ROTHAMSTED RESEARCH

Report for 1959

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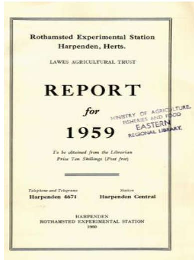
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Plant Pathology Department

P. H. Gregory

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PLANT PATHOLOGY DEPARTMENT

P. H. Gregory

T. Mulligan left to become virologist at The Albert Agricultural College, Glasnevin, Dublin, and was succeeded by R. C. Sinha; A. J. Gibbs was appointed to replace L. Broadbent. The following joined us as temporary workers: H. Dias (Lisbon), J. Gourlet (Paris), Katri Ikaheimo (Helsinki), J. Lacey (Reading), M. A. Ram Reddy (Hyderabad), R. D. Tinline (Saskatoon).

A. Kleczkowski contributed to the Conference on Inactivation of Viruses by invitation of the New York Academy of Sciences. Mary D. Glynne received a grant from the Rockefeller Foundation to study wheat diseases under different climatic conditions in the United States and Canada, and afterwards represented the Department at the 9th International Botanical Congress, Montreal. As the guest of the organisers, B. D. Harrison, Mrs. M. A. Watson and E. W. Buxton also attended the Botanical Congress.

J. M. Hirst became the first recipient of the Jacob Eriksson Gold Medal awarded by the International Botanical Congress for outstanding work in Plant Pathology.

G. D. Heathcote was awarded the Ph.D. degree of London University.

Viruses and Virus Diseases

Studies on the early events in the infection of leaves comparing inocula of nucleic acid preparations and intact virus were extended to include tobacco necrosis virus in addition to tobacco mosaic virus (TMV). Nucleic acid prepared from tobacco necrosis virus by the phenol method has 20–100% of the infectivity of the parent virus compared with 0·1–1% for TMV. As with TMV, some of the changes in leaves infected with tobacco necrosis virus can be detected sooner with inocula of nucleic acid preparation than with intact virus. (Kassanis.)

Ultraviolet-inactivated tobacco mosaic virus is unusual in that it can not be photo-reactivated. By contrast, when infective nucleic acid from TMV is irradiated by ultra-violet, about 50% of absorbed radiation energy causes the kind of damage that can be reversed by exposing inoculated leaves to visible light; the remaining 50% causes irreversible damage. The manner in which the nucleic acid and protein are combined in virus particles protects the nucleic acid from the effect of ultraviolet to such an extent that 90% of the radiation is prevented from causing damage, and this includes all the photo-reactivable damage.

There is much indirect evidence that TMV separates into protein and nucleic acid as an initial step in the infection process, and the discovery that free nucleic acid is photo-reactivable suggested that direct evidence might be gained by exposing leaves to visible light at various times after inoculation with intact virus. Photo-reactivation of the virus would be evidence that the nucleic acid had separated from the protein. The attempt failed because the effect of ultraviolet on the capacity of the leaves to support virus multiplication was so variable and was photo-reactivated to such an extent that any photo-reactivation of the inoculum within the leaves would be masked. The irradiation affected the host so much as to throw doubt on conclusions of previous workers who used radiation to measure the time when virus starts to multiply. (Bawden and A. Kleczkowski.)

Electrophoretic studies of antigen-antibody compounds showed that rabbit antibodies to human serum albumin can differ from one antiserum to another in the degree of firmness and reversibility with which they combine with the antigen. (A. Kleczkowski.)

Bacteriophage

The electrophoretic mobility of bacteria in a constant environment depends on properties of bacterial surface, so alterations in the mobility can be used to detect changes in the structure of the surface. Infection with bacteriophage increased the electrophoretic mobility of pea-nodule bacteria (*Rhizobium leguminosarum*) at pH 7. The increase occurred at about the middle of the latent period, suggesting that the bacterial surface alters at this stage of infection. (A. Kleczkowski and J. Kleczkowski.)

Tobacco rattle virus

Nucleic acid preparations made by disrupting the virus with phenol were about 5% as infective as their parent virus suspensions, and were intermediate between TMV and tobacco necrosis viruses. The properties of the infective nucleic acid preparations differed in many respects from those of their parent virus suspensions, but resembled those of nucleic acid preparations from tobacco mosaic virus. (Harrison and Nixon.)

Virus structure

Similarities in structure between tobacco rattle and tobacco mosaic viruses became evident. Certain preparations of TMV can be stained with phosphomolybdic acid to show a dark central region crossed by a system of bands about 2·5 mμ apart. Reaggregated TMV protein will show a similar staining pattern when mounted by spraying in a solution of neutral phosphotungstate, and whole virus can sometimes be made to show a similar image by treating the dried virus on the mount in hydrochloric acid vapour before adding phospho-tungstate as a fine spray. Although none of these techniques is entirely reliable, the structures observed agree well with the internal structure of tobacco mosaic virus inferred from X-ray-diffraction data. Work is now concentrated on finding a technique which will give reliable results with all samples of TMV and thus permit detailed comparison of strains. (Nixon and Woods.)

Preliminary studies with "spherical" viruses suggested that mounting in neutral phosphotungstate without any other preliminary treatment may give disappointing results, and like TMV they will need some " softening up " before sufficient phosphotungstate to throw the internal structure into relief can be made to enter the particles. For studying details of the external shape of " spherical " viruses shadowcasting, perhaps supplemented by staining, is still the most useful method. Preliminary trials with evaporated carbon/platinum mixtures by Bradley's method (*Nature, Lond.* **181**, 875–877, 1958), in which composite rods made from finely powdered platinum and carbon are used in an evaporation source resembling that usually used for making carbon supporting films and replicas, indicate that this technique is only slightly superior to our pure platinum films and about equal to platinum–iridium alloy evaporated in the ordinary way from a tungsten filament. This work on shadowing has direct bearing on the work on clay mineral morphology, where precise measurements of flake thickness in the 1–2 mμ region of thickness are being attempted, and upon the quality of all the routine shadowed work. (Nixon and Woods.)

