ROTHAMSTED RESEARCH

Report for 1972 - Part 1

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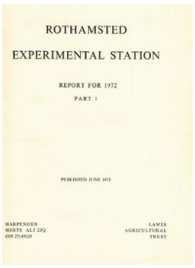

Plant Pathology Department

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PLANT PATHOLOGY DEPARTMENT

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Progress during 1972

Although little of our work escaped the inconvenience of building work and temporary accommodation, the year was an interesting one. We had the unusual combination of a mild, wet winter followed by an unusually dry and rather cool summer. Consequently virus vectors were late and less common than at one time seemed likely. Fungus diseases of cereal leaves were certainly not so inhibited and although mildew was slow to start, it developed quickly. As we expected yellow rust was serious, especially on the small acreage of Joss Cambier wheat that we were committed to grow.

Arranging this report for a varied readership of specialists, advisors and farmers is always difficult. This introductory section is an innovation which mentions scientific and agricultural findings of special interest and changes of emphasis in our programme, which are amplified in the later scientific and 'Crop' sections.

Viruses and virus diseases. Recently acquired apparatus and new techniques are helping us greatly with previously rather intractable problems. A centrifuge with a zonal rotor and associated fraction-collecting apparatus has made it much easier to study differences between virus particles. It seems that many viruses have two (or more) components, differing perhaps genetically, that must both be present for maximum infection. The biological significance of these systems is not yet plain because all components usually coexist. Such investigations demand varied scientific skills as well as sophisticated apparatus and these are often lacking in developing countries. Our work for the Overseas Development Administration not only helps to characterise viruses of tropical crops but has provided us with further interesting examples of the unusual bacilliform virus particles that we have found in ryegrass (p. 124) and previously in Brussels sprout and lucerne.

The specificity of insect vectors to particular viruses is important agriculturally, and differs locally (p. 131). The reasons are as yet little understood, but previous work on helper viruses, and recent advances in transmitting viruses by aphids fed on sap extracts through plastic membranes (p. 123) should help. Studies of plant virus multiplication have always been difficult because plant cells are infected sequentially. Synchronous infection is now possible using naked protoplasts (p. 122) and, although the technique of preparing them requires improvement before it is sufficiently reliable for routine use, it promises to be very valuable to virologists. The infection of yeast protoplasts shows that the method can be applied to viruses of fungi which are at present difficult to transmit in the absence of naked zoospores (p. 123). The effects of virus infection on fungi are difficult to measure (p. 136) but often seem small and could enhance the genetic or biochemical abilities of infected hosts.

Learning how to control virus diseases among crops is a much slower task than working with them in the laboratory. Even in a year when barley yellow dwarf virus was not serious, our work benefited from transferring experiments to sites nearer south-west England. Studies of aphid phenology have shown how infection of autumn-sown cereals can be decreased by delaying drilling, although we realise how difficult this may often be for farmers (p. 131). The weevils, especially *Apion vorax*, that transmit broad bean stain virus and Echtes ackerböhnemosaik virus to field beans seemed even more restricted by

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weather than aphid virus vectors (p. 130). Yields of field beans were generally much greater than in recent years, we think largely because the viruses were spread so much less (p. 144). This limited secondary spread allowed better success than we would usually expect in producing healthy seed crops by roguing infected plants; although we were disappointed with the effectiveness of heat therapy, we hope that we may do better in future, using even higher temperatures (p. 143).

During the year we began fresh work on diseases of pasture plants, too long neglected by pathologists, perhaps because of the obvious difficulties of assessment. With other laboratories we shall first concentrate on diseases of ryegrass. Electron microscope studies connected with initial surveys quickly revealed two unrecorded viruses in addition to ryegrass mosaic, and infections with barley yellow dwarf virus were also detected (p. 145). Mites were prevalent on ryegrass and transmitted ryegrass mosaic virus. We are developing ways to study their biology and it seems likely that the initial stages may be much easier than they were with aphids because the scanning electron microscope is revealing so many details of anatomy, feeding and movement (p. 146).

Fungus and other diseases. Dutch elm disease (the new strain of which appeared at Rothamsted), cereal leaf diseases and claims that blighted potatoes were dangerous to health (p. 152) all brought plant diseases more than usually to public notice during 1972.

Many investigations have stressed the effect that proximity to winter barley has on the development of powdery mildew in adjacent spring barley. Spore trapping has confirmed this (p. 132) and shown how sensitive the catches are to the type and positioning of freely exposed traps (p. 128). Much less has been known of the magnitude and effect of interactions between plots infected with air dispersed pathogens but two experiments on spring barley suggested they are large and may influence incidence, yield and the best timing of fungicide applications (p. 133).

Somewhat regretfully we decided to accept *Gaeumannomyces graminis*, rather than the accustomed *Ophiobolus graminis*, as the correct name of the take-all fungus of cereals (p. 134). Unfortunately, the problems it causes remain and the cause of take-all 'decline' remains unproven despite new suggestions and an examination of the role of virus-like particles in *G. graminis* (p. 135). It is also still difficult to differentiate between several fungi that produce brown runner hyphae on roots and which differ in pathogenicity and probably incidence (p. 134). Although we had less difficulty, than in 1971, in diagnosing diseases of cereal roots, many were again infected with chytrids, *Pythium* spp. and *Endogone* spp. (p. 140), but their effects were often difficult to separate from those of nematodes (especially on other crops, e.g. field beans and potato). Measuring the effects of root lesions or diseases has always been difficult, but we began testing the value of radioactive tracer methods in laboratory and field experiments jointly with the Letcombe Laboratory (p. 126). Progress was also made in measuring the distribution, survival and spread of inoculum of *G. graminis* (p. 136) which we hope will eventually help explain the differences in development and effect of take-all that seem to depend on long-term cropping history and soil types (p. 139).

The multiplication of potato seed stocks from rooted stem-cuttings is now well advanced towards practical testing and is beyond our control. We wish we had better chemicals to prevent the re-establishment of pathogens, especially those causing gangrene and bacterial soft rots. However, detailed chemical studies of uptake and distribution of supposedly systemic fungicides into potato plants may suggest better ways of using them (p. 150). Similarly, detailed studies of the entry of various pathogens into tubers through lenticels (p. 146) and wounds and the effects of seed contamination, weather and tuber maturity are improving our understanding of bacterial rots (p. 147). For example, it seems that prevalent rotting of King Edward potatoes, harvested immature for super-

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markets in July and August, is probably caused by soil inhabiting *Pseudomonas* spp. and not the *Erwinia* spp. which predominate later (p. 147). Until the produce of stem cuttings decreases the incidence of tuber diseases (p. 147), we need to decrease losses in field and storage. Considerable effort has been devoted to a joint investigation with the Potato Marketing Board Experimental Station of the relationships between tuber diseases on seed, during growth and in store (p. 151) which we hope may help define which stocks will store well and which should be marketed quickly. Healthy King Edward crops tend to produce more and rather smaller tubers than existing commercial stocks (although saleable yield is increased by *c.* 6–8%). In 1971 and 1972, experiments testing whether wastage of small tubers could be decreased by altering fertilisers, seed size and spacing surprisingly suggested that, with healthier seed, the same benefit could be achieved by halving the plant population in wider rows, as by doubling (or almost) the fertiliser applied (p. 149).

The Department has for many years applied its familiarity with agriculture, microbiology and aerobiology far outside the boundaries of plant pathology by collaborating in work on human and animal diseases and studying the biology and effects of storage moulds (p. 129). Studies have just begun of the moulding of barley grain, especially by *Penicillium* spp. and preventing hay moulding by applying propionic acid during baling. Other studies have progressed further and have concerned sheep fed mouldy hay, workers in a cork factory in Portugal and the exposure of combine harvester drivers to allergenic dusts. The search for a cause of seasonal asthma associated with ripening barley (p. 141) led to the discovery of an ascomycete in leaves, that pathologists seem not to have noticed.

Properties of viruses and virus diseases

Multi-component viruses

Components of broad bean stain virus (BBSV). When centrifuged, particles of BBSV sediment as three components with sedimentation coefficients of about 60, 100 and 127*S*. The middle (100*S*) and bottom (127*S*) components were separated from one another by centrifuging through a sucrose density gradient in a zonal rotor and in infectivity tests on French bean, bottom component gave an average of 1 lesion/leaf, middle 1·4 and a mixture of the two 104, showing that the two components complement one another. (Govier)

Hybridisation between two strains of radish mosaic virus. Last year we described a virus isolated from a kale plant (KV) which is a strain of radish mosaic virus. Both middle and bottom components of KV are needed to cause an infection as each one separately is not infective. Purified preparations of KV always contain aggregates of 12 particles. Another strain of radish mosaic virus (HZ), obtained from Yugoslavia, does not produce aggregates and lacks an antigen present in KV. KV forms a small spur in immunodiffusion tests when KV and HZ in adjacent wells are reacted against KV antiserum.

Hybrids between the two strains were obtained by inoculating mixtures of bottom component of one strain and middle component of another. Twelve hybrids were isolated from single lesions on *Chenopodium quinoa* plants inoculated with the bottom component of KV and the middle component of HZ and 20 hybrids from the reverse combination. These single lesion isolates were propagated, purified and examined in the electron microscope and in immunodiffusion tests. With one exception in each combination, the hybrids did not form aggregates and lacked the antigen specific for KV. We think that the two exceptions were caused by contamination with KV. By contrast, all of 14 single

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lesion isolates obtained from infections by KV alone formed aggregates and had the KV-specific antigen.

As virus proteins determine both aggregation and antigenicity, our results show that both middle and bottom components are coding for virus coat proteins. KV and HZ are slightly related to cowpea mosaic virus which has two virus proteins with molecular weights of 22 000 and 42 000 daltons present in the virus coat in equimolar amounts (Wu & Bruening *Virology* (1971) **46**, 596). Preliminary work (see below) showed that KV and HZ also contain two proteins, so it seems that the middle and bottom components each code for a different virus coat protein. (Kassanis, White and Woods)

Properties of strains of radish mosaic virus. Many plant viruses have been shown, by SDS-acrylamide gel electrophoresis to have particles constructed from single types of protein subunit (*Rothamsted Report for 1971*, Part 1, 129). Radish mosaic virus and its two strains described above, when analysed by the same procedure, showed two bands corresponding to proteins with molecular weights of approximately 20 000 and 40 000 daltons, and always present in about equal proportions. Carboxymethylation of the viruses did not change the positions or proportions of the bands. These facts suggest that particles of these viruses contain two different proteins, that neither is a dimer of the other and neither arises from the other by proteolytic attack during purification. The suggestion that the three types of particle each contain both proteins was supported by separating the top, middle and bottom components of KV on sucrose density gradients and then analysing them on gels. Each component contained approximately equal amounts of both proteins. (Carpenter and Gianinazzi)

Virus infection and transmission

Infection of protoplasts with TMV. In collaboration with the University of Nottingham, we isolated protoplasts from tobacco plants and infected them with tobacco mosaic virus (TMV) added to the suspension at 1 μg/ml. The amount of virus produced in the protoplasts was estimated in extracts by infectivity tests on tobacco cv. Xanthi, by serological assay and by comparing the number of virus particles seen in the electron microscope with a known concentration of polystyrene latex particles. The maximum yield of virus occurred 48 hours after inoculation and was estimated to be $1{\cdot}4$–$5{\cdot}8 \times 10^6$ particles per infected protoplast, which is almost as many as are found in cells of intact plants. Because over half the protoplasts were infected synchronously, the technique has great potential in plant virus research.

We also infected protoplasts obtained from yeast (*Saccharomyces cerevisiae*) with TMV by adding 125 μg/ml of virus to the suspension. However, little virus was obtained compared with that from tobacco protoplasts. The infectivity of yeast protoplasts decreased during the first 2–3 hours, was maximal after 17–24 hours, and then declined. Infectivity was estimated by inoculating extracts of protoplasts to tobacco cv. Xanthi using carborundum. In one experiment, the numbers of lesions were 42, 19, 570, 386, 379 and 218 respectively for 0, $2\frac{1}{2}$, 17, 24, 48 and 72 hour samples.

Work will continue at Rothamsted to simplify the method of infecting tobacco protoplasts and to standardise conditions for growing suitable tobacco plants. (Kassanis and White, with Mr. R. H. A. Coutts and Professor E. C. Cocking, Department of Botany, University of Nottingham)

***Mechanism of transmission of TNV by* Olpidium brassicae.** Last year we confirmed that tobacco necrosis virus (TNV) particles attach to the surfaces of vector zoospores but subsequently found the association of virus and fungus to be very erratic. Apparently

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the volume of liquid into which zoospores are released affects attachment of virus. A monosporangial isolate of *O. brassicae* was grown on lettuce plants in intermittently irrigated sand cultures. To release zoospores, roots of infected plants kept dry for two days, were washed free of sand and then placed either in just enough dilute nutrient solution (1/20 Hoagland's solution) to cover them (*c.* 10 ml) or a larger volume (*c.* 100 ml). Purified TNV was added to both zoospore suspensions to give concentrations of 2·5 μg/ml and after 15 minutes the zoospores were washed by centrifugation and examined by electron microscopy. More virus particles usually attached to zoospores released into the large volume than the small volume. In one experiment, 80% of zoospores released in the large volume had many virus particles attached whereas 70% of zoospores released in the small volume bore no particles. If zoospores that had been released into a small volume were washed, virus particles became attached; suggesting that some product of fungus or infected root has first to be diluted or leached from the zoospore surface. Behaviour of other fungal zoospores seems to be affected by the volume of medium and washing (Soll, D. R. & Sonneborn, D. R. (1972) *Journal of Cell Science* **10**, 315–333). (Macfarlane and Woods)

Aphid transmission of virus from sap extracts. Potato virus Y was readily transmitted, by aphids that had probed through membranes of stretched Parafilm 'M' into sap extracts made by grinding infected leaves in a solution containing 0·1*M* ammonium acetate pH 9, 0·02*M* EDTA pH 7·6, 0·02*M* DIECA and 20% (w/v) sucrose. Aphids transmitted virus less often when either EDTA or DIECA was omitted, and rarely when both were absent. When extracts were ultracentrifuged, or treated with ammonium sulphate to 40% saturation or with 8% polyethyleneglycol M.W.6000, the infective principle was precipitated and aphids readily transmitted virus from the resuspended precipitates, suggesting that infectivity was associated with whole virus particles.

