ROTHAMSTED RESEARCH

Report for 1962

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Plant Pathology

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PLANT PATHOLOGY DEPARTMENT

P. H. GREGORY

P. H. Gregory visited departments of plant pathology in the United States and Canada, with a travel grant from the Rockefeller Foundation, and attended the First International Conference on Palynology at Tucson, Arizona. B. D. Harrison attended the Third International Conference for the Study of Virus Diseases of the Grape-vine at Lisbon, as guest of the Portuguese Department of Agriculture. A. Kleczkowski worked in the Plant Pathology Department, University of California, Berkeley, California, for six months. D. H. Lapwood was seconded for six months to the Inter-American Potato Improvement Project of the Rockefeller Foundation in Mexico, and later visited research centres in the United States and Canada.

The following joined us as temporary workers: Dr. M. L. V. Borges (Oeiras, Portugal), Dr. A. G. P. Brown (Empire Cotton Growing Corporation), Dr. R. Corbaz (Nyon, Switzerland), Mr. J. Drew Smith (Ruakura, New Zealand), Mr. R. Gamez (Heredia, Costa Rica), Professor B. H. MacNeill (Guelph, Ontario) and Professor G. W. Welkie (Logan, Utah).

P. Babos, R. C. Close, H. F. Dias and M. A. Ram Reddy were awarded the Ph.D. degree of London University. J. Lacey was awarded the Ph.D. degree of Reading University and was appointed to the staff of the department to work on micro-fungi of pasture grasses. G. A. Hide joined the department to work on potato skin-spot and other tuber blemishes, with a grant from the Potato Marketing Board.

Work was greatly helped by the installation of new equipment, which included new analytical and preparative centrifuges, a recording microdensitometer, a monochromator and a set of tanks for growing plants at constant soil temperatures.

Viruses and Virus Diseases

Classification of small viruses. Many plant viruses have spherical or polyhedral particles between 20 and 30 mμ in diameter. When examined in the electron microscope at low magnifications these all look alike, but at high magnification after negative staining by a standardised technique there are distinguishing features that allow most to be grouped according to their size and appearance. The members of virus groups established in this way usually also resemble each other in other properties, such as sedimentation coefficient, thermal inactivation point and nucleic-acid content. Often they also share some important biological property, such as a common mode of transmission. Not all the viruses placed in one group are sufficiently related to share antigens, but so far serological relationships have been found only between viruses that do come within the same

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group. The differences in appearance that determine the groups are qualitative and often difficult to describe, but probably reflect differences in internal structure, which are, as yet, not properly resolved. It has not yet proved possible to allocate every spherical virus studied to a definite group, but the practical value of the partial classification so far achieved is amply evident from the readiness with which electron microscopy and sedimentation behaviour have shown virus preparations to be mixed and placed the different ones in their correct groups. Further progress in classifying spherical viruses depends on the improvement of techniques to permit even finer distinctions to be made. (Gibbs, Nixon and Woods)

The structure of virus particles and the limitations of existing electron-microscope techniques. Of the small polyhedral plant virus particles discussed above, clear pictures of the internal structure of the protein shell have so far been obtained only with turnip yellow mosaic virus. The indications of structure obtained from particles of several other viruses are insufficient to enable the arrangement and shapes of the sub-units which form the protein shell to be determined, even though the size of the sub-units is 2–3 mμ, well within the resolving power of the microscope as determined by the Fresnel fringe test. From a critical study of the many factors that operate to limit the resolution on negatively stained biological objects, such as virus particles, it seems that only marginal improvements will be possible using existing techniques. However, these are worth seeking, because only small improvements may be needed to resolve structure in some virus particles. (Nixon and Woods)

Meanwhile, the growing use of particle-size as a descriptive character makes it urgent that different laboratories should agree in their magnification calibrations. Samples exchanged with Dr. J. Brandes at Braunschweig showed that the calibration of his electron microscope agreed with ours within 2%, an excellent agreement for two instruments of different makes, calibrated by different methods. From published work it is evident that microscopes at Wageningen and Aschersleben also agree closely with our own. (Nixon)

Unstable variant of tobacco necrosis virus. Most viruses exist in infected plants in the form of nucleoprotein particles that are stable in leaf extracts. A variant of a tobacco necrosis virus (TNV) was described last year that was unstable and behaved in a manner suggesting that it exists in plants in the form of nucleic acid. There is now much evidence confirming this suggestion. Extracts of infected leaves prepared with water or pH 8 phosphate buffer in the presence of 3% bentonite (a clay reported to bind and inhibit ribonuclease) were very infective, whereas extracts prepared without bentonite had very little infectivity, even though bentonite was added to them after they were made. Extracts prepared with bentonite and clarified by centrifugation remained infective for several hours at room temperature, but lost their infectivity almost immediately when trace amounts of ribonuclease or clarified sap from healthy leaves were added (but not when the sap had been extracted with bentonite). Further evidence that the infective material in extracts made with bentonite is nucleic acid

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comes from the fact that it behaves like the nucleic acid prepared from stable TNV by phenol, both when centrifuged in sucrose gradient columns and when inoculated to leaves, for the infection centres so initiated almost immediately begin to increase their resistance to inactivation by ultra-violet radiation, whereas leaves inoculated with TNV do so only after a 2-hour lag period. (Kassanis and Welkie)

Destruction of antigenicity by ultra-violet radiation. Studies of the effect of ultra-violet radiation on viruses have been mostly concerned with loss of infectivity, which is destroyed by exposures that leave the structure and antigenicity of virus particles largely unimpaired. Loss of infectivity reflects changes in the virus nucleic acid, and there is no record of the antigenicity of plant viruses being affected by radiation, though there is of other proteins. The effect of large doses of radiation were therefore compared on tobacco mosaic virus and serum albumins.

The ability of the virus to combine with its antibodies, and to be precipitated by the combination, were destroyed simultaneously or nearly so. To halve the ability of the virus to combine with antibody, each mg of virus protein absorbed about 4·5 joules of radiation energy (at 254 mμ). This contrasts with the 0·05 joules of energy absorbed by the virus protein when the infectivity is halved. The original antigenicity of the virus was destroyed when the particles had lost their characteristic rod-shaped structure.

Human and horse serum albumins behaved rather differently. Ultra-violet radiation destroyed their ability to precipitate with their antibodies much sooner than it destroyed their ability to combine with them. Before ability to combine with the antibodies was destroyed their structure was already altered very considerably, as shown by changes in their ultra-violet absorption spectra.

To halve the ability of the albumins to combine with their antibodies to the original albumins, requires 3–5 joules of radiation energy per mg of albumin, a value near that for tobacco mosaic virus and considerably more than the average value of 0·7 joules/mg needed to halve specific activities of enzymes or antibodies. Thus, the structures of proteins determining their antigenicity seem more resistant than structures determining other specific activities to inactivation by ultra-violet radiation.