Soil-borne viruses

Arabis mosaic virus. This name is now used for a virus previously called raspberry yellow dwarf but now known to share many of its antigens with arabis mosaic virus (*Rep. Scottish Hort. Res. Inst.* for 1958–1959, p. 36). The virus was found damaging raspberry, strawberry and clover crops in many parts of England and in Wales. Diseased plants were patchily distributed, and the patches were closely correlated with the presence of a dagger nematode (*Xiphinema* sp.) in the soil. The virus was transmitted to pea seedlings grown in sterilised soil to which hand-picked, washed *Xiphinema* from infective soil were added, but not to seedlings grown in *Xiphinema*-free fractions of infective soil. The infectivity of soil was abolished and *Xiphinema* was killed by air-drying soil at 20° C. for a week or by various chemical treatments. The possibility that other soil-borne viruses have nematodevectors is being studied. (Harrison.)

In collaboration with the Soil Microbiology Department work began on the transmission of tobacco necrosis virus under controlled conditions. (Harrison and Jackson.) Work also began on soil-borne viruses that infect grape vines. (Dias and Harrison.)

Legume viruses

Red clover mottle virus, originally isolated from red clover plants on Rothamsted Farm, seems distinct from previously described viruses of leguminous plants. It was transmitted by inoculation of sap to many leguminous species, but *Gomphrena globosa* was the only non-leguminous species infected. The virus was not transmitted by any of the six aphid species tested or through the seed: its thermal inactivation point is 63° C., and it has " spherical " particles of about 28 mμ diameter.

Similar numbers of local lesions developed in inoculated leaves of French bean at temperatures between 12° and 24° C., but fewer developed at 28° C. and none at 32° C. The rate the virus accumulated in leaves increased with increase of temperature up to 28° C., but then decreased and the virus did not multiply at 32°–34° C.

After disruption with phenol, virus preparations retained 20% of their initial infectivity. The infectivity was presumably conferred by nucleic acid; it was abolished by pancreatic ribonuclease or by storing for a day at 20° C., treatments that have little effect on the infectivity of intact virus. As with tobacco necrosis virus, infection centres initiated by nucleic acid were more rapidly inactivated by ultraviolet-radiation immediately after inoculation, than those initiated by intact virus. Such infection centres also began to increase their resistance to ultraviolet-inactivation without the lag period of 2 hours which occurs at 20° C. with whole virus inocula. (Sinha.)

Field Beans. The spread of virus diseases into an area of field beans grown to compare different seed rates was observed in an experiment by M. J. Way. The number of virus-infected plants in insecticide-sprayed and unsprayed sub-plots did not differ, suggesting that little spread occurred within the plots. The number of plants infected per unit area of land decreased greatly as seed rate increased. The percentage infection on 10 July ranged from 7·7% leaf roll and 6·5% mosaic in sprayed plots with only 50 lb./acre of seed, to 0·3% leaf roll and 0·1% mosaic in plots with 600 lb. Leaf roll was the commonest virus disease, and symptoms were first seen on 4 June. Mosaic appeared later and spread rapidly. By the end of June most of the unsprayed plots were too damaged for the recognition of virus symptoms, but before that date the different viruses showed similar patterns of spread. (Heathcote.)

An experiment tested natural virus infection in field beans sown on 20 February, 3 April and 23 April. Mature beans are not colonised by aphids, and the number of plants naturally infected with virus diseases was highest in the plots sown later in the season. Some rows were watered every 10 days with demeton-methyl so that aphids could not colonise them, but virus spread to them as much as to untreated rows, suggesting that most of the virus spread was by winged aphids. (Heathcote and Gibbs.)

Lucerne mosaic virus. A survey showed that lucerne mosaic virus occurs in lucerne crops in all parts of Great Britain. The virus is more common in old crops, and on average 25% of the plants were infected in those crops sown before 1955.

A small field experiment showed that plots of lucerne in which 75% of the plants were infected yielded 10% less fresh weight in the year than did comparable plots with 18% of the plants infected. Yield was decreased most (20%) in the spring cut, when the diseased plants showed the brightest symptoms and were noticeably stunted. (Gibbs.)

Carrot Motley dwarf virus. No other vector than *Cavariella aegopodiae* has been found for this virus. *Myzus persicae* and *M. cymbellaria* both feed well on carrot but will not transmit. The virus persists in the vector for at least 1 week. It can be acquired by the vector in 7 hours' feeding on infected plants and transmitted during ½ hour's feeding, but a total time of at least 14 hours is necessary between start of infection feed and end of test feed, and 48 hours is optimal. Thus there appears to be a latent, or incubation, period of the virus in the vector, though the times given may not be the minimum possible.

Although the virus cannot be transmitted by sap inoculation

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from carrot to carrot, it can be transmitted by aphids to *Datura tatula* and *Nicotiana clevelandii*, and inoculation of sap from these causes local lesions on *Gomphrena globosa* similar to those caused by feeding of infective aphids on *G. globosa*. Infective aphids will also cause local lesions on *N. tabacum*, from which *G. globosa* and *N. clevelandii* can be infected by inoculation, but the virus in these lesions has not yet been returned to carrot by sap inoculation or by aphids.

The virus was very prevalent in 1959, and a small field experiment at Woburn, to find the effect of infection on the yield of carrots, failed because the carrots were already infested with aphids and infected with the virus before experimental inoculations could be made. (M. A. Watson.)

