# TRANSMISSION STUDIES ON THE POTATO PATHOGENS *FUSARIUM SOLANI* VAR. *COERULEUM* AND *FUSARIUM SULPHUREUM*

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CONTENTS

## DECLARATION

This thesis was composed by myself and describes experimental work which was carried out between August 1974 and August 1977.

ACKNOWLEDGEMENTS

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My sincere thanks are due to Dr A E W Boyd for supervision of the experimental work and to Dr Boyd and Dr J H Lennard for helpful criticism and guidance in the preparation of the manuscript. I am indebted to Dr J Gilmour for help with the analysis of much of the data.

I also acknowledge the technical assistance of Anne Ash, without whose help much of this work would not have been possible in 3 years. Additional technical help, particularly during planting and harvesting of the field experiments was given also by Mr N H Robertson, Mr M Cattell and Mr A Forbes, and for this I am very grateful.

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## SUMMARY

A new selective medium containing pentachloronitrobenzene and 2-aminobutane – the PAB medium – was developed for use with the soil-dilution plate method for the enumeration of fungal propagules of *F. solani* var. *coeruleum* and *F. sulphureum* in field soil. These fungi cause a dry rotting of potatoes in storage. The efficiency of the PAB medium in the measurement of levels of soil contamination was compared with that of other methods. Also described is the PM70 medium, suitable for the isolation of a number of pathogens, including *P. exigua* var. *foveata* from diseased tuber tissue.

The incidence of dry rot after grading was related logarithmically to the number of *F. solani* var. *coeruleum* propagules in progeny tubersphere soil. Highest levels of progeny tuber contamination with *F. solani* var. *coeruleum* were associated with the planting of infected seed. Planting of contaminated seed sometimes gave high levels of transmission, possibly because dry rot developed after planting. Propagule production by infected seed varied between seasons and may be related to soil temperature. Tuber factors, eg variety and seed size, also influenced propagule production. Of the seed treatments tested, only thiabendazole reduced consistently the transmission of *F. solani* var. *coeruleum*.

Levels of soil contamination increased during the growing season but removal of the seed tuber prevented further increase. Highest numbers of propagules were in a 5 cm diam. zone of soil surrounding the seed and spread of propagules was mainly lateral and downwards forming a decreasing gradient of inoculum with increasing distance from the seed tuber. Propagule distribution on progeny tubers

followed the same pattern but harvesting by elevator digger disturbed the soil inoculum, making all progeny tubers highly contaminated.

*F. solani* var. *coeruleum* survived a 6 year rotation in field soil and this soil-borne inoculum is possibly important in the re-contamination of clean seed stocks.

Most varieties, of those tested, were resistant to infection by *F. solani* var. *coeruleum* in November but susceptible by February. Tubers were more susceptible if incubated initially at 4°C rather than at 15°C.

Transmission of *F. solani* var. *coeruleum* was compared with that of *F. sulphureum*. Propagules of *F. solani* var. *coeruleum* were produced in cavities in the tuber and in pustules on the tuber surface but *F. sulphureum* showed little surface sporulation. Thus thiabendazole, which inhibits surface sporulation was inconsistent in reducing transmission of *F. sulphureum*. Moreover, *F. sulphureum* sporulated on stems growing from infected seed tubers in the field.

*F. sulphureum* infected seed usually produced less inoculum than did *F. solani* var. *coeruleum* but in one season the reverse was true and was possibly related to high soil temperatures. *F. sulphureum* does not appear to survive in field soil as well as *F. solani* var. *coeruleum*. Although *F. sulphureum* seems less well adapted than *F. solani* var. *coeruleum* for propagule transmission none of the varieties tested was resistant to infection by *F. sulphureum*.

# SECTION 1

# GENERAL INTRODUCTION

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## GENERAL INTRODUCTION

Dry rot caused by species of *Fusarium* is still one of the major storage diseases of potatoes in Europe and North America. The principal fungi and the countries involved are: *F. solani* var. *coeruleum* (Sacc.) Booth *(F. coeruleum* Lib.) from Denmark, France, Norway, Sweden and Great Britain; *F. sulphureum* Schl. *(F. sambucinum* Fuck. f.6 Wr.) from some European countries, the USA and Canada; and *F. sambucinum* Fuck. *(F. roseum* Lk. var. *sambucinum* Synd. and Hans.) from the USA and Canada. Other *Fusarium* spp. which may also sometimes be implicated are *F. avenaceum, F. arthrosporioides, F. tricinctum, F. sporotrichioides, F. oxysporum, F. culmorum* and *F. merismoides* (Moore, 1945; McKee, 1952; Upstone, 1970a, 1970b; E. Forsund, 1977, personal communication).

The pathogens contaminate progeny tubersphere soil and infect tubers only through wounds caused at or after harvest. Rots develop during storage resulting in loss of saleable yield. If infected tubers are planted as seed, reduced plant emergence may occur, and since the infected seed tuber is the main source of inoculum (Tickle, 1974), disease risk in the resulting crop is increased.

Since about 1950, dry rot *(F. solani* var. *coeruleum)* in Great Britain has decreased in importance. At the present time gangrene caused by *Phoma exigua* var. *foveata* is regarded as the most important storage disease of potatoes. Gangrene is also becoming a problem in other European countries, such as Denmark, France and Sweden (personal communications from J. Bak Henriksen, 1977; B. Jouan, 1977; V. Umaerus, 1977).

The decline in the importance of dry rot in Great Britain has coincided with certain changes in farm practice. (1) More resistant varieties have been introduced and the acreage of the very susceptible variety Doon Star has decreased (Boyd, 1952c). (2) The rate of NPK fertiliser applied to crops has increased (Boyd, 1972). This has been shown to reduce the susceptibility of tubers to dry rot (Ivashchenko, 1963; Boyd, 1967), but increase the susceptibility of tubers to gangrene (MacKenzie, 1968). (3) Rotational requirements have been introduced in the Scottish seed inspection scheme stipulating that potatoes were to be grown in a field no more than once in 7 years (J. L. Hardie, 1977, personal communication). (4) From an awareness that the riddle behaved as an inoculating machine (Foister, Wilson & Boyd, 1952), steps have been taken to reduce the degree of damage by fitting rubber coated meshes. (5) The low temperatures which are being maintained frequently in potato stores today may be less suitable for dry rot development, but more favourable to gangrene development (Booth, 1970; Wellving, 1976).

Despite the decline in the importance of dry rot, it still causes consistent losses. The present policy of encouraging seed growers to destroy the haulm and lift early, in order to reduce the incidence of aphid transmitted virus diseases (Anon, 1976b) and gangrene (Fox & Dashwood, 1977), may alter the position regarding dry rot and gangrene.

In the context of the high standards set for seed potatoes in the Virus Tested Stem Cutting Scheme (VTSC), even small levels of dry rot are important, but it has not been possible to eliminate this disease (Hardie, 1970; Tickle, 1974). Moreover, powdery dry rot caused by *F. sulphureum*, which was first recorded in Great Britain in only 1971 (Boyd & Tickle, 1972), appears to be more widespread now. It is

particularly insidious since the fungus is more virulent than *F. solani* var. *coeruleum* and symptoms may frequently be mistaken for those of gangrene.

Much research work, in Great Britain and Europe, is now directed towards the epidemiology and control of gangrene, but work is still continuing on dry rot. Recent literature reviews on dry rot have been given by Boyd (1972) and Tickle (1974), and in 1975 a bibliography spanning 25 years of research work was published by Miska & Nelson. This work tends to be related to tuber resistance and control measures only. This is not surprising since study of pathogens in the soil is difficult because of the problems associated with quantifying the fungal population.

This thesis is concerned mainly with the transmission of *F. solani* var. *coeruleum*, but where possible parallel studies were made with *F. sulphureum*. The experimental work is divided into four main sections.

Assessments of the levels of soil contamination and their relationship to disease potential (Section 2).

Potato dry rot: soil-borne or tuber-borne? (Section 3).

Factors affecting transmission of *F. solani* var. *coeruleum* from infected seed tubers (Section 4).

Control of potato dry rot (Section 5).

# SECTION 2

# ASSESSMENTS OF THE LEVELS OF SOIL CONTAMINATION AND THEIR RELATIONSHIP TO DISEASE POTENTIAL

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To compare the levels of contamination of soils infested with potato pathogens a number of methods using susceptible potato tubers have been used (Lansade, 1949; McKee & Boyd, 1952; Ayers & Robinson, 1954; Nielsen & Johnson, 1972; Hide, Griffith & Adams, 1977). The infection index found relates to the infectivity of the soil, and may be converted to fungal propagules per gram of soil using a most probable number method (Maloy & Alexander, 1958; Tsao & Canetta, 1964; Hornby, 1969b; Duncan, 1976). This approach, however, does not appear to have been used in research on dry rot.

The bait methods have a number of disadvantages.

1. Seasonal variation in tuber susceptibility (Boyd, 1952b) means that differences in the dry rot index may not always be due to differences in fungal population.

2. Lesions caused by different tuber pathogens may be very similar, making it difficult to determine the index for the pathogen under study.

3. The methods may be insensitive for highly contaminated soils which may all indicate a 100% index even when differences still exist.

4. The methods may not be sensitive enough to detect small but significant changes in the level of fungal contamination, or very low populations of the fungus.

5. In dealing with soils with a very low index there is some uncertainty on the reliability of results because of the possibility of exterior contamination.

Problems associated with bait methods have been avoided for many *Fusarium* spp. by the use of selective media (Tsao, 1970). When used with the soil-dilution plate method, which was originally developed for isolation of soil bacteria (Waksman, 1927), the inoculum density of the pathogen is readily obtained from colony counts. The PM70 selective medium for the isolation of *F. solani* var. *coeruleum* was developed by Tickle (1974); since the logarithmic transformation of the fungal population was proportional to the infection index of soil, the method was judged to be a satisfactory replacement of the bait method.

In this section, the results of further studies on selective media, and on methods for the assessment of fungal contamination of soils are reported under the following headings:-

2.1 Selective media for the isolation of *F. solani* var. *coeruleum* and *F. sulphureum* from soil.

2.2 Relationships between results from methods used to assess the degree of soil contamination with *F. solani* var. *coeruleum* and *F. sulphureum*.

2.3 Relationship between the levels of progeny tuber contamination with *F. solani* var. *coeruleum* and incidence of dry rot.

## 2.1 Selective media for the isolation of *F. solani* var. *coeruleum* and *F. sulphureum* from soil

### INTRODUCTION

Different workers have developed various selective and semi-selective media for the isolation of *Fusarium* species from soil (Tsao, 1970). Of 18 media recommended for the isolation and enumeration of *Fusarium* species from soil, Papavizas (1967) found that, for nine species, the medium of Nash & Snyder (1962), based on pentachloronitrobenzene (PCNB), was superior to the others tested. It was even better in a modified form with oxgall and chlortetracycline HCl added (the Papavizas medium). None of the media had been evaluated for their efficiency in isolating *F. solani* var. *coeruleum* or *F. sulphureum* until Tickle (1974), working with *F. solani* var. *coeruleum*, showed that altering the C:N ratio of the Papavizas medium allowed the characteristic blue colony colour to develop which would enable rapid identification of the fungus: the 1973 medium in Fig. 1. Despite this modification, the medium was very poor at isolating *F. solani* var. *coeruleum* from field soil because of over-growth of the medium by the other soil microflora. It was later improved markedly (Tickle, 1974) by the addition of dodine acetate: the PM70 medium in Fig. 1. However, several years' experience with this medium has shown that, although it was very effective for isolation of *F. solani* var. *coeruleum* from diseased potato tissue, the efficiency of isolation of *F. solani* var. *coeruleum* from some soils, particularly those from paper potato sacks, was severely reduced because of competition from other fungi, notably *Penicillium* spp. In other soils the presence of brown *Humicola* colonies which discoloured the medium made it difficult to observe the *F. solani* var. *coeruleum* colonies.

The aim of the present work was to improve the PM70 medium and develop a selective medium for the isolation of *F. sulphureum* for use with the soil-dilution plate method. Several antifungal chemicals were evaluated for selective isolation of *F. solani* var. *coeruleum* and *F. sulphureum:*

a. benomyl because it has been shown to be more toxic to *Humicola* spp. than some *Fusarium* spp. (Edgington, Khew & Barron, 1971);

b. chloroneb because it has been used in the Nash-Snyder medium to inhibit growth of fungi other than *Fusarium* spp. (Agrawal, Khare & Kushwaha, 1973);

c. 2-aminobutane because, although used commercially to control gangrene caused by *P. exigua* var. *foveata*, it has no effect on dry rot caused by *F. solani* var. *coeruleum* (Graham & Hamilton, 1970) or *F. sulphureum* (Boyd & Tickle, 1972).

## MATERIALS AND METHODS

### Media

Concentrations of fungicide are given in terms of active ingredient (a.i.).

The PM70 medium contained (g/l distilled water): Agar (Davis), 20: sucrose, 20; $KNO_3$,2; $KH_2PO_4$,1; $MgSO_4$,0.5; KCl,0.5; sodium tauroglycocholate (oxgall, from BDH),0.5; pentachloronitrobenzene - PCNB - (Brassicol 50% w.p., from Burts and Harvey),0.375. These were autoclaved at 121°C for 15 min. cooled to 45°-50°C and the following added: dodine acetate (Melprex 65% w.p. from Cyanamid),0.07; streptomycin base, 0.6; and chlortetracycline HCl, 0.05.

The PM70 medium was modified by increasing the concentration of PCNB and oxgall to 1g/l, or by adding 0.001-100mg/l benomyl (Benlate 50% w.p. from Dupont) before autoclaving, or 0.01-1.0g/l chloroneb (Demosan 65% w.p. from Dupont) after autoclaving and cooling.

The fungicide 2-aminobutane (1-10ml/l) was added to (1) PM70 medium, (2) PM70 medium without dodine acetate and (3) PM70 medium without dodine acetate and PCNB. The addition was made after autoclaving and cooling to 45°C since 2-aminobutane boils at 63°C (Anon, 1969).

Media containing dodine acetate or 2-aminobutane were evaluated over the pH range 6-10 since the fungicidal activity of these chemicals is dependant on pH (Byrde, 1967). Adjustments to pH were made using 1M NaOH or HCl.

The media were delivered into Petri plates (9cm diam.) at the rate of 15ml per plate and the surface of the medium dried at 20°C for 3 days before use.

Soil dilution

Samples of air-dried, sieved (0.85mm mesh) soil (each 2g), known to be contaminated with *F. solani* var. *coeruleum* or *F. sulphureum*, were made up to a $10^{-2}$ dilution with a 1:1 ratio of sterile water to 0.15% water agar, and comminuted in a blender (MSE Atomix) at 12000 rpm for 1 min. Aliquots of 1ml were pipetted onto the surface of each plate of selective medium according to the method of Paharia & Kommedahl (1954). Two plates were used per soil and these were incubated at *c.* 20°C in daylight on a laboratory bench as recommended by Nash & Snyder (1962). After 10-25 days incubation the success of the various media were determined by recording the number of *F. solani* var. *coeruleum* or

*F. sulphureum* colonies and the amount of surface cover by 'weed fungi'.

## RESULTS

In initial trials, increasing the concentration of PCNB and oxgall from 0.375 and 0.5g/l respectively to 1g/l, or adding chloroneb (up to 1g/l), did not increase the selectivity of the PM70 medium. The PM70 + benomyl (0.1mg/l) medium, on the other hand, was more selective as it suppressed growth of *Penicillium* spp. which with some soils prevented isolation of *F. solani* var. *coeruleum* on the PM70 medium. No effect on the number of *Humicola* colonies was found and increasing the concentration of benomyl to 1mg/l severely reduced the number of *F. solani* var. *coeruleum* colonies present. The effect of benomyl on selectivity was inconsistent however, and the most promising medium appeared to be one which contained 2-aminobutane and not dodine acetate.

Further trials were carried out with the PM70 medium and media containing 2-aminobutane. Results are shown in Table 1. The selectivity of each medium was assessed on a five point scale where +++++ represents almost total and + represents slight cover of the surface of the medium by 'weed fungi'.

### Isolation of *F. solani* var. *coeruleum*

Increasing the pH of the PM70 medium increased its selectivity but severely reduced the number of *F. solani* var. *coeruleum* colonies present. Increasing the pH of media containing 2-aminobutane had a similar effect, but several combinations of 2-aminobutane concentration and pH were found which gave increased colony counts and greater selectivity as compared with the PM70 medium. Addition of PCNB to the 2-aminobutane media did not markedly improve selectivity.

Table 1. Number of colonies of *F. solani* var. *coeruleum* (*F.c.*) and *F. sulphureum* (*F.s.*) isolated from four different soils and the degree of contamination (C) of the selective media by 'weed fungi'.

| Selective media | pH | Soils: 34b | | D5/8 | | 28b | | | 56 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *F.c.*[a] | C[b] | *F.c.* | C | *F.c.* | *F.s.* | C | *F.s.* | C |
| 1) PM70[c] | 6 | 130 | ++ | 6 | +++++ | 28 | 0 | ++++ | 0 | ++++ |
| | 8 | 131 | + | 60 | +++ | 26 | 0 | +++ | 2 | ++++ |
| | 9 | 75 | + | 5 | ++ | 3 | 0 | ++ | 2 | +++ |
| | 10 | 4 | + | 2 | + | 1 | 0 | + | 1 | ++ |
| 2) PM70 (without PCNB or dodine acetate) +2-aminobutane at 1 ml/l | 6 | ? | +++++ | 0 | +++++ | 0 | 0 | +++++ | 0 | +++++ |
| | 7 | ? | +++++ | 0 | +++++ | 0 | 0 | +++++ | 0 | +++++ |
| | 8 | ? | ++++ | 0 | ++++ | 0 | 0 | +++++ | ? | +++ |
| | 9 | 164 | +++ | >300 | ++ | 11 | ? | +++ | 50 | ++ |
| | 10 | 174 | + | >300 | + | 0 | ? | +++ | 56 | ++ |
| 3) As (2) but 2-aminobutane at 5 ml/l | 6 | ? | +++ | ? | ++++ | ? | 0 | +++++ | ? | ++++ |
| | 7 | 190 | +++ | >300 | + | 25 | 0 | ++++ | ? | ++++ |
| | 8 | 180 | + | >300 | + | 0 | 0 | + | 2 | ++ |
| | 9 | 0 | + | 0 | + | 0 | 0 | + | 0 | + |
| | 10 | 0 | + | 0 | + | 0 | 0 | + | 0 | + |
| 4) As (2) but 2-aminobutane at 10 ml/l | 6 | 162 | ++++ | >300 | ++ | 25 | ? | ++++ | ? | ++++ |
| | 7 | 162 | +++ | >300 | + | 25 | ? | ++ | ? | ++ |
| | 8 | 0 | + | 2 | + | 0 | 0 | + | 0 | + |
| | 9 | 0 | + | 0 | + | 0 | 0 | + | 0 | + |
| | 10 | 0 | + | 0 | + | 0 | 0 | + | 0 | + |
| 5) PM70 (without dodine acetate) +2-aminobutane at 1 ml/l | 6 | 190 | ++++ | ? | +++++ | ? | 0 | +++++ | 170 | ++++ |
| | 8 | 180 | ++++ | >300 | +++ | 48 | 25 | +++ | 170 | ++ |
| | 9 | 180 | + | >300 | + | 55 | 20 | ++ | 175 | + |
| | 10 | 187 | + | >300 | + | 7 | 0 | + | 53 | + |
| 6) As (5) but 2-aminobutane at 5 ml/l | 6 | 150 | ++ | >300 | + | 57 | 0 | + | 180 | +++ |
| | 8 | 170 | + | >300 | + | 0 | 0 | + | 30 | + |
| | 9 | 0 | + | 0 | + | 0 | 0 | + | 0 | + |
| | 10 | 0 | + | 0 | + | 0 | 0 | + | 0 | + |

[a] = Number of colonies per plate (9 cm diam.) or colonies were indistinct and uncountable (?) because of growth of 'weed fungi'.

[b] = +++++ almost total cover of the selective medium by 'weed fungi'

to

+ occasional colonies of 'weed fungi' per plate of selective medium.

[c] = PM70 medium as used by Tickle (1974) not adjusted for pH (normal pH range 5.8-6.4).

Isolation of *F. sulphureum*

The characteristic orange colouration of *F. sulphureum* colonies necessary for rapid identification of the fungus in culture was obtained on the PM70 medium (Fig. 1), but the medium did not allow efficient isolation of the fungus from soil (Table 1) or spore suspensions (Table 3 and Fig. 1). Omission of dodine acetate (the 1973 medium in Fig. 1) gave improved colony counts from spore suspensions, but isolation from soil was poor because of a reduction in the selectivity of the medium. This was markedly improved by the addition of 2-aminobutane. *F. sulphureum* appeared to be more sensitive to changes in toxicity of 2-aminobutane, brought about by pH adjustments, than *F. solani* var. *coeruleum*.

In addition, isolation of *F. sulphureum* from soil, unlike that for *F. solani* var. *coeruleum*, was adversely affected by omitting PCNB from the medium because *F. sulphureum* colonies became less discrete and showed poor colour definition.

The medium selected for use was that which in the results presented gave the most efficient isolation of both *F. solani* var. *coeruleum* and *F. sulphureum*. It was designated the PAB medium (Table 2).

Table 2. The PAB medium for selective isolation of *F. solani* var. *coeruleum* and *F. sulphureum* from soil.

| | | | |
|---|---|---|---|
| Agar | 20 | g/l | |
| Sucrose | 20 | g/l | |
| $KNO_3$ | 2 | g/l | |
| $KH_2PO_4$ | 1 | g/l | |
| $MgSO_4$ | 0.5 | g/l | |
| KCl | 0.5 | g/l | |
| Oxgall (sodium tauroglycocholate) | 0.5 | g/l | |
| PCNB | 0.375 (a.i.) | g/l | (Brassicol) (50% w.p. ) |
| Antifoaming emulsion FC | 1 | ml/l | |
| (Streptomycin base | 0.60 | g/l | |
| *(Chlortetracycline HCl | 0.05 | g/l | |
| (2-Aminobutane | 1 | ml/l | (Butafume -) (BASF ) |

* Add after cooling to 45°C. Adjust pH of medium using IN HCl or NaOH to 9.2.

Silicone antifoaming emulsion was added to the medium because difficulty was encountered with frothing, which made it awkward to pour the Petri plates free from air bubbles. The emulsion did not affect the recovery of *F. solani* var. *coeruleum* or *F. sulphureum* from field soil (Appendix 2). The plates may be used immediately after pouring and no pre-drying of the plates is required.

The percentage germination of washed macroconidia from 3-4 wk old cultures of *F. solani* var. *coeruleum* and *F. sulphureum* on base medium (ie Agar, 20; sucrose, 20; $KNO_3$,2; $KH_2PO_4$,1: $MgSO_4$,0.5; and KCl,0.5; all g/l distilled water), PM70 and PAB selective media is shown in Table 3.

Table 3. Percentage germination at 20°C of *F. solani* var. *coeruleum* and *F. sulphureum* macroconidia on a basal salts medium (BM), PM70 and PAB media after 48 and 72 h.

| Fungus | BM | | PM70 | | PAB | |
|---|---|---|---|---|---|---|
| | 48h | 72h | 48h | 72h | 48h | 72h |
| *F. solani* var. *coeruleum* | 94 | 92 | 40 | 49 | 90 | 87 |
| *F. sulphureum* | 94 | 97 | 46 | 26 | 92 | 97 |

Compared with the germination rate of macroconidia of *F. solani* var. *coeruleum* and *F. sulphureum* on the base medium, germination was significantly reduced on the PM70 ($P<0.001$) but not on the PAB medium. On the PM70 medium, germination percentages of macroconidia for both fungi were similar after 48h incubation, but at 72h the germination of *F. sulphureum* was less ($P<0.001$), because of the formation of clamydospores which did not regerminate.

Figs 1-4 show the isolation of *F. solani* var. *coeruleum* and *F. sulphureum* on four modifications of the Nash-Snyder medium (1962): the Papavizas medium (1967), the improved medium of 1973 (Tickle, 1974), the PM70 medium (Tickle, 1974) and the PAB medium. The isolation of *F. solani* var. *coeruleum* by the PAB and PM70 media from two soils is compared in Figs 5 and 6.

In Figs 1-6, the selective media developed by Tickle are described as the Hedley and Hedley-PM70 media.

Fig. 1.

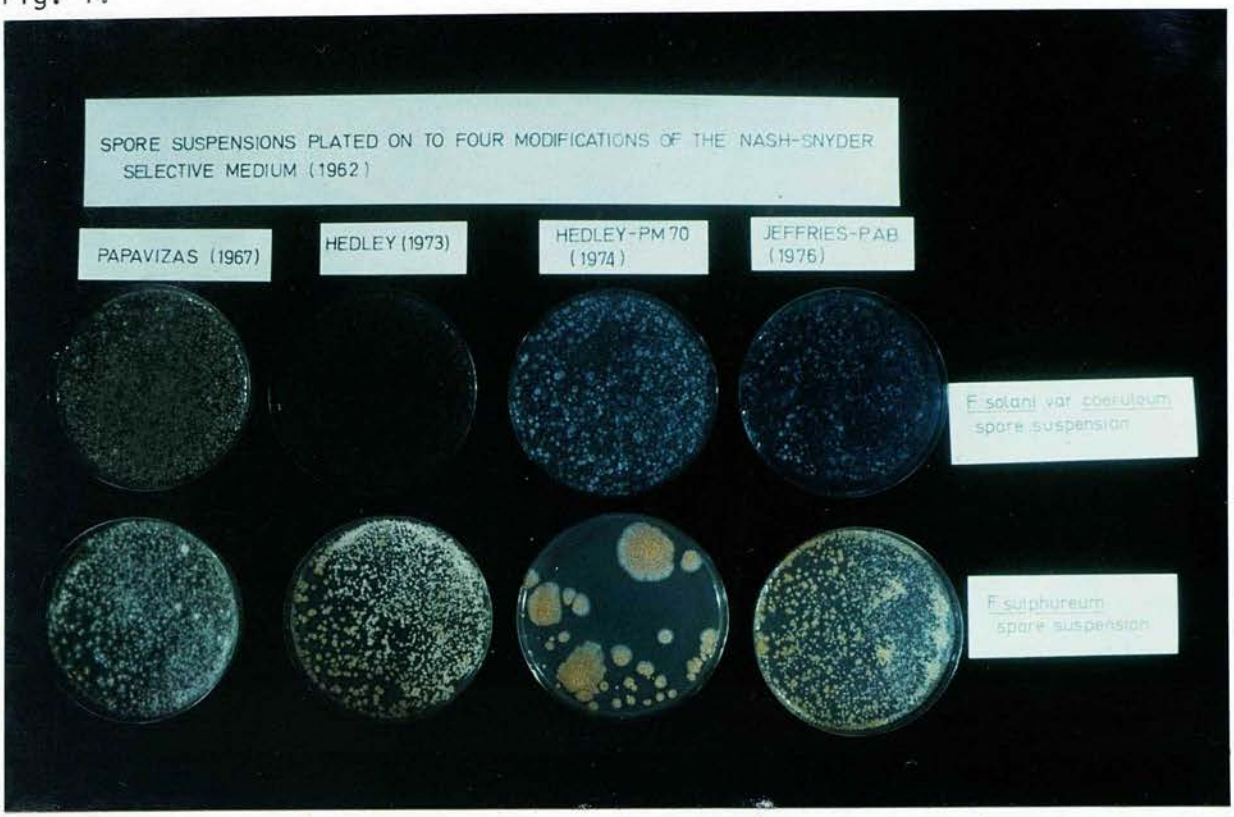

SPORE SUSPENSIONS PLATED ON TO FOUR MODIFICATIONS OF THE NASH-SNYDER SELECTIVE MEDIUM (1962)
PAPAVIZAS (1967)
HEDLEY (1973)
HEDLEY-PM 70 (1974)
JEFFRIES-PAB (1976)
F solani var coeruleum spore suspension
F sulphureum spore suspension

Fig. 2.

