ROTHAMSTED RESEARCH

Report for 1960

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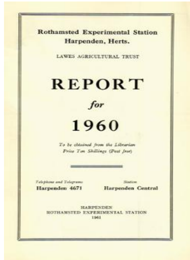
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Plant Pathology Department

P. H. Gregory

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PLANT PATHOLOGY DEPARTMENT

P. H. GREGORY

On 30 September Mary D. Glynne retired after 43 years on the staff of Rothamsted; she is continuing in part-time work in the Department. Her services to agriculture were honoured by the award of the O.B.E.

R. C. Sinha left after gaining the Ph.D. degree of London University, and is now research associate in the Botany Department of the University of Illinois. The following joined us as temporary workers: P. Babos (Birmingham and Mytilene), M. V. Carter (Waite Institute, Adelaide), R. C. Close (Christchurch, New Zealand), R. Kisimoto (Zentsuji, Japan).

A. Kleczkowski attended the 3rd International Congress on Photobiology, Copenhagen, and spent six weeks at Wageningen at the invitation of the late Professor T. H. Thung. B. Kassanis, B. D. Harrison and A. J. Gibbs attended the 4th Conference on Potato Virus Diseases at Brunswick. B. Kassanis was also invited to lecture by the Board of the Agricultural University of Wageningen. B. D. Harrison spent two weeks in Portugal studying virus diseases of the grape-vine. E. W. Buxton was seconded for one year to the United Fruit Company Central Research Laboratories, Norwood, Mass., and spent some time visiting banana plantations in Central America. J. M. Hirst visited New Zealand at the invitation of the Department of Agriculture to advise on research into the ecology of the fungus causing facial eczema in sheep. On his return journey he visited the New South Wales Department of Agriculture, and the Rubber Research Institutes at Kuala Lumpur, Malaya, and Agalawatta, Ceylon.

VIRUSES AND VIRUS DISEASES

Disaggregation and reaggregation of tobacco mosaic virus protein

When purified protein from tobacco mosaic virus (TMV) is disaggregated at pH values over 6·5 it behaves like a mixture of at least seven different antigens. But when reaggregated at pH values below 6·5 it behaves as a serologically homogeneous material, as shown by gel-diffusion precipitin tests, and also by qualitative and quantitative precipitin tests in liquid. Some workers have claimed that a determinant is lost when the virus is broken up into protein and nucleic acid, and is regained when the protein is reaggregated into virus-like particles; although these claims seemed to be supported by some results obtained in the early stages of this work they were finally not confirmed. This cannot, however, be taken as conclusive evidence against the loss and gain of an antigen, because results may depend on the quality of the antiserum used in the tests. (A. Kleczkowski.)

Interaction of viruses

Components of tobacco necrosis virus. The " Rothamsted culture " of tobacco necrosis virus is unique among spherical plant viruses because it consists of large and small particles whose relationship was not understood. We now find that the two sizes of particle are two serologically unrelated viruses, with the peculiar relationship that, whereas the large particles multiply unaided, the small particles multiply only in the presence of the large ones.

Some of the single-lesion isolates from the " Rothamsted culture " gave only large particles, but none gave small particles only. The small particles were separated from the large by centrifuging partially purified preparations in sucrose gradient columns. Three light-scattering zones usually formed during this centrifugation: the top contained the small particles (21 mμ diameter), which alone were not infective, the middle zone contained the large particles (28 mμ), which alone were infective, and the bottom zone (when formed) contained small particles aggregated in groups of twelve. When inoculated together with the large particles, the small particles from the top zone multiplied and the lesions were smaller than those produced by the large particles alone. The number of small lesions produced depended on the ratio of small to large particles in the inoculum. (Kassanis and Nixon.)

Potato aucuba mosaic virus. The twelve strains of potato aucuba virus studied differed in their ability to be transmitted by *Myzus persicae*. No strain was transmitted from plants infected with this virus alone, but some strains were transmitted readily and others occasionally from plants which were also infected with either potato virus A or Y. Some strains were transmitted only from plants also infected with potato virus Y. Although the concentration of potato aucuba mosaic virus in the host is influenced by the presence of viruses A or Y, there is no correlation between its concentration and its transmissibility by aphids. (Kassanis.)

Tissue cultures

When grown under light for 18 hours daily in media containing 2% sucrose, cultures of " tobacco conditioned callus tissue " are white, but when the sugar in the medium is gradually decreased to 0·25% they become green. The phenomenon is reversible; the greenness disappears when the tissues are recultured in high sugar concentration. Tissues infected with tobacco mosaic virus, which had been kept for many years at 2% sugar, become green and their virus content decreases after a few recultures in media containing little sugar. (Kassanis.)

