ROTHAMSTED RESEARCH

Report for 1980 - Part 1

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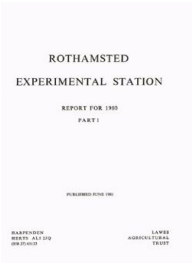

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E. Lester (1981) *Plant Pathology Department ;* Report For 1980 - Part 1, pp 175 - 200 **- DOI: https://doi.org/10.23637/ERADOC-1-137**

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Introduction

After very favourable autumn drilling conditions a mild winter, dry spring and cool wet summer led to a disease spectrum that contrasted with that of 1979. In cereals mildew was generally less severe, much less so in winter wheat which was more heavily attacked by *Septoria* spp. expecially after ear emergence. Barley yellow dwarf virus was recorded on early-sown winter wheat and was widespread on spring barleys in July. Foot rots were much in evidence on the winter wheat multifactorial experiment and sharp eyespot was unexpectedly and markedly decreased by irrigation: with *Septoria* the only serious foliar disease, fungicide, which was only partially successful, increased yield almost as much (0·8 t ha^{-1}) as in 1979 (see pp. 22). Potato blight was more in evidence than for several years and would have been severe but for effective use of fungicide. Stem-base and stolon infections by the skin spot pathogen were rare and stem canker and scab virtually absent. However blackleg and powdery scab, both encouraged by the wet summer, were common, the 'canker' form of the latter occurring on second growth on Pentland Crown tubers.

https://doi.org/10.23637/ERADOC-1-137

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It is a pleasure to record some progress in developing methods for the study of splash-dispersed diseases: these include a new 'head' for the rotorod trap for field use; the use of fixed photographic film for droplet size × spore content which is suitable for direct presentation to the image analysing computer for analysis; and the chemical labelling of non-volatile droplets which promises to assist fundamental physical studies of particle dispersal, impaction and sedimentation in crops. Virus studies have also been substantially aided by the application of new techniques: especially immunosorbent electron microscopy (ISEM). This particularly sensitive and rapid technique is proving very valuable for the study of viruses in low concentration such as the so-called cryptic viruses with which we are becoming increasingly concerned. ISEM has also been shown capable of identifying single aphids carrying barley yellow dwarf virus, a capability that we believe will extend to some other economically important aphid-vectored viruses, to the benefit of forecasting the need for control measures. Another development likely to be important in the control of aphid-vectored viruses is the discovery that one of the newer synthetic pyrethroids, deltamethrin, restricts the acquisition and spread of potato virus Y, a non-persistent virus not amenable to control by aphicides.

Our doubts about a functional relationship between virus resistance and the related Pr-proteins have not yet been resolved. In all our experiments, resistance, no matter how induced, has been accompanied consistently by the appearance of at least one, usually two or more of these proteins, two of which also occur in tobacco leaf callus. We have shown that the plant growth regulators present in the callus medium are able to induce resistance to tobacco mosaic virus and the production of at least one Pr-protein in tobacco plants. Increasing numbers of chemicals capable of inducing Pr-proteins are being identified, including most recently some simple metal salts.

The successful purification of beet mild yellowing virus has enabled preliminary work on serological relationships. The provision of purified virus opens up possibilities for disentangling the complexities of the important sugar beet viruses and potentially brings into use the newer serological techniques already referred to, for diagnostic and forecasting purposes.

The well-known response of cereal yields to the application of fungicides that is not always explained fully by disease control has been paralleled recently by oilseed rape growth responses to sprays of triadimefon. The same fungicide applied to rape stubble in 1978 at several times normal rate was unexpectedly persistent in soil, effectively controlling mildew in a spring barley crop grown on the site in 1980 and increasing yield by almost 50%. The possible significance of this observation to the siting of field experiments deserves attention.

Potential bactericides for the control of soft rot of potatoes have been tested with a new semi-commercial technique with the assistance of the Potato Marketing Board's Sutton Bridge Research Station. Of the two promising chemicals identified, 8-quinolinol and chlorine dioxide, the latter was severely phytotoxic at a concentration below that at which it was bactericidal. Further studies on the epidemiology of potato gangrene have confirmed that the seed tuber is the dominant source of inoculum but that surface contamination is at least as important as the rotting seed tuber. Preliminary experiments indicated that colonisation of stolons could account for large increases in soil-borne inoculum that occur after desiccation of haulm even if this is removed. Of newer fungicides tested for control of tuber-borne diseases some gave better control of specific diseases than thiabendazole but none emerged as more effective against the range of diseases.

The small amount of effort we are able to devote to the study of the taxonomy and interrelationships of the *Gaeumannomyces/Phialophora* group of fungi annually produces some new information. This year we have shown that some isolates of *Phialophora*

https://doi.org/10.23637/ERADOC-1-137

radicicola var. *radicicola* (Prr) have *Gaeumannomyces graminis* var. *graminis* as their teleomorph, though the perithecial form differs (in respects other than morphology) from Australian isolates. The usual negative relationship between the incidence of Prr and of take-all on cereal roots in field experiments at Rothamsted was not maintained. We now have evidence also that small sections of root can carry infection by both fungi, casting some doubt on the reliability of the antagonism of Prr against take-all in field conditions.

Aerobiology

Use of image-analysis in splash-droplet measurement. A new method for collecting spore-carrying splash droplets on fixed photographic film under controlled conditions eliminates the disadvantages of the technique developed by Gregory, Guthrie and Bunce (*Journal of General Microbiology* (1959) **20,** 328–354) and used in previous splash dispersal studies. It has the added advantage that the droplets can be measured by image-analysing computer. Gregory *et al.* collected the droplets on microscope slides coated with gelatine dyed with naphthol green B. However, purification of this dye is tedious and coating the slides uniformly with gelatine is difficult. Photographic film is evenly coated with gelatine during the manufacturing process and the droplets leave stains on the film which are clearly visible with phase-contrast microscopy, making the dye unnecessary. We used FP4 35 mm film (Ilford Ltd) fixed in Amfix (May & Baker Ltd) to remove the silver halides then dried, cut into 5 cm pieces and placed along radii from target spore suspensions. When the splashed droplets had dried the pieces of film gave a permanent record of the number of droplets, their position, size and spore content. Stain size was directly proportional to droplet size with a spread factor (droplet diameter/stain diameter) of 0·56. Pieces of film were placed in a purpose-built holder for presentation to the image-analysing computer (Quantimet), which speeded analysis substantially and improved the accuracy of the results. (Fitt, Lysandrou and Turner)

Mechanisms of splash dispersal. This work (*Rothamsted Report for 1979*, Part 1, 167–168) was extended to investigate the effects of incident drop size and depth of target liquid on the splash dispersal process, using spores and dyes incorporated into either incident drops or target liquids. The results suggest that the splash mixing process, and hence the incorporation of spores into splash droplets, is most efficient when large incident drops strike thin layers of target liquid. When the depth of target liquid was 0·5 mm, a 5 mm diameter incident drop produced more splash droplets and dispersed more spores than a 4 mm diameter drop (Table 1).

TABLE 1

Effect of incident drop size on number of splash droplets and spores dispersed. Depth of target liquid 0·5 mm. Average of two replicates

Diameter of incident drop (mm)	Spore incorporation	Number of droplets	Number of spore-carrying droplets	Number of spores
4	Target liquid*	3068	472	3182
4	Incident drop*	2743	349	1385
5	Target liquid†	6096	1485	18501
5	Incident drop†	5700	650	3949

* 120 000 spores ml^{-1}
† 320 000 spores ml^{-1}

At this depth more spores were dispersed when spores were contained in the target liquid than when the same concentration of spores was present in the incident drop, which suggested that the splash droplets contained more liquid from the target liquid

https://doi.org/10.23637/ERADOC-1-137

TABLE 2

Effect of depth of target liquid (dyed green) on the efficiency of the splash mixing process. Incident drop (dyed red) diameter 4 mm. Average of four replicates

Depth of target liquid (mm)	Colour of splash droplets			
	Red	Green	Brown	Total
0·1	2	3	449	454
0·5	2	55	151	209
1·0	2	43	140	185

than from the incident drop. This greater proportion contributed by the target liquid was confirmed by using dyes (Table 2). The pattern was similar with a target liquid of depth of 1·0 mm but at 0·1 mm, mixing of the liquids was more even, giving more brown splash droplets.

The data in Table 2 give a qualitative estimate of the proportion of splash droplet liquid contributed by the incident drops and target liquid. To obtain a more quantitative estimate a fluorescent dye, magnesium 1-anilinonaphthalene-8-sulphate [Mg(ANS)] is being used in the target liquid and the fluorescence of the splash droplets measured by image analysis. (Fitt, Dance, Lysandrou and Turner)

Splash dispersal of potato blackleg bacteria. The spread of *Erwinia carotovora* var. *atroseptica* by rainsplash, demonstrated by Graham and Harrison (*Phytopathology* (1975) **65,** 739–741), was investigated in raintower and in field experiments.

