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ROTHAMSTED RESEARCH

Report for 1969 - Part 1

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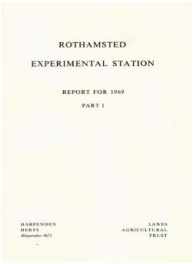

Plant Pathology Department

J. M. Hirst

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PLANT PATHOLOGY DEPARTMENT

J. M. HIRST

Cereal diseases gained increasing attention, especially through a renewed interest in powdery mildew and barley yellow dwarf virus. Systemic fungicides were studied on cereals as well as on potatoes and some work was begun on the effects of air pollution on plants and on diseases previously assumed to be caused by viruses but now thought to be caused by mycoplasmas.

Properties of viruses and virus diseases

For convenience, this section also includes some work on mycoplasma-like bodies and some biochemical investigations not immediately related to viruses.

The effect of temperature on the multiplication of three strains of tobacco mosaic virus (TMV). The multiplication of three strains of TMV, one classed as thermosensitive, was compared in plants kept at 20° and 35°C. Leaves were extracted at different intervals after inoculation in two ways: (1) with phenol, which gives the total nucleic acid (RNA) of the virus; (2) with buffer, which gives whole virus only and inactivates any free RNA. The infectivities of the extracts were compared with those of solutions containing known amounts of RNA or whole virus.

Strain PM_2 produces protein unable to coat the RNA, and only phenol extracts were infective; those from plants at 20° were about 10 times as infective as from plants at 35°C. With the type strain, infectivity of either buffer or phenol extracts was also 10 times greater from plants at 20° than at 35°C. The concentration of either strain reached its maximum in 6–8 days after inoculation, but whereas it then remained constant with the type strain, it decreased with strain PM_2. Strain N-118 has been considered thermosensitive because buffer extracts from plants at 20° are very much more infective than from plants at 35°C. However, phenol extracts were only 10 times more infective from plants at 20° than at 35°C, as with the other two strains. Hence, its RNA is no more sensitive to heat, but at 35°C this strain resembles PM_2 and its RNA fails to become coated with its protein, whereas at 20°C it more nearly resembles the type strain and forms stable nucleoprotein particles.

N-118 causes necrotic local lesions in *Nicotiana sylvestris*, whereas the type strain does not, and so can be assayed in the presence of the type strain. Workers in U.S.S.R. and Germany reported that tobacco plants infected simultaneously with N-118 and the type strain and kept at 35°C gave buffer extracts that produced more lesions on *N. sylvestris* than buffer extracts from plants infected with N-118 alone. We have confirmed this and found that the apparent content of N-118 may be more than 1000 times as much in buffer extract of doubly infected plants as from those

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with N-118 alone, but the mechanism whereby the type strain allows N-118 to form stable particles in plants at 35°C has still to be elucidated. (Kassanis and Bastow)

Relationships of strains of tobacco necrosis (TNV) and satellite viruses (SV). Strains of TNV can be characterised by serological differences, by the symptoms they cause on some plants and by the strain of SV that multiplies in their presence. TNV strains divide serologically into two groups (A and D), but the ease with which they are distinguished depends on the immunization course of rabbits used to produce antisera. With strains of either TNV or SV serological differences were most distinct when the animal was bled 2–4 weeks after a single intravenous injection.

The two serological groups can also be distinguished by the symptoms they produce in tobacco (type White Burley var. Judy's Pride), *Phaseolus vulgaris* (var. Prince) and *Nicotiana clevelandii*. On *P. vulgaris*, group A strains produce lesions that quickly spread along the veins to form a necrotic network whereas strains of group D cause either minute lesions or large round ones that later spread only slowly. Group D strains usually cause many lesions in *N. clevelandii* but few in tobacco and bean, whereas group A strains infect all three plants equally easily.

Three strains of SV have been described in England and three in U.S.A. The English strains SV_1 and SV_2 are identical to the American strains SV_A and SV_B, respectively. The ability of 7 different strains of TNV to allow SV_1, SV_2 and SV_C (American) to multiply was tested. All TNV strains of group A allowed SV_1 and SV_2 to multiply. Some strains of group D allowed SV_1 and SV_2; the others allowed SV_C but not SV_1 or SV_2. (Kassanis and Phillips)

Potato virus X (PVX)

Electrophoretic behaviour. Much PVX often becomes insoluble during purification or storage so its electrophoretic mobility was measured to see whether charge density on the surface of the particles is small, because if it is, the losses might be prevented by maintaining an adequate charge density during purification and storage. Table 1 shows that the electrophoretic mobility of TMV, which remains soluble in the buffer solutions

TABLE 1

Electrophoretic mobilities of two viruses in different buffers at pH 7·5

Virus	Buffer	Specific electrical conductivity in $ohm^{-1}\ cm^{-1} \times 10^3$	Mobility in $cm^2\ sec^{-1}\ v^{-1} \times 10^5$
Potato virus X	0·067*M* phosphate	4·6	0·7
	0·05*M* borate and 0·065*M* NaCl	4·5	1·6
	0·5*M* borate	2·4	2·5
	0·05*M* borate	0·32	10·5
Tobacco mosaic virus	0·067*M* phosphate	4·6	9·0
	0·05*M* borate	0·32	23

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shown in the table, greatly exceeds that of PVX. PVX tends to become insoluble in 0·067 *M* phosphate buffer and in 0·5 *M* borate buffer, in which electrophoretic mobilities are relatively small, but remains dissolved in borate buffer diluted to 0·05 *M* or less, when the electrophoretic mobility is larger. To keep the virus soluble at about pH 7·5 the specific electrical conductivity of the medium should not exceed $0{\cdot}3 \times 10^{-3}$ ohm^{-1} cm^{-1}.

Isolation of the RNA. The 'two phase' phenol method for isolating infective RNA from tobacco mosaic virus gives poor yields (about 5%) of the RNA from PVX. A variant of a 'single phase' phenol method for isolating nucleic acids from viruses, described by Diener and Schneider (*Arch. Biochem. Biophys.* (1968), **124**, 401), was used on 0·5% preparations of PVX. The yield was about 30%, the RNA was infective and, by all the usual criteria, the infectivity was from the RNA and not from any remaining virus particles. The infectivity of the isolated RNA was 0·1–0·9% of whole virus containing the same amount of RNA when the two were compared on *Nicotiana tabacum*, var. Xanthi-nc, and 10–25% when compared on *Chenopodium amaranticolor*. The relative infectivities of tobacco mosaic virus and the RNA isolated from it also differ considerably when assayed on different plants.

Salt concentration and inactivation by ultraviolet radiation. Differences between the susceptibility to inactivation by ultraviolet radiation of virus nucleic acid when free and when in intact particles could depend on the way protein and nucleic acid are combined in the particle. However, to find whether this is so, it is necessary to know whether the susceptibilities of the two depend on the environment in which they are irradiated. Workers in the U.S.A. found that the nucleic acid of TMV is more susceptible in dilute than in concentrated salt solution, and we found this also to be true of PVX nucleic acid which was 1·5 times as susceptible to irradiation at 254 nm in 0·01 *M* as in 0·1 *M* borate buffer at pH 7·5. The respective quantum yields when assay plants were kept in darkness to prevent photoreactivation of the irradiated inocula were 3×10^{-3} and 2×10^{-3}. In contrast, intact virus was equally susceptible in the two buffers, and gave a quantum yield with respect to the energy absorbed by its nucleic acid of 10^{-3}. Hence, the nucleic acid is more protected by the protein in 0·01 *M* buffer than it is in 0·1 *M* buffer. (Kleczkowski and Govier)

Eggplant mosaic virus. The particles of this beetle-transmitted virus which was found in Trinidad and described by Dale (*Ann. appl. Biol.* (1954), **41**, 240), have the same appearance and sedimentation behaviour as those of turnip yellow mosaic virus. Serologically it is closely related to Andean potato latent virus. Its host range is also similar, although it is more virulent in most hosts, and all its other properties indicate that it belongs to the Andean potato latent virus subgroup of the turnip yellow mosaic virus group. (Gibbs with Dr. B. D. Harrison, Scottish Horticultural Research Institute)

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Bees with sacbrood virus (SBV). Attempts to find particles of SBV in thin-sectioned brains and hypopharyngeal glands of SBV infected adult honey bees failed, although serological tests indicated the presence of much virus. Hypopharyngeal glands of some bees did contain flexible rod-shaped virus-like particles (25 × 250 nm) that were grouped in small membrane-bound packets. The arrangement closely resembled that of nuclear polyhedrosis viruses but the particles were in the cytoplasm and polyhedra were not seen. (Milne, with Bailey, Bee Dept.)

Potato mop top virus (PMTV). In further attempts to associate PMTV and its fungus vector (*Spongospora subterranea* (Wallr.) Lagerh.) experimentally, the virus was inoculated to leaves, and zoospores of the fungus to roots of young tobacco and *Nicotiana debneyi*. After the virus had become systemic in the shoots, zoospores from the plants were transferred to roots of tomato and tobacco, which were later tested for PMTV by inoculating sap to *Chenopodium amaranticolor*. Zoospores, even from roots shown by infectivity test to contain virus, failed to transmit it. Likewise, in a similar experiment using rooted cuttings of tobacco stems from systemically infected plants, the virus seemed not to be acquired by fungus infecting their roots. Several such experiments have now failed to associate fungus and virus. Infected potato tubers can produce healthy shoots, which suggests that the virus is often not fully systemic and that it did not reach the cells in the experimental plants that were occupied by the fungus.

Experiments begun in controlled environment cabinets show that temperature and shading affect symptoms shown by mechanically inoculated plants. In tobacco kept continuously at 14°C, PMTV caused necrotic local lesions but not at 24°C nor at 24° by day and 14°C at night. Faint chlorotic lesions formed on *Chenopodium amaranticolor* in shade at 24°C but did not develop to form the characteristic necrotic rings and lines.

Soilborne oat mosaic virus. Mechanical transmission of this virus was greatly improved by using the spray gun method developed by American workers. Infected leaf tissue was ground in ten times its weight of neutral phosphate buffer and the extract, mixed with carborundum, sprayed on the leaves from 2 to 3 cm distance under a pressure of 60 psi (4·2 kg/cm²). Plants with three or four leaves, kept in the dark for a day before inoculation were most susceptible; the variety Powys was more susceptible than Blenda or Condor. Results varied but often 80% of the plants became infected, many more than by rubbing the inoculum over leaves with the fingers, even allowing for the greater volume of liquid used in spraying. To develop symptoms, plants were grown under fluorescent lights either continuously at 13°C or at 15° by day and 10°C by night. Pellets from ultracentrifuged, heat-clarified, extracts of diseased leaves contained many long rods of the type seen before, but they seemed not to be infective. Staining with phosphotungstic acid revealed a central core but no substructure was seen after staining with uranyl formate. (Macfarlane)

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***Tropaeolum* virus.** A virus found in *Tropaeolum* similar in host range, symptoms caused and transmission to tropaeolum ring spot virus (Smith, K. M. (1957), *Text book of plant viruses*, 2nd Ed., Churchill), proved to have an unusual structure and combination of properties. It produced typical ring spot lesions in many kinds of plants, was transmitted by manual inoculation of sap and by the aphids *Myzus persicae*, *Aphis fabae* and *A. craccivora*, in the manner characteristic of a non-persistent virus. It was inactivated in sap within a few days at 20° and 10 minutes at 60°C. The spherical particles were 28 nm in diameter, showed no obvious morphological subunits and separated when centrifuged into three types with sedimentation constants of 113, 98 and 58 S. Thin sections of infected *Chenopodium amaranticolor* showed that 'empty' virus particles (58 S) were much commoner than 'full' particles. Some particles occurred separated from others, but many of all types were aggregated in the cytoplasm into straight tubes 80 nm in diameter, in the walls of which the particles were closely packed hexagonally, showing nine particles in cross section.

The combination of particles of three weights and relative instability, with aphid transmissibility has been described only for broad bean vascular wilt virus, but the *Tropaeolum* virus did not react with antiserum to this virus or to antisera to many other viruses. (Sahambi, Milne and Phillips)

Potato viruses Y and C. Reasons for potato virus C (PVC), which is not transmitted by aphids becoming so from plants also infected with potato virus Y (PVY), were reinvestigated. Mixtures of 1 part PVY and 50 parts PVC sap were inoculated to *Nicotiana glutinosa* and subcultured through five sequences of the following three methods: by aphids from *N. glutinosa* to *N. tabacum*; manually from *N. tabacum* to Majestic potato; manually from single necrotic local lesions in potato to *N. glutinosa*.

