ROTHAMSTED RESEARCH

# Report for 1958

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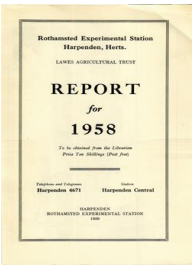


## Plant Pathology Department

### P. H. Gregory

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# PLANT PATHOLOGY DEPARTMENT

P. H. GREGORY

F. C. Bawden was chosen as director when Sir William Ogg retired in September 1958. The staff of this department, which he joined in 1936 and of which he has been head since 1940, are delighted that this leadership is now extended to the whole of Rothamsted. P. H. Gregory was appointed head of the department from 1 October 1958. L. Broadbent left to take up a post at the Glasshouse Crops Research Institute, Littlehampton. G. Salt was seconded for two years as plant pathologist to the Sudan Government Service.

F. C. Bawden, L. Broadbent, E. W. Buxton and J. M. Hirst all spoke by invitation at the Golden Jubilee Meeting of the American Phytopathological Society at Bloomington, Indiana, in August, and E. W. Buxton attended a Conference at Purdue University on Panama Wilt of Bananas. J. M. Hirst was awarded a Kellogg Fellowship and spent three and a half months visiting research stations and universities in Canada and the U.S.A. F. C. Bawden also attended meetings of the Advisory Committee on Agricultural Research in the Sudan at Khartoum in January and of the Technical Co-ordinating Committee for Agricultural and Allied Research in the West Indies in June.

A. Kleczkowski and B. D. Harrison attended the International Congress for Microbiology at Stockholm in August 1958.

D. Perry was awarded the Ph.D. degree of London University and Alison Fletcher the B.Sc. degree.

## VIRUSES AND VIRUS DISEASES

Little is known about what takes place during the first few minutes and hours after a plant is inoculated with a virus. In studying the early events of infection with tobacco mosaic virus in tobacco and *Nicotiana glutinosa* plants, inocula of the whole virus and of the infectious ribonucleic acid obtained from the virus were compared. By infectivity tests new virus was detected about 2 hours earlier in plants inoculated with the nucleic acid than in plants inoculated with the whole virus. Six hours from inoculation with nucleic acid was the shortest period when newly formed virus could be detected. A similar time difference was found when *N. glutinosa* plants inoculated with the two kinds of inocula were dipped in hot water at 50° C. for 30 seconds. This treatment reduced the number of local lesions from either kind of inoculum when it was applied immediately after inoculation, but when it was delayed for 4 hours with the nucleic acid and 6 hours with the whole virus it no longer had any effect.

*Nicotiana glutinosa* plants inoculated with the nucleic acid produced very few lesions when placed at 37° C. immediately after

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inoculation but the usual numbers when the treatment was delayed for about 2 hours. Exposure to 37° C. does not affect the number of lesions produced by plants inoculated with whole virus, and 2 hours may be the minimal time for new virus to be synthesized when nucleic acid is the inoculum. (B. Kassanis.)

Further studies of the decomposition products of tobacco mosaic virus gave evidence that the nucleic acid does not contribute to the surface potential of the virus particle. The fact that the electrophoretic mobility of tobacco mosaic virus is greater than that of the non-aggregated virus protein (obtained by splitting the virus by alkali) seems to result from the way the electric charges are distributed on the surface of the non-aggregated protein particles and from the way these particles are combined either in the native virus or in artificial aggregates of the protein particles. The lack of contribution of nucleic acid to the surface-charge density of the virus particle agrees with the X-ray studies of radial density distribution by Caspar (1956) and Franklin (1956), who showed that the nucleic acid is positioned near the central axis of the virus particle.

Alkali-produced disaggregated protein of tobacco mosaic virus is an antigenically heterogeneous mixture and lacks an antigenic group present in the original virus particle. Recombination of the proteins with nucleic acid seems to restore the lost antigenic group, although the nucleic acid by itself is not antigenic. The recombined nucleoprotein behaves as an antigenically homogeneous material. This is so probably because of aggregation of the protein recombined with the nucleic acid. Aggregation caused by lowering the pH to about 6·5 also leads to the loss of antigenic heterogeneity, although it does not restore the lost antigenic group. (A. Kleczkowski.)

*Electron microscopy*

Some success was obtained in finding methods for mounting plant viruses for electron microscopy at high resolution. The technique of heavy metal staining was successfully applied to particles of a strain of tobacco rattle virus. In normal shadowcast mounts the particles are rods about 21 mμ wide, with a central hole which can be seen when short particles appear " end on ". When mounted on thin carbon films after staining with uranyl acetate, lanthanum nitrate or thorium nitrate, the particles show a dense central thread, about 4 mμ wide, surrounded by an unstained and apparently structureless region. After staining with phosphotungstic acid the central dense region appears about 6 mμ wide, sometimes very densely stained but sometimes appearing as a less-dense region with very heavily stained edges, as though the material which filled it had been partly extracted. When the particles are treated with phosphomolybdic acid, or with uranyl acetate after incubation with trypsin, a regular cross banding with a spacing of about 2·5 mμ becomes visible in the outer region away from the central thread. This is the first time that a structure of this order of size has been seen in a plant virus with the electron microscope, and it may well represent the pitch of a helical protein structure, if, as seems likely, tobacco rattle virus has an internal structure broadly similar to that of tobacco mosaic virus. Attempts are now being



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made to characterize the two parts of the particles as revealed by these staining techniques.

