Wales, and white clover mosaic virus (1% of the total) in two pastures in central England. Other viruses, not yet identified, were isolated from a further 5% of the plants. (Gibbs and Woods)

Virus Identification and Classification

Andean potato latent and dulcamara mottle viruses. Of two viruses, related to ononis yellow mosaic virus (*Rothamsted Report* for 1963, p. 102) and hitherto apparently undescribed, one was isolated from apparently normal plants of *Solanum tuberosum*, group *andigena*, *S. phureja* and *S. chaucha* in the Commonwealth Potato Collection collected from Colombia, Bolivia and Peru; this we call Andean potato latent virus (APLV). APLV was readily transmitted by inoculating sap to some solanaceous species and to *Chenopodium amaranticolor* and *C. quinoa*, and was most conveniently propagated in *Nicotiana glutinosa*, in which symptoms of vein clearing, flecking and mosaic are most evident in cool weather.

The other virus, which we call dulcamara mottle (DMV), was present in a quarter of about fifty plants of *Solanum dulcamara* growing on downland at Streatley, Beds.; it was not isolated from 230 *S. dulcamara* plants collected from 34 other places in southern England. Infected *S. dulcamara* showed a mild mottling and puckering of the leaves, especially in cool weather. DMV was readily transmitted by inoculating sap to other solanaceous species but not to *Chenopodium* species. Infected *N. glutinosa* showed severe chlorotic and necrotic vein clearing, with distortion of the leaves.

Sap from plants infected with either virus contained many polyhedral particles indistinguishable from those of ononis yellow mosaic virus, and in serological tests all three viruses reacted with each other's antisera; the precipitation end point of antiserum titrated against its homologous virus was 8–128 times greater than when titrated against a heterologous virus. Purified preparations of all three viruses contained components with sedimentation coefficients of about 115 S and 55 S, corresponding to the infective nucleoprotein particles and non-infective particles respectively.

Thus these three viruses share many properties with turnip yellow mosaic and cocoa yellow mosaic viruses (*Rothamsted Report* for 1961, p. 106; and for 1962, p. 113). APLV and DMV also resemble turnip yellow

https://doi.org/10.23637/ERADOC-1-57

mosaic, in that when plants infected with these viruses are sprayed with a solution of 2-thiouracil, concentration of the slowly-sedimenting particles rapidly increases and that of the fast-sedimenting intact particles decreases. This effect is also induced by 6-azauracil. (Gibbs, Poinar, Nixon and Woods, with Dr. R. K. McKee, John Innes Institute)

Red clover mottle, cowpea mosaic and squash mosaic viruses. These three viruses all have polyhedral particles 28 mμ in diameter, which are indistinguishable in the electron microscope, but quite distinct from particles of the turnip yellow mosaic and ononis yellow mosaic groups. Purified preparations of red clover mottle and cowpea mosaic viruses each contained three components with sedimentation coefficients of about 125 S, 100 S and 60 S, corresponding with intact, "half-empty" and "empty" particles respectively. These two viruses and squash mosaic virus are distantly serologically related, but no relationship was detected between them and cowpea yellow mosaic virus, turnip yellow mosaic, wild cucumber mosaic or cocoa yellow mosaic viruses. (Gibbs and Giussani)

Cereal and Grass Viruses

Barley yellow dwarf. Differences in susceptibility and tolerance between oat and barley varieties to barley yellow dwarf virus infection were examined by infecting plants growing in pots. Of eleven oat varieties (provided by the Plant Breeding Institute, Cambridge) infected with an isolate transmitted by *Sitobium fragariae* and virulent in Blenda, five did not have their yields of grain and straw significantly decreased. Albion was one of these and was further compared with Blenda, by infection with two other isolates, one transmitted by *Rhopalosiphum padi* and very virulent towards Blenda and the other transmitted by *S. fragariae* and less virulent towards Blenda. The tolerance of Albion was apparent with both isolates. With the more virulent isolate loss of grain was complete in Blenda and 48% in Albion. The less virulent isolate infected only half as many plants of Albion as Blenda; infected plants of Blenda lost 64% of grain yield, and of Albion less than 30%.

A resistant variety of barley (Cb.881, provided by the Welsh Plant Breeding Station) was compared with Proctor by infecting with an isolate transmitted by *R. padi*. Using 10 aphids per plant, only 28% of Cb.881 plants became infected in contrast to 90% of Proctor. Further, *R. padi* transmitted significantly less often from the infected Cb.881 than from the Proctor, in the ratio 61:100. *S. fragariae* also transmitted less often from Cb.881. Thus Cb.881 both resists infection more than Proctor and is a poorer source of virus for aphids. The virulence of the virus towards Blenda oats was not changed by passage through the resistant variety; nor was its transmissibility by different aphids.