While destroying the original antigenicity of horse-serum albumin, irradiation transiently forms a new antigenic determinant. (The work with horse serum was done with Dr. A. H. Gold, University of California.) (Kleczkowski)

Fluorescent antibody studies. Antibody gamma-globulin that is conjugated with fluorescein isothiocyanate can be extremely helpful for locating specific antigens in cells and tissues. However, the conjugates can adhere to tissues non-specifically. The causes of such non-specific staining were studied in collaboration with workers at the London Hospital. Using sections of guinea-pig liver as a test system, it was found that non-specific staining increased with increasing ratio of fluorescein to protein in the conjugate. Much of the material that stained non-specifically could be

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removed or rendered inactive by absorbing the conjugate with mouse-liver powder. This caused little or no loss of antibody. Conjugates of serum proteins other than gamma-globulin also are adsorbed non-specifically. The amount of non-specific staining differs with the tissue and the pH value during staining. (Harrison)

Carrot motley dwarf

Field experiments. Carrot motley dwarf disease caused far less damage than in 1961, because the aphid-vectors appeared later and were fewer. Sticky-trap catches at Rothamsted showed a peak of only 36 *Cavariella aegopodiae* per sq ft in late July compared with over 600 in mid June in 1961. There were even fewer aphids at Woburn, and by August only about 20 plants per plot of 66 ft × 14 ft were infected with the virus in an experiment (var. James Scarlet Intermediate) testing menazon seed dressing and spraying with "Metasystox" to control the spread of naturally introduced virus. These treatments had no effect on yield (as might be expected with few plants infected), which averaged 25 tons/acre. Infection was more frequent in other parts of England, with up to 75% infection reported on untreated carrots in Essex.

At Rothamsted few of the carrots sown on 13 April and sprayed three times with "Metasystox" became infected, but four varieties sown on 4 May and not sprayed had 50–80% infected plants by late August. Those sown on 22 June and 17 July largely escaped infection. James Scarlet Intermediate showed the most conspicuous symptoms and had more infected plants than Elsom's Spalding, Sutton's Early Giant or Autumn King.

At Rothamsted plots of Early Giant artificially infested for a week with aphids that had fed on infected plants in the glasshouse, lost 11·1, 8·4 and 3·6 tons/acre of roots respectively when infected on 5 June, 22 June and 11 July. The healthy plots yielded 24 tons/acre. This contrasts with last year's experiment at Woburn, when deliberate infections had little effect because many plants became naturally infected before it was done. In 1962 an experiment in boxes to find whether treating seed with menazon would protect vars. James Intermediate and Early Giant against deliberate infection, menazon (4% by weight of seed) delayed germination and decreased the yields of plants harvested as seedlings, but slightly increased the final yields. It did not prevent or delay deliberate infection by viruliferous aphids. (M. A. Watson and Serjeant)

Glasshouse experiments. Carrot motley dwarf is a disease caused by simultaneous infection with two viruses, carrot mottle and carrot red leaf. Coriander, but not carrot, can be infected with mottle virus by manual inoculation with sap from diseased plants. Aphids did not transmit mottle virus from plants of coriander manually inoculated, but after such plants were additionally infected by aphids with red-leaf virus, they often transmitted both viruses, confirming that red-leaf virus makes the mottle virus transmissible by aphids. The experiment had the same result whether the coriander were manually inoculated from carrot, or from manually inoculated *N. clevelandii*, thus showing that the symptoms in *N. clevelandii*

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are those caused by mottle virus, and not, as has been suggested, of another virus latent in carrot.

Additional hosts of carrot mottle virus include: *Trifolium incarnatum*, *Nicotiana xanthi* and Prince beans, in which it forms minute, transient, bronze lesions. Extracts from systemically infected leaves of *N. clevelandii* are much more infective when made in pH 9·5 borax buffer with addition of 1 ppm zinc sulphate than in pH 7·3 phosphate buffer. Extracts from recently infected *N. clevelandii* plants are much more infective than those from plants infected for 4 weeks.

Parsnip mottle. Parsnip mottle virus resembles carrot mottle virus in being more readily transmitted by manual inoculation with sap at pH 9·5 than at 7·2, but the two differ in other ways. In particular, it is transmitted by aphids without the aid of red-leaf or other carrier virus. It also infects carrot and other hosts systemically when inoculated manually. Aphids occasionally transmit it from manually inoculated coriander, but less often than from plants infected by aphids, although both methods of transmission produce similar symptoms. Parsnip mottle virus is usually lethal to coriander, whereas carrot mottle virus alone causes mild symptoms, and plants are killed only when they also contain red-leaf virus. Carrots already infected with either parsnip mottle or carrot mottle virus were susceptible to the other viruses introduced by aphids. Carrot mottle virus dominated, whether introduced first or second, but both viruses could be isolated from both lots of doubly inoculated plants.

Parsnip mottle infects *Trifolium incarnatum* and, unlike carrot mottle, also parsnip and celery. (M. A. Watson)

Barley yellow dwarf virus. Spring-sown cereal crops were free from yellow dwarf virus until July 1962, when a large invasion of grass aphids (mainly *Metopolophium dirhodum*) infected many plants, but came too late to affect yield appreciably, and symptoms could not easily be distinguished from natural ripening.

A pot-culture experiment in the open, using nitrogen-deficient soil from Bone's Close sown with Blenda oats, attempted to compare the effects of applying nitrogenous fertiliser at sowing (Early) and at time of symptom-production (Late) on the loss of grain caused by infecting seedlings with a virulent, yellow dwarf virus transmitted by *Rhopalosiphum padi* (RBYDV) and a less virulent virus transmitted by *Sitobium* spp. (SBYDV). The control plants became infected in July. RBYDV inhibited flowering completely. The grain yields of plants early and late-infected with SBYDV were increased by early but not by late nitrogen application, so the practice of applying nitrogen late to crops that become yellowed by virus infection is probably valueless. (Serjeant)

Plant protection tests suggest that RBYDV and SBYDV are probably related strains. Infective *R. padi* often failed to infect SBYDV-infected plants with the virulent RBYDV, in conditions in which they readily infected healthy plants. SBYDV more often infected RBYDV-infected plants, though it is less virulent. *Sitobium* spp. transmitted SBYDV less often from plants infected with both viruses than from plants infected with

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SBYDV alone, and did so increasingly less often the longer plants had been infected with both. (M. A. Watson)

A new virus of cocksfoot. A previously unreported virus, which will be called cocksfoot mottle, was found causing a yellow and necrotic striping and mottling of cocksfoot (*Dactylis glomerata*). It was transmitted by normal inoculation of sap and caused symptoms in Blenda oats and several varieties of wheat, but not in any other graminaceous species tested or any dicotyledon. Oats inoculated when about 4 in. high showed a mottle and large brown systemic lesions which eventually ran together; in the glasshouse flowering was not prevented. Wheat inoculated at a similar stage showed a more severe mottle and was killed before flowering. No vector has been found; nor was there evidence of seed-transmission in oats or cocksfoot.