Potato viruses

Results from 1958 experiments with Insecticides. In the 1958 spraying trial done in co-operation with the Insecticides Department, aphids were few, and examination of plants grown in 1959 from seed tubers showed that viruses spread only little. The percentages of infected plants in the bulk samples, which contained initially 0·8% of plants with each disease were:

	Leaf roll	Rugose mosaic
Unsprayed	4·1	2·8
Sprayed 4 times	1·0	1·2
Sprayed on 2nd, 3rd and 4th occasions ...	1·6	1·1
Sprayed on 3rd and 4th occasion	3·3	1·5
Sprayed on 4th occasion with DDT emulsion ...	3·0	2·2

Spraying dates were 4 and 16 June, 8 July and 6 August (the thiolate isomer of demeton-methyl was added to the DDT on the 3rd occasion).

In tubers taken from plants near to infected ones, the percentage incidence of virus diseases was: unsprayed, 22·4 leaf roll, 15·2 rugose mosaic; sprayed 4 times, 0·8 leaf roll, 2·8 rugose mosaic. As a sample of 642 tubers taken on the same field but away from the plots containing infected plants was free from infection, virus was not spread far from the plots, and little was introduced from other fields. (Broadbent, Burt and Heathcote.)

Although " Rogor " or " Thimet " applied when the seed was planted protected the plants from colonising aphids, they did not completely prevent the spread of viruses. However, this method of application may prove more efficient than foliar sprays, and be more acceptable to the farmer. The percentage of plants with leaf roll and Y-viruses in the five plants on either side of initially infected ones was:

	Leaf roll	Rugose mosaic
" Thimet " (individual dose) ...	2·6	19·5
" Thimet " + fertiliser	0	5·7
" Rogor " (individual dose) ...	0	18·0
Fertiliser alone	8·1	15·8

The yield of plants grown in 1959 was not affected by any insecticidal residues. (Broadbent, Burt and Heathcote.)

Aphid trapping in potato crops in 1959. Nine sticky traps were operated in unsprayed crops near sprayed ones in different parts of England. Aphids were very numerous during June. Six times as many aphids (38,900) were trapped as in 1958 (6,684), although there were two fewer traps operating. *Myzus persicae* were exceptionally common in some areas in September when they were colonising their winter host-plants. (Heathcote.)

Cereal and grass viruses

Wheat striate mosaic was again isolated from oats and wheat at Rothamsted, and it also damaged wheat at Tivetshall, near Norwich, where 10% of the plants in a large area of crop were infected. *Delphacodes pellucida* collected at both sites were carrying the virus. The ability of some of our inefficient vector races to transmit virus can be increased by pricking the anterior abdomen, using the technique described by Storey for converting non-vectors of maize streak mosaic virus into vectors. The virus is optimally transmitted by plant hoppers that feed on infected plants as nymphs or inherit virus from their parents. Females that feed on infected plants as adults transmit only infrequently to plants, and they very rarely transmit to their nymphs through the eggs, even though the interval between virus acquisition and mating is long enough for the virus to become transmissible to plants.

We could not confirm that nymphs or eggs inherit virus from females that are unable to infect plants. If the virus can remain latent in some females for a generation, none of the races we are now using seem to have this ability. (Watson and Sinha.)

Infected eggs and embryos were examined in attempts to ascertain the nature of the damage that often leads to their death. Attention was paid particularly to the mycetome which seems important to the growth of the egg at the stage when infected ones often begin to die, and in which there was some slight evidence of deterioration and deformation of the cells. Attempts in collaboration with the Soil Microbiology Department to culture the mycetome *in vitro* have probably failed, although sterile cultures, in which the cells survived, were made, and a yeast that appears not to have been previously described was isolated. (Watson, Ikaheimo and Parle.)

Barley (cereal) yellow dwarf. Following the *Rev. appl. Mycol.* the name barley yellow dwarf virus will be used in future for the virus previously referred to as cereal yellow dwarf.

The virus was common in field crops, though not so common or so damaging as in 1958. The results of field experiments made in 1956–58 were further examined to try to find the reason for variability in response to infection between seasons and between crops. A suggestion that the time of infection relative to the time of tillering is responsible was not confirmed. In 1957 even the earliest infection (22 April) neither inhibited tillers nor killed the main shoot already formed. In 1958 plots infected around the time of tillering lost yield at the same rate as those infected later. The rate of loss of grain was positively correlated with the severity of leaf symptoms (score for yellowing and measurement of plant height), coupled with the length of time for which the plants were infected. This relationship held for all cereals and for both virulent and avirulent viruses. The loss of yield seems to result from decrease in total leaf area and possibly from a lessened efficiency of assimilation, but not from the loss of any particular flowering organs, though these are decreased in proportion to the rest of the plant. Wheat possibly suffers more than other cereals from later infection because it matures later and more of the contribution of assimilate from the glumes is lost than with the other cereals.

In 1957 we could not transmit virus from an apparently infected oat crop at Hexton in Bedfordshire by *Rhopalosiphum padi*. Samples of grasses, groundkeeper oats and barley from a neighbouring field were tested again in 1959 and found to contain a virulent virus apparently identical with the virulent "Kent" virus isolated previously. This virus was not transmitted by *R. padi*, but was readily transmitted by *Metapolphium dirhodum* and less readily by *Sitobium avenae* and *S. fragariae*. This agreed with the results of workers in the United States, who have reported specific transmissions of individual barley yellow dwarf virus by the "Apple-grain" and "English-grain" aphids, We further found that the avirulent Rothamsted virus was rarely if ever transmitted by *S. avenae* and *S. fragariae*, although it had been so when first obtained in 1954. Another and more virulent isolate from Rothamsted was transmissible by *S. avenae* but not by *R. padi*. The "Kent" isolate was not transmissible by *S. avenae*, though *S. fragariae* and *M. dirhodum* occasionally succeeded, but it was very readily transmitted by *R. padi*. Finally, a plant sent from Bristol early in 1959 contained virus that was readily transmitted by all four aphids, though least successfully by *S. avenae*. General observations suggest that there are many yellow dwarf viruses transmissible by different permutations and combinations of possible vector species and that they may change from one to another when the predominant aphid species changes. Hence the loss of ability of the Rothamsted and the Kent isolates when transmitted only by *R. padi* to be transmitted by the other aphids and the efficient transmission of the Hexton virus by *M. dirhodum*, which was the dominant aphid at the time of the virus outbreak.