The results show that Cb.881 has at least three valuable characters: (1) resistance to infection; (2) ability to tolerate infection; (3) ability to limit virus multiplication, for differences in virus concentration probably explains differences in ability of aphids to transmit from different varieties. (Serjeant)

https://doi.org/10.23637/ERADOC-1-57

Cocksfoot mottle virus. CFMV caused less damage on Rothamsted Farm in 1964, and fewer samples were received from the N.A.A.S. than in previous years. There was little visible evidence that virus had spread from infected plants in the 2nd-year cocksfoot on Long Hoos, and infection occurred only at two places on the edge of the headland. This year, as in 1963, plants exposed in pots became infected only after they and the crop in which they were placed had been cut; only 2 out of 36 plants so exposed became infected. Seedling cocksfoot plants in pots half buried in a 3rd-year field of cocksfoot for the period of cutting became infected with CFMV after cutting either with the flail harvester or an Allen Scythe. (Serjeant)

Nematode-transmitted Viruses

Pea early-browning virus. Preparations of an English isolate of pea early-browning virus, which serological tests show is only distantly related to a Dutch isolate, contain tubular particles, most of which are either about 102 mμ or 207 mμ long, with sedimentation coefficients of approximately 210 S and 286 S respectively. Infectivity is associated only with the long particles. An English and a Dutch isolate affect some pea varieties differently, and only the English isolate could be established experimentally in soil containing the nematode *Trichodorus primitivus*. One of 5 pea varieties did not become infected when grown in soil from the site of one outbreak of the virus in Norfolk, but all were infected in soil from a second site. These kinds of variability in reaction indicate that varieties thought to be resistant should be tested at several sites. (Harrison)

Tobacco rattle viruses. Isolates of tobacco rattle virus from Britain, Continental Europe, and North and South America have particles with very different length distributions. All isolates produce infective particles 182–197 mμ long with sedimentation coefficients of 295–304 S, but different isolates produce non-infective shorter particles of different characteristic lengths and sedimentation coefficients. These differences do not depend on place of origin or degree of serological relationship. Thus an isolate from Florida is distantly related serologically to one from Scotland, yet has a similar particle-length distribution, and an isolate from England is closely related serologically to one from the Netherlands but has a different particle-length distribution. When phosphotungstate-treated preparations are examined in the electron microscope the tubular particles of all isolates appear to be polar, i.e. one end of each particle is different from the other. (Harrison and Nixon)

Interaction of Vector, Virus and Host in the Transmission of Tobacco Necrosis Virus

The transmission of tobacco necrosis virus (TNV) by *Olpidium brassicae* (Wor.) Dang. proves to be more complex than at first thought. Transmission tests were done with: (1) the type strains (A and D) of the two serotypes of TNV; (2) three different isolates of the vector, referred to as *Olpidium* 1, 3 and 4; and (3) seedlings of lettuce (*Lactuca sativa*) and cress

https://doi.org/10.23637/ERADOC-1-57

(*Lepidium sativum* L.), and callus tissues of tobacco (*Nicotiana tabacum*) and of *Parthenocissus tricuspidata* Sieb. and Zucc., growing in agar and liquid medium respectively.

The main results were as follows: *Olpidium* 4 did not transmit either virus strain to any of the hosts. *Olpidium* 3 transmitted both more often than did *Olpidium* 1. Strain D was usually transmitted more often than A, but A was transmitted more readily than D to tobacco callus tissues by *Olpidium* 1.

Differences in transmission of the two strains cannot be explained by differences between hosts in supporting virus multiplication, because D was transmitted better than A to tobacco callus tissue by mechanical inoculation but A better than D by *Olpidium* 1. Nor can specific transmission by one *Olpidium* isolate and not another be explained by different susceptibilities of hosts to different Olpidia. *Olpidium* 4 infects and multiplies profusely in cress but does not transmit TNV, whereas *Olpidium* 3 transmits virus to cress but fails to multiply and produce zoosporangia. Penetration suffices to transmit virus, but there is no evidence that *Olpidium* 3 penetrates more often than *Olpidium* 4; indeed, the opposite seems more likely.