Preparations made from diseased plants by methods customarily used to purify viruses contained many "spherical" particles about 30 mμ in diameter, with the characteristic ultra-violet absorption of a nucleoprotein and the sedimentation constant of 105S. An antiserum was prepared that reacted specifically with the virus, which seems serologically unrelated to brome grass mosaic virus.

In the field the symptoms of cocksfoot mottle were more severe, but otherwise resembled those of cocksfoot streak. Third- and fourth-year crops of cocksfoot severely damaged by cocksfoot mottle were seen in Northampton and Lincolnshire (by Dr. Storey, National Agricultural Advisory Service), and in Berkshire, Norfolk, Hertfordshire and Cambridgeshire. This virus seems the main cause of the decline of cocksfoot crops. (Serjeant)

Turnip mild yellows virus. This virus causes the older leaves of turnips to become yellow, resembling effects in sugar beet caused by beet mild yellows virus, which it also resembles in its method of transmission. It was readily transmitted by *Myzus persicae* that had fed for some hours on infected plants, particularly to *Trifolium incarnatum*, in which it causes dwarfing, chlorosis and reddening of the leaves, symptoms also caused by pea leaf-roll virus. Attempts to transmit it to sugar beet or to *Vicia fabae* failed. (M. A. Watson)

Cocoa swollen shoot virus. Rodlike particles with a most common length of approximately 125 mμ and a width of about 28 mμ were found in a series of preparations made at the West African Cocoa Research Institute, Ghana, and sent to Rothamsted for examination in the electron microscope. These rods were found in preparations from cocoa or *Adansonia* seedlings infected with each of three strains of cocoa swollen-shoot virus, but not in comparable extracts from healthy plants. The number of rods found was usually in good agreement with the infectivity of the extracts, and the length distributions of rods found in extracts from plants infected with each of three strains of CSSV show the main peak at the same length. There was too little material to determine any other physical properties, but these rods resemble those found in extracts of infected cocoa and

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Adansonia made by Tinsley and Martini in Nigeria in 1959 and 1960, and it seems reasonable to think that they are particles of cocoa swollen-shoot virus. They have some features, such as rounded ends, not previously seen in other rod-shaped viruses. (Nixon)

Cocoa yellow mosaic virus. This is the name given to a new virus found in Sierra Leone, which was referred to in the last year's Report. It resembles turnip yellow mosaic virus very closely in most of its physical properties, but some important difficulties delayed the laboratory work. It is serologically related to wild cucumber mosaic virus, which is in turn serologically related to turnip yellow mosaic virus. (Nixon and Gibbs)

Soil-borne Viruses

Tomato black ring virus. The beet ringspot form of the virus was identified as the cause of diseases of onion and leek crops in Northern Ireland. The vector nematode, *Longidorus elongatus*, seems to be extremely long-lived, and live specimens were recovered from soil samples that were stored in polythene bags in room temperature for 29 months. (Harrison)

Pea early browning virus. A virus closely resembling one recently found in the Netherlands, and called pea early browning, was obtained from lucerne and from diseased pea crops on light land in widely scattered parts of East Anglia. Its general behaviour resembles that of tobacco rattle virus, but its rod-shaped particles are slightly longer, and it appears to be a distinct virus. Experiments in the glasshouse showed that it is soil-borne and that it is transmitted by a previously undescribed nematode, a species of *Trichodorus*. (Gibbs and Harrison)

Control of arabis mosaic virus. In field experiments the nematicides "D-D" and methyl bromide again killed the vector *Xiphinema diversicaudatum* and prevented the infection of crops with arabis mosaic virus. Methyl isothiocyanate was less effective. "D-D" gave better results at 800 lb/acre than at 400 lb/acre, and was at least as effective when left in the soil through the winter as when used in summer. (Harrison, in collaboration with J. E. Peachey, Nematology Department)

Docking disorder of sugar beet. Docking disorder was reported from many sugar-beet crops in Norfolk and Suffolk. Outbreaks were of at least three types.

(1) At several sites the patches of affected beet were on slopes or the edges of old marl pits. The soils of these patches contain less organic matter than nearby soils in which beet were unaffected. These outbreaks are similar to those described before (Gibbs, A. J. *Plant Path.* (1959), **8**, 93–4), and perhaps reflect some chemical or physical character of raw soil.

(2) At three sites the patches were of indeterminate shape and not correlated with the relief of the land. The patches were characterised by many gaps between remaining plants of about normal size. Most of the

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beet and weeds in such patches were infected with tomato black ring virus, and the soils were infested with the nematode vector of the virus (*Longidorus attenuatus*). Apparently many infected plants had been killed, but others had suffered only an initial check to their growth.

(3) At two sites on slightly heavier soil the affected plants remained stunted throughout the season. Roots of affected plants were branched and rarely more than 3 in. below the soil surface. The patches of affected beet were 25–100 yards across, and were approximately kite-shaped with concave sides. The axes of the kite were parallel to the principal directions of cultivation, and a symmetrically placed and similarly shaped patch of unaffected beet was in the centre of each patch (i.e., the affected beet were in a kite-shaped fairy ring). This type of disorder could not be correlated with soil composition, or the soil population of nematodes, arthropods, *Fusarium* spp. *Verticilium* spp., actinomycetes or bacteria.

Thus, there appear to be several causes for the stunted growth of sugar beet, that is usually called Docking disorder, in alkaline sandy soils. (Gibbs and Harrison)

Transmission of tobacco necrosis virus by *Olpidium*. Of three isolates of *Olpidium brassicae* (Wor.) Dangeard, two contained tobacco necrosis virus (TNV), but they were freed from it by inoculating the zoospore suspension at dilution 1/100. The three isolates had different host preferences. *Olpidium* cultures were maintained in plants grown in sand. Zoospore suspensions were obtained by placing infected roots in half-strength Hoagland's solution for 10 minutes.

Experiments on the transmission of TNV by *Olpidium* were made using young lettuce and French-bean seedlings grown in sand or water culture, to which was added a zoospore suspension and purified virus at 0·2 μg/ml (final concentration in the water-culture medium). After 4–6 days the roots were washed, crushed and the sap inoculated to the primary leaves of French beans, where the lesions produced gave an estimate of the amount of TNV in the roots. In many experiments made under different conditions sap from roots exposed to zoospores and virus produced many lesions in bean leaves, whereas sap from roots exposed to virus alone produced only a few. The difference between the number of lesions from the two treatments differed between experiments, and the factors responsible are under investigation. Virus was never detected in roots exposed to zoospores alone. These results confirm those reported by Teakle (*Virology* (1962), **18**, 224); they leave no doubt that *Olpidium* greatly aids infection by TNV, but suggest there may be another minor method of transmission.