Aphids rarely transmitted virus from extracts or resuspensions held for 3–4 hours at room temperature or for 24 hours at 4°C. The fact that extracts lose their ability to be aphid-transmitted more rapidly than the virus loses infectivity, suggests that aphid transmission depends on some specific structure of the virus coat-protein or of another viruses-coded component of plant sap, and that this structure is unstable in extracted sap. (Govier and Kassanis)

Purification methods. Filamentous viruses often aggregate during purification. With potato virus X, potato aucuba mosaic virus and henbane mosaic-virus, this was largely prevented by extracting in 0·1*M* EDTA pH 7·6 containing 0·01*M* DIECA, and dialysing against ten volumes of 0·005*M* DIECA before concentrating and purifying the virus by differential centrifugation. The method may also be useful for other viruses. (Govier and Kassanis)

Virus-like particles (VLP) in fungi. The discovery of VLP in fungi suggested they might have a role in biological control of plant diseases. Isometric VLP have been found in *Gaeumannomyces graminis* (see also p. 135), *Colletotrichum lindemuthianum* and *Puccinia graminis*. No VLP were found in isolates of *Cephalosporium gramineum*, *Helminthosporium gramineum*, *Pyrenophora avenae*, *Phialophora radicicola*, *Trichothecium roseum*, three *Phoma* spp. or eight *Fusarium* spp. (Rawlinson)

Gaeumannomyces graminis. Preparations containing both of the isometric virus-like particles (VLP) found in *Gaeumannomyces graminis* were analysed by acrylamide gel electrophoresis. Partially purified mycelial extracts were fractionated on composite gels made from mixtures of acrylamide and agarose and usually showed about five

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bands when stained for protein. Small quantities of each constituent were obtained by extracting the corresponding segments of gels prepared at the same time but not stained. One segment contained large VLP, another the small VLP but the rest contained none. The relative intensities of the bands indicated that the best preparations were impure. The proteins from each segment were analysed on SDS-acrylamide gels and compared with unfractionated preparations. The segment containing large VLP had a single protein of molecular weight 70 000 daltons. Insufficient quantities of the small particle have so far been obtained to characterise its protein separately. (Carpenter and Rawlinson)

Colletotrichum lindemuthianum. Of 33 isolates, representing physiologic races α, β, γ, δ and ϵ of the bean anthracnose pathogen from France, Holland and Britain, only five α isolates contained VLP (*c.* 30 nm diam.). The isolates with VLP were compared with four α isolates lacking VLP; unusual morphology, sporulation and colony appearance were not associated with VLP but four isolates with VLP grew faster on Mathur's medium which favours sporulation. The presence of VLP seemed not to affect pathogenicity although all isolates were much less pathogenic than those of race γ and δ on a range of differential hosts. Isolates neither gained nor lost VLP during frequent subculturing, when hyphae were anastomosed or when hyphal tips were taken from cultures grown near their thermal death point.

The VLP usually sediment in phosphate buffer (pH 7·5) as two components with sedimentation coefficients of 110*S* and 57*S*, but some preparations sediment as three major ultraviolet absorbing components of 110*S*, 85*S* and 78*S* with buoyant densities in cesium chloride of 1·38, 1·35 and 1·31 g/cm^3 respectively. Similar components separated when preparations were centrifuged to equilibrium in rubidium bromide. (Rawlinson)

Puccinia graminis. Twelve cultures derived from wild-type spores of a dikaryotic isolate of *P. graminis tritici* race 126-ANZ-6, 7 that had grown in axenic culture for five years all contained *c.* 38 nm VLP. The cultures were either mono- or dikaryotic and differed in pathogenicity, growth, morphology, texture, colour and mode of senescence (Maclean & Scott *Journal of General Microbiology* (1970) **64**, 19–27). Most particles in mycelial extracts were empty protein shells and even 'full' particles contained little nucleic acid. The VLP sediment in phosphate as two components with sedimentation coefficients of 110*S* and 58*S*, but the VLP may be more complex because three major components with buoyant densities of 1·31, 1·30 and 1·29 g/cm^3, occur when centrifuged in cesium chloride and rubidium bromide. (Rawlinson with Dr. D. J. Maclean, Unit of Developmental Botany, Cambridge)

Viruses from ryegrass

Ryegrass bacilliform virus (RBV). Samples from Wye College sent for assessment of the incidence of ryegrass mosaic virus (RMV) contained bacilliform particles. Viruses of this shape are well known and several occur in cereals although not reported from Britain or previously in grasses. Further samples from Stoneleigh, Warwickshire, showed two distinct leaf symptoms, the flecking and mosaic commonly associated with RMV and a more general chlorosis where RMV and RBV were found. Further samples were correctly classified on this basis but no symptom has been found reliably to indicate infection by RBV alone.

In negative stain the particles measure 220 × 70 nm, show regular cross-banding and are bullet-shaped with one round and one flat end. In infected leaves the virus often occurs in large aggregates enclosed within a membrane, or in the nucleus and between the layers of the nuclear membrane. Particle dimensions in section are 270–300 × 70 nm.

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The morphology of the particles suggests persistent transmission by hoppers or aphids. However, RBV was not transmitted mechanically, or by the aphid *Rhopalosiphum padi* and the plant hopper *Javesella pellucida* given long acquisition and infection feeds. (Plumb and James)

***Ryegrass spherical virus* (*RSV*).** Other plants from Wye developed patchy chlorosis followed by necrosis and contained many spherical particles about 30 nm in diameter besides RMV. Mechanical inoculation did not transmit RSV to wheat, oats, maize, *Chenopodium quinoa*, *C. armaranticolor*, *Nicotiana glutinosa*, *N. tabacum* ('White Burley'), *N. tabacum* var. *Xanthii*, *N. clevelandii*, *Phleum pratense* and *Avena elatior*. Apparently successful inoculations to S.22 Italian ryegrass, are now in doubt because half of the seeds of our stock of S.22 are infected by RSV. Plants grown from infected seed contain particles but show no symptoms unless inoculation with RMV or RMV plus RSV causes the severe symptoms described above. There was no reaction with antisera to cocksfoot mottle virus, Weidelgrass mosaic virus (a strain of brome mosaic virus), Phleum mottle virus, or a spherical component associated with RMV. RSV resembles Lolium mottle virus (A'Brook *Plant Pathology* (1972) **21**, 118–120) but seems to differ in host range, symptoms and in being seed transmitted. (Plumb)

***Electron microscopy of ryegrass mosaic virus*.** Thin sections of RMV-infected leaves of S.22 Italian ryegrass and Blenda oats showed some single particles but more aggregates of virus particles and numerous pinwheels and cell inclusions usually restricted to mesophyll tissue. The presence of pinwheels and inclusion bodies suggests a relationship to the potato virus Y group of viruses although RMV is mite and not aphid transmitted. (Plumb and James)

Virus diseases of tropical crops

***Cocoa necrosis virus* (*CNV*).** Sap from *Phaseolus vulgaris*, infected with CNV and fractionated without butanol, sometimes gave virus preparations containing empty protein shells (54*S*) and small spherical particles (20–30*S*) *c.* 12 nm diameter, in addition to the two nucleoprotein components of 101*S* and 129*S* found in virus preparations made from sap treated with butanol. The 12 nm particles could be polymorphic forms of the outer shell protein subunits. The 54*S* protein shells are precipitated by butanol and so are rarely seen in virus preparations made using it. Butanol does not have the same effect on the empty protein shells of tomato black ring virus, of which CNV is a serotype, or most other nepoviruses. (Kenten)

***Cowpea mild mottle virus* (*CMMV*).** Two cultivars of *Vigna unguiculata*, 'Blackeye' and a white-seeded but unnamed one commonly grown in Ghana usually show a mild systemic chlorotic mottle when infected with CMMV. A brown-seeded Ghanian cultivar develops chlorotic circular lesions on inoculated primary leaves followed by severe systemic necrosis and chlorosis of trifoliate leaves, which soon absciss.

CMMV precipitates with antiserum to carnation latent virus (homologous titre 1/32 000) up to dilutions of 1/64 but not with antisera to ten other 650 nm filamentous viruses, including those known to infect legumes, and so is probably a hitherto undescribed virus. Purification of CMMV is difficult because it readily aggregates and becomes insoluble. Samples of good purity can be got by freezing and thawing infected leaves, then extracting and suspending the virus in 0·01*M* sodium borate (pH 9·5), clarifying the extract with chloroform and concentrating and purifying the virus by two cycles of differential centrifugation and a rate zonal centrifugation through sucrose density gradient

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columns. Such preparations, although partially aggregated, consist largely of straight or slightly flexuous, fragile, filamentous particles *c.* 13 × 650 nm which sediment as a single component of sedimentation coefficient $S^{S}_{20,w}$ of 165 ± 4*S*. They have an ultraviolet absorption spectrum typical of a nucleoprotein containing *c.* 5% nucleic acid and a single polypeptide species with a molecular weight of 32 000–33 000 daltons. (Kenten, with Dr. A. A. Brunt, Glasshouse Crops Research Institute)

***Viruses infecting* Colocasia esculenta.** Leaves diseased with 'Alomae' (see p. 347) contain bacilliform particles of two sizes. The smaller can be partially purified by differential centrifugation of extracts made with 0·05*M* sodium borate (pH 7·5). Such preparations show a single component of sedimentation coefficient 285 ± 10*S* in the analytical ultracentrifuge. The larger particles could not be prepared in partially purified suspensions because they are fragile, tend to aggregate and also appear to absorb on to host material. The small bacilliform particles resembled cocoa swollen shoot virus (125–130 nm × *c.* 30 nm). Staining with uranyl acetate disrupted some particles and some protein sub-units became rearranged as stacked discs. In extracts the larger particles occurred in groups of four to twenty and are now estimated to measure 300–335 × 65 nm. Both particles were found in thin sections of 'Alomae' leaves. Large particles occurred in large aggregates in sieve tubes or in small groups scattered throughout the cytoplasm of parenchyma and cells associated with phloem. They also occurred associated with spherical amorphous bodies or in crystalline arrays between the two nuclear membranes but never within the nuclear matrix. The smaller particles also occurred grouped sometimes with amorphous material but their packing was always haphazard. They were always fewer than the large particles but were commonest in phloem cells. (James, Kenten and Woods)

Uptake of nutrients and water by diseased roots

Measurements of the effects of pathogens on the yield of crop plants are necessary to assess the benefits of control and to decide research priorities for pathologists and plant breeders. Surprisingly, measurements that are scientifically and agriculturally reliable are as difficult to make as any in plant pathology. The difficulties increase as symptoms become less obvious, so that measuring the effects of many root diseases is more difficult than with pathogens that defoliate plants. The discovery that lower fungi, such as chytrids, were often prevalent on roots but ephemeral and symptomless, posed even more difficult problems than lesion-forming root parasites like *Gaeumannomyces graminis* (*Ophiobolus graminis*). Discussing the possibilities of using plant uptake of mineral nutrients to indicate root activity, led to the start of collaborative work with the Letcombe Laboratory, primarily to test the usefulness of radioactive tracer techniques in the laboratory and among crops.

Laboratory tests of the effect of take-all lesions. The uptake of radioactive tracers by short segments of intact wheat seminal root axes and complete root systems was studied using techniques described by Clarkson and Sanderson (*ARC Letcombe Laboratory, Report for 1970*, 16–25). Tracers used included ^{85}Sr (to represent Ca), ^{32}P, ^{59}Fe and ^{42}K. Usually absorption and translocation were greatest with healthy roots but a few take-all lesions caused by *G. graminis* seldom had appreciable effects. Increasing the severity of take-all on root systems decreased both the uptake and translocation of ^{32}P and ^{85}Sr but more interesting differences were found by studying individual diseased root axes. When ^{32}P was applied to slight lesions, much less accumulated in the root than in comparable healthy roots but lesions made little difference to the proportion translocated. When the tracers were applied between the root tip and lesion, little ^{85}Sr or ^{32}P was

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translocated through lesions so severe that they had stopped the elongation of infected axes. The cessation of nutrient supply to the shoot probably coincided with the destruction of phloem in the lesion. Neither ^{85}Sr nor ^{32}P accumulated in the lesion but ^{42}K applied to the lesion was absorbed more and translocated better than from healthy tissue.

These first tests suggested that the position of the lesion is important so efforts were made to produce take-all lesions on predetermined parts of roots. Growing roots of wheat seedlings through pellicles of *G. graminis* mycelium floating on still or aerated nutrient solutions was unsuccessful because few lesions developed at the air/liquid interface, where they were expected. With still cultures few lesions developed and only well above the liquid; whereas in aerated cultures many severe lesions developed, some in, but most above the liquid. Possible explanations are, that oxygen deficits prevented the fungus infecting in still culture, that root exudates in still culture inhibited growth or infection, or that aeration increased growth rate of the fungus and broke off pieces that could infect elsewhere than at the pellicle. Better positioning of lesions was obtained by growing roots down sand columns in glass tubes that were percolated periodically with nutrient solutions, and inoculating roots through side arms when they had reached a desired depth. (Hornby, with Dr. D. T. Clarkson, Letcombe Laboratory, Wantage)

Laboratory tests of the effects of *Pythium* infection. Wheat seedlings (Cappelle-Desprez) were grown singly and aseptically in boiling tubes containing coarse quartz grit and Crones nutrient solution. Half were inoculated with a culture of *Pythium arrhenomanes*. After 16 days at 19°C, the plants were carefully removed from the boiling tubes. The main root axes of infected seedlings were brown and stunted and their cortical cells contained many large resting spores. Lateral roots grew better and many remained white. Inoculated plants yielded 28% less fresh weight of roots, 17% less tops and transpired 25% less water than healthy plants. After 23 hours uptake of radioactive tracers from a nutrient solution at 20°C, shoots from inoculated plants contained less ^{85}Sr and ^{32}P than shoots from healthy plants but amounts in their roots were equal. However, infection increased the amount of ^{59}Fe in both shoots and roots. (Salt with Dr. D. T. Clarkson, Letcombe Laboratory)

Field test of nutrient and water uptake by wheat roots. Radio-isotopes were also used to study root distribution and nutrient uptake of spring-sown wheat in sandy loam at Woburn. On a site free from take-all, 24 of 48 plots 1 m square were inoculated with take-all in April, a month after sowing Maris Ranger wheat. Roots of infected seedlings were pushed into holes, between the crop rows, 15 cm apart and 10 cm deep. By July only 9% of straws from inoculated plots showed roots with take-all, too slight an infection to alter uptake of ^{32}P into the leaves during the nine weeks after it was injected into soil at 20, 30 or 60 cm deep. Soil moisture profiles, measured weekly with a neutron probe at every 10 cm down to 60 cm and then every 20 cm to 120 cm deep were similar in all plots. Yields varied from 1·79 to 5·22 t/ha, mainly as a result of 'scorch' (*Rothamsted Report for 1969*, Part 1, 65) but the symptoms could not be associated with differences in soil moisture profiles or with discoloured straw bases. (Salt with Dr. F. Ellis and Mr. K. R. Howse, Letcombe Laboratory)

***Olpidium* infection of cabbage and iron deficiency.** Earlier observations (*Rothamsted Report for 1965*, 132) were confirmed. When infected by *Olpidium brassicae* in sand culture, cv. January King had worse iron deficiency symptoms and grew less than cv. May Express. Infected plants had, respectively, 44% and 63% of the dry matter of healthy plants but shoot to root weight ratios were not altered by cultivar or infection.