Cocksfoot streak. This virus, supplied by Dr. Kenneth M. Smith from the original stock he described in 1952 was transmitted to several varieties of cocksfoot and to Italian ryegrass by inoculation of sap. *M. persicae* transmitted best after fasting for some hours and feeding for only 2 minutes on infected leaves. The virus persisted less than 1 hour in the vectors when they were feeding on immune hosts. *Hyalopterus humulis*, which appears to have only cocksfoot as a host, is a vector; *S. avenae* and *M. dirhodum* also transmitted in short feeding periods, but *R. padi* and *S. fragariae* did not.

The presence of rod-shaped particles in infected sap, and the similarity of the symptoms to those of ryegrass mosaic, suggested for a time that the viruses might have some relationship, but this seems not to be true.

The virus was isolated from cocksfoot stands at Woburn, at the Grassland Research Institute at Hurley and the Welsh Plant Breeding Station, Aberystwyth. (M. A. Watson and Mulligan.)

Fungi and Fungus Diseases

Cereal diseases

Long-term experiments. Broadbalk was not drilled until December, and was much less affected by eyespot (*Cercosporella herpotrichoides*) than usual. The 1st, 2nd, 3rd, 4th and 8th consecutive wheat crops after a 1-year fallow averaged 8, 20, 32, 21 and 16% straws with eyespot, 2, 10, 11, 12 and 7% with severe lesions; 0, 4·6, 11·3, 7·0 and 9·9% straws had take-all (*Ophiobolus graminis*) on the roots. Take-all was slight, for though many seminal roots were infected, the fungus did not spread to the crown or crown roots. An exceptionally low plant number recorded in spring on some plots of the first crop after fallow did not seem to be caused by fungi. Mildew (*Erysiphe graminis*), unlike last year, was very slight. In the six-course rotation experiment, eyespot incidence was about average for wheat sown in October, and below average in the alternate wheat and fallow experiment sown in late November. Recent changes in the alternate wheat and fallow experiment provide sub-plots with various sequences which show consistent differences in eyespot and take-all. For instance, sown at the normal rate, wheat following two consecutive crops sown at ⅓ bushel/acre had 40% straws infected by eyespot, and 6% with take-all on the roots, whereas those following 1 and 3 years' fallow had respectively 20 and 6% with eyespot, 0·7 and 0% with take-all. The interesting, though economically unimportant, disease caused by *Gibellina cerealis* was more than usually common in this experiment, the only place where it is known to occur in Britain.

Brown foot rot (*Fusarium* spp.) was more common than usual at Rothamsted. In many crops it caused whiteheads and occasionally patches of dwarfed prematurely ripened plants with shrivelled grain. (Glynne and Cox.)

Experiments on factors limiting yield. Cappelle wheat gave very different responses to treatments in two experiments on different fields. One experiment, in which it was hoped to include optimum treatments, followed two consecutive crops of early potatoes which received dung. Virtually free from eyespot and take-all, the crop looked superb until a storm on 10 July caused extensive lodging. The effects of different treatments followed the normal pattern. The mean per cent areas lodged at harvest for plots sown in October, November and January respectively were 94, 66 and 14%, for those sown at 2, 3, and 4 bushels/acre 46, 57 and 70%, and for those receiving respectively 3 and 6 cwt. " Nitro-Chalk " in spring 42 and 73%. The mean yield of grain was 50·6 cwt./acre. Yield responses expected from early sowing and high nitrogen were decreased by lodging, and no treatment significantly affected mean yield. However, plots sown in January were less severely lodged and showed a significant response to seed-rate, 4 bushels seed/acre yielding 5·6 cwt./acre more than 2 bushels. The extra nitrogen tended to decrease yield in the earlier sown crops, but the effect reached the level for significance only in November.

In the other experiment on less fertile land, Cappelle wheat followed two spring-sown barley crops. Eyespot was slight; take-all, observed as early as February, was prevalent and, though it did not cause severe symptoms it is likely to have affected yield. There was negligible lodging. Mean yield of grain was 40·7 cwt./acre. Plots sown in January yielded 5·9 cwt./acre less than those sown earlier, and there was the large response of 7·2 cwt./acre to the heavier dose of nitrogen. Although the highest seed-rate yielded consistently more than the lowest, the effects did not reach the level for significance. (Glynne and Slope.)

Cereal–beans rotation experiment. In this account of the cereal–bean rotation experiment the following abbreviations will be used: W = winter wheat, sW = spring wheat, B = barley, O = oats. In the first series begun in 1957, Koga II spring wheat grown in 1959 after the following crop sequences WW, sWW, OW, WO had respectively 12·2, 8·5, 20·1 and 2·6% straws with take-all in June and yielded 32·0, 32·4, 29·2 and 32·0 cwt. grain/acre. The highest incidence of take-all followed the sequence OW, on which the wheat in June 1958 had only 8·4% of the straws with take-all, whereas the wheat in the sequences WW and sWW had 88·8% infected. This is another example of the tendency for take-all to reach a peak and then decline. In the second series, begun in 1958, Cappelle winter wheat sown in January after W, B, sW and O had 11·4, 41·1, 29·1 and 2·4% straws with take-all and yielded 37·4, 31·9, 37·5 and 41·9 cwt. grain/acre. As in 1958 there was very little take-all in wheat following the non-susceptible oat crop. (Slope).