Epoxy resins ("Araldite") show promise as a medium for embedding plant material before sectioning for electron microscopy. The blocks are less easy to cut than the usual methacrylate mixture, and unlike methacrylate it does not evaporate in the electron beam, so that for a given image contrast the sections must be thinner. These disadvantages are more than offset by the absence of characteristic artefacts produced by methacrylate shrinking during polymerization. When centrifuge pellets of tobacco mosaic virus are embedded and sectioned the virus rods are recognizable in sections thin enough for adequate electron microscopy without removing the embedding resin by solution and then shadowing, a technique liable to introduce gross artefacts. This difficulty could be partly avoided if it were possible to use readily stained virus particles, and this is one reason why time has been spent in a study of possible staining methods rather than on sectioning.

The number of routine examinations made for other members of the department continues to grow. In the past these have usually been of virus particles in clarified sap, and the mounts have required only the minimum of time for their preparation and examination. There is now a tendency for workers to require more highly purified material, and the electron microscopist necessarily has to report on the state of the specimen in at least the final stages of purification. Among work done for other departments progress has been made in finding the conditions which must be met for satisfactory high-resolution electron microscopy of minerals of the montmorillonite group, some of which have so far proved extremely difficult to study in this way (see report of Pedology Department). The problems are somewhat similar to those found in making good preparations of viruses. (Nixon.)

*Soil-borne Viruses*

*Mechanism of soil transmission.* Strong evidence was obtained that soil-inhabiting organisms are concerned in the soil transmission of certain viruses of the ringspot type. Detailed studies were therefore made on the fauna of soils taken from the parts of raspberry plantations where raspberry ringspot virus occurred, and of soils from adjacent virus-free areas. The arthropod populations were analysed by F. Raw, Entomology Department, and the nematode populations by R. D. Winslow, Nematology Department. The virus-containing soils possessed more Collembola and certain types of Acarina, and they sometimes contained more of certain plant-parasitic nematodes than did the virus-free soils. Evidently the composition of the soil fauna in virus-containing and virus-free soils differs in many respects, but the relevance of these differences to the occurrence of the virus is uncertain. (See also reports of Entomology and Nematology Departments.)

*Purification and properties of tobacco rattle virus.* Purified preparations of a strain of tobacco rattle virus, originally obtained from Scotland, contained rod-shaped particles of the same width but whose lengths fell predominantly into two categories. The two types of particles were separated by high-speed centrifugation in a

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sucrose density-gradient. Both types seemed to be nucleoproteins of the same or similar constitution, but only the longer particles were infective. (Harrison.)

## Arthropod-transmitted Viruses

### *Sugar-beet viruses*

The aphid-transmitted yellow-net virus of sugar beet (*Rep. Rothamst. exp. Sta. for 1951*, p. 164) has been studied in pot culture (in collaboration with S. A. W. French of the Botany Department) to determine the effect of spraying with gibberellic acid on susceptibility and symptom production, and the effect of virus on yield and symptom production, the effect of virus on yield and on concentration of carbohydrate in the leaves. The loss in yield caused by yellow-net virus in plants 2 months old was smaller than would be expected for plants similarly infected with necrotic beet yellows virus. Plants treated with gibberellic acid lost about 30 per cent of their root yield as a result of infection, and the untreated ones lost about 20 per cent. The effect of gibberellic acid (applied once during the first month of growth) was a temporary *increase* in yield of tops. Later there was a decrease in yields of laminae, while petioles and roots were not affected.

Plants infected by yellow-net virus, especially middle-aged leaves that show the main yellow-net symptoms, had much less sucrose and reducing sugars than normal plants. The yield of sugars per 1,000 gm. fresh weight of leaf laminae averaged about 3·5 gm. for healthy plants, but only 0·4 gm. for middle-aged leaves of infected plants and 1·5 gm. for other leaves. Yellow-net virus thus contrasts strongly with beet yellows virus which increases the carbohydrate concentration in infected leaves, sometimes to ten or more times normal. (Watson and Chessin.)

### *Cereal viruses*

The host range of cereal yellow dwarf among meadow and hedgerow grasses was investigated by using large numbers of the vector, *Rhopalosiphum padi*, to ensure that the virus was introduced in sufficient quantity. *Festuca pratensis, F. rubra, F. elatior, Poa trivialis, P. pratensis, Dactylis glomerata, Anthoxanthum odoratum* and *Agrostis tenuis* became infected but behaved as symptomless carriers. *Avena fatua, Bromus sterilis, B. arvensis*, and also maize and rice, showed symptoms of leaf discoloration and stunting. *Agropyron repens* and *Arrhenatherum elatior* appeared to be immune. (Mulligan.)

Two yellow dwarf viruses of cereals have now been compared in both glasshouse and in field experiments. They are considered distinct viruses rather than strains of one virus, because plants infected with the avirulent virus become infected with the virulent virus just as severely as healthy plants, and the virus derived from these plants after a few weeks' growth gives virulent symptoms when subinoculated to healthy plants. The avirulent strain does not protect a plant from the virulent infection, and the only evidence of interference seems to be that the avirulent virus is eliminated by the virulent one. (Watson, Govier and Mulligan.)

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In field plots infected with the two viruses at fortnightly intervals between April and June the earliest infection caused loss of 80 per cent of the yield of Blenda oats and about 65 per cent of that of Koga II wheat and Proctor barley. The second and third infections caused losses of about 50 per cent and 15 per cent respectively. With the avirulent virus the loss from the first two infections was 40–50 per cent and the last negligible. Oats were again the most severely affected. (Watson and Mulligan.)