Through the five subcultures some isolates retained the aphid-transmissibility of PVY combined with the ability of PVC to cause only necrotic local lesions in Majestic potato plants. Others that first behaved in this manner later became systemic in potatoes, causing asymmetric necrotic lesions on young leaves. One of the original PVC isolates, PVC^{m}, occasionally became systemic during summer but was not transmitted by aphids. The possibility that this behaviour is temperature dependent is being investigated (see Henbane Mosaic Virus (HMV), below).

Henbane mosaic virus (HMV). A variant (HMBV) of HMV, which was separated by aphids but is difficult to transmit by them, caused necrotic symptoms in *Nicotiana* spp. (*Rothamsted Report for 1967*, 123). It was sub-cultured four times from single local lesions and inoculated to tobacco plants, which were kept for a month in controlled environments ('Saxcil' cabinets, at 20° and 25°C and 75% RH, illuminated for 16 hours per day at 27 500 lx and 11 400 lx (shaded)). The plants were then moved to a glasshouse where lights maintained a similar photoperiod although temperature was variable but usually within the same range. Plants at 20°C showed the usual necrotic lesions. Unshaded plants at 25°C appeared

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almost symptomless and virus could not usually be recovered from their younger leaves. Other environmental changes to plants infected with HMBV have produced variants, some resembling the parent HMV and others less or even more virulent. Plants infected with HMBV and the less virulent variants were readily invaded by HMV, even when the HMV inoculum was diluted near to its infection end point. For this reason, it seems that the original HMBV inoculum was homogeneous but that the variants like HMV arise during its multiplication in tobacco much sooner at 25° than at 20°C. Had they been in the original inocula, they and not HMBV would have become dominant in plants held at 20°C, although they do not arise at that temperature readily. Similarly the variants that have some properties of PVC but are aphid transmitted from plants jointly infected with PVC and PVY may be of only one type initially, but revert from it to forms more nearly resembling PVC or PVY during sub-culturing, especially at different temperatures. (Watson)

European wheat striate mosaic (EWSM). Infection decreased the longevity of nymphs of *Javesella* (*Delphacodes*) *pellucida*. Only 35% of nymphs from congenitally infective mothers of an inbred line (CC) survived to maturity compared to 62% of similar uninfected nymphs and 81% from uninfected outbred mothers with a common parent (CD). The progeny of outbred infected mothers suffered less but still 22% (x^2 9·4) of nymphs died before 7 days old compared with 13% of the control population. The extent of the damage differed between hopper families but it always appeared by the fifth day after hatching. It might occur even earlier in some inbred lines even before hatching as reported by Watson and Sinha (*Rothamsted Report for 1958*, 100). The longevity of congenitally infective adults was 14% less than of non-infective adults in both sexes. (Watson with Dr. E. D. Ammar and Dr. P. W. Murphy, University of Nottingham School of Agriculture)

Yellow net virus (Y or V). Watson (*Ann. appl. Biol.* (1962), **50**, 451–460) reported that *Myzus persicae* would transmit YNV but not yellow net mild yellows virus (YNMYV) from sugar beet to *Nicotiana clevelandii*, but that YNV could not be retrieved from the *N. clevelandii* by *M. persicae*, and suggested that this was because YNV could only be transmitted by aphids in the presence of YNMYV. However, in recent tests both viruses have been transmitted to *N. clevelandii*, though much younger, and perhaps more susceptible, plants were used than in the tests reported by Watson. (Plumb)

Fraction I protein. The chloroplasts of *Salvia* sp. contain rods that resemble the particles of some viruses. However they are composed of protein units similar to those of Fraction I chloroplast protein of other species, and they are probably aggregates of this. Like Fraction I protein, the particles have carboxydismutase activity and single subunits are considerably more active than aggregates. Much of the protein occurs as rods so that reversible aggregation may be important physiologically in regulating the activity of the enzyme.

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Molecular weights of virus proteins. Shapiro *et al.* (*Biochem. Biophys. Res. Commun.* (1967), **28**, 815) have reliably estimated molecular weights of proteins by electrophoresis on polyacrylamide gels in the presence of sodium dodecyl sulphate. The molecular weights of several virus proteins are being reinvestigated by this method because published values are questionable. For PVX protein chemical methods have given molecular weights of 51 000 (Shaw *et al.*, *Virology* (1962), **18**, 79) and 22 300 (Miki & Knight, *Virology* (1968), **36**, 168). Electrophoresis gives a value of 29 000 confirming the smaller chemical estimate. This is also more satisfactory from structural considerations, as it is difficult to visualise how the flexuous PVX rods could be assembled from protein subunits containing as many as 450 amino acid residues. TNV protein has a molecular weight of 28 500 by electrophoresis, which agrees well with the recently published chemical determination of 23 000 (Uyemoto and Grogan, *Virology* (1969), **39**, 79). (Carpenter)

Electron microscopy of virus-infected tissues

Tomato spotted wilt virus (TSWV). It has been thought that this virus may be related to myxoviruses (e.g. influenza), but closer study suggests that it develops differently from these or any other known groups.

Two strains of TSWV from California and two from England were grown in *Lycopersicum esculentum*, *Nicotiana clevelandii*, *Vigna sinensis* and *Chenopodium amaranticolor* in the glasshouse, or in growth cabinets at 20°C. Inoculated leaves were sampled at 8 hour intervals for infectivity assay and fixed for sectioning. Virus particles developed similarly in all virus–host combinations except that one strain from Wye College, Kent, did not produce detectable virus particles in any host.

The sequence of virus particle development is difficult to establish because adjacent cells are not necessarily infected simultaneously or in sequence. The most likely sequence is thought to be:

1. Dark, amorphous patches of 'viroplasm' appear in the cytoplasm and may represent a protein or nucleic acid pool.
2. Small and large pieces of paired and parallel membranes (separated by 12·5 nm of dense material) form in association with the viroplasm.
3. The membranes bud to form spherical particles (100 nm diameter), with two walls spaced 12·5 nm apart. Between 55 and 120 hours after inoculation these particles lie free in the cytoplasm in large numbers.
4. Groups of double-walled particles fuse and their outer membranes form a bag in which the, now single-walled, particles lie free. These 85 nm particles are the only ones found in long-infected tissue and are infective. (Milne)

Potato virus X. Lamellar inclusions in leaf cells seemed diagnostic of PVX in several hosts (*Rothamsted Report for 1968*, Part 1, 126). The lamellae are densely-staining sheets, without visible substructure and 4–5 nm thick. Their appearance and staining suggest they are protein, rather than lipoprotein, carbohydrate or nucleic acid. However, when embedded and sectioned the lamellae resisted attack by pronase, papain,

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pepsin and trypsin under conditions that allowed digestion of some known protein-containing materials. Often virus particles are associated with the lamellae, but more characteristic are the ribosome-like particles that are attached closely but randomly on both sides of the lamellae and not in polyribosome configurations. Although the lamellae and 'ribosomes' are unique structures, they resemble the protein sheets that form 'pinwheels' in plants infected with potato virus Y. (Milne and Turner)

Microscopy of tissues infected with tobacco necrosis and satellite viruses. French bean leaves inoculated with the bean stipple-streak strain of tobacco necrosis virus (TNV) either alone or mixed with satellite virus (SV) were examined with the light and electron microscopes. Light microscopy of epidermal cells stripped from the midrib on the lower leaf surface showed one or more inclusions in each, usually lying against the cell wall. Some inclusions were hyaline and others consisted of very small crystals. Some cells also contained several kinds of crystals that were not in inclusions. Inclusions occurred only in localized areas of tissues infected with TNV or the mixture.

Electron microscopy of parenchyma cells cut at right angles to the leaf lamina showed that both inclusions corresponded to areas in the cytoplasm with well defined edges and containing much apparently pure TNV. The only crystalline forms found were arrays of a few SV particles or small rhombic SV crystals 1–2 μm across, in cells infected with the mixture. The most characteristic abnormality in cells infected with the mixture was the presence of an electron-dense, amorphous material of cytoplasmic origin. When this material occurred in the vacuole, the entire cell was disorganised and contained numerous vesicles of various sizes and apparently formed from disrupted cell membranes. The electron dense material seems connected with the multiplication of the viruses, SV could be certainly identified only when in crystalline arrays, TNV was more easily seen. SV was seen in electron micrographs only when in close proximity to TNV, supporting the fact that it can multiply only when helped by TNV. (Kassanis, Vince and Woods)

Cocoa swollen shoot virus. Thin sections of parenchyma and veins of young leaves of *Theobroma cacao* infected with the severe strain A showed much necrosis but few particles. Such particles as there were measured 26 × 130 nm and were stiff rods in small aggregates in the cytoplasm. (Milne and Kenten)

Virus-like particles in *Javesella pellucida*. Attempts to find the agent of European wheat striate mosaic in this vector failed but showed that the Rothamsted culture of *J. pellucida* contained round-ended virus-like particles (30 × 60 nm). The bacilliform particles seem unconnected with European wheat striate mosaic because they occur in hoppers kept on healthy plants and that did not transmit the agent to healthy plants. The particles appear in the cytoplasm of many tissues but without apparent effect on the insect, and were not found in a hopper culture from Sutton Bonington. (Ammar, Watson and Milne)

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Mycoplasmas. Attempts are being made to find quick methods of detecting mycoplasma-like bodies in diseased plants, using red clover (*Trifolium pratense*) with clover phyllody or clover witch's broom. Mycoplasma-like bodies are easily found in sections of the phloem but not elsewhere; similar bodies have been found but not consistently in negatively stained preparations of extracts from affected plants. (Milne and Grace)

Virus diseases in crops

Carrot motley dwarf. At Woburn in 1968, carrots (cv. Clucas New Model) in some plots were artificially infected on 11 June with the carrot motley dwarf complex of viruses, using 1000 to 2000 *Cavariella aegopodii* Scop per plot (8 ft²). Samples (1 ft length of row) were taken every 2 weeks from these, from similar uninfected plots, and from the areas surrounding both (main plots), to measure growth, to count aphids and to determine virus spread. Half the main plots were sown with menazon insecticidal granules and sprayed with menazon on 17 June, one week after inoculation.

Aphid counts. Most of the introduced aphids had disappeared by 17 June but populations increased on plots without insecticide from 1 or 2 per plant on 2 July to an average of 21 per plant on 30 July. They were erratically distributed and too few to cause direct injury to plants.

Virus infection. Most plants in inoculated sub-plots developed sypmtoms between 2 and 17 July. Infection increased on other areas without insecticide, from 5% of plants on 16 July to 18% on 30 July and 25% on 26 August, when insecticide treated plots had respectively, 3, 2 and 10% of plants infected. At the end of August only 2 of the 12 insecticide treated plots had more than 20% plants infected and only three of the untreated plots had less.

Yields. The weight of roots from uninfected sub-plots (with and without insecticide) increased almost linearly from 2 July (2 tons/acre) to 30 August (15·0 $\pm$ 0·79 tons/acre). Infection seemed quickly but temporarily to interfere with the translocation of metabolites so that on 2 July, 3 weeks after infection but before symptoms appeared, the roots from infected sub-plots weighed only 1 ton/acre. During this period infection had no effect on foliage other than preventing the elongation of petioles. During the remainder of July, infected plants grew but more slowly than uninfected ones; during August their root weight was constant at about 6 tons/acre. Three farm harvests from main plots confirmed that insecticide increased yield by 2·52 $\pm$ 0·626 tons/acre. In contrast, insecticide had no effect on the yield from inoculated sub-plots. The regression of yield (two samples of 9 ft of row from each main plot) on percent plants infected showed a decrease of about 1·5 tons/acre for each 10% increase in plants infected. (Watson and Pullen)

Epidemiology of cereal aphids and barley yellow dwarf virus (BYDV)

Local differences in BYDV. Viruses isolated from plants sent to Rothamsted between 1961 and 1968 and classified as virulent or avirulent

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were divided into those from South and West or North and East Britain. The results in Table 2 support the conclusion that virulent isolates of BYDV are commoner in the South and West and that *Rhopalosiphum padi* is their most important vector, but that some viruses were transmitted by more than one species. (Watson and Plumb)

TABLE 2

Origin, virulence and vectors of BYDV isolates tested at Rothamsted, 1961–68

Origin:	South & West Britain		North & East Britain	
Isolate:	Avirulent	Virulent	Avirulent	Virulent
Vectors:				
Rhopalosiphum padi	5	8	14	7
Sitobion avenae	4	4	17	5
Sitobion fragariae	1	2	8	4
Metopolophium dirhodum	0	0	2	0
Total isolates:	6	9	36	14

Over wintering of cereal aphids. In 1968, fewer *R. padi* and *Sitobion avenae* were found in winter cereals and pastures as autumn turned into winter. Turves (30 × 45 × 10 cm deep) were dug and kept for 4 weeks in warm, lighted aphid-culturing chambers, to allow any aphids sheltering among or near roots to multiply and move to leaves. Many aphids of both species appeared on turves collected on 12 December, only few *S. avenae* and no *R. padi* were found on turves cut on 22 January and none, after prolonged snow and cold weather, on turves cut on 3 March 1969. Samples of all aphid collections were fed on healthy oat seedlings, a few of which became infected with an avirulent BYDV. This decline in aphid populations during autumn may explain why adjacent crops of winter wheat (cv. Cappelle), sown in mid-August and mid-September had, respectively, 6 and 0·1% of BYDV infected plants in spring. (Plumb)

Phenology of BYDV and its aphid vectors in 1969. The Entomology Department aphid survey (p. 237) showed that, despite a cold winter, *R. padi* was common at Rothamsted (86/week, $4{\cdot}5 \times 10^5$ m³ of air sampled) and *S. avenae* just detectable (1 or 2/week) by late May. No species of cereal aphid increased greatly until mid-June, when all increased until the end of July and then became fewer in August. *R. padi* increased again as in 1968 in September and October to a maximum of 770/week.