Pots holding 36 lb. of soil were used in an experiment, begun in 1953, in which wheat is grown either continuously or after different periods under fallow. Take-all has consistently increased in successive wheat crops and been most severe in the 3rd, in which yields have been very low. In subsequent crops the disease has been less severe and yields have risen. Thus in 1959 the 1st wheat crop after 2 years fallow had little take-all and yielded 18·8 g./pot, the 3rd had very severe take-all and yielded 6·6 g., and the 7th with moderate take-all yielded 16·0 g. (Glynne.)

Survival of *Cercosporella herpotrichoides*, estimated by counting the straws on the soil surface which produce spores of the fungus when incubated in the laboratory, was measured on two Broadbalk plots in January and February. On Section II, where wheat was grown in 1958, spore-producing straws averaged 1·4/sq. yd., and 5·8% of the plants in the 1959 wheat crop were infected in May. On Section VA, fallow in 1958, there were only 0·2 spore-producing straws/sq. yd.; no infected plants were found in May. (Cox.)

Incidence of take-all on Broadbalk. The soil microflora associated with wheat was studied to determine whether the low incidence of take-all on Broadbalk results from microbiological activity. The fungi in the soil, in the rhizosphere and on the root surface were surveyed in a pot experiment using soil from: (*a*) the unmanured plot of Broadbalk after the 4th successive wheat crop, which had 5% straws with take-all, and (*b*) an unmanured sub-plot of the Hoosfield Wheat and Fallow Experiment after the 3rd successive wheat crop, which had 31% straws infected. Similar surveys were made in the field on plots with corresponding crop sequences. Take-all developed more rapidly and severely on plants in the pots containing the Wheat and Fallow soil than on those in the Broadbalk soil; in the field there was little take-all on either the Wheat and Fallow or Broadbalk. In pots and the field the total number of fungi in the rhizosphere was higher in Broadbalk soil than in the Wheat and Fallow soil, a difference largely accounted for by the number of Penicillia. (Cox.)

Potato blight

Experiments into the nature of haulm resistance to potato blight were previously confined to the varieties Up to Date, King Edward, Majestic and Arran Viking, which have shown only small differences in the rates of defoliation by blight. The additional varieties, Ackersegen, Ås and Ontario, selected for their blight resistance from varieties grown in small field plots in 1958, were included in experiments in 1959.

Blight was found in some plots on 12 August, and to ensure a more uniform distribution of the disease all plots were infected artificially. Weather favourable for spread occurred sporadically in August, but a dry September stopped further development of the epidemic. In marked leaves frequently examined, destruction followed a similar pattern in all varieties; blight on the leaf lamina advanced through petioles, so causing non-infected leaflets to die into the stem. Some infections in Ackersegen were arrested when still in the leaf lamina. In King Edward and Up to Date, lesions quickly girdled the stem and affected the foliage above, whereas in Majestic, Arran Viking and Ås, although stem lesions occurred, few girdled the stem, and those that did were either near the top of the plant or on the weaker axillary shoots. The 50% level of destruction by blight as assessed on the marked leaves was reached 2, 3, 8 and 14 days later in Majestic, Arran Viking, Up to Date and Ås respectively, than in King Edward. Thirty-three per cent of the leaflets of marked leaves were destroyed by blight in Ackersegen, 63% in Ås and about 85% in the other varieties.

Laboratory studies with Up to Date, King Edward, Majestic and Arran Viking showed that the under surfaces of leaves were usually more susceptible to infection than the upper surfaces. Detached leaves of these varieties differed little in their susceptibility to infection or in the rate at which the fungus advanced in the leaf lamina.

Lesions spreading from the base of the terminal leaflet destroyed the leaflets quicker than those from the tip, and the time for destruction from either site increased in the order King Edward, Up to Date, Majestic and Arran Viking. Infection advanced into and girdled the petiole in Up to Date and King Edward rapidly, but was much slower in Majestic. In Arran Viking, Ackersegen and Ås the infection was arrested within the petiole.

Sporulation on different varieties differed. Up to Date produced more spores more rapidly than did Arran Viking when non-sporing lesions of similar size (10 mm.) were kept at a high humidity. After 5 hours at 15° C. aerial hyphae were abundant in Up to Date and a few sporangia had formed, whereas in Arran Viking hyphae were still fewer. After 10 hours, sporulation was intense in Up to Date but slight in Arran Viking. King Edward behaved like Up to Date, and Ackersegen and Ås like Arran Viking. The varieties slow to produce spores were those whose infected cells died rapidly, and rapid sporulation was correlated with slow death. The rate

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of necrosis may interfere with spore production by limiting the area able to produce spores, and may be a factor in determining the width of the sporing annulus, which is narrow in these resistant varieties.

Plants in pots were used to assess the blight susceptibility of 45 commercial varieties of all maturity groups. Tests included susceptibility of leaf and leaf axil to infection, sporing capacity and the rate of advance of the fungus within leaf tissue. Early varieties were the most susceptible in all respects, and generally resistance increased with the lateness of the variety. (Lapwood.)