The relative ease with which *Olpidium*, naturally contaminated with TNV, was freed from virus, suggests that TNV does not multiply to any great extent in the fungus, if at all. (Kassanis and Macfarlane)

Fungus Diseases of Cereals

Take-all development in 1962. In 1958, 1960 and 1961 development of take-all (*Ophiobolus graminis*) on autumn-sown wheat followed a consistent pattern: plants became infected throughout the winter, but infection of

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the root systems was not extensive until May and June. In 1962, although winter infection of crops sown on infested land was as great as in the previous years (e.g., 60% plants infected at the end of April), the disease developed little during May and June, so that infection on straws in early July was very slight. However, in some crops this "slight" infection appears to have caused as much loss of yield as the more "severe" infection in previous years. Air and soil during late May and June were exceptionally cold in 1962, and this may explain the unusual development of take-all on winter wheat. (Slope and Cox)

Estimation of take-all in field crops. The results of our crop sampling in 1962 emphasised the known defects of the method of measuring take-all by "the percentage straws with take-all on roots". When combined with an arbitrary grading of the severity of the infection, this method can be satisfactory for comparing the relative intensity of attack on different treatments in one experiment when all the diagnosis is by one person. But there are objections to its use for comparing results from different years or from different experiments, or for comparisons between estimates by different people. Not only is the grading subjective, but root recovery differs greatly on different soils, and depends on the weather at the time of sampling; the methods of preparing samples for diagnosis can also greatly influence the result. A more objective, quantitative method of measuring the disease is needed at all stages of crop growth, especially for use in studies of the development of the disease in different seasons and on different soils. Of several methods used at Rothamsted during the past 5 years, the most satisfactory is based on a measurement of: (*a*) the percentage plants infected (incidence), and (*b*) the number of infected roots/infected plant (severity). If the total number of roots/plant is also counted the number of infected roots can be expressed as a proportion of the total. This method of estimating take-all is laborious, especially when more than five roots are infected; but the number of infected roots/plant can then be satisfactorily estimated on a sub-sample of the whole plot sample. Valuable additional information is obtained by counting infection on seminal and crown roots separately. (Cox and Slope)

Effect of chlorinated hydrocarbons on take-all. In a pot experiment in an unheated glasshouse Cappelle wheat, sown in naturally infested soil on 10 November, had 63% crown roots infected on 14 May; adding heptachlor at 0·002, 0·005, 0·01, 0·02, 0·04, 0·05 and 0·08 g active ingredient/pot (1·6 kg soil) brought down the percentage roots infected to 45·1, 36·1, 40·7, 27·8, 11·8, 7·6 and 6·7 respectively (0·01 g/pot is equivalent to approximately 4 lb/acre). In another experiment heptachlor was compared with eight other insecticides applied at 0·005 and 0·05 g active ingredient/1·8 kg soil to soil inoculated with *O. graminis*. None of the insecticides gave better control of take-all than heptachlor. Treating Cappelle seed with heptachlor at rates up to 5 g active ingredient/100 g of seed before sowing in naturally infested soil did not affect the incidence of take-all.

Previous pot experiments on the effect of chlorinated hydrocarbons

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did not indicate whether they act directly on the take-all fungus in the soil or after they are absorbed into roots. To gain information on this point wheat seedlings were grown to the three-leaf stage in uninfested soil, some treated with heptachlor and some untreated, when they were lifted, their roots washed and they were transplanted into naturally infested soil. Eleven weeks later there was less infection of seminal root on plants transplanted from treated soil than on plants from untreated soil, but no difference in crown-root infection. In a second experiment, naturally infected plants with 1–2 cm of lesion on seminal roots were transplanted to non-infested soil, untreated and treated with heptachlor. New seminal roots became infected after transplanting to the same extent in treated and untreated soils, but fewer crown roots became infected in the treated soil. These results need confirmation, but they suggest that heptachlor can act by being absorbed by seminal roots, although they do not exclude direct action on *O. graminis* in the soil. The use of naturally infected transplanted seedlings promises to be a useful technique, not only for experiments with chlorinated hydrocarbons but also to study the effect of other factors on infection of roots by *O. graminis*.

Soil treatments with chlorinated hydrocarbons were tested in two field experiments using October-sown Cappelle wheat. In one experiment, heptachlor was broadcast, combine-drilled and side-placed at 3 and 6 lb active ingredient/acre; in the other heptachlor, aldrin and dieldrin were ploughed in at 2 and 20 lb active ingredient/acre just before sowing. Heptachlor and aldrin slightly decreased infection 5 weeks after sowing, irrespective of method or rate of application, but in the spring the incidence of take-all was similar on all treatments. Subsequent development of the disease was so slight on control plots that no worthwhile measure of the effects of the treatments was obtained. Soil treatment with heptachlor, aldrin or dieldrin had no effect on the incidence of eyespot or sharp eyespot. (Slope and Knight, with R. Bardner, Insecticides Department)

Cereal–bean experiment. This experiment on Great Field I was ended in 1962. The summarised results from the 4th-year test crops of winter wheat (variety Cappelle) on the three series are:

	Previous crops			Grain yield* (cwt/acre)			% Straws with take-all (total)		
				1960	1961	1962	1960	1961	1962
(1)	W	W	sW	27·5	29·3	44·9	77	58	28
(2)	B	W	B	34·2	22·0	39·6	63	76	35
(3)	sW	W	sW	26·8	27·6	39·9	79	71	36
(4)	O	W	sW	25·0	23·7	35·8	85	66	41
(5)	W	O	sW	20·0	25·2	33·0	87	72	45
(6)	W	O	Be	49·9	43·4	54·5	7	7	5

* Yields are mean of two nitrogen rates (0·5 and 1·0 cwt N/acre)

W = winter wheat; sW = spring wheat; B = spring barley; O = spring oats; Be = beans.