The effects of *O. brassicae* on yield and chlorosis were alleviated by supplying more

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iron. Seedlings of May Express grown for three weeks in half-strength Hoagland's solution were then transferred for four weeks to the same containing 2·0, 0·4, 0·08 ppm of iron. The dry weights of uninfected plants were 2·2, 2·3 and 1·7 g respectively compared with 2·2, 1·4 and 0·9 g for infected plants. In units of absorbance the corresponding chlorophyll values for samples of fifth leaves were: uninfected, 1·1, 1·1 and 0·7 and infected, 0·9, 0·4 and 0·2. Roots of inoculated plants grown with 2 ppm iron were densely infected, so increased iron seemed to affect the host and not the fungus. It is not yet known whether other nutrients are affected by *O. brassicae*. (Macfarlane)

Aerobiology

Spore catches within and just above cereal crops. Wind tunnel tests show that, except at very low wind speeds, freely exposed sticky cylinders (5 mm diam.) are more efficient traps than horizontal slides. Air movement within crops is retarded and variable, so traps were exposed (usually for seven hours on dry days), 0, 0·25, 0·5, 0·75, 1·0 and 2·0 m high in and above crops of wheat and barley. The spores counted included some from sources both within and outside the crop and others from outside only.

The cylinders showed abrupt changes in catches just above the crop. As cylinders are being much used to measure dispersal of *Erysiphe graminis* spores (*Rothamsted Report for 1971*, Part I, 137) the vertical profiles were examined in greater detail from crop height −10 cm to +60 cm. At +40 cm above a barley crop (growth stage 9) six times as many *E. graminis* spores were caught as at its top (0 cm) but at +60 cm the catch was only 80% of that at +40 cm. Deposition was greatest at +15 cm above wheat (growth stage 10·5) but only three times that at 0 cm. At +15 cm twice as many wheat pollen and three times as many grass pollen (from outside the crop) were caught as at 0 cm. Cylinders always caught more spores and pollen/unit area than slides above crops. At and below a variable height in the upper half of the crop slides always caught more than cylinders. Progressively fewer spores from outside sources were caught on both traps at decreasing heights within crops, presumably because an increasing proportion were deposited on the foliage above. Spores released within the crops were caught in greatest numbers just below the height at which they were released. (Stedman and Hirst)

Dust hazards during harvesting. Measurements of spore concentrations close to combine harvesters were similar to last year (*Rothamsted Report for 1971*, Part 1, 161) but in cooler, windier weather the driver was exposed to fewer spores (Table 1). Almost as much dust was produced at the front of the machine as at the back. Wheat crops were slightly more dusty than barley. *Cladosporium* spores again predominated, comprising 75% of the total spores in barley dust and 65% in wheat dust, with *Alternaria* (9%; 12%), *Ustilago* (0·5%; 10%), *Cephalosporium*-like spores (3%; 2%), actinomycetes and bacteria (3%; 2%) and uredospores (0·7%; 0·7%). Many of these spores are known to be allergenic. (Lacey, with Dr. C. S. Darke, Sheffield Royal Infirmary)

TABLE 1

Total spore concentration (10^6 spores/m^3) during combining

	Cascade impactor				'Personal sampler' worn by driver	
	near front of machine		behind machine			
	Range	Mean	Range	Mean	Range	Mean
Combining barley	27·1–86·9	51·7	3·5–134·8	54·7	0·5–13·6	4·3
Combining wheat	53·1–71·9	62·5	47·1–212·6	96·4	0·7–14·6	4·0

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Suberosis. Suberosis is a form of allergic alveolitis associated with humans inhaling dust from mouldy cork. In a cork factory near Lisbon, the numbers and predominant types of airborne spores were determined in different departments. Most spores ($54 \times 10^6/m^3$ of air) were found in the small humid warehouses (*fundão*) where the cork was stored between boiling and shaping. Many (up to $22 \times 10^6/m^3$ of air) also occurred where discs were cut from cork strips. *Penicillium frequentans*, *P. granulatum*, *Aphanocladium album* and *Monilia sitophila* were abundant in the warehouse and where the cork was sliced and disced but only *P. frequentans* was widespread through the factory. Airborne cork particles were larger than *Penicillium* spores and fewer (usually $< 1{\cdot}5 \times 10^6/m^3$ air), generally with most where cork stoppers were dried and where granulated cork aggregates were sanded. Precipitins to extracts of *P. frequentans* were present in sera from suberosis patients but their significance is not yet established. (Lacey, with Dr. R. Avila, Faculdade de Medecina de Lisboa)

Biodeterioration

Spoilage of stored crops is important, not only because of the feeding value lost, but also because moulds may produce toxic metabolites in the substrate and their spores may cause allergy or infection when inhaled.

Feeding experiments with mouldy hay. Sheep fed hay made at different water contents (*Rothamsted Report for 1971*, Part 1, 161) showed no symptoms of respiratory disease but post-mortem samples of the lungs of animals with antibodies to *Micropolyspora faeni* showed pathological changes consistent with allergic alveolitis. The changes consisted of interstitial pneumonitis (alveolitis), thickening of the interlobular septa and peribronchial lymphoid hyperplasia, but no granulomata were found.

Further observations were prevented by a severe lung worm infection which made the sheep unsuitable for studying pathological changes in their lungs. Work will continue when sheep born by Caesarian section are available. (Lacey, with Mr. G. A. Embleton, ARC Institute of Animal Physiology, Babraham, Cambridge, and Miss V. Holford-Strevens, Institute of Diseases of the Chest, Brompton, London)

Control of moulding in damp hay. An applicator supplied by BP Chemicals (U.K.) Ltd. was used to apply concentrated propionic acid to hay during baling. Wet weather delayed haymaking so the Italian ryegrass crop was undesirably mature and stemmy, but hay was baled at 41, 28 and 26% water. Propionic acid (applied at 0, 1, 2 or 3% by weight) decreased heating at all water contents and 2% or more restricted the maximum temperature to less than 30°C at 28 and 26% water content (Table 2).

Because the hay was cut late, moulding was not serious, even in untreated bales. In hay with 41% water, both treated and untreated bales had general moulding, but actinomycetes were few in treated bales. Throughout untreated bales of drier hays, Aspergilli of the *A. glaucus* group were predominant forming only conidia. Bales of drier hays treated with propionic acid had few fungi on the outside but toward the centre there were many cleistothecia of the *A. glaucus* group. This distribution persisted even where 3% of propionic acid was added suggesting that its distribution was uneven, with insufficient at the middle. Although dry matter changes were variable, 1 or 2% of propionic acid prevented much loss.

The need to distribute propionic acid uniformly was emphasised using 16 kg lots of hay (36% water) stored in polyethylene drums. Adding 1% of concentrated propionic acid uniformly, prevented any moulding, whether the drums were aerated or not, while including two untreated layers, each *c.* 5 cm deep allowed uniform moulding throughout

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the drum. Moulding occurred in the lower half of a drum uniformly filled with hay containing 0·5% of propionic acid, but aerated slowly (1 air change in about 6 hours). (Lacey)

TABLE 2

Effects of propionic acid application on storage of hay

Water at boiling (%)	Propionic acid applied (%)	Maximum temperature (°C)	Days after baling to maximum temperature	Spore content (10^6 spores/g dry weight)		Dry matter change (%)
				Fungi	Actinomycetes + bacteria	
41	0	58	5	0·1	5·8	−3·3
41	1	46	5	0·9	0·1	−7·0
41	2	39	5	5·7	1·4	−6·8
28	0	46	6	0·3	0·2	−8·1
28	1	35	18	0·2	0·3	−0·3
28	2	28	1	1·6	0·3	−1·2
28	3	25	1	0·3	0·4	−5·5
26	0	42	9	0·1	0·6	−6·3
26	1	31	13	0·8	0·2	0
26	2	26	1	3·4	0·5	+1·1
26	3	30	19	1·3	0·1	−6·5

To test whether propionic acid was redistributed in the vapour phase within baled hay, air was blown through hay into 0·1 ml 0·01*N* NaOH containing a screened indicator consisting of equal parts of bromocresyl green and chlorophenol red. Less than 10 ml of air saturated with propionic acid vapour was needed to bring the NaOH solution to pH 6. However, if tests were made more than one hour after mixing propionic acid with hay in the laboratory, more than 50 ml air was needed to give a similar change. This indicates that propionic acid is rapidly absorbed in the hay. Thus, in practice, redistribution and loss of vapour are likely to be small. (Lacey with Lord, Insecticides Department)

Barley microflora. Methods of assessing the microflora before harvest and during storage were compared. Isolating micro-organisms using the Andersen sampler and wind tunnel (Lacey *Annals of Applied Biology* (1971), **69**, 187) yielded fewer colonies per g dry weight than dilution plating, but gave a greater diversity of species, particularly actinomycetes, *Aspergillus* spp. and *Penicillium* spp. Cascade impactor catches in the wind tunnel provided estimates of the total spore content closer to those estimated by dilution plating. The surfactant Triton X-100 did not consistently increase dilution plate counts. (R. A. Hill and Lacey)

Cereal diseases

Barley yellow dwarf virus (BYDV)

Phenology of BYDV and its aphid vectors. Although *Rhopalosiphum padi* was common in autumn 1971 few alatae carried virus, so infection was only expected to be prevalent where winter cereals were sown by early October. The winter was mild but April, May and June were all colder and duller than usual. At Rothamsted the first infective alate *R. padi* were trapped on 22 May; *Sitobion avenae* on 9 June, and *Metopolophium dirhodum* on 29 June. Although first infections occurred little later than in 1971, the cold spring much delayed aphid multiplication and migration so that aphids increased slowly on crops. By mid-July fewer than 30 *R. padi* had been caught in the Entomology Department suction trap at Rothamsted compared with more than 1000 in 1971. Catches of *Sitobion avenae* were a fifth and of *M. dirhodum* a half of those in 1971.

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Effects of BYDV and aphids. At Rothamsted, Rosemaund Experimental Husbandry Farm (Hereford) and, in conjunction with the National Institute of Agricultural Botany, at Seale Hayne Agricultural College (Devon), insecticides were applied at different dates to test their effects on the incidence of aphids and BYDV. Menazon applied to foliage in June and July decreased aphids in all trials and phorate granules on the seedbed decreased early BYDV at Seale Hayne. The two menazon sprays did not decrease BYDV, and insecticides alone nowhere significantly increased yield. The seed for some plots at Seale Hayne and Rosemaund was treated with ethirimol to control mildew, this increased yield significantly (20%) at both sites and at Seale Hayne there was a further significant increase in yield from plots where ethirimol and phorate were applied at sowing and followed with two menazon sprays.

On a farm near Hereford where 100 ha of winter oats were grown, most (80 ha) were sown early in October, and in March between 0·1–1·0% of Maris Quest plants showed BYDV symptoms, although aphids, mainly *S. avenae*, were infrequent. Glasshouse transmissions confirmed the presence of a very severe BYDV isolate. Oats sown in late October were free from virus. Menazon was applied to parts of early-sown crops late in April to kill overwintered aphids before they multiplied and spread virus and to limit further infestations. Sprayed areas yielded 0·38 to 1·13 t/ha more than unsprayed strips in the same field and, by 29 June, there were 60% fewer virus-infected plants. Best yields came from fields drilled late although these were not sprayed. Delaying drilling to escape autumn infections offers the most effective and cheapest control measure but unfortunately, particularly on heavy land, is often the least practical. We shall test further the value to early sown crops of applying insecticides to the seed bed or spraying immediately after emergence.

Transmission of BYDV isolates. Entomologists in the West Midlands questioned whether *Metopolophium festucae*, not previously reported as a vector, could transmit BYDV. It transmitted mild and severe isolates from Hereford but not isolates from eastern England. Although it was not an efficient vector it is often prevalent and could be important among crops because cereals seem to be damaged more by the feeding of *M. festucae* than of other aphids. (Plumb)

Powdery mildew of cereals (*Erysiphe graminis*)

Response to temperature and relative humidity. *E. graminis* is an ecto-parasite and could therefore be very sensitive to environmental changes. On leaves of barley and wheat it germinated, formed appressoria, infected and grew best at 20°C. Growth rate was approximately halved with each 5°C decrease between 20° and 5°C. Decreasing relative humidity from 95 to 65% only slightly retarded germination and appressorial formation, and did not affect the growth of hyphae. (Bainbridge and J. E. Smith)

Phenology of mildew. In a crop of spring barley (cv. Zephyr) the seasonal periodicities of catches of *E. graminis* conidia were similar in a Burkard suction trap, on freely exposed vertical sticky cylinders (5 mm diam.) and, expressed as pustules, on barley seedlings exposed in the crop. Spore catches began to increase late in May, two weeks later than in 1971, but increased so fast that they were greatest at the same time, early June.

