ROTHAMSTED RESEARCH

Rothamsted Report for 1963

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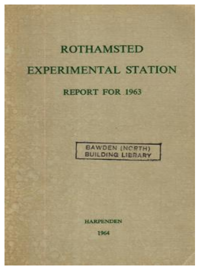

Plant Pathology Department

P. H. Gregory

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PLANT PATHOLOGY DEPARTMENT

P. H. GREGORY

On 30 September Lina Cunow retired after 29 years on the staff. In April Mary D. Glynne and J. M. Hirst attended a symposium on factors determining the behaviour of plant pathogens in soil, sponsored by the United States National Academy of Sciences—National Research Council, at Berkeley, California. After the meeting Hirst visited La Lima, Honduras, to discuss the epidemiology of banana leaf spot, at the invitation of the United Fruit Company.

In June B. D. Harrison was an invited instructor at an Advanced Subject Matter Institute in Plant Virology at the University of Maryland; before this he visited other universities in the United States where viruses are studied.

In September a NATO Advanced Study Institute on Biometeorology and Epidemiology of Fungal diseases of plants, organised by the Pennsylvania State University in connection with the 3rd International Biometeorology Congress at Pau, France, was attended by J. M. Hirst (Co-Chairman) and D. H. Lapwood. Lapwood also contributed to the 2nd Triennial Conference of the European Association for Potato Research at Pisa, Italy in September.

Mr. E. Debrot (Venezuela) joined the department as a temporary worker.

The Department acted as host to an Agricultural Research Council meeting of plant virologists in September.

Properties of Plant Viruses

The inactivation and photoreactivation of virus nucleic acid. There is good evidence that initial stages of inactivation when a bacterial-transforming deoxyribonucleic acid (DNA) is exposed to ultra-violet radiation (UV) depend largely on dimerisation of adjacent thymine residues, and that photoreactivation depends largely on the reversal of this dimerisation. Although UV can also cause uracil to form dimers, no evidence was found that dimerisation plays any part in the inactivation and photoreactivation of the ribonucleic acid from tobacco mosaic virus (abstract 7.24). (Kleczkowski)

Effect of ultra-violet irradiation on susceptibility of serum albumins to trypsin. Ultra-violet irradiation converts bovine serum albumin (BSA) and rabbit serum albumin (RSA) into forms more rapidly hydrolysed by trypsin than the original proteins. The radiation energy (at 2,537 Å) required to be absorbed by each milligram of a protein to convert half of it into a form susceptible to trypsin ($E50\%$) is about 0·32 joules for BSA and 0·62 joules for RSA. The average $E50\%$ for inactivation of enzymes and

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antibodies is 0·7 joules, so the two processes may depend on similar alterations in irradiated protein molecules. The alteration can be considered as denaturation, with protein molecules becoming at least partially unfolded and making the sites susceptible to the attack by trypsin accessible to this enzyme. (Kleczkowski)

A method of overcoming plant inhibitors in virus transmission. Sap of Sweet William plants (*Dianthus barbatus* L.) strongly inhibits the infectivity of viruses, and it is impossible to transmit, for example, carnation ringspot virus from Sweet William to French bean or tobacco plants until the virus is freed from the inhibitor (Kassanis, B., *Ann. appl. Biol.* (1955) **43**, 103–113). Although sap from Sweet William plants infected with carnation ringspot virus is not infective, phenol extracts are and produce over 500 lesions per French bean leaf. Presumably the inhibitor is removed or destroyed by the phenol, and the method is useful in transmitting such viruses as carnation ringspot that give a good yield of infective nucleic acid when disrupted with water-saturated phenol. (Kassanis)

***Ononis* yellow mosaic virus.** A virus was isolated from many plants of Rest harrow (*Ononis repens* L.: Papilionaceae) showing a bright yellow mosaic and growing in different parts of Southern England and Wales. It is readily sap-transmitted to other herbaceous plants of several families, including *Pisum sativum* L. var. Onward, from which it is easily extracted and purified. The virus is distantly serologically related to wild cucumber mosaic virus but not turnip yellow mosaic virus, but is similar to all of these viruses in the size, sedimentation constant and appearance of its particles. Dr. R. MacLeod (Virus Research Unit, Cambridge) also found they are all similarly rich in cytidylic acid. Turnip yellow necrosis virus is transmitted by flea beetles, but flea beetles were not found on *Ononis repens* plants during 1963 at several sites where the virus occurred; the virus was not transmitted by *Acyrthosiphon pisum* (Harris), *Sminthurus viridis* (Linné 1758), *Apion ononis* Kirby and *Macrotylus paykulli* Fall., all of which were common on Ononis plants. None of 50 plants grown from seed collected from infected plants were infected. (Gibbs)

Arthropod-transmitted Viruses

***Cavariella* spp. and carrot motley dwarf and parsnip mottle viruses.** The willow-parsnip aphid (*Cavariella pastinacae*) transmitted parsnip mottle virus, but not carrot motley dwarf. Although it feeds for a few days on carrots, it will not colonise them, and is either unable to transmit motley dwarf viruses or does so very inefficiently. *C. aegopodiae* can transmit both these viruses, which resemble each other in many properties, but differ, like individual barley yellow dwarf viruses, in vector specificity. (M. A. Watson)

During the 2 weeks ending 24 June sticky traps at Woburn and Rothamsted caught 438 and 634 *Cavariella* spp. respectively. Although these equal catches during June in 1959 and 1961, years when carrot crops were badly damaged by motley dwarf disease (*Rothamsted Reports* for 1959 and

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1961), carrot crops were not damaged by the disease in 1963. This may be because: (1) there was no early spring peak invasion in May 1963; (2) the summer peak lasted only 2 weeks in 1963, contrasted with 4 weeks in 1959 and 5 weeks in 1961. *C. pastinacae* were abundant on willow on 7 June, and on sticky traps near to willow two weeks later, when *C. aegopodiae* were rare. *C. aegopodiae* were found on carrots, but many fewer in relation to the numbers of *Cavariella* spp. caught on the traps than in other years. (Watson and Heathcote)

Control of carrot virus diseases. Because of small natural spread of carrot motley dwarf virus in 1963, yields of plots not treated with insecticides were around 20 tons/acre at both Rothamsted and Woburn. At Woburn menazon granules sown beneath the seed-drills and spraying twice in early June with "Metasystox" decreased the aphids/100 plants on 25 June from 1,026 on untreated plots to: menazon only 216, "Metasystox" only 206, menazon plus "Metasystox" 93. These differences, however, did not affect the proportion of virus-infected plants recorded in early September (12·5% motley dwarf and 8% red leaf).