Throughout the experiment take-all was the most prevalent disease, and weeds flourished only where crops were severely stunted by this disease. Eyespot (*Cercosporella herpotrichoides*) occurred each year, but was too slight to cause serious damage to the three test crops. Sharp

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eyespot (*Rhizoctonia solani*) was prevalent in 1960 and 1962 (59% and 34% straws infected respectively), but most of the lesions were superficial. There was as much sharp eyespot in the wheat after oat–beans (sequence 6) as after other crop sequences, and it is unlikely that the disease decreased yields appreciably. Brown Foot-Rot (*Fusarium* sp.) was rare each year, and its incidence did not differ with different crop sequences. Wheat grown after wheat or barley yielded much less grain than wheat grown after beans, and in each year the yield differences were correlated with the incidence of take-all measured by the total percentage of straws with take-all on roots in early July. However, in 1962 70% of the total infection was "slight" (i.e., infection confined mostly to seminal roots), whereas only 15 and 25% of the total infection was slight in 1960 and 1961 respectively. These results suggest that the small proportion of roots infected in 1962 was as damaging as the much greater proportion infected in the two previous years. Unfortunately the method of estimating take-all by counts of roots infected (see above) was used only on crops in sequence 1 (W W sW), where least take-all occurred, and the yield was only 17% less than sequence 6 (W O Be). It is not known, therefore, whether the estimate of straws with take-all in July gives a true picture of take-all development in treatments that suffered severe damage in 1962. It is of considerable interest that, in each year, the 4th consecutive wheat crop in sequence 1 yielded more grain and had less take-all than the 3rd and 2nd consecutive crops in sequences 4 and 5. This difference was greatest in 1962. (Slope)

Take-all on Broadbalk. Winter wheat grown on Broadbalk for 4 years out of 5 is not severely damaged by take-all, although routine sampling in the spring shows that plants with take-all occur throughout the crop. For the past 3 years the incidence and development of take-all has been studied in the 4th wheat crop on Broadbalk and in the 4th wheat crop on the Cereal–Bean Experiment on Great Field I.

In 1960 and 1961 the numbers of early infections were similar on the two fields; in mid-May 1960 the percentage roots infected on infected plants was 20 on the Cereal–Bean Experiment, 15 on Broadbalk; in May 1961 the percentages were 17 and 24 respectively. However, in both years during late May and June take-all developed more extensively on the wheat in the Cereal–Bean Experiment than in Broadbalk. In July 1960 the percentage roots infected on infected plants was 78 on the Cereal–Bean Experiment and 45 on Broadbalk; in July 1961 the percentages were 43 and 20 on Broadbalk. In 1962, by contrast, the percentage roots infected did not increase between May and July on either site. Moreover, on the cereal–bean plots used for this comparison the estimated loss of yield caused by take-all was only 13%. Two possible explanations of the 1962 observations are: either take-all development was suppressed on both sites by some climatic factor or the factor which normally suppresses take-all on Broadbalk was also active on the cereal–bean plots. As take-all caused severe damage on other plots in the Cereal–Bean Experiment and elsewhere on the farm, the latter explanation seems more likely.

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Because of the unexpectedly similar development of take-all on the two sites in 1962, it was unfortunate that the cereal–bean plots were used for comparison with Broadbalk in the study of the relationship between take-all development and the microflora associated with wheat roots. Studies in previous years have not included an estimate of actinomycetes. These were sampled on three dates in May and June in the rhizosphere (R) and the other soil (S) from Broadbalk and the Cereal–Bean Experiment. The total numbers of actinomycetes in R and S from Broadbalk were fairly constant and the R/S ratio was 1 on all sampling dates. In the rhizosphere soil of the Cereal–Bean Experiment, however, the number of actinomycetes increased 10 times between mid-May (R/S ratio 0·3) and the end of June (R/S ratio 9·6). Each field had a distinctive actinomycete flora, and only a small proportion of species were frequent on both. Preliminary tests showed that 3 of 11 isolates were antagonistic towards *Ophiobolus graminis* growing on agar plates. (Cox)

Eyespot on Broadbalk. Routine sampling on Broadbalk in 1962 showed that on plots 2B, 3 and 7 the 1st, 2nd, 3rd, 4th and 11th consecutive wheat crops after 1-year fallow had respectively 38, 38, 50, 52 and 37% straws with eyespot, of which 13, 17, 17, 20 and 14 had severe lesions. (Cox)

***Cephalosporium* Stripe of wheat.** Last year's conclusion that this disease was too rare to be important may prove too optimistic. In 1962 infected plants were found in six wheat crops at Rothamsted and one at Woburn; in most infections were rare, but in one crop 7% of the plants were infected. This crop was in a field that had grown oats, beans and grass in 1961; in the 1962 wheat crop, plants with *Cephalosporium* Stripe occurred only in the area after grass. Similarly, plants with *Cephalosporium* Stripe were found in wheat after grass in both the Rothamsted ley–arable experiments, but not after other crops. This apparent association of *Cephalosporium* Stripe with previous grass crops may be coincidental, as it was observed that seedbeds were "puffy" after grass leys and that the wheat sown on this land was damaged by winter frosts. Evidence from inoculation experiments suggests that *Cephalosporium gramineum* infects wheat roots only through wounds, and the occurrence of *Cephalosporium* Stripe in 1962 may have been associated with frost damage. Naturally infected and adjacent healthy wheat plants were labelled in the field and harvested when ripe. Infection decreased total grain yield by 78% and yield of dressed grain by 92%. (Slope)

Fungus Diseases of Potatoes

Potato blight. Blight was first seen in uninoculated potato crops on August 16, just early enough to allow completion of the full treatments in an experiment designed to define the best time to destroy the haulm. The greatest yield was from plots of King Edward sprayed first with dithio-carbamate (maneb) and later twice with copper oxychloride ("Coppesan"), but not sprayed to destroy their haulm; the total yield of 17·09 tons/acre was 2·39 tons/acre more than from untreated plots. But,

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once again, some of this benefit was illusory, because the sprayed plots lost an estimated 2·6 tons/acre from blight-infected tubers, whereas untreated plots lost 1·6 tons/acre. The loss from blight-infected tubers was only 0·17 tons/acre where sulphuric acid was used on 7 September to destroy fungicide-sprayed haulm, when only 1% had been killed by blight. This treatment was premature because the total yield (14·34 tons/acre) was 2·75 tons below the maximum. The best treatments, which did not differ significantly from one another, all had the three fungicidal sprays and an acid spray when blight had destroyed either 7 or 50% of the foliage. They yielded approximately 16 tons/acre and lost 1 ton/acre from blighted tubers. (Hirst and Stedman)

Soil activity of *Phytophthora infestans*. Potato blight was first seen at Rothamsted on 8 August in an inoculated crop of Up-to-Date. The first lesion was on a leaf resting on the soil surface, close to a young tuber that had been inoculated with *P. infestans* and covered with soil 2 weeks previously. Infection could have been caused by spores being washed the short distance from tuber to soil surface by 0·09 in. rain on 3 August. No other blight had been seen in the area at this time, and it was not seen in nearby crops until a week later.

Daily soil sampling began on a neighbouring plot of Majestic on 13 August and continued until 20 October. The soil was infective to tuber slices from 19 August to 24 September, and the surface soil was most infective when the epidemic was advancing most rapidly (between about 20 and 70% destruction of the haulm). On 2 days during this period 1,300 spores/ml surface soil were detected; concentrations were much smaller when rain occurred, and at 2, 4, 6 and 8 in. below the crest of the ridge there were never more than 100 spores/ml soil.