*Ryegrass mosaic.* Ryegrass mosaic virus was transmitted by *Abacarus hystrix* (Nal.) to ten out of twenty S22 Italian ryegrass test plants when each was tested with ten mites immediately after feeding for 3 days on infected ryegrass leaves. Such mites lost half their infectivity in about 3 hours while feeding on Capelle wheat and almost all infectivity after 12 hours. Healthy mites fed on infected leaves for 12 hours transmitted as readily as those fed for 24 hours or 48 hours, but those fed for only 6 hours did not transmit. Freshly extracted sap of infected S22 Italian ryegrass plants at pH 7 lost most of its infectivity in 10 minutes at 55° C. At room temperature half the infectivity was lost in about 3 hours and most in 24 hours. In addition to species mentioned in last year's report the following developed streaking when infected: *Alopecurus agrestis, Avena fatua, Avena sativa, Dactylis glomerata* and *Oryza sativa*. The streaking in Blenda oats becomes rusty in the upper leaves, and a naturally infected plant has been identified from the field. The streaking in cock's foot S143, S37 and S26 resembles that caused by cock's-foot streak virus, and the ability of *Myzus persicae* to transmit ryegrass mosaic is being tested. Cock's-foot streak virus transmitted by *M. persicae* to cock's foot has been transmitted by sap into S22 Italian ryegrass, in which it gives symptoms like ryegrass mosaic. Rod-shaped particles like those of ryegrass mosaic (*Rotham. exp. Sta. Rep. for 1957*) were found in two out of five samples examined by the electron microscope. (Mulligan.)

*Wheat striate mosaic.* The three races of hoppers isolated last year were used to find out the relation of virus inheritance to the vector-efficiency of the parental strain of hopper and the time of parental virus acquisition.

Efficient races transmitted to a high proportion of the nymphs, but inefficient races to very few. Nymphs from females of efficient races frequently transmitted on the day of hatching, so if the virus multiplies in the tissues of the inheriting nymphs it presumably does so in the egg during the development of the embryo. This view is strengthened because many infected embryos die before hatching. For this reason the progeny of infective females from efficient races are fewer than those of non-infective mothers or mothers from inefficient races (nymphs did not inherit virus unless their mothers or grandmothers had fed on infected plants). Because infective races of hoppers reproduce more slowly than non-infective ones, there is a population pressure to eliminate infective individual lines from mixed cultures, with a result that most become healthy when maintained in conditions where they cannot become infective by feeding on infected plants. (Watson and Sinha.)

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*Non-persistent viruses*

It was reported last year that the non-aphid-transmissible potato virus C (from Edgecote Purple potatoes) can readily be transmitted by *M. persicae* if it is first inoculated to *N. glutinosa* in combination with potato virus Y. Viruses transmitted by aphids from such mixed infections in *N. glutinosa* frequently give black, necrotic local lesions, characteristic of potato virus C when inoculated to Majestic potatoes, but potato virus Y alone has never given such lesions, and neither have isolations from it. Combined viruses that gave local lesions followed by necrotic systemic infection in Majestic potatoes retained their character in *Nicotiana glutinosa*. Some have been maintained by sap inoculation through *N. glutinosa* and single-lesion isolates from Majestic potatoes for 18 months. Although such combined viruses may be changed by systemic passage through Majestic potatoes (as was the original aphid-transmitted potato virus C), their behaviour suggests that they may be " hybrids ".

Attempts to transmit either potato virus C or tobacco mosaic virus as contaminants of henbane mosaic virus have failed. So also have attempts to transmit the non-aphid transmissible strain of cucumber virus 1 as a contaminant of yellow cucumber virus 1.

A virus occurring naturally in Jersey and sent by Mr. D. E. Richardson of the National Institute of Agricultural Botany was very like the aphid-transmissible C virus mutant, except that its aphid-transmissibility did not disappear after passage through Majestic potatoes; also an isolate from it caused necrotic systemic lesions in Majestic potatoes resembling those caused by isolations from mixtures of potato viruses C and Y. (Watson.)

*Tobacco mosaic.* Attempts to transmit tobacco mosaic by using *M. persicae* whose stylets had been exposed and coated with tobacco mosaic nucleic acid before feeding on healthy seedlings were unsuccessful. Ribo-nuclease was applied to the exposed stylets of *M. persicae* before feeding on plants infected with henbane mosaic, and also after feeding. Both treatments reduced the probability of subsequent infection, but similar treatments with distilled water did so also. (Watson and Delobbe.)

*A virus disease of carrots.* An aphid-transmitted persistent virus was isolated from yellow, stunted carrot plants at Kennett, Cambs. The symptoms resembled those described by L. Stubbs from Victoria, Australia, as " carrot mottley dwarf virus ". The vector is the carrot aphid (*Cavariella aegopodeae*), and the virus is not sap-transmissible. (Watson.)

*Potato virus diseases*

Aphids were exceptionally numerous on potato crops in May and June 1957, but numbers then declined rapidly, and there was no summer migration during July and early August. The effect of these infestations on spread of viruses in the field was revealed during 1958 on plants grown from seed tubers saved from experimental plots of the previous year. An experiment on aphid control, done with P. E. Burt of the Insecticides Department, showed that spraying with DDT and " Metasystox " on 3 June 1957 reduced the

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spread of leaf roll to less than a quarter of the amount on unsprayed plots, but that sprays applied on 19 June, 3 July or 30 July had no significant effect. Spread of rugose mosaic was not reduced by any of the four applications. Thus, although the plants were infested with aphids before any spray could be applied, the first spray applied when about 80 per cent of the plants had emerged almost stopped the spread of leaf-roll virus within the plots. The lack of effect from later sprays in 1957 supports previous evidence that the early sprays are the most important, and that the spread of virus Y is attributable to the great activity of winged *Myzus persicae* while the plants are emerging. (Broadbent and Heathcote.)

