ROTHAMSTED RESEARCH

Report for 1954

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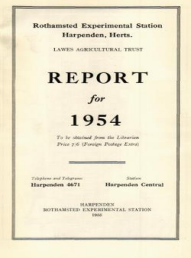

Plant Pathology Department

F. C. Bawden

F. C. Bawden (1955) *Plant Pathology Department ;* Report For 1954, pp 80 - 95 **- DOI: https://doi.org/10.23637/ERADOC-1-76**

PLANT PATHOLOGY DEPARTMENT

F. C. Bawden

New laboratories for the mycologists were built but not finished in time to be occupied during the year. The glasshouses and insectary occupied late in 1953 proved a valuable addition for virus work, though the summer of 1954 did not subject the ventilating system to a severe test.

P. H. Gregory left at the end of January to become Professor of Botany at the Imperial College, London University, and Brenda M. Hamlyn in June to be married. New appointments during the year were E. W. Buxton, T. Mulligan and D. M. Firth (Dunholme Field Station). G. E. Russell of the Plant Breeding Institute, Cambridge, worked in the department for six months.

As the guest of the Indian Science Congress Association, F. C. Bawden attended the Science Congress at Hyderabad in January and spent three weeks afterwards visiting agricultural research stations and universities in various parts of India. B. Kassanis worked for two months at the Station Centrale de Pathologie Végétale, Versailles, learning the techniques of plant-tissue culture. F. C. Bawden, L. F. Gates, Mary D. Glynne, J. M. Hirst, B. Kassanis and F. T. Last attended the International Botanical Congress at Paris, and A. Kleczkowski the International Congress of Photobiology at Amsterdam. R. Hull attended the winter and summer meetings of the International Institute of Sugar Beet Research held respectively in Brussels and Eire and, with J. Blencowe, the Virus Yellows Colloquium at Bergen-op-Zoom.

Viruses and Virus Diseases

Strains of tobacco mosaic virus

We have previously reported that extracts from plants infected with tobacco mosaic virus (TMV) contain a range of specific particles that differ greatly in length and in their ability to cause infection, the shortest being almost or wholly uninfective, though they all seem to contain the same antigens. The proportion of short particles is now much less than we used to find; we cannot account for this except by postulating that our stock culture of TMV has changed. Some indirect support for this idea was obtained by studying several strains of TMV and finding quantitative differences, but with none has the ratio of short to long particles been as great as it was some years ago. The small yield of uninfective particles has prevented critical work on their chemical constitution, and we have not been able to conclude whether they contain nucleic acid or not.

The particles of all the strains are indistinguishable by electron microscopy, but the strains differ from one another in many ways. One from the United States seems not to infect tomato, is precipitated with much less salt than other strains and, a confirmation of American work, is more readily inactivated by ultra-violet light.

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This one, and another from India, although serologically related to our stock culture of TMV, seems to share no antigens with cucumber viruses 3 or 4, which are serologically related to the stock culture. Should further work confirm this, it will be the first example known of viruses showing this type of relationship; that is virus A sharing antigens with B and B with C, but not A with C. (Bawden.)

Inactivation by ultra-violet radiation

One character shared by the four strains of TMV we have studied is that preparations partially inactivated by exposure to ultra-violet radiation have the same infectivity, relative to unirradiated preparations, whether inoculated plants are kept in the light or dark after inoculation. With six other viruses, tomato bushy stunt, Rothamsted tobacco necrosis, cucumber mosaic, tobacco ringspot, cabbage black ringspot and potato virus X, irradiated preparations produce more lesions when inoculated plants are placed in the light than when they are placed in the dark. The quantitative response to visible light differs considerably with the individual viruses and is greatest with potato virus X, irradiated preparations of which may give twenty or more times as many lesions on plants in the light as in the dark. The response with this virus is large enough to study the conditions that give the response. Bright light is not necessary, a good response being given at 40 foot candles. The length of time needed in light depends on the time between inoculation and the first exposure to light. Most of the response happens within 2 hours of inoculation, but the numbers of lesions increase with exposure to light up to 6 hours after inoculation. Fifteen to 30 minutes immediately after inoculation has little effect, but if plants are placed in the dark for the first hour after inoculation, then 15 and 30 minutes exposure to light greatly increases the number of lesions. After 2 or 3 hours in the dark, exposure to light has little effect. It seems that, during the first hour or so after inoculation to leaves, the virus particles undergo some change and enter a state in which the ability of those damaged by ultra-violet light to multiply is affected by visible light. This sensitive state is transitory, lasting for about an hour, and if during this time virus particles do not start to multiply, they seem to be destroyed. (Bawden and Kleczkowski.)

A change in the condition of Rothamsted tobacco necrosis virus (RTNV) during the first hour or two in inoculated French-bean leaves is also suggested by experiments in which inoculated leaves were irradiated with ultra-violet light. At times up to 1 hour after inoculation, a constant amount of irradiation gives a constant decrease in the numbers of lesions produced by a given inoculum, but after that it has less effect. At 25° C. the results are compatible with the hypothesis that substances that absorb ultra-violet start to be synthesized about 1 hour after cells become infected, that new virus particles start to appear between 2 and 4 hours, and that some of these move from the epidermis to the palisade cells by 6 hours after infection. Similarly, experiments with inhibitors of infection, like ribonuclease, suggest changes in infected cells after about 1 hour. Up to 1 hour, applying ribonuclease to inoculated leaves decreases the numbers of lesions produced, but not afterwards. However, the interpretation is uncertain, for it may be that during

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this hour inoculated cells recover from injuries made when they were inoculated with the virus and become impermeable to such large particles as ribonuclease. Inhibitors with small particles, e.g., trichothecin with French bean and thiouracil with tobacco, have effects if applied many hours after inoculating the viruses, but these can penetrate deeply into uninjured leaves and may act by preventing the spread of virus from cell to cell rather than by affecting virus multiplication in cells where it has already been initiated. (Bawden and Harrison.)

