ROTHAMSTED RESEARCH

# Report for 1961

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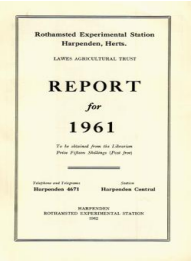


# Bee Department

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had abundant thermophilic moulds. The stack of this batch of moist hay (which was sampled through a horizontal tube in the stack) differed in appearance from the bales; it was dark, sour-smelling and brittle. Micro-organisms developed more slowly, the peak concentration of spores being counted visually on the 16th day, with 5 million mould spores per g. (mainly *Humicola* (*Monotospora*) *lanuginosus*) and 37 million bacteria and actinomycete spores per g. In culture the few bacteria mostly developed at the lower incubation temperatures, and the actinomycetes mainly at the higher temperatures (e.g., 4,800 per g. at 60° C.).

In the dry hay temperatures rose to maxima of only 23° C. in the bales and 24° C. in the stack, the microbial content in both remaining more or less stationary, with only *Aspergillus glaucus* increasing appreciably; the maximum in the bales of total mould spores was 0·07 million per g. and in the stack 0·1 million per g. (except for one count of 6 million spores per g. from a sample on the 9th day). The bacteria and actinomycetes were very variable, but never exceeded 1 million per g. in either bales or stack.

In an attempt to obtain more evenly dried moist hay, grass from Great Knott I was baled at an average water content of 35%. The wetter patches of this heated to 60° C. and moulded. A maximum of 184 million bacteria and actinomycetes per g. was counted visually on the 10th day after baling. A few bacteria grew at all temperatures, and numerous actinomycetes were obtained in culture: 360 per g. at 24° C., 1,300 at 40° C. and 10,600 at 60° C. A maximum of 40 million mould spores per g. were counted on the 11th day, mainly *Mucor pusillus*, with some *Humicola lanuginosus*. The usual thermophilic species grew at 40° C., with *Paecilomyces* developing after 2 weeks. The dry hay (20% moisture content) heated to a maximum of 35° C., and produced *Aspergillus glaucus* spores with a maximum of 35 million per g. on the 11th day. On the same day the maximum of 2 million bacteria and actinomycetes per g. was counted.

Although the hay in 1961 was variable, it confirms the conclusion from the two previous years that the microbial content of hay is determined mainly by the moisture content at baling. (Gregory and M. E. Lacey, with Festenstein, Biochemistry Department, and Skinner, Soil Microbiology Department.)

*Occurrence of* Pithomyces chartarum *in Britain*

This fungus is a saprophytic mould, known to be widely distributed in the tropics and sub-tropics. In New Zealand it produces the toxin, sporidesmin, which causes " facial eczema " of sheep. While examining Hirst trap slides, exposed at Imperial College Field Station in 1958, six spores of *Pithomyces chartarum* were seen, and O. J. Stedman observed one spore on a slide exposed at Rothamsted in September 1960. In an attempt to find the fungus in the field a whirling-arm air-sampler was used to sample air in several areas in Surrey and Berkshire during September 1961. Spores were caught in all the areas tested, the most at Virginia Water, where 426 spores/cu. m. of air were detected and where the fungus was found growing on the remains of mown grass (mainly *Holcus lanatus*). Isolations were obtained from the air by exposing Petri dishes in the Andersen

*Sitka seedling diseases*

To serve as a background for future work, soils from five forest nurseries were analysed in detail using a modified dilution-plate method. Fungi isolated are arranged in five groups—*Penicillium* spp., *Phycomycetes*, *Sphaeropsidales*, *Fusarium* spp. and others, including *Paecilomyces* spp., *Cephalosporium* spp., *Chaetomium* spp. and less commonly *Wardomyces anomala* Brooks and Hansf. and *Emericellopsis minima* Stolk.