When strain D is transmitted to cress by *Olpidium* 3 infection by the virus can be inhibited by inoculating the roots with *Olpidium* 1 or *Olpidium* 4. Neither of these Olpidia transmits D to cress, but, whereas *Olpidium* 4 does not transmit TNV to any host tested, *Olpidium* 1 transmits to the other three hosts. The inhibitory effect of the second inoculation with *Olpidium* 1 or 4 on virus infection can be overcome by heating the roots at 50° C for 10 seconds, which inactivates the fungus but not the virus. The differences in transmission of the two strains of TNV to different hosts by different Olpidia, and the result of inoculating twice with the fungus, suggest that the specific effects in virus transmission reflect changes following fungus infection that render the host cell suitable or unsuitable for the virus to infect. (Kassanis and Macfarlane)

Fungus Diseases of Cereals

Eyespot on Broadbalk. Routine sampling on plots 2B, 3 and 7 showed that in 1964 the 1st, 2nd, 3rd, 4th, 6th and 13th consecutive wheat crops after fallow had respectively 68, 70, 68, 79, 69 and 74% straws with eyespot, of which 26, 35, 30, 40, 36 and 38% had severe lesions. Thus for the first time since 1957 the amount of eyespot was the same on Section 1A (13th successive wheat crop) as on the other sections after crop. Since 1957 herbicides have been applied annually to the continuous wheat crop on Section 1A, while the other sections were sprayed for the first time in 1964. Only further records can show whether the use of weedkiller decreases eyespot infection on Broadbalk. (Cox)

Continuous wheat growing and the decline of take-all. During 1963 perennial grass weeds became so prevalent on the Decline of Take-all experiment that it was necessary to spray with aminotriazole after harvest.

https://doi.org/10.23637/ERADOC-1-57

This satisfactorily controlled the weeds but delayed sowing until 13 November. By 31 March only 1·3% plants had take-all. Although many plants became infected during spring and early summer, fewer roots were infected by July than in 1963. Nevertheless, the 6th successive wheat crop had fewer infected plants and yielded more than the 4th or 3rd crops:

	6th crop	4th crop	3rd crop
% plants infected, 6 July	52	64	74
Grain yield, cwt/acre	38·3	36·4	35·4

Take-all decline has been evident on this site in each of the past 5 years, and its reality can no longer be doubted, although the associated increase in yield has been statistically significant in only one year (1962). Unfortunately the cause of take-all decline is still unknown. The practical implications of these results are considerable, as they indicate that a short break in otherwise continuous winter wheat has no value where take-all is the main factor restricting yields. However, this must not be used as an argument against crop rotation: the yield of the 6th crop was at least 10 cwt/acre less than the usual yield of winter wheat on this soil when eyespot and take-all are eliminated. (Cox)

Varietal susceptibility to take-all. All commercial varieties of wheat and barley are susceptible to take-all, but even small differences in susceptibility could be important when wheat and barley are grown intensively. To test whether such differences exist, four varieties of winter wheat and three of spring barley were sown in pots of naturally infested soil on 30 October. The pots were kept in an unheated glasshouse until 20 May, when take-all on roots was estimated. The wheat varieties, Heines 4013, Rothwell Perdix, Cappelle Desprez and Prestige, had 35, 43, 61, 74% seminal roots and 13, 22, 41, 53% crown roots infected respectively. Despite large variations between replicates, the differences between Prestige and Rothwell Perdix and Heines 4013 were statistically significant, a result that justifies experiments in the field. The barley varieties Proctor, Cambrinus and Maris Badger did not differ in the proportion of their roots infected. (Cox)

Intensive barley growing experiment. The mean yields and incidence of take-all in spring barley grown continuously on the same plots in the 3 years 1961–63 were:

	Nitrogen cwt/acre			
	0	0·3	0·6	0·9
Grain yields, cwt/acre	27·3	36·9	39·2	41·8
% straws with take-all (July)	23	12	13	11

An average yield of more than 2 tons/acre is very satisfactory and demonstrates why intensive barley growing is so popular and successful in south and east England.

However, in 1964 take-all and eyespot were both more prevalent than in recent years, and the maximum yield of the 4th successive barley was only 34 cwt/acre. In contrast the 1st and 2nd crops after an oats–beans break and the crop after potatoes all yielded more than 2 tons/acre (Table 1).

https://doi.org/10.23637/ERADOC-1-57

TABLE 1

	Previous crops				Nitrogen (cwt/acre) 0	0·3	0·6	0·9
Grain yield, cwt/acre	sW	B	B	B	18·5	27·6	29·5	34·2
	sW	O	Be	B	18·5	37·3	37·7	44·8
	sW	sW	O	Be	43·4	45·6	49·8	48·1
	sW	Be	wW	P	41·6	48·3	46·1	44·6
% Straws with Take-all (July)	sW	B	B	B	68	57	52	43
	sW	O	Be	B	40	31	26	17
	sW	sW	O	Be	2	1	3	2
	sW	Be	wW	P	3	3	1	2
% Straws with Eyespot (July)	sW	B	B	B	22	36	43	44
	sW	O	Be	B	20	48	27	34
	sW	sW	O	Be	2	6	6	3
	sW	Be	wW	P	11	4	26	15

sW = spring wheat; wW = winter wheat; B = spring barley; O = spring oats; Be = beans; P = potatoes.