Raintower experiments. Incident drops, diameter 4 mm, were allowed to fall 13 m down the raintower on to a 0·5 mm thick layer of Mg(ANS) solution, containing bacteria, in still or moving air. The resulting splash droplets were collected on fixed photographic film, Petri plates containing pectate medium and on potato slices. The number of splash droplets carrying sufficient bacteria to form craters on the pectate medium was much smaller than the number detected on the photographic film by fluorescence of the Mg(ANS). Fewer still contained enough bacteria to initiate rots on the potato slices. In still air more splash droplets, many of them small, were detected closer to the target than further away. However, the proportion of splash droplets containing sufficient viable bacteria to initiate crater formation increased as distance from the target increased (a result of the greater distance travelled by larger droplets) and as the concentration of bacteria in the target liquid increased from 10^2 to 10^8 cells ml^{-1}. In moving air both splash droplets and bacteria were transported to greater distances than in still air; in still air no bacteria-containing droplets were detected beyond 100 cm from the target whereas in moving air (0·88 m s^{-1}) they were detected up to at least 275 cm from the target. (Fitt, Lapwood and Dance)

Field experiments. In attempts to detect airborne *Erwinia carotovora*, samplers were placed near infected potato plants on the Rothamsted farm. Liquid samplers for splash-dispersed spores (*Rothamsted Report for 1979*, Part 1, 166–167) contained 0·85% sterile saline and a Casella bacterial slit sampler (sampling rate about 200 litres min^{-1}) contained pectate plates and was connected to a rain-activated switch. However, few bacteria were detected, possibly because methods of handling the samples were not sensitive enough. Further evaluation of samplers for splash-dispersed bacteria is required. (Gosling, Fitt and Lapwood)

Trapping of splash-dispersed spores of *Rhynchosporium secalis*. Sticky discs on modified rotorods (Stedman, *Annals of Applied Biology* (1980), **95,** 163–175) were unsatisfactory

https://doi.org/10.23637/ERADOC-1-137

traps of *R. secalis* spores because, although operated under covers, spores were washed from the surface during intense rain. After tests of non-adhesive surfaces which could be used without covers, a glass fibre pad (1 mm thick) overlying a 24 mm diameter millipore filter (1·2 μm pores) was selected. Pad and filter were supported on wire gauze and held by a rubber O ring in a 4 mm long brass tube attached to each rotorod arm. After exposure both fibre and filter were washed and spores were filtered and stained for counting. Six traps, activated by a rain switch were operated at 800 rev min^{-1} in barley (cv. Maris Otter) during the summer. From mid-May to harvest weekly assessments were made of spore distribution on the crop; sample plants (20 on each occasion) were divided into fractions (initially 10 later 15 cm lengths measured from the base) which were washed and spores counted in a haemocytometer. Traps were operated at heights corresponding to the mid-point of each fraction and also above the crop.

Spring months were generally dry and in late May 90% of spores recovered from plants were below 10 cm; none was detected above 40 cm (crop height 55 cm). By contrast, concurrent catches in traps at six heights (up to 55 cm) showed a less steep vertical gradient, with only 64% of the total catch at 5 cm. From early July to harvest most spores were recovered from the 15–30 cm fraction of the sample plants and during this period spores in the third fraction increased as those in the first declined. Throughout however more spores were caught on the lowest trap than on any other and the proportion remained relatively constant. During June and July the uppermost trap at 20 cm above the crop always detected spores but the catch represented only *c.* 1% of the total caught by five traps within the crop. (Stedman)

Chemical labelling of drops. Studies on particle dispersal in crops may be simplified by a technique we have developed, whereby drops of polyethylene glycol 30–40 μm diam. labelled with thiabendazole and generated in the field using a spinning disc, can be detected on barley leaves at concentrations of about 30 drops cm^{-2} of leaf surface. If this technique models the dispersal of inoculum effectively, quantitative estimates of inoculum movement will be much simplified. (Bainbridge, with G. R. Cayley, CLU, and H. A. McCartney, Physics Department)

Transport of spores blown from surfaces by wind. Theoretical calculations suggest that spores which are released by wind will be deposited on surfaces of plants close to the point of release with efficiencies greater than would be predicted from the mean wind speed. Experiments done so far seem to support the calculated predictions. (Bainbridge, with McCartney, Physics Department (full report), and Dr D. E. Aylor, The Connecticut Agricultural Experiment Station, New Haven, Connecticut, USA)

Gradients of spore dispersal away from small plots. Spore concentration (C) probably decreases exponentially with distance from a source according to the form $C = ae^{-bx}$, where a and b are constants, x = distance and e is the base of the natural log system. The steepness of the gradient, which could be important in determining the extent of cross-contamination between plots in field experiments, will depend on a number of factors including the size (shape and area) of the source.

On six occasions during the summer spores were trapped on sticky cylinders and horizontal slides, on lines downwind from two plots of mildew susceptible barley, cv. Zephyr, of different dimensions, set in a field of the mildew resistant cv. Simon. Plots were the same length (9 m) but differed in width (2 and 8·5 m) and trapping occasions were selected so that the wind was blowing approximately at right angles to the length of the plot. A preliminary examination of our results suggests that the spore concentrations decreased as described by the above expression. Although more spores were

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caught downwind of the large than the small plot, there was no evidence that the rates at which concentrations declined (*b*) differed for the two plots. It is possible that our ability to detect effects of plot width was hindered by plots having the same crosswind length. (Bainbridge, Jenkyn and Hanacziwskyj)

Properties of viruses and virus diseases

Induction of resistance to tobacco mosaic virus (TMV) in tobacco

Stimulation of resistance-associated Pr-proteins by aspirin. We reported (*Rothamsted Report for 1979*, Part 1, 29) that when 830mM-aspirin was injected into five cultivars of tobacco it induced up to 100% reduction in the number of lesions formed following inoculation with a necrotic local lesion-forming virus and stimulated production of the resistance-associated Pr-proteins. TMV infects the tobacco cultivar Samsun systemically without causing local lesions. We assessed whether previous injection with aspirin 7 days before inoculation could reduce the amount of virus produced in the injected half of the leaf compared with that found in the water-injected control half. Counts of virus particles by electron microscopy showed a reduction after 7 days multiplication of 60%, whilst infectivity tests showed a 65% reduction in infective virus. When aspirin-treated Xanthi-nc tobacco plants are held at 32°C, a breakdown in the induced resistance occurs. However aspirin-injected half leaves of Samsun plants held at 32°C showed a reduction in virus concentration after 7 days, compared with the water-injected controls, of 46 % as assessed by microscopy and 47% as assessed by infectivity and large amounts of Pr-proteins were found in the aspirin-injected half leaves. When Xanthi-nc leaves were injected with aspirin and the plants placed at 32°C, large amounts of Pr-proteins were produced despite the fact that TMV-inoculated plants placed at the same temperature showed systemic infection with no necrotic local lesions and contained only very small amounts of Pr-proteins. (White and Woods, with Antoniw, Biochemistry Department)

Resistance-associated Pr-proteins in tobacco callus tissue. Callus grown from leaves of healthy Xanthi-nc tobacco plants and maintained on Murashige and Skoog medium contained Pr 1a and 1b, proteins associated with resistance to virus infection. Injection of leaves with each of the three plant growth regulators present in the growth medium, IAA, 2,4-D acid and 6-benzylaminopurine induced Pr 1a but little or no Pr 1b, and also caused a significant reduction in the number of necrotic lesions of, respectively, 44, 41 and 67% on infection with TMV. (White, with Antoniw and Kueh, Biochemistry Department, and Dr D. G. A. Walkey, National Vegetable Research Station)

Location of Pr-proteins in plant tissues. Protoplasts derived from Xanthi-nc tobacco leaves previously injected with 830mM-aspirin contained only very small amounts of protein Pr 1a and no other Pr-proteins. When the enzyme solution used to prepare the protoplasts was examined, a large amount of Pr 1a and some Pr 1b was found. It appears, therefore, that Pr-proteins are present mainly outside the cell or in the plasmadesmata and are released into the enzyme solution in the course of protoplast preparation. (White, with Antoniw, Biochemistry Department)

Pr-proteins are induced by some metal salts. The chloride and sulphate salts of Ba, Ca, Co, Cu, Fe, Mg, Mn and Zn at concentrations from 1 to 100mM were injected into leaves of Xanthi-nc and 7 days later the leaves examined for the production of Pr-proteins. The highest concentrations of all these salts except Ca and Mg were phytotoxic and at lower concentrations produced chlorosis similar to that observed in TMV-infected or aspirin-injected leaves. However, Pr-proteins were found only in the leaves injected

https://doi.org/10.23637/ERADOC-1-137

with the Ba, Co and Mn salts. This is a specific response to these three metal ions and demonstrates that the induction of Pr-proteins is not a consequence of the phytotoxicity of injected compounds and does not correlate with their ability to induce chlorosis in a leaf. (White, with Antoniw, Biochemistry Department)

The role of ethylene and a peroxidase isoenzyme in virus resistance. The formation of necrotic lesions on infection of tobacco leaves with TMV is accompanied by a large burst of ethylene release and the production of a new peroxidase isoenzyme as well as the synthesis of Pr-proteins and the induction of resistance. Leaves injected with salicylic acid (SA) to induce Pr-proteins and resistance were also examined for ethylene release and peroxidase activity. Although both TMV inoculation and SA injection of tobacco cv. Samsun NN leaves induced Pr-proteins and resistance, SA treatment did not produce ethylene release or peroxidase isoenzymes different from the control water-injected leaves. These observations confirm the accepted idea that peroxidase is not directly involved in the resistance mechanism and that ethylene may be an intermediate in the induction of Pr-proteins and resistance to TMV. However, if salicylic acid mimics a natural intermediate in the mechanism of induction of Pr-proteins and resistance, it is a more immediate elicitor of the response than either TMV or ethylene. (White, with Antoniw, Biochemistry Department, and Dr L. C. Van Loon, Agricultural University, Wageningen, The Netherlands)