Insecticide trials on potatoes in co-operation with the National Agricultural Advisory Service were continued in 1958, when most ended. Despite the spread of virus in 1957 in many parts of Britain, few of the sprayed stocks that had been kept for several years deteriorated badly, and the recommendations to farmers to keep their sprayed stocks for 3, 4 or 5 years have been justified. The high cost of certified potato-seed tubers has stimulated interest in our work, and some growers are endeavouring to keep their stocks longer. (Broadbent and Heathcote.)

To obtain further information on the relative ability of winged and wingless aphids to transmit potato leaf roll and rugose mosaic (virus Y) an experiment was done in 1957 in collaboration with P. E. Burt of the Insecticides Department on small plots applying " Phosdrin ", a systemic insecticide that remains active for only 2 days, at intervals to prevent wingless aphids becoming numerous but to leave the plants open to visits of winged aphids during most of the season. One set of plots contained no virus-infected plants, and the other had one plant with leaf roll and one with rugose mosaic in each half plot. Haulms were removed at different dates: 15 July, 19 August or when dead. " Phosdrin " was first applied on 6 June 1957, 2 weeks after the first plant emerged. The percentage of plants infected in the central rows of these plots were:

| | + " Phosdrin " | | — " Phosdrin " | |
|---|---|---|---|---|
| | LR | RM | LR | RM |
| No infectors ... ... ... | 2·5 | 61·2 | 12·5 | 56·2 |
| Autumn harvested ... ... | 10·0 | 87·5 | 37·5 | 97·4 |
| Mid-season harvested ... | 8·1 | 94·7 | 30·0 | 92·5 |
| Early harvested ... ... | 10·0 | 77·5 | 34·2 | 59·0 |

As in the other Rothamsted experiments, most of the spread occurred early. Aptera apparently played little or no part in spreading virus Y (RM). If the incidence of LR in the plots treated with " Phosdrin " represents that spread by alatae, there must have been considerable spread of LR virus by aptera before mid-July.

During the extremely wet season of 1958 aphids in general and *Myzus persicae* in particular were many fewer than in 1957.

Eleven sticky cylindrical aphid traps were operated in unsprayed potato crops near to sprayed ones in different parts of England. *M. persicae* were few during May and June, but became numerous in the Wash area during July. Elsewhere they remained few throughout the summer. (Broadbent and Heathcote.)

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## Fungi and Fungus Diseases

### *Potato blight*

*Phytophthora infestans* attacked potato crops unusually early in 1958. It was first found on commercial crops on 16 July, following a 19-day period when conditions within crops continuously satisfied the Beaumont criteria. The fungus spread rapidly in persistently wet weather, and unsprayed Majestic crops were almost destroyed at the end of August. Majestic crops sprayed twice with copper oxychloride yielded only 1 ton/acre more than unsprayed plots. The first spray applied in mid-July delayed the epidemic considerably, but later sprays did little good, presumably because the residues were too rapidly removed by rain.

In continued observations on the early stages of blight epidemics 700 King Edward and 140 Up to Date tubers naturally blighted in 1957 were planted among a similar number of healthy Majestic tubers. The first above-ground symptoms were discovered on 18 June on one of seven King Edward plants whose stems were invaded by the fungus growing from the seed tubers. The first local lesions on foliage were found on 27 June, and almost all the plants in the plot had become infected by 29 July. This series of experiments, in which only diseased tubers with live sprouts have been planted, cannot be used to assess the importance of naturally infected ground-keepers as sources of blight. To simulate these we planted 300 naturally infected tubers at each of two depths in November 1957. The winter was comparatively mild, and by July 1958, 37 per cent of those placed 4 inches deep had produced plants compared with 30 per cent of those at 8 inches, but on only one plant (King Edward, at 4 inches) were stems above ground invaded by the fungus.

Last year we reported the occurrence, in an irrigated plot, of foci of infected plants which we were unable to associate with any nearby invaded stem. It seemed possible that the fungus, introduced in diseased tubers, might be able to start epidemics without invading any shoots. To test this we planted a plot in which over 600 healthy Majestic tubers, 24 inches apart, alternated with Pentland Ace tubers. The Pentland Ace tubers were artificially infected with Race 3,4 of *P. infestans*, and all the eyes were removed to prevent growth. As in 1957, the plot was irrigated to make good any deficit of rain below 1 inch/week. Little irrigation was required in June because 4½ inches of rain fell, a third of it during 2 days at the end of the month caused water to stand in the furrows. Six days later, on 4 July, blight lesions were found on 27 Majestic plants, all on the lowest leaves which were hanging over the furrows. Isolates from each of 25 lesions found on 4 July were tested on Pentland Ace foliage (which is attacked by Race 3,4 but not by the common Race 4) and 80 per cent caused infections. By contrast, none of 30 isolates from King Edward foliage in the adjoining experiment, mentioned above, infected Pentland Ace. At this early stage of the epidemic, there would have been little spread of the fungus between experiments. The high rate of recovery of Race 3,4 from the plot containing " blind " Pentland Ace tubers suggests that, under wet conditions, attacks of blight can be started, without

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the invasion of stems, by *P. infestans* introduced into unsterile soil on an appreciable food base. (Hirst and Stedman.)