Sticky cylinders and seedlings were also exposed on a laboratory roof. Catches on cylinders on the roof were variable and increased more slowly than in the crop, to a maximum in mid-July but then decreased at a rate similar to traps in the crop. Seedlings exposed on the roof developed many fewer pustules than those in the crop and the seasonal periodicity differed from that on the adjacent cylinder. Perhaps the differences result from seedlings detecting only some of the viable spores of the physiologic races able to attack

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cv. Zephyr, whereas all spores of other races, formae speciales and perhaps some other species would be recognised on cylinders.

Cumulative total catches of spores on sticky cylinders were smaller in 1972 than in 1971, by 10% within the crop and by 50% on the laboratory roof. The latter probably reflect differences between years more reliably than comparisons within crops. (Jenkyn and Banfield)

Sticky cylinders were again exposed in spring-sown barley at ten NIAB centres and Rothamsted (see *Rothamsted Report for 1971*, Part 1, 137) but, unlike 1971, maximum catches at different sites occurred between late May and late June. Catches on cylinders exposed for three or four days could not be used to analyse the detailed effects of temperature, wind and rain on spore dispersal but clearly indicated major trends in the quantity of spores liberated. Long wet spells decreased catches but did not prevent spore liberation or fresh infections. The spore catches on cylinders promise to be useful in defining the time to begin fungicide spraying, so far deposits of 300–500 conidia/cm²/day have usually heralded rapid disease increase.

Cylinder traps in winter barley at seven centres showed that, through late winter and spring, spores could be released in great numbers, with some traps accumulating over 1000 conidia/cm²/day during March and April. (Bainbridge and Alsop)

Timing of fungicides and their effects on yield. Table 3 shows the effects of ethirimol seed dressings or tridemorph sprays, applied at predetermined growth stages or at the onset of rapid increase in spore catches in a crop of Zephyr barley, between 28 May and 7 June. Yield was related to the mean area of green leaf/plant between 9 June and 5 July but not to the area of mildew on any one date. (Bainbridge)

TABLE 3

Effect of fungicides at different dates on mildew and yield of spring barley

	Treatment*					
	Nil	D	1	2	3	T*
Mean mildew (% of total area green leaf)						
9 June (G.S. 8)	5·6	1·3	0·3	5·4	5·7	2·2
22 June (G.S. 10·1)	13·0	5·3	2·9	6·5	12·8	2·6
5 July (G.S. 10·5)	29·9	17·8	18·8	3·8	7·7	10·4
Area of leaf remaining green (cm²/plant)						
9 June	84·8	85·5	86·1	84·5	84·5	85·6
22 June	68·9	76·0	83·9	76·2	67·6	84·3
5 July	39·7	56·4	53·3	68·6	60·2	63·6
Mean	64·4	72·6	74·4	76·4	70·8	77·8
Yield (t/ha)	5·13	5·53	5·89	5·86	5·63	6·00

(S.E.D. 0·22 D.F. 25)

* Nil = untreated
D = ethirimol dressed seed (0·54 kg/ha)
1 = tridemorph (0·7 litre/ha) spray, applied at G.S. 5 (22 May)
2 = tridemorph (0·7 litre/ha) spray, applied at G.S. 8 (12 June)
3 = tridemorph (0·7 litre/ha) spray, applied at G.S. 10·1 (22 June)
T = tridemorph (0·7 litre/ha) spray, applied at rapid increase in spore count (2 June)

In its final year, the experiment testing effects of mildew at different stages of growth was less interesting than in 1971 because there was less mildew early in the season. Nevertheless, seed dressings again increased height and the number of fertile tillers; more with the large seed dressing in the treatment aimed to give full-season protection than the

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small dressing intended only to prevent early mildew (Table 4). The experiment will be modified to test the economic benefit of using both seed dressings and sprays at recommended rates because results in the past two years have shown considerable improvement from combining them. (Jenkyn)

TABLE 4

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

Protection intended	Seedlings/m (G.S. 1)	Fertile tillers/m (G.S. 11·2)	Crop ht. (cm) (G.S. 11·2)	Mildew[1] (G.S. 5)	Mildew[1] (G.S. 11·1)	Yield (t/ha) ±0·076
None	53·6	135	96	0·8	55·0	5·46
Early[2]	51·7	138	98	0·3	43·2	5·75
Late[3]	51·2	137	97	0·7	6·8	6·03
Full season[4]	50·6	144	100	0·0	1·9	6·59

[1] Percentage area of second youngest leaf infected
[2] Seed dressed with ethirimol at 0·22 kg/ha. a.i., about one-third recommended commercial rate
[3] Two sprays with ethirimol 0·9 kg/ha a.i. (at G.S. 10·1 and G.S. 10·5)
[4] Seed dressing 1·8 kg/ha a.i. and two sprays both as above

Problems of designing field experiments with air-dispersed pathogens. Spore deposition gradients decrease quickly close to sources and then more slowly; changes that have long been suspected, but never proved, to be important causes of interaction between neighbouring experimental plots. Two similar barley experiments, with Julia and Zephyr, aimed to measure the effect of distance from sources on disease incidence. Both were square and had an unsprayed border (11 m wide) surrounding a core (*c.* 170 m square) sprayed twice with tridemorph (0·53 kg in 438 litre/ha). Within each, small plots (6·4 m square) differently sprayed, were used to measure mildew and yield. In the frame the small plots were unsprayed or sprayed once, within the core they were sprayed once or twice.

The lay-out provided plots, sprayed once on 1 June, at minimum distances of 0, 2, *c.* 40 and *c.* 80 m from the unsprayed frame which became a potent source of mildew. Table 5 shows that although one spray in the frame area delayed the development of

TABLE 5

Mildew and yield of unsprayed crops, and once sprayed crops at various distances from unsprayed crops

Treatment and minimum distance from unsprayed crop		Mildew[1] 22 May[2] (G.S. 5)	12 June (G.S. 9)	29 June (G.S. 10·4)	17 July (G.S. 11·1)	Yield (t/ha)
Julia unsprayed	—	tr	1·0	1·5	12·5	4·60
Once sprayed	0 m	tr	0	0·3	5·6	5·01
Once sprayed	2·1 m	tr	0	0·1	2·1	4·85
Once sprayed	42·7 m	0	0	tr	1·2	5·65
Once sprayed	83·2 m	tr	0	tr	0·6	5·44
Zephyr unsprayed	—	0·2	1·2	6·6	35·4	4·41
Once sprayed	0 m	0·1	0	1·3	18·0	5·50
Once sprayed	2·1 m	0·1	tr	1·0	12·2	5·60
Once sprayed	38·4 m	0·3	0	0·3	6·5	5·40
Once sprayed	74·7 m	0	0	0·2	5·0	5·74

[1] Percentage area of second youngest leaf: tr = trace (<0·05%)
[2] Dates and growth stages quoted are those for Zephyr. Julia was always assessed within two days of Zephyr and was at a later growth stage on all except the first occasion when mildew was assessed

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mildew and increased yield, both effects increased with distance from the source. Cylinder spore traps in selected plots caught less as distance from the frame increased. Proximity to the source increased the rate and amount of reinfection after spraying. The results stress the caution necessary in interpreting the results of small-plot experiments, especially when some plots or surrounds provide much air-dispersed inoculum. They also emphasise the need for special experimental designs, and to consider the proximity of sources before using experiments to advise on spray-timing. (Jenkyn and Bainbridge)

Take-all. Loath though pathologists are to change the names of important pathogens, there now seems justification for accepting the proposal that the wheat take-all fungus should be *Gaeumannomyces graminis* (Sacc.) Arx & Olivier var. *tritici* (J. Walker) rather than the familiar *Ophiobolus graminis*. Unfortunately, the disease still presents much more intractable problems than the taxonomy of the pathogen, some of these were outlined last year (*Rothamsted Report for 1971*, Part 1, 140). In this report we amplify studies into the nature of take-all inoculum and of the 'decline' of the disease that frequently occurs in land consecutively cropped with susceptible cereals. We also mention studies of the early stages of the spread of take-all through cereal populations on fields previously free from the fungus and some exceptions to the decline sequence, important to farmers but as yet neither understood nor predictable.

***Comparison of isolates of* Gaeumannomyces graminis *and* Phialophora radicicola.** Only isolate 77 (*Rothamsted Report for 1971*, Part 1, 140) and a similarly avirulent isolate (81, probably *Phialophora radicicola*), from a first spring wheat crop after fallow, have now failed to produce microconidia. Isolates 43 and 59, the only ones to produce mature perithecia on seedling roots grown in agar or grit in test tubes, recently produced microconidia directly from ascospores.

Walker's recent classification of *G. graminis* varieties (*Transactions of the British Mycological Society* (1972) **58**, 427–457) depends much on characteristics of hyphopodia. Only isolate 43 had mild swellings of the penetrating hyphae that resembled Walker's simple hyphopodia, although isolates 43, 59 and 76 all caused many conspicuous lignitubers in wheat coleoptiles.

The isolates classified as *G. graminis* (43, 59, 76) all caused lesions on roots, lignitubers on coleoptiles and had characters not found in *P. radicicola* [perithecia (43, 59), microconidia from ascospores (43, 59), hyphopodia (43) and virus-like particles (59)]. Some *P. radicicola* isolates resembled *G. graminis* isolates in producing microconidia from hyphae (78, 79), in colony appearance on agar (78, 79) and runner hyphae on roots (79, 81). Only isolate 77 had no similarities. (Hornby)

Effect of ploughing and consolidation on the distribution of plant debris and* G. graminis *in soil. Soil samples to estimate *G. graminis* inoculum in plant debris have usually been taken in cores 15 cm deep. Most of the fragments of plant debris ($> 150\ \mu m$) are extracted automatically (*Rothamsted Report for 1969*, Part 1, 157), the remainder are elutriated manually and the total volume measured by displacement. At Woburn, debris volumes are greatest at harvest but then decrease. However, in 1970 and 1971 the volume and the number of infective fragments increased, by as much as 30%, between January and March. It seemed probable that these changes were affected by twice ploughing (23 cm deep) and subsequent consolidation; so soil was sampled both from 0–15 cm deep and 15–23 cm. Table 6 shows that between November and March the volume of fragments ($> 150\ \mu m$) decreased but the proportion in the upper stratum increased.

Later examinations showed a narrow band with much coarse debris in soil cores (12·5–15·0 cm, March to August 1972) and in a profile pit (11·5–13·0 cm, May 1972).

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Take-all infectivity has been assessed, by seedling infection tests, for each 2·5 cm interval down to 23 cm. Until just before harvest the segment 12·5–15·0 cm deep was always most infective, but thereafter two maxima occurred above and below, presumably as a result of infections developing on the growing crop. Ploughing decreases the bulk density of soil and buries some of the superficial debris. We think that the stratum with most debris probably lies beneath our standard 15 cm sample until the soil consolidates again after ploughing, and that the increase in infectivity detected in mid-winter is the result of this artefact. (Hornby, Henden and S. M. Hill)

TABLE 6

*Volumes of plant debris (>150 μm) in winter samples of Woburn (Butt Furlong) soil twice ploughed**

	Debris (cm^3/2·8 kg: air-dry soil) in depth intervals			
Strata sampled	A 0–15 cm	B 15–23 cm	C 0–23 cm	A : B
Sampling date				
29 November	22	36	58	0·6
20 December	26	32	58	0·8
10 January	25	39	64	0·6
2 February	24	26	50	0·9
24 February	18	18	36	1·0
16 March	21	15	36	1·4

* Ploughed 27 September and 7 November 1971

Attempts to explain the nature of 'decline' of take-all. This work (*Rothamsted Report for 1971*, Part 1, 141) continued with studies of how to assess the pathogenicity of *G. graminis* isolates, whether or not virus-like particles (VLP) affect their pathogenicity or growth, the role of VLP in take-all decline, the effect of consecutive cereal cropping on soil N and microbes and how inhibition of *G. graminis* develops in soil repeatedly infested with it.

There is no satisfactory standard test for the pathogenicity of *G. graminis* isolates. We assessed the severity of disease in wheat-seedling infection tests in four ways (percentage seminal roots infected; percentage total length of root axes with lesions; percentage seedlings with stunted and yellowed shoots, and the number of discrete lesions per root system, expressed as a percentage of an arbitrary upper limit) and used the mean of the assessments as an index of pathogenicity. (Pearson and Hornby)

Cellulose decomposing ability might also be used to indicate pathogenicity, because the cellulolytic abilities (Garrett, *Transactions of the British Mycological Society* (1963) **46**, 572–576) of two isolates of *G. graminis* highly pathogenic on wheat and one highly pathogenic on barley were about twice as great as that of an isolate weakly pathogenic on both cereals. (Pearson)

Genetic recombination and segregation complicate testing whether freeing isolates from VLP by ascospore culture affects their growth and pathogenicity. Nine complete ascospore sets, comprising 72 progeny cultures freed from VLP, were examined for differences from the parental cultures. Three exhibited segregation (usually 1 : 1) for colony appearance and growth rate and the ability to form perithecia on agar. Two other sets from the same perithecium differed morphologically, but did not segregate. The pathogenicity of two parent and 40 F1 colonies (five ascospore sets) was compared in seedling infection tests. Three ascospore sets segregated for pathogenicity (1 : 1 or 1 : 3, weakly : strongly pathogenic) and the most pathogenic offspring were equally or more

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pathogenic than their parents. Where segregation did not occur, all progeny were less pathogenic than the parent. (Hornby, Pearson and Rawlinson)

In Yorkshire and at Rothamsted two VLP (27 and 35 nm diam.) were common in *G. graminis* var. *tritici* (see p. 123) isolated from second and subsequent susceptible cereal crops. Isolates from first wheat or barley after fallow or non-susceptible crops rarely contained VLP and none were found in isolates from Barnfield where take-all is increasing in cereals grown after over 100 years without them. Elsewhere at Rothamsted, VLP occurred where take-all was slight, severe in patches,, generally severe or in 'decline'. *G. graminis* isolates from sites where decline had occurred were less pathogenic than from other sites but the occurrence of VLP was not consistently associated with this, with weak pathogenicity or with isolates of unusual growth, morphology, pigmentation, lysis or readiness to form perithecia. However, two trends seemed consistent; first, isolates with either particle alone were more pathogenic and those with both particles less pathogenic than isolates without VLP, second, the proportion of isolates with both particles increased during consecutive cereal cropping. Isolates did not gain or lose VLP during infection and re-isolation from wheat seedlings grown in infested sand, but were freed from VLP either by culturing ascospores or growing hyphal tips excised from colonies kept near their thermal death point. Colonies acquired VLP after anastomosis with 'infected' isolates. Very few VLP were seen in extracts from uninvaded parts of take-all infected wheat roots from crops in any stage of consecutive cropping or from seedlings grown in the glasshouse, suggesting that the VLP do not multiply in wheat cells. Attempts to infect mechanically VLP-free isolates and a range of virus-indicator plants were unsuccessful. We conclude that VLP in *G. graminis* are not the sole cause of take-all decline. (Rawlinson, Pearson and Hornby)

Soil N content was not related to severity of take-all or the number of wheat and barley crops grown consecutively on different plots on Little Knott I. Closer relationships existed between take-all, NH_4^+–N and NO_3^-–N in rhizosphere soil and microbial populations on the rhizoplane. Loss of 'decline' during soil sterilisation did not support Gerlagh's hypothesis (*Laboratorium voor Fytopathologie, Wageningen, Mededeling* 241, 1968). Our results suggest that 'decline' results from changes in the root microflora that accompany developing take-all and eventually limit the disease nutritionally.