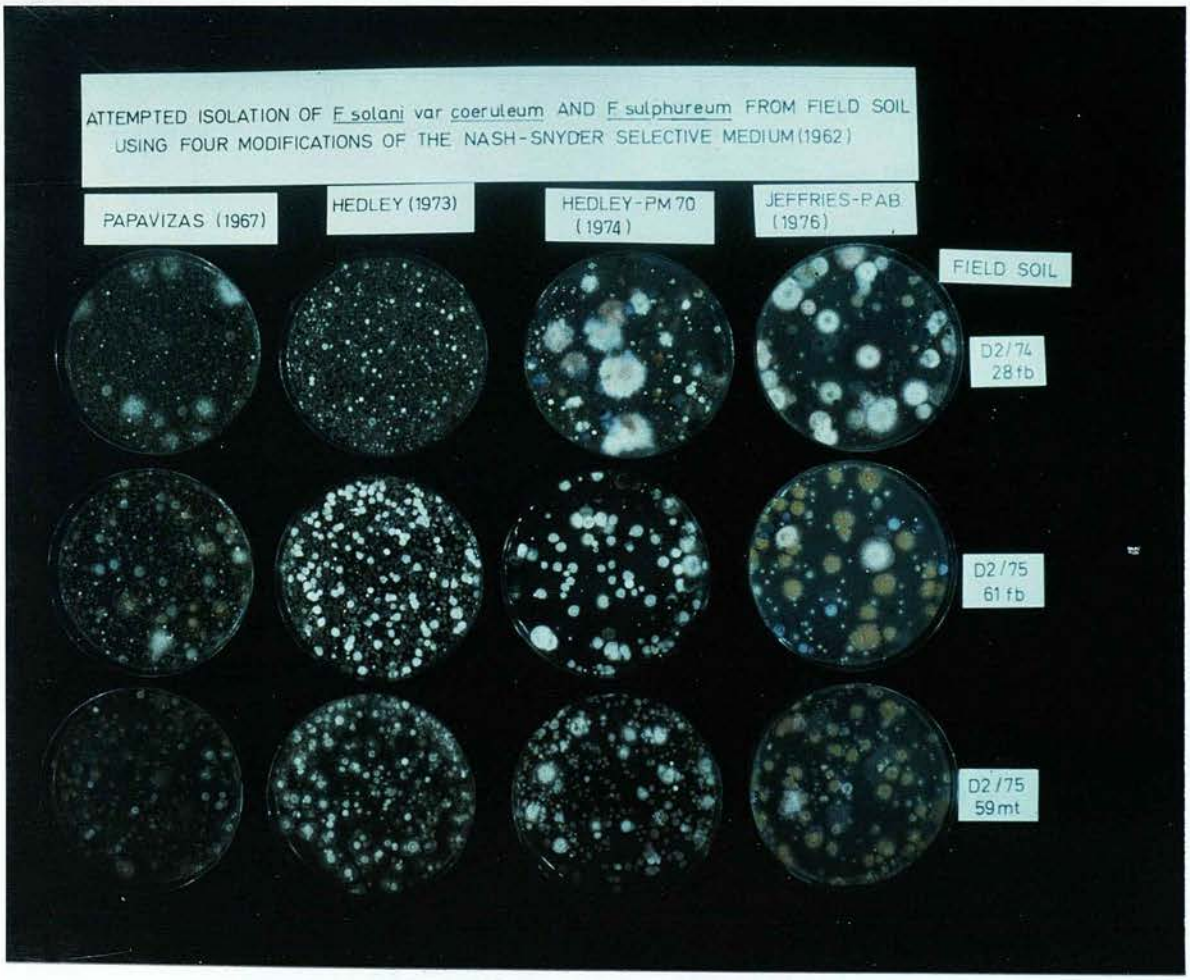

ATTEMPTED ISOLATION OF F solani var coeruleum AND F sulphureum FROM FIELD SOIL USING FOUR MODIFICATIONS OF THE NASH-SNYDER SELECTIVE MEDIUM (1962)
PAPAVIZAS (1967)
HEDLEY (1973)
HEDLEY-PM 70 (1974)
JEFFRIES-PAB (1976)
FIELD SOIL
D2/74 28 fb
D2/75 61 fb
D2/75 59 mt

Fig. 3.

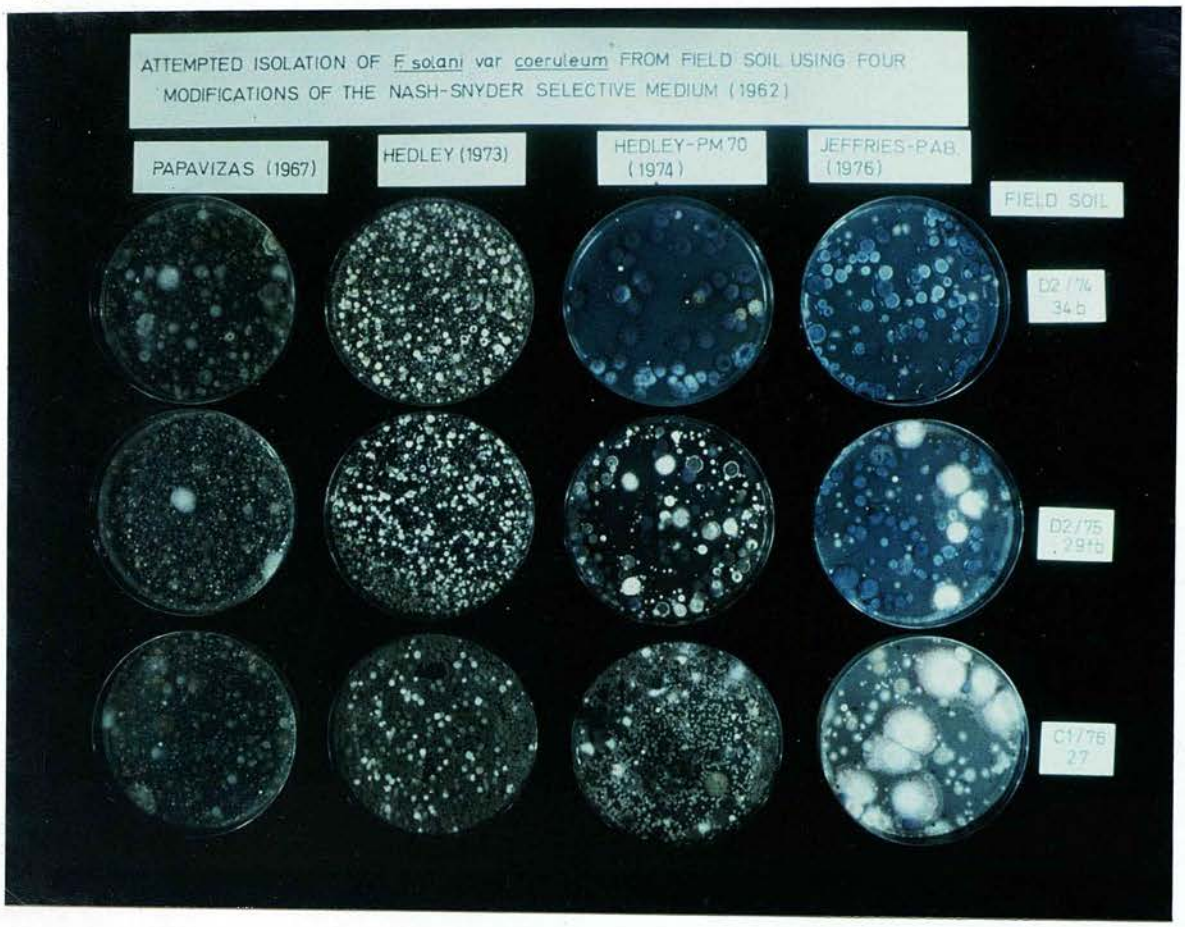

ATTEMPTED ISOLATION OF F. solani var coeruleum FROM FIELD SOIL USING FOUR MODIFICATIONS OF THE NASH-SNYDER SELECTIVE MEDIUM (1962)
PAPAVIZAS (1967)
HEDLEY (1973)
HEDLEY-PM 70 (1974)
JEFFRIES-PAB. (1976)
FIELD SOIL
D2/74 34b
D2/75 29+b
C1/76 27

Fig. 4.

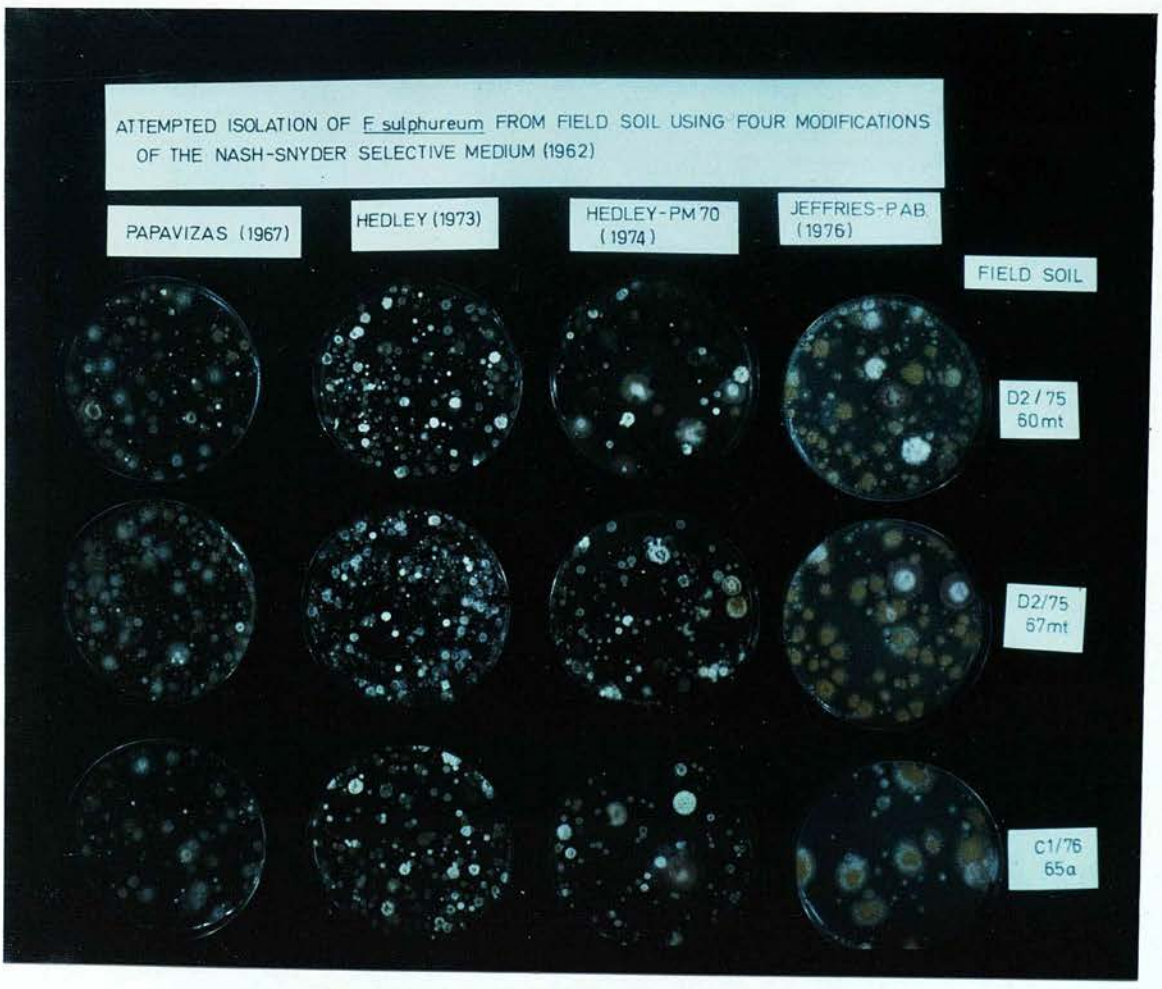
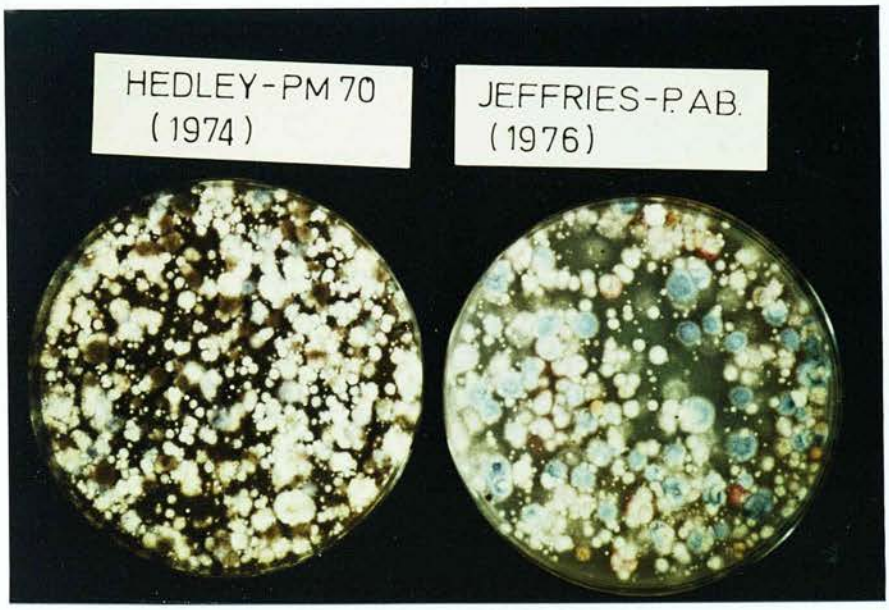

ATTEMPTED ISOLATION OF F. sulphureum FROM FIELD SOIL USING FOUR MODIFICATIONS OF THE NASH-SNYDER SELECTIVE MEDIUM (1962)
PAPAVIZAS (1967)
HEDLEY (1973)
HEDLEY-PM 70 (1974)
JEFFRIES-PAB. (1976)
FIELD SOIL
D2/75 60mt
D2/75 67mt
C1/76 65a

Fig. 5.


HEDLEY-PM 70
(1974)
JEFFRIES-P.AB.
(1976)

Fig. 6.


HEDLEY-PM 70
(1974)
JEFFRIES-P.AB.
(1976)

## DISCUSSION

Disease severity is equivalent to inoculum potential x disease potential (Baker, Maurer & Maurer, 1967). More specifically disease severity is a product of (inoculum density x capacity) x (proneness x susceptibility of the host), where capacity refers to the effect of the environment on the energy for colonisation and proneness relates to the effect of the environment on the host (Baker, 1968). An estimate of the inoculum density or fungal population is thus required not only for predicting disease severity, but to follow interactions between the pathogen and its environment.

*F. solani* var. *coeruleum* and *F. sulphureum* are suitable for quantitative assays from soil with the soil-dilution plate method because they possess several kinds of discrete units to enumerate (eg chlamydospores and macroconidia). They have the disadvantages, however, in that their growth rate is slow and their population, relative to the other soil microflora, is small. Thus McKee & Boyd (1952) were unable to isolate *F. solani* var. *coeruleum* on Waksman's Agar, despite using soil of high infectivity. Selective antimicrobial chemicals are required for the isolation of most plant pathogenic fungi from soil; the present work has shown that the newly developed PAB medium (containing PCNB and 2-aminobutane) was more selective than the PM70 medium (containing PCNB and dodine acetate) for the isolation of *F. solani* var. *coeruleum* from soil at a $10^{-2}$ dilution. This not only makes colony counts easier, but the medium may be used on soils where isolation of *F. solani* var. *coeruleum* was not previously possible because of growth of 'weed fungi' not inhibited by the antimicrobial agents present in the PM70 medium. The diversity of the soil microflora means that the PAB medium will possibly not be effective for

the isolation of *F. solani* var. *coeruleum* from all soils, but at present efficient isolation has been obtained from all soils examined (>3000). Even lower soil dilutions ($10^{-1}$) have now been used successfully.

*F. sulphureum* and *F. culmorum* were also isolated successfully on the PAB medium, but in some soils there were often difficulties in obtaining accurate colony counts of *F. sulphureum* because of the presence of fungi (eg *F. dimerum* and *F. lateritium)* with similar colony characteristics. However, no better alternative selective medium for the isolation of *F. sulphureum* from soil is at present available. No attempt was made to isolate *F. sulphureum* from soils using a $10^{-1}$ dilution.

*In vitro* studies suggest that dodine acetate (a major constituent of the PM70 medium) markedly inhibits germination of *F. solani* var. *coeruleum* macroconidia and particularly *F. sulphureum*, whereas constituents of the PAB medium do not affect germination. It is therefore tempting to suggest that the PAB medium allows the maximum possible recovery of *F. solani* var. *coeruleum* and *F. sulphureum* propagules from soil. Some differential sensitivity of spore forms to toxicants might exist, but no attempt in this study was made to identify visually the type of propagule from which the *Fusarium* colonies arose. Where the deposition of propagules from a food base into soil is followed through the season, it is possible that colonies may arise from macroconidia or mycelia which have not lysed or been converted into chlamydospores. Work, elsewhere, has shown that many *Fusarium* spp. survive in natural soil as chlamydospores (Booth, 1971) and it is from these which colonies develop on soil-dilution plates (Warcup, 1955; Nash, Christou & Snyder, 1961). In the present studies, colonies most probably arise from chlamydospores since the soil was air-dried and stored for a time before assessments were made.

Differential sensitivity of spores and mycelia to various toxicants is known (Singh & Nene, 1965). This appears to be shown by *F. sulphureum* when grown on the PM70 medium. Although germination of *F. sulphureum* on the PM70 medium was reduced markedly compared with germination on the PAB medium, mycelial growth rates were similar on both media (unpublished data).

In addition to soil isolation, methods are required to isolate potato pathogens from tuber tissue. A comparison of the PM70 and PAB selective media showed that they both effectively isolated *F. solani* var. *coeruleum*, *F. sulphureum* and *F. culmorum* from diseased tissue. However, the PM70 medium isolated in addition *P. exigua* var. *foveata* (gangrene) and *F. avenaceum*, although growth of the latter was often obscured by growth of other fungi. *P. exigua* var. *foveata* may be identified by the yellow fluorescent anthraquinone crystals (Bick & Rhee, 1966; Logan & Khan, 1969) deposited in the medium under the colony. With the selective media up to 16 tissue segments may be plated per dish (9cm diam.), whereas with non-selective media only a few segments, which require surface sterilisation, may be plated per dish. The PM70 medium offers a fairly rapid method of distinguishing between similar rots caused by the most important fungal pathogens of potato tubers.

The PM70 medium is therefore most suitable for isolation of pathogens from diseased tuber tissue, whereas the PAB medium is recommended primarily for determining the inoculum density of *F. solani* var. *coeruleum* and *F. sulphureum* in soil, and may be also used for studies on *F. culmorum*.

## 2.2 Relationships between results from methods used to assess the degree of soil contamination with *F. solani* var. *coeruleum* and *F. sulphureum*

### INTRODUCTION

Assessments of soil contamination with *F. solani* var. *coeruleum* have been made using the tuber inoculation method of McKee & Boyd (1952) and the soil dilution/PM70 selective medium method of Tickle (1974). The PAB medium, which has now been developed (Section 2.1), is more selective than the PM70 medium in the isolation of *F. solani* var. *coeruleum* and *F. sulphureum* may be isolated also.

The present section covers studies on:-

i. the *F. solani* var. *coeruleum* population of soils assessed on PM70 and PAB media;

ii. the relationship between populations of *F. solani* var. *coeruleum* and *F. sulphureum* in soils and soil infectivity.

### MATERIALS AND METHODS

Air-dried, sieved soils (0.85mm mesh), naturally contaminated with *F. solani* var. *coeruleum* or *F. sulphureum* propagules, were used. An experiment was conducted from 11-17 May, 1976, and repeated from 24-30 November, 1976. Between these times, soils were stored at $4^{o}C$. Tubers used for tuber inoculation in May and November were harvested on 14 October, 1975 and 4 October, 1976 respectively. Assessments of fungal population and soil infectivity were carried out as described below.

#### Fungal population

For each soil, a 2g sample (on an air-dry basis) was made up to a $10^{-2}$ dilution (using 100ml sterile distilled water and 100ml 0.15%

water agar) and comminuted at 12000 rpm in a 400ml reservoir of a MSE Atomix blender for 1 min. Mixing time does not affect recovery of *F. solani* var. *coeruleum* or *F. sulphureum* (Appendix 3). Aliquots of soil suspension were removed to small beakers using 5ml pipettes which had enlarged apertures (3mm diam.) to avoid excluding larger soil particles from the sample (Hornby, 1969a). From the beakers 1ml aliquots were pipetted, using an Eppendorf automatic pipette, onto the surface (Paharia & Kommedahl, 1954) of each of 10 plates of PM70 and/or PAB selective medium. The plates were incubated on a laboratory bench and *F. solani* var. *coeruleum* colonies counted after 14 days (PM70) or 20 days (PAB). *F. sulphureum* colonies were counted after 15 days' incubation.

Soil infectivity

For each soil sample, 20 seed-size, surface sterilised (0.8% formalin dip for 1 min.) tubers, var. Catriona, were wounded (7mm diam. x 7mm depth) four times around the circumference midway between the heel and rose ends with a sterilised glass rod (McKee & Boyd, 1952). Soil was pressed into two opposite wounds in each tuber, using the flattened end of the glass rod, until the soil was wet with potato juice. The two remaining holes were left unfilled. The inoculated tubers were placed on damp peat in small cardboard boxes, 10 tubers per box. These boxes were then placed in larger boxes lined with damp paper.

Tubers inoculated with soil contaminated with *F. solani* var. *coeruleum* were incubated at 4°C for 6 wk followed by 2-4 wk at 15°C as the dual temperature regime increases the susceptibility of tubers to *F. solani* var. *coeruleum* infection (Boyd, 1974). Tubers inoculated with soil contaminated with *F. sulphureum* were incubated at 15°C as initial incubation of tubers at 4°C did not appear to increase the

frequency of *F. sulphureum* lesions (Section 5.2). After incubation the tubers were cut open. Tubers with lesions associated with the uninoculated control holes were discarded since this was an indication of contamination of the tuber surface. The remaining tubers were used to calculate the 'soil-infectivity index' which is the number of dry rot lesions expressed as a percentage of the total number of soil inoculations.

RESULTS

In considering the results the following relationships were examined by calculating linear regressions in the form $y = a + bx$ where a = intercept on y axis and b = regression coefficient (slope of line):-

a. populations of *F. solani* var. *coeruleum* assessed on the PM70 and PAB selective media;

b. soil infectivity and population of *F. solani* var. *coeruleum* assessed on the PM70 and PAB selective media;

c. soil contamination with *F. solani* var. *coeruleum* assessed at different times by two different methods;

d. soil infectivity and population of *F. sulphureum*;

e. soil contamination with *F. sulphureum* assessed at different times by two different methods.

Before regression analysis, data for propagules were transformed to $\log_{10}(x+1)$, where x = number of propagules per g soil from colony counts and data for soil infectivity were transformed to arcsin.

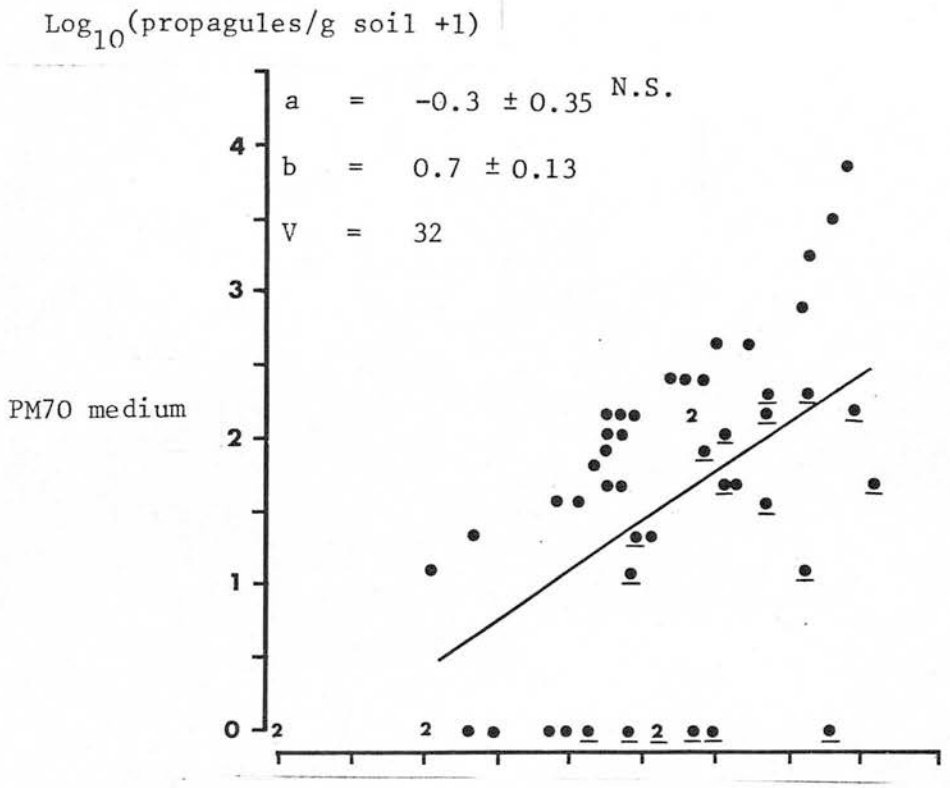
All regression coefficients were highly significant. In some cases intercepts were not significantly different from zero ($P>0.05$).

a. The linear regression of the number of *F. solani* var. *coeruleum* propagules (PM70 medium) on the number of *F. solani* var. *coeruleum* propagules (PAB medium) is shown for two assessment dates, May and November, in Figs 7.1 and 7.2 respectively. In May a poor relationship was obtained between propagule assessments on the different media because the PM70 medium was ineffective in isolating *F. solani* var. *coeruleum* from a number of soils: only 32% of the variance was accounted for. Poor isolation was observed to correspond with growth of 'weed fungi', notably *Penicillium* spp., on the surface of the medium. The soils in which this happened are indicated as ⊛ in Fig. 7.1. The relationship was improved in November when the PM70 medium was more effective in isolating *F. solani* var. *coeruleum*. Profuse growth of 'weed fungi' was no longer observed. The regression coefficient was 1, and 78% of the variance was accounted for. At population levels above 100 propagules per g soil the PAB medium detected consistently more propagules per unit weight of soil than the PM70 medium.