Lucerne mosaic virus

Purified preparations of lucerne mosaic virus contain bacilliform-like particles, quite unlike any other known virus in shape and apparent structure. Electron micrograms of mounts in neutral phosphotungstate show particles 18 mμ wide and of various lengths. All particles had the same electrophoretic mobility, serological specificity and nucleic acid content, but they differed in their sedimentation rates. Most of the particles were either 36, 48 or 58 mμ long, and correspond with the components having sedimentation rates of 73 s, 89 s and 99 s reported by other workers for this virus. There was no evidence that particles of different lengths differed in infectivity. (Gibbs, Nixon and Woods.)

Three strains of lucerne mosaic virus were isolated, and all were transmissible by *Myzus persicae*, *Neomyzus circumflexus* and *Acyrthosiphum pisum*. Two of these (strain I avirulent and II necrotic to *Nicotiana xanthi*) caused necrotic local lesions when manually inoculated to French bean var. Prince. Strain III produced necrotic ringspots on *N. xanthi* and systemic symptoms instead of local lesions on bean. *M. persicae* was more successful than *A. pisum* as a vector of strain II, and both vectors transmitted best when given a period of fasting followed by 2 minutes' feeding on infected leaves. (Attafuah.)

Potato virus diseases

Paracrinkle virus in King Edward potatoes. In a survey for paracrinkle virus in commercial stocks of King Edward potatoes all tubers tested proved to be infected, some with paracrinkle virus alone, and some also with potato virus S. The strains of paracrinkle virus differ in virulence towards the variety Arran Victory and in their ability to be transmitted by *Myzus persicae*. Aphid-transmitted strains were common in all the commerical stocks of King Edward tested.

The virus-free stock of King Edward, produced by excising the apical meristem of a potato sprout as reported in 1955, has been increased in Northern Ireland by the National Institute of Agricultural Botany, and stocks are now large enough to start supplying potato-seed growers. In 1958 the virus-free stock was compared with seven commercial stocks in a trial organised by Mr. J. C. Cullen in different parts of England and outyielded them on average by 10%. (Kassanis.)

Control by insecticides. In co-operation with the Insecticides Department a trial was started at Efford Horticultural Station, Lymington, on the control of potato leaf and rugose mosaic by insecticides, including one (a granular formulation of "Rogor" prepared by Pest Control Ltd.) applied to the soil. Their effects on virus spread will not be known until tubers from the plots are grown in 1961, but the soil insecticides kept the plants free from aphids throughout the growing season, whereas a DDT spray did not. Aphids will be reared in the spring on tubers from the treated plants to detect any insecticidal residues. (Heathcote and Burt.)

Effect of age of infector on virus spread. Pots containing young potato plants infected with leaf roll or Y viruses were sunk in ridges of a potato crop (replacing healthy plants) at the end of May. In mid-July and mid-August they were moved to new sites, and at the same time younger infected plants were exposed at other sites. Tubers from the initially healthy plants adjacent to each infector were taken to detect spread of virus. Many plants adjacent to infectors exposed from 26 May to 14 July became infected, 83% with leaf roll and 78% with virus Y (including 6% leaf roll and 25% Y acquired from sources other than the adjacent infector). As in

1957, viruses spread little later in the season, regardless of the age of the infector plants. (Heathcote.)

Soil-borne viruses

Arabis mosaic virus. The ecology of the nematode vector, *Xiphinema diversicaudatum*, in relation to outbreaks of arabis mosaic virus, was studied in collaboration with R. D. Winslow (Nematology Department). The highest populations of the nematode were found in soil from woodlands and hedgerows. The virus was isolated from wild trees, and an outbreak in strawberry was seen on land newly taken into cultivation. Arabis mosaic virus was obtained from almost all sites where *X. diversicaudatum* was found, but the proportion of infective nematodes at different localities varied greatly. Males, females and larvae could all transmit the virus, and adults transmitted after being denied access to plants for a few weeks, indicating that the virus persists for long periods in the nematodes. (Harrison.)

Tomato black ring virus. Further work, in collaboration with the Scottish Horticultural Research Institute, showed that field outbreaks of the beet ringspot strain of this virus in raspberry and strawberry are associated with the presence in soil of the nematode *Longidorus elongatus*. The virus was transmitted to sugar-beet and spinach seedlings by larvae (but not by adults in the few experiments when they were tested separately) of *L. elongatus* hand-picked from virus-infested soil. (Harrison.)

Tobacco rattle virus. Sandy soil, taken from a field of potatoes severely affected by spraing disease, proved to be infested with tobacco rattle virus, which is thought to cause the disease. In tests with nematodes extracted from this soil, the rattle virus was transmitted by *Trichodorus primitivus*. (Harrison.)