For the first time attempts were made to supplement the studies of cereal aphids with information on the proportion of alatae that were viruliferous. Between May and mid-August, 6 in. suction traps (4 ft a.g.l.) at Rothamsted; Broom's Barn, Suffolk; Starcross, Devon and Gleadthorpe, Notts., caught live *R. padi*, *S. avenae* and *Metopolophium dirhodum* that were tested for BYDV. Of 634 aphids caught and tested at Rothamsted, 48 were infective most with avirulent isolates, elsewhere catches were too few for regular tests. At Rothamsted the first viruliferous aphid was caught on 13 June and the percentage carrying BYDV increased until late July when the proportions of vectors were approximately 20% *R. Padi*, 15%

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S. avenae and 3% *M. dirhodum*. The proportion of aphids flying just above crops that are viruliferous may provide more reliable estimates of the prevalence of BYDV in cereal crops than testing transmission by aphids fed on bulk leaf samples from crops, which records only the proportion of crops in which the disease occurs. (Plumb and Cook)

At Rothamsted, central areas of plots of barley (cv. Zephyr) were artificially infested with infective and non-infective *R. padi* and *S. avenae* on 23 May. There was little spread of vectors or virus into the surrounding parts of the plots. Wet and cold weather in late May and early June decreased populations, especially of *R. padi*. As a result few plants became infected on the areas infested with *R. padi* and virulent virus, whereas all did in the areas infested with *S. avenae* and mild virus. In July nearby crops of oats, wheat and barley had between 5 and 8% plants infected with BYDV.

On unsprayed plots aphids were commonest in mid-July when there were 80 *S. avenae*, 14 *R. padi* and 383 *M. dirhodum*/1000 tillers. Parasites were common, perhaps as a result of the many aphids in 1968. In mid-July there were two or three syrphid larvae per 90 cm of barley row and by the end of the month many had eaten enough aphids to pupate. During the same period, mummified aphids (mostly parasitised by *Aphidius* spp.) increased from 25 to 73/1000 tillers; Staphylinid beetles were also present.

Effect of BYDV. As infective aphids appeared late and mostly transmitted avirulent BYDV, little damage was expected. Some plots of the above experiment were sprayed with Menazon 12 days after infestation so that virus spread would be predominantly by aphids from outside. Table 2 shows little difference between yields of plots with or without insecticide, both in the central infested areas or in the plots surrounding each. In the infested areas the very few infections by virulent strains transmitted by *R. padi* slightly decreased yield, whereas the almost complete early infection by the avirulent strain with *S. avenae* almost halved yield. This happened equally on sprayed and unsprayed plots and was therefore attributable to virus infection and not to aphid feeding. Yields in areas surrounding the infested areas were not affected either by aphids or virus, supporting the opinion that unless artificially introduced early, BYDV and aphids caused little harm in this locality.

TABLE 3

Effect of aphids, BYDV and insecticide on yield of barley (cv. Zephyr), Rothamsted 1969

Introduced:	Grain yield (cwt/acre)					S.E.
Aphid	None	*R. padi*	*S. avenae*	*R. padi*	*S. avenae*	
Virus isolate	None	None	None	Virulent	Avirulent	
Infected central sub-plots						
No insecticide	48·8	42·8	49·1	42·1	24·6	±1·72
Insecticide	48·2	45·1	45·0	39·6	27·1	
Non-infected surround						
No insecticide	43·0	46·1	44·4	44·4	41·9	±3·81
Insecticide	46·3	48·4	49·2	41·4	43·5	

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The Mexican dwarf wheat varieties, Mexico 120 and Lerma Rojo 64, grown in 'Saxcil' controlled environment cabinets at 20°C (with 20 000 lx for 16 hours per day at 75% R.H.) yielded, respectively, 45 and 20% less grain when infected with a virulent BYDV isolate at the first leaf stage. At 25°C the corresponding decreases were only 18 and 17%. Comparable oats (cv. Blenda) and barley (cv. Zephyr) yielded, respectively, 79 and 83% less grain than uninfected plants at 20°C, and 5 and 56% less at 25°C. In the glasshouse, infection decreased yield of the dwarf wheats by 40%, oats by 77% and barley by 26%.

Tests of vectors. *Metopolophium festucae*, a common grass aphid did not transmit virulent or avirulent isolates of BYDV but *Myzus circumflexus* was confirmed as an efficient vector.

Nearly all the *R. padi* tested transmitted a virulent BYDV isolate and symptoms were equally severe whether 1, 3, 5 or 10 *R. padi* were used to infect healthy oat seedlings. (Watson, Plumb and Cook)

Multiplication of aphid vectors. The spread of BYDV isolates may be partially or entirely specific to individual aphid species, so it is necessary to know how each species multiplies. Between 1 and 10 *R. padi* or *S. avenae* were placed on single oat seedlings (cv. Blenda) at 15°C and 3000 lx and in the glasshouse (average 25°C).

The first experiment showed that individuals differed in fecundity, so clonal populations were used in later experiments. *R. padi* began reproducing sooner and faster than *S. avenae*, especially in the glasshouse, but crowding caused multiplication to slacken sooner. *S. avenae* eventually produced more progeny and individuals lived longer. Both species reproduced faster in the glasshouse and produced most progeny when plants were initially infested with only one aphid. In contrast to *R. padi*, *S. avenae* produced very few alatae at 25°C. (Cook)

European wheat striate mosaic (EWSM). Very stunted plants (0·10%) showing symptoms were found in one crop of Capelle winter wheat at Rothamsted in mid-June and were probably infected in April. *Javesella pellucida*, the leafhopper vector, was not found in the field but was hatched from grass turves brought into the laboratory (p. 148) on 3 March. These were not already naturally infective but became so when fed on infected plants.

Cocksfoot mottle virus (CFMV). A cocksfoot-lucerne sward in its fourth year at Rothamsted had 25% of Cocksfoot (*Dactylis glomerata*) plants killed by CFMV and 70% of the remainder were infected. Insecticide (Metasystox) sprayed regularly (*Rothamsted Report for 1967*, p. 189) throughout the 4 years did not decrease the incidence, which supports the earlier conclusion that CFMV is prevalent in old swards probably because of transmission by grass-cutting machinery. (Plumb)

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Sugar beet. Sugar beet on Stackyard Field, Woburn and Barnfield, Rothamsted, had, respectively, 10 and 22% of plants with yellows most with mild yellowing viruses.

Mineral deficiency symptoms can be confused with those of yellowing diseases and, perhaps because the summer was unusually dry, the distribution of these was interesting. At Woburn, there was much heart-rot caused by boron deficiency. Increasing nitrogen fertiliser increased heart-rot, as did additional P, K and Mg unless they were accompanied by peat or FYM, when there was less heart-rot. At Rothamsted most of what little boron deficiency there was, occurred with most added minerals. An average of 5% of plants showed magnesium deficiency symptoms, with most on plots given P without Mg or other minerals. (Watson and Plumb)

Potato. Winged aphid vectors of potato viruses were rare in the seed crops at Rothamsted in 1968 and neither leaf roll nor potato virus Y was found in 650 plants examined in the stocks used in experiments in 1969. Similarly scarcity of winged aphids in 1969 suggests that the stocks intended for experiments in 1970 should be equally healthy.

Serological tests showed that potato virus X was prevalent in Majestic with 20% in seed crops and 50% in experiments, but not in other varieties. (Govier)

Diseases of cereals

Epidemiology of cereal powdery mildew (*Erysiphe graminis*). As a start to this work, plots of Cappelle winter wheat were sown at the middle of August, September, October, November and March to note the time when mildew appeared and spread. In one series, unreplicated plots were 'isolated' (no mid-August sowing) from one another and from another series where successive plots were 'adjacent' to their immediate predecessors. The August plot became severely infected and adjacent plots sown later became infected but less so with delay in sowing from September to November. By comparison isolated plots sown in autumn developed little mildew but spring-sown Cappelle was infected almost equally in the two series.

At intervals mildew infection was assessed (per cent of area) on the three leaves then uppermost, but these leaves change through growth and senescence so the results are not easy to interpret or explain. However, mildew became much less prevalent between November and April because the severely infected lower leaves died. Thus infection of the spring sowings was little affected by the large source of infection that had existed in the 'adjacent' series during autumn. Assessment on specified leaves is convenient once the flagleaf has emerged but a better method is needed for earlier assessments. Plant spacing seems an important factor in spore supply. Usually the number of green leaves is almost the same over a long period because lower leaves die almost as fast as new ones are formed. In a wide-spaced crop (10 in. row) there were more green leaves and much more mildew than in crops close-spaced (5–7 in. row). (Jenkyn)

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Fungicides and powdery mildew of cereals. Ethirimol, one of the promising new systemic fungicides, was used in several experiments (as wettable powder 80% a.i.) to test its effects on different crops and varieties, its influence on the use of nutrients by the crops and to modify mildew attacks so that their effect at different stages of growth could be studied.

At Woburn there was little mildew and although there was more at Rothamsted the attack was not severe, because weather did not favour the disease. At both places mildew was less in spring barley varieties with ethirimol, which significantly increased the average yield of four varieties given three amounts of applied nitrogen fertiliser (Table 4). Average yields of the four varieties showed a significant interaction between the effect of ethirimol and amount of nitrogen. At 0·3, 0·6 and 0·9 cwt N/acre, respectively, the differences between plots with and without fungicide were −0·1, +1·0 and +3·3 cwt of grain/acre at Rothamsted and −0·7, +1·2 and +2·3 cwt/acre at Woburn. The amount of lodging was not affected by the fungicide, but it differed between varieties. Sultan, which lodges rather easily and resists mildew, and the susceptible variety Zephyr represented somewhat erratic extremes (Table 5). Benefits from the fungicide were usually greatest with most nitrogen, and where little nitrogen was applied the fungicide was often slightly detrimental. As increasing nitrogen increased mildew, the greater benefits from the fungicide could be attributed to preventing more disease. Equally, if the fungicide slightly damages barley this could show as smaller yield where mildew was slight, as with the resistant varieties or with little nitrogen. If confirmed, the greater benefit of fungicide when much nitrogen is applied will increase the profit from using it where cereals are grown intensively.

Ethirimol was confirmed as less effective on wheat than on barley. At Rothamsted, it decreased the average area infected by mildew on the 2nd leaf of six spring wheat varieties only from 3·3 to 1·8% and usually decreased yield, but not significantly. (Jenkyn and Moffat)

TABLE 4

Effect of ethirimol on mildew and grain yield of spring barleys, 1969

	Woburn				Rothamsted			
	% Mildew[1]		Yield (cwt/acre)		% Mildew[1]		Yield (cwt/acre)	
Fungicide[2]	−	+	−	+	−	+	−	+
			±0·57				±0·87	
Variety								
Julia	0·3	0·0	42·7	43·6	0·6	0·2	49·6	51·5
Maris Badger	0·8	0·2	34·4	35·6	1·5	0·3	41·4	43·6
Sultan	0·1	0·1	43·2	43·7	0·1	0	49·5	48·4
Zephyr	1·0	0·4	43·2	44·3	6·1	1·1	48·6	51·2
			±0·28				±0·43	
Mean	0·6	0·2	40·9	41·8	2·1	0·4	47·3	48·7

[1] % area of 2nd youngest leaf infected with mildew at growth stage (G.S.) 11·1.
[2] ± ethirimol seed dressing (as 1 lb/acre of 80% a.i. wettable powder).