At Rothamsted, to compare loss of yield resulting from motley dwarf infection with loss from aphid infestation, small plots of carrots, protected by (nominally) aphid-proof cages, were experimentally infested with either viruliferous or non-viruliferous aphids on 30 May. The aphids were killed by spraying either 7 or 48 days later, but some aphids and plants outside the cages became virus-infected, and spread from these contaminated cages initially infested with virus-free aphids. The experiment therefore did not discriminate accurately between the two causes of loss, but it demonstrated that where aphids are very numerous introductions of virus too small to affect yield directly soon lead to all plants being infected and to much loss. Yields based on four replicates are:

Aphids left to multiply:	Tons/acre (7 days)		Carrots (48 days)
Treatment			
Control: no aphids	22	S.E. 1·6	—
Virus-free aphids	21		10
Infective aphids	5		2½

† = mean of 3 plots contaminated by virus.

Plants infected as seedlings lost over 75% of yield when the aphids were killed early, and 87% when aphids were not killed. With a less-virulent strain of virus in 1962 the loss was 50%.

Plots where aphids were left to multiply had massive aphid-populations when the covers were removed on 17 July; one nominally virus-free plot was already infected and two showed symptoms within a few weeks. The fourth, which was among treatments not involving infection, remained almost uninfected and yielded 17 tons/acre, compared with 7½ tons for the others. Probably 20% represents the loss that can be attributed to direct damage by aphids.

For the first time in these experiments (*Rothamsted Reports* for 1959–62) carrot root-fly (*Psylla rosae*) was troublesome. It did not attack plants that were caged from 24 May to 17 July, and their roots at harvest showed no

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obvious damage. Carrots surrounding the cages were sampled on 17 July and 20 September. Those around artifically infected plots obviously had motley dwarf before the end of June, and those around healthy plots remained green. On 17 June roots of plants with motley dwarf seemed more frequently and more severely damaged than others, but this was probably because they were smaller and the tunnels more conspicuous. At harvest 36% of roots from infected and uninfected plants were damaged. Leaves of virus-free plants remained green and turgid even when the roots were badly damaged. Virus-infected plants were dwarfed and discoloured whether tunnelled or not, but the vascular supply to the tops was easily severed, and more virus-infected plants wilted and died than healthy plants. This may explain the tendency to associate symptoms of motley dwarf virus with carrot root-fly injury. (Watson and Serjeant)

Turnip mild yellows. Symptoms of this disease, described last year (*Rothamsted Report* for 1962, p. 112), resemble those of potash or phosphate deficiency. The failure of the virus to infect *Physalis floridana* distinguishes it from the turnip latent virus described from Canada; similarly, its failure to infect sugar beet or *Claytonia perfoliata* distinguishes it from beet mild yellows virus, and its failure to infect radish distinguishes it from American radish yellows (Western X virus of sugar beet). It was isolated from *Brassica chinensis* and cabbage var. King of Hearts on which infective *Myzus persicae* had been fed. Cabbage and turnips showed yellowing of cotyledons and first leaves. It was retained for a week by aphids feeding on henbane, which is not susceptible. It has been twice isolated from garden crops grown in the open in Harpenden. (Watson)

Viruses of Cereals and Grasses

European wheat striate mosaic virus. Further experiments (*Rothamsted Report* for 1961, p. 108) injecting leaf-hoppers (*Delphacodes pellucida* Fab.) with extracts of infective hoppers or infected plants have failed to associate infectivity with any particle identifiable by electron microscopy. The infectivity can be sedimented in an ultracentrifuge, but is lost when extracts of hoppers or plants are treated with ether or carbon tetrachloride. Hopper extracts heated to 30° C for 10 minutes remain infective, but those heated to 40° C do not. Infected plant extract (in 0·2*M*-phosphate buffer with 0·01*M*-diethyl-dithio-carbamate) were infected when diluted $\frac{1}{20}$ but not $\frac{1}{40}$. Extracts of thoraces and abdomens of infective hoppers, injected at 0·01 g/ml into healthy hoppers, caused them to become infective, but the extracts of severed heads at 0·1 g/ml did not. In hoppers feeding on infected plants infectivity was first detected by injection into healthy hoppers after 8 days; this is the same period required for infectivity to be detected by feeding hoppers on test plants. (Serjeant)

Cocksfoot virus diseases. Cocksfoot mottle virus was transmitted to barley (var. Proctor) by sap inoculation; it caused systemic necrotic lesions and stripes, but did not prevent plants flowering in the glasshouse. It caused similar symptoms in 13 varieties of cocksfoot inoculated.

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In the field it was common again during 1963 in older crops, and about 50% of suspected cocksfoot mottle samples sent in by the National Agricultural Advisory Service contained the virus. Cocksfoot streak virus occurred in about 20%. A unique field-infected wheat plant was found in June in a crop planted after four years of cocksfoot/lucerne ley, in which cocksfoot mottle had been abundant. Previous glasshouse experiments in which wheat was planted in soils from such fields had not produced infection. Cocksfoot mottle has not been found on the Park Grass Experiment, or on Highfield, the oldest grazed pasture on the Rothamsted Farm. Young cocksfoot seedlings in pots which were sunk in the soil of a field carrying a cocksfoot crop with many infected plants were left in the field for periods of 2 weeks and then moved to the glasshouse; several of these seedlings exposed in July and August became infected with cocksfoot streak virus, but only when they and the crop had been cut during their exposure did any of them become infected with cocksfoot mottle virus.

Measurements on electron micrographs of shadow-cast preparations of cocksfoot mottle virus showed the particles to be 30·5 mμ in diameter. Micrographs of stained preparations showed that some particles were penetrated by the stain and others were not, suggesting some did not contain nucleic acid, but in the analytical ultracentrifuge the preparations gave a single peak and no sign of containing particles of two different weights. The electrophoretic mobility in $M/15$-phosphate buffer was $-0{\cdot}75$ μ/sec/V/cm.