Effect of temperature on virus multiplication

Virus multiplication was estimated by assaying the infectivity of sap expressed from leaves at intervals after inoculation. From 1 to 3 days after inoculation, the optimum temperature for multiplication of RTNV in French-bean leaves was 22° C. Three days after inoculation, the virus content of leaves kept at 22° C. was 4,000 times greater than in leaves at 10° and 1,000 times greater than at 30° C. The factor by which virus concentration increased between 1 and 3 days after inoculation, decreased as the temperature increased from 10° to 30° C. When leaves kept at 22° C. for 2 days after inoculation were put at 30° C. for a day, their virus content dropped to one-fifth; it increased again when they were returned to 22° C. It is thought that RTNV is simultaneously synthesized and broken down at all these temperatures, but that the ratio of breakdown to synthesis increases as the temperature increases.

For 5 days after inoculation, tomato aucuba mosaic virus increased in inoculated *Nicotiana glutinosa* leaves more rapidly at 27° and 30° C. than at 33° and 36° C. At 27° and 30° C. most lesions were necrotic, whereas at 33° C. nearly all were chlorotic. Only a few chlorotic lesions developed at 36° C.

The period after inoculation during which irradiating leaves with ultra-violet decreased the number of lesions produced by RTNV was shortened by increasing temperature up to 22° C., but not between 22° and 31° C. This period is thought to be that needed for virus to multiply in and move out of epidermal cells into deeper tissues. (Harrison.)

Inhibitors of infection

The way in which substances like ribonuclease and chymotrypsin inhibit infections by plant viruses is still uncertain. Both these enzymes also inhibit the infection of *Rhizobium* sp. by bacteriophage, and in this system it seems that the two act in different ways. Ribonuclease acts by preventing the phage from combining firmly with the bacteria. Chymotrypsin does not affect this combination, but apparently inactivates the phage when it combines with the bacterium, although it does not inactivate free phage. It was thought that experiments resembling those with bacteriophage might throw light on the way in which inhibitors affect plant viruses, but before they could be made it was necessary to have some method of measuring the amount of virus that combined with host cells or fragments of them. For this purpose tobacco leaves were ground and then suspended in solutions of TMV. After incubating the mixtures, the solids were removed and the virus in the supernatant

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fluid was assayed. Although the ratio of the weight (dry) of the ground leaves to virus was over 10^7, the infectivity of the supernatant fluid did not differ from that of the control in water. Hence, it seems that freshly ground leaves contain no large particles with which the virus combines specifically, as do bacteriophages with their host bacteria. (Kleczkowski.)

The statistical analysis of local-lesion counts

As numbers of local lesions produced by plant viruses are not normally distributed and their standard errors vary with their magnitude, they are not amenable to customary methods of statistical analysis. A transformation $z = \log_{10}(x + c)$, in which x is the number of lesions per leaf or half leaf and c a constant, has already been proposed that is appropriate when mean values of x exceed 10. In many kinds of work, however, fewer lesions than this are unavoidable, and for these a new transformation has been derived. It is $z = \log_{10} \frac{1}{2}(x + c + \sqrt{x^2 + 2cx})$, and can be used when mean numbers of lesions exceed about 1·5. The results of the two transformations converge as x increases. A table of values of z has been calculated that makes the new transformation as easy to use as the old. (Kleczkowski.)

Tissue cultures

A series of tissue cultures, using virus-infected roots and callus tissue, was produced as a preliminary to starting work on the factors that affect virus multiplication. In France virus-free potato plants have been produced by culturing the excised stem meristems from infected plants. This suggests that the ability of viruses to give systemic infections does not extend to invading these meristems, and work was started to find how virus concentration varies through plants. Tomato roots infected with TMV were grown in White's medium, then cut into pieces, macerated and the extracts assayed for their virus content. Although all contained virus, the older parts contained very much more than the younger ones. Similarly, the enclosed buds of systemically infected tobacco plants contained much less virus than the young leaves, and these less than the well-developed ones. In these tests the youngest tissues used were not exclusively meristematic, but further tests with more refined techniques will be needed before deciding whether or not the apical meristems contain virus. (Kassanis.)

Carnation latent virus

The virus described last year as latent in many stocks of carnation has now been found to be serologically related to one that often occurs in normal-looking plants of many potato varieties. All plants of some varieties, for example King Edward and Arran Victory, that have so far been tested contain it. Although serologically related, the viruses from carnation and potato are far from being antigenically identical. Also, they are transmitted in different ways and they infect different plants. The strain from carnation is transmitted by *Myzus persicae* and infects sweet william, *Chenopodium amaranthicolor* and sugar beet, but not potato or tomato. The strain from King Edward potatoes has not been transmitted by *M. persicae*; it infects tomato, but not the other species listed.

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The strains in different potato varieties also seem to differ, for one in Arran Victory has not been transmitted to tomato. The potato strains are almost certainly the same as the viruses described in Holland as potato virus S. (Kassanis.)