Relative importance of mildew attacks at different growth stages. The yield of cereals depends greatly on contributions from the flag and second upper-

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TABLE 5

Effect of ethirimol and nitrogen on mildew and grain yield of barley

	Woburn				Rothamsted			
	Mildew[1] %		Yield cwt/acre	Increment[2] from ethirimol cwt/acre	Mildew[1] %		Yield cwt/acre	Increment[2] from ethirimol cwt/acre
Ethirimol[3]	−	+	−		−	+	−	
Variety (N cwt/acre)								
Sultan (0·3)	0·1	0·1	35·0	+1·8	0	0	45·1	−3·8
Sultan (0·6)	0·2	0·1	45·9	−0·3	0·1	0	52·9	−1·2
Sultan (0·9)	0·1	0·1	48·7	0	0·1	0	50·6	+1·5
Zephyr (0·3)	0·6	0·2	36·9	−2·8	4·6	0·9	47·3	+0·3
Zephyr (0·6)	0·7	0·2	45·1	+3·1	5·8	1·0	50·0	+3·4
Zephyr (0·9)	1·8	0·7	47·5	+3·1	7·8	1·3	48·6	+4·1

[1] % area of 2nd youngest leaf infected with mildew at G.S. 11·1.
[2] Yield of fungicide treated minus untreated plots.
[3] ± ethirimol as seed dressing (1 lb/acre, 80% a.i.).

most leaves, so infection of these is probably particularly damaging. However, little is known of the relative importance of attacks at different stages of growth, for example, how much early attacks decrease the size or efficiency of the root system and of the leaves just below the ear. Infector plants and ethirimol were used to modify mildew attacks on spring barley at different stages of growth. The treatments (Table 6) were intended to compare full control of the disease with severe attacks early, late or during the full growing season. Despite early inoculation of relevant plots, cold spring weather so delayed mildew development that in June there was little contrast between 'full season' and 'late' attack. The maximum increase in yield was 4·2 cwt/acre but neither early nor late control was very beneficial. (Jenkyn)

TABLE 6

Effect of times and intensity of mildew attack on barley (cv. Zephyr)

Intended attack	Ethirimol[1] treatment	Mildew (%)[2] 9th June G.S. 8 to 9	25th July G.S. 11·2	Grain yield cwt/acre ±1·05
Full season[3]	None	0·1	18·2	48·4
Late	Seed dressing, ¼ lb/acre	0	16·6	48·6
Early[3]	Two sprays, 1 lb/acre	0·1	4·5	49·1
'None'	Seed dressing, 2 lb/acre + 2 sprays, 1 lb/acre	0	0·6	52·6

[1] All as wettable powder, 80% a.i.
[2] % area, of 2nd youngest leaf, mildew infected.
[3] Included infector plants.

Control of mildew on cereals in glasshouses. Cereals in the glasshouses are often severely attacked by powdery mildew, which not only complicates work with the fungus, but makes it difficult to maintain aphid cultures or work with cereal viruses (*Rothamsted Report for 1968*, Part 1, 130).

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Fungicides could sometimes be used so long as they do not influence other pests or pathogens, but for other purposes healthy plants must be produced without fungicides.

Ethirimol controlled mildew well on barley (cv. Zephyr) but was less successful on oats (cv. Blenda) or wheat (cv. Cappelle). It seemed not to affect the development of symptoms caused by barley yellow dwarf virus but there was evidence, from an unreplicated trial, that it decreased the fecundity of aphids. Viruliferous *Rhopalosiphum padi* that had fed either on ethirimol-treated or untreated Blenda or Cappelle were transferred (30/pot) to untreated seedlings of the same varieties. After 13 days on oats or 24 on wheat, when symptoms began appearing, the progeny of aphids that had previously fed on untreated plants numbered, respectively, 1135 and 1340/pot compared with only 612 and 435 from aphids that had fed on plants given ethirimol. Although a disadvantage for experiments with aphids, a similar effect in field crops might mean an additional benefit from its use. (Jenkyn and Plumb)

An apparatus was built in which pots with cereal plants can be grown isolated from one another and in spore-free filtered air. It is hoped it will produce healthy test plants for use in various infection studies, and for testing possible phytotoxicity of systemic fungicides. (Jenkyn, Hirst and King)

The effects of frequent cropping with wheat and barley. The effects of growing crops not susceptible to *Ophiobolus graminis* ('break' crops) on take-all and yield in winter wheat were studied in 'Intensive Wheat Experiments' at Woburn, Rothamsted and Saxmundham. There were large differences between experiments in yield, response to nitrogen and the incidence and effect of take-all; unfortunately, the causes of some remain difficult to explain.

At Woburn, wheat after ley and potatoes and given ample nitrogen fertiliser yielded 40 cwt/acre of grain, 10 cwt/acre more than the most from wheat after wheat, where take-all was always common (Table 7). The fourth successive wheat crop had much less take-all than the third, but

TABLE 7

Grain yields and incidence of take-all in winter wheat (Cappelle) at Woburn, 1969

Previous crops	Nitrogen, cwt/acre 0·5	1·0	1·5	2·0
	Grain yield, cwt/acre			
Wheat, ley, potatoes	33·0	41·6	43·6	39·6
Ley, potatoes, wheat	23·3	28·6	24·6	26·6
Potatoes, wheat, wheat	17·0	24·8	23·4	25·0
Wheat, wheat, wheat	23·3	28·1	31·8	29·6
	% plants with moderate and severe take-all, early July			
Wheat, ley, potatoes	0	0	0	0
Ley, potatoes, wheat	47	39	49	38
Potatoes, wheat, wheat	57	48	63	49
Wheat, wheat, wheat	31	31	25	19

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the difference in yield was not commensurate with the difference in disease, probably because grass weeds were more prevalent in the fourth crop. Before the experiment began in 1966, the site had been free from susceptible crops for 4 years (oats, fallow, beans, fallow), but previously had been in wheat almost continuously for 85 years. If inhibition of *O. graminis* developed during this long wheat sequence, it seems not to have survived the 4-year break.

The occurrence of 'take-all decline' in the fourth successive wheat crop at Woburn agreed with previous experience that inhibition to *O. graminis* infection of winter wheat develops only after 1 or 2 years of very severe take-all and small yield. However, on the Intensive Barley Experiment at Rothamsted take-all declined after the disease was common but without causing small yields of barley during the formative years (*Rothamsted Report for 1968*, Part 1, p. 137). In 1968–69 this experiment was sown to winter wheat instead of barley, to study the incidence of take-all in winter wheat and especially to see whether the inhibition of *O. graminis* generated by barley crops would extend to winter wheat. Take-all proved less common and severe in wheat after continuous barley than in wheat after three barley crops following a 2-year break (Table 9), so it seems that a sequence of barley may be a less costly way than continuous wheat to establish inhibition to *O. graminis* in soils. Take-all obviously affected growth of the wheat soon after ear emergence, yet unless nitrogen limited growth even the worst attacked crops yielded more than 40 cwt/acre, as much as the crops free from take-all at Woburn.

TABLE 8

Grain yields and incidence of take-all in winter wheat (Cappelle) at Rothamsted, 1969

Previous crops	Nitrogen, cwt/acre 0·6	1·0	1·4	1·8
	Grain yield, cwt/acre			
Fallow 1968 after 7 wheats	53·9	55·4	50·5	55·0
One barley after oats–beans	42·3	51·9	52·9	56·0
Three barleys after oats–beans	38·7	46·9	44·0	48·3
Five barleys after oats–beans	43·9	50·7	52·1	54·0
Continuous barley since 1961	42·6	51·2	56·1	53·1
	% plants with moderate and severe take-all, mid-July			
Fallow 1968 after 7 wheats	0	1	1	0
One barley after oats–beans	40	25	50	11
Three barleys after oats–beans	80	65	63	49
Five barleys after oats–beans	40	27	21	31
Continuous barley since 1961	33	24	21	9

At Saxmundham take-all was slight in all crops, winter wheat after a 2-year break yielded no more than the 2nd wheat crop and little more than the best yields of 3rd and 4th wheats (Table 9). All yields were poor, the best much smaller than the worst in the experiment at Rothamsted.

Of the several other diseases found at each site, only mildew was likely to have decreased yield appreciably. It was most severe at Woburn and Saxmundham and with most nitrogen, yet other evidence suggests it was

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TABLE 9

Grain yields and incidence of take-all in winter wheat (Cappelle) at Saxmundham, 1969

Previous crops	Nitrogen, cwt/acre 0·6	1·2	1·8
	Grain yield, cwt/acre		
Wheat, ley, beans	26·3	29·9	31·4
Ley, beans, wheat	24·7	31·7	31·4
Ley, wheat, wheat	20·4	27·5	28·9
Wheat, wheat, wheat	20·8	26·9	29·3
	% plants with moderate and severe take-all, mid-June		
Wheat, ley, beans	0	0	0
Ley, beans, wheat	7	8	1
Ley, wheat, wheat	5	2	16
Wheat, wheat, wheat	1	1	1

unlikely to explain the very large differences between experiments. The soils at Rothamsted, Woburn and Saxmundham are very different, so was the weather, but we cannot yet relate these to the differences in yield of wheat and in the prevalence and effect of take-all. (Slope, Etheridge and Palmer)

Estimating the number of infective units of *O. graminis* in soil. Attempts were made to define optimum conditions for measuring λ, the number of infective units of *O. graminis* in soil (*Rothamsted Report for 1968*, Part 1, 135).

Temperature. In soil temperature tanks and 'Saxcil' growth cabinets the proportion of seminal and crown roots of Cappelle wheat seedlings infected at different temperatures showed a unimodal curve with an optimum at 18–20°C. The shape of the curve was usually preserved when the soil was diluted by sand to one-sixteenth. The proportion of infected roots reflects the severity of infection on the test plants, but λ, the number of infective units/unit volume (150 cm^3) of soil, is estimated from presence or absence of infection, not its severity. Estimates of λ at different temperatures sometimes showed a similar optimum between 18 and 20°C, but at other times increased almost linearly from 10 to 25°C. Reasons for the difference are not yet fully understood so the best temperatures for the tests are difficult to choose.

Moisture. A stepped sand bench provided three soil moisture tensions for estimating λ in glasshouse tests lasting 4 weeks at about 15°C. Previously, it has been suggested that moisture contents between 30 and 80% of saturation most favour take-all infection. Estimates of λ for soil from Butt Furlong, Woburn, with moisture contents (at the end of the experiment and averaging gradients within pots) of 30% (pF 2·1), 25% and 7% of saturation were, respectively, 10·7, 8·6 and 0·9. A second experiment with comparable moisture contents of 12, 24, 39, 50 and 62%

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of saturation estimated λ, respectively, at 4·7, 1·6, 2·0, 4·4 and 3·2. These values are close enough to regard them as samples from the same population. The two experiments agree that moisture contents between 25 and 60% of saturation are unlikely to greatly affect estimation of λ.