Gel-diffusion tests using antisera to tomato aspermy, broad bean mottle, red clover mottle and tobacco necrosis showed no serological relationship between these viruses and cocksfoot mottle. (Serjeant)

Soil-borne Viruses

Raspberry ringspot virus. Cucumber, French bean, sugar beet, *Chenopodium quinoa* and *Petunia hybrida* but not turnip plants became infected with the English form of raspberry ringspot virus when grown in soil containing viruliferous *Longidorus macrosoma*; cucumber and French bean were infected the most readily, cucumber when exposed to the nematodes for only 4 days. Like other nematode-transmitted viruses, raspberry ringspot seems to persist in its vector for some weeks. Seedlings became infected when grown in soil containing viruliferous *L. macrosoma* that had been kept without plants for 34 days. Virus was once detected by inoculating plants with extracts of crushed nematodes. (Debrot)

Specificity of vector nematodes. The form of raspberry ringspot virus known from southern England is serologically related to the form known from Scotland, but only distantly, and it seems to have a different vector. A similar situation exists with tomato black ring virus. *Longidorus elongatus*, which transmits the forms of both viruses occurring in Scotland, was not found where infected plants occurred in England. Instead, *L. macrosoma* occurred where raspberry ringspot virus was spreading and *L. attenuatus* where tomato black ring virus was spreading. In laboratory tests, *L. macrosoma* readily transmitted the English but not the Scottish

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form of raspberry ringspot virus, or either form of tomato black ring virus. *L. attenuatus* transmitted the English form of tomato black ring virus, but in the one test made failed to transmit the Scottish form. Ability to be transmitted by a given species of nematode appears correlated with the antigenic constitution of the virus. (Harrison)

Pea early browning virus. Viruses obtained from pea crops in East Anglia affected by early browning disease were closely related to one another in serological tests, but only distantly related to a culture of pea early browning virus from The Netherlands. The British and Dutch viruses, however, caused similar symptoms in many herbaceous hosts. In several plants these symptoms resembled those caused by tobacco rattle virus, but in some (e.g., French bean) they differed. Some fields carrying diseased pea or lucerne crops contained *Trichodorus viruliferus*, shown last year to be a vector, but more contained *T. primitivus*, sometimes together with other *Trichodorus* spp.

Some isolates of pea early browning virus were readily transmitted by manual inoculation when the inocula were leaf extracts made with phenol, but not when crude sap was used. (Gibbs and Harrison)

Viruses in sugar beet. Tomato black ring virus occurred in several poorly growing sugar-beet crops in East Anglia. A nematode vector of this virus, *Longidorus attenuatus*, occurred at all such sites. The virus also was seed- and pollen-transmitted in sugar beet. A second nematode-transmitted virus, tobacco rattle, was found at some of the sites; it was often restricted to the roots of infected plants, but when leaves were infected they showed bright yellow blotches and ring patterns. These symptoms could not always be distinguished from those caused by tomato black ring virus, although they were usually more severe. *Trichodorus pachydermus* occurred in all the beet fields infested with tobacco rattle virus and transmitted the virus in laboratory tests. (Gibbs and Harrison)

Transmission of Tobacco Necrosis Virus by *Olpidium*.

Experiments on transmission of tobacco necrosis virus (TNV) to roots of lettuce plants by the zoospores of the fungus *Olpidium brassicae* (Wor.) (*Rothamsted Report* for 1962, p. 114) were continued using young lettuce and Mung bean seedlings, grown in Hoagland's nutrient solution diluted $\frac{1}{20}$, and strain D of TNV. The seedlings were grown in small vials containing 5 ml of nutrient solution, and to inoculate them 1 ml of a zoospore suspension and 0·5 ml of purified virus were added to each vial. Virus transmission was favoured by small salt concentration and neutral or slightly alkaline reaction of the nutrient solution, and depended also on concentration of virus and zoospores. When the zoospore concentration was about 10^5/ml transmission to lettuce was obtained with as little as 0·05 μg/litre of virus. With more concentrated virus (about 5 μg/litre), it was transmitted by 50–100 zoospores/ml. Fungus infection, as measured by the number of zoosporangia in the roots, was not correlated with virus infection. Perhaps penetration of zoospores that fail to develop is sufficient to transmit

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virus. This is indicated by the fact that virus was transmitted when the fungus was killed 2 hours after inoculation by dipping roots for 10 seconds in water at 50°C; zoospores penetrate roots within 2 hours.

There is evidence that *Olpidium* causes TNV superficially: (1) some *Olpidium* isolates were naturally contaminated with TNV, but, from these, virus-free cultures were obtained by inoculating roots with dilute zoospore suspensions; (2) zoospores that had been mixed with TNV were partially freed from it by centrifugation; (3) virus transmission was prevented by adding antiserum to zoospores that had already been exposed to virus.

TNV was not only transmitted by *Olpidium* to roots but also to tobacco callus tissues, in which the fungus produced mature sporangia. This discovery may aid work both on the fungus and virus multiplication. Previously only roots have been known to be susceptible to *Olpidium*, and callus tissues may be a better medium in which to study this obligate parasite. Also, callus tissue is very difficult to infect by mechanical inoculation with any virus, but it is very susceptible to TNV transmitted by *Olpidium*. (Kassanis and Macfarlane)

Fungus Diseases of Cereals

Take-all development in winter wheat in 1963. Growth of winter wheat at Rothamsted was retarded by the exceptional cold lasting from early December to late February. Most crops were sown in November, but as germination and growth were very slow, plants were still at the 2-leaf stage in early March. Very few plants became infected with *Ophiobolus graminis* during the winter, but take-all later developed rapidly and became severe, as shown on a typical third successive wheat crop:

	1 Apr.	3 May	27 May	17 June	4 July
% plants infected	1	31	66	—	73 (straws)
% seminal roots infected	0·2	7·1	19	50	68
% crown roots infected	0	0·7	8	22	52

Thus, in sharp contrast to 1962, take-all progressed without a check throughout the summer, but although 66% plants were infected by the end of May, the crop showed no obvious damage until many of the crown roots were infected in the second half of June. This conforms with observations of previous years, and it seems likely that if crown-root infection could be prevented by fungicidal sprays applied in late April or early May damage from take-all might be largely prevented, even though most of the crop might be infected—a more promising possibility than attempting to eradicate *O. graminis* before sowing or preventing infection throughout the winter. (Slope and Cox)

Take-all on Highfield ley–arable experiment. Take-all was recorded for the first time on winter wheat in the Highfield ley–arable experiment, now in its 15th year. Small, widely separated patches of plants with severe root infection were associated with patches of *Agropyron repens* and showed up strikingly as whiteheads in July when the rest of the crop was green.