Electron microscopy

The electron microscope has been much used for routine examinations of virus preparations, but it has been mainly occupied by the study of thin sections of infected plants and by developing methods for counting virus particles. Our machine is not well adapted for such kinds of work, because it does not permit large areas to be first surveyed at a low magnification as a preliminary to selecting areas for detailed examination at high magnification. One modification that has helped to speed up the work is the addition of a " Stigmator ", a device that allows the residual astigmatism of the objective lens to be compensated while the machine is operating instead of needing to open the microscope and remove the pole piece. In studying the changes in tobacco leaf cells caused by infection with TMV, the most suitable material seems to be the youngest leaves as they become invaded by virus coming from older inoculated leaves. This method provides a successive series of leaves of approximately the same size and avoids many of the difficulties experienced in preparing sections from fully-expanded leaves. No morphological changes have been detected during the early stages of infection, even though the virus is multiplying rapidly. It is impossible to be sure whether no changes occur or whether, at this stage, only a few of the cells are infected and these have not been encountered. At about the time inclusion bodies begin to form, some of the chloroplasts change in appearance, and the usual layer structure of the grana is replaced by granular vacuolate material. The thinnest sections show that the granules have a similar cross-section to TMV particles; the cut surfaces of such chloroplasts, when sectioned, resemble the cut surfaces of virus particles in the inclusion bodies. This suggests that the plastids may be one site where the virus multiplies, but is far from conclusive.

To develop the spray-drop technique of Backus and Williams into a routine method for counting virus particles, many technical difficulties have had to be overcome. When dealing with impure or partially purified virus preparations, the highest resolution is needed; this requires smaller drops than those used by Backus and Williams, which in turn calls for smaller standard particles than the Dow polystyrene latex spheres. The Plastics Division of Imperial Chemical Industries has kindly prepared for us a polyvinyl chloride latex, with particles about 0·1 μ diameter. The particles vary more than the Dow latex, but are sufficiently uniform for errors to be removed when enough are counted to give good statistical accuracy in determining the volume of droplets. The spheres aggregate at below pH 5·5, and virus preparations must be above pH 6·0 when mixed with the latex. Using the polyvinyl latex, droplet traces can be made small enough to be photographed at the highest magnification, thus making the best use of the microscope's resolving power. The method works well with purified virus preparations, but is unlikely to do so with impure preparations of viruses that have spherical particles, for it is difficult to identify these particles when

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surrounded by other material. Another difficulty is that the 60-kV electrons in our microscope do not give a picture bright enough to count all the particles on the screen; to ensure accuracy, therefore, all droplet traces have to be photographed.

The rapid freeze-drying method described by Williams for preventing specimens from flattening during drying has been tested, but has given many difficulties. Our atmospheric humidities are so high that the air carrying the droplets into the freezing zone must be dried, or the mounts become covered with "snow", which prevents the drops from impacting on them. Also, our supplies of volatile salts are impure, and residues from these obscure the pictures. Some success was achieved by a less elaborate form of freeze drying. The specimen is mounted, as a large drop or as a spray droplet, on a grid held in a deep recess of a block cooled to —70° C. by a mixture of dry-ice and alcohol, when the block is placed in a vacuum and the ice sublimed. (Nixon and Fisher.)

Potato virus diseases

Field experiments at Sutton Bonington were made to find how varying the times of planting and lifting potatoes (King Edward) affects their yield of tubers, the proportion of "seed" infected with leaf roll and virus Y, and the cropping power of virus-free "seed". Results from a trial started in 1953 are shown in the table, the figures in brackets being the yields in tons of tubers per acre and the others being the percentage of healthy "seed". When grown to maturity, the biggest yields came from the earliest planting, but when grown for 12 weeks only they came from plantings in May and June; plantings in these two months and April, however, gave the smallest proportion of healthy "seed". The viruses became most prevalent in the crop planted in June, because the plants were young and susceptible during June and July when aphids were most active. Virus-free "seed" from the crop planted in August yielded significantly less than that from earlier plantings.

The yields and health of tubers from potato crops planted and lifted at different times

	Time of Planting				
	April	*May*	*June*	*July*	*August*
Lifted					
12 *weeks after planting*	*100(4)	95(9)	23(7)	46(4)	93(4)
After death of haulm	35(26)	37(21)	7(10)	43(7)	93(4)

* Figures in brackets are yields of tubers in tons per acre: others are percentages of healthy "seed" tubers.

In co-operation with the Insecticides Department experiments on the use of persistent insecticides to control the spread of potato virus diseases were continued in several localities. The results of the 1953 experiments again indicated that insecticides effectively prevented the spread of leaf roll from infected to healthy plants in the same crop, and they usually decreased the spread of virus "Y". They did not prevent viruses from being introduced into the crops from outside sources, but, unless there are severely affected stocks near, few plants usually become infected in this way compared with those infected by spread within a crop. (Broadbent.)