Carrot motley dwarf virus

Many samples of carrots with " purple top " from different parts of England were tested, and all contained carrot motley dwarf virus. Previously we had been able to transmit the virus mechanically only after it had been transmitted by aphids to some host outside the *Umbelliferae*. We now find that it is transmissible from carrots by manual inoculation provided the clarified sap is buffered at pH7; it is also transmissible in water–phenol extracts that presumably contain mostly virus nucleic acid. The infection end-points with both kinds of preparation are around 1/1,000. (M. A. Watson, Sinha and Serjeant.)

In a field experiment at Woburn spraying with " Metasystox " on 13–14 June and again on 4 July decreased infection of carrots with motley dwarf virus by 21% and increased yield of roots by 2·02 ± 0·526 tons/acre. An earlier spraying on 26 May, soon after germination, had no effect (the virus entered the crop much later than in 1959, when all spray treatments were given too late). The vectors (*Cavariella aegopodiae*) migrated in large numbers from the beginning of June onwards, but were slow in establishing on the plants, probably because of heavy thunderstorms. Plant number, and apparently plant nutrition, varied across the experimental area, but had no effect on percentage infection or yield of roots; 75% of the variance in total fresh weight was accounted for by the regression on the percentage of plants infected. (M. A. Watson and Serjeant.)

Barley yellow dwarf

When the virulent barley yellow dwarf virus " B " (which inhibits flowering and is transmissible by *Rhopalosiphum padi*, *Sitobium avenae* and *S. fragariae*) was inoculated to oat seedlings, together with the avirulent strain " X " (which does not alter plant habit, is transmitted only by the *Sitobium* spp., and produces characteristic red discoloration of foliage), the two viruses remain together in the plants for at least 3 months, and could be isolated by their vectors with little change in their proportions. But when the two viruses were inoculated to the plants in succession—one 14 days after the other—the first virus introduced gradually established supremacy over the other and, though they could be re-isolated easily within 30 days of the second inoculation, the challenging virus was rarely obtained after 60 days, even if it was the more virulent virus and had produced symptoms.

Rhopalosiphum padi, which was unable to transmit the original " X ", could transmit avirulent viruses resembling " X " from plants with mixed infection. These avirulent forms came particularly from plants infected by *S. fragariae* that had fed on a leaf infected with both viruses. (M. A. Watson.)

True broad bean mosaic

Plants infected with this seed-borne virus were found in crops of broad beans in several places in southern England. Most crops had 0·1% or less of plants infected, but a few crops had up to 10%. The spatial distribution of infected plants in the field, and the time of appearance of the symptoms, suggested that all the primary infections were from the seed, which is imported principally from North Africa. In late-sown crops secondary spread was observed over distances of 25 yards. Broad bean plants infected late in the season yielded as much seed as healthy plants, but early infection decreased the number of pods per plant and lowered the yield of seed by 20%.

Purified preparations of the virus contained " spherical " particles about 28 mμ in diameter, similar in appearance to those of the soil-borne ringspot viruses or red clover mottle virus. An antiserum prepared against true broad bean mosaic did not react with any of these viruses. Attempts to transmit the virus by *Xiphinema diversicaudatum*, *Ditylenchus dipsaci* (giant race) and *Sitona* spp. failed. (Gibbs.)

Spread of legume viruses in field bean crops

Use was made of experiments by M. J. Way (Insecticides Department) at Rothamsted and Woburn to compare the natural spread of legume viruses in plots of field beans sown at widely different seed rates. Results resembled those obtained in 1959. There was no significant difference between the number of virus-infected plants in insecticide-sprayed and unsprayed plots. Infection decreased with increased seed rate: for example, at Rothamsted on 27 June, plots sown with 50 lb./acre had 2·7% plants with leaf-roll, and plots sown with 600 lb./acre only 0·1% leaf-roll. Leaf-roll, caused by a persistent virus, was the commonest disease early in the season, but the "non-persistent" mosaic virus spread later in the season. (Heathcote.)

In an experiment at Rothamsted with field beans sown on three occasions, 9 March, 27 April and 3 May, leaf-roll spread early in the season and mosaic later, the number of infections increasing with later sowing dates. Some rows were regularly treated with demeton methyl without affecting virus spread. Losses of yield depended on time of infection; early infection by leaf-roll decreased yield by over 75%, but late infection with mosaic had no appreciable effect. (Heathcote and Gibbs.)