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Random samples showed that too few plants were infected to cause detectable loss of yield; wheat after 3 years lucerne had 3·2% straws infected, compared with 2·8% in the arable rotation and none after cut grass, or grazed ley. (Salt)

Effect of chlorinated hydrocarbons on take-all. Cappelle wheat, sown in pots of naturally infested soil in an unheated glasshouse on 12 February, had 71% crown roots infected on 11 June. Adding heptachlor at 0·001, 0·002, 0·003, 0·004, 0·005, 0·01, 0·02, 0·04 and 0·08 g/pot (1·8 kg soil) brought the percentage roots infected down to 68, 50, 46, 37, 42, 32, 22, 7 and 1 respectively, resembling results obtained in 1962.

Cappelle wheat sown in the field on 13 November had 42% straws with severe take-all on 8 July on untreated control plots; spraying the soil, 3 weeks before sowing (after ploughing but before rotovating), with 4 or 8 lb heptachlor per acre decreased the percentage straws with severe take-all to 31 and 20 respectively, and increased yield, though not significantly. Control plots yielded 24·5 cwt/acre, plots treated with 4 or 8 lb heptachlor/acre yielded 27·2 and 27·5 cwt/acre respectively. (Slope, with R. Bardner, Entomology Department)

Continuous wheat growing and the decline of take-all. Last year we reported that in the cereal–bean experiment the 4th successive wheat crops had less take-all and yielded more grain than the 2nd or 3rd crops, and suggested that in the 4th-year crops the disease was partially suppressed by an inhibitory factor similar to one that appears to control take-all on Broadbalk field. To investigate this idea further, the 1962 cereal–bean plots were again sown with Cappelle wheat. The 5th crop was always slightly less infected than the 3rd or 4th crops, and at the final sampling on 4 July the percentage straws with take-all was 64, 75, 73, 75 in the 5th, 4th, 3rd and 2nd crops respectively. The "decline" of take-all in the 5th crop was similar to that in the 4th crops of the cereal–bean experiment in 1960 and 1961, but very much less than that in 1962. Differences in grain yield were small and not significant, the 5th crop yielding 28·1 cwt/acre compared with 27·0, 24·4, 25·5 cwt/acre from the 4th, 3rd and 2nd crops. Clearly, whatever its cause, the extent to which take-all diminishes in continuously grown wheat differs greatly in different years; whether it will become more constant after a longer period of continuous wheat remains to be seen. Of more immediate interest is the fact that in four consecutive years the 4th or 5th wheat crops have yielded as much, and sometimes more, grain than the 2nd or 3rd crops. (Slope and Cox)

Inhibition of take-all on Broadbalk. In a study of take-all inhibition on Broadbalk, plants of Cappelle wheat with at least two seminal roots naturally infected with *Ophiobolus graminis* were transplanted from Highfield into small plots on Broadbalk on 10 May. New crown roots were produced soon after transplanting and the plants grew well. On 23 May, 7 June, 27 June and 10 July these plants had 5, 15, 35 and 58% crown roots infected, whereas plants uninfected when transplanted remained largely uninfected.

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Similar infected plants were also transplanted into Long Hoos field (4 years free of wheat) on 10 May; they grew poorly and many died, but the survivors developed the same amount of take-all as the transplants on Broadbalk, and as a drilled (2nd wheat) crop on Great Field I, which had 0, 11, 22 and 49% crown roots infected on 3 May, 27 May, 17 June and 4 July respectively. In contrast, take-all was much less severe on the drilled Broadbalk crop, where infected plants from the second wheat crop after fallow had 6, 3, 14 and 16% crown roots infected on the same dates. Hence the factor that seems to inhibit take-all in wheat drilled on Broadbalk was not effective in preventing its further development in already infected plants. A complicating factor in interpreting these results is difference of variety—Squarehead's Master on Broadbalk, Cappelle on the other fields, but in previous experiments on other fields Squarehead's Master has been severely attacked by take-all, so there is no reason to suppose that take-all is unimportant on Broadbalk because the variety grown is resistant. (Cox)

Eyespot on Broadbalk. Routine sampling on Broadbalk showed that, on plots 2B, 3 and 7, the 1st, 2nd, 3rd, 4th, 5th and 11th consecutive wheat crops after fallow had respectively 16, 52, 48, 38, 31 and 26% straws with eyespot, of which 3, 19, 12, 11, 12 and 8% had severe lesions. (Cox)

Intensive barley growing experiment. Intensive barley growing carries less risk of loss from disease than intensive wheat growing, but there is little information on the effect of growing successive barley crops on yield and incidence of soil-borne diseases, and in 1961 a long-term experiment was started at Rothamsted to give such information. In 1963 Proctor barley after oats in 1961 and beans in 1962 yielded 35·0, 41·8, 40·5, 33·1 cwt grain/acre when given 0, 0·3, 0·6, 0·9 cwt nitrogen/acre, whereas the 3rd successive barley crops yielded 26·2, 33·3, 35·9, 39·7. Thus the value of the 2-year break of oats–bean was little more than the cost of an extra 0·6 cwt nitrogen/acre. Response to nitrogen in the crops after oats–beans was limited by severe lodging, and a stiffer-strawed variety might have shown more advantage from the break. On 6 June 33, 23, 15, 12% plants had take-all in 3rd barley crops fertilised with 0, 0·3, 0·6, 0·9 cwt nitrogen/acre; the crops after oats–beans had fewer than 2% plants infected. (Slope)

Brown foot-rot associated with poor growth of cereals on light soils. In recent years there have been frequent reports of poor growth in cereals grown on light shallow soils. Establishment and early growth are good, but in late May or June crops suddenly stop growing and soon wither. These losses are enhanced by large amounts of nitrogen fertilisers and are usually associated with shortage of soil water (see report of work by members of the Chemistry Department, *Rothamsted Report* for 1962, pp. 55–60). However, in July and August many straws in affected crops show symptoms of brown foot-rot (*Fusarium* spp.) (*Rothamsted Report* for 1961, p. 112), and this infection may be partially responsible for the crop failures. To investigate this possibility spring wheat, sown in light sandy soil at Woburn on 3 March 1962 was fertilised with 0 (N0), 0·3 (N1), 0·6 (N2),

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0·9 (N3) or 1·2 (N4) cwt nitrogen/acre broadcast on the seedbed. Plants were sampled on 25 April, 22 May, 26 June and 24 July. Fertilised crops grew well until mid-June, when growth stopped and many plants suddenly withered and died; in contrast, N0 plots continued to grow and matured normally, showing only symptoms of severe nitrogen deficiency. In April and May few plants were infected by *Fusarium* and on 26 June only 1, 1, 5, 8, 8% straws had symptoms of brown foot-rot on plots N0, N1, N2, N3, N4 respectively. However, isolations from straws free from symptoms of brown foot-rot indicated that an additional 4, 16 and 14% were infected by *Fusarium* on treatments N0, N2 and N4 respectively. By 24 July the percentage straws with brown foot-rot had increased to 15, 36, 58, 77 and 74 on N0, N1, N2, N3 and N4. In this experiment, as elsewhere, crop failure was associated with drought; only 0·35 in. rain fell between 28 May and 8 July.