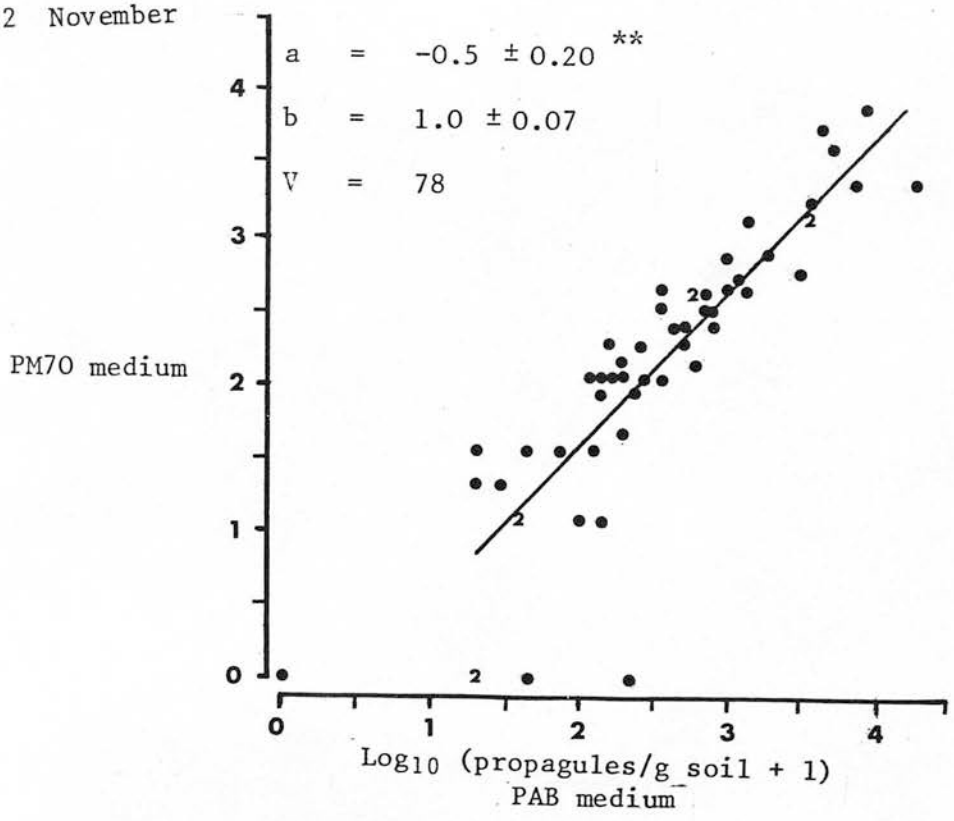
b. The linear regressions of soil infectivity on the number of *F. solani* var. *coeruleum* propagules (PM70 medium) and on the number of *F. solani* var. *coeruleum* propagules (PAB medium) are shown respectively in Figs 8.1 and 8.2 for the May assessment and in Figs 9.1 and 9.2 for the November assessment. In May, the better linear relationship between soil infectivity and propagule assessments was obtained where propagule assessments were made on the PAB medium because isolation of *F. solani* var. *coeruleum* from a number of soils on the PM70 medium was not successful: 84% of the variance was accounted for on the PAB medium compared with 35% on the PM70 medium. The soils from which poor isolation of *F. solani* var. *coeruleum* was obtained are indicated as ⊛ in Fig. 8.1. In November, a linear regression fitted the data well, irrespective of

Fig. 7. Number of *F. solani* var. *coeruleum* propagules (PM70 medium) regressed on number of *F. solani* var. *coeruleum* propagules (PAB medium) at two assessment dates, May and November.

7.1 May




7.2 November



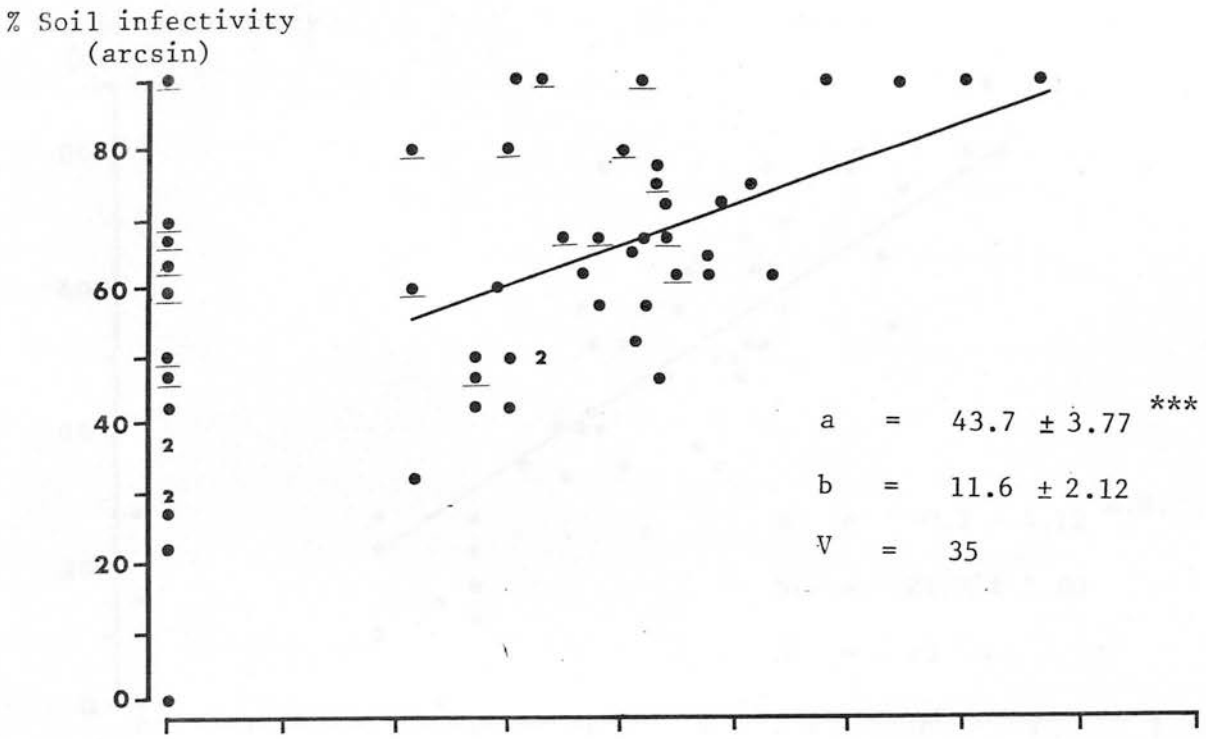

** Intercept significantly different from 0 at P = 0.01.

V = % variance accounted for.

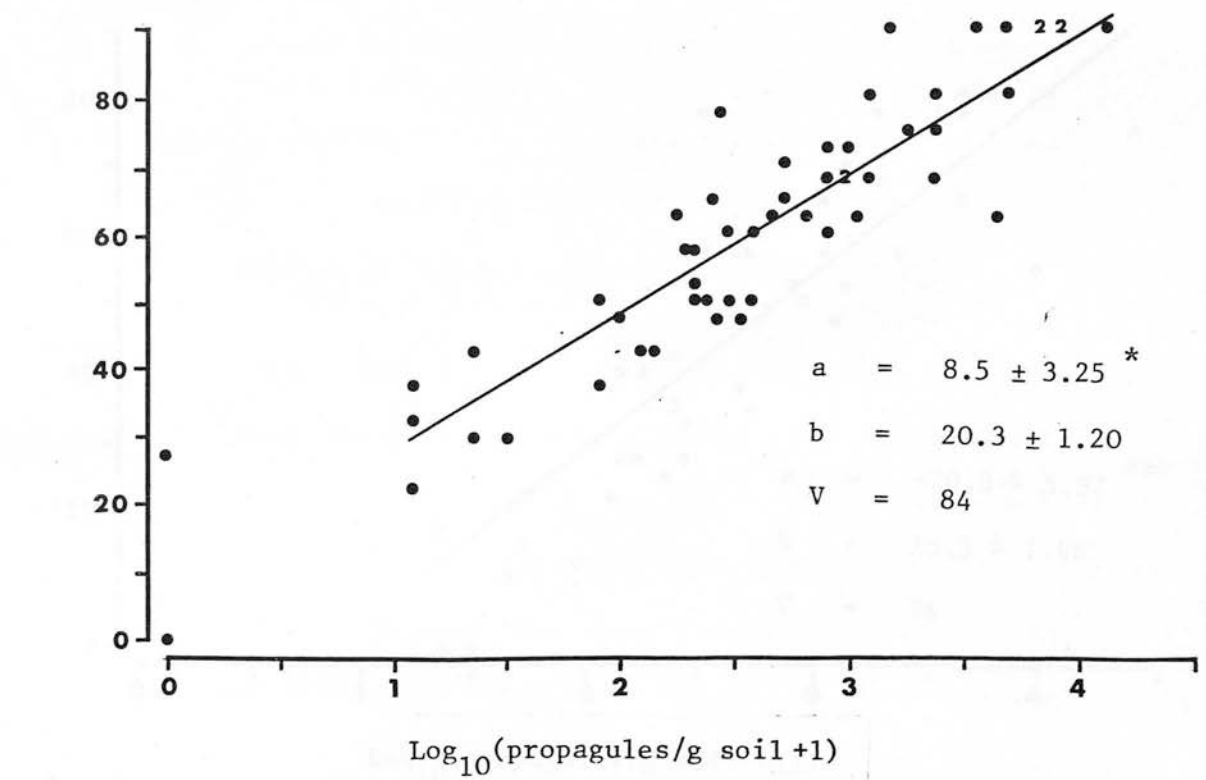
• Poor selectivity shown by the PM70 medium because of growth of *Penicillium* spp.

Fig. 8. Regressions of soil infectivity on (.1) number of *F. solani* var. *coeruleum* propagules (PM70 medium) and (.2) on number of *F. solani* var. *coeruleum* propagules (PAB medium). Assessment date, May 1976.

8.1 PM70 medium




8.2 PAB medium



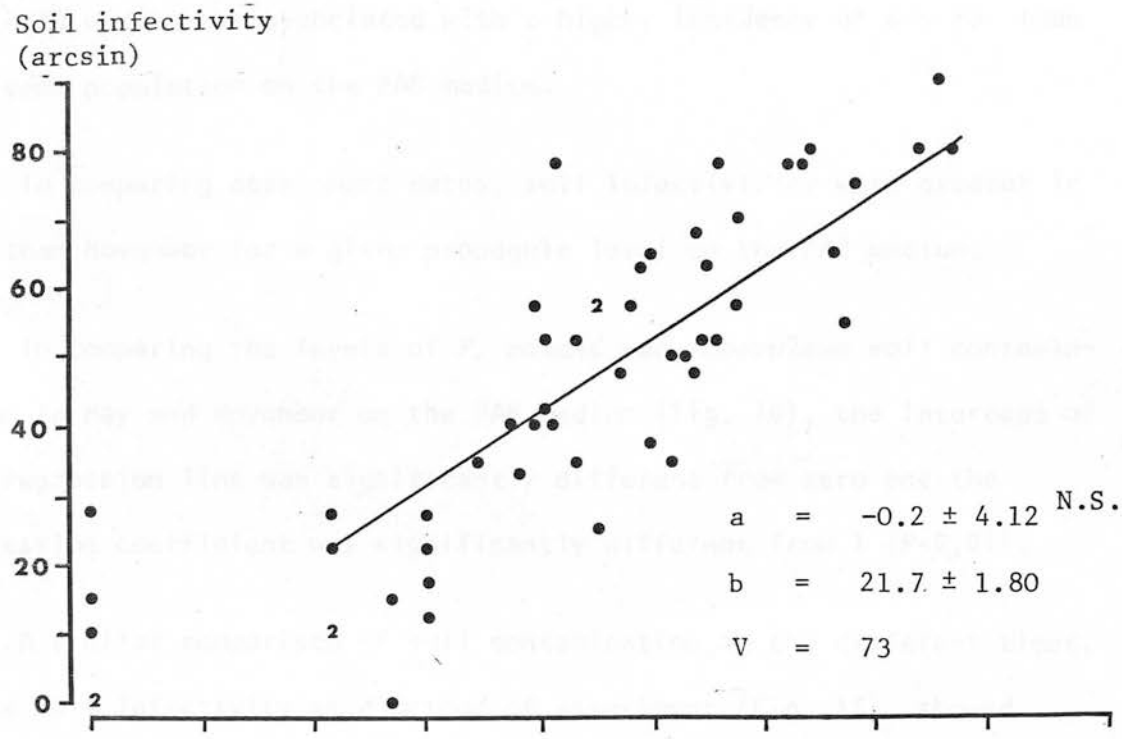

*, *** Intercept significantly different from 0 at P = 0.05 and P = 0.001 respectively.

V = % variance accounted for.

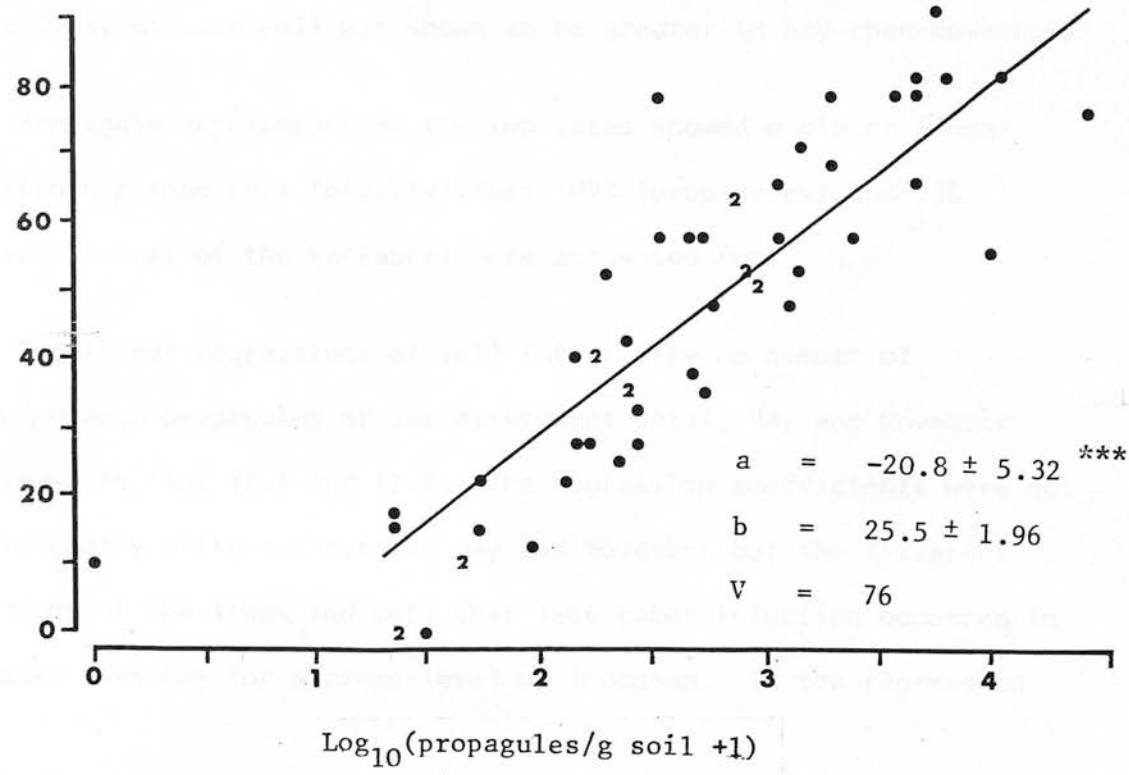
● Poor selectivity shown by the PM70 medium because of growth of *Penicillium* spp.

Fig. 9. Regressions of soil infectivity on (.1) number of *F. solani* var. *coeruleum* propagules (PM70 medium) and on (.2) number of *F. solani* var. *coeruleum* propagules (PAB medium). Assessment date, November 1976.

9.1 PM70 medium




9.2 PAB medium




*** Intercept significantly different from 0 at P = 0.001.

V = % variance accounted for.

whether the fungal population was determined on the PM70 or PAB media: respectively, 73% and 76% of the variances were accounted for. Regression coefficients for both media were similar, but because the positions of the lines were different, fungal population assessed on the PM70 medium was associated with a higher incidence of dry rot than the same population on the PAB medium.

In comparing assessment dates, soil infectivities were greater in May than November for a given propagule level on the PAB medium.

c. In comparing the levels of *F. solani* var. *coeruleum* soil contamination in May and November on the PAB medium (Fig. 10), the intercept of the regression line was significantly different from zero and the regression coefficient was significantly different from 1 ($P<0.01$).

A similar comparison of soil contamination at the different times, using soil infectivity as a method of assessment (Fig. 11), showed that the regression coefficient was not significantly different from 1. However, since the intercept was significantly different from zero the infectivity of each soil was shown to be greater in May than November.

Propagule assessments at the two dates showed a closer linear relationship than soil infectivities: 84% (propagules) and 73% (infectivities) of the variances were accounted for.

d. The linear regressions of soil infectivity on number of *F. sulphureum* propagules at two assessment dates, May and November are shown in Figs 12.1 and 12.2. The regression coefficients were not significantly different between May and November but the different positions of the lines indicate that less tuber infection occurred in November than May for a given level of inoculum. In the regression

Fig. 10. Number of *F. solani* var. *coeruleum* propagules (November 1976) regressed on number of *F. solani* var. *coeruleum* propagules (May 1976). Propagule assessments made on the PAB medium.

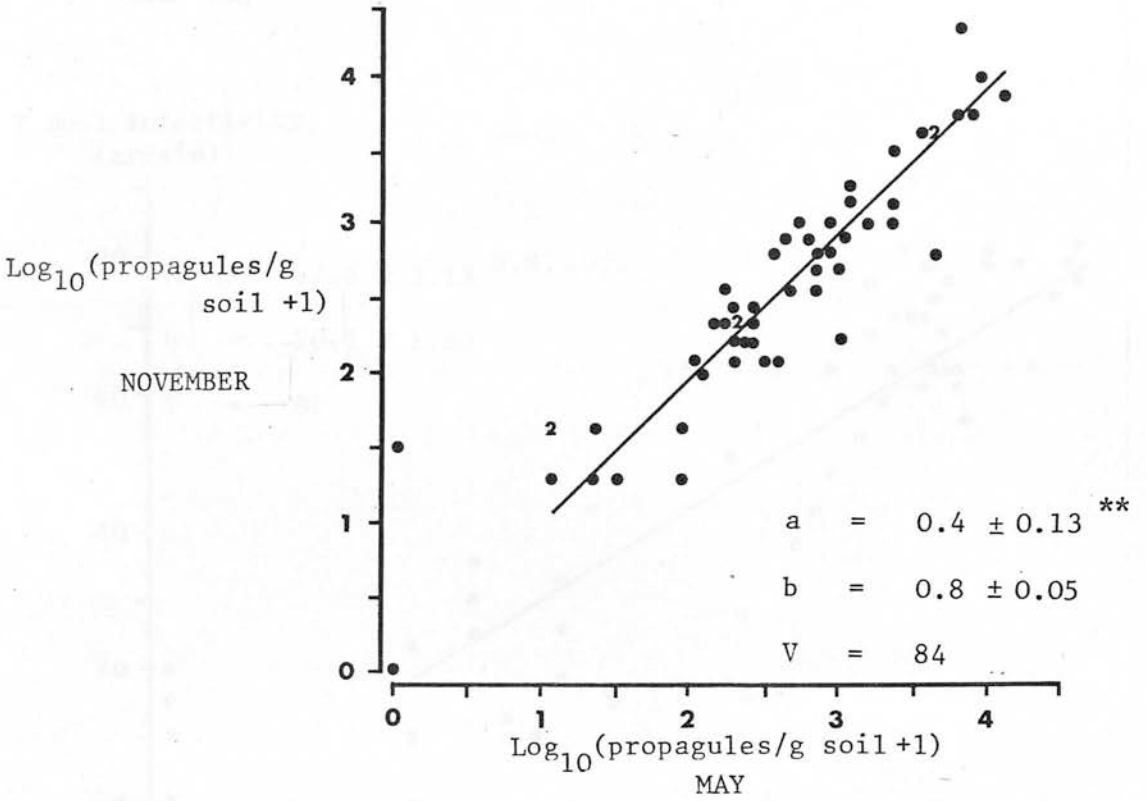
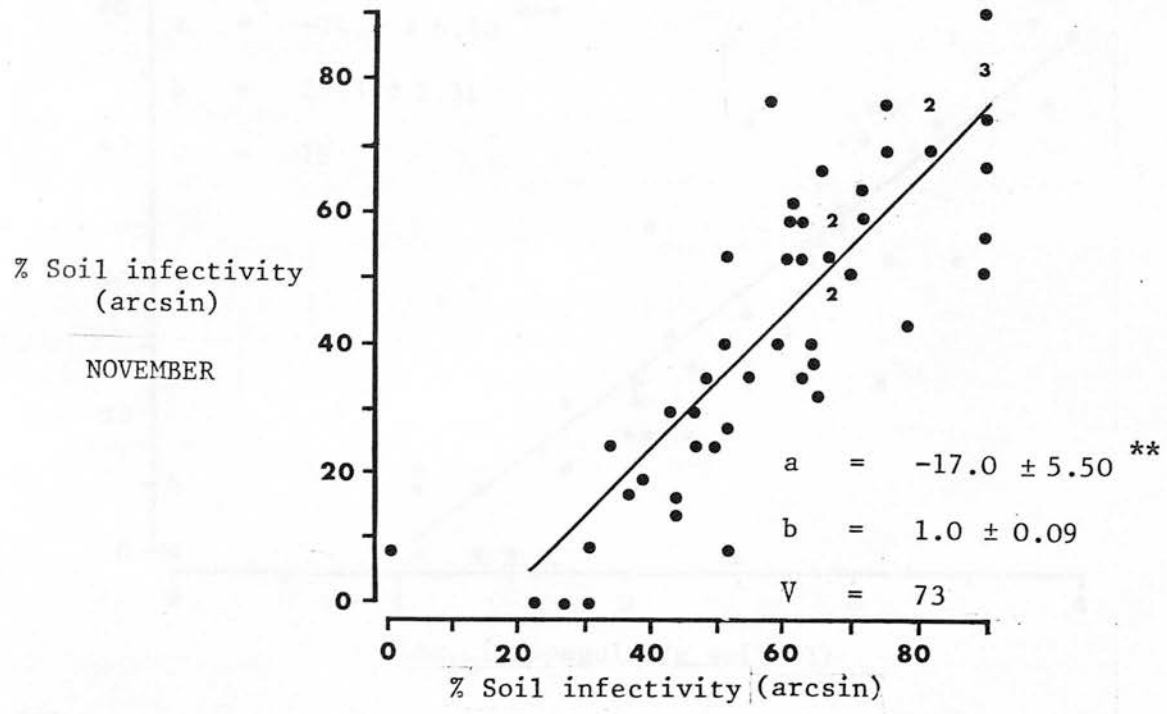


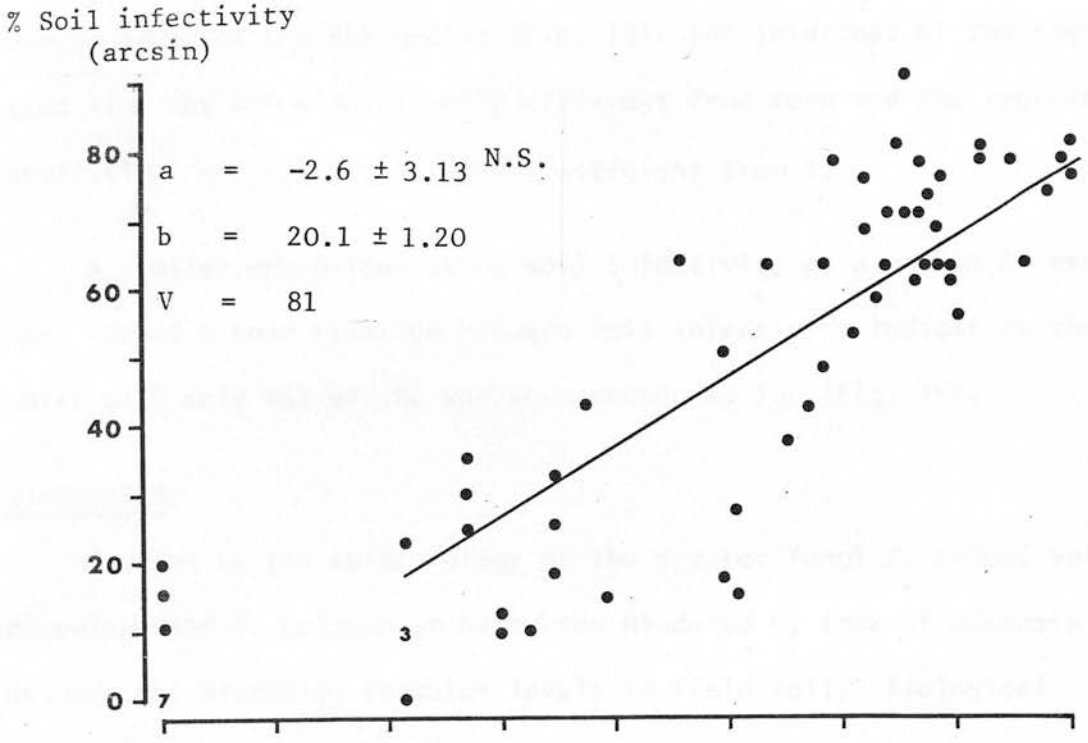

Fig. 11. Soil infectivity (November 1976) regressed on soil infectivity (May 1976) for *F. solani* var. *coeruleum* contaminated soils.



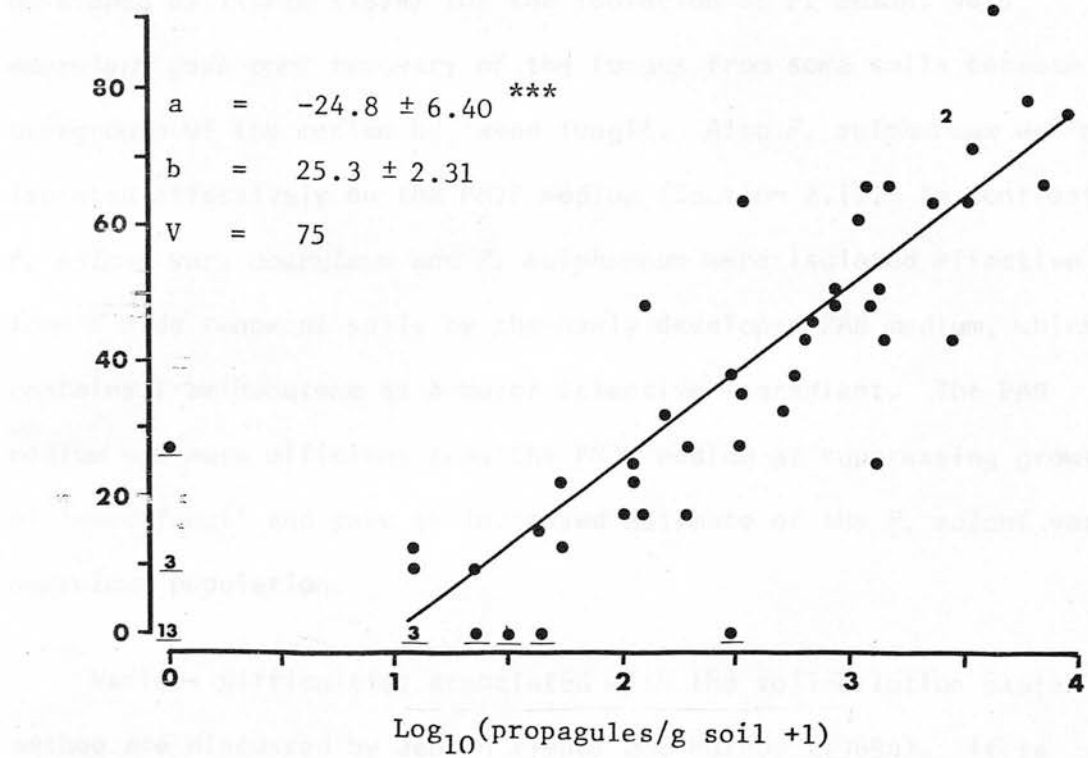

** Intercept significantly different from 0 at P = 0.01.
V = % variance accounted for.

Fig.12. Regressions of soil infectivity on number of *F. sulphureum* propagules (PAB medium) at two assessment dates, May and November 1976.

12.1 May




12.2 November




*** Intercept significantly different from 0 at P = 0.001.
V = % variance accounted for.
• Data omitted from regression calculation.

for November, data from certain soils, showing zero infectivity and/or zero levels of inoculum, illustrated as ⊙ in Fig. 12.2, were omitted from the regression calculations.

e. In comparing the levels of *F. sulphureum* soil contamination in May and November on the PAB medium (Fig. 13), the intercept of the regression line was not significantly different from zero and the regression coefficient was not significantly different from 1.

A similar comparison using soil infectivity as a method of assessment showed a poor relation between soil infectivity indices at the two dates with only 65% of the variance accounted for (Fig. 14).