Beet mild yellowing virus (BMYV). BMYV was purified from aphid-inoculated plants of *Claytonia perfoliata* using enzyme-assisted extraction, butanol–chloroform clarification and concentration by polyethylene glycol precipitation and differential centrifugation. After sucrose-density-gradient centrifugation to remove remaining host material, virus yields approached 1 mg kg^{-1} of plant tissue. The virus particles were about 27 nm in diameter and, in immunosorbent electron microscopy (ISEM) (Roberts & Harrison, *Annals of Applied Biology* (1979) **93,** 289–297), reacted strongly with an antiserum to beet western yellows virus provided by Dr J. E. Duffus indicating a close serological relationship, but did not react with antisera to barley yellow dwarf or beet cryptic viruses. (Govier and Woods)

Serological diagnosis of barley yellow dwarf virus (BYDV). The use of sensitive serological techniques to diagnose BYDV has been extended to include ISEM. The method, which has been modified slightly from that described by Roberts and Harrison, readily detects virus in leaf extracts diluted 10× using antisera diluted 1000× and distinguishes the two virus strains as well as does the enzyme-linked immunosorbent assay (ELISA) method (*Rothamsted Report for 1979*, Part 1, 173–174). ISEM will also detect BYDV in single aphids, whereas so far, ELISA has detected virus only in bulk samples of aphids (*Rothamsted Report for 1978*, Part 1, 211). BYDV isolate B has been detected in single *Rhopalosiphum padi* tested immediately after 24 and 96 h acquisition feeds as well as in aphids that had been stored in 95% alcohol for 2–3 h after an acquisition feed. However, when aphids were allowed an infection feed between acquisition and ISEM testing there was not always good agreement between those shown to carry virus and their transmission of virus to test plants. While there are many questions still to answer the method has great potential, expecially in providing a rapid test of alate aphid infectivity with a consequent improvement in forecasting virus occurrence. (Plumb and Lennon)

Resistance to potato virus X (PVX) in *Solanum berthaultii*. *S. berthaultii* is a wild potato being used in breeding programmes to produce pest-resistant cultivars. A clone has been

https://doi.org/10.23637/ERADOC-1-137

found which is hypersensitive (field-immune) to PVX strains 1–3. Thus, no permanent infection occurred after manual inoculation and plants grafted to infected *S. tuberosum* became top-necrotic. PVX strain 4 was lethal to plants of this clone, a feature which would also help protect crops. When sap from the clone was mixed with PVX inocula, infectivity was greatly reduced but whether this effect is associated in any way with sensitivity to PVX is unknown. (Gunenc and Gibson)

Pyrethroid insecticides can prevent transmission of potato virus Y (PVY). Starved *Myzus persicae* given 2½ min access to PVY-infected potato leaves treated with deltamethrin (0·001% a.i.) were only one-third as effective in transmitting virus to *Nicotiana tabacum* seedlings as aphids allowed access to untreated leaves. Spraying plants to run-off with 0·001%, and especially 0·01% deltamethrin also helped protect against infection by viruliferous aphids. The older-generation aphicide 'Metasystox' (0·1% a.i.) had no such effect on either acquisition or infection, probably because it does not have the rapid knockdown effect of the pyrethroid. (Gibson, with Rice and Sawicki, Insecticides and Fungicides Department)

Cereal diseases

Barley yellow dwarf virus (BYDV)

Aphid infectivity. 1979 and 1980 were two contrasting years for aphid numbers and infectivity with BYDV and illustrated the relevance, for any scheme that attempts to forecast virus incidence, of measurements of both parameters.

To the end of June 1979 few aphids were caught in the Rothamsted Insect Survey (RIS) suction traps and few were present on crops. During July, in settled weather, aphids, especially *Metopolophium dirhodum*, reached plague proportions. However, the first infective aphid, a *Macrosiphum* (*Sitobion*) *avenae*, was not caught until 25 July and the first infective *M. dirhodum* on 30 July, too late to cause much crop infection or yield loss. No *Rhopalosiphum padi* were infective during the spring and summer.

In 1980 infective aphids occurred much earlier; *R. padi* and *M.*(*S.*) *avenae* were first found to be infective on 4 June and the first infective *M. dirhodum* was caught on 7 July. To the end of August 8·5% of *R. padi*, 7·4% of *M.*(*S.*) *avenae* and 6·3% of *M. dirhodum* were infective. When expressed as the proportion of all species infective/week, 20–25% were infective from 11 to 25 August; this probably reflects the percentage plants infected by BYDV in cereal crops. This incidence was much greater than usual, not only because infective aphids were relatively common and early but also because a cool, wet spring delayed sowing. At Rothamsted on 17 July spring barley cv. Wing sown on 24 March had 4·0% shoots infected but 12·7% when sown on 28 April.

During the autumn migration of cereal aphids, most of which are *Rhopalosiphum* spp., the result of multiplying the number caught in the local RIS trap by the proportion infective which we have called the Infectivity Index (II) has given a good indication of the risk of autumn infection by BYDV. While this is only one of many factors that need to be considered when assessing disease risk, it is fundamental. In autumn 1978 and 1979 the II was 27 and 42 respectively and little infection of autumn-sown wheat was seen the following summer. In 1981 we expect much more infection on early sown crops because the 1980 II was 160, the second largest index recorded. The largest II was 161 in autumn 1973 when pesticides applied to September- and October-sown oats increased yield (*Rothamsted Report for 1974*, Part 1, 220). (Plumb, Lennon and Gutteridge)

The fate of aphids caught in suction traps. BYDV is persistently transmitted and a minimum of 48 h feeding is required for all infective vectors to transmit. As a result

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aphids caught in suction traps are allowed a minimum of 48 and a maximum of 96 h feeding time on test plants. After the feeding period the aphid is recorded as dead, missing, present or present and reproduced with the number of nymphs produced. These records indicate the suitability of cereals as hosts for the potential vectors which can be correlated with BYDV transmission success rate. Such information might be especially important in the autumn when many, if not most, of the cereal aphids are sexual forms seeking woody, rather than cereal hosts. In autumn 1980 of 278 *R. padi* tested, 94 (34%) survived the infection feeding period and 33 (11%) reproduced. Of 23 *R. padi* that were infective, 12 were alive at the end of the test feeding period and six had reproduced. Those that survived the test period were twice as likely to have infected test plants as those that had died or were missing. Nine out of 13 aphids of *Sitobion* spp. tested, were alive and all had reproduced as had one other before it died. All three aphids that transmitted BYDV reproduced. (Lennon and Plumb)

Vector specificity of isolates introduced by infective aphids. Of isolates transmitted to test plants by aphids caught in suction traps 98% were most efficiently transmitted by the introducing species in subsequent tests. Vector specificity of the isolates followed the established pattern: 64% of the *R. padi*-transmitted isolates were classified as 'severe' while 89% of those transmitted by *Macrosiphum* (*Sitobion*) spp. were 'mild'. Of the *Macrosiphum* (*Sitobion*) spp.-transmitted isolates 80% were not transmissible by *R. padi*, whereas most of the *R. padi*-transmitted isolates were transmitted albeit less efficiently by other species. (Lennon and Plumb)

Minimum cultivations and eyespot. Investigations into the effect of direct drilling and straw burning on diseases of wheat and in particular on eyespot have continued. To obtain a range of plant growth and density Armada winter wheat was sown either after ploughing or by direct drill at three times (mid-September, -October and -November) at three seed rates (100, 150, 200 kg ha^{-1}) on land where the straw had either been burnt or baled and carted. The incidence of disease in the crop was recorded and also every week from October to May seedling plants were exposed on each plot for a week and then transferred to a humid glasshouse and assessed for eyespot after 9 weeks.

The number of exposed plants which were infected increased in late (1978) or early (1979) autumn and remained near maximum for much of the winter and spring only declining in April (1980) or May (1979). In only 3 separate weeks in the 2 years, when no rain fell, were no plants infected. Particularly interesting was the considerable infection which occurred in January 1979 when the ground was lightly snow-covered for much of the time. Treatments had little effect on the number of plants infected in winter and spring but in autumn, plants exposed in ploughed, burnt plots had fewer infections (60% in 1978, 19% in 1979) than those in plots ploughed, unburnt.