The relation between water shortage and infection of spring wheat by *Fusarium* was also studied in plots in an unheated glasshouse. Jufy wheat was sown on 9 April 1963 in a mixture of 1 part John Innes potting compost to 3 parts coarse sand. After sowing, straws naturally infected with *Fusarium* were buried close to the seeds in half the pots. All pots were kept adequately watered until 24 May. Three watering treatments were then started: treatment A—pots watered with measured amounts to maintain adequate soil moisture; treatment B and C—pots watered with similar amounts on every second and third occasion respectively. Soon after plant emergence half the pots were given 1·5 g ammonium sulphate/pot. On 17 June the percentage plants infected by *Fusarium* was 17, 46, 63 on treatments A, B, C without nitrogen, and 29, 96, 96 on treatments A, B, C with nitrogen. Inoculating the soil with infected straws did not increase infection. Water deficiency soon checked growth and produced symptoms similar to those occurring in affected field crops. It clearly predisposes plants to infection by *Fusarium*, but it has not yet been established that water shortage would be less damaging if *Fusarium* infection was prevented. (Slope)

***Cephalosporium* stripe of wheat.** Reports by N.A.A.S. plant pathologists of at least two cereal crops severely damaged by *Cephalosporium* stripe in 1963 have given new zest to investigations on this disease. Infected plants were found in most winter wheat crops at Rothamsted this year, but in none did the proportion of infected plants exceed 2%. However, even at this level of infection interesting differences were observed between wheat crops on the ley–arable experiments. In similar areas the numbers of infected plants in wheat after:

	Fosters	Highfield
12 years reseeded grass:	643	172
3 years cut grass	651	410
3 years grazed grass	25	35
3 years lucerne	37	4
3 years arable cropping	52	8

It is difficult to explain these differences, especially as the reseeded grass had been grazed more than cut. (Slope)

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Fungus Diseases of Potatoes

Potato blight. After late planting (10 May) and slow growth King Edward potato crops at Rothamsted suffered a massive aphid (*Aphis* (*Doralis*) *rhamni*) infestation in late July. Although blight appeared on 26 July, it developed patchily, and even a month later it had destroyed, on average, only 0·1% and 10·0% of the foliage of plots with and without protective fungicides respectively. By the end of August the crops had lost at least half of their foliage, mostly as a result of dry weather and aphid attack.

During early September more rain fell and the fungus multiplied quickly destroying almost half the remaining foliage on unprotected plots but no more than a fifth of that on protected plots. The total tuber yield on unsprayed crops was only 9·05 tons/acre. Some protective spray programmes appeared to decrease yield and others to increase it, but only a few of the differences were even barely significant. However, when allowance was made for losses through infected tubers (ranging from 3 to 25% by weight) some of the differences became significant. Unprotected crops yielded about 7·2 tons of healthy tubers/acre (least 6·9 tons/acre) compared with 8·2 tons of healthy tubers/acre (largest 9·4 tons/acre) from protected crops.

The proportion of infected tubers seemed to depend less on the date at which the haulm was destroyed than on the amount of foliage attacked by the fungus. For the first time in this series of experiments, the benefit of protecting King Edward foliage with fungicides came less from increased total yield than from decreased tuber infection. (Hirst, Hide and Stedman)

Haulm and tuber resistance to blight (*Phytophthora infestans*). Field studies on tuber infection were continued with two tuber-susceptible varieties, Up-to-Date (UD) and King Edward (KE), and two tuber-resistant varieties, Majestic (MJ) and Arran Viking (AV). Plots were arranged in a 4 × 4 Latin square with two rows of MJ planted between each column for inoculation, to ensure that blight arrived in all plots at the same time. Blight was introduced on 8 July, but because of dry weather did not spread from this, or from a further seven inoculation occasions, until 6 August, and even then the attack developed slowly and lesions were not widespread until 27 August.

On 31 August 80–90% of the KE foliage was dead, mostly from aphid attack and only 3–4% could be attributed to blight. On 17 September the haulm was dead and 30% tubers were blighted. Lesions, although few on leaves, were abundant on stems and this, and the fact that most infected tubers were clustered at the stem bases, suggests that spores from the stem lesions were transported in water running down stems (see Lacey, Ph.D. thesis, University of Reading, 1962) to infect the tubers. Thus, losses of tubers can be large, even when the foliage shows little blight.

UD was less affected by aphids, and on 12 September 60% of the haulm was destroyed and 60% tubers infected by blight. Infection did not increase after this date (final sample 25 September) and the main times of infection, determined from new lesions on tubers, were detected in samples on 29 August, 3 and 10 September.

Tuber infections of AV and MJ were first found on 3 and 5 September

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respectively, when there was about 5% infection on leaves. Blight destroyed 80–90% of the foliage of both varieties, but less than 10% of the tubers were infected. Of the tubers recorded as newly infected on lifting, only a third developed on further incubation, as in 1961.