## DISCUSSION

Studies on the epidemiology of the dry rot fungi *F. solani* var. *coeruleum* and *F. sulphureum* have been hindered by lack of adequate methods for assessing inoculum levels in field soil. Biological methods using the potato tuber to assess soil infectivity have not been entirely satisfactory (Section 1) and the PM70 selective medium developed by Tickle (1974) for the isolation of *F. solani* var. *coeruleum* gave poor recovery of the fungus from some soils because of overgrowth of the medium by 'weed fungi'. Also *F. sulphureum* was not isolated effectively on the PM70 medium (Section 2.1). In contrast, *F. solani* var. *coeruleum* and *F. sulphureum* were isolated effectively from a wide range of soils by the newly developed PAB medium, which contains 2-aminobutane as a major selective ingredient. The PAB medium was more efficient than the PM70 medium at suppressing growth of 'weed fungi' and gave an increased estimate of the *F. solani* var. *coeruleum* population.

Various difficulties associated with the soil-dilution plate method are discussed by Jensen (1968) and Hornby (1969a). It is

Fig.13. Number of *F. sulphureum* propagules (November 1976) regressed on number of *F. sulphureum* propagules (May 1976). Propagule assessments made on the PAB medium.

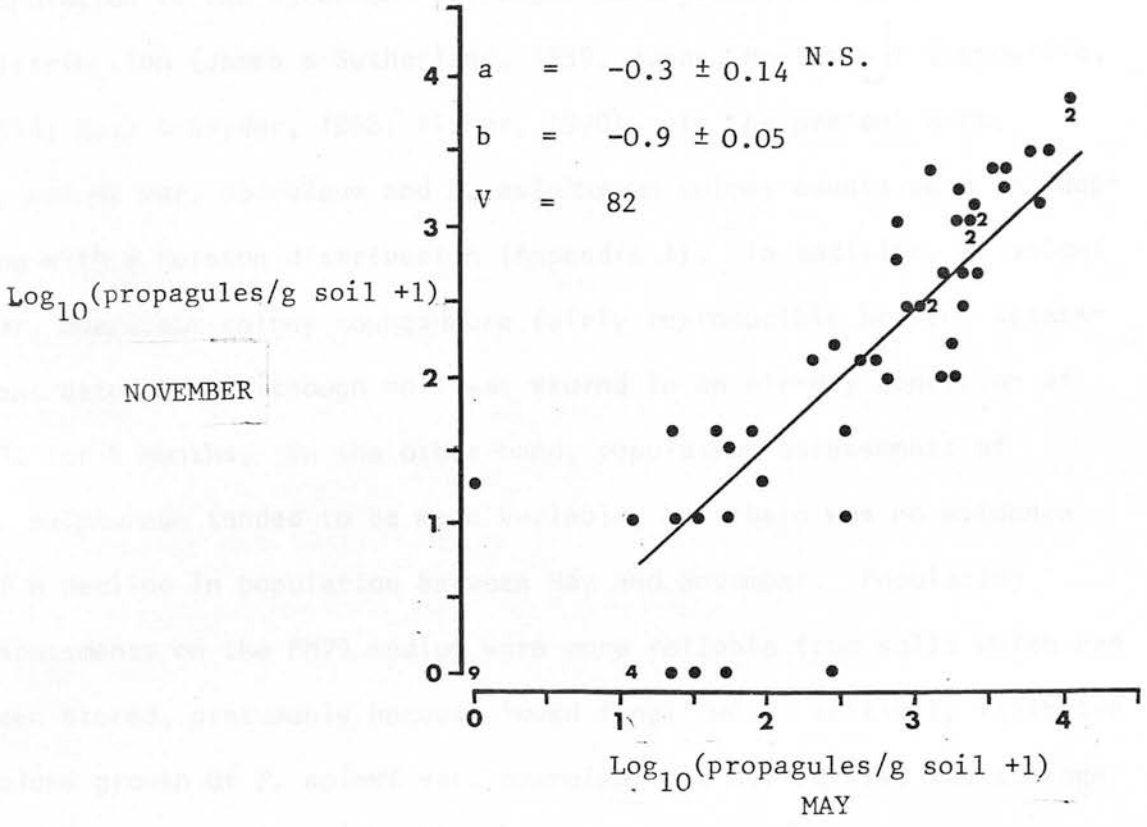
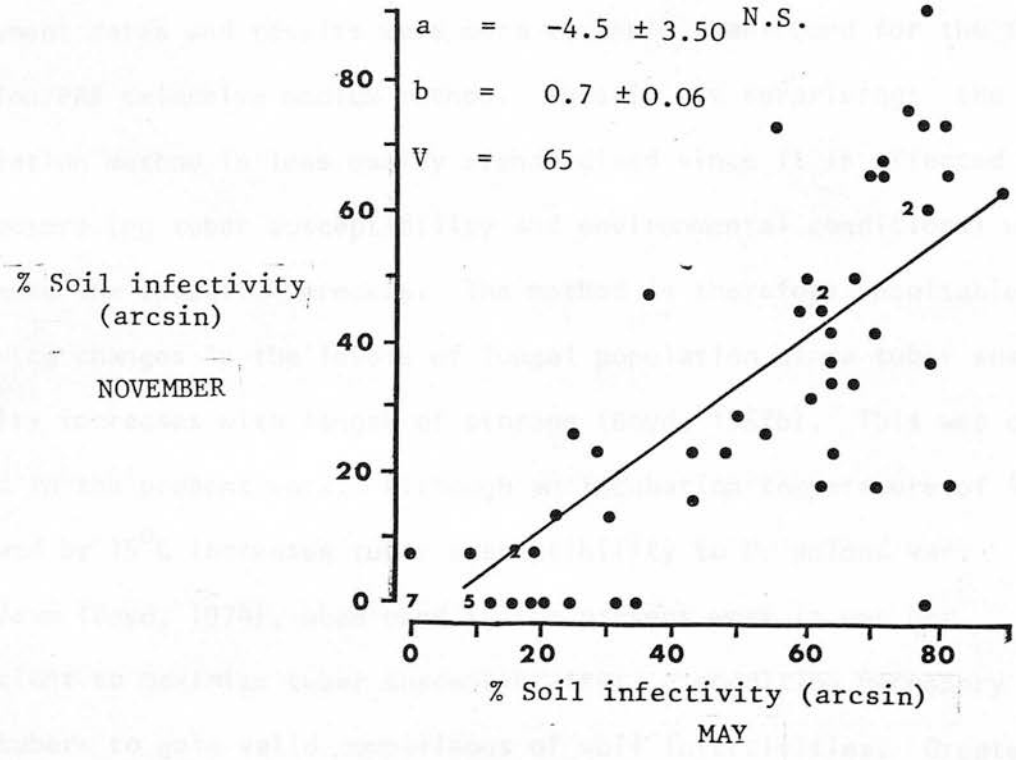



Fig.14. Soil infectivity (November 1976) regressed on soil infectivity (May 1976) for *F. sulphureum* contaminated soils.




V = % variance accounted for.

generally agreed that a suitable test to ascertain the suitability and accuracy of the soil-dilution plate method for measuring the fungal population is the agreement of fungal colony counts with the Poisson distribution (James & Sutherland, 1939; Jones, Mollison & Quenouille, 1948; Nash & Snyder, 1962; Fisher, 1970). In the present work, *F. solani* var. *coeruleum* and *F. sulphureum* colony counts were in keeping with a Poisson distribution (Appendix 1). In addition, *F. solani* var. *coeruleum* colony counts were fairly reproducible between assessment dates even although soil was stored in an air-dry condition at 4°C for 6 months. On the other hand, population assessments of *F. sulphureum* tended to be more variable, but there was no evidence of a decline in population between May and November. Population assessments on the PM70 medium were more reliable from soils which had been stored, presumably because 'weed fungi' which initially inhibited colony growth of *F. solani* var. *coeruleum* did not survive the storage period.

Results for the assessment of soil infectivity with *F. solani* var. *coeruleum* from the tuber inoculation method were not reproducible between assessment dates and results were more variable than found for the soil dilution/PAB selective medium method. This is not surprising: the tuber inoculation method is less easily standardised since it is affected by all factors (eg tuber susceptibility and environmental conditions) which influence the infection process. The method is therefore unsuitable for following changes in the levels of fungal population since tuber susceptibility increases with length of storage (Boyd, 1952b). This was confirmed in the present work. Although an incubation temperature of 4°C followed by 15°C increases tuber susceptibility to *F. solani* var. *coeruleum* (Boyd, 1974), when used in the present work it was not sufficient to maximise tuber susceptibility: a condition necessary in test tubers to gain valid comparisons of soil infectivities. Greater

variation in soil infectivities between the two assessment dates were found for *F. sulphureum* than *F. solani* var. *coeruleum*.

The soil infectivity index was related logarithmically to the number of *F. solani* var. *coeruleum* or *F. sulphureum* propagules in the propagule range 1-4.5. However, the *F. sulphureum* populations obtained tended to overestimate the infectivity of some soils. This may be due to the incorrect assumption of a linear relationship between soil infectivity and the logarithmic transformation of the number of *F. sulphureum* propagules, although a linear relationship appears to be correct for *F. solani* var. *coeruleum*. Alternatively non-pathogenic *F. sulphureum* propagules may have been isolated on the PAB medium. This requires further investigation particularly since Booth (1971) suggested storage of *Fusarium* spp. in soil cultures to prevent loss of pathogenicity.

In cases where the intercept of the linear regression of soil infectivity on the *F. solani* var. *coeruleum* or *F. sulphureum* population was significantly different from the origin several explanations are possible.

1. Where the intercept is positive this suggests that the tuber inoculation method is detecting soil contamination where the soil dilution method is not.

2. Where the intercept is negative this suggests that a threshold level of inoculum is required for infection to take place.

3. Alternatively, these explanations may be inadequate since regressions in the population range log0-1 are extrapolations. Further work is thus required to investigate the relationship between soil infectivity and inoculum levels in this range.

According to Dimond & Horsfall (1965) the disease inoculum curve is analogous to the fungicide dosage response curve and differences in the characteristics of any two curves will indicate quantitative or qualitative differences in resistance or pathogenesis. In the present work the slopes of the disease inoculum curves for *F. solani* var. *coeruleum* and *F. sulphureum* were the same at two assessment dates (May and November), when respectively mature and relatively immature tubers with respect to length of time in storage, were inoculated with the different pathogens. This would suggest that the mode of pathogenic action for *F. solani* var. *coeruleum* and *F. sulphureum* is unlikely to be different and the mechanism of resistance is the same for mature and immature tubers var. Catriona. It would be useful to obtain disease inoculum curves for other potato varieties since they may prove helpful in discriminating between similar and dissimilar mechanisms of disease resistance.

The positions of the disease inoculum lines, although the same for each assessment date, were different between dates, ie a given level of inoculum caused a higher incidence of rotting in mature tubers than in immature tubers. This indicates a similar relationship between disease and inoculum for *F. solani* var. *coeruleum* and *F. sulphureum* in the var. Catriona. The reason for the difference in susceptibility between mature and immature tubers is not known but is probably related to the changing physiology of the tuber (Boyd, 1967) and not to differences which may have been caused by using tubers of two stocks (ie tubers inoculated in May and November grown in 1975 and 1976 respectively), or a loss of fungal pathogenicity between assessment dates. The increase in susceptibility of mature tubers to infection by *F. solani* var. *coeruleum* has been shown by other workers (Boyd, 1952b; Langerfeld, 1977) but the position regarding *F. sulphureum* is less clear as later

experiments revealed, in contrast to the present work, no differences in the susceptibility of those varieties tested to *F. sulphureum* infection during the storage season (Section 5.2).

## 2.3 Relationship between the levels of progeny tuber contamination with *F. solani* var. *coeruleum* and incidence of dry rot.

### INTRODUCTION

The risk of disease development during storage varies in different potato stocks with such factors as the level of fungal and bacterial contamination of the tuber surface and the degree of damage sustained at harvest or during riddling. Attempts (Anon, 1974; 1975a; 1976a; 1977; Meijers, 1975a) have been made with varying degrees of success to predict the suitability of stocks for long-term storage on the basis of:

1. visual or microscopic assessments of disease or pathogen levels (eg skin spot (*Polyscytalum pustulans*), silver scurf (*Helmintho-sporium solani*) and bacterial soft rots (*Erwinia* spp.)) before storage;

2. the incidence of disease (eg dry rot and gangrene) which develops in a sample of tubers selected from the stored stock after standard wounds;

3. the incidence of disease (eg dry rot and gangrene) which develops in susceptible test tubers after inoculation of soil taken from the stock.

This preliminary investigation was conducted in small plot experiments over 3 seasons to determine the relationship between the incidence of dry rot and the results from two test methods, ie soil dilution/selective medium and tuber stab methods.

### MATERIALS AND METHODS

From 1974-1976 *F. solani* var. *coeruleum* contaminated progeny tubers, var. Catriona, were obtained by planting different proportions of

*F. solani* var. *coeruleum* infected seed tubers with contaminated stock (Section 3.2). The progeny were lifted in October by single-row elevator digger, stored in new paper sacks (*c.* 10kg tubers per sack) until February when the contents of each sack were tipped onto clean paper. Levels of tuber contamination were assessed by the soil dilution/selective medium and tuber stab methods. Incidence of dry rot was determined after riddle abrasion.

In the soil dilution/selective medium method, soil which remained in the paper sack after removal of tubers was air-dried, sieved (0.85mm mesh) and the *F. solani* var. *coeruleum* population per g progeny tubersphere soil determined as described in Section 2.2. The PM70 selective medium was used 1974-1975 and the PAB medium 1975-1976 and 1976-1977.

In the tuber stab test 20 seed-size tubers visually free from lesions were selected from the contents of each sack. Each tuber was given four standard wounds (7mm diam. x 7mm deep) around the circumference midway between the heel and rose ends. Tubers were incubated at 4°C for 6wk followed by 15°C for *c.* 4wk. The number of dry rot lesions were expressed as a percentage of 80 inoculations: the progeny tuber contamination index (PTCI).

The riddle abrasion test aimed to simulate the type of damage which occurs in practice during riddling. Tubers from each sack (after removal of tubers showing dry rot lesions) were damaged on a circular hand riddle (3.15cm wire grid) which was rotated 25 times. The riddle was sterilised by washing with methanol. Damaged tubers were stored in new paper sacks in a farm store for 4 months, then washed and the percentage infected tubers determined.

Data were examined by regression analysis. Propagules per g soil were transformed to $\log_{10}$ (propagules/g soil +1) and percentages to arcsin.

## RESULTS

Linear regressions of the incidence of dry rot after riddling on the number of propagules per g progeny tubersphere soil and on the progeny tuber contamination index are shown in Figs 15 and 16 respectively. All the regression coefficients were highly significant ($P<0.001$).

Regression coefficients for dry rot incidence regressed on propagules were significantly different between 1974-1975 and 1976-1977 ($P<0.01$), but not significantly different between 1975-1976 and 1976-1977. The positions of the regression lines were the same for 1975-1976 and 1976-1977 but the regression obtained in 1974-1975 indicated that more dry rot developed in that season than in 1975-1976 for a given level of inoculum.

Regression coefficients for dry rot incidence regressed on PTCI were not significantly different between 1975-1976 and 1976-1977 and the positions of the lines were the same. These regression coefficients were, however, significantly different from the coefficient for 1974-1975 indicating a difference in the rate of increase of disease incidence between seasons.

## DISCUSSION

The Potato Marketing Board of the UK has shown interest in the possibility of selecting ware crops with low disease risk for storage late into the season (eg stocks of low potential for silver scurf infection for the late or end of season pre-pack trade) and 'high risk' stocks for early dispersal.

FIG.15. Dry rot incidence regressed on number of *F. solani* var. *coeruleum* propagules per g progeny tubersphere soil.

| Season | Regression data | | |
|---|---|---|---|
| | a | b | % variance accounted for |
| 15.1) 1974-75 | 6.4 ± 5.26 | 11.6 ± 1.65 | 64 |
| 15.2) 1975-76 | -18.8 ± 6.80 | 14.6 ± 2.40 | 71 |
| 15.3) 1976-77 | -26.2 ± 4.89 | 18.9 ± 1.60 | 86 |

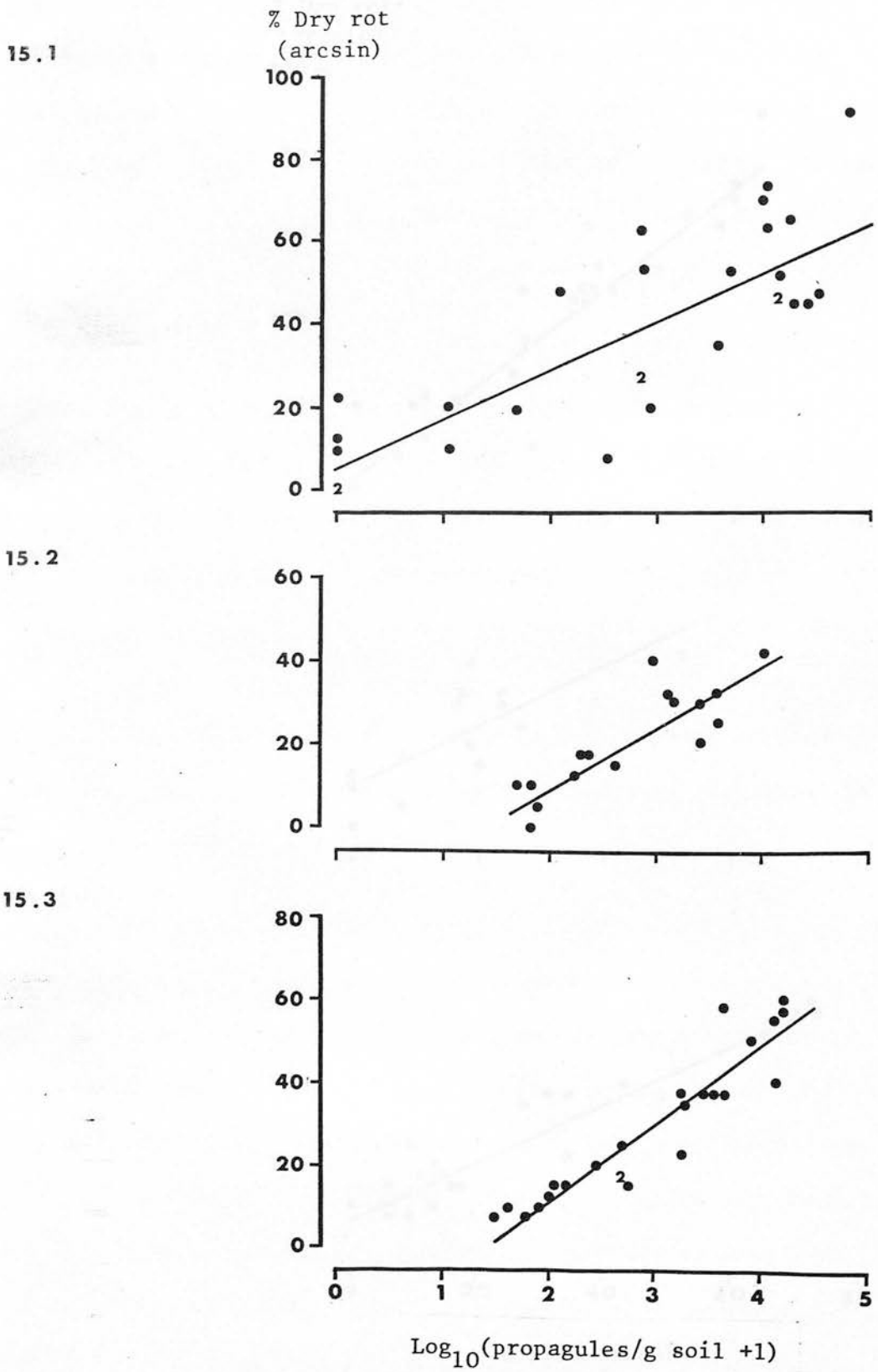
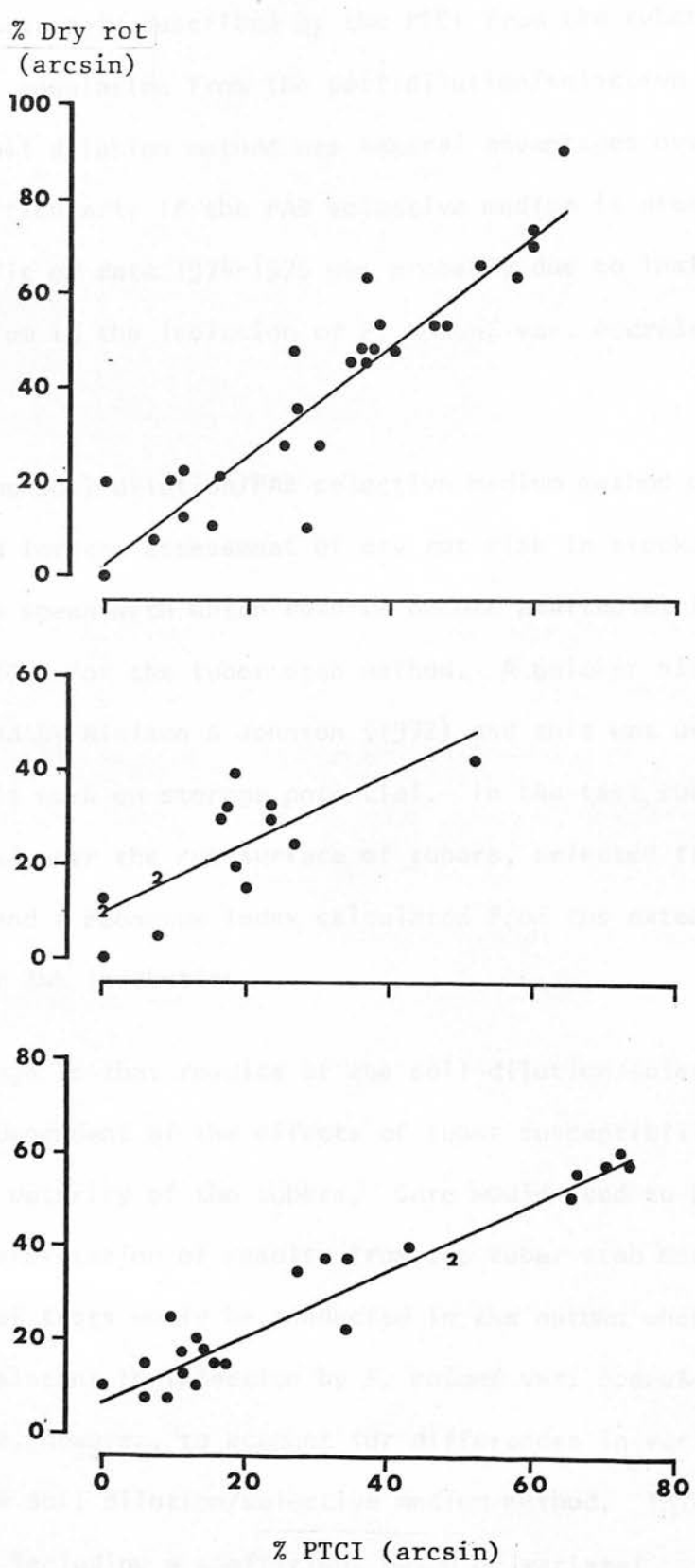


Fig.16. Dry rot incidence regressed on progeny tuber contamination index (PTCI).

| | Season | Regression data | | |
|---|---|---|---|---|
| | | a | b | % variance accounted for |
| 16.1) | 1974-75 | 2.4 ± 3.59 | 1.2 ± 0.10 | 83 |
| 16.2) | 1975-76 | 10.4 ± 2.93 | 0.8 ± 0.15 | 62 |
| 16.3) | 1976-77 | 7.6 ± 1.79 | 0.7 ± 0.05 | 91 |



The work described shows that two simple test methods (ie soil dilution/selective medium and tuber stab methods) may be of use in selecting potato crops of high or low dry rot disease risk. Results from the test methods were linearly related to the incidence of dry rot after transformation of data. Although dry rot incidence in 2 out of 3 seasons was more accurately described by the PTCI from the tuber stab method than by fungal population from the soil dilution/selective medium method, the soil dilution method has several advantages over the former method particularly if the PAB selective medium is used. The relatively poor fit of data 1974-1975 was probably due to inefficiency of the PM70 medium in the isolation of *F. solani* var. *coeruleum* from soil (Section 2.2).

Advantages of the soil dilution/PAB selective medium method over the tuber stab method for the assessment of dry rot risk in stocks include, firstly, the speed with which results become available, ie 2-3wk compared with 10wk for the tuber stab method. A quicker biological test was described by Nielsen & Johnson (1972) and this was used by Meijers (1975a) in his work on storage potential. In the test, tubersphere soil was spread over the cut surface of tubers, selected from those to be stored, and a *Fusarium* index calculated from the extent of tissue browning after 2wk incubation.

A second advantage is that results of the soil dilution/selective medium method are independent of the effects of tuber susceptibility which increases with maturity of the tubers. Care would need to be exercised in the interpretation of results from the tuber stab method since in practice most tests would be conducted in the autumn when tubers are fairly resistant to infection by *F. solani* var. *coeruleum*. It would be desirable, however, to account for differences in varietal susceptibility in the soil dilution/selective medium method. This might be achieved by including a coefficient based on varietal

susceptibility in the regression equation which describes the relationship between dry rot incidence and propagule number. It would not, however, allow for variation in susceptibility caused by different growing conditions (Boyd, 1952b, 1967) which could only be determined in biological tests where tubers are selected from the crop to be stored.

Finally, the PAB medium is able to distinguish between *F. solani* var. *coeruleum* and *F. sulphureum* whereas this may not be possible from lesion symptoms in the tuber stab or Nielsen & Johnson (1972) methods. The tuber stab method can also prove unreliable because of the decay of test tubers by soft-rot bacteria. Identification of *F. sulphureum* is important since UK varieties do not appear to be resistant to this fungus (Section 5.2). Identification of seed stocks contaminated with *F. sulphureum* may help contain its spread.

The value of the test methods in predicting storage potential, depends on the relationships obtained from small plot experiments being repeated for the whole crop. At present, most attempts in the UK to predict storage potential, involving other diseases under commercial conditions, have been disappointing. This is not surprising since disease levels depend on many interrelated factors. In the case of dry rot, for example, the disease level may be affected by the degree of tuber damage, which may be affected by maturity of the crop and environmental conditions at harvest (Hunnius & Fuchs, 1970), the type of harvester and grader (Foister, Wilson & Boyd, 1952) and the temperature at which grading is carried out. Even in the small plot experiments which have been described, the relationship between dry rot incidence and the results from the test methods were not the same over the 3 seasons. The relationship was different for the 1974-1975 season and was possibly caused by lack of standardisation in treatments between seasons. Tubers in 1974 were lifted under very wet conditions

and a fairly high level of tuber infection and sporulation occurred before riddling. The 1975 and 1976 crops were lifted under fairly dry conditions and although low levels of infection were noticed, no surface sporulation was found.

Because of the variation in the relationship between dry rot incidence and results of the test methods, it may not be possible to give a precise index of the level of contamination at which a crop should or should not be stored. It should be possible, however, to locate those crops of lowest and highest disease risk and then appropriate management action could be taken. In Holland, the Nielsen & Johnson test (1972) has been used on a limited scale to select crops of low dry rot disease risk (Meijers, 1975a) and in Scotland a ware crop of Home Guard was identified as of high powdery dry rot disease risk because progeny tubersphere soil was highly contaminated with *F. sulphureum* propagules (unpublished). A sample of the crop showed 30% powdery dry rot after 4 months storage.

Besides use on ware crops, the soil dilution/PAB selective medium method could be used to keep a check on the *F. solani* var. *coeruleum* or *F. sulphureum* level of contamination of seed stocks, since high levels will ultimately lead to an increase in the numbers of infected tubers.