Final incidence of eyespot was not greatly affected either by cultivation or straw disposal treatments although direct drilled unburnt plots tended to give least infection. As expected delayed sowing and decreased seed rate each decreased eyespot significantly. However in 1979 the ratio of slight infections to moderate + severe infections was little affected by sowing date or seed rate whilst in 1980 early sowing and high seed rate each greatly increased the proportion of moderate + severe infections. Other diseases present were also affected by the cultivation treatment; there was less sharp eyespot but more brown foot rot (*Fusarium* sp.) on direct drilled than ploughed plots. Take-all was more prevalent in direct drilled plots in the first year but less by the third year than in ploughed plots. (Bainbridge and Prew)

https://doi.org/10.23637/ERADOC-1-137

Take-all disease. In these reports the following abbreviations are used for simplicity:

Gaeumannomyces graminis var. *tritici* = Ggt
G. graminis var. *graminis* = Ggg
Phialophora radicicola var. *radicicola* = Prr
P. radicicola var. *graminicola* = Prg

Couch and take-all in Great Harpenden. The origin of take-all in old arable land after a period without susceptible crops is usually unknown but grass weeds are frequently suspect. In the first 3 years (1978–80) of an experiment on seasonal changes in soil-borne inoculum of the pathogen (Ggt), couch (*Agropyron repens*) was widespread, unevenly distributed but never dense. With one possible exception (plot 10) subjective estimates of the amount of couch were unrelated to soil infectivity or disease in winter wheat crops. Although the soil was infectious (15% of roots infected in a bioassay), couch rhizome taken from plot 10 in March 1980 was not infectious and did not produce Ggt on agar but after a period in moist sand one piece of discoloured rhizome developed perithecia which produced virulent progeny. In July, when soil infectivity had increased (39% roots infected in bioassay), couch roots, with or without discolourations, were effective as inoculum and sources of perithecia (Table 3), but only discoloured rhizomes were infectious and none of these produced perithecia. Couch material from the field edge was more discoloured, but much less infectious. In plot 10 changes in the infectivity of couch seemed to correspond to changes in soil infectivity, but there is no clear indication that couch increased infection of wheat or survival of inoculum. (Hornby and Henden)

TABLE 3

Take-all and the production of perithecia of Ggt from couch samples from Great Harpenden

Location	Discoloured tissue sample (%)	No. of pots with take-all (out of 14)	Category		Infectivity in bioassay (% roots infected)	Proportion tests with perithecia (%)*
Plot 10 (80/R/CS/212)	22	8	Roots	C	14·8	100
				D	12·9	100
			Rhizome	C	0	0
				D	30·4	0
Field edge	70	1	Roots	C	0	0
				D	1·3	0

C Roots without discolourations
D Discoloured roots
* Roots of bioassay seedlings rotted down in light

Studies of a site with little take-all. Added inoculum but not temporary breaks from monoculture, increased take-all in spring barley in the 'Effects of Breaks on Take-all' experiments at Woburn (*Rothamsted Report for 1979*, Part 1, 171–172). Soils from continuous barley plots and plots that had been without susceptible crops for 2 or 3 years were mixed with artificial inoculum and incubated for 4 weeks before testing in host-infection assays. All developed similar, high levels of infection (82–90% roots infected in bioassay plants), showing that continuous barley soil did not suppress added inoculum any more than the others may have done. By contrast the infectivities of the soils without added inoculum were very low (continuous barley soil, 4·8% roots infected; 2-year break soil, 0·1%; 3-year break soil, 0·05%), continuing the situation that developed 1 year after a peak of take-all in 1969. (Hornby, Henden and Bedford)

https://doi.org/10.23637/ERADOC-1-137

Perithecia from Prr isolates. Because of similarities in culture and in growth on wheat seedlings we have suspected our Rothamsted isolates of Prr (*sensu* Deacon) to be anamorphs of Ggg but we have failed to induce formation of perithecia in pure culture or on infected wheat seedlings kept moist in plugged test tubes for up to 9 months, a method which usually induces perithecial production by Ggt, Gg var. *avenae* and *G. cylindrosporus*. However, in 1978 and 1979 we isolated Prr from wheat at Abbots Ripton, Huntingdon, and eight of 27 isolates produced perithecia in tube tests. Average dimensions of the perithecia (310 μm diameter, neck 340×103 μm), asci (91×10 μm) and ascospores ($79 \times 2{\cdot}7$ μm) were within the range described for Ggg and ascospore isolates resembled the originals in colony appearance, phialospores and the formation of lobed hyphopodia on coleoptiles of infected wheat seedlings. Thus there seems little doubt that at least some of the Prr isolates from Abbots Ripton are anamorphs of Ggg. However, Miss M. Holden (personal communication) reports that these isolates and the Rothamsted isolates do not grow on oat leaf agar, on which Australian isolates of Ggg grow well: clearly further clarification of the relationships of these fungi is needed. (R. J. (Gutteridge)

Lack of effect of Prg on take-all in winter wheat. Further studies on the retained phases of the Rothamsted Ley-arable experiments during 1976–78 confirmed our earlier report (*Rothamsted Report for 1975*, Part 1, 255) that the incidence of take-all in second wheat crops after leys seemed to be inversely related to the incidence of Prg, thus supporting Deacon's suggestions (*Plant Pathology* (1973) **22,** 88–94) that grass leys increase populations of Prg and consequently inhibit the development of take-all in subsequent wheat crops. We now report on two field experiments in which populations of Prg were not consistently enhanced by grasses and the incidence of take-all was not related to the incidence of Prg. In the first experiment (R/CS/145) three successive winter wheat crops (cv. Cappelle-Desprez) were grown during 1976–78 after 2 years in different graminaceous species: *Agrostis tenuis*, *Dactylis glomerata*, *Festuca rubra*, *Holcus lanatus*, *Lolium perenne*, *Poa trivialis*, spring barley and spring wheat. Wheat seedling assay of soils sampled before ploughing the grasses and direct examination of the 1976 wheat crop showed that Prg was common in only one of the four replicate blocks and, within this block, populations were substantial only after *L. perenne* (68% of root pieces infected in July). This population decreased in 1977 but, in the same block, Prg increased on wheat after *F. rubra*, so that in July 1977 the percentages of root pieces from the second wheats after *L. perenne* and *F. rubra* were 22 and 52 respectively. There was little take-all on the winter wheat in 1976 and 1977 except after spring wheat and barley and after *H. lanatus*, known to be an efficient carrier of the take-all fungus. In 1978 all the wheat crops were attacked and the severity of infection was not less in the plots with the large populations of Prg after *L. perenne* in 1976 or after *F. rubra* in 1977.

In the second experiment (R/CS/202) three successive winter wheat 'test' crops (cv. Flanders) were grown during 1978–80 after the 'treatment' crops ryegrass (cv. RvP), oats (cv. Manod) and wheat (cv. Sappo) sown in spring 1977, following spring barley in 1976. The treatment crops, which grew for 4 months, did not differentially affect the incidence of Prg on roots of assay wheat seedlings grown in soils sampled before ploughing or on roots of field plants in the subsequent winter wheat test crops. However, populations of Prg were consistently much less in one block than in the other two, a difference that existed before the treatment crops were sown and persisted at least until July 1979, the date of our last available estimate. Inoculation of soil with Prg (agar plate cultures macerated in sand and broadcast before the final cultivation for the treatment crops) increased the incidence of Prg on assay seedlings and field plants only in the block with least Prg initially. On some plots the incidence of Prg on field plants (up to 65% of 15 mm

https://doi.org/10.23637/ERADOC-1-137

root pieces infected in July) was similar to that recorded in the Ley-arable experiments but despite this there was no apparent inverse relation between the incidence of Prg and of take-all. Moreover, microscopic examination of roots from field plants in July 1979 showed that Prg and the take-all fungus were simultaneously present on up to 18% of root pieces from the second winter wheat after spring wheat, showing that invasion of tissue by one fungus does not preclude invasion by the other.

The discrepancy between these observations and those from the Ley-arable experiments casts doubt on the general application of the thesis that grass leys encourage large populations of Prg which delay the development of take-all in subsequent wheat crops. Until this discrepancy is explained farmers should not expect the control of take-all to last longer after leys than after other break crops. (Slope and R. J. Gutteridge)

Residual effect of systemic fungicide on barley mildew. Triadimefon and benomyl were applied as sprays at unusually high rates (2 kg a.i. ha^{-1}) to soil (Great Field I) on 22 August 1978 prior to a winter oilseed rape crop. Triadimefon, but not benomyl, gave almost complete control of powdery mildew throughout the growth of a subsequent crop of spring barley cv. Georgie sown (18 April) and harvested (2 September) 2 years later, in 1980. Barley mildew was assessed as percentage leaf area infected on all leaves of ten plants from eight plots of each treatment on four occasions through the growing season (5 June 1980, GS (Zadoks) 30; 26 June, GS 51; 11 July, GS 65; 28 July, GS 75); components of yield were measured at harvest.

Mildew developed quickly in the untreated and benomyl-treated plots and was severe from June onwards. However in triadimefon-treated plots the leaves and stems of 84% of all plants sampled at the end of July (including main stem and tillers) remained completely healthy; the few infected plants had pustules mostly confined to the oldest leaf.