Sensitivity of assays. The usual assay procedure (see reference 7.12, p. 390), but with capillary watering, was used to estimate λ in three replicate dilution series of a sand containing on average four artificially infected wheat straws per 150 cm^3. The dilutions used only a small proportion of the sand/straws mixture. Two of the three estimates of λ (1·34, 1·29 and 3·94) were smaller than they should have been, but agreed with the disease severity as measured by roots infected (respectively 12·4, 12·9 and 22·1%). The errors are thought to result from non-random distribution of straws rather than failure of straws to cause infections, because tests showed that straws tended to migrate to the outside of the heap during mixing. (Hornby)

Extraction of organic debris from soil. An apparatus incorporating six sieves of decreasing mesh size extracted different sizes of plant debris from soil samples for infectivity assays of extracted debris (*Rothamsted Report for 1968*, Part 1, 135). Spouted buckets interposed between the meshes allowed mineral matter to sediment so that later sieves were not choked. To prevent debris being trapped in mineral sediments, without increasing water flow, the contents of each bucket were agitated by compressed air. Soil was introduced to the cascade either from a bucket where it was agitated by a high-pressure water jet or from a modified root-washing can (Cahoon, G. A. & Morton, E. S., *Proc. Am. Soc. hort. Sci.* (1961), **78**, 593). The methods are not yet suitable for routine use because neither extracted all of the smaller grades of debris (Table 10). (Hornby)

TABLE 10

Extraction of organic debris by a cascade of six sieves from 2·3 dm^3 of Kettering loam

Mesh size (μm)	4000	2000	707	420	297	149
(% of total organic debris, extracted automatically)						
Soil introduced by:						
Water jet	100	97	80	51	41	8
Root-washing can	100	89	82	53	40	75

Seasonal fluctuations of *O. graminis* inoculum. Sequential estimates of λ were made in barley on Butt Furlong at Woburn (3rd successive cereal, sown 11 March, on sandy loam) and wheat on Harwoods Piece at Rothamsted (4th successive cereal, sown 4 April, on clay loam). Inoculum decreased from before sowing to minima, respectively at Woburn and Rothamsted, in May or early June (λ = 4 and 10), followed by a rapid increase to maxima in late June or early July (λ = 36 and 59). At Rothamsted the number of units decreased continuously until harvest late in August (λ = 24). At Woburn the June–July peak was briefer, perhaps because the barley was sown earlier and harvested early in August

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($\lambda = 15$). However, a mid-August sampling suggested a large but transient increase ($\lambda = 33$) before a further decrease by the end of the month ($\lambda = 8$). The mid-August estimate may be erroneous or may be real, reflecting infection of the copious volunteer barley that emerged soon after harvest.

Up to harvest the fluctuations were similar to those reported last year. The mid-summer maxima in inoculum frequency are attributed to fungus that was actively parasitic when the samples were collected. The peaks coincide with maximal root mass in cereals (Jonker, *Van. Zee. tot. Land.* (1958), No. 25) so the decreasing infectivity before harvest possibly reflects the death of small roots and the inoculum they carried. (Hornby and Henden)

Take-all development and decline. Winter wheat from all plots of the previous Intensive Barley Experiment (Little Knott, Rothamsted) was examined on three dates to trace how take-all developed in soils where *O. graminis* was and was not inhibited. Table 11 shows the incidence of take-all (average of treatments with 0·6, 1·0, 1·4 and 1·8 cwt/acre N) and the 'infection indices' (*Rothamsted Report for 1968*, Part 1, 134) of assay plants grown in whole soil, sampled in October 1968 from all plots to receive 1·0 cwt/acre N.

Take-all developed faster on winter wheat after two or three barley crops than after four or more. Thus, the severity of take-all on 10 July was not closely correlated with the 'infection index' in the previous October or the percentage of plants infected in April. Therefore, the assays of infection index may not accurately predict the probable severity of take-all in crops, but comparing them with pre-harvest examinations may give a valuable indication of the degree of inhibition or 'decline'. (Slope, Etheridge and Henden)

TABLE 11

Infection index and % plants and roots with take-all in winter wheat (Cappelle); Little Knott, Rothamsted

Samples collected	λ/150 cm³ soil Oct. 1968	% assay roots infected Oct. 1968	Infection index Oct. 1968	Crop samples, 1969 % plants infected/ % roots infected 14 April	2 June	10 July
Previous crops						
7 wheats, Fallow (1968)	0	0·3	1·4	3/0·3	5/ 0·3	4/ 0·5
Oats, beans, 1 barley	0·6	3·9	5·5	14/1·9	32/ 3·5	56/18·9
Oats, beans, 2 barleys	3·3	10·7	24·2	39/6·5	80/14·3	91/38·4
Oats, beans, 3 barleys	6·9	39·6	34·8	44/7·0	84/14·8	95/33·5
Oats, beans, 4 barleys	3·4	19·3	26·3	31/4·4	70/ 8·4	80/17·3
Oats, beans, 5 barleys	6·2	16·0	27·0	31/4·2	67/ 6·3	77/14·7
Oats, beans, 6 barleys	3·6	14·1	19·5	29/4·0	62/ 6·1	75/15·2
8 barleys	2·7	12·1	14·3	21/2·8	48/ 3·7	64/12·0
8 wheats	1·9	8·0	16·6	31/4·7	59/ 5·8	68/13·6
Bean, wheat, potatoes barley (Fallow in 1969)	6·0	16·7	23·7	—	—	—

The two left-hand columns of Table 11 show estimates of λ and per cent roots infected on assay plants (average of all dilutions) grown in the soils used, to estimate 'infection index' (i.e. plots to receive 1·0 cwt/acre N).

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Estimates of λ reflected the pattern of incidence of take-all in winter wheat after the various crop sequences rather less closely than did the other October assays. The meaning of these differences cannot be assessed until the tests are replicated or repeated over several years. For example, in October 1968 soil from first barley crops (second and last lines of Table 11), following a 2-year break or alternating non-susceptible crops, differed considerably but consistently in all three assay parameters suggesting real differences in the distribution or behaviour of inoculum that no method explained. (Hornby)

Bioassays offer one of the few ways of measuring how the infectivity of *O. graminis* varies seasonally and from crop to crop. The controlled environments should eliminate variation in host susceptibility, but the tests are certainly influenced not only by the number, size and vigour of infective units, but also by their durability and spatial distribution. The dilution method estimates how many units are infective, and the proportion of roots infected gives a measure of severity, but neither estimates the extent to which infection is inhibited. As suggested last year the enquiry promises to be lengthy, but some patterns are becoming plainer; for example seasonal changes in infectivity are correlated with numbers of infective units, which also become fewer when soils carry a long sequence of susceptible cereals.

Tests of chemicals to control take-all. Cappelle wheat sown in October was sprayed on 10 April (growth stage 3, 2–3 tillers/plant) with benomyl, oxycarboxin or Dow 'M2452' (each at 1 and 5 lb/acre a.i.). In July unsprayed plots had 58% plants with take-all, 10% straws with eyespot (*Cercosporella herpotrichoides*) and 36% straws with sharp eyespot (*Rhizoctonia solani*), they yielded 47 cwt/acre of grain. No spray damaged the wheat, increased yield or decreased take-all or sharp eyespot, but benomyl decreased eyespot to less than 1%. (Prew, with A. H. McIntosh, Insecticides Dept.)

***O. graminis* on maize.** Maize has been claimed as a suitable 'break' crop in sequences of wheat and barley, despite a report that it is attacked by *O. graminis* and is less effective for controlling take-all than an oat crop (Robinson & Lucas, *Pl. Path.* (1967), **16**, 75–77). The susceptibility of maize was tested in two experiments with plants in pots. Wheat and maize were first grown separately and together in naturally infested soil during summer in an unheated glasshouse. The wheat was slightly attacked (11% of crown roots infected), the maize was not. Later, in growth rooms at 19°C, wheat and maize grown separately in sand artificially infested with *O. graminis* or *O. g. var avenae* were both attacked by both strains (> 50% of crown roots infected). Maize and wheat roots infected by *O. g. var avenae* caused new infections on wheat and oats whereas only wheat was reinfected from roots with *O. graminis*. (Prew)

Effect of direct seeding on take-all of winter wheat. Take-all is sometimes less prevalent on winter wheat drilled into unploughed land sprayed with

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paraquat than when drilled into ploughed land (*Rothamsted Report for 1965*, 125; Brooks & Dawson, *Ann. appl. Biol.* (1968), **61**, 57–64). Apparent effects at Woburn this year depended on whether take-all was estimated by examining the growing crop or by assays of the infectivity of the soil after harvest (Table 12). Direct seeding after paraquat alone had no effect on the proportion of plants infected in July unless accompanied by insecticides, which had no effect on take-all on ploughed plots. In contrast, after harvest the infection index of soil was less in direct-seeded than in ploughed plots. The insecticides had no effect on the infection index.

The experiment was designed to study changes in soil fauna, and the insecticide treatment comprised fresh applications and residuals from earlier ones (10 lb/acre thimetphorate before sowing in 1968, 8 lb/acre chlordane spray in April, plus residues of chlordane, diazinon, 'Zinophos' and D.D.T.). Chlordane has decreased take-all in plants grown in pots (*Rothamsted Report for 1961*, 111), but whether the effects in the field are attributable to it is unknown. Also there is no obvious explanation for the interaction between sowing methods and insecticides on take-all, or the different results from soil assay and examining crop plants. (Prew)

TABLE 12

Incidence of O. graminis *on the Direct Seeding Experiment at Woburn, 1969*

	% crop plants infected July		Infection index September	
Insecticides:	−	+	−	+
Paraquat and direct seeding	64	19	26·5	29·6
Conventional cultivation	54	64	43·2	49·2

Host nutrition and take-all. Huber (*Phytopathology* (1969), **59**, 12) suggested that the form of nitrogen influenced take-all more than the amount, but experiments seem not to have been made with nitrogen supplied entirely as the nitrate or ammonium ion. The nitrification inhibitor, 'N-Serve' was therefore used to maintain the two forms of nitrogen in the presence of equal amounts of all other nutrients; 'N-Serve' has also been reported to inhibit *Rhizoctonia solani* in soil (Papavizas, G. C., *Phytopath. Z.* (1969), **64**, 101–111). Nitrogen was supplied as NO_3^- or NH_4^+ to Cappelle wheat grown in pots in the glasshouse for 38 days in leached sandy loam at approximately 20% of saturation and containing organic debris infected with *O. graminis*. Table 13 shows that nitrogen, as NO_3^-, not only increased growth and root number more than as NH_4^+, but also made root infections fewer. Where comparison was possible (T_2 *v.* T_3) and (T_4 *v.* T_5), 'N-Serve' made take-all less severe.

T_1 plants had more K and P (in foliage) and N (in foliage and roots) than T_2 plants and these had more than T_3. Tops of plants from T_7, T_6 and T_2 had most Ca and Mg, and T_1 plants early showed signs of Mg deficiency. (Hornby with C. A. I. Goring and J. Bolton, Chemistry Department)

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TABLE 13

Growth and infection of Cappelle wheat with differing nitrogen nutrition

(all data/pot, with 5 seedlings)

Treatment	Nutrients added[1]			other amendments		Oven dry wt (g) Roots	Oven dry wt (g) Foliage	No. of main roots	% roots with take-all	Rating[4]
T_1	NH_4^+	P	K	NS[2]	*O.g.*[3]	0·30	0·55	37·2	11·3	0·96
T_2	NO_3^-	P	K	NS	*O.g.*	0·41	0·83	44·4	1·8	0·16
T_3	NO_3^-	P	K	—	*O.g.*	0·43	0·86	50·8	2·5	0·29
T_4	—	P	K	NS	*O.g.*	0·18	0·22	25·0	6·7	0·37
T_5	—	P	K	—	*O.g.*	0·18	0·22	32·2	34·2	2·84
T_6	—	—	—	—	*O.g.*	0·15	0·21	29·7	18·4	1·45
T_7	—	—	—	—	—	0·18	0·20	27·8	0	0
S.E. of treatment means						0·016	0·020	0·47	5·22	0·479

[1] 0·01 g N, 0·033 g P, 0·069 g K/100 g of soil using combinations of $(NH_4)_2HPO_4$, $K_3PO_4.H_2O$, K_2HPO_4, KH_2PO_4, KNO_3.
[2] 0·001 g/100 g soil of 'N-Serve' (2-chloro-6(trichloromethyl) pyridine, Dow Chemical Co) a nitrification inhibitor.
[3] Organic debris infested with *O. graminis* extracted from similar soil at twice the rate of natural occurrence.
[4] Root discoloration, 0 (none) to 5.

Serological identification of *O. graminis*. The antiserum prepared last year from fungus extracts reacted positively with fungi other than *O. graminis*, probably because of the many injections needed to produce a usable titre of antibodies. However, we now have better methods of preparation that may produce an antiserum specific to *O. graminis*. (Govier and Hornby)

Effect of aureofungin on *O. graminis* in vitro. The antibiotic aureofungin (Hindustan Antibiotics Ltd., Pimpri, Poona, India) is reported to be active against many fungi and to be absorbed and translocated by leaves and roots. Growth of two isolates of *O. graminis* was inhibited at 40 μg/ml and retarded at 20 μg/ml but smaller concentrations had no more effect than could be attributed to the solvent (dimethylformamide). Lack of inhibition of *O. graminis* at recommended concentrations might be attributable to the difficulty of completely dissolving our sample in any of several solvents. (Hornby)

Microbiology of *O. graminis*. Work in collaboration with the Soil Microbiology Department is described on p. 98. (Hornby, with M. E. Brown)

Dual infection of wheat by *O. graminis* and *Heterodera avenae*. Work in collaboration with the Nematology Department is described on p. 197. (Hornby with T. G. Williams)

Eyespot on dwarf wheat. The dwarf wheat Gaines (see p. 108) had 37% of its straws infected with the eyespot fungus (25% moderate and severe) whereas Cappelle had only 14% (5% moderate and severe). Eyespot also caused more whiteheads on Gaines but there was less lodging. (Prew)

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Residual effects of soil fumigation on cereals

Wheat. Soil fumigation experiments, begun on Little Knott and Pastures in 1965 and on Claycroft in 1967, ended by growing Cappelle winter wheat to measure residual effects. On Little Knott, areas respectively with and without formalin in October 1967 yielded 26·6 and 30·8 cwt/acre of grain and had 24 and 17% of straws with take-all at harvest. On Pastures and Claycroft there was no measurable effect from formalin, 'D-D', or dazomet applied in previous years.