This hypothesis depends on complex interactions between host and environment where each factor needs investigating. Like Gerlagh and others we found inhibition of *G. graminis* increased in soil repeatedly infested with the fungus, although we grew non-susceptible oat seedlings rather than leaving the soil fallow. The tests were atypical of field conditions because a blue-green alga (*Oscillatoria* sp.) increased and may have inhibited the disease abnormally. (Pearson and Hornby, with M. E. Brown, Soil Microbiology Department)

Spread of take-all. The distances to which *G. graminis* may grow, or be carried, help to determine how quickly it can spread from a few sources to infect all plants in a cereal crop. Plots of wheat on sites initially containing little or no inoculum were infested in winter with line sources of take-all infested soil so that during growth spread could be detected both along and between crop rows. Infections were only increased significantly in samples taken within 18 cm of the sources, although take-all was not severe in the crops of 1971 or 1972.

The short spread observed in these experiments presumably resulted from hyphal growth along and between host roots, because machines were excluded during the test period. Their effects were measured in another experiment where a line source of infective soil was placed 6 cm below the soil surface in a trench 30 cm wide and across the direction in which the plots were always power-harrowed, drilled and rigid-tine harrowed. Again

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no spread occurred further than 18 cm, in the direction contrary to machine movement but infections were found erratically distributed up to 3·5 m from the source in the direction where machines could have moved soil and infective fragments. (Prew)

Take-all on Barnfield. Barnfield, the site of the 'classical' experiment begun by Lawes and Gilbert to measure the effects of fertilisers and manures on root crops, carried no cereal crop from 1856 to 1967. In 1968 a four-course rotation (spring barley, sugar beet, spring wheat, potatoes) was introduced on part of the field, with each of the fertiliser plots split to carry two phases of the rotation each year, the phases paired so that wheat and barley were grown in 1968, 1970 and 1972, and sugar beet and potatoes in 1969 and 1971. To measure how soon take-all appeared and developed on land free from cereals for 112 years, plants from six 30 cm lengths of row from 14 plots of wheat and barley given different fertiliser treatments were examined in early July in 1968, 1970 and 1972. None of the plots was close to hedgerows or grass paths which might harbour grass hosts of the take-all fungus, and the area was less infested by grass weeds than most old arable fields. No take-all symptoms were seen in 1968 (1451 plants examined) but 4% of plants had take-all in 1970 (1183 examined) and 10% in 1972 (1750 examined). Wheat and barley were equally attacked, and the fertilisers seemed not to affect the incidence of take-all, although our sampling was not enough to measure this precisely because the distribution of take-all was very patchy.

Although none of the wheat or barley crops was seriously damaged by take-all, the disease became common much sooner than expected, especially as cereals were not grown in successive years. The amount of infection in the 1972 crops was similar (though less evenly distributed) to that expected in crops grown after one year without wheat or barley on old arable fields previously cropped frequently with wheat or barley. On such fields at Rothamsted a second wheat crop is usually severely attacked by take-all, so it seems likely that it would now be at risk on Barnfield. To obtain more information on this, 20 soil cores (5 cm diam. × approximately 12 cm deep) were taken in September, before ploughing, from four plots on Barnfield. The cores were inverted into pots, sown with ten chitted seeds of Cappelle-Desprez wheat and these were grown for 35 days under artificial light, at 15°C for a 16 hours day and 10°C at night. Table 7 shows the proportion of cores showing take-all, and the average percentage of roots infected. Similar assays on other soils at Rothamsted suggest that the inoculum in Barnfield soil was enough to cause severe take-all in a field crop of winter wheat. We cannot explain why soils were less infested after barley than after wheat, though the crops seemed equally infected in the field. (Slope and Gutteridge)

TABLE 7

Occurrence of take-all on wheat assay seedlings grown in Barnfield soil

Fertiliser to field crop	Crop in 1972	No. of cores infecting assay seedlings/20	% roots infected	
			all cores	infective cores
Nil	Wheat	13	29	45
Nil	Barley	9	15	34
NPKNaMg*	Wheat	19	48	50
NPKNaMg	Barley	10	18	37

* NPKNaMg = 96 kg N, 73 kg P_2O_5, 275 K_2O, 90 kg Na, 22 kg Mg/ha

'Break-crops' as a preparation for wheat. The effects of different non-susceptible ('break') crops between sequences of cereals susceptible to take-all and eyespot were assessed in three experiments, the yields from which are in *Rothamsted Report for 1970*,

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Part 1, 236, *Rothamsted Report for 1971*, Part 1, 258, and Field Experiments Section Report Table 21 (p. 255). Take-all was abundant and severe after barley in all experiments. After break crops take-all was not serious, and prevalent only on Geescroft where *Agrostis gigantea* was common. Type of break crop or undersowing with trefoil seemed not to affect take-all (see p. 255). In 1970 and 1971 break crops did little to decrease eyespot but in 1972, although eyespot was more severe generally, there was less after oats than barley (Table 8). Doubling the amount of nitrogen fertiliser given to cereals in the treatment ('break-crop') year had no effect on wheat diseases or yields the following year. By contrast, increasing nitrogen to the test wheat crops increased mildew and number of shoots/plant but decreased the severity of eyespot and take-all (Table 9). On Long Hoos field the effects of nitrogen were negligible perhaps because it was applied late and was followed by dry weather. (Prew and G. V. Dyke, Field Experiments Section)

TABLE 8

Effect of previous cropping on the incidence of take-all and eyespot on winter wheat test crops (results from the smaller N dressing to treatment crops only are included)

	Long Hoos 1970		Geescroft 1971		Fosters West 1972	
Treatment cropping	% plants with take-all	% straws with eyespot	% plants with take-all	% straws with eyespot	% plants with take-all	% straws with eyespot
Barley	91 (43)	10 (3)	83 (51)	51 (27)	78 (43)	89 (74)
Barley u/s Trefoil	93 (52)	12 (4)	89 (58)	49 (26)	81 (48)	91 (78)
Oats	6 (1)	9 (2)	17 (5)	37 (17)	4 (2)	70 (49)
Oats u/s Trefoil	4 (0)	6 (2)	24 (9)	23 (11)	2 (1)	66 (46)
Beans	6 (0)	8 (2)	14 (3)	40 (21)	6 (2)	85 (68)
Clover	9 (2)	8 (1)	16 (5)	38 (20)	6 (3)	88 (72)
Maize	—	—	13 (2)	25 (13)	2 (0)	81 (59)

Figures in brackets are percentage plants or straws moderately and severely infected

TABLE 9

Effect of nitrogen applied to winter wheat on take-all and eyespot

(Means of all cropping treatments)

	Geescroft 1971 Nitrogen kg/ha				Fosters West 1972 Nitrogen kg/ha			
	0	50	100	150	0	50	100	150
Take-all[1] Total	42	34	32	28	24	22	18	16
Moderate + Severe	21	17	15	12	14	12	9	7
Eyespot[2] Slight	16	21	21	20	13	16	19	22
Moderate	16	17	16	14	29	40	46	41
Severe	7	5	4	4	35	30	22	17

[1] Percentage plants infected
[2] Percentage straws infected

Effects of frequent cropping with wheat and barley. The 'Wheat after Intensive Barley' experiment on Little Knott field ended in 1972 with the second of two crops of Joss Cambier winter wheat grown on all sequences. After harvest in 1970 soil pH had been

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measured on each half plot (range, pH 5·6 to 7·4, in water) and 12·6 t/ha of ground chalk was applied to half of every plot before ploughing. Table 10 shows the grain yield and take-all disease of wheat grown after the ten crop sequences. Both in 1971 and 1972 the third successive wheat (W3) yielded least and had most take-all. Yields increased and take-all declined where more than three wheat or barley crops had been grown without a break; in 1971, no other sequence yielded as much as wheat after fallow (W1). The incidence of take-all was estimated from eight samples of 30 cm of crop row (100–120 plants) from each of 16 plots per sequence. Estimates for individual plots differed greatly with some W12 plots having much more than some W3 plots. The sampling was too little to estimate take-all on single plots accurately so we do not know if these differences were real, but there is no doubt that on average take-all became less severe in wheat following many susceptible crops, or that some plants in these crops were severely infected.

TABLE 10

Effect of the number of previous wheat or barley crops on grain yield (t/ha, 85% DM) and on the percentage of plants with take-all in winter wheat

Previous cropping			1971			1972		
1968	1969	1970	Crop	Yield	% take-all	Crop	Yield	% take-all
sB.6	W.7	F	W.1[1]	6·87	2 (1)[2]	W.2	5·05	49 (23)
sB.1	F	W.1	W.2	6·14	80 (54)	W.3	4·45	89 (54)
F	W.1	W.2	W.3	5·31	84 (52)	W.4	4·54	78 (38)
sB.1	W.2	W.3	W.4	5·77	75 (43)	W.5	4·63	77 (33)
sB.2	W.3	W.4	W.5	5·91	61 (24)	W.6	4·93	64 (23)
sB.3	W.4	W.5	W.6	6·16	58 (21)	W.7	4·95	60 (19)
sB.4	W.5	W.6	W.7	5·87	56 (24)	W.8	5·00	61 (21)
sB.5	W.6	W.7	W.8	6·09	50 (20)	W.9	5·18	51 (14)
sB.9	W.10	W.11	W.12	6·06	48 (23)	W.13	4·83	50 (17)
sW.9	W.10	W.11	W.12	5·96	52 (18)	W.13	4·77	53 (18)

[1] Crop symbols: W, winter wheat; sW, spring wheat; sB, spring barley; F, fallow. Numbers after crop symbols indicate the number of successive wheat or barley crops

[2] Numbers without brackets are total infection, within brackets moderate plus severe infections

Lodging was unimportant. Eyespot was little affected by crop sequences but was prevalent, affecting 43% of straws even after fallow (W1) in 1971 and averaging 46 and 43% of straws infected in 1971 and 1972 respectively. Take-all, eyespot and grain yields were little affected by the residues of different amounts of nitrogen applied between 1961 and 1970 or by liming in 1971, although lime increased average yield by 0·3 t/ha in 1972. Attacks of yellow rust on Joss Cambier were slight in 1971 but more severe in 1972. Blackgrass (*Alopecurus myosuroides*) was common despite pre-emergence applications of terbutryne.

A caution to farmers. In experiments in Little Knott and Great Field at Rothamsted, Stackyard field at Woburn, and Harwood Field at Saxmundham, take-all infection became maximal in the second, third or fourth successive wheat or barley crops but with more successive crops take-all declined and yields usually increased. All these fields had long been in arable crops, over half of them wheat or barley and some severely attacked by take-all. Thus the take-all fungus was general in the soil when our experiments began and populations soon increased when successive susceptible crops were grown. By contrast, where wheat or barley have not been grown for many years (e.g. after old pasture or long leys) the take-all fungus may be rare and erratically distributed and so may

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increase only slowly during the first few susceptible crops, especially if these are spring sown. In such circumstances, the worst take-all may not occur until the sixth to eighth successive crop, and then decline a year or two later still. For example, in 1972 winter wheat had to be grown (against our advice) in a field at Rothamsted where it was the sixth successive susceptible cereal after old grass. Take-all attacked 80% of the plants moderately or severely and yields were small (average 3·4 t/ha). On farms on other soil types we often find take-all developing in this way. However, it is difficult to predict the timing of decline. We also know puzzling exceptions where the disease has not developed as expected or has become prevalent but failed to decrease yield proportionally. Farmers who wish to grow mostly wheat or barley may benefit from harnessing take-all decline, but the venture may be hazardous, especially with winter wheat, unless it is certain that the 'decline' situation is established. Farmers who decide to grow wheat in this way should seek specialist advice and begin cautiously. (Slope and Broom)

Other diseases of cereals

Chytrid and other fungi on roots of winter wheat. Table 11 shows that the mycorrhizal *Endogone* spp. were usually the most prevalent group colonising 'root segments' of winter wheat (Cappelle-Desprez) from Broadbalk plots in sections following wheat, field beans and fallow, and were commonest in starved soil (plot 3). *Pythium* spp. and *Olpidium* spp. were both commonest in June and *Olpidium* spp. favoured generously manured plants. Roots from plots continuously cropped with wheat had more *Pythium*, *Olpidium* and *Endogone* than from wheat after fallow or beans. The identity of 'brown runner hyphae' was not determined by isolation in pure culture, but mycelial characters suggested that at least three different fungi were represented, *Gaeumannomyces graminis*, *Aureobasidium bolleyi* and *Phialophora radicicola*, in this order of frequency. The association of runner hyphae with vascular discolouration of the root was regarded as reasonable evidence for the presence of *G. graminis*, but in the absence of vascular symptoms the three fungi could not be distinguished confidently.