Once again the response of barley to nitrogen fertiliser was greatly affected by previous crops, and more attention must be given to this factor if fertilisers are to be used with maximum efficiency. Barley grown continuously may respond to much larger applications than are normally given. (Slope)

***Cephalosporium* stripe of wheat.** Cappelle wheat was sown in John Innes compost in pots under glass and some pots inoculated with *Cephalosporium gramineum*. Four inocula were compared: a 3-week-old culture on autoclaved sugar-beet seed (B), a 3-week-old culture on potato–dextrose–agar (A), naturally infected wheat straws (S) and artificially infected green plants (P). In half the pots five wireworms (*Agriotes* larvae) were placed on the soil surface immediately after sowing and again on 13 March. Plants showing symptoms of *Cephalosporium* stripe were counted at the end of May, and all plants were grown to maturity and the yield of each pot determined.

	Inoculum None	B	A	S	P
% plants infected, + wireworms	0	56	12	35	15
no wireworms	0	0	0	2	2
Grain yield, g/pot, + wireworms	17·5	12·6	16·6	12·2	15·8
no wireworms	19·2	17·0	19·0	18·6	19·6

Clearly wireworms can induce infection of wheat grown in pots, presumably because they wound roots while feeding, but whether they also do in the field has to be established. Even if they do, they are not necessarily the only agents that can cause wounds and thus allow infection. In this experiment the loss of yield caused by *C. gramineum* was much greater than the loss directly by wireworm feeding, which was slight: fewer than a tenth of the plants were killed in the seedling stage when field plants are most vulnerable to wireworm attack. (Slope, with R. Bardner, Entomology Department)

Fungus Diseases of Potatoes

Potato blight. Potato blight occurred unusually early in S. and E. England in 1964, but dry weather soon followed, and so restricted the

https://doi.org/10.23637/ERADOC-1-57

development of the disease that in experiments at Rothamsted, Woburn and Terrington St. Clement, Norfolk, respectively, it killed only 20, 1 and 25% of the foliage of unsprayed plots. At these sites any benefit from spraying was certain to be small and no plot had more than 0·4% of King Edward tubers blighted.

By contrast on Black Fen soil at Mepal, Cambs., early occurrence and favourable weather allowed *Phytophthora infestans* to destroy more than 80% of unsprayed foliage, mostly during late July and early August. Fungicides were unusually effective, and at the end of September less than 5% of sprayed foliage had been killed by blight. Even allowing for death from all causes, this delayed the date when half the foliage was dead from late July on unsprayed to late August on sprayed plots. The surprising consequence was that, in a year when there was little blight, spraying increased the total yield of this crop from 8·5 to 14·2 tons/acre (ware on 1½ in. riddle, from 5·1 to 12·8 tons/acre), by far the greatest increase we have measured in any experiment. (Hirst and Stedman)

Leaf infection by zoospores. Further work on the effects of zoospore concentration on leaf infection confirmed results obtained in 1963, and showed more clearly that the number of spores required to infect half the leaves inoculated (ED 50) differs greatly between varieties. More spores were necessary to infect the upper leaf surface of Libertas and Zeeburger, than Pimpernel (all resistant varieties), whereas fewer were required to infect either the moderately susceptible Arran Viking and Majestic, or the susceptible Up-to-Date and Bintje. (Lapwood with Dr. R. K. McKee, John Innes Institute)

Haulm and tuber resistance to blight. Field studies 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). *Phytophthora infestans* was introduced artificially into guard rows during late June and early July. Spread into the plots was first seen on 15 July, and the attack developed rapidly, so that by 31 July about 1% UD and KE, and 0·5% MJ and AV foliage was affected. Further spread was stopped by dry weather, but defoliation continued because leaf lesions extended into stems, so that by 10 August 24, 54, 42 and 27% of the stems of UD, KE, MJ and AV respectively were infected. In 1963 the many infected tubers in KE were attributed to spores washed into the soil from stem lesions, so the situation in 1964 appeared particularly dangerous. However, from 11 to 18 August, the only rainy period before the KE haulm died, only 0·33 in. fell. During this period traces of sporulation were seen, and viable spores were not detected in the surface soil or at 5 in. below plants. The clay soil was very dry, and both ridges and furrows were cracked, in places exposing tubers.