Residual triadimefon significantly increased grain (42%) and straw (38%) yield, 1000 grain weight (12%) and grain size (75% increase in grain >2·8 mm), but had little effect on the number of ears or grain per metre row or number of grains per ear (Table 4). In previous experiments at Rothamsted yield increases of this magnitude in spring barley, from control of mildew, have been achieved only after intensive routine use of foliar fungicides. The results indicate that triadimefon may remain active in soil for long periods and that future siting of experiments on barley mildew may need to take this into account. (Rawlinson and Muthyalu)

TABLE 4

Residual effect of triadimefon on mildew and yield of spring barley. Great Field 1980

	Fungicide			SED
	None	Benomyl	Triadimefon	(n=8)
% mildew on leaf 2 GS75	35·9	35·3	0·7	*
Grain yield (t ha^{-1})	2·5	2·6	3·5	0·16
Straw yield (t ha^{-1})	2·6	2·6	3·7	0·24
1000 grain weight (g)	31·7	32·3	35·6	0·96
% grain >2·8 mm	24·3	26·7	42·8	2·76

* Analysis of variance of transformed data (logit) shows a variance ratio with a probability of <0·005

Observations on *Rhynchosporium secalis*. To study the effects of herbicides on the number and viability of spores on barley volunteers 25% dinoseb (as 'Farmon Desicoil 25') was sprayed on to barley volunteers (cv. Georgie) on 24 October 1979 and the number of spores of *R. secalis* was assessed weekly. Although much of the exposed green tissue was destroyed plants were not killed and regrowth had started after 7 days. There were fewer

https://doi.org/10.23637/ERADOC-1-137

spores on treated than untreated plants for 6 weeks after spraying but after new leaves became infected spore numbers differed little. The viability of spores from volunteers sprayed with glyphosate on 24 October was measured weekly until early February. Viability generally declined from 80% 1 week after treatment to 4% 14 weeks later but throughout did not differ significantly from that of spores from untreated plants. (Stedman)

Diseases of grasses and forage legumes

Ryegrass seed-borne virus (RGSV). This virus occurs in very low concentration in plants infected through seed and cannot readily be detected by electron microscopy. When infected plants are subsequently infected with ryegrass mosaic virus (RMV) the multiplication of RGSV is apparently stimulated and particles *c.* 30 nm in diameter can readily be seen in leaf-dip preparations. Using this method RGSV infection was detected in *Lolium multiflorum* cvs S 22 and RvP, *L. multiflorum* × *perenne* hybrids cvs Grasslands Manawa and Sabrina and *L. multiflorum* var. *westerwoldicum* cv. Baroldi. The method was time consuming and only plants infected with RMV could be diagnosed as infected with RGSV. However, the development of ISEM allowed RGSV infection to be diagnosed using an antiserum prepared to RMV and RGSV. Whereas with conventional methods it was almost impossible to find particles in leaf sap, using ISEM the presence of up to 200 particles per field allowed ready diagnosis and the screening of many cultivars. RGSV is most readily detected in older leaves and can be detected in *L. multiflorum* cvs RvP, S 22, Combita, Tiara, Delecta, Lema, Florida Rust Resistant, and Optima but not in Asso or Gulf; in *L. multiflorum* × *perenne* cvs Sabrina, Grasslands Manawa and Augusta and in *L. perenne* cvs Reveille and Monta but not S 24. Up to 86% of plants of some cvs were infected, although RvP plants grown from five different seed lots had infection ranging from 10 to 70%. The virus seems more widespread in *L. multiflorum* than *L. perenne*.

Attempts to transmit the virus mechanically to *L. multiflorum* cv. S 22 have failed whether the source of RGSV was plants infected with RGSV alone or with RMV and RGSV. However, there was a suggestion that the use of jointly infected plants as a source of RMV slightly increased the rate of transmission of RMV to a range of *Lolium* and *Avena* spp. Experiments have so far failed to demonstrate any additional adverse effect on the yield of cv. S 22 of infection with RGSV as well as RMV.

When RGSV-infected and healthy plants of cv. S 22 were allowed to cross-pollinate in the glasshouse, 20% of the seeds from healthy plants produced plants infected with RGSV. This compares with *c.* 70% infection from the original infected stock. (Plumb and Lennon)

Red clover necrotic mosaic virus (RCNMV). An experiment, begun in 1978, investigated the transmission of RCNMV and its effect on yield. Boxes of soil-less compost were each planted with a 7 × 7 lattice of red clover seedlings cv. Hungaropoly. Disease was established by sap-inoculating either 20 or 50% of plants at random, or the plants along one edge of the box; some boxes were not inoculated. The crop was cut with reciprocating electric shears three times in 1979 and once in 1980.

There was no difference in yields at the first two cuts even though 10% of plants in boxes with 50% initial infection had died before the first cut and 30% before the second. The mean yields for the last two cuts from boxes with 20 and 50% initial infection were respectively 18 and 27% less than yields from uninoculated boxes. After the final cut, 53% of plants in boxes with 50% initial infection, 44% in boxes with 20% initial infection, but only 12% in uninoculated boxes were dead. Obviously surviving healthy plants partly compensated for the missing plants. However, the experiment was hand-weeded

https://doi.org/10.23637/ERADOC-1-137

and less compensation may occur in crops, where weeds can invade. In boxes with 50% initial infection, 23% of surviving plants were infected by RCNMV compared with 12% in boxes with 20% initial infection.

In boxes with inoculated plants along one edge, direction of cut (to or from the infected row) made no difference to final percentage plants infected (21%). Surprisingly, infection did not always spread to plants adjacent to those initially infected and is therefore difficult to attribute to root contact or mechanical transmission. No infection occurred in uninoculated swards which were cut without treatment of the shears immediately after those with much infection, so it seems that although machinery may transmit RCNMV within a sward infection is unlikely to be carried from field to field on the cutter bar. (R. A. Gutteridge)

Putative vectors of RCNMV. In the glasshouse in mid-winter, clover, sown in soil from a disease site and kept wet, developed symptoms of RCNMV infection after 4 weeks. Such infected plants in field soil were placed under a continuous drip of water and the drainage conducted to pots of sterilised soil in which healthy clovers were growing. Within 4–6 weeks, these, in turn, showed symptoms of RCNMV. Fungi seen in their roots included *Olpidium brassicae*, *O. radicale*, *Rhizophydium* (? *graminis*) and a *Ligniera* sp. In roots from several RCNMV soils, the *Ligniera* was the most abundant.

A culture of *Olpidium brassicae* from a virus-diseased plant was established on clover which, however, remained free from virus. A Scandinavian report, unsupported by clear evidence, that both these *Olpidium* species transmitted RCNMV then drew our attention to *O. radicale*, a species probably more widespread than hitherto recognised. Fragments of root from a diseased field-grown plant containing, apparently, sporangia only of *O. radicale* were placed alongside clover roots growing in sand. Three months later, in winter, zoosporangia and zoospores of *O. radicale* were seen in and around these inoculated roots. No other fungal parasites were observed. In summer the fungus became inactive. From these inoculations we now have plants infected by *O. radicale* and RCNMV. This suggests that *O. radicale* may be the vector. (Macfarlane)

Effects of aldicarb on local spread of ryegrass mosaic virus (RMV). In 1979, short-distance spread of RMV from infected and mite-infested ryegrass to newly sown plants greatly exceeded long-distance spread by mites blown into plots from outside the experiment (*Rothamsted Report for 1979*, Part 1, 174). In 1980, plots of ryegrass were either treated with aldicarb (10 kg a.i. ha^{-1}) or not before sowing and a row of old infected grass was transplanted to the central row of some plots 1 month later. In plots without aldicarb planted with old sward, mites spread rapidly to adjacent rows and within 2 months 50 and 20% of tillers in 1st and 2nd rows next to the old grass showed symptoms of RMV. In plots without old grass or with old grass but treated with aldicarb, <1% of tillers had RMV in any row. (Gibson)

Diseases of grain legumes

Vicia cryptic virus (VCV). This seed- and pollen-borne virus (*Rothamsted Report for 1979*, Part 1, 176) was readily detected in sap of individual plants of several field bean and broad bean cultivars by ISEM using an antiserum to VCV from cv. Maris Bead. By ISEM, no serological relationship was found between VCV and several other legume viruses with isometric particles of similar size (*c.* 30 nm), nor between VCV and beet cryptic virus. No obvious reaction was observed between VCV and its antiserum in double-diffusion and microprecipitin tests but when a partially purified preparation of the virus was mixed with antiserum diluted to 1 : 100 and was then treated with phospho-

https://doi.org/10.23637/ERADOC-1-137

tungstate, particle aggregates up to 1 μm in diameter were seen in the electron microscope.

VCV was detected in five broad bean seedlings that had been kept at 32–38°C for four 1-week periods with 1 week at normal temperatures between each treatment. Thus it seems that VCV cannot be eliminated from plants by high temperatures.

The results of two plot experiments again suggest that VCV has little or no effect on yield. Yields of six inbred infected lines (*c.* 66% plants infected) of field bean cv. Minden ranged from 14·5 to 40·6 g per plant (mean, 28·8 g); yields of six inbred healthy lines ranged from 15·6 to 40·1 g per plant (mean 29·6 g). Corresponding results for inbred lines of broad bean cv. Triple White (*c.* 78% plants infected) were 24·0 to 45·5 g per plant (mean, 36·1 g) and 28·6 to 54·4 g per plant (mean, 40·0 g). Some of the lines proved to be very susceptible to aphid-borne bean leaf roll virus but susceptibility to this virus was not related to infection with VCV. (Kenten, Cockbain and Woods)

Bean yellow vein-banding virus. This virus is transmitted by aphids in a persistent manner, but only in the presence of helper viruses—pea enation mosaic and bean leaf roll (*Rothamsted Report for 1977*, Part 1, 221–222). In glasshouse tests it was transmitted readily by *Acyrthosiphon pisum*, less readily by *Myzus persicae* and not at all by *Aphis fabae*. The virus is transmissible by mechanical inoculation but usually only a small proportion of plants is infected unless phenol or bentonite is used in extraction of sap. No virus-like particles were seen in infective clarified preparations from plants infected with vein-banding virus alone but membrane-bound particles up to 90 nm in diameter were seen in sections of infected-leaf cells. The particles seem to be similar to those found in other species infected with two other dependent viruses—carrot mottle and lettuce speckles mottle. (Cockbain and Jones)

Pea early-browning virus. This nematode-borne virus was found in *c.* 30% of shoots taken from a field bean crop at Woburn but it was not detected in shoots from an adjacent crop of leafless peas. Infected plants showed a very mild mottle or were without symptoms. The possibility of seed transmission in field beans is being investigated. (Cockbain and Woods)

Chocolate spot of winter beans. Four fungicides were assessed for control of chocolate spot on winter beans (cv. Throws MS). In laboratory tests prochloraz was the most effective in decreasing mycelial growth on Last's agar. Growth after 6 days on agar containing 0·1 ppm prochloraz was only 21% of control whereas on iprodione, benomyl and thiabendazole at the same concentration it was 31, 54 and 85% respectively. When tested in thin aqueous film benomyl at 0·1 ppm either prevented spore germination or caused distorted germ tube growth, while thiabendazole needed 0·3 ppm and iprodione and prochloraz 3 ppm to achieve the same effect.