Rye. In the Woburn Ley-Arable Experiment rye yields were increased by chloropicrin applied for potatoes in 1968 (*Rothamsted Report for 1968*, Part 1, 150 and this report pp. 171 and 191). The increase was largest (32%) on the continuous arable (C) series where take-all was decreased from 75 to 23% of straws at harvest. Not all the benefit can be attributed to control of take-all because there was none in the other series, where previous chloropicrin increased yield by 12% on the ley-arable and 9% on the continuous arable (H) series. Although rye is not important locally, the benefits exemplify how fumigants given for one crop can aid others in a rotation. They also contrast with the detrimental effects usual where cereals follow cereals on fumigated land. (Salt)

Diseases of field beans (*Vicia faba L.*)

Wilting. A pythiaceous fungus isolated from beans with a black spongy root rot (*Rothamsted Report for 1968*, Part 1, 141) failed to reproduce the symptoms in pots, either when a maizemeal–sand culture was added to Kettering loam or an agar culture was applied to wounds in the stem cortex at soil level. (Hornby)

The wilt described last year was widespread in the third consecutive crop of beans on part of Barnfield. It appeared on 19 June 1969 before any wilt or black rot was seen elsewhere at Rothamsted or Woburn. The symptoms somewhat resembled those caused by broad bean wilt virus, but attempts to transmit the virus from them failed. Nor were they caused by simazine because dead and discoloured roots were common on all Barnfield plots.

Beans grown in pots containing Barnfield soil, with or without chopped bean stems and roots, remained healthy where the soil and plant debris had been steamed, but developed root rot where unsterilised plant debris was mixed with steamed soil and where the soil had not been steamed. (Hornby, Salt and Phillips)

Soil fumigation and nitrogen treatments. On light land at Woburn beans wilted through drought and not root rot. Discoloration of roots was much less than on Barnfield and was unaffected by soil fumigation but yields were increased both by fumigating and applying nitrogen (see p. 294). (Hornby and Salt with J. McEwen, Field Experiments Section)

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Potato diseases

Survey of fungal diseases of seed tubers. There was more blight and powdery scab and less common scab than average in King Edward seed tubers produced in 1968 (Table 14). Pentland Crown grown in 1967 and 1968 had less skin spot, blight, common scab and powdery scab than other varieties examined but more gangrene and black scurf. (Hide and Griffith)

TABLE 14

Survey of fungal diseases of seed tubers, 1968–69

(% tubers infected / % stocks with infected tubers)

Examined	Disease	King Edward	Majestic	Pentland Crown
R	Skin spot (*Oospora pustulans*)	51/100	44/100	35/100
P	Gangrene (*Phoma* spp)	12/88	10/76	12/83
P	Dry rot (*Fusarium caeruleum*)	1/47	5/64	3/53
R	Blight (*Phytophthora infestans*)	2/57	1/19	1/20
R	Black scurf (*Rhizoctonia solani*)	24/100	27/96	29/100
R	Powdery scab (*Spongospora subterranea*)	21/88	17/69	2/43
R	Common scab (*Streptomyces scabies*)	18/96	36/98	15/100
Number of stocks examined		49	48	30

R = at receipt P = at planting

Prevalence of gangrene and yield. Field experiments on the effects of gangrene present many problems, not the least of which are the difficulties of finding suitable seed stocks and the time it takes for lesions to appear. Comparing yields from seed of individual stocks graded into different severities of gangrene (*Rothamsted Report for 1968*, Part 1, 142) does not quickly show the least proportion of gangrene-infected tubers that causes measurable loss. In 1967 and 1968 we planted identical stocks ranging in gangrene incidence from 0 to 90% on the Experimental Husbandry Farms at Terrington St. Clement, Norfolk, and Mepal, Cambs.

In 1967, different stocks were collected and planted in replicated pairs of plots either without selection or after removing all tubers showing gangrene lesions. At both sites yields of unselected stocks were only significantly less when more than half the tubers bore lesions. Regression analysis of per cent gangrene in the original stocks on yield showed that at Terrington and Mepal, respectively, stocks with 90% of tubers showing gangrene yielded 74 and 62% of stocks with only 10% of seed tubers infected. Surprisingly there were still decreases in yield after the removal of all tubers showing lesions from stocks that initially had 90% of gangrene, respectively, to 86% at Terrington and 79% at Mepal. We cannot tell whether the smaller yield from seed of stocks that originally showed much gangrene resulted from cryptic infection of growing plants by *Phoma exigua* var. *foveata* or from diseases, such as blackleg, that tend to be

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associated with gangrene. It is improbable that it was caused by using smaller seed tubers after rejecting all those showing gangrene, because the weight of seed planted in each treatment did not show the reverse trend to yield. Eight of 11 stocks were common to both sites. After storing the harvested tubers for 6 months the produce from lesion-free seed had almost as much gangrene as the produce from unselected seed, respectively averaging 34·7 and 37·2% of tubers at Terrington. At Mepal this was also true but the comparable figures (6·8 and 7·2%) show that much less gangrene developed there although we do not know whether this resulted from differences in growth, handling or storage. Although the proportion of seed tubers with lesions ranged from 10 to 90%, infection of their produce increased only about twofold over the same range.

In 1968 we could not obtain a suitable range of diseased stocks and so from one infected stock we constituted stocks with from 0 to 90% of tubers with gangrene lesions of various severity. Errors of yield estimates were large but it again seems unlikely that total yield was significantly decreased unless more than half the tubers had lesions. Unfortunately this experiment could not provide further information on possible yield effects of latent gangrene but it showed that widely different amounts of visible gangrene on seed did not significantly affect the percentage of produce infected when all the seed came from the same stock. After 3 months storage only 2·9% of tubers were infected from either farm but following uniform wounding and a further 3 months at 5°C this increased to 29·8% from Terrington and 32·5% from Mepal.

These experiments suggest that seed with 90% tubers infected seldom decreased plant populations by more than 15% and that more than half the seed had to bear lesions before yield was measurably decreased. Furthermore, rejecting lesion-bearing tubers did not make the produce any better or fully restore the yield of the remaining seed. (Griffith)

Gangrene infection and date of lifting. In 1966 and 1967, progeny tubers from gangrene infected seed were dug at intervals between mid-July and mid-October, then uniformly wounded and stored at 5°C, to develop gangrene. This showed that the fungus was always present but increased greatly during August and September and, in 1967, sooner on King Edward and Red Craigs Royal than on Majestic or Pentland Crown. Almost all tubers dug in mid-October became infected. In 1968, this was repeated, but the lifted tubers were washed and surface sterilised (3% hypochlorite) before they were wounded and stored at 5°C until February. Few tubers (max. 11%) became infected and none of those lifted before mid-August. In contrast soil taken from the tubers at lifting, air-dried and inoculated to Arran Banner test tubers in February was always infective, but much more so from lifting after mid-August than before. Averaging eight occasions, soil from Red Craigs Royal was most infective (100%) compared to King Edward 63%, Majestic 58% and Pentland Crown 42%. Skin parings taken in February from unwounded areas of surface-sterilised tubers of the four varieties failed to infect Arran Banner tubers confirming the paucity of inoculum suggested by the wound test. Thus, the results confirmed that the amount of fungus increases as the crops mature. They

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also suggest that until mid-October most of it is in soil or so superficially in the tubers that it can be killed by surface sterilants. (Griffith)

Pathogen, wound type, temperature and gangrene infection. The different extents to which gangrene develops is no doubt explained largely by differences in damage to tubers and whether storage conditions allow the fungus present on (or in) tubers to produce lesions. However differing abilities of varieties of *Phoma exigua* to infect different kinds of wounds could also be a factor. Hence, equal numbers of spores of *P. exigua* var. *foveata* or *P.e.* var. *exigua* (Syn *P. foveata* and *P. solanicola*) and of a half-strength mixture of each were placed on intact skin or various wounds in King Edward tubers. The mixture behaved as though it had contained only *P.e.* var. *foveata* which usually was the only one that could later be isolated. Table 15 shows results with the two varieties separately. Neither infected through intact skin. *P.e.* var. *foveata* caused more and larger lesions and its greater ability to colonise shallower and cleaner wounds together with its dominance over *P.e.* var. *exigua*, may help explain why it is much the more prevalent pathogen.

TABLE 15

Wound type and the pathogenicity of two varieties of Phoma exigua

(King Edward: March–May 1968)

Wound type	*P.e.* var. *foveata*		*P.e.* var. *exigua*	
	% wounds infected	% area[1] of lesion	% wounds infected	% area[1] of lesion
Crushed hole (6 mm diameter, 10 mm deep)	100	36	100	14
Clean cut hole (6 mm diameter, 10 mm deep)	98	28	34	4
Crushed hole (4 mm diameter, 5 mm deep)	98	22	6	1·5
Superficial scuffing	34	6	4	1·0
Unwounded tuber eye	0	0	0	0

[1] The proportion of tuber cross section, through infection point, that was rotted.

In 1968 and 1969 controlled temperature and humidity cabinets at the Potato Marketing Board Experimental Station at Sutton Bridge, were used in infection tests in which *P.e.* var. *foveata* spores were placed in various kinds of wounds (Table 16). Fewer infections occurred through cuts than crushes, and fewer through shallow than deep wounds. Microscopy of wounds showed that complete wound periderms formed quickly only beneath 'cut' wounds at 12°C. When colder or beneath crush wounds the periderm was incomplete and it formed deeper beneath crushes than cuts. Contrary to widely accepted opinion very humid air increased infection, but the tests were with artificially inoculated wounds and need repeating with natural inocula.

In a more practical test of temperature 'curing', lesion-free progeny tubers of infected seed were slightly or severely crush wounded. Slight, V-shaped crush wounds (12·5 mm long by 4 mm deep) produced few lesions on 'cured' or 'uncured' tubers stored for 12 weeks at 5°C after wounding. On average, 54% of uncured King Edward and Majestic tubers became

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TABLE 16

Effect of wound type, temperature and relative humidity on gangrene infection, 1968 and 1969*

Temperature °C	Relative Humidity %	Wound type: Severe crush[1]	Slight crush[2]	Deep cut[3]	Shallow cut[4]
		(% wounds infected)			
2	95	100	98	34	32
	85*	100	98	29	20
	75	100	86	14	2
6	95	98	98	6	2
	85*	100	70	2	7
	75	98	58	6	0
8	95	98	80	2	2
	85*	100	61	0	2
	75	90	34	4	0
12	95	98	65	0	0
	85*	100	16	0	0
	75	38	22	2	0

* Tests at 85% RH were in 1968 on King Edward tubers using 6×10^3 spores/wound. Other tests were in 1969 using Arran Banner tubers and 4×10^3 spores/wound.

1968	1969
[1] U shaped 5 mm deep 1·5 cm long.	Crushed hole 6 mm deep 4 mm wide.
[2] V shaped 5 mm deep 1·5 cm long.	Crushed hole 3 mm deep 4 mm wide.
[3] U shaped 5 mm deep 1·5 cm long.	Cut hole 6 mm deep 4 mm wide.
[4] V shaped 5 mm deep 1·5 cm long.	Cut hole 3 mm deep 4 mm wide.

infected on severe crushes (approximately a half-hemisphere depression 17·5 mm long and 6·0 mm deep), compared with 31 and 25% of tubers 'cured' by keeping at 10°C for 1 or 2 weeks respectively before storage at 5°C for 12 weeks. Comparable figures for 'curing' at 15°C were 31 and 13% and, at 20°C, 21 and 7%. These results support those in Table 15 (12°C) that temperature curing may not be fully effective against deep crushes even by keeping at temperatures costly to maintain. (Griffith)

***Verticillium dahliae* and nematodes.** Further work on the interactions between these parasites on potato crops is described on p. 198. (Hide, with D. C. M. Corbett, Nematology Department)

Fungicidal control of tuber diseases. Last year (*Rothamsted Report for 1968*, Part 1, 144 and see reference 7.10, p. 389) we reported on two benzimidazoles that act both during storage and crop growth as alternatives to the organo-mercurial disinfectant fungicides. Unfortunately the seed stock used had too little gangrene to test their value against this disease, so an organo mercurial fungicide was compared with thiabendazole and benomyl on stored tubers (see reference 7.10, p. 389). Thiabendazole was as good or better than the organo-mercurial but benomyl was a little less effective. Dipping was perhaps more effective than dusting but a 1% thiabendazole (lactate formulation) dip killed many eyes.