Experiments had shown unexpectedly little difference in the dates at which the varieties Up to Date, King Edward, Majestic and Arran Viking became defoliated by blight. Experiments were continued in 1958 using a 4 × 4 Latin square in which the plots were surrounded by a non-susceptible guard. As non-susceptible guards to reduce possible spread from plot to plot, which was suspected in previous years, kale was planted between the columns of the potato plots and mangolds between the plots within columns. On 18 July blight was found in one King Edward plot, and all plots were then infected artificially so that the difference in the time of arrival of natural blight in plots found in 1957 could be avoided. The epidemic was particularly rapid and severe. Once again only small differences in the rate of destruction of varieties were recorded. In Arran Viking 50 per cent defoliation was reached 4 days later than Up to Date and King Edward and 2 days later than Majestic. Other varieties showing more resistance are to be included in future trials.

When leaves were marked to assess the progress of blight, defoliation resulted mainly from the direct infection of leaflets and not from their indirect destruction through stem or petiole lesions. Indirect destruction was more frequent in 1957, and the effect in 1958 can probably be explained by the prolonged periods of weather favourable for spread of the fungus. Lesions appeared every day and many marked leaflets became " peppered " with lesions and were destroyed before infection of stem and petiole could have an effect.

When leaf axils of glasshouse plants were inoculated with a droplet of spore suspension, the varietal differences in susceptibility of stem and petiole observed in 1957 were confirmed. In the field, stem lesions were abundant in King Edward and Up to Date, fairly frequent in Majestic and few in Arran Viking, and as a result of the agreement between field and laboratory observations the test was extended to thirty-three varieties of all maturity groups. Varieties differed in susceptibility but further experiment is required to assess the reliability of such a test.

There is evidence that some varieties produce more spores than others. In Arran Viking in the field the width of the zone producing spores changes as the lesion grows. In the susceptible Up to Date the average width of the sporing zone was similar (3·2–3·9 mm.) irrespective of the size of the lesion. In Arran Viking the sporing zone of lesions 5 mm. diameter was only 1 mm. wide, but in large lesions it was almost as wide as in Up to Date. As a result, Arran Viking produced relatively few spores on the smaller lesions, a feature seen also in the laboratory in the resistant varieties Ackersegen and Ås. Although, with suitable temperature and humidity, the sporing zones of Arran Viking can be the same as a more susceptible variety, in a field crop these conditions may be reached only at the lowest level in the canopy.

Of thirty-two commercial varieties grown for observation in small field plots, Ackersegen, Ås and Ontario remained green longest, and gave reduced sporulation. In another experiment with

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plots of the hypersensitive varieties Orion, Ulster Torch and Pentland Ace, only Orion showed some foliage and tuber resistance when inoculated with its specialized race. Ulster Torch gave the impression of being very susceptible because leaves of the middle and upper canopy abscissed readily when only partially infected, leaving stems green and bare. The fallen leaves remained green and spored profusely in the furrows, and at lifting 19 per cent of the tubers were blighted.

To investigate the effect of age on the susceptibility of potato foliage to blight, the variety Majestic was planted at fortnightly intervals from 30 April to 16 June. The last plot to be planted was 50 per cent defoliated only 9 days later than the first, and defoliation in plantings on other dates was intermediate between these two extremes.

In a field trial of chemicals for the destruction of potato haulm, plots 10 rows by 30 feet of Ulster Supreme in a 2 × 5 randomized block were sprayed when 75 per cent defoliated by blight. The rate of application was equivalent to 40 gal./acre of sulphuric acid 50 per cent B.O.V., sodium arsenite 1 gallon, sodium chlorate 24 lb. and FB2 2 lb./acre (FB2 is 1 : 1′-ethylene-2 : 2′-dipyridylium dibromide, supplied by Plant Protection Ltd.). Sulphuric acid killed both foliage and stems most rapidly. FB2 was a little faster than arsenite in the initial killing of leaves, but slower in killing the stems. Chlorate was slow killing both leaves and stems. All chemicals gave good control of weeds compared with the unsprayed plots. (Lapwood.)

*Apple Scab*

Tests of surface-wetness recorders designed to assist the accurate timing of curative sprays were continued in conjunction with the Meteorological Office and the Ministry of Agriculture.

Trapping of ascospores was continued in orchards at Wisbech and in Kent and Suffolk, but the results have not yet been analysed. We continued a series of measurements designed to test whether it is possible to forecast the number of ascospores to be expected during spring. The two methods being tested both rely on estimating the weight of dead leaf remaining in orchards shortly before bud-burst. In the first method the extent of this source would be related to tests of the ascospore productivity of leaf samples, of known area, in which the maturation of perithecia has been hastened artificially. Trials in 1958 suggest that this is feasible but that the number of ascospores liberated would be considerably less than from replicate samples left to mature naturally. The second method would also relate the amount of dead leaf in spring to ascospore productivity but indirectly through the regression of percentage of leaves infected in autumn on the subsequent ascospore productivity of samples of the same leaves the next spring.

Tests of ascospore productivity were made on uniform area samples of leaves formed on the extension growths of unsprayed Laxton's Superb in May, June, July or August 1957. The later formed leaves were most heavily infected and produced most ascospores, and the first leaves to fall in autumn produced fewer ascospores than those which fell late. (Hirst and Stedman.)

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*Fungi on hay*

Work on the mycofloral succession on hay was started because mouldy hay is thought to have a role in the etiology of some human and animal diseases (e.g., " farmer's lung ", bovine mycotic abortion, aspergillosis in poultry), and also for its importance as an agricultural problem.