The haulm of KE was dead by 23 August, and MJ and AV by 6 September, but some UD haulm remained until the crop was lifted on 22 September. Stem lesions on UD continued to be a potential danger, and as late as 20 September viable spores were detected in water from stem traps. In spite of this only two blighted tubers were found in the whole experiment;

https://doi.org/10.23637/ERADOC-1-57

these were already well rotted when dug on 14 August. The tests in this season are of interest, because without summer rain tuber blight was negligible, despite the considerable haulm destruction from infection early in the season. In another experiment near by, despite the use of a fungicide, blight became epidemic on foliage and many tubers became infected, in irrigated but not in non-irrigated plots. (Lapwood)

Common scab (*Streptomyces scabies*). To test the importance of seed tubers as the origin of infection of common scab, tubers of the variety Majestic were separated in 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 that was duplicated on Highfield and Fosters (Highfield consistently produces more scabbed tubers than Fosters). At harvest the mean percentage area of tubers affected was estimated by the key developed by Large and Honey (*Pl. Path.* (1955) **4**, 1). On Highfield 13% was recorded on the progeny of severely affected seed tubers, and 7–8% for all other treatments; on Fosters all treatments gave about 4% scab. (Lapwood)

Tubers examined from another experiment on Highfield testing four nitrogen, two phosphate and two potash treatments showed a tendency for scab to increase with increasing nitrogen: 5·1, 7·2, 10·5 and 11·5% respectively for nil, 0·75, 1·5 and 3·0 cwt N as "Nitrochalk 21"/acre. (Lapwood, with P. W. Dyson, Botany Department)

The effects of irrigation on the prevalence of scab on the susceptible varieties Majestic and Redskin were studied using four treatments: (1) "dry" plots received only rain; (2) "wet" had rain supplemented by overhead irrigation to keep the soil at 4 in. deep at field capacity; (3) "wet-dry" had the rain supplemented to maintain field capacity only during the time tubers were forming; and (4) "dry-wet" had irrigation only after the tubers had formed. Two planting dates were 30 April and 29 May. Whereas June was wetter than usual, August and September were drier, and this distribution of rain was reflected in the results. "Wet-dry" tubers had more scab than "dry-wet" ones. "Dry-wet" and "wet" both diminished scab. In the "dry" plots scab was more prevalent on tubers from the plots planted on 29 May than for the plots planted on 30 April, but the reverse was true for "wet-dry" plots. Redskin and Majestic were about equally susceptible. (Lapwood, with Dr. B. G. Lewis, University of Nottingham School of Agriculture)

Other potato tuber diseases. The health of King Edward seed tubers to be grown in England and Wales was again assessed from samples of 200 stocks supplied by the Potato Marketing Board. This year the examination of 20,000 seed tubers from 200 stocks was hastened by scoring both visual and microscopical examinations directly on to punched tape for analysis by the Orion computer. Compared with 1962–63, tubers with skin spot (*Oospora pustulans*) decreased from 78 to 30%, and dead eyes infected with *O. pustulans* from 16 to 3%. In contrast, the percentage of tubers with black scurf (*Corticium* (*Rhizoctonia*) *solani*) sclerotia increased from 8 to 22%. Dry rot (*Fusarium caeruleum*), gangrene (*Phoma solanicola*) and

https://doi.org/10.23637/ERADOC-1-57

common scab (*Streptomyces scabies*) decreased slightly; blight (*Phytophthora infestans*) and powdery scab (*Spongospora subterranea*) increased a little.

Majestic seed tubers extracted from a bulk store at the High Mowthorpe Experimental Husbandry Farm and boxed (chitted) at monthly intervals confirmed the previous result that boxing before January prevented *O. pustulans* from killing infected buds. Dipping Majestic seed in "Aretan" temporarily decreased *O. pustulans* infection, but by April the fungus was reappearing, and on plants produced from dipped seed the fungus was almost as prevalent at harvest as on plants from undipped seed. A second dipping in January gave a small additional decrease. Disinfection is as effective as early boxing in preventing the death of eyes, but seems unable to eradicate the fungus.