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Viruses of cruciferous crops

The transmission of cauliflower mosaic virus (CLMV) by aphids differs from other non-persistent viruses, and the manner in which it behaves differs with different aphids. Previously fasted *Myzus persicae* give as many or more infections after infection feeding times of 2 minutes as after 24 hours, whereas *Brevicoryne brassicae* give more after 24 hours, and a previous fast does not increase the number. To see whether these differences could be correlated with differences in feeding behaviour, the feeding habits of these aphids and *Myzus circumflexus* were compared. The average times for which fasted *M. persicae*, *M. circumflexus* and *B. brassicae* remained with their stylets in leaves at the first penetrations were 37, 30 and 210 seconds; for unfasted individuals the times were 65, 36 and 360. When fed on plants infected with cabbage black ringspot virus (CBRSV), the penetration times that made individuals infective were nearly all between 15 and 120 seconds, and mainly between 60 and 120. Although the proportion of unfasted aphids that penetrated for these periods was almost as high as of fasted ones (the average penetration time was higher because the scatter was greater and some unfasted aphids penetrated for long periods), only few of the unfasted aphids became infective, seven *M. persicae* compared with eighty-five fasted ones. *M. circumflexus* behaved very similarly to *M. persicae*, but transmitted only a quarter as often. *B. brassicae* gave only one-tenth as many transmissions as *M. persicae*. We have previously given evidence that CBRSV is more concentrated in the epidermal than in other cells of infected leaves, and these results suggest that fasted *M. persicae* imbibe fluid from the epidermis more readily than do the other aphids. If aphids carry CBRSV in drops of sap contaminating their stylets, then these drops seem to be carried inside the stylets, for exposing the beaks of infective *M. persicae* to ultra-violet light did not affect their ability to transmit. Irradiating leaves infected with CLMV had less effect on the numbers of *B. brassicae* that became infective after 2-minute infection-feeding times than with *M. persicae*, though both aphids behaved similarly with CBRSV. With 24-hour infection-feeding times, both species behaved alike with both viruses, as many transmitting from irradiated as from unirradiated leaves. Aphids feeding on healthy plants remained infective with CLMV for longer than with CBRSV. (Watson, Hamlyn and Mulligan.)

The main conclusions from a field trial made to study how mosaic and manuring affect two varieties of cauliflower (St. George and Early Extra Roscoff) were: (*a*) the incidence of CLMV and of "tip-burn" ("scorch") increased with increasing amounts of nitrogen, but "tip-burn" was not caused by CLMV; (*b*) the number of plants that died during winter was increased by nitrogen, but in St. George was unaffected by CLMV; (*c*) CLMV decreased curd size and increased the number of "bracty" and loose curds; (*d*) increasing nitrogen also increased the "bracty" curds and accentuated the loss of yield by CLMV; (*e*) moderate dressings of nitrogen increased the yields from uninfected plants, but high levels decreased them; (*f*) the number of marketable curds was much decreased by CLMV and less by high nitrogen; (*g*) CLMV and high levels of nitrogen made plants mature a few days earlier; (*h*) the adverse

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effects of mineral nitrogen were more pronounced with hoof than with farm-yard manure; (*i*) filling up gaps a month after planting the crop was not worth while, as fewer than half those planted late produced marketable curds.

The effect on the incidence of cauliflower mosaic of surrounding cauliflower seedbeds with single rows of barley was again tested. At Efford there were too few aphids and infections for results to be significant. At Luddington early-sown barriers decreased the incidence to one-quarter of that in control plots in four-row and to one-half in twenty-two-row seedbeds. When barley was drilled at the same time as the cauliflower seed, the decrease was slightly less.

Aphid infestations and the movement of winged aphids was studied by operating twenty-two sticky traps in different parts of England. Of 278 winged *M. persicae* bred on plants infected with CLMV and tested for their ability to infect healthy plants, 51 did so: of 45 winged aphids from plants with CBRSV, 12 caused infections. (Broadbent and Heathcote.)

A strain of turnip yellow mosaic virus found damaging cruciferous crops near Newcastle differs from the strain prevalent near Edinburgh. The two are both transmitted by flea-beetles and are serologically related, but they are far from antigenically identical and do not crystallize in the same ways. The Newcastle strain also differs from the Edinburgh one in readily infecting and severely affecting cabbage, cauliflower and kale. Another flea-beetle-transmitted virus was found at Edzell (Scotland) affecting turnips in 1953. It is not serologically related to turnip yellow mosaic virus and has provisionally been called turnip crinkle virus. It has a thermal inactivation point around 80° C. and infects cabbage and cauliflower, but does not damage them severely. (Broadbent and Blencowe.)

Sugar-beet virus diseases

The susceptibility of various varieties of wild and cultivated beet to yellows virus (SBYV) was tested. All proved equally easy to infect with aphids, but they showed symptoms of different severity when infected. *Beta maritima* varieties were more tolerant than crosses between *B. maritima* and *B. vulgaris*, and these more than *B. vulgaris*, some varieties of which, and particularly a Klein "D" non-bolter, were very severely affected. The severity of symptoms shown by varieties under glass was correlated with loss of yield in the field. The severity of symptoms was also correlated with the antigen content of plants, estimated by titrating the sap against SBYV antiserum. In all varieties the antigen content was highest about 3 weeks after infection, when the content started to fall, falling more rapidly with tolerant than with intolerant varieties.

The seedlings of one variety often show more severe symptoms if colonized by several infective aphids than if colonized by single aphids. This may explain a German claim, contrary to our experience, that *Aphis fabae* transmits only avirulent strains of SBYV. When seedlings were colonized with 3 aphids, conditions in which *M. persicae* infected 50 out of 50 plants, *M. circumflexus* infected 46, *Macrosiphum pisi* 31, *A. fabae* 13 and *B. brassicae* 0. The average severity of symptoms shown by the 13 plants infected by *A. fabae*

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was less than those shown by plants infected by *M. persicae*, but plants colonized with 20 *A. fabae*, when all the plants became infected, developed the same severe symptoms as those infected by *M. persicae*. The apparent virulence of a culture of SBYV can often be increased by serial passages using many aphids per plant and decreased by serial passages with single aphids. Some of these differences may reflect an effect of the amount of virus initially introduced into seedlings, but infected plants may also contain several virus strains which are sometimes transmitted separately by single aphids. (Watson and Russell.)