Preliminary studies on the difficult problems arising in sampling hay to assess mould content have involved: (1) shaking a weighed specimen of hay in air in a small wind tunnel to find what respirable spore and dust load was set free into the air; and (2) subsequently washing the samples to estimate the spore load entering the digestive tract of farm animals. The respirable and ingested fractions have been estimated by use of the Cascade impactor, haemocytometer counts and by dilution cultures.

The samples of normal hay examined contained little dust and relatively few fungus spores. The spores put into the air were typical of the dry air spora of this country (e.g., *Cladosporium*, *Helminthosporium*, *Alternaria*, *Botrytis*, *Polythrincium*, *Ustilago*) and support the view that the outdoor air spora is derived from above-ground vegetation rather than from the soil. Specimens of mouldy hay put very many spores into the air, classifiable by direct visual examination and in culture as predominantly species of *Aspergillus*, *Penicillium* and related moulds, *Monotospora lanuginosa*, *Absidia* and *Actinomycetes*. Many isolates were thermophilic, growing well at 40° C. The total numbers of spores removed from four samples varying from normal to increasingly mouldy hay were as follows in millions/gm. dry weight: in air—3, 5, 67, 342; in washing—48, 89, 306, 526.

The rate of spore removal from samples of mouldy hay shaken in the wind tunnel for an hour decreased rapidly. Cumulative percentage output was approximately linear when plotted against time on log. probability paper, and in a wind of 1 m./second (2·2 m.p.h.) 50 per cent of the spores were removed during the first 2 minutes.

In the samples examined there was no evidence that plant material had been broken down into dust by moulds. Almost all the dust in the size range 1–15 μ consisted of fungus and actinomycete spores. (Gregory.)

*Air-borne spore trapping*

Since 1956 we have been co-operating with the Meteorological Office in studying variations in spore concentration with height. Suitable apparatus has now been developed for collecting and examining catches. While it is too early for definite conclusions, it seems probable that the distribution of air borne spores with height is much affected by lapse-rate. A wide range of fungus spores and pollen grains have been caught, and at times in high concentration, for example, 53,000 *Cladosporium* conidia/cu. m. of air at 2,000 feet on the afternoon of 18 July 1957.

Part of the work, done in collaboration with workers at Bristol and Cardiff, has been to test whether attacks of *Puccinia graminis* can be attributed to uredospores dispersed over long distances.

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Results from several years will be necessary in view of the few occasions and spores involved, but it is encouraging to have found single uredospores, indistinguishable from *P. graminis*, in four catches between 4,000 and 9,000 feet over the English Channel (30 miles south of Portland Bill) on 4 July 1957. These catches indicate a concentration of the order of 30 uredospores/cu. m. of air. (Hirst and Stedman.)

Eyespot (*Cercosporella herpotrichoides*), lodging and take-all, (*Ophiobolus graminis*), favoured by wet summer weather, were unusually severe in 1958. On Broadbalk the 1st, 2nd, 3rd, 4th and 7th consecutive wheat crops after 1 year fallow on plots 2B, 3 and 7 averaged: 23, 68, 79, 77 and 60 per cent straws infected by eyespot; 9, 40, 49, 43, and 30 per cent with severe eyespot lesions; 26, 35, 41, 36 and 18 per cent of their areas were lodged; 0·1 8·6, 5·5, 7·1 and 2·4 per cent straws had take-all on the roots. The lower incidence of eyespot and take-all on the 7th as compared with the 2nd, 3rd and 4th consecutive crops may indicate a decline in disease under continuous cropping, but was also associated with control of weeds by spray, which was applied only to this section. Take-all caused greying of straws and ears within the small areas on which the more severe " patch " form of the disease had been recorded for the first time on Broadbalk plots in 1957. The patch form did not recur on Broadbalk plots in 1958, but was found on a non-experimental part of the field in the second wheat crop after 2 years fallow. Loss in grain of about 40 per cent was associated with the greying, and of about 80 per cent with the severe patch form of the disease. (Glynne and Cox.)

Recent changes in the alternate wheat and fallow experiment showed the dramatic effects of: (1) substituting a wheat crop for fallow; and (2) thin seeding. Sown at 3 bushels/acre, the first wheat crop after fallow had only a trace of take-all, the second was very severely stunted by the disease; but when sown at $\frac{1}{3}$ bushel/acre the second wheat crop was lightly, and the third severely infected. Eyespot was severe after a 1-year fallow when sown at 3 bushels/acre, but only moderate when sown at $\frac{1}{3}$ bushel/acre. A wheat crop sown at $\frac{1}{3}$ bushel/acre in 1957 was as effective as one sown at 3 bushels in carrying both diseases to the subsequent wheat crop. On the Rothamsted six-course rotation experiment, wheat was more severely infected by eyespot than ever before recorded, having 99 per cent straws infected, 81 per cent with severe lesions; this caused early lodging, and loss in grain, the yield being only 18·7 cwt./acre, as compared with the previous 20-year average of 30·4 cwt./acre. Spring-sown barley and autumn-sown rye in this experiment had respectively 22 and 15 per cent of their straws infected. These cereals grown in alternate years with non-susceptible crops carry enough infection to cause serious loss to winter wheat in the wetter years.

Spring-sown barley grown continuously on Hoos Field, usually only lightly infected, this year had eyespot causing lodging in small areas and take-all causing greying in larger areas. Spring-sown wheat, the fourth wheat crop in 5 years, was seriously affected by take-all in an experiment (Great Knott Field III) on levels and dates of application of nitrogen. On plots receiving 0, 2, 4, 6

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cwt./acre " Nitro-chalk " 86, 29, 22, 14 per cent respectively of their areas were grey with take-all, and the very low yields of 8·5, 16·2, 20·2, 21·3 cwt./acre showed the depressing effects of this disease, which causes most loss in poorly nourished crops, and so increases the apparent response to nitrogen.