The obvious damage caused by *O. pustulans* is to kill the eyes of seed tubers, and so lead to a gappy or uneven stand. The fungus also attacks the roots, stolons and underground stem bases, but whether this affects yield has never been determined. Therefore half of the stock of tubers free from *O. pustulans*, produced in 1963 by rooting stem cuttings, was inoculated with the fungus before the tubers were planted in a small field trial. The crop from the fungus-free seed tubers yielded 25% more than the one from the inoculated seed. Further evidence of losses caused by infection of stems and roots was obtained by planting seed tubers selected from commercial stocks for their different degrees of infection with *O. pustulans*, for yield decreased as the degree of infection increased. Yield was also decreased when the amount of underground infection was increased by inoculating seed tubers from commercial stocks before they were planted.

Microsclerotia were found in cultures of *O. pustulans* resembling those previously described only by Russian workers. Conidiophores of *O. pustulans* were also seen growing from similar bodies extracted from potato roots buried in soil for one year, but whether the fungus can survive the break of at least 3 years between potato crops in most rotations is unknown.

Seed tubers of King Edward and Majestic were also selected for different degrees of infection with *R. solani*. Clean tubers outyielded those severely infected by a third and also had more ware tubers/plant. Infection on the progeny tubers depended on the amount on the seed planted. (Hirst, Hide and Stedman)

Fungi of Other Crops

Control of pea wilt by chitin. The ability of chitin when added to soil to lessen the severity of pea wilt, caused by *Fusarium oxysporum* f. *pisi* (*Rothamsted Report* for 1961, p. 118; for 1962, p. 121), reflects its ability to change the soil microflora. It does not affect the growth of uninfected pea seedlings, but encourages the growth of organisms antagonistic towards *F. oxysporum*.

Chitin mixed with naturally infested soil in the glasshouse 4 weeks before transplanting pea seedlings increased the numbers of rhizosphere fungi, actinomycetes, bacteria and chitinovorous micro-organisms by 61, 50, 41

https://doi.org/10.23637/ERADOC-1-57

and 48% respectively, and decreased numbers of *F. oxysporum* f. *pisi* by 43%. Even larger effects were obtained in the field, where chitin, added at different rates 4 weeks before sowing, increased rhizosphere actinomycetes by 29–92% and fungi by 60–95%, whereas the *Fusarium* population was halved. These microbial changes were much greater in the rhizosphere than in soil away from the roots, but as similar changes occurred when chitin was added to infested soil without any growing plants, root exudates and debris in the rhizosphere intensify rather than determine the changes brought about by chitin. Moreover, although chitin did not alter the pH of pea rhizosphere or non-rhizosphere soil, it greatly decreased the number of nodules on pea roots in both glasshouse and field, further illustrating its effect on the rhizosphere microflora.

Most of the actinomycetes and bacteria, and a few of the fungi isolated from the rhizospheres of chitin-treated soils in the glasshouse and in the field, were more antagonistic towards *F. oxysporum in vitro* than were non-rhizosphere isolates; antagonism was intensified by adding sterile, concentrated, pea-root exudate. The germination of *F. oxysporum* spores was inhibited by sterile concentrated extracts of rhizosphere soils given large amounts of chitin, whereas it was slightly stimulated when only small amounts were given. Thus the effect of chitin on pea wilt seems an example of biological disease control, the chitin increasing micro-organisms that are antagonistic to or lyse *F. oxysporum*. (Khalifa)

Diseases of Sitka spruce in forest nurseries. In collaboration with the Research Branch of the Forestry Commission, work continued on seedling losses and poor growth in forest nurseries. Effects of several partial soil sterilants with a wide biocidal action were compared with more selective fungicides. Experiments at Ringwood and Kennington, some of which have continued on the same plots for 5 years, showed that the partial sterilants formalin, dazomet, metham-sodium and chloropicrin, and the fungicides nabam and quintozene usually increased seedling survival, but only the partial sterilants appreciably increased growth. The beneficial effects of these materials did not decrease with successive annual treatments of the same soil.

Partial sterilants and fungicides increased emergence and decreased seedling mortality in 1964. From 1,800 viable seeds sown per square yard on 6 April an average of 1,407 emerged at Ringwood, and of these 887 survived in untreated soil, and 1,322, 1,380, 1,086 and 1,272 in soils treated with formalin, dazomet, nabam and quintozene respectively. At Kennington only 893 emerged, of which 497 survived on untreated soils, and 830, 661, 766 and 639 respectively in treated soils. Only formalin and dazomet appreciably increased growth. The loss of over 50% of viable seeds sown at Kennington in treated soils was not obviously caused by pathogens. Pathogenic fungi were rare on the roots of emerged seedlings in treated soils. Also, applying nabam or quintozene in spring to soils that had been partially sterilised during winter did not further increase emergence. Thiram seed-dressing had little or no effect on the emergence of seed sown in either treated or untreated soils.