Tuber infection in fungicide-sprayed and unsprayed plots of King Edward were compared using the method described by Lapwood (*Rep. Rothamst. exp. Sta.* for 1960, p. 123). It was first seen in unsprayed plots after rain on 4–5 August, and new infections occurred after rain on 10–11 August, after which the first infections were seen in the sprayed plots. Infection also occurred on the sprayed plots after rain on 28–29 August, by when the haulm of the unsprayed plots was killed by blight. The fungicidal sprays delayed haulm destruction and tuber infection, but also prolonged the period when there was blight on the haulms. At the end of the season there were more tubers infected in sprayed than in unsprayed plots, suggesting that haulm destroyers should be used early in the epidemic.

Tubers of four varieties were inoculated while growing, lifted at intervals from 7 to 21 days afterwards and incubated at 15° C in humid conditions. Sporulation was greatest on Ulster Ensign tubers, intermediate on Up-to-Date and King Edward and least on Majestic. The infectivity of soil around the tubers similarly decreased from Ulster Ensign to Majestic.

Soil was taken from unsprayed and fungicide-sprayed plots of King Edward at intervals after the haulms had been sprayed with concentrated sulphuric acid (BOV). The concentration of viable spores in the soil decreased rapidly during the first 7 days after acid-spraying, but then

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declined much slower. Some infectivity (6–14 spores/ml) remained 32 days after acid-spraying on 7 September. Plots without fungicide but sprayed with acid on 14 September had 1,000 spores/ml surface soil, which declined to about 100 spores/ml by 25 September, much the same as on similar plots acid-sprayed on this date, suggesting that few spores were added to the soil after the haulm had been two-thirds killed by blight. (J. Lacey)

Skin spot of potato (*Oospora pustulans*). The unusual severity of this disease on seed tubers in 1962 led us to resume work on its epidemiology, with a research grant from the Potato Marketing Board. We are particularly interested to define whether the pathogen grows and survives in soil or is introduced annually with seed tubers; how infection spreads in crops; what damage it does and how this may be prevented.

During 1962 potatoes were grown on half of each plot on Barnfield, for the first time since at least 1843. The remainder of the field was cropped with mangolds (the classical treatment). Examination of the Majestic seed tubers at planting showed that 96% had visible skin-spotting and 39% of them had more than a tenth of their surface covered with spots. On Majestic such symptoms usually indicate severe damage to buds, and a detailed examination of excised eyes held in damp storage for 5 days at 15° C showed that 51% were infected (had *O. postulans* sporing on them) and 46% appeared dead. Lesions appeared on the underground parts of these plants as expected, and the "percentage disease ratings" (Hirst and Salt, *Trans. Brit. mycol. Soc.* (1959) **42**, 59–66) was 69% on 10 July, 82% on 7 August, 92% on 27 August and 96% on 20 September. Of the 714 stem bases examined in these assessments, none was healthy. Examination of excised eyes showed considerable infection of the buds and bract on the "eyebrow" as early as 30 July, while by 20 September almost half of the eyes had at least one of their three buds infected. By this time, however, very few eyes had been killed, and infection of the skin was only just becoming common.

Soil of sub-plots growing potatoes and mangolds was compared either by growing rooted Majestic stem cuttings or by growing tomato seedlings, which can be used to assess *O. pustulans* populations in soil. All methods detected the fungus in soil of the potato plots, but none did in mangold plots. Tomato seedling tests were started approximately monthly and showed increasing amounts of fungus as follows: no infection on 9 July, 6% on 31 July, 15% on 27 August, 29% on 20 September. Admittedly Barnfield is unique, but the clear suggestion that the *O. pustulans* was introduced on the seed tubers and may arrive in this way each year was supported by the failure of tomato seedlings to detect the fungus in a nearby plot of fallow land where potatoes were grown the previous year.

Rooted potato stem-cuttings proved much more sensitive than tomato seedlings in detecting *O. pustulans* either before, during or after growing potatoes. This is by no means the only use of rooted stem-cuttings: on the mangold plots of Barnfield they produced the only Majestic plants completely devoid of *O. pustulans* we have ever seen. Cuttings can be used

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to produce truly "spot less" tubers, which will make it possible to do critical experiments on the damage caused by stem infection, but more important, they may provide a method of re-establishing foundation stock of seed-tubers free from at least some of the tuber fungi. That this is necessary was shown by examining the eyes of (15 samples to date) King Edward or Majestic tubers harvested in 1962 in various parts of the United Kingdom. Infection of eyes by *O. pustulans* averaged about 40% and ranged from 9 to 80%. (Hirst, Salt and Hide)

Fungi of Other Crops

Isolating micro-fungi from soil. To counteract some of the deficiencies in the soil-water dilution technique, which is standard practice for isolating micro-organisms from soil, a new method was devised making use of an Andersen air sampler. When air-dried soil is drawn through this instrument six dishes of agar each become inoculated with 400 equally sized units of soil. These are transferred to agar media that selectively encourage growth of different fungi. Using 0·1-g samples of soils from Essex that contain the pea-wilt fungus (*Fusarium oxysporum* f. *pisi*) 6,909 *Fusarium* propagules/g were detected, whereas only 3,250/g were found by using the soil-water dilution technique. In forest nursery soils *Pythium ultimum* was detected at a rate of 360/g by the sampler method, compared with numbers ranging from 0 to 10/g with dilution. Similar increases in efficiency of detection were found for these two fungi in several soils from fields in which they had caused diseases.

Calibrating sterilised soils with a series of numbers of *Fusarium* conidia showed that nearly all were recoverable and that they were evenly distributed among the soil units. Results were always more reproducible than those obtained by soil-water dilution procedures, because dilution errors cause by soil settling in pipettes and dilution tubes are eliminated in the sampler method. In addition, the sampler disperses soil uniformly over agar media in Petri dishes, and many non-pathogenic fungi whose spores normally predominate in water suspensions do not have the same opportunity of crowding out others of more interest to pathology. This method usually detected 1·7 times as many fungi in soils as the dilution method, moreover, it gave 0·65 of the theoretically expected number of fungi instead of 0·45 with dilution. The error does not increase as sample size decreases, whereas it usually does with the dilution technique.