In the field benomyl decreased disease most. In early July, after two applications of fungicide, at early flowering (25 May) and toward the end of flowering (28 June), percentage leaf area diseased on upper leaves was 6·2, 11·8, 18·7 and 19·5 on the benomyl-, iprodione-, prochloraz- and thiabendazole-sprayed plots respectively, compared to 23% on untreated plots. Neither thiabendazole nor prochloraz increased yield but benomyl and iprodione gave a 7·1 and 9·6% increase respectively (control = 3·97 t ha^{-1}). Of this increase most was contributed by the later of the two sprays. (Bainbridge with Cayley)

Root disease of field beans. In several field experiments in 1976–78 investigating factors affecting yield, large seedbed dressings of benomyl (20 kg ha^{-1}) only slightly reduced root-rot and had little effect on yield. By contrast relatively small amounts (0·6 kg ha^{-1}) applied as foliar sprays improved yields significantly without affecting root-rot, an

https://doi.org/10.23637/ERADOC-1-137

effect not attributable solely to control of foliar diseases. In 1978, 1979 and 1980 the effects on root-rot and yield of seed treatments and foliar sprays of benomyl, fosetyl-Al and metalaxyl were studied. Late foliar sprays of benomyl again increased yield most and there were smaller increases in yield and decreases in root rot by fosetyl-Al applied as a seed treatment or as an early post-emergence spray and by benomyl + thiram seed treatments. Relatively small amounts of fungicide applied to seed were as effective as, or more effective than, much larger dressings to the seedbed and mixtures, e.g. benomyl + fosetyl-Al and benomyl + thiram were better than fungicides applied singly. The sticker 3% methyl cellulose, applied alone to seed, also seemed to have a fungicidal effect. The results suggested that a combination of seed treatment and late foliar spray of benomyl might give the best yield but when this was tested experimentally in 1980 the yield was no greater than where foliar benomyl alone was applied. (Salt)

Other work on grain legumes is reported in Multidisciplinary Activities, pp. 26–33.

Biodeterioration

Fungicidal control of the microflora of ripening grain. Between ear emergence and harvest of untreated ears of spring wheat (cv. Timmo), populations of fungi increased from $3{\cdot}5 \times 10^5$ to $4{\cdot}0 \times 10^7$ propagules g^{-1}. The predominant fungi were *Aureobasidium*, *Hyalodendron* and yeasts up to GS 75 but subsequently *Cladosporium*, *Verticillium* and *Alternaria*. The microflora of flag leaves was similar except that pink yeasts were more numerous and *Verticillium* was absent. At harvest, at least half the grains were contaminated with *Alternaria*, *Cladosporium* and *Epicoccum* but fewer than 5% with storage fungi such as *Aspergillus* and *Penicillium*.

Carbendazim + maneb was applied to plots at GS 38–39 and again at GS 50 or GS 60 or was replaced for the later sprays by benomyl, imazalil, prochloraz or captafol. The early spray had no effect on ear microflora but carbendazim + maneb, benomyl, imazalil and prochloraz significantly decreased the numbers of fungi on treated ears within 24 h at both GS 50 and 60. However, numbers of fungi on ears after 14–28 days were significantly smaller only on captafol-treated plots. By harvest, populations were 75–90% of those of untreated control plots. Effects on flag leaves were similar. Populations of *Aureobasidium*, *Hyalodendron* and yeasts were most affected by fungicides but only captafol decreased numbers of *Cladosporium* markedly. Prochloraz was the only fungicide to decrease *Alternaria* but the difference was significant on only one sampling occasion. Only the early carbendazim + maneb treatment increased yield significantly.

TABLE 5

Inhibition of mycelial growth and spore germination of field fungi by fungicides

Mycelial growth	Minimum inhibitory concentration (ppm)				
Fungicide Fungi	Prochloraz	Benomyl	Carbendazim + maneb	Captafol	Imazalil
Alternaria tenuis	10	>500	>500	100	>500
Cladosporium cladosporioides	10	10	100	100	200
C. herbarum	10	10	200	100	500
Epicoccum purpurascens	100	100	200	500	200
Fusarium culmorum	1	10	10	500	500
Spore germination					
A. tenuis	>500	>500	>500	100	500
C. cladosporioides	200	500	10	100	200
C. herbarum	>500	500	500	200	500
E. purpurascens	200	500	100	200	200
F. culmorum	10	10	100	10	500

https://doi.org/10.23637/ERADOC-1-137

Mycelial growth of five field fungi was usually more sensitive to a range of fungicides *in vitro* than spore germination (Table 5). Prochloraz was the most effective of the fungicides in inhibiting mycelial growth of all fungi but was less effective than captafol in inhibiting spore germination. *Alternaria* was tolerant of benomyl and carbendazim + maneb but spore germination was inhibited by 100 ppm captafol and mycelial growth by 10 ppm prochloraz or 100 ppm captafol. (Magan and Lacey)

Water activity, spore germination and growth in field and storage fungi. Linear growth and spore germination were studied in sealed Petri dishes or test tubes using wheat extract agar with the water activity (a_w) modified in the range 0·70–0·97 by adding glycerol. Five field fungi required >0·85 a_w for spore germination, >0·88 a_w for linear growth and *Alternaria* and *Cladosporium* required >0·90 a_w for sporulation. The minimum a_w for germination varied in a group of *Penicillium* species between 0·79 (*P. piceum*) and 0·83 (*P. roqueforti*) and in a group of *Aspergillus* species between 0·71 (*A. amstelodami*) and 0·86 (*A. fumigatus*). Changing the pH from 6·5 to 4 increased the minimum a_w for germination by 0·02 at optimum temperatures and by 0·05 close to the maxima and minima. The period required for germination was also markedly increased.

Changing the a_w of media can have pronounced effects on colony growth and appearance. *Aspergillus aurantiobrunneus*, isolated from Iranian grain, formed yellowish colonies, on malt extract or Czapek agar, with few or no conidiophores but composed of a mass of hulle cells surrounding cleistothecia containing typical *Emericella* ascospores. On hay-infusion agar, more conidiophores were formed among the hulle cells, colouring the colonies avellaneous. Decreasing the a_w of these media with 10% NaCl, 40% sucrose or 2·5M-KCl largely inhibited hulle cells and ascospores but allowed more rapid linear growth with a dense stand of conidiophores giving plane, velvety, ochraceous buff to ochraceous orange colonies. At the optimum temperature (30°C), colonies covered a 9 cm Petri dish in 3–4 weeks. The minimum a_w for spore germination was 0·8. (Lacey and Magan)

Selective isolation of actinomycetes. Further tests (*Rothamsted Report for 1979*, Part 1, 169) have shown that *Actinomadura* spp. can be selectively isolated from soil by incorporating 5 μg rifampicin ml^{-1} yeast extract glucose agar. Isolations from a group of tropical soils using this medium have shown *Actinomadura* spp. to be more common than previously thought with up to 360 000 propagules g^{-1} soil. Heating the air-dry soil at 100°C for 15 min greatly decreased bacterial contamination. Although numbers of *Actinomadura* and other actinomycetes were also decreased by heating, this method facilitated the isolation of *Actinomadura* spp. in pure culture.

Addition of rifampicin to half-strength nutrient and tryptone soya agars was also useful for the isolation of *Thermomonospora chromogena*, *Saccharomonospora viridis* and *Streptomyces albus*. Eubacteria and the actinomycetes *Thermoactinomyces vulgaris*, *Micropolyspora faeni* and *Thermomonospora fusca* were mostly inhibited by 5 μg rifampicin ml^{-1} medium. (Lacey, with Miss M. Athalye and Dr M. Goodfellow, Newcastle University)

Penetration of respirator filters by actinomycete spores. Penetration of *Thermoactinomyces vulgaris* spores through respirator filters was assessed using sedimentation chambers or a small wind tunnel, Andersen air samplers and isolation on medium containing 25 μg novobrocin ml^{-1}. The best cartridge filters constructed to British Standard BS 2091 Type B permitted only 0·2–0·3% of the spores to penetrate while those constructed to BS 2091 Type A allowed 1·9–2·8% penetration. Some disposable respirators gave variable performance but overall none performed as well as the Type A cartridge filters. The

https://doi.org/10.23637/ERADOC-1-137

standard bag filter from a ventilated helmet gave a penetration close to that of Type A filters, although a heavier-duty version performed as well as the Type B. Nuisance dust respirators with fibre or foam filters allowed penetration of 14–50% of *Thermoactinomyces* spores. Care is thus necessary in choosing a suitable respirator for protection against the actinomycete spores causing farmer's lung disease. (Lacey)

Diseases of winter oilseed rape

Disease control and crop growth response to fungicides. A single autumn foliar spray (500 g a.i. ha^{-1}, 13 November 1979) of benomyl, prochloraz or imazalil, on a heavily diseased crop of Primor winter oilseed rape on Summerdells I, halved the incidence of light leaf spot (*Pyrenopeziza brassicae*) in spring 1980 and greatly reduced the severity of the disease compared to untreated plots which had >70% plants infected and >3 infected leaves per plant. A second fungicide spray (27 February) maintained the low level of light leafspot, which increased in untreated plots to >90% infected plants. None of these fungicides, nor thiabendazole or metalaxyl, applied at the same times and rate, had significant effects on the incidence of the leafspot stage of canker (*Leptosphaeria maculans*) and a single application on 27 February had little effect on either disease.