The relationship of yellow net virus (YNV) to SBYV remains uncertain, but its multiplication is decreased by the presence of strains of SBYV, and more so by virulent than by avirulent strains. On its own, it cripples beet plants and turns them almost wholly yellow, and the characteristic yellow lines following the veins may be typical of dual infections with SBYV. YNV does not readily invade plants already infected with avirulent strains of SBYV and produces only a few scattered spots on plants with virulent strains. Plants already infected with YNV, however, are not protected against infection with virulent SBYV, which seems to multiply normally and produces its typical symptoms on young leaves; the yellow net symptoms persist on the old leaves, but do not develop on leaves produced after the plant becomes infected with virulent SBYV. By contrast, avirulent strains of SBYV protect plants from normal invasion by virulent strains; not only do the symptoms remain mild when plants are inoculated with virulent strains, but the antigen content of the plants also remains well below that typical of plants infected with the virulent strain alone. Both SBYV and YNV infect *Nicotiana clevelandii* and *N. biglovia*, but yellow net is difficult to recover from them by aphids; SBYV but not YNV infects *Chenopodium amaranthicolor*. (Hull and Watson.)

Uniform crops of three commercial beet varieties were inoculated in June with a strain of SBYV that causes etch symptoms, a strain that causes only chlorosis or a mixture of the two. The aphids used to inoculate the plants were killed by spraying, and the plots were later sprayed at regular intervals. The varieties suffered equally from infection and the yields, compared with 19·02 tons/acre for the healthy plots, were 10·85 with the etch strain, 9·08 with the other and 8·80 with the mixture. There was less difference in the appearance of the plots in the field than was expected from the behaviour of the strains under glass. Each plant was "scored" for the severity of symptoms in August, using a scale from 0 for a healthy plant to 5 for one severely stunted and with all its leaves showing some yellowing or necrosis. Most infected plants were in category 3, but the range on plots inoculated with the etch strain was wider than on the others. With the yellowing strain, the range was 1–4, with more in category 2 than 4; the range with the etch strain was 0–5, with most in 3 and 4, and with the mixture was similar, except that all plants were infected and fewer came in categories 1 and 2. There was no necessary correlation between severity of leaf symptoms and yield, but plants with slight symptoms usually gave large roots, and those with severe symptoms small ones. Infected plants of one variety gave many more large roots than the others, and although in total it was not more tolerant

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than the others, this suggests that it contains tolerant genotypes that might be useful in a breeding programme.

Differences in tolerance were also found in experiments comparing the effects of SBYV on seven inbred lines of sugar beet, but the most tolerant variety yielded less than the others when uninfected. Measuring tolerance by effects on yield, however, is complicated by the fact that some varieties suffer relatively more loss than others if infected early in life but less if infected late. Progenies from self-pollinated plants selected in earlier years for tolerance were again tested, and showed great differences in severity of symptoms. The size of roots did not always reflect severity of leaf symptoms, but some of these progenies gave a larger yield of roots than comparably infected commercial varieties. Three polyploid lines derived from Klein E by the Plant Breeding Institute, Cambridge, yielded as much sugar per acre as commercial varieties when uninfected, but suffered twice the loss of sugar when infected with SBYV. (Hull and Firth.)

Experiments to test how spraying with systemic insecticide affects the incidence of yellows in root crops were made on six crops in Lincolnshire and Norfolk. The proportion of plants with yellows at the end of the season was more than halved by one spray with "Systox" at 400 g./acre, applied either in mid-June or early July; spraying on both occasions had little additional effect. A third spray at the end of July decreased the proportion of infected plants by one-third. Although less than 40 per cent of the plants on the unsprayed plots became infected, and many of them only after mid-August, spraying increased yield by from 1·75 to 3·5 cwt./acre of sugar.

An experiment at Dunholme tested the effect of sprays on early- and late-sown plots, with wide and narrow spacing, and with different amounts of fertilizer. Fertilizer and spacing did not affect the incidence of yellows, but early sowing increased it, and spraying decreased it to one-fifth. A similar experiment at Sprowston has not yet been analysed.

In co-operation with the Sugar Corporation's agricultural staff, replicated 1-acre plots were sprayed by contractors with the systemic insecticide "Metasystox". At seven of the eighteen sites yellows did not spread enough for spraying to have effects. In two fields near Colchester, where half of the plants on unsprayed plots contracted yellows, one spray decreased the incidence to one-quarter and increased yield by $\frac{1}{2}$–$\frac{3}{4}$ tons/acre of roots. All the plants in two other crops in the same district became infected whether sprayed or not. However, the sprayed plots took longer to become 100 per cent infected, and yielded an extra 3 tons/acre of roots. A second spraying gave no additional benefit. Spraying also decreased the incidence of yellows in crops near Bury St. Edmunds and Peterborough, but effects on yield were not measured. *Aphis fabae* infested the sugar beet in Suffolk and Norfolk. These were killed by "Systox" and "Metasystox", and it is not possible to say what proportion of the increased yield should be attributed to the control of these pests. (Blencowe, Gates, Hull and Firth.)

Seed crops planted with unsprayed stecklings had 73 per cent infected plants whereas the percentage of infected plants in crops planted from stecklings sprayed three times in the autumn was:

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" Systox ", 30; " Hanane ", 28; " Schradan ", 34; and " NC7 ", 22.