At Bagley Wood and Ringwood the dominant species of *Penicillium* were *P. daleae* Zal. and *P. janthinellum* Biourge, respectively. At two Kennington nurseries, Old and Extension, *P. daleae* Zal. and *P. restrictum* Gilm. and Abb. were equally abundant, whereas at Wareham, *P. melinii* Thom., *P. spinulosum* Thom. and *P. restrictum* Gilm. and Abb. predominated. Many phycomycetes were isolated, including species of *Mortierella*, *Zygorhynchus* and *Absidia*. *Mortierella macrocystis* Gams. was found in great numbers, but only at Bagley Wood; *M. vinacea* Dixon-Stewart and *M. ramanniana* (Moeller) Linnemann were numerous at Ringwood.

*Phoma* spp., and *Coniothyrium* spp., of the Sphaeropsidales, were common and *Pyrenochaeta* spp. occasional. The ratios of the numbers of isolates of *Phoma* to *Coniothyrium* were greater at Kennington than at Ringwood. *Fusarium oxysporum* Schlecht. ex Fr., *F. solani* (Mart.) Sacc., *F. sambucinum* Fuckel, *F. avenaceum* (Fr.) Sacc. and *F. merismoides* Corda were isolated at all sites, with *F. sambucinum* Fuckel particularly common at Kennington Extension.

The two groups of fungi most often isolated from diseased Sitka seedlings, without using chemical surface sterilants, were rarely isolated from soil. *Cylindrocarpon radicicola* Wollenweber was the fungus most frequently isolated at Kennington Old and Ringwood. Next was a group of four species of *Pythium*, including *P. ultimum* Trow and *P. irregulare* Buisman, but the two most abundant, RR 493 and RR 494, seem not to have been described before. (Ram Reddy and Last.)

*Mycofloral succession in hay*

Grass from Great Field I was again cut for experiments on moulding of hay, but the product was very unevenly dried, and the mean water content lower than aimed at. The practice of crushing cut grass to aid drying of the stalks needs constant tedding to get even drying. The moist hay (average water content approximately 30%) and dry hay (approximately 15%) were both baled and stacked. The moist bales reached a temperature maximum of 55° C.; the most spores, recorded visually on the eighth day after baling, were 21 million moulds and 42 million bacteria and actinomycete spores per g. dry weight of hay. Using the Andersen sampler, 2,900 colonies of moulds per g. were obtained in culture, mainly *Aspergillus fumigatus*, *Mucor pussilus*, *Absidia ramosa* and *Aspergillus nidulans*, all of which grew at 24° and 40° C. (*Paecilomyces* developed after 3 weeks). Few bacteria grew at the lower temperature, but actinomycetes grew at all three incubation temperatures: 1,200 colonies per g. at 24° C., 1,000 at 40° C. and 3,200 at 60° C. The bales of this batch were not particularly rich in actinomycetes, but

linked. The ability to form perithecia was not always inherited through ascospores of the crosses, even though the *Nectria* itself was 100% homothallic. This situation, already recorded in *Aspergillus* spp., might indicate either a cytoplasmic control of perithecial formation or the presence of a dominant " non-perithecial " gene in the Fusaria used in these experiments.

The fact that the *F. oxysporum* isolates did not cross with those of *F. solani*, yet each separately and easily crossed with the *Nectria*, suggests a close phylogenetic relationship between the two. Their well-defined difference in ability to rot or wilt plants seems the only reliable means of telling them apart, for physical characteristics such as spore shape are not always sufficiently consistent for definite identification. (Buxton.)

*Control of* Fusarium *wilt*

Flaked chitin has been reported to deter *Fusarium* wilt (*United Fruit Company Reports*, 1960–61). It was used on a *Fusarium*-infested area in Essex and in experiments on pea wilt in the glasshouse. In the field a 7 × 7 Latin-square design, with treatments of soil at six concentrations of chitin, showed that applying it at 8 oz./7-foot row prevented pea wilt in variety Onward. De-acetylated chitin had the same effect as pure chitin.