The most effective treatments for control of light leaf spot (autumn + February applications of prochloraz or benomyl) decreased the amount of leaf area occupied by lesions from 20% in untreated plots to <3%. Growth analyses during April and May showed that the autumn application of fungicide contributed most to disease control and crop development; crop growth rate (CGR) and leaf area index (LAI) were approximately double those of untreated plots and flowering was earlier. Untreated plots had a CGR of 6·31 g m^{-2} day^{-1} compared to 11·58, 13·35 and 13·15 for plots given an autumn spray of prochloraz, benomyl or imazalil respectively. CGR increased only slightly (up to 15·96 with imazalil) following the second (February) spray. LAI for untreated plots at the end of April was 0·98 compared with 1·95–2·62 for autumn-sprayed plots and 2·44–2·55 for plots given both autumn and February sprays. A single spray in February gave a less marked response in CGR (range 9·69–11·31) and little effect on LAI (range 0·74–1·53).

Although no fungicide tested significantly affected the incidence of the leafspot stage of canker, the autumn + February sprays of benomyl, prochloraz or imazalil approximately halved the incidence and severity of stem cankers in mid-May but such differences were not maintained subsequently.

Effects of chemicals applied to rape stubble. An adjacent field experiment tested the effectiveness of a range of herbicides and fungicides, applied as sprays (29 August 1979, 10 days before sowing) to rape stubble, in suppressing disease development in a subsequent crop of Primor rape. Triadimefon (1 kg a.i. ha^{-1}) gave 40% plants infected with light leafspot in spring compared with 80–100% in plots given other treatments or none: by mid-May stem infections were <10% and 40–70% respectively.

Triadimefon had little effect on the leaf spot stage of canker early in the season, but decreased the proportion of stems severely lesioned by stem cankers in May. By mid-May triadimefon-treated plots had nearly double the dry matter per unit area and 70% greater leaf area than untreated plots. This growth response almost matched that achieved by two carefully timed foliar sprays of fungicide in the nearby experiment.

Autumn-applied foliar sprays appear to be more beneficial than those applied later, and the persistent effects of triadimefon sprayed even earlier on stubble/soil before sowing (see also p. 186) are being investigated further. The remarkable growth responses recorded, some of which may not be due to disease control alone, have complicated the

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interpretation of disease effects: experiments are in progress to investigate the nature of these responses.

This year's results indicate that there are several promising fungicides available for disease control in oilseed rape, other than those currently approved for use on the crop. However, further work is needed to confirm their effectiveness, especially on other cultivars and under conditions of less severe disease pressure. (Rawlinson, Muthyalu and Cayley)

Potato diseases

In a season very different from 1979, potato yields in 1980 were well above average and the spectrum of disease also differed markedly. There was little stem-base or stolon damage from *Rhizoctonia solani* and stem-base browning by *Polyscytalum pustulans* was scarce. The June rain coincided with the early stages of tuber formation and there was little or no scab even in experiments where severely scabbed seed from Woburn 1979 had been planted. The cool weather favoured natural blackleg which was commonly seen during July but, in experiments where seed tubers had been inoculated with *Erwinia carotovora* var *atroseptica*, there was none, presumably because the initiated rots had become arrested and dried out during the unusually dry weather which followed planting. Late blight (*Phytophthora infestans*) was first seen in the region on 12 August but not until 22 August on Long Hoos at Rothamsted. Lesions could be found readily and a few blighted tubers were seen in hand-dug samples at harvest but generally the fungicide sprays kept the disease in check. Powdery scab (*Spongospora subterranea*), not seen in August was prominent on tubers in September. Typical pustular lesions were seen on King Edward, Maris Bard and Ulster Sceptre but the severe 'canker' form developed on secondary growth on Pentland Crown tubers.

Disease caused by bacteria

Spread and survival of* Erwinia carotovora *varieties. In order to simulate the movement of bacteria from stems to progeny tubers and to study survival in soil, inoculum of either var. *carotovora* or var. *atroseptica* was introduced on two occasions over a 72 h period by squirting 3 ml of a suspension of 10^{15} cells ml^{-1} from a syringe around the stem bases of plants, cv. Pentland Crown, that had had their seed tubers removed soon after emergence. Inoculation was done on five dates between June and September and the fate of the introduced inocula was monitored serologically (*Rothamsted Report for 1976*, Part 1, 271).

Generally var. *atroseptica* was recovered from soil more frequently and after more of the inoculation dates than var. *carotovora* but usually neither was detectable 2 weeks from introduction, except occasionally from soil adhering to progeny tubers. Both varieties could be recovered from induced progeny tuber rots within a week of inoculation provided sufficient rain had fallen to saturate the soil. During the dry September it proved difficult to induce rot in progeny tubers: after October rain more rotted but never in the numbers found earlier in the season. At harvest the introduced strains were isolated only from some of the induced rots. The results suggest that single introductions survive for a limited period and that a continuing source of inoculum, e.g. stem lesions or rotting seed tuber, may be essential to maintain a population sufficiently large to pose a risk of rotting in store. (Lapwood, Gosling and Read)

Susceptibility of stems to* Erwinia carotovora *varieties. Experiments to study the spread of bacteria from inoculated stems to progeny tubers (*Rothamsted Report for 1979*, Part 1, 178) showed differences in the type and severity of lesions produced on the different inoculation dates and it was not clear whether this was due to changes in

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inherent host susceptibility or to the weather following inoculation. In 1980 two stems of each of ten field-grown plants of cv. Pentland Crown were injected with a concentrated bacterial suspension, through the wound left after removal of a leaf, weekly from mid-June to late August. Two isolates of var. *carotovora* and two of var. *atroseptica* were used and symptoms recorded after 1 and 2 weeks. Both the var. *atroseptica* isolates produced lesions typical of blackleg but one isolate proved more aggressive than the other both in the speed at which symptoms were produced and in severity. The var. *carotovora* isolates produced much drier internally brown lesions which caused stems to split and again one isolate proved more aggressive than the other. The most severe symptoms from all isolates cocurred from inoculations made from mid-June until mid-July. The weather then became drier and symptoms much less severe until rain in mid-August encouraged more aggressive lesions, indicating that, with the method of inoculation used, the weather was the dominating influence on lesion severity. (Lapwood and Gosling)

Potential bactericides. Attempts have been made in collaboration with the Potato Marketing Board Research Station, Sutton Bridge, to develop a more practical evaluation of the potential bactericides selected in small *in vitro* tests (*Rothamsted Report for 1977*, Part 1, 224). After the 1979 harvest nets of tubers containing 25 deliberately damaged and 25 'as lifted' tubers of cv. Desiree were dipped for 1 min in water, dichlorophen (2000 ppm), chlorine dioxide (750 or 1500 ppm) or 8-quinolinol (1500 ppm). Each of three nets per treatment was allocated to a different level within a 1 tonne box which contained all five treatments. In an attempt to provide environments conducive to rotting, some boxes were lined with black plastic and some of these were filled with pre-wetted tubers. When emptied after 3 months storage, damaged tubers showed marked differences in percentage rots. In the unlined, lined and lined + wetted treatments, water-dipped tubers showed 0, 3 and 30% rots; dichlorophen 7, 3 and 40%; chlorine dioxide (1500 ppm) 11, 1 and 15%; and 8-quinolinol 2, 2 and 16% respectively. Chlorine dioxide had no effect on soft rotting at 750 ppm but was severely phytotoxic at both concentrations, causing deep necrotic lesions. (Lapwood and Read)

Gangrene (*Phoma exigua* var. *foveata*)

Sources of inoculum. To determine the relative importance of seed tubers and stems as sources of inoculum for progeny tubers, different levels of inoculum were established in field experiments between 1977 and 1979 by planting rotting or contaminated seed tubers and by inoculating stems shortly before haulm desiccation. On plants grown from contaminated seed, incidence of pycnidia on desiccated stems increased with increasing inoculum on the seed tubers and with increasing time after desiccation. Stem infection was probably derived from inoculum on seed tubers spreading via the soil to stem bases because the pathogen was occasionally isolated from within green stems in June and July although the seed tubers remained sound. Contamination of progeny tubers after desiccation was correlated with contamination levels on seed tubers and some transmission also occurred from rotting seed to progeny tubers. Inoculum levels around progeny tubers increased rapidly after desiccation even where stems had previously been cut at soil level and removed to eliminate pycnidial development above ground as a source of inoculum. The results indicated that inoculum on seed tubers (whether from rots or surface contamination) contributed more to the contamination of progeny tubers at harvest than did inoculum from pycnidia on stems following desiccation.