To compare the rate at which different insecticides kill aphids, plants were sprayed and then later colonized with winged *M. persicae* and covered with tumblers. When colonized 24 hours after spraying, " Malathon ", " NC7 ", " Metasystox " and " Hanane " killed aphids within 5 hours, and " Parathion " and " Systox " between 5 and 17 hours. When colonized 4 days after spraying, the first four still killed aphids in periods of 5–17 hours. The kill is too slow to prevent infective aphids from infecting healthy seedlings, though the proportion that become infected was slightly decreased by " Malathon ", " NC7 " and " Parathion ".

Beet seed was soaked in various insecticides to see whether this would protect emerging seedlings against aphid infestations. When seed soaked in 2 per cent " Hanane " was sown thickly the seedlings had fewer aphids a month after emerging than did control seedlings, but at the seed rate normal for root crops the treatment had no effect. " Systox ", " Metasystox ", " Malathon " and " NC7 " gave less protection against aphids, killed some seedlings and stunted others. " Pestox III " did not protect against aphids. (Gates.)

Further tests with " Systox ", " Parathion " and " Pestox 14 " sprayed on mangolds and fodder beets a few days before harvest showed that, to kill aphids and prevent infestation in clamps, all three need to be applied at three times the concentration normally used on sugar beet in the summer.

Maleic hydrazide, a growth-regulating substance, was again tested for its ability to check sprouting of stored mangolds and fodder beets with the idea that this might decrease aphid infestations. Sprouting was almost completely halted on clamped Orange Globe mangolds sprayed on 16 October with 0·25, 0·5 and 1·0 per cent maleic hydrazide, and on Hunsball fodder beet sprayed on 2 September with 1 per cent maleic hydrazide, and 4 November with 0·25 and 0·5 per cent maleic hydrazide.

When stored at about 40° F. in a cellar, unsprayed mangolds sprouted rapidly, and by 5 January had many sprouts 7 inches long. Those sprayed with maleic hydrazide on 16 October produced shoots 2 inches long by 5 January, but the shoots soon died; more appeared, and some were 3 inches long on 15 February, but these also died. On 29 March three-quarters of the sprayed roots had no shoots, and those on the others were probably too small to support a population of *Myzus persicae* over the winter. (Cornford.)

Samples from commercial sugar-beet stecklings planted at Dunholme varied more in the percentage of plants with yellows than in the previous three years. More beds were rejected this year for commercial planting because they contained more than 1 per cent infected plants in the autumn of 1953. Rejecting five beds decreased the mean percentage of infected plants in beds from the north of England from 10·5 to 7·8; rejecting twenty-six beds from eastern England, where control of yellows depends upon spraying with insecticides, decreased the mean percentage of infected plants from 25·2 to 4·1. The average percentage of infected plants in crops from all steckling beds was 17·4, but in those certified for planting it was 5·4. The mean percentage of plants with yellows in seed crops planted from beds raised under cover crops was 2·5. The

https://doi.org/10.23637/ERADOC-1-76

consistently good control of yellows obtained by growing stecklings under barley is increasing the use of this measure.

In the autumn of 1954 the mean percentage of infected plants in eighty-three sugar-beet steckling beds was only 0·17, equal to the previous lowest, in 1951. All steckling beds were certified as being usable. Mangold and fodder-beet steckling beds, especially those in south Lincolnshire, were less healthy, containing up to 8·5 per cent infected plants, and the average for 137 beds was 2·9 per cent. (Hull and Firth.)

Of 200 plants from thirteen different weed species collected in February from sites where they had opportunity to contract yellows in 1953, six contained viruses transmissible by *M. persicae* to sugar beet. Two plants of *Senecio vulgaris* and one of *Capsella bursa-pastoris* contained SBYV. One *C. bursa-pastoris* and one *Stellaria media* contained what are tentatively diagnosed as strains of cucumber mosaic virus. One *Rumex crispus* contained an unidentified virus that stunted sugar beet and produced a mottle and yellowing of the leaves. (Hull.)

Mycology

Potato diseases

To study the origin and course of outbreaks of potato blight, tubers infected with *Phytophthora infestans* were planted among healthy ones. Only two out of 246 such tubers produced plants with stem lesions, and in only one could the fungus in the lesion be certainly traced to the tuber. This one produced a lesion on 28 May, the earliest date that blight has been noted in the field at Rothamsted. Blight did not appear generally in the crops until the end of July, by which time there had been six periods at crop level corresponding to those specified as the "Beaumont warning" for blight. On each of these occasions blight spread to plants near the one with the initial lesion. Thus, the use of a "zero" date, before which "Beaumont warnings" are ignored in forecasting outbreaks, is explained by the need for about six "generations" of infections from over-wintering sources before the fungus is plentiful enough to affect crops generally.

Although blight became general at about the same time and developed at the same rate as in 1953, in 1954 two sprays with a copper fungicide increased yield by 2·4 tons/acre, compared with only 0·5 tons in 1953. The difference can be explained by the cooler summer of 1954, which meant that the crop was later in maturing. When blight appeared in 1953 about two-thirds the final yield of tubers on sprayed plots had already been formed, whereas only one-third was formed at the same time in 1954.

Spore traps operated in potato fields showed the greatest concentration of spores of *P. infestans* yet measured, 14,700 sporangia per cubic metre on 21 August. (Hirst and Stedman.)