Emergence differed greatly at different sowing dates. At Kennington 726

https://doi.org/10.23637/ERADOC-1-57

seedlings emerged in plots sown early in April when the rest of the nursery was sown, whereas 1,221 and 1,326 seedlings emerged in plots sown on 4 and 26 May respectively. By contrast, at Ringwood emergence decreased with delay in sowing from 6 April (1,188 seedlings) to 27 May (646 seedlings). During the past 5 years the earliest sowings always produced the tallest plants; sowings in May to July sometimes suffered from drought, but otherwise there was no consistent relationship between sowing date and optimum emergence. These wide differences in emergence from the same seed stock, and the observation that formalin had no greater effect where emergence was poor than where it was good, indicate that plant numbers were affected more by soil conditions during germination than by the activity of soil or seed-borne pathogens.

To evaluate the importance of seed-borne pathogens, seed with a germination capacity of 69% was compared in split plots with seed of only 18% germination. Each was sown at a rate to give 1,800 viable seeds per square yard, and although 10 times more dead seed was sown with the poor quality than the good, this had no effect on emergence in either treated or untreated soils. There was therefore no indication of spread of pathogens from dead to living seed in the soil, or of any increase in virulence of soil-borne pathogens that might have been able to use dead seed as food base.

Formalin, metham-sodium and dazomet applied in December 1962 to plots cropped in 1963 also increased numbers and growth of the next crop sown in April 1964 by similar amounts to formalin and dazomet applied to adjacent experiments in December 1963. For example, untreated plots and those treated with formalin, metham-sodium and dazomet respectively yielded an average of 929, 1,316, 1,346 and 1,248 seedlings, measuring 0·84, 1·50, 2·50 and 1·62 in. at Ringwood, and 497, 544, 478 and 552 seedlings, measuring 1·07, 1·43, 1·48 and 1·57 in. at Kennington. (Metham-sodium increased growth more in the second than in the first year at both nurseries, suggesting that toxic residues may have been slow to disperse.) In the same experiment the fungicides quintozene and nabam applied in spring 1963 had no effect on growth in 1964, but respectively yielded 1,180 and 1,046 seedlings at Ringwood and 369 and 644 at Kennington.

At Ringwood and Wareham half of the plots treated with formalin in December 1963 were re-inoculated in March with soil and root residues taken from adjacent untreated plots, at the rate of about 0·3 kg/square yard. The residues were forked into the top 6 in. of soil with the basal fertilisers and the plots sown a month later. The re-inoculation had no detrimental effect on either numbers or growth of seedlings. These results, supported by the observed long persistence of growth responses in partially sterilised plots, open to continuous recontamination from adjacent untreated soil, suggest that either micro-organisms are not primarily responsible for stunting and death of seedlings or, if they are, they do not readily recolonise treated soils, even in the presence of the host crop. The same conclusion is suggested by the results of transplant experiments where seedlings, stunted after being grown for a season in untreated soil, recovered as soon as they were transplanted into formalin-treated soil.

Fusarium oxysporum and *F. solani* were more prevalent on seedling roots, and *Cylindrocarpon*, *Pythium* and the nematode *Rotylenchus* (*Haplolaimus*)

https://doi.org/10.23637/ERADOC-1-57

uniformis less prevalent than in 1963, possibly because the soil was drier. Isolates of *Pythium*, *Cylindrocarpon* and *Fusarium oxysporum* were tested for pathogenicity towards Sitka seedlings grown in a mixture of 3 g fresh weight of chopped root residues from Ringwood and Kennington and 300 g quartz grit/pot, to which nutrient solution was applied weekly. All seedlings died in the pots containing autoclaved residues inoculated with *Pythium ultimum* and *P. irregulare*, whereas *Cylindrocarpon*, and *F. oxysporum* were only weakly pathogenic. Unsterilised residues and autoclaved residues inoculated with *Cylindrocarpon* and *F. oxysporum* singly and together did not decrease seedling growth. (Salt)

Air-borne Moulds and Actinomycetes from Crops in Storage

Moulding of hay in vacuum flasks. Moist hay has been allowed to mould spontaneously in wide-mouthed cotton-plugged vacuum flasks to obtain more precise information on effects observed previously in baled hay. The micro flora developed depended on the moisture content of the hay at the start of the experiment, as also did the amount of self-heating (see Report of the Biochemistry Department, p. 123). The inoculum necessary for any particular type of moulding thus seems to be widely distributed through hay. Most organisms found grow over a range of moisture contents, but best starting points for different species are shown below.