# SECTION 3

# POTATO DRY ROT: SOIL-BORNE OR TUBER-BORNE?

## SECTION 3

## POTATO DRY ROT: SOIL-BORNE OR TUBER-BORNE?

Soil adhering to the surface of progeny tubers may be contaminated with *F. solani* var. *coeruleum* spores (Small, 1944; Cunningham & Reinking, 1946) and when removed by washing with water the incidence of dry rot which follows damage may be reduced (Boyd, 1960; Nielsen & Johnson, 1972). Alternatively, when the natural contamination of the tuber is reinforced by application of *F. solani* var. *coeruleum* spores the incidence of dry rot is increased (Small, 1946). Since the natural level of contamination of the tuber surface is a factor affecting disease incidence (Schippers, 1962; also Section 2.3) the source of this contamination is important.

Usually progeny tubers are contaminated with *F. solani* var. *coeruleum* propagules during growth of the plant (Small, 1944; Lansade, 1949; Tickle, 1974) but lesser levels of contamination may also occur, through contact with dust containing the fungus, in potato stores, sprouting trays and potato bags (Pethybridge & Bowers, 1908; Small, 1944; Cunningham & Reinking, 1946). Possible sources of propagules during growth of the plant are, (a) field soil contaminated before planting (Pethybridge & Lafferty, 1917; Small, 1944; Foister, Wilson & Boyd, 1945), (b) infected seed tubers (Boyd & Logan, 1967; Boyd & O'Donnell, 1968; Tickle, 1974) and (c) contaminated seed tubers (Ayers & Robinson, 1956).

This section is concerned with the relative importance of soil-borne and tuber-borne transmission of *F. solani* var. *coeruleum*. It also includes studies on *F. sulphureum*. The section is sub-divided into investigations on:-

3.1 The longevity of *F. solani* var. *coeruleum* and *F. sulphureum* in soil under field and laboratory conditions.

3.2 The transmission of *F. solani* var. *coeruleum* from infected and contaminated seed tubers.

## 3.1 The longevity of *F. solani* var. *coeruleum* and *F. sulphureum* in soil under field and laboratory conditions

### INTRODUCTION

At present, it has not been possible to eliminate dry rot caused by *F. solani* var. *coeruleum* from high grade potato stocks derived from virus tested stem cuttings (VTSC), (Hardie, 1970; Tickle, 1974). This may be because of insufficient attention to hygiene where high and low grade stocks are grown on the same farm. Dust from *F. solani* var. *coeruleum* contaminated tubers may contaminate potato trays and bags, and grading machinery (Pethybridge & Bowers, 1908; Cunningham & Reinking, 1946).

Another possible reason for the failure to maintain dry-rot free stocks is planting into field soil which is already contaminated with *F. solani* var. *coeruleum*. The fungus is known to have survived for at least 10 yr in air-dried field soil stored in the laboratory at 0°C (Boyd, 1970) but information on its survival under field conditions is less certain. Although Small (1944) suggested that *F. solani* var. *coeruleum* could remain viable under field conditions for 9 yr after the last potato crop, his experimental methods are suspect since soil samples were collected from around harvested tubers which might have been grown from infected seed. Foister, Wilson & Boyd (1945) showed that *F. solani* var. *coeruleum* could survive for at least 2 yr in field soil. On the other hand neither *F. solani* var. *coeruleum* nor *F. sulphureum* appeared to over-winter in soil in Canada (Ayers, 1972). In the studies described the tuber inoculation method (McKee & Boyd, 1952) was used to assess the level of soil infectivity.

Since evidence on the longevity of *F. solani* var. *coeruleum* and *F. sulphureum* is controversial, further studies were undertaken to determine longevity of these fungi using a soil dilution/selective

medium method. In addition, information is presented on the effect of some of the laboratory storage conditions on the survival of both fungi, since this relates to the maximum time soils for analysis may be stored before any decrease in the fungal population occurs.

## MATERIALS AND METHODS

### Field Experiments

Two field experiments with the following treatments were conducted, each with a randomised block design of five (Experiment 1) and four (Experiment 2) replicates.

Experiment 1 (planted April, 1971 in previous work /Tickle, 1974/).

1. Catriona tubers surface sterilised with 0.8% formalin for 1 min. then inoculated with *F. solani* var. *coeruleum* (lesions *c.* 2.5cm diam. at planting).

2. Catriona tubers visually free from lesions but tubersphere soil naturally contaminated with *F. solani* var. *coeruleum*.

3. As 2 but disinfected in a 150 ppm mercury (methoxyethylmercuric chloride - MEMC -) dip for 1 min. The formulation used was Agallol.

Experiment 2 (planted May, 1976)

1. Catriona tubers surface sterilised with 0.8% formalin for 1 min. then inoculated with *F. solani* var. *coeruleum* and *F. sulphureum* (lesions *c.* 3.5 cm diam. at planting).

2. As 1 but sprayed several days before planting with thiabendazole at 2g a.i./100 ml water/50.8 kg potatoes. The formulation used was Tecto RPH, 60% a.i.

Each plot consisted of two adjacent drills, 0.7m apart and 4m length planted with 20 tubers (10 per drill). After harvest (Expt 1 by hand-fork; Expt 2 by elevator digger) the trial areas were sampled at intervals. Location of plots was done by measurements from the field perimeter.

Five soil samples, each 0.3m apart, were taken with a soil corer (20cm x 1.5cm diam.) from the centre 1.5m length of each drill. Soil from each plot (ie two drills) was bulked. Soil from Experiment 1 was air-dried for 3 days at room temperature and sieved (0.85mm mesh). Soil from Experiment 2 was left at field moisture, sieved (3.15mm mesh) and its moisture content determined by oven drying (Johnson & Curl, 1972).

Soil contamination was assessed quantitatively using the soil dilution/selective medium method: 5g samples of soil (on an oven-dry basis) were used to make a $10^{-2}$ dilution by comminuting the soil with 100ml sterile water and 100ml 0.15% water agar in a blender (MSE Atomix) at 12000 rpm for 1 min. Two 5ml aliquots of suspension soil were withdrawn and added to 15ml of sterile water to complete the dilution. Aliquots of dilution soil (1ml) were then pipetted onto each of 10 plates of PAB medium. Colony counts were made after 20 days incubation on a laboratory bench.

In Experiment 1 the infectivity of soil was assessed by the tuber inoculation method as described on pp 23-24. An initial $4^{o}C$ incubation temperature was used to increase tuber susceptibility (Boyd, 1974).

Laboratory Experiments

Experiments were started in 1975 (Expt 1) and 1976 (Expt 2) to determine the longevity of *F. solani* var. *coeruleum* and *F. sulphureum* in stored field soil. In a third experiment the effect of air drying on the viability of various *F. solani* var. *coeruleum* spore forms was investigated.

In Experiment 1 three separate soil samples (*c.* 1kg) were collected (October, 1975) from around *F. solani* var. *coeruleum* infected seed tubers. The same day each soil sample was sieved (3.15mm mesh), thoroughly mixed and divided into four lots, each of which received one of the experimental treatments described in Table 4. A randomised block design was used with the three soil samples as replicates. The *F. solani* var. *coeruleum* population was assessed in the soil before and after air drying and after 2, 4, 16 and 54 wk storage.

In Experiment 2 separate soil samples (*c.* 2kg) were collected (October, 1976) from around *F. solani* var. *coeruleum* or *F. sulphureum* infected seed tubers. The same day each sample was sieved (3.15mm mesh), thoroughly mixed and divided into 16 sub-samples. These samples were allocated at random to the four treatments shown in Table 4. A fully randomised design was used with four sub-samples per treatment. The fungal populations were determined in soil immediately after air drying and in the corresponding soils left moist and then again after 4 months storage.

Table 4. Treatments given to soil samples collected from around seed tubers infected with *F. solani* var. *coeruleum* or *F. sulphureum*.

| | |
|---|---|
| 1. | Soil kept moist and stored[a] at 4°C. |
| 2. | " " " " " " 15-28°C (room temperature). |
| 3. | Soil air-dried at room temperature for 3 days then stored at 4°C. |
| 4. | " " " " " " 3 " " " " 15-28°C. |

[a]Soils were stored in sealed double polythene bags each of wall thickness 0.35mm.

For Experiment 3, two investigations were conducted. In the first investigation the survival of *F. solani* var. *coeruleum* from two sources, infected seed tubers and soil artificially contaminated with macroconidia were compared after air drying. Two 0.2kg soil samples, which had been air-dried and sieved (0.85mm mesh), were contaminated respectively with *c.* $1 \times 10^4$ and $1 \times 10^5$ washed *F. solani* var. *coeruleum* macroconidia per g soil. The macroconidia were devoid of chlamydospores and were obtained from 3wk old cultures by washing with sterile distilled water, filtered twice through fine nylon gauze to remove mycelial fragments and then washed three times by centrifugation at 2500 rpm. The *F. solani* var. *coeruleum* contaminated soil was adjusted to 15% moisture and 30g (oven-dry basis) added to each of four nylon gauze bags (mesh 50μ). Into another four bags, each of which contained a desprouted *F. solani* var. *coeruleum* infected seed tuber, was added 30g (oven-dry basis) of sieved (0.85mm mesh) field soil (15% moisture) which contained no detectable *F. solani* var. *coeruleum* (<1 propagule per g). The bags were sealed and arranged 0.4m apart in a four replicate randomised block pattern along one drill on 9 June, 1976. The bags were harvested 17 September, 1976 and the fungal population in the soil determined before and after air drying.

A second investigation compared the survival of *F. solani* var. *coeruleum* macroconidia and hyphae. Washed macroconidia were obtained as described previously. Hyphae devoid of chlamydospores were obtained from 3wk old cultures which were not sporulating, suspended in sterile distilled water, fragmented in a blender and then washed as described for the macroconidia. The macroconidia or hyphal fragments were added respectively to two 500g soil samples at the rate of *c.* $3 \times 10^3$ propagules per g soil. The soil was adjusted to 25% moisture, divided into eight portions and air-dried. The fungal population was determined before and after air drying.

For assessment of the fungal population the PM70 medium was used in Experiment 1 and the PAB medium in Experiments 2 and 3. Samples of 2g soil (on an oven-dry basis) were used to make $10^{-2}$, $10^{-3}$ and $10^{-4}$ soil dilutions, depending on the expected fungal population. Aliquots of dilution soil (1ml) were pipetted onto each of five plates of selective medium for each soil. Colony counts were made after incubation for 14 (PM70) or 20 (PAB) days at room temperature.

## RESULTS

### Field experiments

Experiment 1 The crop rotation which followed potatoes was barley 1972, grass 1973, barley 1974, barley 1975 and grass 1976. The level of soil contamination by *F. solani* var. *coeruleum* on 19 March 1976, was higher in plots originally planted with *F. solani* var. *coeruleum* infected seed tubers than in plots planted with contaminated seed (Table 5). The difference, however, was not significant at the P = 0.05 level.

Table 5. Levels of *F. solani* var. *coeruleum* contamination of field soil 5 yr after harvest of plots planted in 1971 with tubers infected and contaminated with *F. solani* var. *coeruleum*

| Treatment of planted seed tubers | Levels of soil contamination in March 1976 | |
|---|---|---|
| | Propagules per g soil[a] | Soil infectivity index (%)[b] |
| Infected | 2.29 | 28.3 |
| Contaminated | 1.64 | 21.5 |
| Contaminated dipped in MEMC | 1.81 | 17.1 |
| S.E.D. | 0.329 | 5.70 |

[a] $Log_{10}$ (propagules/g soil +1) and [b] arcsin transformed data from five replicates.

An area planted in 1971 with farm stock (var. Pentland Crown) and at least 100m distant from the experimental area was also contaminated with *F. solani* var. *coeruleum* (mean of 15 soil samples = 37 propagules per g, equivalent to 1.60 after $\log_{10}$ (propagules +1) transformation; range = 0-80 propagules per g).

Experiment 2 Before planting, 52 soil samples, each *c.*500g were taken from the area of the field to be planted which had last cropped potatoes in 1970. Experiment 2 occupied a small proportion of this area. Samples were air-dried, sieved (0.85mm mesh) and 10g samples (on an air-dry basis) used to make a $10^{-1}$ dilution. Aliquots of soil suspension (1ml) were plated onto each of 10 plates of PAB medium for each soil sample. A mean of 3 *F. solani* var. *coeruleum* propagules per g of soil was detected. The range of 0-31 propagules and only two of the soil samples were contaminated with more than 10 propagules per g.

After harvest of Experiment 2 the field was cultivated with a 'Triple-K' harrow and planted with winter wheat. Results presented in Table 6 show that more *F. sulphureum* propagules than *F. solani* var. *coeruleum* propagules were present in field soil after harvest. However, the *F. solani* var. *coeruleum* population remained fairly steady for the next 12 months, whereas the *F. sulphureum* population declined during the winter and then remained at a level slightly below that for *F. solani* var. *coeruleum*. Thiabendazole significantly reduced the number of *F. solani* var. *coeruleum* propagules which were present in field soil after harvest and this reduction was still evident after 12 months. Thiabendazole had no significant effect on the *F. sulphureum* population.

Table 6. Number of *F. solani* var. *coeruleum* and *F. sulphureum* propagules[a] per g field soil at intervals after harvest on 20 October 1976

| Treatment of planted seed tubers | Date of sampling | | |
|---|---|---|---|
| | 5.11.76 | 9.5.77 | 21.10.77 |
| | *F. solani* var. *coeruleum* population | | |
| Untreated | 2.52* | 2.47 | 2.31* |
| Thiabendazole | 1.78* | 2.02 | 1.87* |
| S.E.D. | 0.144 | 0.199 | 0.138 |
| | *F. sulphureum* population | | |
| Untreated | 2.70 | 1.95 | 1.93 |
| Thiabendazole | 2.29 | 1.74 | 1.94 |
| S.E.D. | 0.512 | 0.434 | 0.224 |

[a] $Log_{10}$ (propagules/g soil +1) transformed data from four replicates.

* Significantly different from untreated at P = 0.05.

Additional evidence of the ability of *F. solani* var. *coeruleum* and *F. sulphureum* to over-winter came after sampling a field which had grown a commercial crop of var. Home Guard which subsequently developed severe powdery dry rot (*F. sulphureum*) during storage. Twelve, *c.* 0.5 kg soil samples, were collected on 15 February, 1977, 4 months after harvest of the crop. The field area was contaminated moderately (mean = 36 and range = 0-190 propagules per g soil) and heavily (mean = 348 and range = 100-1700 propagules per g soil) with *F. solani* var. *coeruleum* and *F. sulphureum* respectively. An adjacent area, which in the previous year had grown the seed with which the above field was planted, was contaminated only slightly with *F. solani* var. *coeruleum* (mean = 5 and range = 0-30 propagules per g soil) and *F. sulphureum* was detected in one soil sample only at 10 propagules per g.

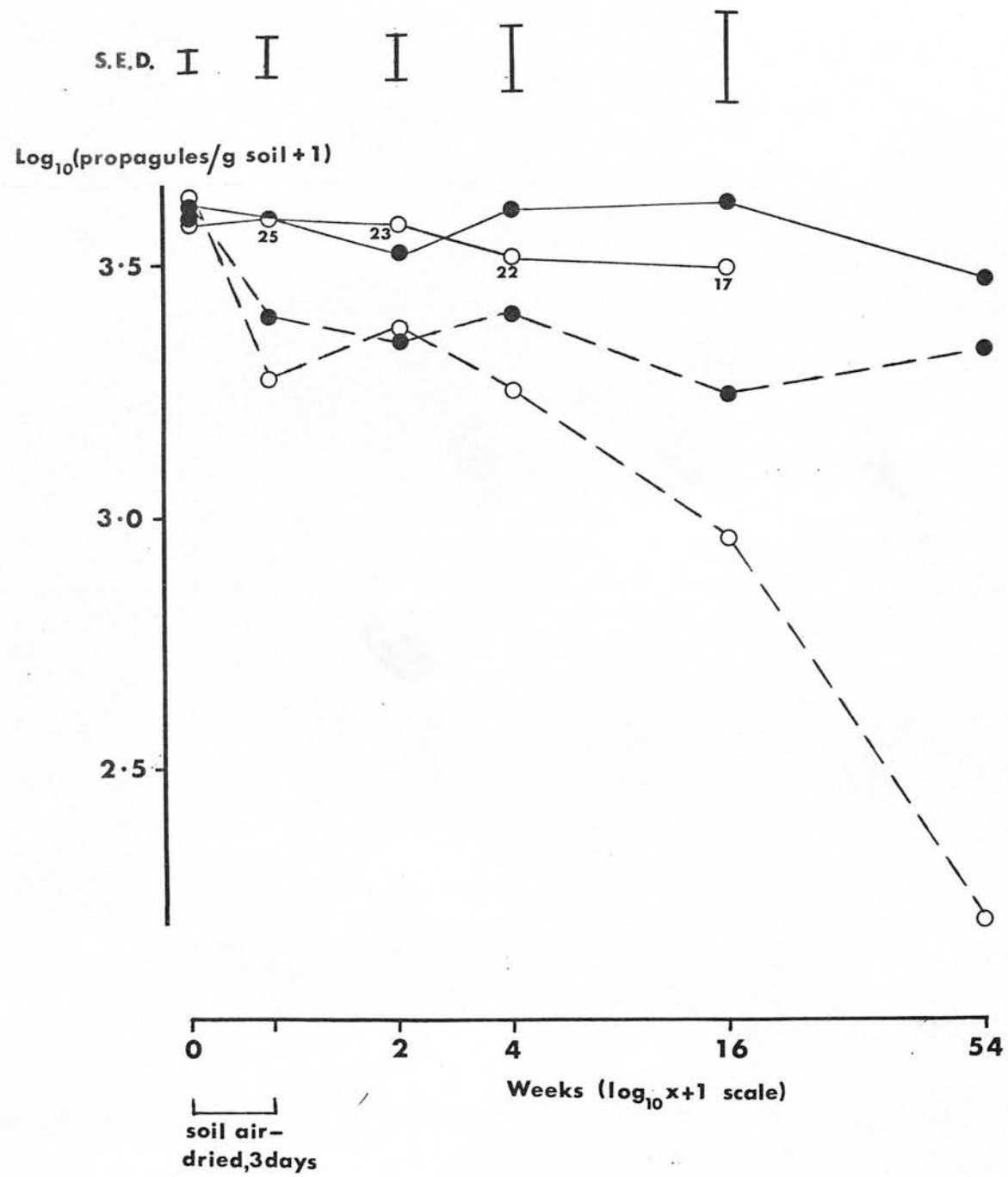
Laboratory experiments

Experiment 1 The results of the work on the effects of air drying and storage temperature of soil collected from around infected seed tubers on the survival of *F. solani* var. *coeruleum* are illustrated in Fig. 17.

Air drying the soil significantly reduced ($P<0.01$) the population of *F. solani* var. *coeruleum*. Storage temperature had very little effect on the population for the first 4 wk following air drying. Subsequently the fungal population remained at a fairly constant level over the period of observation but decreased rapidly in soil stored at 15-28°C.

The effect of temperature on the population in moist soil was more difficult to ascertain since by 16 wk two of the polythene bags containing soil stored at 15-28°C had been accidentally punctured and the contents had air-dried (Fig. 18). These replicates were treated as missing plots and computer assigned values used. The results suggest that storage at 15-28°C had little effect on the population in moist soil for 16 wk. After this time no reliable data were available since soil from the remaining replicate began to air dry even although no perforations were detected in the bag.

Fig.17. Survival of *F. solani* var. *coeruleum* propagules in field soil stored under various conditions.




●——● Moist soil (25% water by weight) stored 4°C.

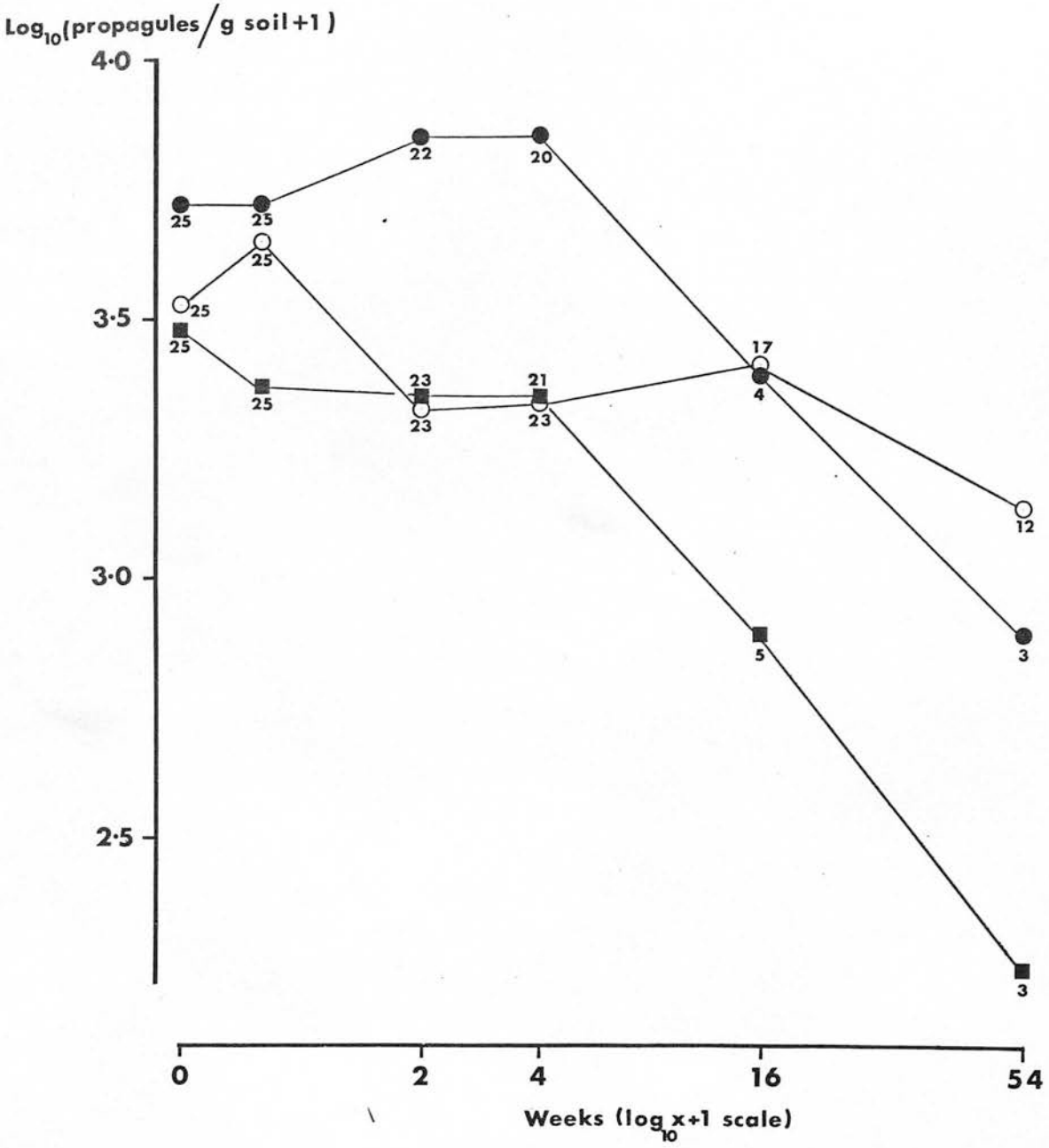
○——○ Moist soil (25% water by weight) stored 15°-28°C.

●- - -● Air-dried soil (2% water by weight) stored 4°C.

○- - -○ Air-dried soil (2% water by weight) stored 15°-28°C.

Numbers in graph = % soil-water by weight.

Fig.18. Survival of *F. solani* var. *coeruleum* propagules in three samples of moist soil stored at 15°-28°C.




Numbers in graph = % soil-water by weight.

Experiment 2 The population of *F. solani* var. *coeruleum* and *F. sulphureum* in field soil collected from around infected seed tubers (soil moisture content *c.* 25%) was significantly reduced ($P<0.01$ and $P<0.001$ respectively) by air drying the soil to a moisture content of *c.* 2% (Table 7).

Table 7. Effect of air drying on the population of *F. solani* var. *coeruleum* and *F. sulphureum*

| Soil treatment | $\text{Log}_{10}$ (propagules/g oven-dry soil +1)[a] | |
|---|---|---|
| | *F. solani* var. *coeruleum* | *F. sulphureum* |
| Moist | 4.48 | 4.22 |
| Air-dry | 4.40 | 4.07 |
| S.E.D. | 0.023 | 0.038 |

[a]Mean of eight replicates.

After 4 months storage of the soils contaminated with *F. solani* var. *coeruleum* and *F. sulphureum* there was a significant interaction ($P<0.05$ and $P<0.001$) between soil treatment and storage time (Tables 8 and 9). The populations in air-dried soil at 15-28°C were significantly less than the other treatments, the populations of which were not significantly different.

Table 8. Effect of moisture and temperature on the propagule level /$\text{Log}_{10}$ (propagules/g oven-dry soil +1)/ of *F. solani* var. *coeruleum* in field soil after 4 months storage

| Temperature (°C) | Soil moisture level | | |
|---|---|---|---|
| | Moist | Air dry | Mean |
| 4 | 4.47 | 4.41 | 4.44 |
| 15-28 | 4.45 | 4.27 | 4.36 |
| Mean | 4.46 | 4.34 | |

S.E.D. Temperature and moisture means 0.024
Temperature x moisture 0.033

Table 9. Effect of moisture and temperature on the propagule level /$\log_{10}$ (propagules/g oven-dry soil +1)/ of *F. sulphureum* in field soil after 4 months storage

| Temperature (°C) | Soil moisture level | | |
|---|---|---|---|
| | Moist | Air dry | Mean |
| 4 | 4.19 | 4.14 | 4.16 |
| 15-28 | 4.10 | 3.43 | 3.76 |
| Mean | 4.15 | 3.78 | |

S.E.D. Temperature and moisture means 0.039
Temperature x moisture 0.056

In 4 months there also appeared to be no decline in the *F. solani* var. *coeruleum* and *F. sulphureum* population in air-dried soil at 4°C, a slight decline in moist soil at both 4°C and 15-28°C and a marked decline in air-dried soil at the higher temperature.

Experiment 3 The results of studies on the effect of air drying on propagules produced in soil from infected seed and macroconidia from axenic culture are shown in Table 10. The number of propagules from infected seed was reduced significantly ($P<0.001$) by air drying, whereas macroconidia, after they had been in field soil for *c.*3 months were unaffected by this procedure. If macroconidia were air-dried immediately after addition to soil then their survival was very poor as was the survival of the hyphae (Table 11).

Table 10. The effect of air-drying on the survival of *F. solani* var. *coeruleum* in field soil: contaminated with propagules from growing infected seed tubers or contaminated at two concentrations with macroconidia from axenic cultures

| Source of propagules (Planted 14.6.76) | $Log_{10}$ (propagules/g oven-dry soil +1) Treatment of field soil 18.9.76 | | S.E.D. |
|---|---|---|---|
| | Left moist (25% moisture) | Air-dried (2% moisture) | |
| Infected seed | 5.19 | 4.97 | 0.035 |
| Macroconidia $1 \times 10^4$ propagules per g soil | 3.81 | 3.81 | 0.027 |
| Macroconidia $1 \times 10^5$ propagules per g soil | 5.58 | 5.58 | 0.041 |

Table 11. The effect of air drying on the survival of *F. solani* var. *coeruleum* macroconidia and hyphae in soil

| Propagule | Initial number of propagules per g in soil before air drying | | % Decrease in propagule number after air drying (arcsin) | |
|---|---|---|---|---|
| | Mean | S.E. | Mean | S.E. |
| Macroconidia | 1595 | ± 53.6 | 63.7 | ± 1.87 |
| Hyphae | 4926 | ± 262.4 | 57.8 | ± 2.16 |

## DISCUSSION

Using a new selective isolation medium (the PAB medium) it was possible to detect *F. solani* var. *coeruleum* at low populations (1 propagule per g soil) hitherto undetected by previous methods (McKee & Boyd, 1952; Tickle, 1974). The fungus was found to survive the rotation required for potato certification in all European countries (Table 12).