Runner hyphae were commonest in unmanured soil, and surprisingly more abundant in wheat following a potato–beans break (17% of roots) than after wheat (3%) or fallow (3%), on each of the four sampling dates. Because the runner hyphae on wheat after beans were associated with vascular discolouration many of them probably were *G. graminis*.

TABLE 11

Root segments[1] *(%) of Broadbalk winter wheat colonised by fungi*[2]

	Date sampled				Manures			
	10 May	19 June	12 July	9 Aug	FYM+N (Plot 21)	FYM (Plot 22)	Nil (Plot 3)	NPK (Plot 8)
Brown runner hyphae	5	11	4	11	3	8	12	8
Pythium spp.	22	41	31	24	20	35	29	33
Olpidium spp.	26	35	29	26	31	33	22	31
Endogone spp.	39	44	36	48	30	45	57	34

[1] See *Rothamsted Report for 1970*, Part 1, 135
[2] A total of 360 root segments were examined on each date and from each plot

Polymyxa graminis occurred again on winter wheat on the same two plots of Pennel's Piece as last year (*Rothamsted Report for 1971*, Part 1, 147), indicating that its distribution probably depends more on local soil conditions than previous oat crops. (Salt)

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***Eyespot on* Triticale.** If forms with fatter grain can be selected, *Triticale* wheat-rye hybrids might provide useful alternatives to wheat and barley, especially if they were not susceptible to the same diseases. Plants of Cappelle-Desprez winter wheat and *Triticale* were grown in an unheated greenhouse and inoculated with the eyespot fungus *Cercosporella herpotrichoides* by splashing spores from discs of agar cultures during watering. Isolates from wheat and oats, but not from rye, were tested.

At flowering, *Triticale* was less attacked than Cappelle-Desprez by both isolates (percentage straws infected by wheat isolate, Triticale 55, Cappelle-Desprez 91; by oat isolate, Triticale 0, Cappelle-Desprez 37). The isolate from oats produced fewer spores than that from wheat and this may explain the less frequent infection of both hosts. (Gutteridge and Slope)

Airborne ascospores from a barley leaf fungus. A Burkard model of the Hirst spore trap was operated 50 cm above ground in a garden near Blandford, Dorset, to investigate the air-spora in the vicinity of a patient who suffered asthma when close to large areas of ripening barley. During July and August many hyaline, 1-septate ascospores occurred regularly at night, reaching a maximum concentration of approximately $2 \times 10^6/m^3$ of air early in August. The ascospores resembled those reported by Last (*Transactions of the British Mycological Society* (1955) **38** 453–464) at Rothamsted, and by Corbaz (*Phytopathologische Zeitschrift* (1969) **66**, 69–79) in Switzerland.

The ascospores apparently orginated from a fungus with hyaline, immersed perithecia abundant in every senescent barley leaf examined. Perithecia with asci occurred in the flag leaves of barley collected at Blandford on 3 August. Perithecia were irregularly distributed over leaves and averaged $100/cm^2$ at Rothamsted and $300/cm^2$ near Blandford. The identity of the fungus and its significance to allergy and plant pathology are being investigated. (Gregory)

Fungicide experiments. Dressing seed of Cama winter wheat with a thiophanate fungicide ('NF 48') at 12 g a.i./kg seed, decreased mildew greatly until mid-June but not a month later. Few other chemicals tested have had such a large and lasting effect on wheat.

Spraying 'BAS 3170 F' on Cama winter wheat on 23 June when yellow rust (*Puccinia striiformis*) was prevalent, decreased the infected area of the second youngest leaves from 15·4% to 7·5% on 21 July (G.S. 11·1). 'BAS 3170 F' also almost eliminated brown rust (*Puccinia hordei*) from top leaves of spring barley although little rust occurred on unsprayed plants (1% of area of the top two leaves on 25 July).

A stock of spring barley seed that produced 3200 smutted ears/100 m^2 produced only 80/100 m^2 after seed was treated with 'Vitavax' (carboxin) and thiram or 40/100 m^2 with 'BAS 3302 F'. (Jenkyn and Prew)

Effects of formalin soil fumigation on wheat. A three-year comparison of the effect of formalin soil fumigation on winter and spring wheat was completed. Effects on yield were smaller than in previous experiments (*Rothamsted Report for 1970*, Part 2, 138). Crops at Rothamsted yielded more than those at Woburn, where disease and pest attacks were more severe and formalin significantly increased yield. However, fumigating soil never produced yields equal to those harvested on disease-free fields of the same farm.

Table 12 shows that in 1972, at Woburn, formalin failed to decrease damage from cereal cyst nematodes or numbers of their eggs in soil at harvest, but halved take-all in winter wheat and significantly increased grain yield. Once again the tests showed that *G. graminis* redevelops much more quickly after fumigating and subsequent crops may be as badly or worse attacked than untreated crops. (Salt)

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TABLE 12

Effect of formalin on yield, diseases and pests of winter and spring wheat

Site	Rothamsted				Woburn			
Crop	Winter wheat		Spring wheat		Winter wheat		Spring wheat	
Time of sowing	Autumn	Spring	Spring	Spring	Autumn	Spring	Spring	Spring
Formalin applied	Autumn	Autumn	Autumn	Spring	Autumn	Autumn	Autumn	Spring
Yield, t/ha								
No formalin	4·57	3·51	3·82	4·05	1·96	1·51	1·63	1·61
Formalin in 1972	4·84	3·59	4·34	4·28	3·29	1·78	1·77	1.49
No formalin	4·82	3·50	4·05	4·25	2·96	1·66	1·82	1·55
Formalin in 1971	4·59	3·60	4·11	4·09	2·29	1·63	1·57	1·55
S.E.D.	±0·233 (V)*		±0·482 (HI)*		±0·193 (V)*		±0·191 (HI*)	
% take-all (straws in July)								
No formalin	15	5	13	4	44	2	1	2
Formalin in 1972	8	4	4	11	22	2	11	3
No formalin	10	6	13	4	26	0	2	2
Formalin in 1971	12	3	4	11	40	3	10	4
% plants with roots deformed by cereal cyst nematodes								
No formalin	0	0	0	0	0	11	26	29
Formalin in 1972	0	0	0	0	3	6	11	33
Post-harvest count of nematode eggs/g soil								
No formalin	—	—	—	—	1·2	4·2	9·4	12·3
Formalin in 1972	—	—	—	—	1·4	7·8	13·2	12·6

* Standard error of differences: V for vertical comparison, HI for horizontal and interactions

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

Virus diseases

Incidence in 1972. At Rothamsted, viruses were less common and damaging than in the three previous years. Several crops had fewer than 4% of Maris Bead plants infected with the aphid-transmitted bean leaf roll virus (BLRV) by July, and less than 3% with the weevil-transmitted broad bean stain (BBSV) and Echtes Ackerböhnemosaik (EAMV) viruses. Adult *Apion vorax*, the main vector of BBSV and EAMV, were found in mid-May, but, in June, their number was only 7% of that in 1971 and 2·5% of that in 1970, possibly because cold weather in spring and early summer prevented movement to crops.

Viruses seemed scarce elsewhere. During July, incidence of BLRV in nine spring varieties grown by the National Institute of Agricultural Botany ranged from 1–5% at Cambridge, 2–10% at Sparsholt, Hants, and 2–18% at Terrington, Norfolk, and BBSV/EAMV ranged from 2–11%, 2–24% and 0·4–3%, respectively, at the three sites.

Effects of BBSV and EAMV on yield. Maris Bead plants that grew from seed infected with BBSV or EAMV yielded, respectively, 94 and 89% less than uninfected plants. In the same crop, initially healthy plants that developed symptoms before flowering yielded 70 and 89% less than uninfected plants, and plants that developed symptoms after flowering 46 and 39% less.

Transmission of BBSV and EAMV by weevils. Glasshouse tests confirmed that *Apion vorax* is the best vector known and that weevils transmit EAMV more readily than BBSV. BBSV was transmitted by 13% of *A. vorax* and 2% of *A. aestivum*, but not by *A. aethiops*, *A. apricans*, *A. assimile*, *A. pisi* or *S. lineatus*, when caged for five days on infected beans and then five days on healthy seedlings. EAMV was transmitted by 44% of *A. vorax*, 7% of *A. aethiops* and 3% of *S. lineatus*, but not by *A. aestivum*, *A. apricans*,

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A. assimile or *A. pisi*. *A. vorax* were fed for four days on infected plants and then transferred to healthy seedlings every four days. BBSV was transmitted by 12% in 0–4 days, 8% in 4–8 days, 4% in 8–12 days and none thereafter. Comparable figures for EAMV were 28%, 8%, 12% and 0. We do not know if the viruses persist on the mouthparts or are regurgitated from the gut of the weevil but the results are important because they suggest that migrating weevils remain infective long enough to spread the viruses over long distances.

Although *S. lineatus* is the most common weevil on field beans it rarely transmits EAMV and has only twice transmitted BBSV in glasshouse tests. It seems unlikely that the viruses are inactivated in its body because BBSV was recovered from 15–50%, and EAMV from 25–60%, of *S. lineatus* caged for 1–16 days on infected plants, ground in phosphate buffer and manually inoculated to healthy seedlings. *S. lineatus* fed for similar periods on infected plants did not transmit BBSV, but 3% transmitted EAMV within five days.

Catches of air-borne* Apion *spp. and* Sitona *spp. Between March and October all species of *Apion* and *Sitona* caught in 14 of the suction traps operated in different parts of the country by the Rothamsted Insect Survey (p. 195) were identified and counted. Although there were few *Apion* on bean crops in 1972, 12 species were caught, the commonest *A. aestivum* at five sites. Only two *A. vorax* were caught, one at Broom's Barn in May and one at Rothamsted in August. *S. lineatus* was the commonest of four *Sitona* spp. and was trapped at eight sites but most often at Broom's Barn and Rothamsted where it was caught each month.

Seed transmission of BBSV and EAMV. BBSV is more often transmitted by seeds than EAMV but the proportion of seeds infected with either virus seems to decrease during maturation. Immature seeds that were approximately 2·5, 5·0, 7·5 and 10·0 mm wide were taken from infected Maris Bead plants grown in the glasshouse; respectively, 54, 37, 29 and 23% were shown to be infected with BBSV, and 7, 3, 2 and 2% with EAMV, when ground in phosphate buffer and manually inoculated to French beans. Mature seeds from the plants produced 7% of seedlings infected with BBSV and 1% with EAMV. Unfortunately the decrease ceases at maturity because seed kept for a year at 15°C produced as many infected seedlings as seed just matured. So seed stocks cannot be freed from infection by storage.

Heat therapy of BBSV/EAMV infected seed. Attempts to prevent the emergence of seedlings infected with BBSV or EAMV were less promising than in 1971. Without treatment, one stock of Maris Bead produced 2·4% of infected seedlings, whereas seed kept for one to eight days at 44 to 48°C produced 1·4 to 2·0% of infected seedlings when grown in the field; comparable figures for a second stock were 0·6% unheated and 0·2 to 0·5% from heated seeds.

However, laboratory tests have recently shown that field bean seeds tolerate temperatures close to the thermal inactivation points (ten minutes) of BBSV and EAMV, respectively 60–65° and 65–75°C. Germination of Maris Bead seed (initial water content 11%) after one to eight days at 60° or 65°C ranged from 98–100% and 91–97% respectively, compared with 98% germination of unheated seed. Comparable figures for seed of Throws MS (14% water content) were 69–77% and 65–75%, compared with 75% germination of unheated seed. One day at 70°C killed almost all seeds. Several days at 60–65°C may rid seed stocks of infection more completely than the lower temperatures used previously.

Effects of roguing on seed infection. Samples of 1600 seeds were germinated from plots of Maris Bead (0·6% seed infection) which were untouched or from which infected

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plants were rogued once in mid-May or seven times between May and August; the number of infected seedlings were, respectively, 5, 2 and 0. During July, once-rogued and unrogued plots had, respectively, 1·3 and 1·4% of plants infected. Intensive roguing may be effective and practical for elite stocks with few infected seeds, in years when *A. vorax* is as uncommon as in 1972, but in many years may be ineffective and uneconomic. (Cockbain, Cook and Vorra-urai)

Effects of chemicals on virus diseases and yield. Viruses spread little even in untreated plots of Maris Bead sown with seed of which about 0·6% were infected with BBSV/EAMV or in similar plots treated with aldicarb (4·5 kg/ha a.i.), γ-BHC (2·2 kg/ha) 'Dexon' (78 kg/ha) or dieldrin (2·2 kg/ha). *A. vorax* was rare, only 5/100 m of untreated row in June, and the viruses infected only 1·1% of untreated plants by July. BLRV-infection ranged from 0·6% in plots with aldicarb to 3% in plots with 'Dexon' or no treatment. Aldicarb increased yield from 3·43 to 3·95 t/ha (P = 0·01), possibly because it controlled *Ditylenchus dipsaci* (p. 167) and 'Dexon' increased yield to 3·81 t/ha (P = 0·02), possibly because it contained nitrogen equivalent to 13 kg/ha, although not all may be in an available form.

Viruses were also scarce, and wilt and *D. dipsaci* lacking, in a similar experiment sown with virus-free seed of Minor. Here, no plants showed BBSV/EAMV symptoms in July and less than 0·1% in August 'Dexon' increased yield from 3·58 to 3·90 t/ha (P=0·01) and dieldrin to 3·92 t/ha. These increases are difficult to explain because BHC was as effective as dieldrin in controlling larvae of *Sitona* spp. (p. 217) but, like aldicarb, did not increase yield significantly. (Cockbain, Salt and Hornby)

During 1970, 1971 and 1972 several chemicals, but especially aldicarb, increased yields from field beans but it was impossible to apportion the benefit between several possible causes. It now seems that decreasing pests and disease must be entirely responsible because aldicarb had no effect on growth and yield of healthy beans grown in pots containing aldicarb equivalent to 0, 2·8, 5·6 and 11·2 kg/ha a.i. (Cockbain and M. F. Smith)

Other pests and diseases. Field beans have been grown on part of Barnfield each year since 1967, with the intention of increasing pathogen populations. However, in 1972 virus diseases, root-rots and wilting were less serious than previously. The experiment described in *Rothamsted Report for 1971*, Part 1, 152, was repeated. In untreated plots wilt was first noticed on 3 July and increased until early August when 12% of plants were wilted, compared to 45% in 1971, and yielded twice as much (Table 13).