Aerobiology

The work on the mechanisms of distant air-dispersal of fungus spores was continued in conjunction with the Meteorological Office. On several occasions traps operated in aircraft showed that the maximum concentrations of *Puccinia graminis* spores were at 3,000–5,000 feet. The catches were closely correlated with air trajectories from Southern Europe and support the conclusion that before mid-July uredospores caught in Britain are of foreign origin. Previously our spore-trapping methods were restricted to the visual recognition of air-dispersed spores, but we are developing cultural methods to measure the proportion of viable spores and details of their movement in splashed or dripping water. (Hirst and Stedman.)

Clubroot of crucifers

A study begun this year seeks: (1) better differential hosts for the three races of *Plasmodiophora brassicae*—(i) our standard strain, (ii) from Agdell Field and (iii) from Norway; and (2) different types of response to infection as influenced by host species or variety.

Thirty-five sorts were tested, but so far the search for better differential hosts has been disappointing. The three races behaved much as before; race (i) had little or no effect on turnips and swedes, which were, however, severely clubbed by races (ii) and (iii). No turnip or swede variety tested showed any pronounced resistance to (ii) and (iii). Race (iii) was usually more virulent than the others but not always so. Race (i) tended to club cabbage rather more than (ii), and (iii) was intermediate. A series of strains of several cultivated mustards, from various parts of the world (ex Colman's), were uniformly and highly susceptible to all races. Strains of *Brassica nigra* were exceptional in that the population seemed to be heterogeneous in their response to Plasmodiophora, and some plants became severely diseased whereas others were unaffected. The proportion of susceptible plants differed with host strain. An English variety of rape was severely clubbed by all three races; a New Zealand club-resistant rape was unaffected by (i), a few plants were moderately diseased by (ii) and all plants were severely diseased by (iii). This was the most striking effect observed. Another differential effect was that a few plants of *Matthiola bicornis* were clubbed by race (ii) but were unaffected by (i) and (iii). *Matthiola annua* remained unaffected by all three.

Most plants, other than those of the cabbage group and the mustards, formed galls that were nodular in appearance, although the nodules were often fused and irregular. *Malcolmia maritima*, however, tended to form many separate nodules on short laterals. An interesting feature of some swede varieties was that with race (i) fairly numerous very minute galls were formed on fine rootlets. This type of symptom has been mentioned in the literature and warrants further study. (Macfarlane.)

A series of complexes of copper, zinc and cobalt with gluconic acid were tested on clubroot. Plants were grown in small, white, polythene pots, which rested inside glass beakers, leaving gaps between the bases of the pots and the bottoms of the beakers. In this way leachings containing fungicide could be conserved and returned to the soil. Increasing doses of copper compound tended to decrease dry weight of clubs but increased weight of fibrous roots, tops and total dry weight. The zinc compound apparently hydrolysed in water and zinc was probably present in the soil as hydroxide. This completely suppressed clubroot with yields of tops closest to those of uninoculated controls. The cobalt compound was about as effective as copper or zinc in preventing clubroot, but was more toxic. Effects differed slightly according to whether the host plant was cabbage or brown mustard (*Brassica juncea*). (Macfarlane and Gourlet.)

Fusarium diseases of peas

Varietal resistance. Further tests for wilt-resistance in peas, made in fields at Yaxley, Peterborough and at Coggeshall, Essex, divided 35 pea varieties into three groups: 8 were very susceptible, 4 susceptible and 23 were resistant. In earlier work, isolated healthy plants appeared among some susceptible varieties, and seed from these was sown at Yaxley and Coggeshall. 47% of the plants from these were wilt-resistant, and only three were not true to varietal type. Selection, therefore, quickly provides resistant stocks from important susceptible varieties, such as Feltham First, Lincoln, British Lion and Little Marvel. In tests designed to control pea-seedling losses, seed of varieties Onward and Dark Skin Perfection were treated with " Captan ", " Thiram " or " CoBH " (quinone-oxime-benzoylhydrazone). " Thiram " increased emergence by 30%, " Captan " by 46% and " CoBH " by 24%. Subsequent yield was as much as 89% higher in the wilt-resistant Dark Skin Perfection. None of the treatments affected wilt in the variety Onward.

Rhizosphere studies. Extending the work on interactions between rhizosphere micro-organisms which grow on pea roots revealed that the most common fungi were *Gliocladium roseum*, *Penicillium* spp., *Fusarium oxysporum*, *Fusarium solani*, *Mortierella* spp., *Rhizopus stolonifer* and *Trichoderma viride*. From 137 morphologically different isolates, 14% strongly inhibited pathogenic *Fusarium oxysporum*, 42% were considerable inhibitors, 29% only slightly so and 15% not at all. *Gliocladium roseum*, some bacteria and other strains of *Fusarium oxysporum* were among the strongest inhibitors. When grown in media containing rhizosphere extract, pea root exudate or both, their ability to inhibit the pathogenic

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Fusarium greatly increased. Different isolates of *Gliocladium roseum* and of *Fusarium oxysporum* inhibited the pathogenic *Fusarium* to different extents, showing that they contain physiologic strains that may act differentially towards *F. oxysporum* in the rhizosphere. Although there were more micro-organisms/g. of dry soil in the rhizosphere of the wilt-susceptible variety Onward than of the wilt-resistant variety Alaska, there was no difference, either quantitative or qualitative, between the two varieties in the organisms that could inhibit the *Fusarium*. Neither those rhizosphere micro-organisms that were more prevalent during the time that *Fusarium* invades the host roots nor the ones themselves most strongly stimulated by pea-root exudate were prominent among the group most antagonistic towards the *Fusarium*. This implies that competition between pathogenic *Fusarium* and the other rhizosphere flora for nutrients in root exudates may be at least as important as overcoming antibiosis in maintaining successful growth of the pathogen near host-root surfaces. Although these results were obtained *in vitro*, they suggest that rhizosphere soil extract and microbial metabolites together deter the growth of *F. oxysporum* f. *pisi* near pea roots, and that the growth-promoting effects of root exudate from the wilt-susceptible pea variety Onward are partially offset by its ability to increase inhibition by some of the other rhizosphere inhabitants. (Buxton.)