In glasshouse tests, adding chitin to infested soils 4 or more weeks before introducing pea seedlings prevented wilt. Dilution plates of soils, to which chitin had been added 1–8 weeks previously, revealed a decrease in populations of *Fusarium* as the association of soils and chitin increased. Using a selective agar medium containing colloidal chitin, soil-borne actinomycetes were isolated from treated soils. As the number of actinomycetes increased in soils containing chitin, the numbers of Fusaria decreased. Tests show that many of these actinomycetes antagonised at least five different *Fusarium* strains, including three physiologic races. This probable mechanism of the mode of action of chitin in preventing wilt is being studied in relation to other factors that are already known to operate in the host root surface zone. (Buxton.)

*Banana fruit rots*

Serious losses are incurred in transporting bananas from Jamaica to Britain. Infection can occur either in the field, during shipment or in ripening rooms in this country. Fourteen different species of fungi were isolated from fruit variety " Lacatan "; most frequent were *Gloeosporium musarum*, *Thielaviopsis basicola* and *Fusarium roseum*. *Deightoniella*, *Botryodiplodia* and *Pestalotia* were common. Seventy-eight isolates were tested for pathogenicity. *Gloeosporium* and *Fusarium* caused most damage and, unlike *Thielaviopsis* and *Pestalotia*, they caused lesions on unwounded fruit. All seventy-eight isolates caused more damage at 80° F. (ripening temperature) than at 58° F. (refrigeration temperature in ships' holds during the 9-day voyage). Some, notably *Thielaviopsis*, caused considerable damage at 58° F. Sources of infection are banana trash in the field, decaying florets on stems during shipment and the air of ships' holds and ripening rooms (*United Fruit Company Report*, August 1960). (Buxton.)

field, the two susceptible varieties Up-to-Date (UD) and King Edward (KE) were compared with two more resistant varieties, Arran Viking (AV) and Majestic (MJ), in plots arranged in a 4 × 4 Latin square. Sampling methods were as described in 1960. The detection of blight and the detailed inspection of tubers were made difficult on UD and MJ because of the high proportion of tubers severely affected by scab (*Actinomyces scabies*); tubers of KE and especially AV were much less affected, but unfortunately there were few tubers infected with blight on KE, because of the rapid natural death of the haulm early in the epidemic. The first blighted tubers were found on 12 September on UD, KE and AV (with about 5% blight on haulm), and 1 week later on MJ (blight developed more slowly on the haulm of this variety). The number of infected tubers from later samples increased after periods of rain, except in MJ, which had few infected tubers. Infections on about half the AV tubers (recorded as newly infected on lifting) failed to develop on further incubation; this also happened with MJ and agrees with laboratory results reported in 1960. Unlike 1960, most infections on UD were on the body of tubers, and few infections occurred at the heel end of any variety.

Laboratory experiments using wound-free tubers were continued, and the relative susceptibility of fifteen varieties was assessed on the number of tubers successfully infected and the amount of rotting after 14 days at 15° C. Populair, Robijn and Pimpernel proved most resistant, and Ulster Ensign, Duke of York and Bintje most susceptible. The resistance of Majestic showed clearly, but Arran Viking proved more susceptible in these tests than expected from field experiments. (Lapwood.)

Fusarium *wilt*

The *Fusarium* state of an isolate of *Nectria haematococca* was crossed with several cultures of wilt-inducing *Fusarium oxysporum* to seek genetic relationships between them. Further crosses involving *F. solani* were made to see whether Fusaria that caused wilt could be mated with any that caused foot-rot. Heterokaryons (colonies with hyphae containing nuclei of different genetic origins) were separately established between isolates of *F. oxysporum* f. *pisi*, *F. oxysporum* f. *lycopersici*, *F. oxysporum* f. *cubense*, *F. solani* f. *pisi*, *F. solani* f. *phaseoli* and the *Fusarium* state of the *Nectria*. The first three cause wilt of pea, tomato and banana respectively, and never cause foot-rot; the other two cause foot-rot of pea and French bean, and never cause wilt. Heterokaryons were also made between all the isolates of *F. oxysporum*, but none could be made directly between *F. oxysporum* and *F. solani*.