The severity of powdery mildew (*Erysiphe graminis*), on wheat was measured on randomly selected plants on Broadbalk plots by estimating the percentage area of the top two leaves covered by mildew pustules. The disease was most severe in the first crop after fallow of plots which had received nitrogen and phosphate; it was rather less severe where only nitrogen had been applied, and relatively slight on plots which had received potash as well as nitrogen and phosphate. (Glynne.)

Wheat grown in small pots containing Broadbalk soil, either untreated or with the pH altered by adding lime or sulphuric acid, had large root systems with negligible take-all in soil taken from Section II after a year under fallow. In soil which had carried four wheat crops (section VA and VB) there was much take-all with smaller root systems, except in acidified soils with pHs of about 4–5 in which take-all was negligible and root systems as large as those after fallow. At lower pHs the roots were damaged by acidity.

*Cercosporella herpotrichoides* isolated from wheat, barley, oats, rye and bent grass (*Agrostis stolonifera*) showed wide differences between isolates from either the same or different hosts in: (1) the numbers of spores produced on disks cut from pure cultures on potato dextrose agar; (2) in the time taken to produce eyespot lesions on wheat seedlings; and (3) in their effects on yield of grain. (Glynne and Cox.)

Survival of *Cercosporella herpotrichoides*, estimated by counting the number of straws on the soil surface which produced spores of the fungus incubated in the laboratory, was measured on two Broadbalk plots. In February to March on Section IV, where wheat had been grown in 1957, there were an average of 4·4 potentially infective pieces of straw per sq. yd. and 11 per cent of the plants in the 1958 wheat crop were infected in March, 17 per cent in April. On Section II, which had been fallow in 1957, there were only 0·2 pieces of infected straw per sq. yd.; no plants were found infected in March, and 1 per cent in April. (Cox.)

In the third and last year of a small-plot experiment measuring effects of sowing date, seed rate and nitrogen, Cappelle wheat was grown on rich land free from appreciable eyespot and take-all. The relative yield of each row was determined by sampling and the plot yields corrected to allow for the much bigger yield of the two outside rows. As in previous years, the corrected yields decreased with each delay in sowing and were 50·5, 47·7, 37·0, 35·6 cwt./acre for plots sown 26 September, 11 October, 8 November and 6 December respectively. The highest seed rate and higher levels of nitrogen increased lodging and therefore depressed yields. Mean yields of plots sown at 1½, 3, 4½ bushels/acre were 42·6, 44·1 and 33·6 cwt./acre. The effect of nitrogenous manuring is shown by plots sown at 4½ bushels/acre, where plots receiving sulphate of ammonia at 0, 3, 6 and 9 cwt./acre yielded respectively 42·4, 39·6, 32·0, 20·6 cwt./acre

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with 0, 5, 20, 62 per cent of their areas lodged. Nitrogen had little effect on yields of plots sown at 1½ and 3 bushels/acre. As in previous years, there was a small progressive decrease in yield from the second to the central rows in each plot, and this effect was most marked in the early sowing, so the effect of sowing dates on yields of the middle rows was small; sowings in successive months gave yields in the central rows (5 and 6) of 42·9, 43·2, 39·2 and 39·6 cwt./acre respectively. The wheat was attacked in spring by the larvae of dipterous insects, mainly *Opomyza florum*, and as in previous years the attack was heaviest on the early sown wheat. Wheat sown in September, October, November, December had respectively 58, 13, 4, 3 per cent plants and 18, 3, 1, 1 per cent shoots infested on 5 May, but as the larvae mostly attacked late-formed tillers, the effect on yield was not serious.

Results of this small-plot experiment were used to plan a larger replicated field experiment in which Cappelle wheat was sown at three dates, three seed rates with three levels of nitrogen on land virtually free from eyespot and take-all. The land was richer than that of the small-plot experiment, and in early June the crop looked better than any others at Rothamsted, but the rapid growth in spring and the continuous wet weather produced such lush growth that lodging began early, was very extensive, all plots were flat by harvest, and yield averaged only 30·7 cwt./acre. Here lodging limited yield, although Cappelle normally resists lodging. But the six discard rows separated from the harvested area of each plot by a blank row stood up well till just before harvest, suggesting that this device might enable us to obtain high yields on very fertile plots with less risk of lodging. (Glynne and Slope.)

The first series of the rotation experiment started last year provided a comparison of the incidence of eyespot and take-all in winter wheat (variety Heine 7) grown after winter wheat (W), barley (B), spring wheat (sW) and spring oats (O). The results are summarized in the table:

| | % plants infected | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Eyespot | | | | Take-all | | | |
| Previous crops... | W | B | sW | O | W | B | sW | O |
| December 1957 | 0·3 | 0 | 0·7 | 0 | 23·6 | 17·1 | 44·9 | 0 |
| March 1958 ... | 7·4 | 12·4 | 0·6 | 0·5 | 26·8 | 25·7 | 51·8 | 0·6 |
| May 1958 ... | 4·9 | 14·8 | 0·8 | 0·5 | 52·8 | 44·4 | 74·0 | 0·9 |

The high percentage infection by take-all in December emphasizes the unusually favourable conditions for autumn survival and infection. The disease increased throughout the year, and at harvest the wheat after wheat, barley and spring wheat was very severely damaged. In contrast, the wheat after oats was a good crop. Reliable yields were not obtained because of a severe attack by birds; this attack was worst on the wheat after oats, which ripened several days later than the other crops. The percentage tailcorn (i.e., the grain that passed through a sieve with mesh size 20 × 2·2 mm.) was 14·7, 11·2, 11·1 and 5·7 for wheat after wheat, barley, spring wheat and oats respectively.