Effects of previous cropping on pea wilt and on F. oxysporum *in naturally infested soil.* The rate physiologic races of *F. oxysporum* f. *pisi* increase in pea rhizospheres in naturally infested soil was studied by growing peas up to different ages and by growing them repeatedly in the same soil. Rates of decline of soil populations of the *Fusarium* in the absence of peas after different cropping treatments were also investigated. In rhizospheres of the wilt-susceptible variety Onward the *Fusarium* increased from 10,000/g. dry soil on 2-week-old, to 195,000/g. on 5-week-old plants, falling to 150,000/g. after 7 weeks. In the variety Alaska, which resists race (i), the corresponding figures were 6,000, 263,000 and 142,000. Of the total rhizosphere flora isolated, *Fusarium oxysporum* formed 8, 35, 54, 61, 60, 55, 32 and 61% after each week up to 8 weeks on Onward, and 12, 19, 41, 71, 59, 60, 35 and 69% on Alaska. The effect of pre-cropping with Onward or Alaska on subsequent wilt in Onward depended on the age and type of the previous crop. Wilt occurred more rapidly in soils pre-cropped with Onward than with Alaska, but wilt developed most rapidly in soils which had previously grown Onward for 2 or 6 weeks. Inoculation tests using the rhizosphere isolates showed that only a small proportion were pathogenic, indicating that the rhizosphere effect can act equally on pathogenic and saprophytic Fusaria. High soil populations of *F. oxysporum* were also produced by repeated cropping of soil with peas of either variety. From soils in which pea cropping had been repeated from once up to six times the decline in populations took 8 weeks to fall from 123,000 to 25,000/g. of dry soil whether the cropping was with Onward or Alaska. The effect on wilt and *Fusarium* of crops other than peas laid out as a four-course rotation between wheat, a root crop, barley and fallow or peas, in outdoor microplots containing naturally infested soil, is being studied over a long period. (Buxton.)

Sitka-seedling diseases

In experiments by the Chemistry Department, done in co-operation with the Research Branch of the Forestry Commission, on the growth of seedlings of Sitka (*Picea sitchensis*), difficulties have arisen from disorders not correlated with changes in levels of the applied nutrients. These troubles are partially controlled by such materials as formalin and chloropicrin, which has led to the supposition that they may be caused by soil-borne plant pathogens. To study this possibility, the development of micro-organisms on roots of Sitka seedlings taken from an experiment at Old Kennington, near Oxford (a nursery started in 1925), was examined on 15 occasions through the growing season. Three distinct diseases occurred; first, a pre-emergence and immediately post-emergence damping-off, which was followed in mid-summer by a sudden browning of the plants; during the early autumn the plants became stunted but with no discoloration. Emergence in plots treated with formalin was 44%, with chloropicrin 33% and in untreated was only 16%. Counts of browned plants later, on three occasions, showed 6·4% on formalin-treated, 10·5% on chloropicrin-treated and 24·7% on untreated plots. The autumn stunting was confined to plots treated with formalin.

All three diseases can occur on plots that have received formalin annually for the past 5 years, so any pathogenic organisms responsible may be brought in with the seed, or re-invade the soil from adjacent land or both. Plating-out seed on agar medium revealed the presence of nearly all the organisms also found on roots of plants from the field, so the rhizosphere flora may be derived from seed alone. Whether rapid re-invasion depends on adaptation in some of the soil fungi towards tolerance of high levels of formalin and chloropicrin is being studied. The prevalence of the 18 different micro-organisms found both on roots and in soil away from roots during the growing season fluctuated considerably. Striking changes occurred in *Pythium*, abundant at first but almost absent later, in *Fusarium oxysporum*, rare at first and abundant later, and in *Trichoderma viride*, which reached a peak in later August. Other fungi also changed in abundance as the season advanced. From the isolates made from the 13,200 root fragments plated out during the season, 96 fungi and bacteria were tested for pathogenicity to Sitka seedlings. Of these, *Pythium* spp. *Fusarium oxysporum* and an unidentified bacterium caused symptoms similar to those seen in the field. A higher percentage of the *Pythium* isolates made earlier in the season were pathogenic than of those made later on, and the *F. oxysporum* isolates made in mid-summer contained more pathogens than at other times. This evidence, together with the symptoms observed, strongly suggests that *Pythium* causes most of the damping-off, and that the browning is mostly caused by *F. oxysporum*. From the later samples, several isolates of *F. oxysporum* and *Fusarium roseum* stunted seedlings inoculated in the glasshouse. (Buxton.)

Sitka-seedling diseases also occur at the nurseries in Ringwood, Hants, and Wareham, Dorset. Damping-off has previously been recorded at Ringwood, but browning in the newest part of Wareham was noticed only during 1959. At Wareham plots treated with compost (made from bracken and hop waste) had many more browned plants than did fertiliser-treated plots. Plants taken from both localities had disease symptoms similar to those seen at Old Kennington, and plating out roots on agar media revealed that by far the most predominant fungus was *Fusarium oxysporum*.

In a new section at Kennington, Oxford, started in 1951, similar plots had browned plants for the first time on both compost- and fertiliser-treated plots. At both Wareham and Kennington healthy plants from all plots were examined and none had *Fusarium* on the roots except from one fertiliser-treated plot at Kennington, which had plants with roots yielding 12·5% *Fusarium*.