Fungi and Fungus Diseases

Diseases of cereals

The six-course rotation experiment. In this experiment, begun in 1930 and ended in 1960, six crops were grown each year in the sequence: potatoes, rye, sugar beet, barley, clover, wheat. Annual surveys in winter wheat (variety Yeoman) in the last 24 years showed a trace of take-all (*Ophiobolus graminis*), with an annual mean of less than 1% of plants with lightly infected seminal roots. Eyespot (*Cercosporella herpotrichoides*) occurred each year, more than half the straws being infected in 5 out of 20 years (1938–57) (whereas in the four-course rotation experiment—potatoes, barley, ryegrass, wheat—this level was reached in 9 of the same 20 years). Eyespot was least in 1947 (2·3%), and most severe in 1958, when 98·7% of the straws were infected so that the entire crop lodged. In the few years when lodging occurred it was associated either with unusually severe eyespot or high yield of straw.

Barley and rye both became lightly infected by eyespot, but had fewer infected straws and fewer severe lesions than winter wheat. The disease appeared to have a negligible effect on barley and rye, though both helped to carry it through the rotation to subsequent crops. (Glynne.)

Effect of previous crops on winter wheat. Effects of date of sowing, seedrate and nitrogen were compared in two adjacent experiments in which Cappelle wheat was grown after two non-cereal crops (beans and potatoes) and after three cereal crops (spring wheat, barley, winter wheat). Sown at 2 and 4 bushels/acre on 2 October, 21 October, 23 November and 18 December, yields of wheat after potatoes were respectively 51·4, 46·0, 47·6 and 47·7 cwt/acre, with 34, 79, 60 and 20% of their areas lodged and with very little eyespot or take-all. Wheat following wheat, however, yielded only 32·1, 33·3, 34·6 and 33·0 cwt./acre, and in May about 34% of the plants were affected by take-all and 20% by eyespot. Seedrate had no effect on mean yields, but in both experiments the optimum seed-rate depended on the sowing date; 4 bushels/acre yielded significantly less than 2 bushels at the second sowing, but more than 2 bushels at the last sowing.

Increase in the amount of nitrogen applied as "Nitro-Chalk" in spring from 0·46 to 0·92 cwt.N/acre had no significant effect on yield of wheat after potatoes, and increased the area lodged from 33 to 64%. But the extra nitrogen added 6 cwt./acre to the mean yield of wheat after wheat, with no lodging; the effect was greatest in the plots sown on 2 October and at the lower seedrate. (Glynne and Slope.)

Effect of missing rows on non-parasitic lodging. Cappelle wheat was grown on fertile land which had been freed from the fungi causing eyespot and take-all by growing non-susceptible crops for two years. The drill was set to sow 2 and 4 bushels (R1 and R2) seed/acre at 7 inches spacing with all rows sown (D1), one row in four blocked (D3) and alternate rows blocked (D2). "Nitro-Chalk" was applied in spring at 0·5 (N1), 1·0 (N2), 1·5 (N3) cwt. N/acre. A clear pattern of lodging was apparent by the third week in June, when 84% of the area was lodged in D1 R2 N3, about half that area in D1 R2 N2, D1 R1 N3 and D3 R2 N3, so showing a similar decrease in lodging produced by omitting one dose of N, by halving the seedrate, or by missing one row in four. There was then little lodging in other plots, but prolonged wet weather, which delayed harvest till 31 August, led to lodging in all plots and decreased yield to an average of only 37·1 cwt./acre. Each dose of nitrogen increased lodging and decreased yield. Four bushels of seed produced less grain, with more lodging, than 2 bushels. The highest yield, 43·8 cwt./acre, was obtained with all rows sown at 2 bushels/acre, with least nitrogen. At this seedrate missing rows lowered yield, and missing two rows yielded less than missing one. When sown at 4 bushels, missing rows had little effect on yield at N1 and N2, but at N3 missing rows increased yield, D1, D3, D2 yielding respectively 30·3, 36·6 and 36·0 cwt./acre. D3 sown at 2 bushels yielded less than D2, but sown at 4 bushels yielded more. With a seedrate of 2 bushels/acre, 7-inch spacing yielded about 3 cwt./acre more than 14-inch spacing, but the effect of the wide spacing was lessened by increasing the amount of nitrogen. (Glynne and Slope.)

Broadbalk, 1960. On plots 2B, 3 and 7, the 1st, 2nd, 3rd, 4th and 9th consecutive wheat crops after 1 year fallow had respectively: 70, 51, 81, 79 and 47% straws with the eyespot fungus, of which 35, 28, 56, 54 and 26% had severe lesions; 0, 11, 17, 27 and 21% straws had take-all on the roots. It is difficult to explain the unusually high incidence of eyespot in the first crop after fallow, especially as at the end of March the same crops had only 4, 10, 26, 20 and 14% plants with eyespot. (Cox.)