In further studies of haulm resistance the behaviour of spores on leaves of Bintje and Pimpernel were observed to try to explain differences in the minimum infection period, reported in 1961. On Bintje encysted zoospores germinated to produce an appressorium and then penetrated the host; few spores were seen with germ-tubes. On Pimpernel the pattern was completely different; few spores produced appressoria, and most had germ-tubes ranging in length from two to three times the diameter of the spore. Assuming that spores with germ-tubes penetrate, then the time taken for the germ-tube to grow could explain the difference in the minimum infection time between the two varieties. (Lapwood)

Preliminary work on the effects of zoospore concentration on leaf infection showed: (*a*) that the number of zoospores in an inoculation droplet affected the generation time and, within certain limits, as the number decreased the generation time increased; (*b*) one zoospore in a droplet could cause an infection; (*c*) that the ED 50 (number of spores required to infect 50% replicates) differed in different varieties, e.g., twice as many spores were required to infect the upper surface of Pimpernel leaves as of Majestic. (Lapwood and Dr. R. K. McKee, John Innes Institute)

Common scab (*Streptomyces scabies*). A survey on the incidence of common scab in ley–arable rotations and other potato fields at Rothamsted and Woburn was started in 1960, using a modification of the Key devised by Large and Honey (*Plant Path.* (1955), **4**, 1) to estimate the mean percentage area of tubers affected (see table below). Some fields on both farms consistently produce more scab than others, but within a field no fertiliser, cultural treatment or crop rotation, consistently affects the disease. For example, at Rothamsted the incidence of scab in the ley–arable experiment on Highfield (an old permanent pasture) is consistently greater than on Fosters (old arable land), but within each experiment the amounts of scab on potatoes grown in the 3-year ley and in the 3-year arable rotations were similar. Other fields where scab is prevalent had all been pastures; at Rothamsted Stackyard (grass 1928–45) gave more affected tubers than other fields, and at Woburn Broadmead III (recently out of grass) many more than Warren Field South. The summarised results of the experiments are:

Mean % surface affected with scab in 1961

	Field			
Variety	Highfield	Fosters	Stackyard	Others
(*a*) Rothamsted				
Majestic	8·6	1·8	11·1	6·1 (2 fields)
King Edward	—	—	5·6	2·3 (2 fields)
Ulster Supreme	—	—	15·4	2·1 (1 field)
(*b*) Woburn	Broadmead III		Warren Field South	
King Edward	22·8		3·1	

(Lapwood and Salt)

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Experiments were started to investigate the effects of irrigation on the incidence of common scab. A small trial at Rothamsted with two blocks of four treatments compared: (1) "Dry"—plots received normal rainfall; (2) "Wet"—rainfall was supplemented by overhead irrigation to keep soil at a depth of 4 in. at field capacity; (3) "Wet-Dry"—rainfall supplemented to maintain field capacity during tuber formation only; (4) "Dry–Wet"—irrigation after tuber formation only. King Edward tubers had little or no scab with any treatment. Majestic also was not badly affected, but there was much less with the "Wet" treatment than with the other three.

The results of a further trial at the Nottingham University School of Agriculture, Sutton Bonington are not yet known. (Lapwood and Dr. B. G. Lewis, University of Nottingham, School of Agriculture)

Skin spot (*Oospora pustulans*) and other tuber-blemishing diseases. The relative importance of fungus in the soils where potatoes are planted and on the seed tubers planted in determining the incidence of skin spot was tested by several methods: (1) examining progress of differently infected stocks grown in one soil; (2) examining progenies of a single stock grown at the widely different sites of the National Institute of Agricultural Botany maincrop variety trials; and (3) measuring the decline in infectivity of soils after potato crops are lifted, to determine the proportion of residual virus that survives or exists saprophytically.

There is little doubt that the important source of the fungus is infected tubers. Potatoes were again planted on Barnfield on land free from potatoes for over 100 years, and the planting of infected tubers again made the soil infective, with its infectivity depending on the extent to which the seed tubers carried *O. pustulans*. The area where infected potatoes were planted in 1962 still contained some *O. pustulans* in the spring of 1963, but the amount was much less than in the autumn of 1962. The fungus also overwintered in other field soils where potatoes were grown in 1962, but the amount that did so was small, and whether any would survive a milder winter, except on diseased tubers that also survived, is uncertain.

That diseased tubers are an abundant source of the fungus was shown by a survey of 200 stocks of King Edward tubers supplied by the Potato Marketing Board. Every stock was infected with *O. pustulans*. On average it occurred on about three-quarters of the tubers and on about half the eyes. It was the most important cause of dead buds, sporing on about 90% of them. A single Majestic stock grown on High Mowthorpe Experimental Husbandry Farm, and stored in bulk, was equally infected. Samples of this stock boxed for chitting before January had only about 5% of buds dead at planting time, whereas when boxing was delayed until mid-January or mid-February there were 15 and 27% dead buds. This suggests that the gappy crops commonly produced by planting tubers with skin spot can be prevented by early chitting. (Hirst and Hide)

Fungi of Other Crops

Apple scab. As part of a programme assessing levels of scab in orchards studied for the last 10 years, "ascospore dose" (Hirst and Stedman, *Ann.*

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appl. Biol. (1962), **50**, 551–567) was again measured in one orchard at Wisbech (H), and "ascospore productivity" at Wisbech (H and G) and at Sudbury. The values of both in 1963 were the largest recorded since 1959 ("dose" in Wisbech H = 120; "productivity" in Wisbech G = 155), but neither reached $\frac{1}{20}$ of the largest recorded in the 10-year survey. An increase of "dose" over recent seasons was expected because an unusually large amount of leaf survived after the exceptionally severe winter. Weights of dead leaf per unit area of ground were estimated at bud burst, which was 2 weeks later than the average date for the previous five years. Although much of the surviving leaf was removed by earthworms and gales during these 2 weeks, 21, 44 and 29% still survived to bud burst at Sudbury, Wisbech H and Wisbech G respectively, compared with the average of 6, 13 and 15% over the previous six seasons.

Measuring the number of ascospores released after intermittent wetting in field and laboratory has not explained how temperature affects the number matured and liberated. Probably this results from the release, at each wetting, of a variable part of an accumulation of mature ascospores (Hirst and Stedman, *Ann. appl. Biol.* (1962), **50**, 525–550).

Apparatus was therefore designed to eliminate accumulated ascospores, by uniform wetting every hour at constant temperature. The few tests completed show that the apparatus can bring release to a constant rate, which presumably represents maturation, and hence should allow the influence of temperature to be studied. It has already shown that perithecia are unexpectedly sensitive to rapid temperature changes, because a rise of approximately 10° C lasting only 1 minute gives a large transient increase in ascospore liberation. (Hirst and Stedman)

Diseases of Sitka spruce seedlings in forest nurseries. Work continued in collaboration with the Research Branch of the Forestry Commission on fungi associated with emergence losses and poor growth of Sitka spruce seedlings, and several different partial sterilants, fungicides and seed dressings were tested as methods of control.