The sampler is also proving useful for examining the form in which fungi occur in soil. Thinly dispersed soil units contain recognisable spores and fragments of mycelium, some of which are blown clear of soil particles. Chlamydospores transferred to culture media usually grew as colonies of *Fusarium*. By adding mixtures of genetically different cultures to soil and recovering chlamydospores with the sampler, attempts are being made to establish whether or not the several nuclei in each chlamydospore are genetically different. (Buxton, Kendrick and Brown)

The effects of chitin on pea wilt. Adding chitin, an amino-polysaccharide, to soil diminished the amount of pea wilt caused by *Fusarium oxysporum*

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f. *pisi*, both in glasshouse and field (*Rep. Rothamst. exp. Sta.* for 1961, p. 118). In glasshouse tests different amounts of chitin were added to pots of John Innes compost inoculated with *F. oxysporum* f. *pisi* race 1. Eight weeks later pea seedlings var. Onward were transplanted into the pots. Wilt indices, recorded weekly for eleven weeks, showed that chitin levels A (38·5 g/8 pots) and A/2 (19·25 g/8 pots) decreased wilt. Soil samples collected at the end of the experiment were plated out by the dilution technique on colloidal chitin medium to count actinomycetes, and the modified Andersen Sampler technique was used to count *Fusarium*. Increasing the amounts of chitin increased actinomycetes from 186,000/g of soil with no chitin to 964,000/g of soil with chitin level A, and decreased *Fusarium* from 7,440 to 2,960/g of soil. This suggests an antagonism by the actinomycetes towards the *Fusarium*, which may explain the decrease in wilt. Actinomycetes collected from soil used in this experiment differed in the extent of their antagonism towards the pathogenic *Fusarium* and other *Fusarium* isolates.

Similar experiments in which chitin was added at weekly intervals to inoculated soil showed that the earlier the chitin was added, the less wilt developed. The wilt indexes when chitin was added 8 weeks and 1 week before transplanting were 31% and 48%. This may mean that chitin acts, probably indirectly, by suppressing the *Fusarium*, for less fungus remained in the soil the earlier chitin was applied.

The effect of five levels of chitin was investigated in microplots containing wilt-sick soil from Yaxley, Peterborough. Peas, var. Onward, were sown 6 weeks after the chitin was added. Eight rhizosphere and non-rhizosphere soils were analysed at weekly intervals for *Fusarium*, actinomycetes and other soil-borne fungi. The *Fusarium* population was larger in the non-rhizosphere soil than in the rhizosphere soil of chitin-treated plots, the reverse of the position in control plots, as found in the glasshouse experiments. Actinomycete populations were proportional to the amount of chitin applied, whereas the amount of *Fusarium* was inversely proportional to it. Actinomycetes were more abundant in the non-rhizosphere than in the rhizosphere soil, but those from the rhizosphere were more antagonistic towards *Fusarium*. (Khalifa)

Diseases of Sitka spruce seedlings. Seed and soil treatments were used in experiments on the control of losses of Sitka spruce (*Picea sitchenis*) in five nursery seedbeds. The number of seedlings obtained from 1,800 viable seeds sown/square yard differed greatly between nurseries, although all were sown under ideal conditions in March. Untreated seed sown in untreated soil yielded only 346 seedlings at Old Kennington and 745, 815, 1,083 and 1,360 respectively at Kennington Extension, Ringwood, Bagley Wood and Wareham. Seed treatment with methoxyethyl mercury chloride lessened pre-emergence loss and increased numbers to 422, 914, 860, 1,207 and 1,474 respectively, but had no effect on post-emergence damping-off, or on seedling height, which ranged from an average of 1·23 in. at Ringwood to 2·41 in. at Wareham.

In contrast, partial soil sterilisation with formalin and chloropicrin controlled damping-off and substantially increased heights at all nurseries

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except Wareham (where seedlings usually grow well). Soil and seed treatments together produced the best results; the most outstanding improvement was at Ringwood where the combined treatments increased numbers from 815 to a mean of 1,318, and heights from 1·23 to 2·56 in. At all nurseries pre-emergence loss was more serious than post-emergence damping-off, for it was not controlled by partial sterilisation, and even where both seed and soil treatments were applied about $\frac{2}{3}$ of the viable seed sown failed to emerge at Old Kennington, $\frac{1}{2}$ at Kennington Extension, $\frac{1}{4}$ at Bagley Wood and Ringwood and $\frac{1}{5}$ at Wareham.

A wider range of soil treatments were applied at Ringwood and Old Kennington nurseries, where healthy seedlings usually cannot be raised without chemical treatment of the soil. At Ringwood seedling numbers were increased most by formalin, "Vapam" and "Mylone" applied as pre-sowing drenches, and less by pentachloro-nitrobenzene (PCNB) broadcast as a dust and forked in, and maneb applied as post-sowing drenches at monthly intervals. Height also was increased most by "Mylone", formalin and "Vapam", and less by maneb, but was not affected by PCNB.

At Old Kennington fewer seedlings died in untreated soil than at Ringwood, and only formalin and "Mylone" increased numbers and heights. The other three chemicals increased seedling mortality and failed to increase height. The different behaviour of "Vapam" at the two nurseries was particularly striking.

Applications of PCNB and maneb to soils already treated with formalin, "Vapam" and "Mylone" were no more effective than formalin alone in increasing numbers and heights.

Isolates of fungi from the roots of seedlings growing in treated and untreated plots show that several different species of *Pythium* were common early in the season; in pure culture these killed seedlings before they emerged. Later in the season *Cylindrocarpon radicicola* was the most frequent species, but *Fusarium* and *Phoma* were also common; the pathogenicity of these still has to be determined. Soil treatments affect different fungi differently; for example, all treatments affected *Phoma* less than *C. radicicola*, which was decreased most by formalin and "Vapam", less by "Mylone" and hardly at all by PCNB and maneb.

Beneficial effects of partial sterilisation were found 15 months after treatment in other experiments designed to measure its persistence. Soil treated with formalin in January 1961 and fallowed until sown in March 1962 yielded seedlings with mean heights of 2·40 in. at Ringwood and 2·60 at Old Kennington, whereas the mean from untreated soil was 1·66 and 2·31 in. respectively. Numbers also were increased by the treatment at Ringwood. Seedling numbers in untreated plots, fallowed for one season, exceeded those in plots that were cropped by 20% at Ringwood and 26% at Old Kennington. Fallowing also improved height slightly. (Salt)

Genetic variation in *Colletotrichum atramentarium*. Irradiating isolates of *Colletotrichum atramentarium* (cause of black dot of tomato fruit) with ultra-violet light produced morphological and nutritionally deficient mutants, which formed heterokaryons grown together on agar media. Mutants plated singly did not grow on basal media, on which the

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heterokaryons all resembled wild-types. Several uninucleate conidia from heterokaryons gave rise to colonies with wild-type growth on basal media, indicating their probable diploid nature. Single conidial progeny from suspected diploids gave rise to cultures either of parental or recombinant genotype. Many of the recombinants proved to be haploid, indicating that genetical recombination had probably occurred through a parasexual cycle.