Studies of soil microflora are being continued to explain the unexpectedly small amount of take-all on Broadbalk despite continuous cropping with wheat. No correlation was apparent between the total numbers of fungi or bacteria in either the soil or rhizosphere, and the incidence of take-all in the Broadbalk, Wheat and Fallow, and Cereal–Beans experiments. The low incidence on Broadbalk seems to be caused by factors preventing the development of the disease *after* infection has taken place. On the unmanured plot of Broadbalk, 21% of plants were infected on 28 March in the fourth successive wheat crop, but at harvest only 19% straws showed any crown-root infection. By contrast on the 4th wheat crop in the Cereal–Beans experiment, 59% of plants were

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infected on 5 April, and at harvest 73% of straws had severe crown-root infection. The numbers of infected roots per plant were 1·5 on Broadbalk and 1·9 on the Cereal–Beans Experiment at the end of March, but 1·8 and 4·1 respectively at the end of May. (Cox.)

Cereal–Beans rotation. In the fourth and final year of the first series of this experiment, Cappelle winter wheat was grown after the following crop sequences: W Ws W, B W B, sW W sW, O W sW, W O sW, and W O Be (using the abbreviations: W = winter; sW = spring wheat; B = spring barley; O = spring oats; Be = winter beans). After these respective sequences plots yielded 27·5, 34·2, 26·8, 25·0, 20·0 and 49·9 cwt. grain/acre; they had respectively 77, 63, 79, 85, 87 and 7% of straws with take-all at harvest. Two years with the non-susceptible crop, oats and beans (sequence W O Be) nearly eliminated take-all, and wheat after this sequence yielded twice as much as after a susceptible crop. However, growing a non-susceptible crop of oats in 1957 and 1958 (sequences O W sW and W O sW) had no beneficial effect in 1960—in fact, there was more take-all and less yield after these sequences than after continuous wheat.

Eyespot was not prevalent; its incidence was less than 10% after all these sequences, and less than 1% after oats–beans. The incidence of Sharp Eyespot was unusually high (mean 59·3% of straws infected), but there was no difference between the six crop sequences. (Slope.)

Cephalosporium stripe. In mid-June occasional wheat plants with yellow-striped leaves were found in several field at Rothamsted. Dr. L. J. Tyler (visiting from Cornell University) diagnosed the symptoms as stripe caused by *Cephalosporium gramineum*. A *Cephalosporium* was isolated from affected leaves, nodes and internodes, but the isolates have not yet been tested by inoculation. (Slope.)

Potato diseases

The study of how potato blight epidemics originate, of haulm resistance and of control by spraying, have all stressed the need for more information about tuber infection and the activity of *Phytophthora infestans* in soil. This has now become a major aim of our work, and the weather of 1960 was unusually suitable for the study.

Development of potato blight in 1960. The first 3 months after planting were warm and dry and potatoes grew slowly. After the end of June frequent rain encouraged rapid growth of haulm and tubers, so that yields were potentially high, despite tubers forming somewhat late. Blight was first found occurring naturally in an invaded stem on a King Edward plant in a spraying experiment on 24 July, by mid-September the haulm was completely killed. Zineb (Z) or copper oxychloride (C) were applied on four dates (14, 25 July, 13, 27 August). The total yield from spray with an early application of Zineb (Z, C, C, —) was 18·46 tons/acre, compared with 14·43 tons/acre from unsprayed plots (—, —, —, —). Plots with sprays (—, C, C, —) and (—, Z, C, C) yielded respectively 16·66 and 16·76 tons/acre. Initially there were large differences between treatments in tuber infection, but these differences eventually disappeared with just over 25% of tubers infected in all treatments. (Hirst and Stedman.)

Effect of nitrogenous fertilisers on blight. An unsuccessful attempt was made to confirm Lowings's (*Trans. Brit. mycol. Soc.* 1959, **42**, 491–501) observations of how the amount and timing of nitrogenous fertilisers may affect the development of potato blight. Unsprayed Majestic plots received similar treatments to those used by Lowings, but differences in the rate the foliage was destroyed were small and inconsistent, and they seem unlikely to have had any effect on yield or to offer hope of a useful and reliable control practice. (Hirst and Stedman.)

Haulm and tuber resistance to Phytophthora infestans. In a field experiment the haulm resistance of Pimpernel (the most resistant commercial variety in laboratory tests in 1959) was compared with Ackersegen, Majestic and Arran Viking. Majestic and Arran Viking leaflets were half killed 15 days sooner than comparable leaflets of Pimpernel and ten days sooner than Ackersegen. Pimpernel was defoliated more slowly because leaflets were infected more slowly and also died more slowly after infection. Sporulation differed significantly on the four varieties: the mean width of the sporing annulus, measured in the field for 13 days, was 3·2, 2·1, 1·7 and 1·1 mm. for Majestic, Arran Viking, Ackersegen and Pimpernel respectively.