Effect of seed dressings. The severe winter and late spring delayed sowing until the last week in April, but in response to mild weather and adequate soil moisture seedlings emerged within 4 weeks of sowing instead of the usual 6–8 weeks. Moreover, percentage of seeds that germinated or emerged was large in all nurseries, and seed dressings had little or no effect, in contrast to 1962, when, with a cold dry spring associated with a slower and smaller emergence, seed dressings were beneficial. Poor-quality seed (21% germination) sown at 27 g and normal seed (67% germination) at 7 g to give 1,800 viable seeds per square yard both produced similar, even stands on untreated or on partially sterilised soil, and were unaffected by thiram seed dressing.

Effect of partial sterilisation. As usual partial sterilisation stimulated growth. At Ringwood a mean height of 0·42 in. in unsterilised soil was increased to 1·81, 1·92 and 1·97 in. respectively by formalin, metham-sodium and dazomet (= "Mylone") applied 4 months before sowing, and

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to 0·87 in. by a single drench of nabam at sowing time. Quintozene had little beneficial effect. At Kennington seedlings grew much taller than at Ringwood in untreated soil (2·36 in.), so benefits of formalin (2·87 in.), dazomet (3·02 in.) and nabam (2·51 in.) were small, and metham-sodium (2·03 in.) and quintozene (1·80 in.) both depressed growth.

The contrast in growth between plants at Ringwood and Kennington is striking, especially as the pH of Ringwood soil is lower and nearer the optimum for growth than that at Kennington. Biological factors seem to affect growth at both nurseries, but they differ in that at Kennington growth can be improved either by increasing soil acidity or by partial sterilisation, whereas at Ringwood seedlings are stunted even at optimal pH, and only partial sterilisation improves growth.

For many years formalin has been the most convenient sterilant for use in forest nurseries; its main disadvantage is the amount of water needed to drench the soil. Dazomet has therefore a practical interest, as its beneficial effect at least equals that of formalin, and it is applied as a dry powder lightly forked into the top 6 in. and left to weather without a surface seal.

Fungi in root debris and seedlings. Quantities of root debris often survive in seedbeds from one year to the next, and might be a source of infection. Accordingly, the fungi present in the debris at sowing time, on seedlings just after emergence in June, and again in August were determined on 20–50 pieces of root per treatment. These pieces were cut to about 1-cm lengths, surface sterilised in sodium hypochlorite and plated first on water agar and then on 2% malt or acidified potato dextrose agar.

Cylindrocarpon spp. were isolated from debris in unsterilised soil more often than any other fungus and occurred on 98% and 66% of roots from Ringwood and Wareham respectively. It was equally abundant on debris from soil treated in the previous season with quintozene and maneb, but was decreased by December application of formalin to 12% and 8% respectively at the two nurseries, and was not isolated from debris treated with dazomet. A species of *Phoma* on 40% of roots from untreated soil at Ringwood was not found on roots from soils treated with dazomet or formalin; nor was it isolated from untreated or treated soils at Wareham. *Fusarium* spp. occurred on 20% of untreated debris at Ringwood, but on only 6, 0, 8 and 6% of roots from soils treated with formalin, dazomet, quintozene and maneb respectively. *Pythium* spp., relatively uncommon on root debris, were isolated from 2–4% of roots, except those treated with quintozene (12%) and dazomet (0%). Although partial sterilants almost eliminated these three genera from debris, the material was colonised by other fungi rare or absent in isolations from untreated material. Thus debris from formalin-treated soil yielded 42% *Coniothyrium* spp., 36% black sterile mycelium and 26% *Botrytis* at Ringwood, and 88% *Penicillium* spp. at Wareham. Debris treated with dazomet gave 48% T.209, another unidentified fungus with dark mycelium.

To select potential pathogens, moist finely chopped debris in Petri dishes was sown with surface sterilised spruce seed and incubated at 20° C. *Pythium* and *Cylindrocarpon* were the only two genera consistently isolated from diseased seedlings, 23% of which yielded both fungi, 32% *Pythium*

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only and 27% *Cylindrocarpon* only. *Cylindrocarpon*, *Fusarium* and *Pythium* were also the most prevalent fungi on seedlings sampled from nurseries. At Ringwood in June, 85% yielded *Cylindrocarpon*, 50% *Fusarium* and 20% *Pythium*. These fungi were almost eliminated after treatment with formalin, chloropicrin, metham-sodium, dazomet and nabam, but quintozene controlled only *Fusarium*. By mid-August *Cylindrocarpon* was the dominant fungus and *Fusarium* spp. had increased on seedlings in all treatments except dazomet. *Pythium* was abundant (55%) on seedlings from untreated soil and from that treated with quintozene (45%) and nabam (35%), but was still rare or absent in partially sterilised soils.

In untreated soil at Kennington in June *Fusarium* spp. were more abundant (35%) than *Cylindrocarpon* (20%) or *Pythium* (5%), but by August the numbers were 35, 65 and 25% respectively. All three were greatly decreased by partial sterilisation, but not by quintozene or nabam.

Tests for pathogenicity. The pathogenicity of pure cultures was tested at 20° C in oven-sterilised quartz grit, fertilised with Hoagland's nutrient water-culture solution, giving a pH between 6·0 and 7·0, which would favour fungal invasion in unsterile soil. All species of *Pythium*, including those from Wareham, killed most seedlings before emergence. *Cylindrocarpon*, several species of *Fusarium*, *Phoma* and *Botrytis* behaved as weak pathogens by decreasing emergence and causing some post-emergence deaths. The roots of survivors (except survivors from *Botrytis*) were darker brown than those of uninoculated seedlings, and the original fungi were recovered from them after surface sterilisation, but rotting was insufficient to destroy the root cortex. *Trichoderma viride* and *Penicillium canescens*, often the dominant fungi in partially sterilised soil, and *Aspergillus niger* and *Rhizopus stolonifera*, common seed contaminants, were not pathogenic.