Table 12. Requirements for potato certification: minimum interval (yr) between two potato crops (J.L. Hardie, 1977, personal communication)

| | | | |
|---|---|---|---|
| 2 | Finland | 4 | Czechoslovakia |
| | Spain | | Denmark |
| | Sweden | | Netherlands |
| | Switzerland | | Rumania |
| 2-4 | Yugoslavia | | West Germany |
| 3 | Austria | 4-7 | Northern Ireland |
| | Belgium | 5-7 | Scotland |
| | Ireland | | |
| | Norway | | |
| | Poland | | |
| 3-7 | England | | |

It is also likely to survive the 8 yr rotation required for Scottish Virus Tested Stem Cutting (VTSC) stocks since the potential for survival in the laboratory has been shown to be at least 10 yr (Boyd, 1970). Recently Boyd (unpublished), using the PAB medium, found *F. solani* var. *coeruleum* in soils in Maine, USA, which had been in grass for 14 yr. The presence of soil inoculum is possibly the reason for the disappointing performance of the VTSC scheme in decreasing the prevalence of most fungal tuber-borne pathogens (Hirst, 1977). Despite the lack of information on the distribution and levels of *F. solani* var. *coeruleum* soil inoculum in these seed growing areas, studies of the contamination of the surface of VTSC tubers (Tickle, 1974) would suggest that some fields are contaminated thus presenting

a difficulty in the maintenance of stocks free from *F. solani* var. *coeruleum* (Hardie, 1970).

Pethybridge & Lafferty (1917) and Booth (1971) respectively conclude that *F. solani* var. *coeruleum* is a normal soil inhaibitant and is ubiquitous wherever plants are grown. This would perhaps suggest that the fungus is present in soils which have not grown potatoes. In this case survival would depend on the fungus being able to colonise other plant species or dead organic material. Work on *P. exigua* var. *foveata* (potato gangrene) has indicated that the fungus may be a non-pathogenic parasite on a number of crop plants and weed species (Fox, Dashwood & Wilson, 1970).

Although *F. solani* var. *coeruleum* has been reported causing crown and root rot of lucerne in Italy (Booth, 1971), guar wilt in India (Singh, 1951; Vir & Grewal, 1973) and a root rot of peas in the USA (Harter, 1938) there is no evidence relating these strains to those causing potato dry rot. In nature *F. solani* var. *coeruleum* causing potato dry rot is believed to be able only to infect potato tuber tissue. It is unable to colonise potato sprouts stems or roots (Pethybridge & Lafferty, 1917; Lansade, 1949) mangels, carrots or apples (Pethybridge and Lafferty, 1917). Despite having a high competitive saprophytic ability (Rao, 1959; Wastie, 1961) the ability of *F. solani* var. *coeruleum* to colonise dead organic material has never been shown in nature. Recently the methods used to determine the competitive saprophytic ability of an organism have been criticised (Dhingra, Tenne & Sinclair, 1976).

In view of the evidence presented it would seem that *F. solani* var. *coeruleum* is unable to maintain itself in an active condition in soil and is therefore not a soil inhabitant but a soil invader

(Waksman, 1917) or allochthonous fungus (Saitô, 1955) or exochthonous fungus (Park, 1957). If this is the case, *F. solani* var. *coeruleum* is introduced to field soil on potatoes only and after harvest remains in an inactive condition in the form of chlamydospores as do many other *Fusarium* spp. (Booth, 1971). Hence *Fusarium* colonies on soil dilution plates usually arise from chlamydospores (Warcup, 1955; Nash, Christou & Snyder, 1961). Whether *F. solani* var. *coeruleum* is a natural soil inhabitant or soil invader ultimately depends on the isolation of the fungus from soils which have not been cropped with potatoes. This is difficult since detection depends on the sensitivity of the isolation method.

Once established, a chlamydospore population in soil does not remain at a constant level but gradually declines because of germination of chlamydospores and lysis of germlings. Persistence of dormant chlamydospores of *F. solani* in soil depend on the *forma specialis* (Nash & Alexander, 1965) or variety. With *F. solani* var. *coeruleum* the population showed little decline after removal of the host. If one expects a logarithmic decline in population (Park, 1965; Griffin, 1972) then the half-life of the fungus, a more meaningful expression than total longevity (Yarwood & Sylvester, 1959), since the latter depends on the initial population and sensitivity of the isolation method, will be considerable. Thus the length of rotation employed at present will have little effect on decreasing the fungal population unless a means can be found to stimulate chlamydospore germination and lysis.

The mechanism of lysis remains to be solved. Lloyd & Lockwood (1966) suggested that fungal lysis in soil is induced by a combination of starvation conditions and antibiotics. Ko & Lockwood (1970) concluded that lysis of fungal hyphae in soil is an autolytic process

based on starvation only. On the other hand there is evidence that lysis of fungi in soil may be of heterolytic origin. Old & Robertson (1969) and Clough & Patrick (1972) noticed micro-organisms penetrating cell walls of conidia and resting structures of *Cochliobolus sativus* and *Thielaviopsis basicola* respectively. Bacteria and actinomycetes able to lyse fungal propagules or fungal cell wall preparations, have been frequently isolated from soil (Mitchell & Alexander, 1963; Skujins, Potgieter & Alexander, 1965; Potgieter & Alexander, 1966). Biological control of *F. solani* f.sp. *phaseoli* by use of soil amendments suggests that lytic micro-organisms are involved in biodegradation of fungal structures in soil (Maurer & Baker, 1964). The effect of cropping with millet in reducing the incidence of dry rot (*F. solani* var. *coeruleum*) has been noted in the USA (S. Leach, 1977, personal communication), but whether this is an effect on the survival of the fungus or some residual effect which persists in soil to inhibit propagule production by infected seed-pieces is unknown.

At present there seems little hope in the UK of being able to reduce the survival of *F. solani* var. *coeruleum* by inhibiting conversion of macroconidia and mycelia to chlamydospores or stimulating chlamydospore germination. To maintain seed stocks free from *F. solani* var. *coeruleum* they should be treated, as are Irish seed (Morrow, 1976) with thiabendazole soon after lifting. If the thesis that the fungus is a soil invader proves tenable then health could be maintained by planting in soil which has never cropped potatoes.

Surprisingly, Boyd (unpublished) could find no evidence in the USA that close cropping with potatoes increased the levels of soil contamination. However, much depends on the level of inoculum from infected seed pieces, of which there was no record. If this varies greatly between fields then inter-field comparisons of soil contamination will prove difficult.

The irregular distribution of *F. solani* var. *coeruleum* in field soil after harvest (Schippers, 1962) probably coincides with the distribution of infected seed tubers and even after 5 yr of rotation, despite cultivations, plots planted with infected seed tubers still showed higher *F. solani* var. *coeruleum* population levels than those planted with contaminated seed (Table 5). The high level of *F. solani* var. *coeruleum* contamination found in other parts of the same field originally planted with a commercial crop of Pentland Crown suggests that a high proportion of infected tubers had been planted.

From a study of the survival of *F. solani* var. *coeruleum* and *F. sulphureum* at Charlottetown in Canada, Ayers (1972) concluded that neither fungus overwintered in field soil but survived on the surface of stored potatoes. The inability to overwinter was not supported by the present results although *F. sulphureum* did not appear to survive as well as *F. solani* var. *coeruleum*. Propagule death due to freezing injury has been discussed by Mazur (1968) but this does not appear to be responsible for the difference in results. Boyd (unpublished) was able to isolate the fungus from soils in Maine which had experienced much lower winter soil temperatures than soils in Charlottetown. Possibly the isolation method used by Ayers was too insensitive to detect low populations of either fungus.

Although *F. solani* var. *coeruleum* may survive in the field by infecting damaged ground-keeper tubers its survival is believed to depend mainly on the chlamydospore. This also appears to be the case for *F. sulphureum*, but whereas there is no evidence to suggest that *F. solani* var. *coeruleum* is able to colonise alternate hosts, *F. sulphureum*, which is pathogenic to potato tubers, may infect damaged subcrown internodes of wheat (*Triticum aestivum*) under laboratory

conditions (Tinline, 1977). Whether infection of wheat occurs in the field, to aid the survival of *F. sulphureum*, remains to be investigated but the observation of *F. sulphureum* sporulating on stems in the field (p 136) indicates that *F. sulphureum* may have a wider pathogenic potential than *F. solani* var. *coeruleum*.

The population of *F. solani* var. *coeruleum* and *F. sulphureum* in soil from around infected seed tubers was reduced by air drying but *F. sulphureum* was more affected (Section 3.1). McLennan (1928) argued that differences in population which developed after desiccation of soil were due to death of hyphae which were less resistant to water loss than spores. The present work has shown that the survival of both hyphae and macroconidia were severely reduced by air drying. Warcup (1960) also suspected the accuracy of the desiccation method since he found that drying killed some fungal spores. Air drying of soil may, however, be a useful way of determining the *F. solani* var. *coeruleum* chlamydospore population since this, which developed after macroconidia were added to soil, was unaffected by air drying. If this proves tenable, then the *F. solani* var. *coeruleum* population in soil surrounding infected seed tubers at the end of the growing season is composed of a number of propagule types which differ in their response to air drying. The differences in survival of *F. solani* var. *coeruleum* and *F. sulphureum* after air drying probably reflects differences in survival potential of the respective chlamydospores rather than differences in the proportion of chlamydospores to macroconidia or hyphae, since storage of air-dried soil at 15-28°C reduced the population of *F. sulphureum* more than for *F. solani* var. *coeruleum*.

Various electron microscopy studies have been made on chlamydospore formation in *Fusarium* spp. *in vitro* and *in vivo* (Stevenson &

Becker, 1972; Griffiths, 1973; Old & Schippers, 1973; Campbell & Griffiths, 1974; Van Eck & Schippers, 1976) and it may be that the newly formed cell wall makes the chlamydospore more resistant to desiccation than the macroconidium, possibly because of the presence of lipid material in the chlamydospore cell wall (Griffiths, 1973). The contention that the lipid content of the cell wall is important in survival has been refuted by Schneider & Barran (1977).

The reasons for the apparent differences in the persistence of *F. solani* var. *coeruleum* and *F. sulphureum* have not been investigated. If autolysis of chlamydospores is triggered by starvation conditions then studies of the respiration rate and loss of nutrients to the exterior may reveal differences between the two pathogens. Electron microscopy studies have been made on *F. sulphureum* chlamydospores (Schneider & Seaman, 1974a; 1974b; 1977) and comparison with *F. solani* var. *coeruleum* chlamydospores may reveal differences in cell wall structure or the size of lipid bodies which have been suggested as causing differences in the survival of *F. solani* f.sp. *cucurbitae* and *F. solani* f.sp. *phaseoli* (Nash & Alexander, 1965; Van Eck, 1976). Lipids have been judged to play an important role in the survival of chlamydospores since lipids are catabolised in endogenous respiration and during spore germination (Cochrane, Cochrane, Colling & Serafin, 1963). Recently, however, experiments have shown that persistence of chlamydospores seems to be independent of the lipid content of the chlamydospore cell (Van Eck, 1978).

Considering the ecological significance of lysis of cell walls very little work has been done on the chemical analysis of chlamydospores of *Fusarium* spp. In certain basidiomycetes resistance to mycolytic actinomycetes was attributed to chemical components of the cell wall (Ballesta & Alexander, 1972). Chemical studies may throw

some light on the differences in persistence shown by many *Fusarium* spp.

In practice the results suggest that low temperature potato storage would favour survival of both *F. solani* var. *coeruleum* and *F. sulphureum* in air dry or moist soils, whereas survival of the fungi, particularly *F. sulphureum*, would be severely affected in air dry soil on potato handling equipment or in potato boxes for example.

Several recommendations can be made regarding the storage of soil samples for analysis:

1. Estimations of the maximum soil population may be obtained by using soil at field moisture but preparation of soil for dilution tends to be time-consuming. Also there are difficulties with sieving and storing soil without dehydration even at 4°C.

2. Very reproducible results are obtained using air-dried soil but because the procedure affects the population of *F. solani* var. *coeruleum* and *F. sulphureum* differently this has to be borne in mind when making population comparisons between the two fungi.

3. Moist or air-dried soil may be safely stored at 4°C for at least 4 months without significant change in the population.

In most of the work to be described in later sections air-dried soil stored at 4°C was used.

## 3.2 The transmission of *F. solani* var. *coeruleum* from infected and contaminated seed tubers

### INTRODUCTION

Ayers & Robinson (1956) and Ayers (1972) recognised that an increase in population levels of *F. solani* var. *coeruleum* and *F. sulphureum* occurs in soil during the growing season. Planting tubers infected with *F. solani* var. *coeruleum* gave rise to a greater level of propagule transmission than contaminated seed (Boyd & Logan, 1967; Boyd & O'Donnell, 1968). Subsequently Tickle (1974), from detailed soil population studies, showed that transmission occurred only from infected seed tubers. In larger scale field experiments, however, disinfection of apparently healthy contaminated seed tubers with an organo-mercury compound (Boyd & Logan, 1967; Tickle, 1974) or dusting with benomyl (Tickle, 1974) reduced the incidence of dry rot in the progeny, suggesting that in commercial practice contaminated seed stocks might be still important in transmission.

The present work examines the relative importance of infected and contaminated seed in relation to the contamination of field soil and progeny tubers.

### MATERIALS AND METHODS

The experiments were conducted over 3 seasons. Eight adjacent drills, 0.7m apart and 18m long were planted with 60 seed tubers. The drills contained either 50, 10, 3 or 0% *F. solani* var. *coeruleum* infected tubers arranged between apparently sound tubers naturally contaminated with the fungus. Two drills were planted at each infection level and they were arranged in a series from no dry rot tubers in two outer drills to 50% dry rot tubers in two adjacent central drills. The progeny tubers were harvested in October with a single-row

elevator digger in order of expected increasing tuber contamination, beginning with those drills which had no known infected seed tubers planted. This was done so as to minimise cross contamination during harvesting as it was impractical to sterilise the digger.

After harvest, soil samples to a 10cm depth were collected at 1m distances along each drill (*c.* 1kg soil per drill, air-dry basis), air-dried, sieved (0.85mm mesh) and the *F. solani* var. *coeruleum* population determined as described in Section 2.2. The PM70 selective medium was used in 1974-1975 and the PAB medium in 1975-1976 and 1976-1977. The potatoes from each drill were stored in new paper sacks (*c.* 10kg per sack, 3-4 sacks per drill). In February the tuber contamination of each sack was assessed by determining: (1) the fungal population in air-dried progeny tubersphere soil; (2) the progeny tuber contamination index (PTCI) and (3) the disease incidence after riddling. The relevant methods are described in Section 2.3.

RESULTS

Results for the mean levels of contamination with field soil (propagules per g soil) and progeny tubers (propagules per g soil, PTCI, percentage dry rot) at each planted infection level are shown in Table 13. The original data for each drill are presented in Appendix 4.

Levels of contamination of progeny grown from 0 and 3% infected tubers were similar but above this increasing the proportion of infected tubers planted usually increased: (1) the number of *F. solani* var. *coeruleum* propagules left in field soil after harvest; (2) the fungal population in progeny tubersphere soil; (3) the PTCI and (4) the incidence of dry rot developing in the progeny after riddling.

Table 13. Contamination of field soil (A) and progeny tubers (B, C and D) grown from seed stocks var. Catriona containing different proportions of tubers infected with *F. solani* var. *coeruleum*

| Percentage infected tubers planted | 50 | 10 | 3 | 0 |
|---|---|---|---|---|
| Season | (A) Propagules per g field soil after harvest in October | | | |
| 1974-1975 | 625 | 42 | 16 | 11 |
| 1975-1976 | 510 | 15 | 100 | 165 |
| 1976-1977 | 895 | 250 | 135 | 10 |
| | (B) Propagules per g progeny tubersphere soil in February after tuber storage | | | |
| 1974-1975 | 29846 | 13804 | 6185 | 1583 |
| 1975-1976 | 4295 | 993 | 98 | 1318 |
| 1976-1977 | 10940 | 2552 | 244 | 213 |
| | (C) Progeny tuber contamination index (%) in February | | | |
| 1974-1975 | 70.3 | 40.7 | 14.1 | 13.0 |
| 1975-1976 | 25.3 | 8.2 | 0.4 | 6.0 |
| 1976-1977 | 80.4 | 37.5 | 4.2 | 3.8 |
| | (D) Dry rot (%) after riddling in February | | | |
| 1974-1975 | 76.9 | 55.3 | 23.4 | 13.7 |
| 1975-1976 | 28.8 | 17.0 | 4.0 | 15.6 |
| 1976-1977 | 64.5 | 32.6 | 6.4 | 7.0 |

For a given seed tuber infection level most propagules were found in field soil in 1976 but contamination of progeny tubersphere soil was highest in 1974. The high level of tubersphere contamination in 1974 occurred because some tubers developed dry rot before the levels of tuber contamination were assessed.

## DISCUSSION

The importance of the infected seed tuber as a source of *F. solani* var. *coeruleum* propagules was demonstrated in these experiments, thus supporting evidence presented elsewhere (Boyd & Logan, 1967; Boyd & O'Donnell, 1968; Tickle, 1974). However, transmission also occurred from contaminated seed and this was similar to stocks

with 3 % infected tubers. Since Tickle (1974), in a detailed study of *F. solani* var. *coeruleum* populations in soil around single plants, could find no evidence of transmission from naturally contaminated seed, other factors were probably responsible for transmission in the present experiments.

The low number of *F. solani* var. *coeruleum* propagules in field soil after harvest in 1974 and 1976 may reflect the *F. solani* var. *coeruleum* population surviving from a previous crop since the fungus has been shown to survive the rotation, albeit at low levels (Section 3.1). In 1975, however, the population was higher than expected and more consistent with levels associated with infected seed tubers. Gangrene (*P. exigua* var. *foveata*) has been shown to develop from latent infections (Todd & Adam, 1967) which would be unobservable at planting, but this has not been demonstrated for *F. solani* var. *coeruleum*. Instead, it is more likely that the seed tubers were damaged shortly before or at planting and became infected by propagules which contaminated the tuber surface. The reduction in transmission of *F. solani* var. *coeruleum* by treating contaminated seed with fungicides (Boyd & Logan, 1967; Tickle, 1974) may therefore be explained as reducing the amount of inoculum available for infection.

Contamination of soil adhering to the harvester before lifting, 70 and 260 propagules per g soil in 1975 and 1976 respectively, may also have affected transmission from contaminated seed since progeny tuber contamination over the 3 seasons was consistently greater in the first drill lifted than the second (Appendix 4). Contamination of field soil did not show the same trend, possibly because the bulk of field soil diluted changes in the population caused by the harvester, whereas progeny tubers would come into direct contact with harvester soil

as they pass up the elevator. Such reasoning, however, does not explain the higher first drill levels of tuber contamination in progeny grown from various proportions of infected seed, since in these cases harvester contamination would be less than tuber contamination because tubers were lifted in increasing order of expected level of progeny tuber contamination.

Practically, the results show that planting of high proportions of infected seed (10-50%) is to be avoided since this markedly increases transmission of *F. solani* var. *coeruleum* and therefore the disease potential of the crop and the population of the fungus available for survival in field soil. Although 50% may appear to be an excessively high figure, apparently healthy seed tubers contaminated with *F. solani* var. *coeruleum* damaged before planting, at a time when most varieties are very susceptible to infection (Section 5.2), may develop extensive dry rot after planting. The procedure of seed removal from the stock late in the storage season and associated sprout removal is therefore to be avoided. In addition, the use of chitted seed without the machinery to ensure gentle handling of the seed might be expected to increase the transmission of *F. solani* var. *coeruleum* but this requires further investigation.

# SECTION 4

# FACTORS AFFECTING TRANSMISSION OF *F. SOLANI* VAR. *COERULEUM* FROM INFECTED SEED TUBERS

## SECTION 4

## FACTORS AFFECTING TRANSMISSION OF *F. SOLANI* VAR. *COERULEUM* FROM INFECTED SEED TUBERS

Tickle (1974) indicated the importance of *F. solani* var. *coeruleum* infected seed, as compared with healthy contaminated seed or field soil, as a source of inoculum, and this was confirmed in the present studies (Section 3.2). Little, however, is known about factors which affect inoculum production and levels of progeny tuber contamination. Some of these factors are considered in the following two sub-sections:-

4.1 Factors affecting transmission of *F. solani* var. *coeruleum* from infected seed tubers in the variety Catriona.

4.2 The effect of tuber variety on the transmission of *F. solani* var. *coeruleum* from infected seed tubers.

## 4.1 Factors affecting transmission of *F. solani* var. *coeruleum* from infected seed tubers in the variety Catriona

### INTRODUCTION

During the growing season the *F. solani* var. *coeruleum* infected seed tuber is a potential food base for soil micro-organisms. No work has been conducted on the interaction between the pathogen and secondary invaders, but premature decomposition of the seed tuber by *Erwinia* spp., which is more common in cold wet seasons, might be expected to affect propagule production.

In this investigation the level of transmission was assessed in relation to, seed tuber treatments devised to affect the availability of nutrients (Experiment 1) and the distribution of tubers in the ridge (Experiment 2).

### MATERIALS AND METHODS

#### Experiment 1

Seed tubers, var. Catriona, were surface sterilised in 0.8% formalin and inoculated several weeks later at the heel end with *F. solani* var. *coeruleum* macroconidia. Tubers were incubated at 4°C for 4wk, 15°C for 2wk, then 4°C for 1-4wk until planting. Infected tubers with lesion diameters of about 2cm were planted in two trials on 24 April 1974 and 4 May 1976, at a 0.4m spacing, in drills 0.7m apart. Two adjacent drills were planted per plot and each drill contained 10 tubers. The experimental layout was a design of four randomised blocks. Treatments are shown in Table 14.

Table 14. Treatments of infected seed tubers

| | Treatments | Season | Mean wt of each tuber in 1976 (g) |
|---|---|---|---|
| 1. | Tubers left *in situ* throughout growing season | 1974, 1976 | 73 |
| 2. | Tubers removed 25.6.74 and 28.6.76 after plant establishment | 1974, 1976 | 83 |
| 3. | Tubers inoculated with *Erwinia carotovora* var. *carotovora*, 28.6.76 | - 1976 | 75 |
| 4. | Small tubers planted | - 1976 | 38 |
| 5. | Eyes of tubers excised before planting | - 1976 | 81 |

With treatment 5, eyes were excised 2wk before planting and the wounds allowed to heal at 15°C.

In 1974, an attempt was made to reproduce in the field conditions thought to be necessary for the premature decomposition of seed tubers by soft-rot bacteria. Plants were watered on 11 June at the rate of 4.5l per plant. It soon became apparent that this method was impractical on a field plot scale because of difficulties encountered handling large amounts of water. Instead, to simulate early decay, the seed tubers were carefully removed from the ridge after plant establishment. In 1976, the seed tubers were inoculated with *E. carotovora* var. *carotovora* through glass tubes (30cm long x 0.5cm diam.) which had been inserted into the seed tubers at planting.

Tubers from the two trials were lifted by a single-row elevator digger on 10 October 1974 and 20 October 1976, respectively. It was not practical to sterilise the digger between plots. The tubers were stored in a farm store until required.

Treatment effects were assessed by determining the population of *F. solani* var. *coeruleum* in soil samples and the progeny tuber contamination index.

Soil samples Before harvest, progeny tubers from four plants were collected. In addition, soil (*c.* 50g) was collected from around the remains of each seed tuber and bulked. Where seed tubers had been removed, soil was collected, in 1974 from the region of the stem base and in 1976, from around polystyrene markers inserted in place of the seed tubers. In 1976, soil samples were also collected at horizontal distances from seed tubers of treatments 1, 2 and 5 using the following procedure. At planting, a Perspex template with 1.2cm diam. holes drilled at 0.5-4.5, 5.5-9.5 and 10.5-14.5cm from the edge of the plate (Fig. 19) was placed touching a *F. solani* var. *coeruleum* infected seed tuber. The template was inserted into a slit made in the soil parallel to the line of the drill, so that the horizontal line of holes was adjacent to the seed tuber. Four seed tubers from each plot were prepared in this way. The seed tubers and templates were covered with soil and then a ridging machine formed the drills. Soil samples were collected on 28 June and 7 September. Soil was removed from one side of the template (Fig. 20) and a 1cm diam. soil corer used to obtain samples, through the template, to a depth of 7cm. The corer was sterilised between each sampling distance with methanol. At the June sampling, waterproof tape was stuck over the sampling holes to avoid introduction of exterior contamination, before re-covering the template with soil.

After harvest, soil samples were collected on 18 January 1977 from the base of sacks containing potatoes from the 1976 experiment.

Fig. 19. Arrangement of sampling holes on Perspex template.

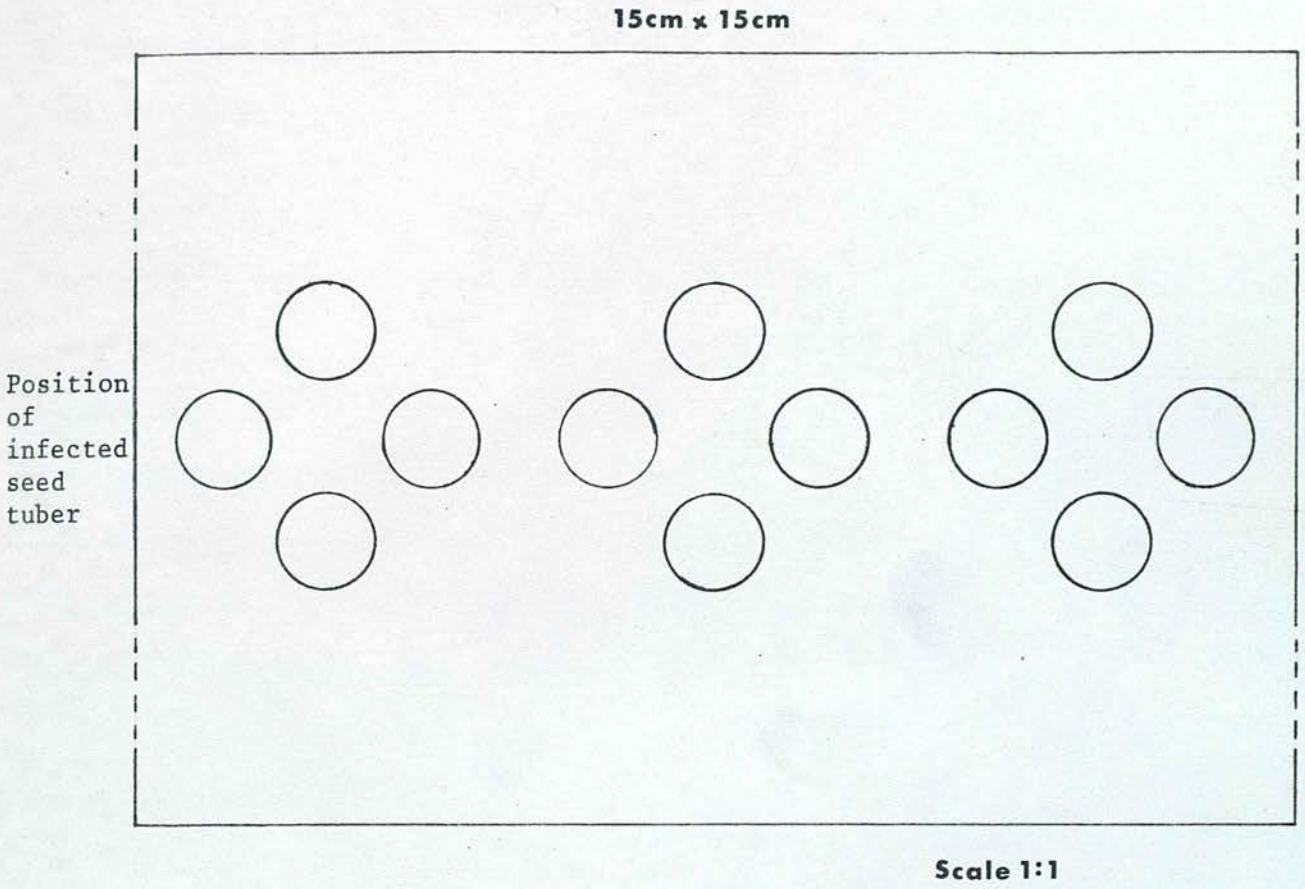



Fig. 20. Sampling at set distances from infected seed tuber using a Perspex template and soil corer.