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TABLE 17

Effect of fungicides on yield and diseases of King Edward

(Rothamsted 1969: Once-grown seed)

Treatment	Time	Yield, (ton/acre > 1¾ in.)	% tuber eyes infected		
			Oospora pustulans	*Rhizoctonia solani*	*Helminthosporium solani*
Untreated		13·18	27	31	31
Agallol dip	Lifting	11·01	15	13	6
Dusted (% a.i. in kaolin 10 lb/ton)					
Nil	Planting	10·44	25	20	29
Benomyl					
1%	Dormant	11·70	14	2	11
10%	Dormant	11·17	4	6	3
30%	Dormant	9·95	4	1	0
1%	Planting	9·88	6	18	5
10%	Planting	9·61	3	9	1
30%	Planting	7·93	1	0	1
Thiabendazole					
0·1%	Dormant	11·25	21	23	36
1·0%	Dormant	11·45	10	14	8
10·0%	Dormant	11·65	5	1	1
0·1%	Planting	9·82	16	13	34
1·0%	Planting	9·17	4	12	4
10·0%	Planting	6·72	7	3	1
		±0·524	±2·6	±5·4	±3·8

Thiabendazole and benomyl were applied at various times and in different amounts to tubers planted in a field experiment (Table 17). Unlike 1968, all fungicides decreased yield, especially the large amounts used to see whether they would have harmful effects. Treatment at planting decreased yield more than treating dormant tubers in late January, but treating only with the inert carrier (kaolin) showed that much of this damage was attributable to removal of sprouts during dusting. Other than yielding less, the plants showed no symptoms. The experiment was intentionally planted with a seed stock on which blemishing pathogens were common. *Oospora pustulans* and *Helminthosporium solani* were again controlled well but *Rhizoctonia solani* less well than in 1968. These were controlled better the more fungicide was used, but only amounts that affected yield approached complete control. Less fungicide may be adequate on healthier seed stocks. For example, in Perthshire the incidence of *O. pustulans*, *R. solani*, and *H. solani* on seed initially only lightly infected, was decreased from, respectively 39, 51 and 16% of eye plugs infected on untreated tubers to 1, 39 and 1% with 1% benomyl (in 10 lb of kaolin/acre) and to 1, 40 and 4% with the same amount of thiabendazole, each applied as dusts to seed tubers.

Only further experiments can decide whether these fungicides will usually increase or decrease yield but because a main objective of their use is to improve or maintain the health of high grade seed stocks their cost may be justified even without increased yield. (Hide, Hirst, Griffith and Stedman)

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Agronomic effects of healthier seed potatoes. The experiments where we first measured the effects of tuber-borne fungi (*Rothamsted Report for 1968*, Part 1, 142) used tubers graded according to the severity of macroscopic symptoms but often all were infected microscopically. These experiments could not test the full effects of individual pathogens or measure interactions between the several pathogens that occurred together. Multiplying progenies of stem cuttings has provided healthier seed of several varieties, and this was compared with commercial stocks with and without benomyl (10% a.i. in 10 lb of kaolin/ton of tubers). Also tested were the effects of chitting on healthy and diseased seed and of introducing *O. pustulans* and *R. solani* separately and together.

Yields of the varieties Pentland Crown, Pentland Dell, King Edward and Majestic in similar experiments at Rothamsted and Woburn were increased more by chitting than by any other treatment and the yield of the healthier seed was increased more than that of the once grown. Healthier seed of some varieties (especially Pentland Crown) yielded more than once-grown seed but with King Edward and Majestic this difference was small or even reversed. Until pathological analyses are complete the effects of the fungicide or fungi cannot be explained, but neither significantly affected yields. The healthier King Edward stock often produced more and smaller tubers, so that pathogens such as *O. pustulans* and *R. solani*, which make tubers fewer sometimes increased ware yield, whereas fungicides had the opposite effect. Other varieties behaved differently and responses differed between the two sites so it would be unwise to draw conclusions until more information is available. (Hide, Hirst, Stedman and Griffith)

Common scab (*Streptomyces scabies*)

Tuber development and the duration of susceptibility. At Sutton Bonington the scab-infested soil under glass (*Rothamsted Report for 1967*, 135) was again used to measure the scab resulting from similar periods of drying soil at different stages of tuber development. Plants (Majestic, chitted and planted 30 April) were trickle irrigated so that soil moisture tension did not exceed 15 cm Hg except during consecutive periods (approximately 7 days) when irrigation was stopped on different treatments (Table 18). Drying the soil again resulted in discrete bands of

TABLE 18

Dry periods, tuber growth and common scab: Glasshouse, Sutton Bonington, 1969

	Approx. 7 day dry period from: May 28	June 4	June 11	June 18	June 25	July 2	July 9	July 16	No dry period
Scab %[1]	2·6	8·5	14·3	8·6	3·7	3·6	2·2	1·6	1·8
Zone infected[2]	0–3	0–3	1–5	3–8	5–9	5–10	7–13	8–12	—

[1] Mean scores (4 replicates of 50 tubers) of % surface area scabbed, after lifting (4 Sept.) S.E. for 'No dry period' and 28 May to 25 June, ± 1·34. S.E. for 2 to 16 July, ± 0·68.
[2] Mean zone to the nearest eye (node) along the phyllotactic spiral from the stolon attachment = 0.

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scab infection. Because nine treatments were needed and the site only accommodated six with the required plot area, the three first exposed to drying (28 May, 4 and 11 June) were exposed to second dry periods (respectively from 2, 9 and 16 July). These treatments had two distinct infection bands which were assessed separately, after lifting on 4 September when all assessments were made.

Continuous irrigation prevented scab developing on all but a few tubers, probably where trickle nozzles had been temporarily blocked. Tuber initiation started between 28 May and 4 June and the areas scabbed were greatest during the first half of June, especially during the dry period that began on 11 June. The areas scabbed can be expressed in terms of internodes by following the phyllotaxis of eyes (nodes) spiralling around the tuber. The lower half of Table 18 shows the nodes (from the stolon attachment) that limited each infection band. Dry periods occurring later during tuber growth allowed infection progressively further from the stolon. Most dry periods encouraged infection of four or five internodes but, because later internodes expanded little, infection of the earlier internodes accounted for most of the area scabbed.

A similar experiment on the same site in 1968 gave less clear results because soil moisture tension was kept below 10 cm Hg and with predominantly dull weather the soil remained too wet, during the 7-day periods without irrigation for many scab infections. However on the same dates the area scabbed (per cent) showed a similar trend to that in 1969 (1·8, 0·9, 5·1, 2·0, 0·4, 0·8, 0·3, 0·4 and 0·5 (continuously irrigated)) with most scab from drying started on 11 June. (Lapwood with Dr. T. F. Hering, University of Nottingham School of Agriculture)

Irrigation practice for scab control. To prevent scab, irrigation needs to start earlier in the season than is usually needed for maximum yield. However, there would be little profit in irrigating to increase the yield of potatoes already severely scabbed. Four potato varieties were grown in different irrigation regimes to find the most profitable ones for decreasing scab and increasing yield. The experiment, in conjunction with National Agricultural Advisory Service was at Gleadthorpe Experimental Husbandry Farm, near Mansfield, in the sandland area of Nottinghamshire, where scab is a problem and irrigation is frequently used for potatoes.

Only the effects on common scab are described in Table 19. Two regimes (A and B) were intended primarily to control scab, two (D and E) to increase yield. There was also a compromise regime (C) and one without irrigation (F). The watering regimes started when the first tubers began to form on Majestic plants from chitted seed. Soil moisture deficits were calculated from local meteorological data.

Tubers began to form in mid-June, and a week later wet weather gave way to a dry spell lasting to late July. Technical difficulties delayed the first irrigation but even so, at lifting (Table 19) there was little infection of the more susceptible variety Majestic, under regimes A and B. Regime C protected tubers much better than did D, E or F, although early rain had made it unnecessary to irrigate to field capacity. Chitting slightly advanced the date of tuber formation on Majestic, so this year produce of

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TABLE 19

Scab control on main crop potatoes by irrigation: Gleadthorpe EHF, 1969

Irrigation regimes[1]:	A	B	C	D	E	F
No. of irrigations:	6	3	5	2	1	0
	mean % tuber surface scabbed[2]					
Variety:						
Majestic (chitted seed)	1·6	3·8	2·5	8·1	16·4	25·9
Majestic (unchitted seed)	2·4	3·4	6·9	12·1	29·6	30·3
King Edward	0·6	0·7	1·5	3·0	9·5	8·4
Record	0·8	1·0	1·1	2·7	5·5	8·8
Pentland Crown	0·05	0·04	0·3	0·3	0·4	1·3

[1] A – 0·6 in. soil moisture deficit before irrigation allowed for 6 weeks after tuber initiation of MJ (CH), and then 1·5 in. deficit.
B – 0·6 in. deficit for 3 weeks and then 1·5 in. deficit.
C – Irrigation to field capacity at 20% crop cover and then at 0·8 in. deficit.
D – Irrigation when 1·5 in. deficit.
E – Irrigation when 2·25 in. deficit.
F – No irrigation to supplement rain.

[2] S.E. between irrigation regimes ±1·89.
S.E. between varieties ±1·28.

unchitted seed tended to be formed in drier soil and to be infected more under regimes C to F. Regimes A to D gave valuable control on the moderately resistant King Edward and Record. Pentland Crown is so resistant that the irrigation given was unnecessary to control scab. (Lapwood, Mr. W. R. Rosser, National Agricultural Advisory Service, Shardlow and Mr. L. W. Wellings, Experimental Husbandry Farm, Gleadthorpe)

Bacterial soft rot. Work on these rots, caused mostly by *Erwinia carotovora* var. *atroseptica* and *E.c.* var. *carotovora*, began with two small field experiments, one with irrigation and the other without, to test how organism, potato variety and inoculation affected blackleg, seed tuber disintegration and contamination of the produce. Some of the Virus Tested seed tubers (derived from stem cuttings) of Majestic and Pentland Crown were chitted, some not and some stab inoculated with either bacterium (or none), at the middle, rose or heel ends of tubers a day before planting on 2 May.

In mid-July all the seed tubers inoculated with *E.c.* var. *atroseptica* had disintegrated, whereas only 10% of those inoculated with *E.c.* var. *carotovora* had and only 3% of tubers stabbed but not inoculated with bacteria. At the end of August, on the unirrigated and irrigated experiments respectively, 57 and 93% of Majestic and 26 and 50% of Pentland Crown tubers inoculated with *E.c.* var. *carotovora* had disintegrated, as also had, respectively, 78 and 69% of Majestic and 26 and 35% of Pentland Crown tubers stabbed but not inoculated. At the final harvest in mid-October, only a few Majestic seed tubers survived, mostly in the unirrigated experiment. A very few Pentland Crown seed tubers survived in the irrigated experiment, but more in the unirrigated one, 13% of those stabbed only and 8% of those inoculated with *E.c.* var. *carotovora*.

Inoculating Majestic tubers with *E.c.* var. *atroseptica* produced most plants with blackleg when chitted seed was inoculated at the rose end

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(24%); inoculating chitted seed at the middle or inoculating unchitted seed gave from 8–14% infected plants. Unchitted seed of Pentland Crown produced blackleg stems (4%) only when inoculated at the rose end; chitted seed gave up to 10% of plants with blackleg. Some shoots that developed curled and yellowed leaves late in June recovered to produce healthy shoots in July, which was dry. Blackleg did not show in plants from tubers inoculated with *E.c.* var. *carotovora* or from tubers stabbed but not inoculated. (Lapwood and Martin)

Effect of soil fumigant and nematicide on the Woburn Ley-Arable Experiment. Chloropicrin, which gave unexplained increases in the yield of potatoes last year, was used again, on different plots, with and without the nematicide 'Temik'. Table 20 shows that chloropicrin (400 lb/acre injected) and 'Temik' (rotavated-in at 100 lb/acre of 10% granules just before planting), increased potato yield, and more so together than when separate. As in 1968, chloropicrin was most beneficial in the two continuous arable series; benefits from 'Temik' were smaller but consistent in both the continuous arable and the alternate ley-arable series.