Of the fungi prevalent on seedlings, *Pythium* is specially interesting because it was the most virulent pathogen and was controlled throughout the growing season by partial sterilants but not by the fungicides nabam and quintozene. This result is consistent with the effects of these chemicals on growth, but unfortunately growth increases cannot be correlated with the control of pathogen alone; partial sterilisation also changes the nutritional status of the soil, and other methods will have to be used to measure the effects of pathogens independently. Whatever the causes of stunting they were not transferred to partially sterilised soil by inoculating it with untreated soil. At Ringwood stunted plants from untreated soil recovered when transplanted to formalin-treated soil and grew as strongly as larger plants transplanted from sterilised seedbeds. The possibility that saprophytic fungi prevalent in partially sterilised soil may inhibit reinvasion by pathogens requires further investigation. (Salt)

Effect of urea on pea wilt. The effect on pea wilt of urea applied with "Tween 80" wetter and spreader to the foliage was investigated in the glasshouse. Four sprays at 3-day intervals were applied by dipping seedlings in urea solution for 1 minute, care being taken not to spill urea on to the soil. Controls were sprayed similarly with distilled water, with "Tween

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80". After spraying, the plants were covered with polythene cages for 24 hours. Urea at 0·05*M* decreased pea wilt by 19·6%, but smaller concentrations (0·01–0·03*M*) had little effect, and larger ones (0·1*M*) were phytotoxic.

The effect of 0·05*M* urea foliar spray was also tested in eight 9-in. × 12-in. micro-plots of wilt-sick soil, each alongside a control. Four spray applications were given with an atomiser late in the afternoon at 3-day intervals. Wilt was recorded, and the effect on vegetative growth of the pea plant estimated by counting the number of leaves/plant. The spray decreased pea wilt by 60% and increased vegetative growth by 40%.

Rhizospheres of sprayed (0·05*M*-urea solution, with "Tween 80" as wetter and spreader) and unsprayed pea seedlings, growing in the glasshouse, were analysed at different stages of growth. Qualitatively *Verticillium* spp. and *Penicillium* spp. were greatly decreased, and *Mucor* spp. and *Rhizopus* spp. were greatly increased. The spray decreased the total number of rhizosphere fungi by 15%, but the most interesting observation was that, while the actinomycete population increased by 13%, *Fusarium oxysporum* f. *pisi* decreased by 44%. As actinomycetes are antagonistic towards the *Fusarium in vitro*, it seems likely that they also antagonised or lysed *Fusarium* in the rhizosphere, thus lessening wilt.

Urea is readily absorbed and metabolized by plants and increases the amount of root exudate. Hence the spray may have influenced the rhizosphere microflora directly, and favoured species antagonistic to pathogenic *Fusarium*. However, it also increases the vigour of the seedlings, and this may increase their resistance to infection. (Khalifa)

Inhalation diseases associated with moulds and actinomycetes on vegetable products. Dust from numerous samples of hay, straw and grass, associated with farmer's lung disease in man, pneumonic conditions in cattle resembling fog fever, mycotic pneumonia in pigs, broken wind in horses and other animal diseases are being examined by the wind-tunnel technique (see Abstracts of Papers, No. 7.17).

Work on mouldy hay associated with farmer's lung progressed to the stage where antigens reacting with patients' serum, and producing typical symptoms when inhaled, were obtained from pure cultures of *Thermopolyspora polyspora*, and to a lesser extent from *Micromonospora vulgaris* (see Abstracts of Papers, No. 7.26). (Gregory, J. Lacey and M. E. Lacey, in collaboration with G. N. Festenstein, Biochemistry Department and F. A. Skinner, Soil Microbiology Department, and with Dr. J. Pepys and Mr. P. A. Jenkins, Institute of Diseases of the Chest)

Because bagassosis, a disease resulting from inhalation of dust from mouldy bagasse, shows clinical resemblances to farmer's lung, samples of bagasse are also being examined by the wind-tunnel technique. Up to 45 million fungus spores and 106 million bacteria and actinomycetes/g air-dried bagasse were found on cascade impactor slides. The Andersen sampler showed that many of the thermophilic organisms in hay are also present in bagasse, including: *Aspergillus fumigatus*, *A. terreus*, *Humicola lanuginosa*, *Scopulariopsis* spp., *Paecilomyces* spp., *Micromonospora vulgaris*, *Streptomyces fradiae*, *S. thermoviolaceus*, *Thermopolyspora glauca* and *T.*

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polyspora. Several other unidentified actinomycetes were also common. (J. Lacey, with Dr. J. Pepys and Mr. P. A. Jenkins, Institute of Diseases of the Chest)

Micro-fungi of pastures. Spores of *Pithomyces chartarum*, the cause of facial eczema of sheep in New Zealand, were found regularly on Harpenden Common between early April and mid-October. *P. chartarum* was first recorded in Britain on a Hirst trap slide exposed in 1958 (*Rothamsted Report* for 1961, p. 120), but there was no reason to regard it as a new-comer. Dr. R. G. Pawsey has since kindly lent us a Hirst trap slide showing 330 spores/m^3, caught when a nearby grassy slope at Nottingham University was mowed on 12 July 1956. This was nearly two years before the discovery that *P. chartarum* is associated with facial eczema. (J. Lacey)

"Yellowses". Scottish hill farms were visited in June to examine pastures associated with "yellowses" of sheep, a hepatogenous photosensitisation (*Rothamsted Report* for 1962, p. 125). The disease is commonest in the Cape Wrath area, and on some hillsides up to 50% of the lambs become affected. These areas, like the neighbouring hillsides, are dominated by *Calluna vulgaris*, with abundant *Trichophorum caespitosum*, *Erica* spp., *Narthecium ossifragum* and *Sphagnum* spp. One area, said to be unaffected, was more grassy, but *N. ossifragum* grew over much of the area and appeared to have been grazed. *N. ossifragum* was often abundant on other hillsides where yellowses occurred infrequently.

In Perthshire, where usually fewer than 10% of the lambs developed yellowses on affected pastures, affected and unaffected pastures differed little, and were dominated by *Molinia caerulea*, with *N. ossifragum*, *C. vulgaris* and *Erica* spp. occurring frequently. Pastures associated with the disease in South-West Scotland were similar, except for one farm on the Ayrshire coast, which had relatively good permanent pastures with no evidence of *N. ossifragum*.

Examination of spore traps has not revealed any fungi that might be involved, and yellowses does not seem to be associated with any one pasture type or with the occurrence of *N. ossifragum*. (J. Lacey)

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 (Festenstein, Skinner and Gregory); Entomology (Cockbain and Gibbs); Insecticides (Etheridge and Gibbs); Nematology (Hooper and Gibbs); Soil Microbiology (Skinner, Festenstein and Gregory).

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