Using markers induced by ultra-violet irradiation and the specific pathogenicity towards different hosts showed that heterokaryons between each isolate and the *Nectria* led to genetic recombination during ascospore formation. With electrical and mechanical micromanipulation, 931 single ascospores were isolated from crosses and analysed genetically. Most characteristics were isolated in new genetic combinations, but the ability to cause foot-rot was never found recombined with ability to cause wilt. This suggests that genes governing these abilities may be allelic or very closely

estimated. Maximum spore production occurred 8–13 days after inoculation, when up to 40,000 sporangia/tuber were produced. Maximum spore concentrations estimated to be up to 2,300 spores/c.c. soil were found around these tubers 9–18 days after inoculation. *P. infestans* spread from an inoculated tuber to healthy tubers of Ulster Ensign where tubers grew close together.

Estimation of spore profiles below the crest of the ridge, in Majestic potatoes, was difficult in 1961, because blight appeared late and destroyed less than half the foliage before the plants died naturally. Sporangia were most abundant in the surface soil on 13 September, when the concentration rose from an estimated 560 sporangia/c.c. to 2,100 sporangia/c.c. after 0·77 inch rain. This coincided with an increase from 45 to 1,600 sporangia/c.c. at 2 inches, 0 to 1,600 at 4 inches, 0 to 670 at 6 inches and from 30 to 1,070 at 8 inches. Infectivity declined afterwards as the haulm died, but infectivity was still detectable nearly 3 weeks after a crop of Up-to-Date, 95% of whose haulm was destroyed by *P. infestans*, had been burnt off with concentrated sulphuric acid (BOV). (J. Lacey.)

*Haulm and tuber resistance to blight* (Phytophthora infestans)

The value of the 1961 field experiments on the nature of resistance of different varieties was decreased because dry weather early in the season retarded development of the crop and because the disease, although introduced in August as soon as weather was favourable, failed to become epidemic until early September. Thus, in a field experiment where ten varieties (selected because they varied greatly in haulm susceptibility in laboratory tests) were planted in small (twenty-plant) replicated plots, the haulm destruction of only four later-maturing varieties could be studied in detail by leaf tagging. Voran haulm was half defoliated (50% marked leaflets dead) by blight 6 days, Libertas 11 days and Zeeburger 14 days later than Majestic. The delay in Voran and Libertas was explained by the slower rate leaves were destroyed after infection, whereas in Zeeburger this was combined with a delay in the initial infection of the leaves (50% leaflets infected 9 days later than in Majestic). Sporulation differed on the different varieties, and the mean width of the sporing annulus measured in the field for 6 days was 4·4, 1·7, 1·6 and 1·1 mm. for Majestic, Zeeburger, Voran and Libertas, respectively.

Umaerus (*Sverig. Utsädesfören. Tidskr.*, **70**, 59, 1960) reported that the minimum infection period differed between varieties, for the susceptible Anna it was 3 hours and for the resistant Ackersegen 8 hours. To investigate this, experiments were done with varieties of differing susceptibility. Leaflets of detached leaves in boxes were inoculated with droplets of sporangial suspension, and after 1, 3, 5, 7, 10 or 24 hours' incubation at 15° C., leaves were removed, the inoculation droplets dried and the incubation continued at a low relative humidity until lesions appeared (the petioles were dipping in water). Ackersegen behaved like the susceptible Bintje, but Pimpernel differed. Although some Pimpernel leaves, like Bintje, became infected in 3–5 hours, some took 24 hours, whereas all Bintje were infected within 7 hours.

Continuing the study, begun in 1960, of tuber infection in the