Work was begun to assess the acuracy of estimating haulm resistance from a single chemical test—peroxidase activity. Of 41 clones tested the 10 Mexican selections of Niederhauser, reported to have high levels of " field resistance ", had the greatest peroxidase activity.

In laboratory tests wound-free tubers (grown in peat overlying soil in large porcelain pots) were infected through eyes and lenticels by spraying the tuber surface with inoculum. Preliminary tests showed that Arran Banner and Ulster Ensign were more readily infected by this method than Up-to-Date and King Edward, and that infections on Arran Viking and Majestic tubers were often arrested at an early stage.

Infection of tubers in the field was studied in plots of the tuber-susceptible varieties Up-to-Date, King Edward, Arran Banner and Ulster Ensign, arranged in a 4×4 Latin square. Two rows of Majestic, planted between each column of plots, were inoculated on 4 July to ensure that blight arrived in all plots at the same time. Samples were taken each week from predetermined areas within plots and tested in two ways. The tubers were lifted at once from half the plant; the haulms were removed from the other, when the ridge was covered with " Polythene " sheeting and the tubers allowed to " incubate " for 5 days in the ground before lifting. The first blighted tubers were found in the " incubated " samples of all varieties lifted on 3 August; these must have become infected when less than 5% of the haulm was blighted. The numbers of infected tubers from later samples increased after periods of rain, and most new infections occurred after rain which fell when the haulm was about 50% defoliated.

Most infections on Arran Banner, King Edward and Up-to-Date occurred at the rose end or on the body of the tuber, and were

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associated about equally with eyes or lenticels (though in King Edward the eyes were invaded rather more often than lenticels). On Ulster Ensign most infections were in lenticels on the body of the tuber. During the epidemic the fungus was found sporing from lenticels and eyes of blighted tubers of these varieties while still in the soil.

Blight susceptibility of haulm and tuber in different genotypes. In co-operation with the John Innes Institute, the differential reactions of haulm and tuber were studied in genotypes based on genes R1, R2, R3 and R4 to six races of *Phytophthora infestans*. The races either caused a hypersensitive reaction on leaves of the clone or could infect and spread in their leaves. Gene R1 conferred to the tuber a hypersensitivity comparable with that in the leaves, whereas genes R2, R3 and R4 often did not. Races giving a hypersensitive reaction in the leaves of R2, R3 and R4 clones grew in the tubers, although more slowly than in clones without these genes. Tubers of most clones, except Orion (R1) and Reaal (R1R3), were highly susceptible, often more so than King Edward, to races of the fungus which could spread in their leaves. (Lapwood.)

Activity of Phytophthora infestans *in soil.* Unless haulm is destroyed very early, usually with some sacrifice of yield, a proportion of growing tubers usually becomes blighted in the soil. A method of assessing the concentration of *P. infestans* spores in the soil is needed to study conditions and routes by which tubers in soil are infected from the haulm. Plating soil dilutions on media containing antibiotics proved unsatisfactory, as did attempts to separate sporangia by preferential movement in an oil/water emulsion. The use of tuber-tissue as a selective medium to " bait " the fungus out of soil is a promising method. Slices 5 mm. thick from washed tubers are covered with 0·75 ml. of soil, wetted and stored in humid chambers at 20°. After 24 hours each slice was cut radially into octants, and a week later octants bearing sporangia of *P. infestans* were counted. The method was calibrated with soil to which known numbers of sporangia had been added. After applying the multiple-infection transformation, there was a significant linear relation between the number of spores per slice and the number of octants infected; 1,000 sporangia/slice caused about 150 infections per 100 octants (or about 78% infection); 50% of the octants were infected by about 500 sporangia/slice. The method should be capable of detecting as few as 10 sporangia/ml. of soil.

In 1960 the technique was used to estimate spore concentration at various depths from the crest of the ridge beneath Majestic potato plants with blight on the haulm. When approximately half the crop had been destroyed the concentration was estimated at 2,000/ml. in the surface soil, 440 at 2 inches and 50 at 4 inches. (Lacey.)

Aerobiology

Black rust of wheat. The number of airborne *Puccinia graminis* uredospores within spring wheat crops often increased at the start of, or an intensification of, rain. Similar behaviour has been noticed previously for spores of *Cladosporium*. (Hirst and Stedman.)