TABLE 13

Effects of chemicals on yield, pests and diseases of field beans, Barnfield 1972

Treatment		Yield t/ha	% plants wilted 4 August	% plants infected with *D. dipsaci* 9 August	% lateral roots discoloured 2 August
Nil	(−)	2·98	11·8	81	53
'Dexon'	(F)	3·31	6·7	89	23
aldicarb	(N)	3·84	1·4	2	8
γ-BHC	(I)	3·51	8·7	78	21
formalin	(B)	3·46	8·5	87	14
F + N + B		4·37	1·2	7	30
Standard error of difference		±0·303			

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Aldicarb again increased yield most, about a quarter of the difference was because individual beans grew larger. No treatment greatly increased the number of pods per plant or of stems per plot. However, aldicarb, 'Dexon' and formalin produced plants that were 8% taller than the others on 20 July, and had thicker tap roots. Plants treated with aldicarb had the thickest stems and bushiest root systems, and were the ones with least stem eelworm (*D. dipsaci*) and least leaf damage by *S. lineatus*. The whitest tap roots and healthiest laterals were on plants treated with aldicarb alone, but where this was combined with 'Dexon' and formalin they were more discoloured (Table 13). By contrast, 'Dexon' and formalin decreased mycorrhizal infection by *Endogone* spp. 'Dexon' decreased root rot by half but increased yield only slightly, whereas in other experiments (p. 144) where root rot was absent, it increased yield significantly. Mycorrhizal infection was least and yield was greatest where the three chemicals were combined. (Salt and Hornby)

Diseases of grasses

Grassland occupies approximately two-thirds of the agricultural land of England and Wales. Pathologists have studied diseases of pasture plants quite extensively but have never studied their effects or agriculture importance in proportion to the extent or value of the grass crop. This may be explained partly by the facts that much grassland is 'rough grazing' and until recently little has been managed intensively; much grass is grown in mixed communities where it is very difficult to measure the effect of diseases and where diseases may not be so devastating as in single species crops. Intensively managed temporary grassland usually has swards of one or few species so these limitations apply much less. As management improves and nutrition becomes more generally adequate, diseases will increasingly become the factor limiting yield. As Italian and perennial ryegrass comprise over 80% of all the grass seed sown in Britain, virus diseases and fungi attacking their roots and foliage were obvious starting points for work planned jointly with the Grassland Research Institute and the Welsh Plant Breeding Station early in 1972. In this report we can mention little but initial observations, some promising, some less so.

Virus diseases of ryegrass

Viruses occurring in ryegrass. For descriptions, see p. 124.

Incidence of ryegrass mosaic virus. Reports suggest that the disease may have become more prevalent and severe in recent years. Collaborative measurements of incidence were made at a number of trials by the National Institute of Agricultural Botany and at grassland manuring trials done jointly by the Agricultural Development and Advisory Service and the Grassland Research Institute.

Rothamsted staff examined manuring trials in Devon, Somerset and Hereford and found less than 10% of plants infected on plots that received 160 or 320 units of nitrogen. At Seale Hayne, Devon, varieties differed little and the worst had 10% of plants infected. There was rather less infection in plots sown in 1971 than in 1970 but all the symptoms were mild and lacked necrosis. (Plumb)

Mite transmission of ryegrass mosaic virus. Mulligan (*Annals of Applied Biology* (1960) **48**, 575–579) reported transmission of RMV by mites. This has been confirmed with an eriophyid mite tentatively identified as *Abacarus hystrix* which transmitted the disease to healthy Italian rye grass after being reared on infected plants.

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The mite feeds mainly on the epidermal cells lining interveinal grooves on the adaxial leaf surface. The scanning electron microscope shows tracks made by the mite in the leaf surface waxes and, where it fed, roughly circular areas 5 μm across with a small central hole where the cheliceral stylets had been inserted through the epidermal cell wall. (Gibson and Turner)

Other diseases

Fungicides applied to old pasture. If fungicides increased the yields from pasture it would suggest that they suffered from fungus diseases. To test this and gain experience in examining grass roots, we measured the effects of three fungicides (benomyl, carboxin and mancozeb) and two amounts of nitrogen fertiliser on an old pasture (sown in 1945; now with predominant grasses: *Alopecurus pratensis*, *Dactylis glomerata*, *Agrostis* spp., *Festuca rubra*, *Poa* spp.). Details of treatments and yields are published in *Yields of the Field Experiments for 1971* and *for 1972*. Serious diseases of roots or foliage seemed absent and this may explain why no treatment increased average yield. After the first cuts, patches of grass, larger with most nitrogen (total of 414 kg N/ha), failed to regrow. None of the fungicides decreased these patches.

Examining grass roots from old swards proved very difficult. Sample soil cores were taken during 1971 but separating live roots from the debris accumulated in the surface mat was laborious. Roots of *F. rubra* and *A. pratensis* were red-brown in colour so that it was difficult to see lesions on them; other young roots seemed free from lesions but most old roots were dark brown or black. (Broom, Slope and Gutteridge)

Potato diseases

Development and infection of tuber lenticels. Proliferation of lenticels may increase the susceptibility of tubers to several pathogens. For laboratory tests it is induced by wrapping tubers in wet paper towelling, but lenticels are more difficult to proliferate as growing tubers age and storage continues. Proliferation was always faster on tubers of Majestic and King Edward than Pentland Crown; on tubers with developing sprouts than tubers from which the eyes had been removed; and was hastened by raising temperature between 10 and 20°C.

A glasshouse experiment and data from previous field experiments suggested that *Streptomyces scabies* could not cause common scab infections on the two apical internodes of young tubers but that this was possible on the third to fifth internodes. The susceptible region corresponds to about a week's growth and, on Majestic, King Edward and Pentland Crown, represents the interval in the transition of a stoma to a lenticel from the loss of the guard cells to the suberisation of the walls of the outermost cells plugging the lenticel. The results of field trials with common scab may be further explained by evidence that other soil micro-organisms decrease the growth of *S. scabies*, especially in wet soil.

Tubers were lifted at intervals throughout growth without pretreatment, after three weeks protected from rain or after three weeks watering to keep soil moisture tension < 10 cm Hg. Lenticels were then tested for permeability to fluorescein solution and susceptibility to *Phytophthora infestans* and *Erwinia carotovora* var *atroseptica*. As the tubers aged, fewer were penetrated by fluorescein or infected by the pathogens, but altering soil moisture during the previous three weeks had no effect, despite proliferation of lenticels in early samples from wet soil. The results suggest that wet soil affects these diseases more by influencing the growth and transport of the pathogen than by altering the susceptibility of the host. (Adams)

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Bacterial soft rots. After April the growing season of 1972 was cooler, duller and drier than average, with heavy rain only early in August and on 8 September. The season was unusual because soil moisture deficits increased after June in soils that were cooler than average. Only during late July and late August were temperatures for long over 16°C, a limit said to decrease populations of *Erwinia carotovora* var. *atroseptica* in soil. Blackleg was prevalent but the pathogen spread little.

In the last of a series of experiments aimed to explain why not all infected seed tubers that produce plants also show blackleg, the yield from Majestic and Pentland Crown (initially believed free from *Erwinia*) was smaller when stab-inoculated at the heel end. Most of the difference (*c.* 10–12 t/ha for both varieties) could be explained by the failure of different proportions of the plants to emerge. However infected plants without obvious blackleg symptoms also probably affected yield, for although chitted Majestic tubers inoculated at the middle or heel end produced equal numbers of plants that emerged and showed blackleg, those inoculated in the middle produced more weak plants than heel end inoculations and yielded *c.* 5 t/ha less.

The bacterial decay of seed tubers is thought often to be a major source of contamination to their progeny. Tubers initially free from *E.c.* var. *atroseptica* were inoculated as in 1970 and 1971, to test when decay occurred and its consequences. Most inoculated seed tubers had rotted by late July but the bacterium could be found in only a few immature progeny tubers, although many of them rotted with soil-inhabiting *Pseudomonas* spp. which may be more important in these circumstances than thought previously. Many uninoculated seed tubers remained intact until lifting because, unlike 1971, *Phytophthora erythroseptica* was absent.

Merchants who have to maintain supermarket contracts complain that during July and August when they are forced to lift, wash and pack immature King Edward potatoes into polythene bags, losses from soft rot can be severe. To study this problem, crops from 'healthy' and commercial seed were tested periodically for susceptibility to soft rots. Many tubers rotted when lifted in July and stored wet, in conditions conducive to soft-rotting, but the proportion decreased as the crop matured. Comparable samples were similarly lifted and tested ten days after the haulm was removed, when rotting was similar; or dug and stored for ten days before wetting and testing, when rotting was largely avoided. Isolations made from rots showed a few to be caused by *Erwinia* spp. but most by *Pseudomonas* spp.; the numbers of tubers rotted by *Pseudomonas* spp. was independent of the health of the stock. (Lapwood and Legg)

Field experiments with healthier seed. Since we showed that 'healthier' seed tubers can be produced from rooted stem cuttings we have made many experiments to measure their effects on crops. Yield increases have not been so dramatic as some who complained about diseased stocks had predicted. However, the real benefits also include maintaining the health of seed stocks, and decreasing losses in store, at dressing or planting.

Maintaining freedom from fungal pathogens. On a farm in Scotland we have used organo-mercurial fungicide dips since 1965 and later benomyl and thiabendazole dusts to delay the re-introduction of fungal pathogens to VT seed tuber stocks derived both by tuber selection and from stem cuttings rooted in 1964 and 1965. *Oospora* and *Helminthosporium* were virtually eliminated and *Phoma exigua* var. *foveata* very nearly so. Soil inhabiting pathogens were controlled less (*Rhizoctonia solani*) or not at all (*Streptomyces scabies*, *Spongospora subterranea*). Similar results have been achieved with 'VTSC' (virus-tested stem cutting) stocks during the four years since they were first released by the Department of Agriculture for Scotland.

In comparable tests at High Mowthorpe Experimental Husbandry Farm, Yorkshire,

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benomyl has been applied annually since 1970 to commercial and healthier seed. During 1972 the differences noted in *Rothamsted Report for 1971*, Part 1, Table 21, 155, were maintained. However, results now available for gangrene in 1970 and 1971 show that this was less well controlled and the disease became equally common on the two stocks. Benomyl seemed to decrease gangrene on the variety Record but to increase it on Majestic and Pentland Crown. (Hide and Griffith)

Effect of chitting, fungicide and tuber diseases. The final experiment of a series which has compared the effects of chitting and fungicide (benomyl, 0·23 kg a.i./t of tubers) on commercial seed, healthier seed and healthier seed re-inoculated with *Oospora pustulans* and *Rhizoctonia solani*, gave different results from earlier experiments. Since 1969, chitting always increased yield more than other treatments but healthier seed averaged 6% more saleable yield than commercial seed (*Rothamsted Report for 1971*, Part 1, 156). Table 14 shows that in 1972 healthier seed out-yielded commercial by 10% at Rothamsted and 19% at Woburn, when unchitted but, abnormally, when chitted, healthier seed produced 3% less at Rothamsted and 12% less at Woburn than commercial.

TABLE 14

Effects of seed health, chitting and benomyl on saleable tubers (t/ha >4·4 cm)

	Rothamsted				Woburn			
	K.E.[1]	Mj.	P.C.	Rec.	Mj.	P.C.	Rec.	M.P.
Commercial not chitted	24·2	33·3	39·3	23·7	35·1	39·7	28·0	23·2
Healthier, not chitted	27·1	37·2	41·1	28·1	42·0	49·0	37·1	27·3
Commercial, not chitted, benomyl	17·4	37·6	37·3	22·8	41·8	38·8	28·9	21·4
Commercial, chitted	31·2	38·9	46·6	28·2	37·2	49·0	32·4	32·1
Healthier, chitted	29·3	39·9	45·2	26·1	39·7	46·4	22·2	23·6
Commercial, chitted, benomyl	29·8	40·1	44·6	29·4	38·0	46·0	30·1	32·7
Healthier, chitted, benomyl	22·8	37·8	39·2	26·0	50·5	49·1	29·1	28·8

[1]K.E. King Edward, Mj. Majestic, P.C. Pentland Crown, Rec. Record, M.P. Maris Piper
S.E.D. Rothamsted 2·57 (1·92 for comparison between varieties of same treatment); Woburn 3·78 (2·56 for comparison between varieties of the same treatment)

On both farms crops from chitted seed began maturing in August because large soil moisture deficits had accumulated in June and July. Crops from unchitted seed were later developing, withstood the dry soil better and so benefitted more from rain in August and September than plants from chitted seed. The anomalous results are therefore attributed to the unusual weather. (Hide, Bell, Griffith and Hirst)

Comparison of commercial and healthier seed stocks. Each year since 1967 experiments have compared a few stocks of up to five varieties at Rothamsted and Woburn. Even the commercial grade was probably superior to the national average because we were anxious not to incur the bias from badly diseased stocks which have recently been few. During 1972 we grew ten certified and ten once-grown stocks of both King Edward and Pentland Crown in comparison with healthier stocks. Table 15 shows that yields from the duplicated plots of some commercial stocks were as large as from healthier seed but total and saleable yields from King Edward commercial certified stocks averaged 8% and commercial once-grown 10% less than the healthier seed. The corresponding differences with Pentland Crown were much smaller, 4% and 1%. (Hide and Bell)

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TABLE 15

Comparison of yields from healthier, certified and once-grown seed (t/ha)

	Healthier	Certified		Once-grown	
	mean	mean	range	mean	range
King Edward					
Total	45·7	42·4	39·0–45·3	41·4	36·8–46·8
>4·4 cm	30·7	29·3	24·6–33·8	27·5	22·7–31·4
Pentland Crown					
Total	41·0	39·2	34·6–45·2	40·4	34·2–44·4
>4·4 cm	35·0	33·9	29·6–41·3	34·8	31·1–39·1

Effects of irrigation on tuber numbers, size and diseases. Early irrigation is necessary to control common scab and has been said to increase tuber numbers and decrease the proportion of ware tubers. Also, healthy plants of some varieties produce more tubers than diseased plants. The effect of irrigation on yield and tuber numbers was tested on healthier King Edward seed and seed once-grown from a certified stock. Plots were planted with large (120 g) or small (23 g) seed from each stock. Irrigation treatments were: (1) none; (2) 'early' from 75% plant emergence (19 May) at 15 mm soil moisture deficit until 'marble' stage (19 June) and then at 38 mm s.m.d.; (3) 'normal', from marble stage at 38 mm s.m.d.; (4) 'late', from 15 August at 38 mm s.m.d.