A fungus indistinguishable from *Oospora pustulans*, the cause of skin spot of tubers, was found frequently in brown lesions on the roots, stolons and stems below ground. Isolates from typical skin-spot lesions on tubers and from brown lesions both increased the number and size of such lesions when added to soil at the time the potatoes were planted. Tests to see whether the fungus from stems will cause skin spot are not yet complete. (Hirst and Salt.)

https://doi.org/10.23637/ERADOC-1-76

Spore trapping

Automatic spore traps were again used to study the dispersal of spores of *Venturia inaequalis*, the cause of apple scab. Conidia are usually assumed to be dispersed only in wet weather, but most were caught on dry afternoons, and there is evidence that rain washes them out of the air.

Changes in the spore content of the air in the open are difficult to interpret because of interacting biological and physical factors. Trapping in the open is therefore being accompanied by tests in the partially controlled conditions of the wind tunnel. After a thorough wetting, scabby dead apple leaves ejected ascospores freely, the number liberated reaching a peak between 1½ and 3 hours after wetting. After atomizing water equivalent to 0·2 mm. of rain evenly over the leaves, few spores were caught, suggesting that dew is unlikely to liberate many. Ascospores of *Ophiobolus graminis*, the cause of take-all of wheat, were liberated in the same conditions as *V. inaequalis*, but more quickly, the peak catches coming in less than an hour after thoroughly wetting infected straws. (Hirst and Stedman.)

Spores of *Sporobolomyces roseus* are often the predominant type caught in spore traps operated in the open. This species seems a normal contaminant of leaf surfaces, reaching large numbers on ageing leaves, particularly at high humidities. It seems not to influence the development of leaf pathogens or to be influenced by them, but *Tilletiopsis minor*, another innocuous fungus that occurs on leaves during July and August, does suppress it. (Last.)

Cereal diseases

Further evidence was gained that the optimum seed rate for wheat depends on whether or not the land is infested with eyespot (*Cercosporella herpotrichoides*) and take-all. If it is, then a seed rate of 1½ bushels of Squarehead's Master yields a better crop than a seed rate of 3 bushels, but on clean land the higher seed rate gives as good as or better yield. Seed rates in bushels that are appropriate for one variety are not necessarily appropriate for others, because grain sizes differ. Holdfast drilled at 1½ bushels, for instance, produced as many plants per foot of row as the larger-grained Cappelle drilled at 3 bushels. The proportion of small grains and weed seeds in harvested grain varied widely in different experiments. In one at Rothamsted, Cappelle sown at 3 bushels on land contaminated with take-all and eyespot gave a threshed yield of 35·3 cwt./acre, but this included 5·7 cwt. of thirds, weed seeds and other impurities and 6·0 cwt./acre seconds, leaving only 23·6 cwt./acre dressed corn. This contrasts with Cappelle in another experiment on land almost free from take-all and eyespot, in which a yield of 49·4 cwt./acre included only 1·2 cwt./acre of small grain, no weed seeds and 2·1 cwt./acre seconds, giving 46 cwt./acre dressed corn.

The effect of seed rate on lodging in spring-sown Proctor barley was tested at three levels of nitrogen. By 22 July plots sown with 1, 2 and 3 bushels had respectively 3, 17 and 36 per cent of their areas lodged in plots receiving 1½ cwt. sulphate of ammonia, 27, 63 and 85 per cent in those receiving 3 cwt., and 33, 88 and 91 per cent lodged in those receiving 4½ cwt. sulphate of ammonia. This

https://doi.org/10.23637/ERADOC-1-76

striking increase in lodging with increased seed rate and nitrogen was evident throughout July, but during the wet August most of the crop fell down and was flat by harvest on 2 September. The total grain harvested averaged 42·9 cwt./acre; a seed rate of 2 bushels yielded 2·5 cwt./acre more than 1 bushel, and 3 bushels gave an insignificant further increase. One dose of nitrogen had no significant effect, but a second decreased yield by 1·5 cwt./acre. The amount of " tail corn " increased with increasing seed rate and nitrogen, and when both this and weed seeds were removed, the yield of dressed grain from plots sown at 1, 2 and 3 bushels/acre was similar; it was decreased by 3 and 3·4 cwt./acre by the first and second doses of nitrogen respectively.

Disease surveys of the classical and other wheat experiments showed an exceptionally high incidence of eyespot. (Glynne, Salt and Slope.)

Continuing experiments to test the effect of date of sowing on the incidence of powdery mildew, wheat was sown on four dates from 6 March to 21 April and barley on 22 March and 5 April 1954. Mildew was not observed until late May, and the last sown crops became the most heavily infected. The barley formed part of an experiment to measure how mildew affects yield. A susceptible (Plumage Archer) and resistant (Haisa II) variety were sown on both dates, and half of each sowing was sprayed with lime sulphur five times from 13 May to 8 July. Plants infected with *Erysiphe graminis* were placed in the plots in mid-May. Unsprayed Plumage Archer had ten times as much leaf infected as the sprayed, and leaves died from mid-June onwards. By 23 June, mildew had decreased the area of leaf on the main stems of the unsprayed plants by 16 per cent in the early-sown crop and by 36 per cent in the late-sown. The unsprayed plots yielded significantly less than the sprayed, 13 per cent less for the early and 22 per cent for the late sowing.

Unsprayed Haisa II developed only few pustules, and spraying did not affect yields. (Last.)