Release of ascospores during dew. Reports on the importance of wetting by dew in release of ascospores of certain Pyrenomycetes are conflicting. To assess " dew-release " of ascospores of *Mycosphaerella pinodes*, about 50 g. of dry pea haulm, heavily infected during summer of 1960, was placed on a frame carried upwind of the orifice of a Hirst spore trap (from which the rain-shield had been removed) and exposed repeatedly to dews during the autumn. Dew records were made with a Surface Wetness Recorder Mk. III beside the spore trap. With the sample 0·5 m. above ground level, and between 10 and 50 cm. from the trap orifice, ascospores were caught on each of eight nights when a full-scale dew reading was shown on the Wetness Recorder. Release began when the trace was half-way up the scale, usually reached a maximum some 3 hours later and declined to zero during the next 6 hours—usually before drying had begun.

The rainfall equivalent to a half-scale deflection is calculated as 0·009 inch. (The amount of rain required to produce measurable ascospore release from the perithecia of *M. pinodes* in wind-tunnel tests at the Waite Institute, Adelaide, has been estimated as 0·007 inch.) (Carter.)

Calibration of whirling-arm air sampler. The Perkins " Rotorod " sampler is claimed to be over 90% efficient for collecting airborne particles in the 20-μ range. A sampler of this design, powered by a 6-volt E.M.I. motor, was calibrated in the wind-tunnel against the isokinetically operated Cascade Impactor with ascospores of *Mycosphaerella pinodes* (12–15 $\times$ 6–8 μ). In a turbulent air-stream efficiency depends on wind speed and varies from 50% of Cascade Impactor efficiency at 1–2 m./sec. to 90% at 4–5 m./sec.

In similar tests with spores of *Lycopodium clavatum* (30 μ) the rod sampler showed a maximum of 80–85% efficiency at 1·5 m./sec. Efficiency declined with increasing wind speed to 60–65% at 9 m./sec. Comprehensive tests with a range of spore sizes are clearly needed so that the " Rotorod " sampler can be used in the field. (Carter.)

Clubroot of Brassicae

In attempts to free *Plasmodiophora brassicae* from contaminating organisms to facilitate physiological studies, young galls were incubated temporarily in solutions containing penicillin and streptomycin in high concentration, and, after washing, inoculated at various dilutions to young cabbage seedlings. After a week the plants were transferred to meat infusion for a few days to detect contaminants. A small proportion of apparently bacteria-free seedlings, each with a few root hair infections, was occasionally obtained. So far, however, a contaminant-free club has not been grown, apparently because of the difficulty of growing enough good plants aseptically. (Macfarlane.)

Fusarium wilt of peas

F^4 lines derived from the second back-cross between the garden pea varieties Greenfeast (syn. Lincoln) and New Era, brought from the Waite Institute, South Australia, were grown at Coggeshall, Essex, in a field heavily infected with *Fusarium oxysporum* f. *pisi* race 1. Of seven lines selected for homozygosity of resistance to South

Australian race 2 of the wilt fungus, six were fully resistant at Coggeshall, and a seventh showed a uniform partial wilting.

An F2 population of 99 plants from the above cross, inoculated in the glasshouse during the summer with an isolate of race 1 taken from wilted Onward plants at Coggeshall, failed to segregate any susceptible plants, although Onward control plants wilted readily. This suggests that the parental strain of Greenfeast (selected in New Zealand for resistance to race 1) shares with New Era a common or closely linked gene for resistance to this race of *F. oxysporum* f. *pisi*. (Carter.)

Variation in Verticillium albo-atrum

The investigation of genetic recombination in *Verticillium albo-atrum* was continued. Using nutritional markers and differences in acriflavine tolerance showed that recombinant genotypes were produced much more frequently than usually observed in other imperfect fungi. Conidia of *V. albo-atrum* are almost always uninucleate, so that novel genotypes formed during development of a colony were readily detected by determining the phenotypes of the spores which the colony produced.

About one per million of the conidia produced in a mixed culture of two complementary auxotrophic strains were prototrophic. These were later shown to be prototrophic, because they contained heterozygous diploid nuclei and the nutritional requirements were recessive to their respective wild type alleles. The selected diploid nuclei were heterozygous at four loci. Samples of conidia harvested from 10-day-old cultures initiated by single heterozygous uninucleate conidia contained fourteen of these sixteen possible classes.

The two remaining classes were not found in a random sample of 2,000 spores isolated directly from cultures segregating for all four markers (i.e., among first-order segregants). However, about 2% of these first-order segregants were still heterozygous with respect to one or more of the marked loci, and a sample of over 2,000 conidia from heterozygous first-order segregants included many spores with the phenotypes not found previously. This delay in the production of certain recombinant classes can be interpreted as an effect of genetic linkage, and the evidence gathered from the analysis of heterozygous first order-segregant nuclei indicated that the four markers were located in one linkage group.