Not all the possible recombinants were recovered. The phenotypic expression of one mutant character in a recombinant persistently emerged only after a mean of 10 subcultures, indicating a possible interaction between the gene controlling the expression of this character and the cytoplasm. Theoretically, cytoplasmic particles conditioning expression of the gene might replicate at a rate which results in dissociation only after 10 divisions. The effect of mixing different cytoplasms from distinct strains on the expression of genotypes is being studied in this fungus and in *Fusarium oxysporum*. (Buxton and MacNeill)

Apple scab. In 1960 and 1961 applying ammonium sulphate in spring to dead apple leaves decreased the liberation of ascospores by *Venturia inaequalis*. In 1962 it did not; nor did the application of granular fertilisers, whether compound or nitrogenous only, affect ascospore liberation. The difference was probably due to the weather after the fertilisers were applied; in 1962 it was cold and wet in contrast to the unusually warm dry days and dew nights of 1961. It will be necessary to continue tests to define the conditions that affect ascospore liberation and to estimate how often the treatment is likely to succeed. (Hirst and Stedman)

Spore-liberating mechanisms. Preliminary micro-wind tunnel tests suggested that the aleuriospores of *Pithomyces chartarum* could be liberated from dead ryegrass leaves at wind speeds as slow as 1 m/sec and over a wide range of relative humidity, but that many are liberated only when the wind exceeded 2·5 m/sec or the specimen was shaken. *P. chartarum* can certainly be added to the fungi listed in last year's Report (p. 114), whose spores can be dispersed by impact of the raindrops on wet spore-bearing surfaces. These tests had to be made with dead spores from cultures on freshly killed ryegrass leaf and require confirmation in more natural conditions. Because winds of 2·5 m/sec will seldom persist among the litter in pastures, the rain-induced "take-off" may be one of the most important ways by which *P. chartarum* spores are air dispersed; it would certainly account for the rapid increases of airborne *P. chartarum* spores at the start of rain recently described in Jamaica by Meredith (*Ann. appl. Biol.* (1962), **50**, 577–594). (Hirst, Stedman and Drew Smith)

Micro-fungi of pastures. The cause of hepatogenous sensitisation of sheep in Great Britain is unknown, but symptoms of conditions known variously as yellowses, head grit, saut or plochteach are somewhat similar to mild cases of facial eczema in New Zealand which is caused by the toxin sporidesmin. The saprophytic fungus *Pithomyces chartarum*, which produces sporidesmin, was recently found in England, growing on debris of *Holcus lanatus* (Lacey, M. E. & Gregory, 1962) and spores were

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trapped in Berkshire, Surrey and Hertfordshire. The occurrence of *P. chartarum* and the possible relationship between it, or other fungi, and photosensitisation of sheep in Britain are being studied, and farms in the north of England and Scotland where these conditions occur annually were visited. These were mostly hill farms. Except for one farm in Northumberland, the condition is noted in lambs in June, and effects described ranged from a mild illness with few deaths to 25% mortality. The incidence and severity increase northwards and westwards in Scotland; they also vary with season, although there is little agreement about the weather that favours its appearance. Often only parts of farms are affected. One well-defined area may be affected, whereas others close by are not.

Narthecium ossifragum has been incriminated as a cause in Norway, but on the farms visited in Britain there was no evidence that it was associated with yellowses. Weather was generally unsuitable for spore trapping, but a single spore of *P. chartarum* was seen on a trap exposed in Kirkcudbrightshire. (J. Lacey)

Actinomycetes in mouldy hay. Many actinomycetes in mouldy hay were isolated, and seven of the most common species were identified. *Micromonospora vulgaris* Waksman *et al.*, *Streptomyces thermoviolaceus* Hens. and *Thermopolyspora polyspora* Hens. grew at 40° and 60°. A new species of *Thermopolyspora* Hens., *Streptomyces fradiae* (Waksman et Curtis) Waksman et Henrici, and *S. griseoflavus* (Krainsky) grew at 40°, *S. olivaceus* (Waksman) Waksman et Curtis at 28° and 40°, and *S. griseus* (Krainsky) Waksman et Henrici at 28°.

The numbers of different species isolated by the Andersen sampler method from any one sample of hay depends on the medium used. *Micromonospora vulgaris* and the *Thermopolyspora* species are most easily isolated on nutrient agar, *S. fradiae* on yeast agar, and *S. thermoviolaceus* and *S. griseoflavus* on V8 (vegetable juice) agar. (Corbaz, Gregory and M. E. Lacey)

Banana fruit rots. Attempts to control crown rot (*Rep. Rothamst. exp. Sta.* for 1961, p. 118) by applying "Dithane M.22" treatments to bananas in the Cameroons before shipment failed, despite reported success in similar circumstances in Central America. "Dithane" concentrations were 1, 2 and 3 lb/gal, ensuring not more than the permitted maximum of 7 ppm residue in the fruit. Fruit rots and crown rots, caused mainly by *Botryodiplodia*, *Thielaviopsis* and *Gloeosporium*, were recorded 1, 4 and 8 days after the fruit arrived in Britain and were equally common on fruit treated with "Dithane", water or sodium hypochlorite.

Some shipments of bananas, var. Poyo, from the Cameroons showing unusual rots were examined at the request of the importers. The trouble was caused by the fungus *Trachysphaera fructigena*. First recorded in 1923 as a pathogen of coffee and cacao fruits, the fungus has also been occasionally reported as causing a slight rot near the distal end of bananas. On the Cameroons fruit it caused a severe crown rot, which developed so rapidly that boxed or stem-shipped fruit was unsaleable within a few days of arrival.

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The fungus grows and sporulates on the surface of unwounded fruit, but causes damage only when it infects through a wound. Brown lesions up to 3 in. diameter develop in 3 days at 70–80° F (ripening temperature) and sporulate profusely. At 58° F (shipping temperature) lesions develop at about two-thirds this rate, and do so on unripe green fruit. Var. Lacatan, shipped from the West Indies, is susceptible. Spores are released in slight air movements and occur in the air of ripening rooms. West Indian fruit becomes contaminated from Cameroon fruit after arrival in Britain. The disease is unknown in the West Indies, but the possibility obviously exists that the fungus might be taken there by ships that have been to the Cameroons.

Fruit inoculated with spores or hyphae growing on 1 cu mm agar blocks was treated with "Dithane M.22" at various concentrations and for different times. No treatment that left less than 7 ppm residue, the commercially acceptable concentration, was successful. (Buxton, Ward and Brown)

Conjoint Work with Other Departments

Besides the conjoint work reported above, other work is noticed in the reports of the following departments: Bee (Bailey and Gibbs); Biochemistry (Pierpoint and Harrison; Festenstein, Skinner, Gregory and M. E. Lacey); Insecticides (Bardner and Gibbs); Soil Microbiology (Skinner, Gregory, M. E. Lacey and Festenstein).

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