Pea diseases

From diseased peas a range of Fusaria was isolated that severally and together produced a variety of syndromes. *Fusarium oxysporum* f. *pisi* and *F. oxysporum* var. *redolens* cause wilt, *F. solani* causes foot rot, and *F. oxysporum* and *F. solani* together cause a condition resembling " St. John's disease ", previously recognized only on the continent of Europe. " St. John's disease " is less severe than either foot rot or wilt. In affected plants *F. oxysporum* is dominant in the stele and *F. solani* in the cortex of roots and stems. Inoculating isolates of *F. oxysporum* f. *pisi* to a range of differential pea varieties identified a previously unrecognized race, which is provisionally called 3A.

The pathogenic strains of *F. oxysporum* cannot be distinguished by cultural or taxonomic methods. Three possible ways of identifying them, other than laborious tests for pathogenicity, are being studied. Antisera were prepared by injecting rabbits with spores and macerated mycelium, but detailed agglutination and cross-absorption tests will be needed before their value can be assessed. Pathogenic and non-pathogenic strains are being compared for their resistance to ultra-violet radiation, and the third method, which

https://doi.org/10.23637/ERADOC-1-76

shows promise, is to test their inhibition by growth products of soil actinomycetes. Of twenty actinomycetes so far tested, some consistently inhibited the growth of all eight selected strains and others inhibited none. *Streptomyces albidoflavus*, however, inhibited some strains but not others.

In attempts to gain some information on the genetics of pathogenicity and on the mechanism of recombination in *F. oxysporum*, several thousand conidia were irradiated with ultra-violet light to produce mutants. Cultural tests showed fifty-two mutants with deficiencies in their aminoacid metabolism and thirteen deficient in their nucleic acid metabolism. In addition, fifty-two mutants were obtained which were dwarf or otherwise morphologically different from the wild type. Their ability, and that of the heterokaryons between them, to infect peas is being tested.

Several cultures of *F. oxysporum* isolated from roots of diseased Sitka seedlings in forest nurseries proved pathogenic when inoculated to Sitka seedlings under glass at Rothamsted. They may contribute to the difficulties experienced in raising healthy plants in old nurseries. (Buxton.)

Club-root of crucifers

The technique of infecting seedlings in water-culture to obtain root-hair infections has been mentioned in previous reports, and some of the factors affecting numbers of infections have now been studied. In general, they resemble those affecting infections in soil. Young seedlings were grown singly in small tubes or vials containing spores of *Plasmodiophora brassicae* suspended in a dilute mineral nutrient solution (Hoagland's solution) and incubated at 25° C. in the dark. The logarithm of the mean number of infections per plant is linearly related to the logarithm of the spore concentration. Root-hair infections occurred equally readily at pH 5 and at pH 6, but there were many fewer towards pH 8. The concentration of the culture solution was important. Thus, diluting Hoagland's solution, at pH 5 or pH 6, to one-fifth of the standard concentration considerably increased infections, and it seems that the germination of spores is affected. The number of infections obtained, however, depends on the interaction of at least three factors: numbers of spores, concentration of the culture solution and its pH. With high spore concentrations, changes in pH or concentration have less effect. Similarly, at certain spore concentrations, diluting the solution diminishes the influence of pH. (Macfarlane.)

Sugar-beet diseases

Continued tests with fungicides to control seedling diseases of sugar beet gave further evidence of the benefits derived from soaking seed for 20 minutes in 0·004 per cent solutions of ethyl mercury phosphate; at thirteen out of fourteen sites this treatment produced an average of 25 per cent more seedlings than dressing seed with "Agrosan". In soil artificially contaminated with *Pythium deBaryanum* and *Rhizoctonia solani*, pouring solutions or suspensions of various fungicides along the drills immediately before sowing much increased the number of seedlings that emerged; formalin increased the stand by more than 50 per cent. A drill with spray

https://doi.org/10.23637/ERADOC-1-76

nozzles mounted behind the coulters is being built to test the method further.

In the Docking district of Norfolk, and in other places with light, alkaline soils, sugar beet in June or July often become yellow, their leaf margins scorched and the laminae cupped. Tap roots are small, fanged, become horizontal at 3–4 inches below ground, and have a " beard " of laterals, many of which die. The " Docking disorder " is worse in dry weather and on land containing little organic matter. Severely affected plants are often infected with *Pythium* and *Rhizoctonia* sp., but when inoculated to sugar beet in glasshouses the isolated fungi fail to produce symptoms comparable with those in the field. Sugar-beet plants still grow poorly in affected soil that has been steamed. Both in pots and in the field, the condition can be alleviated by adding organic matter to the soil. The pathogenic fungi so far isolated from affected plants seem insufficiently virulent to cause the serious field condition; the effects on roots of young seedlings resemble those produced by extremely acid soils, and this, combined with the early leaf chlorosis and cupping, suggests a toxin may be the primary cause of the trouble with the fungi secondary. (Gates.)

Work was continued on the rotting of stored beet by *Botrytis cinerea*. This fungus does not rot beet cut during the summer, but after November they become susceptible. When untopped stored roots were cut lengthways and the cut tissue inoculated with *B. cinerea*, the lower parts of the roots became invaded but not the upper. With increasing age, more of the root becomes susceptible, and by about February only a small band of tissue immediately below the crown resists infection. Spore-germination tests with pieces of roots show that the resistant tissues contain a fungistatic substance, whereas the susceptible tissues do not, or at least not in detectable amounts. Bruised tissue provides an easier entry into roots than cuts. *B. cinerea* was also found to be the most important fungus causing rots of clamped mangolds and fodder beet. (Cornford.)

https://doi.org/10.23637/ERADOC-1-76