Stolon infection. In the absence of the host plant, populations of the pathogen in soil usually decline steadily but, in the field, large increases in inoculum occur after

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desiccation. This may be due partly to spores washed into the soil from pycnidia on stems but similar increases occurred when stems were removed before desiccation (see above). To test whether the pathogen might multiply by infecting stolons in the soil, pieces of stolon were added to soil inoculated with *P. exigua* var. *foveata*, incubated at 5 and 15°C and sampled periodically by plating on to semi-selective agar media. In soil with added stolons, inoculum levels were about five times greater than those in soil without additions after 7 weeks (15°C) and 14 weeks (5°C). These differences were then maintained or increased for the duration of the experiment (23 weeks). Pieces of stolon recovered from the soils were black with pycnidia which proved to be *P. exigua* var. *foveata*. (Adams)

Effects of common scab on growth and yield. Seed tubers with severe (71% cover) or slight (12% cover) common scab selected from a badly infected crop of cv. Maris Piper were boxed for sprouting in November and February and planted in field experiments at Rothamsted and Woburn. Severely scabbed seed lost more moisture during sprouting than the slightly scabbed seed and sprouted from a larger number of eyes but total stem numbers were greater on plants from slightly scabbed seed. Plant growth during the first 6–9 weeks after planting was less from the severely scabbed than from the slightly scabbed seed. This was shown by records of ground cover, leaf area index, fresh weights of stems and leaves, total tuber yield (18% lower at 9 weeks) and tuber number. Subsequently these differences became non-significant and it seems unlikely that severe scab infection of seed tubers would significantly decrease tuber yields except perhaps in early potato production. (Adams and Hide)

Wound healing and storage diseases. Wound healing (curing) conditions can decrease the incidence of some storage diseases but the effects on others are uncertain or even detrimental. To investigate effects on dry rot (*Fusarium* spp.), damaged tubers were dipped in conidial suspensions and stored at 7°C for 6 weeks without curing or after periods of 3, 7 and 14 days at 15°C and 95% r.h. Incidence of *F. sulphureum* rot was decreased by 14 days curing but that of *F. caeruleum* was slightly increased by all the curing periods. The effect of storage conditions on the development of bacterial soft rot (*Erwinia carotovora* vars *atroseptica* and *carotovora*) at wounds was studied using tubers damaged and dipped in bacterial suspensions of differing concentrations. Few wounds had rotted 2 weeks after inoculation where tubers had been stored at 7°C and 90% r.h. or at 15°C and 75% r.h. even after dipping in 10^9 bacteria ml^{-1}. Where tubers were dipped in bacterial suspensions of 10^7 ml^{-1} or more and then stored at 15°C a large proportion of wounds rotted at 90–95 and especially at 100% r.h. (Marriott, with Potato Marketing Board, Sutton Bridge)

Tests of fungicides

Sprout treatment for control of stem canker. In previous experiments (*Rothamsted Report for 1978*, Part 1, 225) we found that incidence of stem canker was decreased by treating sprouts on seed potatoes with systemic fungicides. In 1980, sprouts on once grown seed cv. Desiree were treated with 5% a.i. dusts of 11 anilide fungicides and the tubers planted in soil infested with cultures of *R. solani*. In August stem canker was significantly decreased by F849 ('Uniroyal'), benodanil, pyracarbolid, carboxin, oxycarboxin, salicylanilide, mebenil and 2-bromobenzanilide and, except with carboxin, total yield of tubers in October was significantly increased. (Cayley and Hide)

Tuber treatment before storage. The effectiveness of fungicides in controlling gangrene was tested by infesting Pentland Crown tubers with soil slurry containing cultures of

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P. exigua var. *foveata*, wounding them (cut or crush) the following day and treating immediately or 3 or 7 days after storage at 5 or 10°C by dipping in suspensions of thiabendazole, imazalil or prochloraz. After treatment all tubers were stored at 5°C for 12 weeks with appropriate controls. On untreated tubers rots developed at 88% of crush wounds and treatment on day 0 decreased infection to 10%. More wounds became infected when treatment was delayed for 3 days (39%, 5°C; 51%, 10°C). Treatment after 7 days storage at 5°C also decreased gangrene (68% wounds with gangrene) but treatment after 7 days at 10°C did not have a significant effect. Treating tubers with cut wounds (16% with gangrene on untreated tubers) significantly decreased disease only after treatment on day 0 (1%) or after 3 days at 5°C (7%). On both wound types imazalil was more effective than other materials.

Maris Piper tubers similarly wounded after immersion in slurry containing cultures of *Fusarium sulphureum* were treated immediately or after storage at 5°C for up to 21 days or at 10°C for up to 7 days. Dry rot developed at few cut wounds. On crush wounds (24% infected on untreated tubers) infection was decreased to 0·8% by treating on day 0 and to 1, 7, 15 and 18% when treated after 3, 7, 14 and 21 days storage at 5°C respectively. Treatment after storage for 3 days at 10°C decreased infection (4%) but not after 7 days (28%). Prochloraz was the most effective material.

In testing fungicides against skin spot, washed Pentland Crown tubers were wounded with groups of 25 pin pricks (1 mm long, 0·5 mm wide), dipped in a culture macerate of *Polyscytalum pustulans* and stored at 5°C. Two days later tubers were immersed in fungicide suspensions for 5 min, dried and stored at 5°C in sealed plastic bags. Of 14 materials tested the most effective were carbendazim, thiophanate methyl and thiophamine but thiabendazole, prochloraz and imazalil also significantly decreased the incidence of skin spots. In a similar experiment with King Edward tubers treatment with thiabendazole, prochloraz and imazalil was delayed for 1, 4, 9 or 14 days. The disease was decreased most by thiabendazole but the degree of control was consistent irrespective of the interval before treatment. (Hide and Cayley)

Staff and visiting workers

Staff changes during the year were few. R. H. Kenten retired under the ARC's Voluntary Premature Retirement Scheme: Judi Driver and Merle Heger resigned and vacancies were filled by Margaret Ross and Tracey Fox.

J. Lacey was appointed Chairman of the Planning Committee for a mycotoxin symposium at the 4th International Congress of Plant Pathology, Melbourne, 1983. With P. H. Gregory, he was a Keynote speaker at the 2nd International Symposium on Microbial Ecology, University of Warwick. E. Lester was elected Chairman of The British Crop Protection Council and accepted an invitation to chair the Organising Committee for the International Congress of Plant Protection, Brighton, 1983. The film, made by C. C. Doncaster and R. W. Gibson, on aphid-trapping potato plants, noted in last year's *Report* was awarded a Diploma of Honour at the 34th International Scientific Film Association Congress (Cologne).

The Department arranged a 1-day conference on potato diseases for 60 members of the National Association of Seed Potato Merchants in January and was host to 40 virologists from 11 countries who attended the 3rd Conference on Virus Diseases of Gramineae in Europe held in May. R. T. Plumb was elected President of the 4th Conference.

Miss Yildiz Gunenc was awarded an M.Phil. Reading University for her research on preventing spread of potato viruses by means other than aphicides and returned to Turkey in October. K. Delaney (ARC student), N. Magan (United Nations Fellow) and

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Susan Marriott (CASE student, Potato Marketing Board) continued full-time research on root-diseases of legumes, microbiology of cereal grain and wound-healing of potatoes in relation to disease, respectively. R. Brennan (Ph.D. student, Manchester University) and M. Lysandrou (M.Sc. student, Reading University) spent 1 and 4 months respectively working on splash-dispersal of fungal spores. P. H. Gregory continued his work in the Department at the invitation of the Lawes Agricultural Trust. Visiting workers who spent 1 month or more in the Department included Mr M. Ivanovic, University of Belgrade, working on potential virus-vector fungi and Mrs Marjut Kotimaa, Kuopio Regional Institute for Occupational Health, Finland, working on microbial allergens. I. Bedford, S. Collingwood, Sheila Dance, R. Dominy and P. Hanacziwskyj were sandwich course students.

A. Bainbridge and J. Lacey visited India at the invitation of the British Council, to run workshops, deliver lectures and act as consultants on problems of sampling airborne particles concerned in human and plant disease. R. W. Gibson lectured in Tunis on potato pests to students from developing countries. D. Hornby visited France at the invitation of the University of Nancy as an examining juror for a D.Sc. thesis and for discussions on 'Soil Infectivity'. J. F. Jenkyn spent 3 months in Thailand as an FAO consultant attached to the project 'Strengthening Plant Protection Services of Thailand' funded by the United Nations Development Programme. D. H. Lapwood attended and gave a paper at a workshop on '*Phytophthora* Disease of Tropical Cultivated Plants' held at Central Plantation Crops Research Institute (CPCRI), Kerala, India, and visited the Kayangulam station to discuss coconut root (wilt) disease. E. Lester visited Brazil and lectured at a Symposium on the Root/Soil System held in Londrina, Parana, at the invitation of the State Agricultural Research Insitute (IAPAR). R. T. Plumb attended a workshop on barley yellow dwarf virus at El Batan, Mexico, at the invitation of CIMMYT. R. D. Prew saw and discussed systems of wheat production in northern France.

Financial support from the Potato Marketing Board is gratefully acknowledged.

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https://doi.org/10.23637/ERADOC-1-137