The site of the discontinued Deep Cultivation Rotation experiment on Long Hoos Field was used to obtain further evidence on the effect of previous crops on eyespot and take-all of winter wheat.

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Yeoman wheat was sown in October on six areas that had the following crops in the years 1954–57:

(1) oats, sugar beet, barley, hay;
(2) wheat, potatoes, oats, barley;
(3) potatoes, oats, barley, barley;
(4) hay, wheat, barley, barley;
(5) barley, hay, wheat, barley;
(6) sugar beet, barley, hay, wheat.

In July the percentage of the straws with take-all was 2, 3, 2, 14, 21, 20, and with eyespot 44, 14, 8, 14, 39, 10 respectively. Thus take-all was slight only when there had been 2 consecutive years free from susceptible crops (wheat and barley) in the previous 4 years. In this experiment the incidence of eyespot was not related to the frequency of susceptible crops in the rotation. (Slope.)

*Fusarium diseases of peas*

Extensive field tests were made at Yaxley, near Peterborough, and at Rayne, Essex, to find sources of wilt-resistance among the pea varieties grown on a commercial scale in Britain. At both sites, where the soil was naturally infested with the wilt fungus, *Fursarium oxysporum* f. *pisi*, ninety pea varieties were sown in replicated plots. Records taken throughout the season showed that 50 per cent of the varieties were highly susceptible to wilt, among them most of the varieties that are popular and widely grown in this country. Some of the varieties susceptible in our tests had previously been found resistant in Holland and in New Zealand, probably because of differences in the geographical distribution of physiologic races of the fungus. Including evidence from previous observations and isolations from diseased plants, the reaction to wilt of 143 different pea varieties in Britain is now known. Of these, sixty-six varieties are susceptible. There was no correlation between wilt-resistance and any particular morphological feature of any group of varieties. Some susceptible seed stocks produced a few resistant plants, and these could be tested for suitability as breeding material. Tests of resistance in the glasshouse revealed that most methods of inoculation were unreliable guides to what may happen in the field, so it is unlikely that selection of resistant stocks can be speeded up in this way. The problem of control of pea wilt by using sources of resistance can now be handed over to plant breeders.

In further work on the interactions in the rhizosphere between root exudates from growing pea roots, soil micro-organisms and *Fusarium oxysporum* f. *pisi*, ninety-one different species of fungi and bacteria were isolated from the rhizosphere of peas and tested for their ability to inhibit the pea wilt *Fusarium* in culture. Of the sixteen that strongly inhibited the *Fusarium*, three had increased ability to inhibit when root exudate was added to the agar medium. These three organisms may exert an important effect on *F. oxysporum* in nature, in addition to the direct effects that root exudates have already been shown to exert on this pathogen. (Buxton.)

Further work on the mechanisms underlying the decrease in pea wilt that results from the presence of *F. solani* in plants infected

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with *F. oxysporum* showed that water extracts from stems with foot-rot lesions contained two fractions, one stimulatory, the other inhibitory to *F. oxysporum*. When the concentration of the extract was reduced to $\frac{1}{10000}$, the stimulatory effect was nullified, and spore germination of the fungus was inhibited. *F. solani* colonized the epidermis and outer cortex more rapidly and more extensively than did *F. oxysporum*. It discoloured the cortex after 4 days, the invaded cells became packed with hyphae, and the cytoplasm later became granular and stained deeply with cotton blue. On the other hand, the hyphae of *F. oxysporum* passed both inter- and intracellularly through the epidermis and cortex; the root surface was not discoloured, the cortical cells remained intact and there was no obvious reaction in the cortex to invasion. The respiration rate of plants inoculated with *F. solani* was 25 per cent higher than that of uninoculated controls. From this and previous work it is concluded that the interaction between the two fungi occurs during growth in the cortex, where *F. oxysporum* may be delayed in its progress to the vascular track, resulting both in delay and ultimate decrease of wilt. (Buxton and Perry.)

*Clubroot of crucifers*

Studies of resting spore germination were resumed; all stages of germination and the free, presumptive, primary zoospores have been seen, and earlier observations confirmed. The readier tendency of spores from old galls to germinate spontaneously, compared with those from young galls, was again noted, but the level of germination was low. It has now been repeatedly confirmed that diffusate from the roots of cabbage seedlings, grown aseptically either in dilute Hoagland's solution or in dilute calcium chloride solution, stimulates spore germination. In one experiment, with a spore suspension containing initially, 1·5 per cent empty spores, 60 per cent were empty after 2 days incubation in solution containing root diffusate as against 15 per cent in solution alone. Differences in ionic composition of the medium also appear to influence germination, and this factor will require further examination.

Several small crucifers were tested for susceptibility to clubbing by three isolates of *P. brassicae*, but no differential effects were observed. *Arabidopsis thaliana* (L) Hynh. was the most susceptible of the plants tested, 100 per cent having symptoms of clubroot, followed by *Arabis alpina* (L) with 30 per cent, *Alyssum maritimum* Lam. with 22 per cent and *Cheiranthus allionii* Hort. with 10 per cent. *Aubrietia purpurea* (?) remained free from clubbing. (Macfarlane.)

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