All soil samples and those progeny tubers collected before harvest were air-dried at room temperature for 3 days. Tubersphere soil was removed from the progeny tubers with sterilised wire brushes. Soil samples were sieved (0.85mm mesh) and the *F. solani* var. *coeruleum* population determined.

Population assessments Two gram samples of air-dried soil (or 1g in the case of soil from the Perspex templates) were used to make $10^{-2}$ soil dilutions, using a 1:1 ratio of sterile distilled water and 0.15% water agar. In 1976 a $10^{-3}$ dilution was used with soils obtained after harvest. Suspensions of 2g and 1g soil were comminuted at 12000 rpm in respectively, a 200ml reservoir of a MSE Atomix blender and a 100ml reservoir of a MSE vortex blender. Aliquots of suspension (1ml) were pipetted onto the surface of each of 10 plates of PM70 medium (used 1974) or five plates of PAB medium (used 1976-1977). Colony counts were made after 14 (PM70) or 20 (PAB) days' incubation.

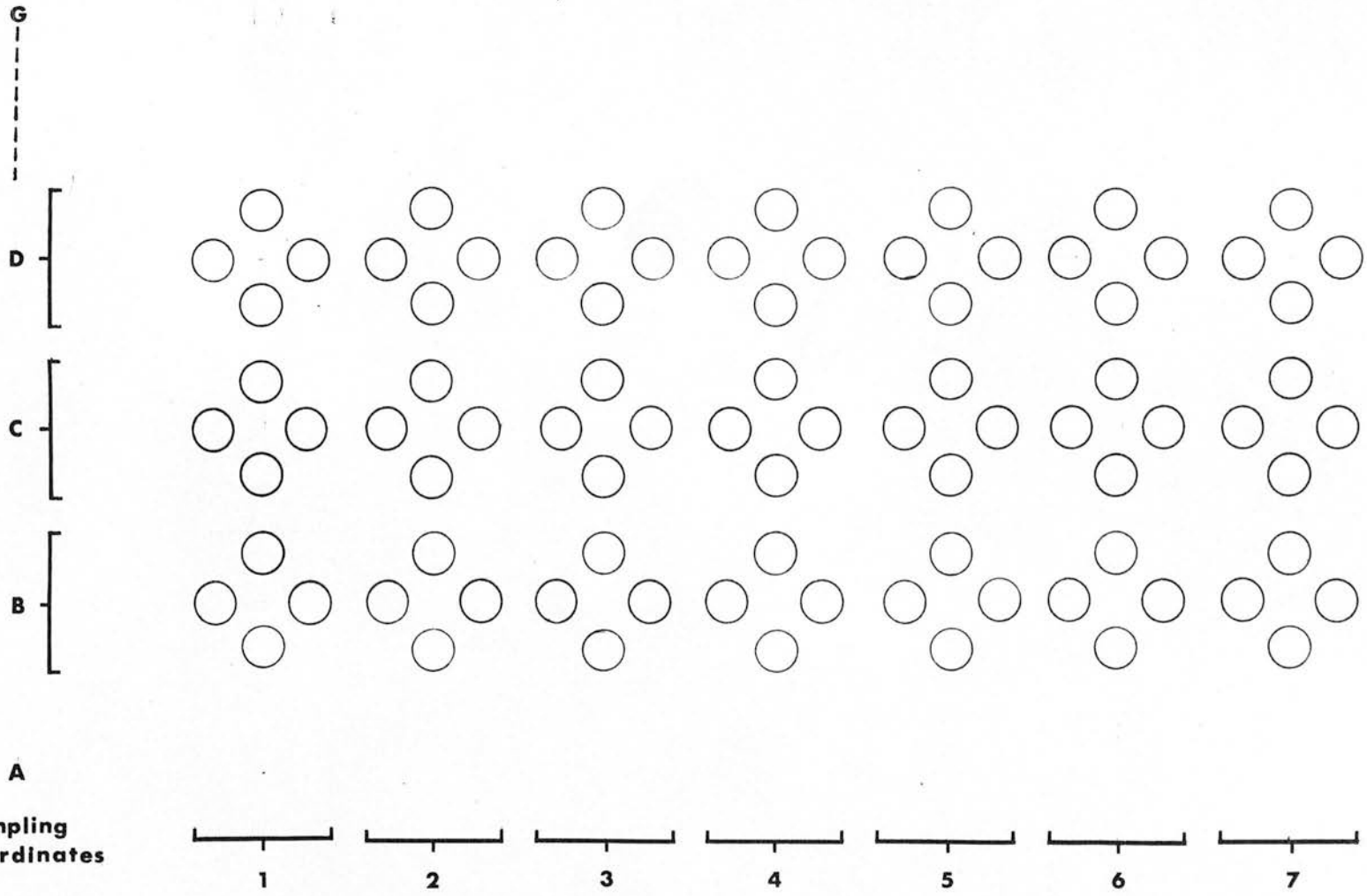
Progeny tuber contamination index (PTCI) On 12 December 1974 and 18 January 1977 the tuber stab method (p 39 ) was used to determine the PTCI.

Experiment 2

The distribution of *F. solani* var. *coeruleum* around infected Catriona seed tubers was determined by examining soil profiles at intervals during the 1975 growing season. At the end of the season the positions of the progeny in relation to the seed were mapped and the number of propagules in tubersphere soil determined. Tubers were planted on 11 June 1975.

Soil profile In order to gain access to the soil profile and yet prevent it collapsing, Perspex templates (45cm x 40cm) with holes (1.2cm diam.) arranged as in Fig. 21 were used. Four holes constituted

Fig. 21. Arrangement of sampling holes on Perspex template.




Scale 1:2

one sampling point and this was located by reference to letters and numbers labelled along the templates vertical and horizontal axis, respectively. Before use, the templates were sterilised in methanol. In the field, an unplanted length of ridge was levelled and a trench was formed to accept the template. A vertical soil face was made at right angles to the axis of the ridge and the template pressed against it so that the third horizontal line of holes from the base (C co-ordinate) were just above soil level. Soil was replaced in the trench. An infected tuber was placed so that its position corresponded to the '4C' sampling position (Fig. 22) and the ridge was built up around the tuber to a depth of *c.* 0.2m. Four soil profiles were prepared in this way.

Three profiles (2, 3 and 4) were sampled 15, 35, 64, 100 and 136 days after planting. Plant 1 was not sampled until 136 days, since it was thought necessary to check whether the contamination profiles from plants 2, 3 and 4 would be affected by disturbance of soil at the previous samplings. To gain access to the sampling holes the trench was reformed. Two steel rods driven into the ground were used to hold each template firmly against the soil profile (Fig. 23). Soil samples were collected to a depth of 7cm using soil corers (1.0cm diam.). In order to minimise cross contamination by soil dropping down between the soil face and the template, only the innermost 5cm of soil was kept for assessment of the fungal population. On completion of sampling, the access holes were sealed with water-proof tape and the trench refilled.

Tuber mapping The positions of the seed and progeny tubers from plants 1, 2 and 3 were mapped using a grid similar to that described by Lacey (1966) and Bannon (1972). The grid (75cm x 60cm), divided by tight wires into 2.5cm squares, was numbered along one axis and lettered

Fig. 22. Infected seed tuber in position next to co-ordinate 4C prior to ridging

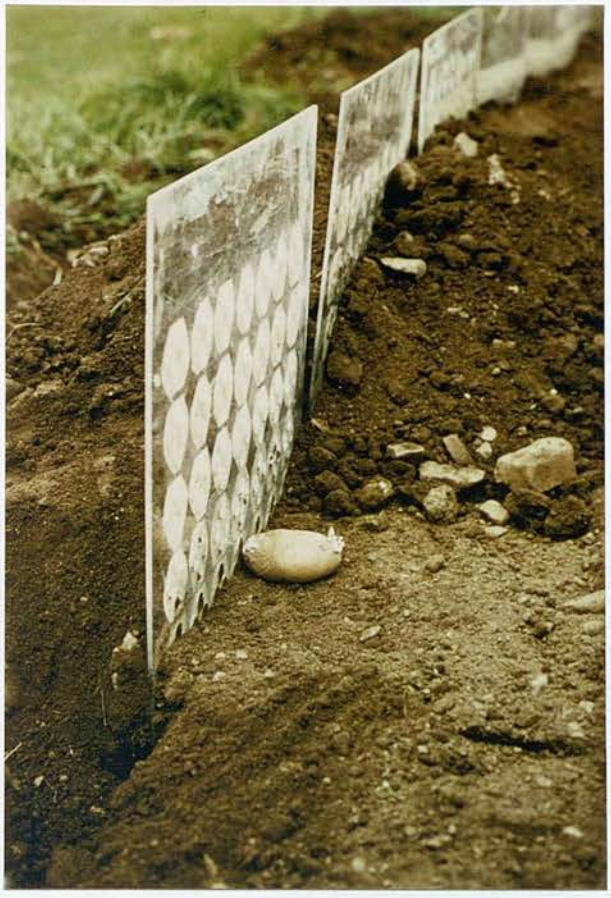
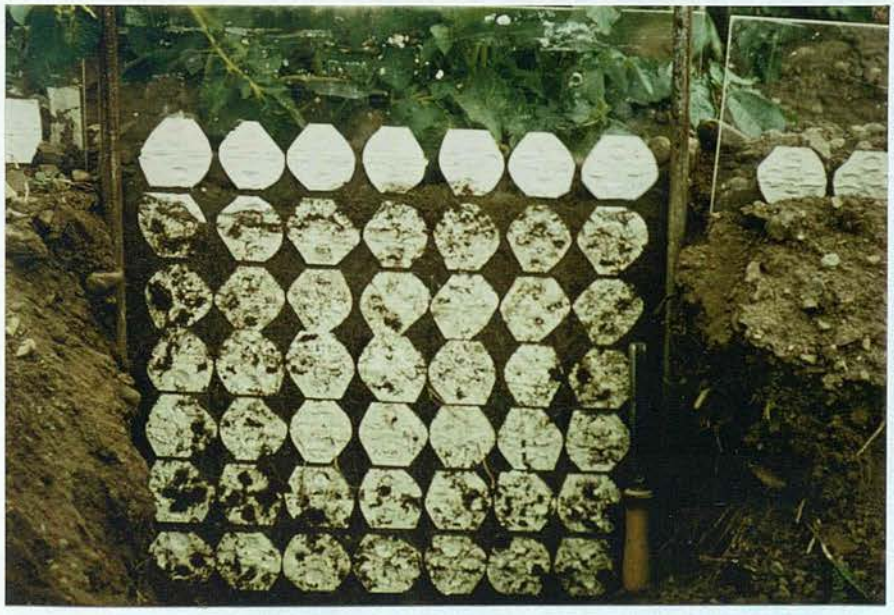


Fig. 23. Soil removed from non-planted side of Perspex template in preparation for sampling



along the other. Retort stands were used to hold the grid which could be swung upwards to make it easier to uncover the tubers. A graduated pointer inserted through the grid was used to measure distances down to the ridge surface and tubers.

In the field, the stems of the plants to be mapped were cut at ground level and the grid supports put in the adjacent furrows. These were made firm by linking them together with lengths of steel rod attached to the supports by clamps. The grid was placed approximately level with the crest of the ridge and made horizontal with the aid of a spirit level. The shape of the ridge was recorded by measuring the distance between the grid and soil surface at 10cm intervals, and the soil was then removed to uncover the uppermost tubers. After several tubers had been exposed they were mapped onto squared paper, marked similarly to the grid at a scale of 1:1. The distances below the grid and the highest and lowest points of each tuber were recorded before further progeny tubers and the seed tuber were uncovered and mapped. Each tuber was placed in a labelled polythene bag and soil samples were sometimes collected from points in the cavity left by the removal of the tuber. Progeny tubersphere soil was removed with sterilised wire brushes after air drying.

Assessment of the population of *F. solani* var. *coeruleum*. Soil samples were air-dried, sieved (0.85mm mesh) and the population of *F. solani* var. *coeruleum* determined as described on p. 82 for 1g samples, using the PM70 medium.

RESULTS

Experiment 1

Results of the experiments in 1974 and 1976 are summarised in Table 15. Inoculating seed tubers with *E. carotovora* var. *carotovora*

Table 15. Number of *F. solani* var. *coeruleum* propagules per g of seed tuber soil and progeny tubersphere soil and the progeny tuber contamination index (PTCI) in relation to the treatment of infected seed tubers

| Treatment of infected seed tubers | 1974 | | | 1976 | | | |
|---|---|---|---|---|---|---|---|
| | Before harvest | | After harvest | Before harvest | | After harvest | |
| | $Log_{10}$(propagules +1) | | PTCI | $Log_{10}$(propagules +1) | | | PTCI |
| | Seed | Progeny | (arcsin) | Seed | Progeny | Progeny | (arcsin) |
| Seed left *in situ* throughout growing season | 3.80 | 3.48 | 55.1 | 4.36 | 4.05 | 4.24 | 78.3 |
| Seed removed on 25.6.74 and 28.6.76. | 2.77 | 3.17 | 36.1 | 3.90** | 3.22 | 3.55*** | 54.6*** |
| Seed inoculated with *E. carotovora* var. *carotovora* on 28.6.76. | - | - | - | 4.41 | 3.51 | 4.15 | 72.7 |
| Small tubers planted | - | - | - | 4.19 | 3.67 | 3.91* | 64.9** |
| Eyes of tubers excised before planting | - | - | - | 4.24 | - | - | - |
| S.E.D. | 0.405 | 0.122 | 7.36 | 0.122 | 0.252 | 0.132 | 3.71 |

*, **, *** Significantly different from control (ie seed left *in situ*) at P = 0.05, 0.01 and 0.001 respectively.

or excising eyes before planting did not significantly affect the number of *F. solani* var. *coeruleum* propagules in soil around infected seed tubers and in the case of the *E. carotovora* var. *carotovora* treatment, progeny tuber contamination was not reduced. Two treatments affected transmission of *F. solani* var. *coeruleum*. Over 2 seasons, removal of the infected seed tuber after plant establishment consistently reduced soil contamination with *F. solani* var. *coeruleum*, but only in 1976 were significant reductions obtained. Also small seed tubers tended to produce less fungal inoculum than larger seed tubers and a significant reduction in the level of progeny tuber contamination was obtained after harvest. Results of an experiment conducted in 1975 (Table 16) also indicate that small seed tubers produce less fungal inoculum than larger seed.

Table 16. Population of *F. solani* var. *coeruleum* in soil surrounding large and small infected seed tubers

| Type of seed | $Log_{10}$ (propagules/g soil +1) |
|---|---|
| Large | 4.17 |
| Small | 2.28 |
| S.E.D. | 1.775 |

Results presented in Table 17 show the population of *F. solani* var. *coeruleum* at horizontal distances from *F. solani* var. *coeruleum* infected seed tubers at two dates. Propagules were present 10.5-14.5cm from infected seed tubers 53 days after planting but were at low levels compared with the population at 0.5-4.5cm. At each sampling distance the number of propagules were not significantly different ($P>0.05$) between treatments or sampling times. However, where the seed tubers

were removed the fungal population at the three sampling distances did not increase, or increased only slightly, between June and September, whereas substantial increases in population occurred when the seed tuber was not removed. The number of propagules in soil 5.5-14.5cm from seed tubers with eyes excised was greater than from intact tubers where progeny had been produced.

Table 17. Number of *F. solani* var. *coeruleum* propagules at horizontal distances from *F. solani* var. *coeruleum* infected seed tubers in June and September, 1976

| Seed tuber treatment | Sampled | $Log_{10}$ (propagules/g soil +1) distance (cm) | | |
|---|---|---|---|---|
| | | 0.5-4.5 | 5.5-9.5 | 10.5-14.5 |
| Left *in situ* throughout growing season | 28.6.76 | 3.40 | 1.27 | 0.59 |
| | 7.9.76 | 3.80 | 1.96 | 1.40 |
| Removed 28 June | 28.6.76 | 3.32 | 1.75 | 1.61 |
| | 7.9.76 | 3.37 | 1.88 | 1.44 |
| Eyes excised before planting | 28.6.76 | 3.38 | 1.96 | 0.83 |
| | 7.9.76 | 3.54 | 2.33 | 1.87 |
| S.E.D. for comparing means at each sampling date | 28.6.76 | 0.240 | 0.437 | 0.467 |
| | 7.9.76 | 0.223 | 0.160 | 0.560 |

Experiment 2

Soil profile Populations of *F. solani* var. *coeruleum* in soil around infected seed tubers, 15, 35, 64 and 100 days after planting are shown in Fig. 24. Few propagules were detected within 5cm of the seed and none were detected further away, 15 days after planting. By 35 days the fungal population had increased markedly and propagules were detected 11-15cm horizontally from the seed tuber. Propagule numbers continued to increase throughout the season, particularly horizontally adjacent to and below the seed. By 136 days (Fig. 25.1), few propagules were detected above the seed but many were present directly below it. The profile examined only at the final sampling (Fig. 25.2) showed the same pattern of propagule distribution as profiles sampled at intervals through the growing season.

Tuber mapping Maps of vertical and horizontal tuber distribution and the number of *F. solani* var. *coeruleum* propagules in progeny tubersphere soil are shown in Fig. 26. The variation in tubersphere propagule numbers was similar to that expected from the distribution of the fungus over the soil profile, ie progeny tubers formed nearest the seed were the most highly contaminated and the tuber surface nearest the seed was more contaminated than the surface furthest away (Fig. 26, plant 2).

DISCUSSION

Since the levels of soil contamination with *F. solani* var. *coeruleum* are very low in field soils under a normal rotation the fungus may be regarded as mainly tuber-borne. The number of propagules in soil adhering to the sound seed tuber are usually not at high enough levels to assure transmission. Only when the fungal population increases to levels normally associated with infected seed does transmission occur.

Fig. 24. Number of *F. solani var. coeruleum* propagules per g soil from soil-profiles around *F. solani var. coeruleum* infected seed tubers at various days after planting. (Mean of 3 replicates).

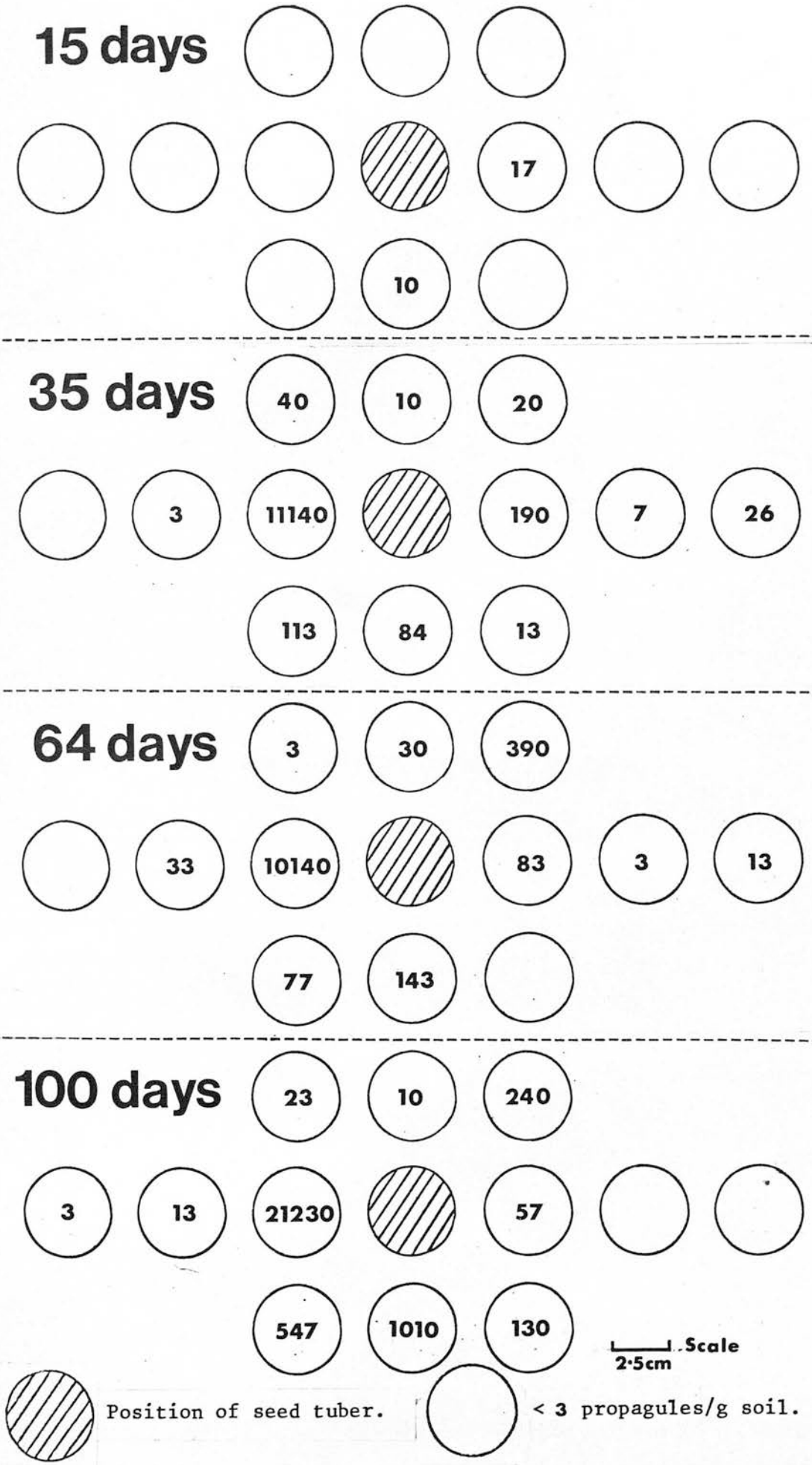


Fig.25 Number of *F. solani* var. *coeruleum* propagules per g soil from soil-profiles around *F. solani* var. *coeruleum* infected seed tubers, 136 days after planting.

Fig.25.1 Sampled previously (see Fig.24). Mean of three replicates.

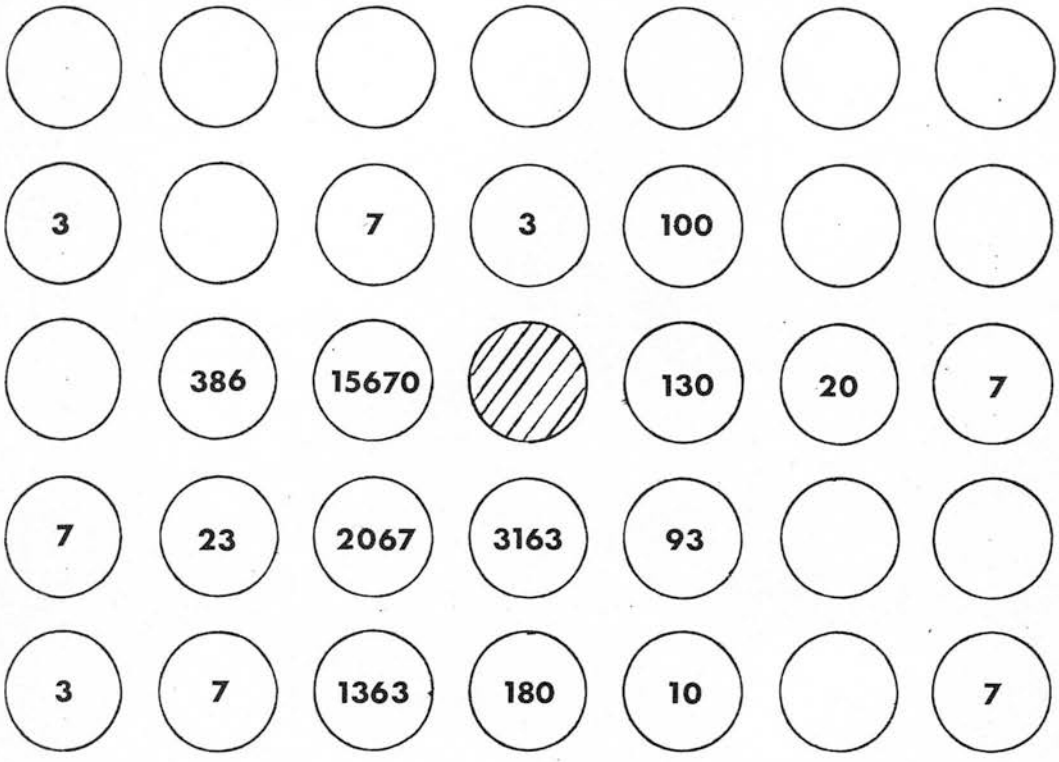
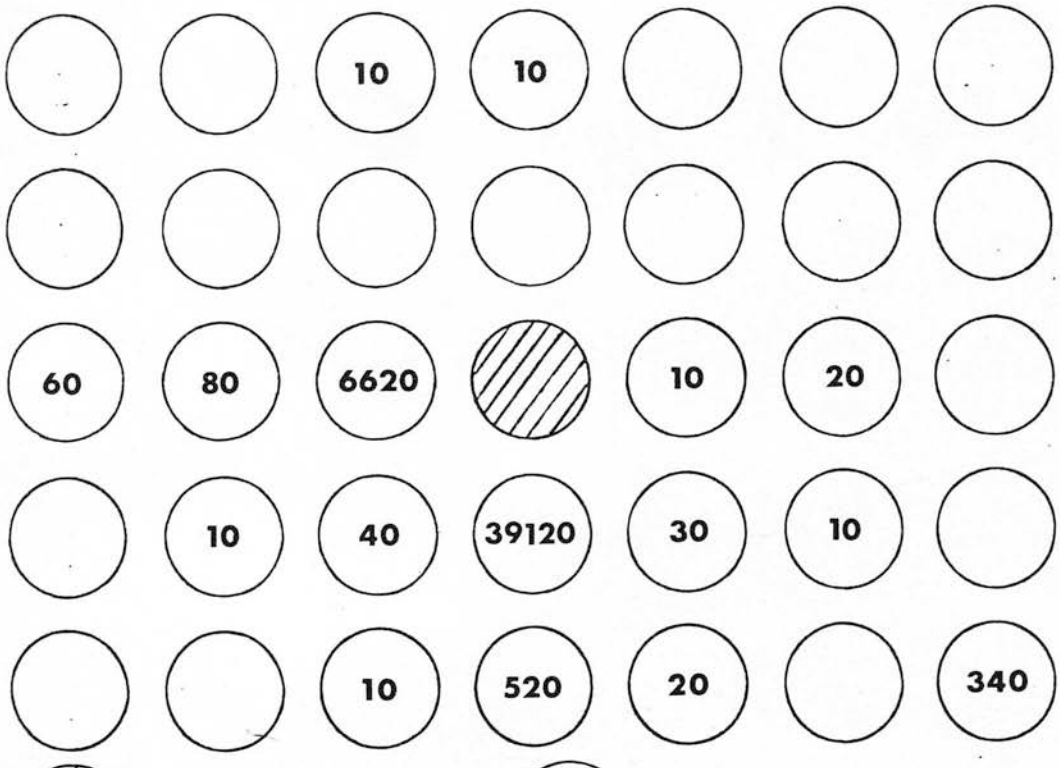


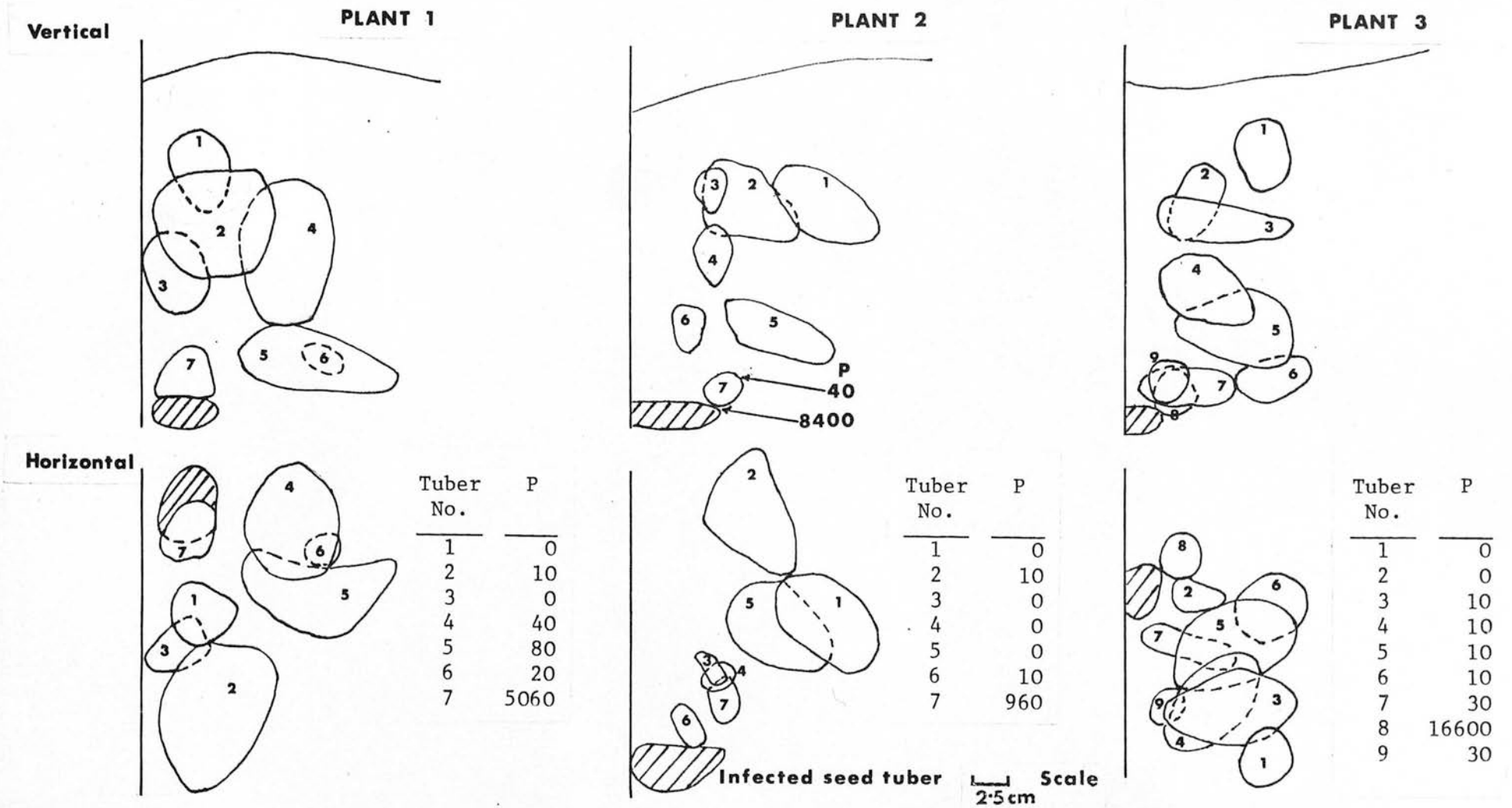

Fig.25.2 Not sampled previously.