In September plants from large healthier seed yielded 10% more and small healthier seed 6% more than comparable once-grown seed. Irrigating increased total and ware yields and decreased total tuber numbers, but there was no difference in yield and numbers of tubers between 'early' and 'normal' irrigation. Irrigating 'late', as expected, increased *Oospora* and *Rhizoctonia* infection with little effect on yield or number of tubers, but it decreased infection by *Helminthosporium solani*. (Hide, Lapwood and Bell)

Agronomic effects of healthier seed potatoes. If more and smaller tubers from healthier King Edward seed meant less saleable yield and more wasted 'ground-keepers', then healthy seed would be a disadvantage to ware growers. Therefore we have tested, for three years, how much yield and tuber size and number can be influenced by planting fungicide-treated healthier seed of different sizes (*c.* 34 g and *c.* 100 g) at different spacings in wide or narrow rows and with conventional and extra fertiliser.

Table 16 shows that in 1972 small seed yielded most saleable tubers. When plant populations were approximately equal, total yields were greater from 71 cm (28 in.) rows than from 91 cm (36 in.) rows; with 34 500 plants/ha (14 000/acre). Saleable yields were greatest from narrow rows but with fewer plants there was no difference between row spacings. Our results in 1971 and 1972 conflict with those from Experimental Husbandry Farms (using commercial seed) because we found that decreasing plant population increased saleable yield. Doubling compound fertiliser (containing 13% N, 13% P_2O_5 and 20% K_2O) from 1500 to 3000 kg/ha (12 to 24 cwt/acre), increased saleable yield by more than 5 t/ha (2 tons/acre), but the increase with plants 30 cm (12 in.) apart was more than double that with plants 61 cm (24 in.) apart.

The greatest mean yield was from seed 51 cm (20 in.) apart in 91 cm (36 in.) rows, given 3000 kg/ha of fertiliser but the most surprising and important result was that in both years halving seed cost and plant population by planting at 61 cm (24 in.) apart in 91 cm (36 in.) rows instead of our conventional 41 cm (16 in.) spacing in 71 cm (28 in.) rows did not affect saleable ware yield. The lower cost wider spacing also increased

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TABLE 16

Effects of seed size, spacing and fertiliser on saleable yield (t/ha >4·4 cm)

	Rows at 71 cm Seed size			Rows at 91 cm Seed size		
	Small	Large	Mean	Small	Large	Mean
1500 kg/ha fertiliser						
Row spacing (cm)						
30	22·9	19·3	(21·1)	23·2	21·1	(22·2)
41	25·8	23·2	(24·5)	28·0	23·6	(25·8)
51	28·3	26·1	(27·2)	29·3	27·0	(28·1)
61	32·5	28·0	(30·2)	30·0	28·1	(29·0)
3000 kg/ha fertiliser						
Row spacing (cm)						
30	32·5	27·2	(29·9)	28·7	28·1	(28·4)
41	32·9	32·3	(32·6)	32·6	27·4	(30·0)
51	31·5	31·8	(31·7)	34·0	33·1	(33·5)
61	34·3	31·9	(33·1)	33·0	32·5	(32·7)

S.E.D. 2·39; 1·69 for means

Theoretical plant populations ('000/ha)

	Spacing within rows (cm)			
	30	41	51	61
71 cm rows	46·2	34·6	27·7	23·1
91 cm rows	35·9	27·6	21·5	17·9

saleable yield as much as the expensive extra application of fertiliser. (Hide with Widdowson, Chemistry Department and Moffatt, Farm)

The uptake and activity of thiabendazole in potatoes. We have used benomyl and thiabendazole dusts because they decrease some pathogens and do not involve wetting tubers which often increases bacterial diseases. However, chemical tests showed that tubers absorbed ten times as much thiabendazole from solution or suspension as from dusts of equal strength. In liquid, most uptake occurred within 5 minutes, the amount was maximal at pH 3 (and was also increased by pretreating tubers with weakly acid solutions) and little fungicide penetrated beneath the skin. This suggests that suppressing the ionisation of acid groups makes the skin more lipophilic and favours the partition of the thiabendazole from water. Concentrations within the skin and tuber were linearly related indicating that inward movement is by simple diffusion. However, very little thiabendazole moved into tubers or decomposed because more than 90% of the original uptake was still in the skin after three months.

Dipping tubers in 0·5% suspensions of thiabendazole at pH 3 in spring prevented more than half from producing plants. In less damaging treatments no thiabendazole was found in haulm or progeny tubers but tuber eyes were infected by *Helminthosporium* in inverse proportion to the fungicide concentration in the seed tuber. (Tisdale and Hide, with Lord, Insecticides Department)

Survey of diseases of seed tubers. Table 17 shows that blight was again scarce, and skin spot and gangrene were less common than since 1964. There were still a few stocks with many tubers affected, and *Phoma exigua* was more often present when it was expressed as gangrene, because sub-samples wounded and stored at 5°C for 12 weeks developed lesions on 19% of King Edward and 24% of Pentland Crown tubers not 5% and 6% respectively as on unwounded samples. Table 17 also shows comparable data for the ten-year period 1963–72. (Hide, Griffith and Bell)

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TABLE 17

Survey of fungal diseases of seed tubers (% tubers infected/% stocks with infected tubers)

Examined	Disease	1971–1972 King Edward	1971–1972 Pentland Crown	1962–1972 King Edward Average	1962–1972 King Edward Range of annual means % tubers
R	Skin spot (*Oospora pustulans*)	32/94	8/74	47/96	31–78
P	Gangrene (*Phoma exigua*)	5/64	6/67	8/68	4–12
P	Dry rot (*Fusarium solani*)	2/28	3/53	2/38	0–6
R	Blight (*Phytophthora infestans*)	1/18	<1/4	2/38	0–3
R	Black scurf (*Rhizoctonia solani*)	22/100	30/100	21/97	9–37
R	Powdery scab (*Spongospora subterranea*)	19/90	5/74	15/77	5–26
R	Common scab (*Streptomyces scabies*)	30/98	19/98	24/98	13–34
Number of stocks examined		50	49	1094	

R = at receipt; P = at planting

Incidence of virus diseases at Rothamsted. When examined in early July, the potato experiments planted with seed grown in 1971 at Rothamsted in isolated, insecticide-treated crops contained few plants infected with potato virus Y (0·04% in Pentland Crown and 0·5% in King Edward) and no leaf roll. By contrast, an experiment planted with seed also grown at Rothamsted for one year but alongside already once-grown stocks, had over 10% infection with potato virus Y—an illustration of the value of isolation to our seed crops. Potato virus Y spread little during the season and only one plant with leaf-drop streak was found in the crops grown to provide seed for the 1973 experiments. (Govier)

Relationship between diseases of seed, plants and stored tubers. Occasionally potatoes rot catastrophically in store while others apparently treated similarly do not. It would help farmers, merchants and processors if it were possible, before lifting, to predict the risk of rots and blemishes developing in different crops during storage. During 1971, we began testing methods for such a 'storage disease prediction survey' with the Potato Marketing Board Experimental Station at Sutton Bridge, Lincs. Fungal and bacterial diseases were assessed on seed of 15 King Edward stocks before planting, on plants in July and on tubers then, at lifting and after four months storage.

Infection by *O. pustulans* and *H. solani* can be measured by incubating on excised eye-plugs so that the fungi sporulate. It is more difficult to estimate gangrene, because *P. exigua* does not produce easily recognised sporing structures quickly and lesions are usually expressed after several months at 5°C. We are developing faster tests from gangrene by inhibiting wound healing, with IPC or agrimycin, to allow faster incubation at 10°C. In these conditions we can have visible lesions in three weeks after inoculating tubers or tuber slices with infective soil or skin parings. At present the difficulty is in recognising which fungus is responsible and we need to confirm by culturing. Like *P. exigua*, the soft rot bacteria are not easily recognised so tuber rots need to be induced and the causal organisms identified with selective media and at least three biochemical tests. Unfortunately bacteria of several genera can rot tubers and which one does depend considerably on the conditions which trigger their multiplication, so the conditions used to induce rots are critical. Promising results are being obtained with artificially contaminated tubers by enclosing them at constant temperature in gastight buckets in an atmosphere much enriched with carbon dioxide. Until the quantities of bacteria necessary to produce

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TABLE 18

Occurrence of gangrene and skin spot in 15 King Edward crops*

Gangrene				Skin spot			
Seed	Growth	Lifting	Storage	Seed	Growth	Lifting	Storage
44	0	0	0	66	3	2	0
90	20	0	1	28	1	3	0
8	0	2	1	38	6	4	0
28	0	0	1	4	1	7	0
2	0	2	2	18	3	9	0
30	0	1	3	66	3	16	0
2	3	0	3	10	9	18	0
78	0	6	5	74	27	41	1
5	0	10	6	70	21	13	1
3	3	0	6	54	8	33	3
36	35	33	14	52	40	64	3
47	20	9	15	72	42	38	3
19	18	10	23	44	13	24	5
22	20	10	23	51	30	67	8
70	0	49	32	62	14	37	9

* 'Seed', 'growth' and 'lifting' indices, for gangrene are percentage test wounds infected, for skin spot percentage eyeplugs infected
'Storage' values are percentage tubers showing the diseases

rots have been calibrated experimentally, it will remain difficult to discriminate between error and real differences between naturally contaminated stocks.

Table 18 shows results for gangrene and skin spot in 1971–72. In neither, does the index of seed tuber infection predict risk at lifting or disease in store but correlations improve later. Of five stocks predicted as 'most risky' in growth (July), four were among the most troublesome through gangrene in store. Few tubers developed skin spot in store but of the eight stocks with any, five had been predicted as the 'most risky'. July examinations were too early to predict silver scurf because few *H. solani* infections were then established. (Hide, Lapwood, Less, Griffith and Bell)

Potato blight. During 1972, a dramatic and quite unproven hypothesis (Renwick, J. H., *British Journal of Preventive and Social Medicine* (1972) **26**, 67–88) directed public attention to diseases of potatoes and especially to blight. Even a possibility that the claim is true merits careful medical investigation because of the tragic consequences of spina bifida and ancephaly to infants. Nevertheless, from an agricultural viewpoint it is regrettable that the suggestions were not substantiated experimentally. Until this has been done and a cause identified there is little that plant pathologists can do except continue their efforts to decrease the incidence of diseases for economic reasons. However, the general scarcity of blighted tubers during the past four years is worth mention. It is well illustrated in Table 19 which records incidence of blighted tubers in the susceptible variety King Edward. The long series of experiments at Rothamsted begun to study blight epidemiology and tuber infection has been continued to monitor the disease, tuber bulking in relation to weather and more recently to measure the effects of aphids on crops sprayed or not with insecticides or fungicides. It is well known that crops sprayed with fungicide may have as many or more infected tubers than unsprayed crops when spraying prolongs growth into weather favouring tuber infection. Most tuber infection can be prevented by destroying the haulm before more than 5% of the foliage has been blighted (Hirst, Stedman, Lacey & Hide, *Annals of Applied Biology* (1965) **55**, 373–395) and the right-hand column of Table 19 illustrates that most tuber infection is preventable. (Hirst and Stedman)

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TABLE 19

Percentage (by weight) of King Edward tubers blighted in spraying experiments at Rothamsted

	Unsprayed	Fungicide sprayed only	Fungicide sprayed + early haulm killing
1955	0·4	0	(0)
1956	4·2	2·8	(0)
1957	17·6	9·6	(0·2)
1958	10·3	7·5	(0·1)
1959	0·9	0·1	(0)
1960	26·6	26·1	(0·8)
1961	4·7	0·7	(0·7)
1962	8·5	8·8	1·2
1963	18·0	9·9	0
1964	0·2	0·1	0
1965	21·2	12·3	0·5
1966	1·7	0·3	(0)
1967	8·9	5·8	(0·6)
1968	7·1	11·1	(1·2)
1969	0	0	—
1970	0	0	—
1971	2·9	1·4	0
1972	0·4	0	0

* For further explanation see text, figures in brackets are estimated from 'blight progress curves' and tuber samples, others by killing haulm early with sulphuric acid

Staff and visiting workers

The following changes occurred in research staff: R. W. Gibson transferred from the Entomology Department. Mari James and M. J. Tisdale left. D. J. Banfield, Janet E. Smith and M. F. Smith worked in the department from April to September as 'sandwich course' students.

Visiting workers included Dr. R. Azcon (Granada, Spain), Mr. S. Chareonridhi (Thailand), Dr. Jyotsna Chatterjee (McMaster University, Ontario), Dr. S. Gianinazzi (Dijon, France), Mr. D. Gollifer (Solomon Islands), Mr. S. Vorra-urai (Thailand). R. A. Hill began an Agricultural Research Council Scholarship, Mr. M. J. Adams continued his Potato Marketing Board Scholarship and P. H. Gregory continued working at the invitation of the Lawes Trust Committee.

Scandinavian aerobiologists invited J. M. Hirst to a symposium at Stockholm in April, J. Lacey spent parts of March and April in Portugal measuring the air-spora of a cork factory and I. Macfarlane attended the 2nd International Symposium on Marine Mycology in Bremerhaven in September.

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