Analysing soils from Old Kennington and Ringwood by the dilution plate method shows that soils treated with formalin contain no *Fusarium oxysporum*, but untreated soils, in all of which seedlings develop browning, contained an average of 150,000 propagules of the fungus/g. dry soil At Ringwood one untreated area has grown Sitka seedlings for 6 years, from repeated spring sowings, without a break, and without disease. No *Fusarium oxysporum* was found in soil samples or on roots of plants from this area. Microscopic examination of roots of diseased plants shows the presence of several fungi, both as mycelium and spores. *Fusarium roseum*, *Cylindrocarpon radicicola* and *Rhizoctonia* sp. are prevalent, yet the two last-mentioned fungi are only rarely revealed by plating out roots on agar media. In addition, direct examination of soil after centrifuging suspensions and filtering micro-organisms through a Seitz filter on Oxoid membranes also shows fungi that might be missed by plating techniques. These direct observation methods are being extended and standardised. (Buxton and Ram Reddy.)

Variation in Verticillium albo-atrum *from hop*

Verticillium albo-atrum has no known sexual stage, but it seems to vary pathogenically, and to understand how it produces novel progeny work was begun to see whether genetic recombination occurs without sex. Both dark and hyaline wild-type strains of the fungus can be isolated from infected hop plants, and cultures established from single hyphal tips yield conidia that produce either dark or hyaline colonies. Other authors have demonstrated that over 98% of the conidia produced by their isolates were uninucleate, so the hyphal tips seemed likely to be heterokaryotic, but the term should not be applied until it has been confirmed that colour is determined by the nucleus. It was therefore necessary to synthesise heterokaryons composed of strains labelled with known nuclear markers, and to determine whether heterokaryosis would recombine colour with these markers.

Nutritionally deficient mutants were isolated after exposing cultures to ultraviolet radiation. The mutants were characterised by replica plating and auxanography, and almost all the conidia were uninucleate. When conidia of a hyaline strain requiring methionine and biotin were mixed with spores from a black isolate that required adenine, and incubated on Czapek–Dox agar medium which supplied none of these compounds, the mixed inoculum produced slow-growing sectors. Some hyphal tips taken from these areas produced conidia which required either adenine or both methionine and biotin, but a sample of about 1,000 spores contained none able to grow on unaugmented media. The double auxotrophs were always hyaline like the parent, but the adenine requirers from the heterokaryon varied from light grey to black, a greater colour range than was observed in single-spore isolates from the original pure culture of the adenine-dependent strain. The results confirmed that heterokaryosis occurred, but did not prove that the colour was controlled at nuclear loci.

A sample of several million conidia from the heterokaryon included a few able to grow on unaugmented Czapek–Dox agar (prototrophs). If these colonies arose from heterokaryotic conidia they would be expected to produce further heterokaryotic spores at the low frequency observed in the first selection. However, they produced only prototrophic conidia when a spore suspension was spread on Czapek–Dox, but a similar sample of spores incubated on a weakly augmented medium produced an equal number of wild-type colonies; they also produced a few which were slow growing. These had nutritional requirements and included some recombinant phenotypes. By using such a selective system all three nutritional markers were recovered. Because they all came, indirectly, from a single prototrophic conidium which was almost certainly uninucleate a heterozygous diploid phase could be inferred, suggesting that a system resembling parasexuality allows genetic recombination in *V. albo-atrum*.

Certain adenine-requiring mutants rarely produce aerial conidiophores unless they are grown on a medium containing a high adenine concentration. These produce conidia in pionnotes, and it can be inferred that this is a pleiotrophic effect of mutations which block adenine synthesis at a stage preceding the synthesis of inosine. There was no evidence in the parental strain of morphological variation directly attributable to mutations affecting methionine and biotin synthesis, but these mutations may have such effects when combined with other genes, and this may account for certain recombinants having characters common to *Cephalosporium* species. (Buxton and Hastie.)

Mycofloral succession in hay

Work on the mycoflora of hay was restricted to the study of the air-borne fraction, as inhalation of spores appears to be the cause of certain human and animal diseases associated with mouldy hay. This fraction is assessed by shaking a known weight of hay for 3 minutes at a wind speed of about 4 metres/second in a small wind tunnel. The spore-laden air is sampled by several methods. The Cascade Impactor gives a visual estimate of the total number of spores (expressed as millions/g. dry weight of hay). Viable fungi, actinomycetes and bacteria are isolated in culture with the Henderson Impinger or the Andersen Sampler. Malt and nutrient agar plates are incubated at both 24° C. and 40° C.

Samples of hay and straw from different parts of the country were examined for their spore content. Good hays had as few as 0·4 million spores/g.; poorer hays had up to 20 million *Aspergillus* *glaucus* spores. Hays associated with human and animal diseases had up to 85 million fungus spores or 1,500 million bacteria and actinomycete spores/g. The fungi present in these bad hays are generally thermophilic species, such as *Absidia ramosa*, *Mucor pusillus*, *Aspergillus fumigatus* and *Monotospora lanuginosa*.

Farmers know that, when hay is baled too wet (moisture content much more than 20%), it will become hot, lose moisture and go mouldy. There is also some rise in temperature during the normal maturation of hay. To study the succession of the microbial flora on hay during maturation, hay from the mixed ley on New Zealand field at Rothamsted was baled at different moisture contents. After baling at 42% moisture the temperature rose to over 60° C. in 3 days and remained steady for over a week, during which moisture was lost, fungi, including *Aspergillus fumigatus* and *Absidia ramosa*, developed up to 6M spores/g. and actinomycetes and bacteria up to 500 M. Baled at 28% temperatures rose to over 40° C. and 40 M *Aspergillus glaucus* spores developed. Baled at 16% moisture it reached 30° C., but very few spores of any kind developed. (Gregory and Bunce.)