TABLE 20

Effect of chloropicrin on potato yields: Woburn Ley-Arable Experiment 1969

Total yield (ton/acre)

Treatment	None	Temik	Chloropicrin	Temik & Chloropicrin
Rotation				
Ley-arable[1]	17·93	20·68	18·97	22·44
Sainfoin-arable[1]	18·61	20·34	22·72	23·66
Continuous arable (H)[2]	13·81	16·98	20·93	22·05
Continuous arable (C)[2]	13·75	16·48	17·56	18·75
Mean	16·03	18·62	20·04	21·73

[1] Potatoes every 10th year.
[2] Potatoes every 5th year, and 1 year hay (H) or carrots (C) every 10th year.

Once again the differences in yield could not be explained by the occurrence of fungi on roots in May or August. *Verticillium* spp. were scarce, *Oospora pustulans* was ubiquitous and *Rhizoctonia solani* commoner after fumigation. Other fungi isolated from roots in May included a fine sterile mycelium (from 27% of roots) *Mortierella* (14%), *Pythium* (9%) and occasional *Phoma*, *Fusarium* and *Cylindrocarpon*; in August, *Fusarium* (22%) was most common with *Cephalosporium* (13%), *Pythium* (12%) and *Cylindrocarpon* (9%). Temik had less effect than chloropicrin on fungi in the roots, and the mixture had more effect than either alone. Only *Fusarium*, mostly *F. avenaceum*, seemed affected by crop sequence; untreated plots on the ley and sainfoin-arable sequences had 14 and 16% whereas there were 33 and 27% in the H and C arable sequences. *Pythium ultimum* developed profusely in roots of tomato and potato plants grown in the glasshouse in soil from the plots but was equally abundant in both fumigated and unfumigated soils from both ley-arable and continuous arable sequences. (Salt, and see also Nematology Dept. Report, p. 191)

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Biodeterioration

We began to study the moulding of stored products because our experience in agriculture, microbiology and aerobiology had fitted us to integrate diverse techniques. Spoilage by moulding is important not only by the losses it causes, but also because it can cause respiratory diseases in man and animals known collectively as allergic alveolitis. Fine dusts that penetrate deeply into the lungs are responsible and can be of various types, but those that most interest us are the small spores of fungi and actinomycetes. New machinery has often aggravated these problems, both because rougher handling disperses more spores and, for example, because it allows hay to be baled wetter than previously and so encourages moulding. The introduction of new machines or techniques often needs studies not only of how they affect the harvested produce but also of possible health hazards to workers and livestock.

Deterioration of tropical products

Sugar cane bagasse. Last year's Report (p. 133) gave preliminary results from examining mouldy bagasse (the waste cane fibre after extracting sugar), which is associated with the respiratory disease, bagassosis. Economic factors in Trinidad make it increasingly important to use bagasse to make hard particle board, but moulding threatened to prevent it both by rotting the fibres and by making bagassosis prevalent among the workers. However, our results suggest that moulding can be prevented chemically and produce better quality particle board without risk to the workers.

Fresh green bagasse baled and stacked in Trinidad heated spontaneously to 54°C after 5 days, then cooled to 40°C before heating again to 49°C after 33 days when temperature began declining slowly to near ambient. Bagasse, dried to 27% water content, heated to 49°C within 3 days but then cooled to ambient without secondary heating. However, bagasse treated with propionic acid (British patent application No. 49162/68) neither heated nor lost sugar during 3 months' storage, whether it was dried or not. Untreated bagasse produced 50×10^6 spores/g, mostly actinomycetes and bacteria, without drying and 62×10^6 spores/g, mostly fungi (particularly *Paecilomyces varioti*) when pre-dried. Wet or dry bagasse treated with propionic acid yielded very few organisms.

During trial manufacture of particle board in a German factory, from bagasse treated with propionic acid up to $2{\cdot}3 \times 10^6$ spores/m^3 of air were caught along most of the production line, and up to $15{\cdot}4 \times 10^6$ spores/m^3 at the mattress-forming machine. The fact that a bagassosis sufferer felt no ill effects, although often covered with dust, is explained by the very few actinomycetes. The most common spores were fungi, especially *P. varioti*, and may well have grown after accidental wetting of the bagasse in Germany. (Lacey)

An unusual species of *Nocardia* is widespread and sometimes abundant in bagasse. It produces sparse white aerial mycelium with bead-like chains of hairy-walled spores, grows at 25 and 40°C and sometimes produces yellow soluble pigment. Two isolates that were tested decomposed casein,

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xanthine, hypoxanthine and tyrosine, but did not produce acid from inositol. Whole-cell hydrolysates contained the meso-form of diaminopimelic acid, arabinose and galactose. The isolates thus have a cell-sugar pattern characteristic of the *N. asteroides* group (Lechevalier, M. P., *J. Lab. Clin. Med.* (1968), **71**, 934) but differ from the other species placed in this group in other features. (Lacey and Carpenter)

Cocoa. Samples of fermented cocoa beans from Trinidad yielded actinomycetes and bacteria (up to 31×10^6 spores/g) and fungi (up to 9×10^6 spores/g). All samples produced *Thermoactinomyces vulgaris* and half produced *Streptomyces griseus* but none was prolific. *Thermomonospora viridis*, *Streptomyces albus* and *Micropolyspora faeni* were occasional, as were *Penicillium* spp., the *Aspergillus glaucus* group (mostly *A. chevalieri* and *A. restrictus*), *A. fumigatus*, *A. niger* and *Paecilomyces varioti* among the fungi. In a few samples *Absidia* spp., *Aspergillus ochraceus*, *A. candidus*, *Dactylomyces crustaceus* and *Scopulariopsis brevicaulis* were moderately abundant.

Coffee. *Aspergillus niger* and *Penicillium* spp. were abundant in half the samples of coffee and dust from coffee, as occasionally were *Streptomyces* spp. *Thermoactinomyces vulgaris* was present but infrequent in all samples.

Copra. Only one sample was examined in which *Aspergillus niger* and *Streptomyces albus* were abundant together with fewer *Penicillium* spp., *A. fumigatus* and yeasts. (Lacey)

Control of moulding in damp hay. In Dewar flask tests (*Rothamsted Report for 1968*, Part 1, 132) up to 2% 'Hay Guard' (Bray Developments Company, Sherborne, Dorset) had no effect on heating or moulding of hay containing between 20 and 40% water.

To design effective yet economical ways of applying propionic acid during hay baling, it is necessary to find whether it inhibits moulding at a distance from deposits. Dewar flasks were therefore loaded with three zones of damp hay. When only the middle zone was treated with 2% by weight of propionic acid, it was invaded by moulds from above and below. By contrast, there was no moulding when all zones were treated uniformly with only 1% of acid. When only the middle zone was treated with 4% of acid there was little moulding of this zone except for a profuse growth of *Paecilomyces varioti* hyphae adjacent to the untreated layers. Only adding 10% of acid to the middle layer prevented moulding for a distance of about 5 cm into untreated hay and again *P. varioti* seemed the most tolerant mould. It therefore seems necessary to apply the acid uniformly, possibly even adding some water as a diluent to achieve this. (Lacey and Hill)

Methods of examining mouldy hay. Gregory and Lacey (M.E.) (*J. gen. Microbiol.* (1969), **30**, 75–88) found, but did not explain, large differences between estimates of the number of spores released from mouldy hay as

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estimated by the cascade impactor and Andersen sampler. Tests of six alternative media for fungi and 13 for actinomycetes produced none better than the present standard media, respectively, 2% malt extract agar with streptomycin and penicillin, or half-strength 'Oxoid' nutrient agar and actidione.

Discrepancies in estimating the number of spores on hay samples were also examined by comparing cascade impactor and Andersen sampler catches of spores blown-off in the wind tunnel, with haemocytometer and dilution-plate estimates of spores removed by washing. Cascade impactor estimates averaged 19% of those from the haemocytometer and cultural methods such as the Andersen sampler and dilution-plating recorded only a quarter of the fungus spores seen in visual counts from the cascade impactor or haemocytometer. Discrepancies in estimating actinomycetes and bacteria were even greater; sampling airborne spores with the Andersen sampler favoured actinomycetes whereas dilution plating of washings estimated more bacteria. Other errors resulted from clumping of airborne spores and deposition on the inside walls of the Andersen sampler. (Lacey and Dutkiewicz)

Aspergillus flavus. Some strains of this fungus are important because they produce aflatoxins, so it is important to know how they multiply and disperse. More spores were produced on malt extract agar than on nine other culture media. Hay was the most prolific natural substrate found, followed by wheat straw and then wheat grain. Straw with 50–60% moisture grew many more spores than when wetter or drier.

Spore release was encouraged when the substrate was dry and air movement fast. However, the number released from undisturbed hay or straw was small compared with the number released by vigorous shaking, yet even this detached only 10–33% of the total. Terminal velocity of fall in air was estimated from the rate of decline of spore concentrations within a settling chamber, measured by periodic samples with a cascade impactor. Single spores fell at 0·07 cm/sec, pairs at 0·08 cm/sec and clumps of three or more at 0·09 cm/sec. (Ramalingam)

Air pollutants at Woburn. Last year we questioned whether air pollutants were partially responsible for the premature death of potatoes at Woburn which happened between mid-July and mid-August. Analysis of air samples taken there showed that the daily average sulphur dioxide concentrations exceeded 100 $\mu g/m^3$ on 13 of the 17 days between 24 July and 9 August, with the largest, of 727 $\mu g/m^3$, on 8 August. Potatoes can be damaged by SO_2, as can barley, lucerne and hawthorn. During the same period very extensive necrosis of hawthorn foliage was noticed on the Woburn farm.

During a brief visit in July 1969, Ir. A. van Raay of the Institute of Phytopathological Research, Wageningen, diagnosed damage from both SO_2 and fluorine in the Woburn area, although there had been unusually few occasions when pollution from the local brickfield was noticeable. Fluorine analyses in 1968 showed 20–30 ppm in needles of conifer seedlings and 270–390 ppm F in foliage of artichokes (in 1969 170 ppm in leaf and

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30 ppm in stems, weighted mean 120 ppm). Collections of fog droplets had up to 20 ppm F and 150 ppm sulphur.

These observations were made to assess the need for research on damage to crops by air pollutants, and suggest there may be, as it now receives little attention. (Hirst, with G. V. Dyke, Field Experiments Section, R. J. B. Williams, Chemistry Department and Mr. A. C. Chamberlain, A.E.R.E., Harwell)

Staff and visiting workers

A. J. Gibbs continued to work in Australia and J. Waller in Kenya. R. H. Kenten returned from Ghana. G. A. Hide was awarded the Ph.D. degree of the University of London. E. W. Broom, Sara M. Cook, N. F. Martin, R. D. Prew and Doreen A. Vince were appointed and Judith Etheridge and Margaret Pullen left.

Visiting workers included Miss U. Allitt (Botany School, Cambridge); Dr. E. D. Ammar (Cairo University); Dr. J. Dutkiewicz (Witold Chodzko Institute of Occupational Medicine and Rural Hygiene, Lublin, Poland); Dr. T. D. C. Grace (C.S.I.R.O., Canberra); Mr. M. M. Hussein (Sudan); Dr. A. Ramalingam (University of Mysore, India); Dr. H. S. Sahambi (Indian Agricultural Research Institute, New Delhi); Mr. B. Srinivasan (Centre for advanced study in Botany, Madras); Mr. H. Zengin (Ministry of Agriculture, Turkey). During 1969, R. A. Hill (Reading University) and R. H. Turner (Liverpool College of Technology) worked in the department as 'sandwich course students'.

In September, A. Kleczkowski attended a NATO International Advanced Study Institute on 'Photodynamic action' in Alghero, Sardinia, Italy. J. Lacey visited the Bähre Metallwerk K.G. Springe, Germany, on behalf of the Trinidad Government to sample air-borne spores during the manufacture of particle board. P. H. Gregory visited several countries in West Africa, America and the West Indies to study black pod disease of cocoa.

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