Reciprocal phenotypes tended to be produced as often as expected if the recombinant genotypes were produced at meiotic divisions. However, this expectation was not supported by a further statistical analysis, and the recombinants were probably produced at irregular mitotic divisions as in the parasexual cycle.

V. albo-atrum occurs naturally in two colour forms. Most isolates produce black torulose hyphae, but strains unable to do so are often isolated from them. Previous work (*Rep. Rothamst. exp. Sta.* for 1959) suggested that cytoplasmic factors are involved in the inheritance and/or expression of the black character. The heterozygous lines selected for studying genetic recombination were established by mating black and white hyaline auxotrophs. These heterozygotes, and over 5,000 isolates from them, all formed some black torulose hyphae on augmented prune extract agar medium, and none was identical with the hyaline parent. Therefore, the colour difference was not determined at a single chromosomal locus. Although the evidence does not exclude the possibility of it being inherited as a polygenic character, cytoplasmic factors affecting expression and perhaps inheritance still need to be invoked.

The development of black hyphae depended strikingly on incubation temperature. Heterozygous isolates, and recombinants derived from them, were examined after incubation for 2–3 weeks at 23°, 20°, and 15° on augmented prune extract medium. The optimum temperature for growth of *V. albo-atrum* on this medium is 23°, and all isolates so tested formed intense black hyphae, whereas all replicates at 20° were hyaline. The cultures at 15° contrasted with the previous two series in showing, with one exception, enormous variation in colour between replicates of each isolate. The exceptional isolate was a single auxotroph requiring only adenine, and this was the nutritional phenotype of the original black parental culture. All replicates of this isolate incubated at 18° were black. These observations do not allow any conclusion to be drawn about the inheritance of the colour difference, but emphasise the need for a precisely defined environment for determining an isolate's capacity to produce black hyphae.

The two auxotrophs crossed in studying genetic recombination were both derived from parental strains causing fluctuating hop-wilt, but none of the recombinants tested showed enhanced virulence towards hops of the variety Fuggle. Only one of the parental strains wilted tomatoes (variety Klondine Red), and this only mildly, but a few of the stable prototrophic recombinants recovered from the unstable heterozygous lines caused much more severe wilt in tomato. (Hastie.)

Mycofloral succession in hay

During 1960 hay was again baled experimentally at different moisture contents to study the mycofloral succession. Attention was concentrated on hay with the kind of moulding previously found to be associated with "farmer's lung". Hay from Great Field, baled at a moisture content of 46%, behaved in the same way as hay from New Zealand Field in 1959 which had been baled with 42% moisture. The temperature rose to 60° in 3 days, by the 11th day it was still 55°, but by the 21st day it had fallen to 20°, after which it remained more or less steady near the mean air temperature. The moisture content fell gradually from the start, and by the 16th day it had reached 20%. During this period the mould content increased to about 10 million spores/g. dry weight of hay, and the bacteria and actinomycetes increased to over 100 million spores/g., conspicuous among which were thermophilic actinomycetes growing at 60°. The pH rose from about 5·5 to 7 as the hay became very mouldy with a whitish covering consisting mainly of actinomycete spores. (Gregory and Bunce.)

Sitka-seedling diseases

The effects of treating seed and soil in various ways on numbers and sizes of Sitka spruce (*Picea sitchensis*) were investigated at five Forestry Commission nurseries where the incidence of disease varies greatly. (See Reports of Chemistry and Insecticides Departments.) Treating seeds with TMTD, "Captan" and an organo-mercury compound sometimes increased the numbers both of germinating seedlings and survivors, but did not affect their mean heights. In contrast, partial soil sterilisation with formalin and chloropicrin did not increase the numbers of seedlings that emerged but increased the numbers of survivors and their mean heights.

In an attempt to identify the causes of these effects, detailed analyses for fungi and bacteria were made at frequent intervals, of soil, of rhizospheres, of rhizoplanes and of diseased seedlings. Excepting *Trichoderma*, formalin and chloropicrin usually decreased the numbers of fungi in soil, including possibly pathogenic Phycomycetes and strains of *Fusarium*, but had little effect on their numbers in the rhizosphere.

Although soil sterilisation had little effect on the rhizosphere flora, it had, surprisingly, a big effect on the fungi, excepting *Fusarium*, isolated from the root surface-"rhizoplane". Both formalin and chloropicrin increased the frequency of *Trichoderma* and decreased the frequency of Phycomycetes. In addition, some Phycomycetes were fewer after seed treatment, suggesting, because the seed-coat is carried above ground by germinating seedlings, that the microflora of root surfaces may be determined at an early stage in development. (Ram Reddy and Last.)