Moisture % fresh weight	
26	*Aspergillus glaucus* group
28–29	*Asp. versicolor*, *Scopulariopsis brevicaulis*.
31	*Asp. nidulans*, *Absidia ramosa*, *Streptomyces fradiae*.
40	*Asp. fumigatus*, *Thermopolyspora glauca*.
47	*Humicola lanuginosa*, *Thermopolyspora polyspora*.
57	*Thermoactinomyces* (*Micromonospora*) *vulgaris*.
68	(bacteria predominate)

At 40% moisture content *T. polyspora* started to sporulate after 4 days (soon after the maximum temperature was reached). Faint reactions in serological tests were obtained after 3 days, and a day later the precipitin lines were strong and typical of farmer's lung hay. (Lacey, with Festenstein, Biochemistry Department, Skinner, Soil Microbiology Department, and with Dr. J. Pepys and Dr. P. A. Jenkins, Institute of Diseases of the Chest)

Fog fever in cattle. Hays associated with a pneumonic condition in cattle resembling fog fever were examined in the wind tunnel by the method developed for examining farmer's lung hays (which they usually resemble). *Thermopolyspora polyspora* was isolated from most of these hays, but the numbers of colonies obtained by the Andersen sampler were not related to the severity of outbreaks. Precipitin reactions typical of farmer's lung disease in man were obtained with cattle sera when tested against *T. polyspora*. (Lacey, with Dr. J. Pepys and Dr. P. A. Jenkins, Institute of Diseases of the Chest)

https://doi.org/10.23637/ERADOC-1-57

Moist storage of barley grain. The recently introduced method of storing moist barley grain in open concrete tower silos carries a risk, unless anaerobic conditions are achieved, not only of the grain deteriorating because of microbial attack but also of hazards to human and animal health. When conditions are correct grain can be stored at up to 40% moisture content; in hay this would lead to extensive moulding with a flora including potentially pathogenic and toxic fungi. In the stored barley fungal growth should be restricted by anaerobic conditions, but some air exchange is difficult to avoid at the top of an open silo; also even with a hermetically sealed silo, the amount of grain will not always be enough to maintain anaerobic conditions.

Silos so far examined have ranged from one from which grain was being extracted daily, and which had apparently clean grain with only occasional white patches of yeasts, to others with grain covered with a weft of mycelium of *Sporotrichum pruinosum* and *Fusarium* spp., or yellowed by *Aspergillus flavus*. Two of the latter type, which had been covered with plastic sheeting, had layers of grain up to 6 in. thick rich in actinomycete spores. In one of these *T. polyspora* (active in farmer's lung disease) predominated, and in the other *Streptomyces fradiae*. *Aspergillus fumigatus*, *Asp. terreus*, *Humicola lanuginosa*, *Mucor pusillus* and *Absidia ramosa* were also isolated in abundance. Temperatures up to 58° C were recorded in the top foot of grain in one silo.

The atmosphere in the silos was sampled with the Cascade impactor and Andersen sampler. In one, while grain was being removed, the concentration of actinomycetes and bacteria in the air increased from 4 to 1,800 million and fungi from 5 to 1,100 million per m^3. Concentrations were still 18 million and 26 million per m^3 respectively 20 minutes after the grain had been disturbed. *Aspergillus fumigatus*, *Asp. flavus* and *Mucor pusillus* were the most abundant fungi. Bacteria obscured most actinomycete colonies, but *T.* (*M.*) *vulgaris*, *T. polyspora* and *Str. fradiae* were isolated from the air. Workers exposed to inhalation of such concentrations should clearly be protected by efficient dust respirators (simple fabric masks do not protect against the very small particles encountered). (Lacey, with Dr. J. Harrison, N.A.A.S., Shardlow)

Conjoint Work with Other Departments

Besides the conjoint work reported above, other work is noticed in the Reports of the following Departments: Bees (Bailey with Gibbs); Chemistry (Widdowson with Slope); Entomology (Cockbain with Gibbs); Soil Microbiology (J. Kleczkowski with A. Kleczkowski; Skinner with Lacey); Biochemistry (Festenstein with Lacey); Broom's Barn (Dunning with Watson, Serjeant and Lack).

https://doi.org/10.23637/ERADOC-1-57