Position of seed tuber. < 3 propagules/g soil.

Scale 2·5cm

Fig. 26 . Maps of vertical and horizontal tuber distribution, var. Catriona, in relation to the number of *F. solani* var. *coeruleum* propagules (P) per g progeny tubersphere soil.




| Tuber No. | P |
|---|---|
| 1 | 0 |
| 2 | 10 |
| 3 | 0 |
| 4 | 40 |
| 5 | 80 |
| 6 | 20 |
| 7 | 5060 |

| Tuber No. | P |
|---|---|
| 1 | 0 |
| 2 | 10 |
| 3 | 0 |
| 4 | 0 |
| 5 | 0 |
| 6 | 10 |
| 7 | 960 |

| Tuber No. | P |
|---|---|
| 1 | 0 |
| 2 | 0 |
| 3 | 10 |
| 4 | 10 |
| 5 | 10 |
| 6 | 10 |
| 7 | 30 |
| 8 | 16600 |
| 9 | 30 |

From the present work, it is clear that soil contamination from infected seed increases throughout the growing season, the level depending on the amount of substrate available to the fungus. Therefore small infected seed tubers tended to produce fewer propagules than large infected seed tubers. In addition, removal of the substrate after plant emergence prevented further increase in soil contamination.

Competition for nutrients with other micro-organisms might also be expected to affect production of *F. solani* var. *coeruleum* propagules. Sometimes, particularly in cold wet years and on heavy soils it has been reported that planting seed affected by dry rot tended to increase the incidence of black-leg (Boyd, 1970; Pett & Kleinhempel, 1976). Occasionally complete decomposition of the seed tubers by soft-rot bacteria occurred by the time of plant establishment (Perombelon, 1974). However, in the experiment reported here, inoculating *E. carotovora* var. *carotovora* into *F. solani* var. *coeruleum* infected seed tubers did not reduce propagule production. Inspection of a sample of seed tubers late in the growing season showed that few tubers were decomposing because of soft rotting. Clearly the method of initiating bacterial infection requires further study.

Marked differences in the number of *F. solani* var. *coeruleum* propagules produced by infected seed occurred between years, ie the number of propagules detected were higher in 1976 than 1974. This may be due to the higher temperatures recorded in 1976 (Section 5.1 , Fig. 35) favouring production of *F. solani* var. *coeruleum* propagules. Warm, dry conditions also appear to favour propagation of *F. sulphureum* (Ayers & Ramsay, 1961; Section 5.1).

Dispersal of the fungus from the infected seed tuber occurred fairly rapidly after planting and continued throughout the growing season so that the population in soil steadily increased. The spread of the fungus was mainly lateral and downwards forming a decreasing gradient of inoculum with increasing distance from the seed tuber. Because of the restricted vertical spread of the fungus and because most progeny tubers were formed above the seed few progeny tubers from each plant were highly contaminated. These tubers were formed within a zone of highly contaminated soil which surrounded the seed tuber. Usually, only part of a progeny tuber was in this zone and considerable variation was found in the number of propagules in progeny tubersphere soil from different parts of the tuber.

Some progeny tubers grew in contact with the seed tuber and these were usually the most highly contaminated. However, the method by which *F. solani* var. *coeruleum* is able to spread through soil is uncertain. Whereas some fungi (eg *P. pustulans*, *P exigua* var. *foveata*) may achieve lateral or vertical dispersal by colonising and sporulating on stems, stolons or roots (Todd & Adam, 1967; Bannon, 1972), *F. solani* var. *coeruleum* is unable to colonise these tissues (Lansade, 1949). Dispersal of the fungus from tubers with eyes excised would tend to support the view that other mechanisms are involved with dispersal. From an experiment (not described) using the method of Vujicic & Park (1964) *F. solani* var. *coeruleum* was able to spread by mycelial growth only 0.2cm across non-sterilised soil from a 0.4cm diam. disc of Czapek Dox agar. Extensive mycelial growth from the infected seed tuber is therefore unlikely. Although the extent of growth may depend on the size of the food base (Vujicic & Park, 1964), growth is probably restricted by competition

from other soil organisms and the distance the fungus is able to translocate nutrients through the mycelium from the food base (Schutte, 1956; Lucas, 1977). In view of the high competitive saprophytic ability of *F. solani* var. *coeruleum* (Rao, 1959; Wastie, 1961), growth by utilisation of organic substances in soil might have been expected. There was no evidence of this. Indeed, removal of the infected seed tuber prevented an increase in propagule numbers. Methods used to determine competitive saprophytic ability have been criticised by Dhingra, Tenne & Sinclair (1976). Moreover, if extensive mycelial growth from the food base or saprophytic growth had occurred then a more uniform spread of *F. solani* var. *coeruleum* over the soil profile might have been expected. Instead the patterns of propagule distribution, with few propagules above the seed tuber but with many below and with the greatest lateral spread near the source of the inoculum suggests the passive movement of propagules in soil water (Park, 1959). Initially the propagules would be from pustules on the surface of the tuber (Fig. 27) and then as the tuber disintegrates, from pustules previously contained within the tuber.

Fig. 27. Pustules of *F. solani* var. *coeruleum* surrounding inoculation hole of seed tuber removed from the drill 34 days after planting

Similar patterns of propagule distribution would have occurred if, during sampling, propagules were dislodged from the seed tuber and fell to contaminate the soil profile. However, soil most likely to be affected in this way was excluded from the sample.

Whereas experiments on uniformly compacted soil, where spore size to soil-pore size is important (Dickinson & Parkinson, 1970), have shown that movement of fungal propagules tends to occur only when the soil is saturated (Hepple, 1960), in the potato ridge spore movement may occur in soil under unsaturated conditions in channels caused by stem movement, earthworms and dead roots (Griffin, 1963). Lateral and possibly vertical dispersal may be aided by the compaction of soil under the seed tuber caused by the ridging machine. This would also tend to hinder downward movement of spores and root penetration. From the present work, growth of the plant appeared to hinder lateral dispersal. Possibly progeny tubers are a physical barrier to dispersal. Alternatively, root growth may aid penetration of the compacted soil under the seed tuber, improving downwards movement of fungal propagules but reducing lateral dispersal since fewer propagules would be available for movement in this direction. Occasionally, it has been noticed that during heavy rain the furrows between the drills fill with water, increasing the possibility of vertical and lateral spore movement as water drains into the drill.

The results obtained in 1975 may underestimate the degree of vertical spread of *F. solani* var. *coeruleum* which occurs in some years since Tickle (1974) found evidence of at least 10cm vertical movement in 1973. Data obtained in 1976 (Section 4.2, Fig. 33) showing relatively high levels of progeny tubersphere soil contamination on most tubers tends to support this observation. The differences

in vertical transmission between 1975 and 1976 may be partly accounted for by the greater production of inoculum by the seed tuber in 1976, but this does not appear to be the case in 1973. It was also thought unlikely that continuous sampling through the growing season would have markedly affected dispersal of propagules since the patterns of distribution were not so different from those formed when the plant was sampled at the end of the growing season only. Possibly earthworms, shown to be involved in the dispersal of soil fungi (Hutchinson & Kamel, 1956; Khambata & Bhat, 1957; Edwards & Lofty, 1972) including *F. solani* var. *coeruleum* (Tickle, 1974) were responsible for these differences. Very few earthworms or their casts were found in 1975 whereas Tickle (1974) noted considerable earthworm activity and in the 1976 many casts were found adhering to progeny tubers growing more than 10cm from the seed.

## 4.2 The effect of tuber variety on the transmission of *F. solani* var. *coeruleum* from infected seed tubers

### INTRODUCTION

The decline in the importance of dry rot (*F. solani* var. *coeruleum* in Great Britain since 1950 coincided with the reduction in acreage of the highly susceptible variety Doon Star, the introduction of some rotational requirements for Scottish seed, and changing agronomic practice.

Since infected seed tubers are primarily responsible for the contamination of progeny tubers and field soil, planting resistant varieties will reduce transmission of *F. solani* var. *coeruleum* insofar as the proportion of infected to healthy tubers will be probably less than for a susceptible variety. During the growing season, the degree of contamination of progeny tubers grown from infected seed, depends partly on the amount of inoculum produced by the seed (Section 2.3). It depends also on the position of the progeny tubers in relation to the seed (Section 4.1), which is mainly a varietal characteristic.

The work to be described considers these two aspects of transmission in Experiments 1 and 2 respectively, for two varieties Catriona and Pentland Crown. The dispersal of propagules in harvesting is investigated in Experiment 3.

### MATERIALS AND METHODS

#### Experiment 1

Two field trials were planted, on 10 May 1975 and 4 May 1976, with *F. solani* var. *coeruleum* infected seed tubers of the varieties Catriona and Pentland Crown. Tubers of similar size were selected and prepared as described on p 78 . A 0.4m spacing was used between tubers and

drills were 0.7m apart. Two adjacent drills were planted per plot and each drill contained 10 tubers. The experimental layout was a design of four (1975) and three (1976) randomised blocks. Progeny tubers from the 1976 trial only were harvested (15 September) for further study. Harvesting was done by single-row elevator digger which it was not possible to sterilise before digging each plot. Progeny tubers were stored in new paper sacks in a farm store.

The effect of variety on transmission of *F. solani* var. *coeruleum* was assessed by determining the fungal population in soil samples collected before and after harvest. Before harvest soil samples were collected from around seed tubers and progeny tubers as described on p 80 and p 82. After harvest and tuber storage soil was collected on 21 January 1977, from the base of sacks containing progeny tubers from the 1976 trial. The fungal population was determined in air-dried, sieved soil (0.85mm mesh) as described on p 82 for 2g soil samples. The PAB medium was used.

Experiment 2

*F. solani* var. *coeruleum* infected Catriona and Pentland Crown seed tubers were planted on 10 May 1975, in separate drills, 0.7m apart. Tuber spacing was 0.4m.

Because adverse weather conditions made it difficult to map the position of progeny tubers in the field, whole plants contained in the ridge were taken back to the laboratory on 24 October using a method similar to that described by Bannon (1972). For each plant, a steel box (35cm x 35cm x 21cm deep) without a top and with a sliding base plate was used. The box was sterilised with methanol and sealed in a plastic bag for transport to the field. In the field, soil was

removed from at least 1m length of the ridge next to the selected plant down to several centimetres below the level of the adjacent furrows. The steel box, without the sliding base plate, was placed in the gap in the ridge with the open end facing the selected plant and pushed into position (Fig. 28). The base plate was re-positioned and forced horizontally through the soil (Fig. 29). Finally a spade was used to make a vertical cut in the ridge and the box was lifted clear (Fig. 30). To inhibit movement of soil in the box, paper was packed between the sides of the box and ridge surface. In the laboratory, the positions of the tubers were mapped as described previously (p 84 ). The progeny tubers were collected, air-dried and tubersphere soil removed with sterilised wire brushes. Soil was also collected from various positions within the rhizosphere. The population of *F. solani* var. *coeruleum* was determined in air-dried, sieved soil (0.85mm mesh) as described on p 82 for 1g soil samples. The PAB medium was used.

Experiment 3

Four adjacent drills, 0.7m apart and 0.2m deep, were planted with *F. solani* var. *coeruleum* infected Catriona and Pentland Crown tubers (lesion diam. *c.* 1.1cm) on 4 May 1976. Two drills were planted per variety with eight tubers per drill at a 0.4m spacing. On 16 October 1976, one drill of tubers of each variety were lifted by hand. Each tuber was placed in a separate polythene bag. The numbers of progeny tubers making contact with the seed were recorded. Several days later (20 October), the remaining tubers were harvested by single-row elevator digger which deposited the tubers onto the ground in which they had grown. Each tuber was placed in a separate polythene bag. The tubers were air-dried for 3 days at room temperature and the tubersphere soil removed with sterilised wire brushes. The population of *F. solani* var. *coeruleum*

Fig. 28. Soil box in position around selected plant.

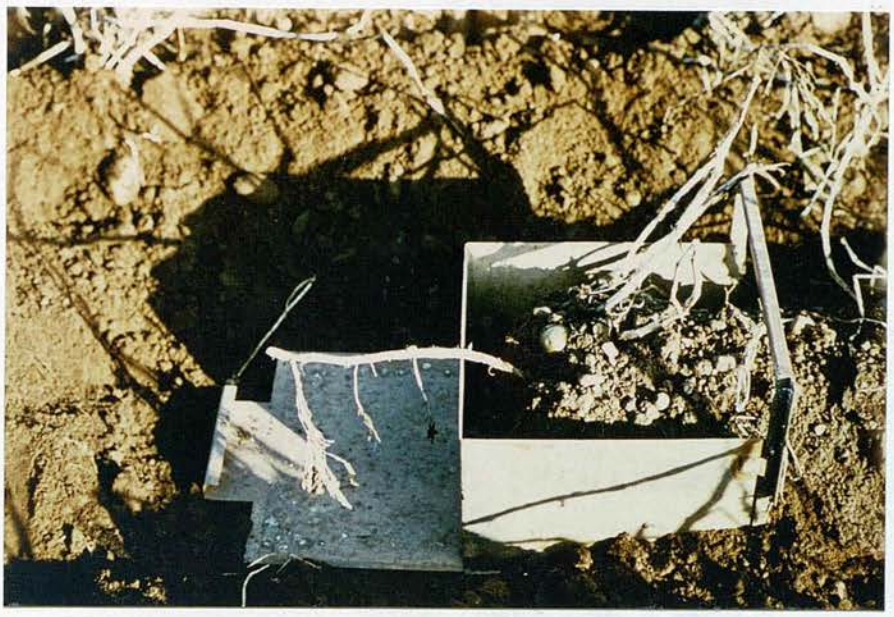


Fig. 29. Base plate forced horizontally into box.

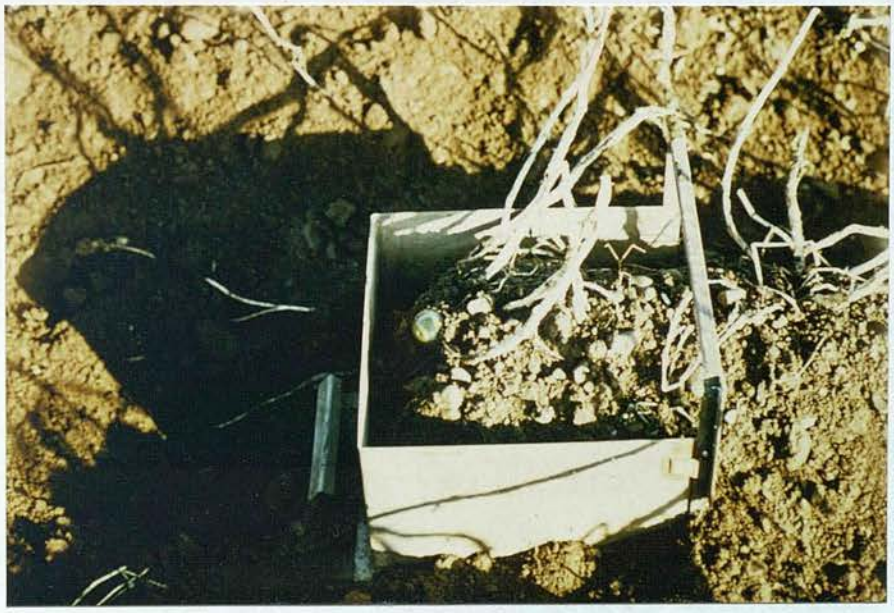
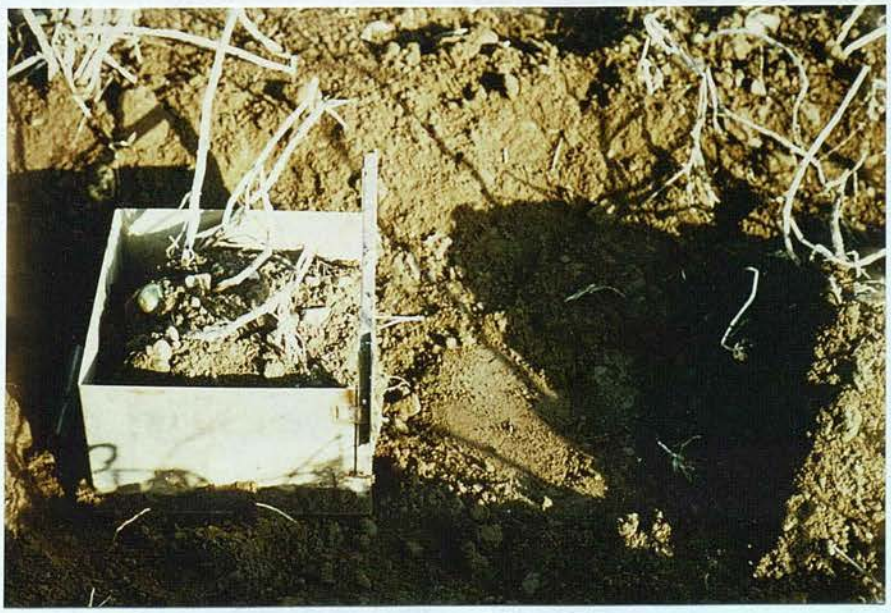


Fig. 30. Single plant removed from ridge.



was determined in air-dried, sieved soil (0.85mm mesh) as described on p 82 for 1g soil samples and a $10^{-2}$ soil dilution. Five plates of PAB medium were used for each soil.

## RESULTS

### Experiment 1

Before planting lesion diameters were recorded. For 1975 these were 3cm (Catriona) and 2cm (Pentland Crown) and for 1976 2cm (Catriona) and 1cm (Pentland Crown). Results presented in Table 18 show that larger *F. solani* var. *coeruleum* populations were present in soil surrounding infected Catriona than Pentland Crown seed tubers in both years. Only in 1976, however, were the increases statistically significant. Before harvest, contamination of progeny tubersphere soil was not significantly different between varieties in either year. After harvest, significantly more *F. solani* var. *coeruleum* propagules were present in Catriona tubersphere soil.

Table 18. Number of *F. solani* var. *coeruleum* propagules per g seed tuber and progeny tubersphere soil of two varieties[a]

| Variety | 1975 | | 1976 | | |
|---|---|---|---|---|---|
| | Before harvest | | Before harvest | | After harvest |
| | Seed | Progeny | Seed | Progeny | Progeny |
| Catriona | 3.73 | 1.90 | 4.51 | 3.95 | 4.24 |
| Pentland Crown | 3.30 | 1.06 | 4.14* | 3.84 | 3.62* |
| S.E.D. | 0.203 | 0.485 | 0.083 | 0.183 | 0.111 |

[a] Results are $Log_{10}$ (propagules/g soil +1) transformations from four (1975) and three (1976) replicates.

* Significantly different from Catriona at P = 0.05.

From results shown in Table 19, the highest number of propagules in tubersphere soil, before harvest in 1976, occurred when progeny

tubers were formed in contact with the seed. The contamination of tubersphere soil did not appear to depend on the level of inoculum surrounding the seed.

Table 19. Relationship between progeny tuber contamination and the number of contacts made between progeny and infected seed tubers of the varieties Catriona and Pentland Crown

| | Replicates I | II | III | IV |
|---|---|---|---|---|
| | | var. Catriona | | |
| Propagules/g seed tuber soil | 41440 | 39120 | 27880 | 2440 |
| Propagules/g progeny tubersphere soil | 14360 | 3860 | 5500 | 21060 |
| No. of progeny-seed tuber contacts | 1 | 0 | 0 | 2 |
| | | var. Pentland Crown | | |
| Propagules/g seed tuber soil | 19620 | 10840 | 18740 | 86 |
| Propagules/g progeny tubersphere soil | 23320 | 5540 | 2860 | 5900 |
| No. of progeny-seed tuber contacts | 2 | 0 | 0 | 0 |

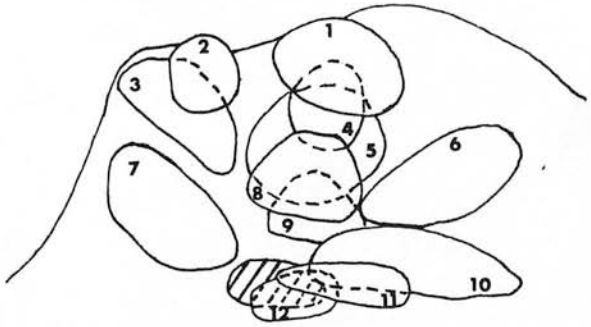
Experiment 2

Maps of tuber distribution with numbers of *F. solani* var. *coeruleum* propagules in progeny tubersphere soil are presented in Figs 31 and 32. Propagule numbers were dependent on the position of the progeny relative to the seed as those formed closest were generally more contaminated than those formed further away. On individual progeny tubers the population over the tuber surface varied (eg 0-1050 propagules on tuber 4, Plant 1 in Fig. 32). Highest levels were found on the tuber surface closest to the seed tuber.

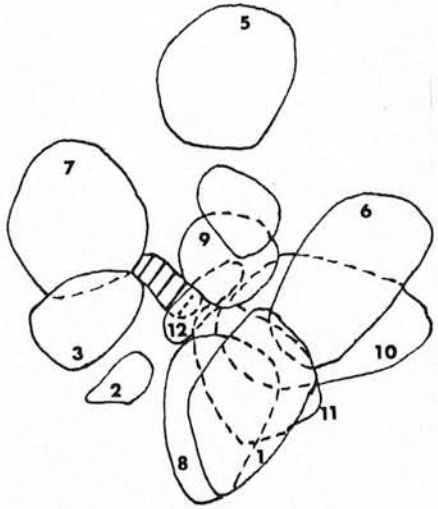
Fig. 31. Maps of vertical and horizontal tuber distribution, var. Catriona, in relation to the number of *F. solani var. coeruleum* propagules (P) per g progeny tubersphere soil.

**PLANT 1**

**Vertical**

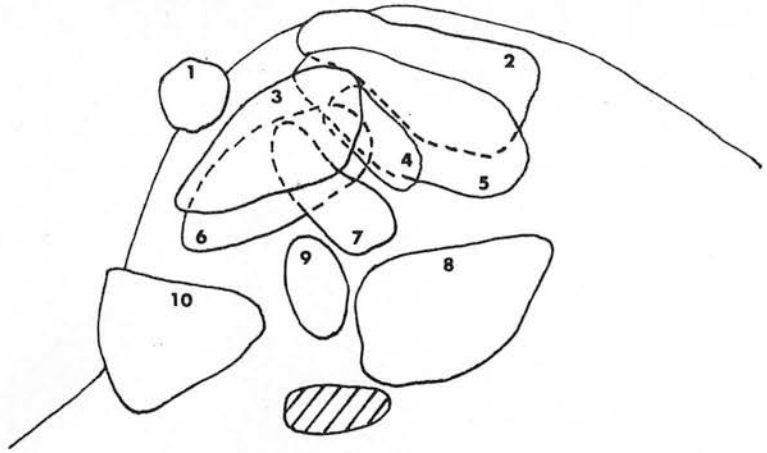


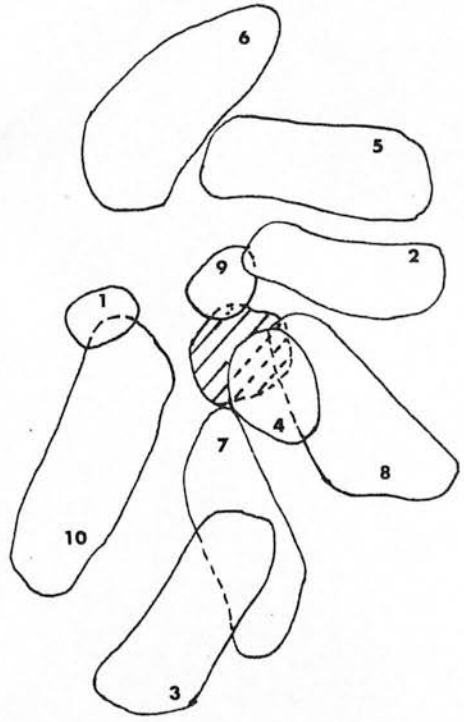

**Horizontal**




| Tuber No. | P |
|---|---|
| 1 | 0 |
| 2 | 0 |
| 3 | 0 |
| 4 | 0 |
| 5 | 30 |
| 6 | 10 |
| 7 | 7500 |
| 8 | 0 |
| 9 | 12170 |
| 10 | 390 |
| 11 | 4540 |
| 12 | 5620 |

**PLANT 2**



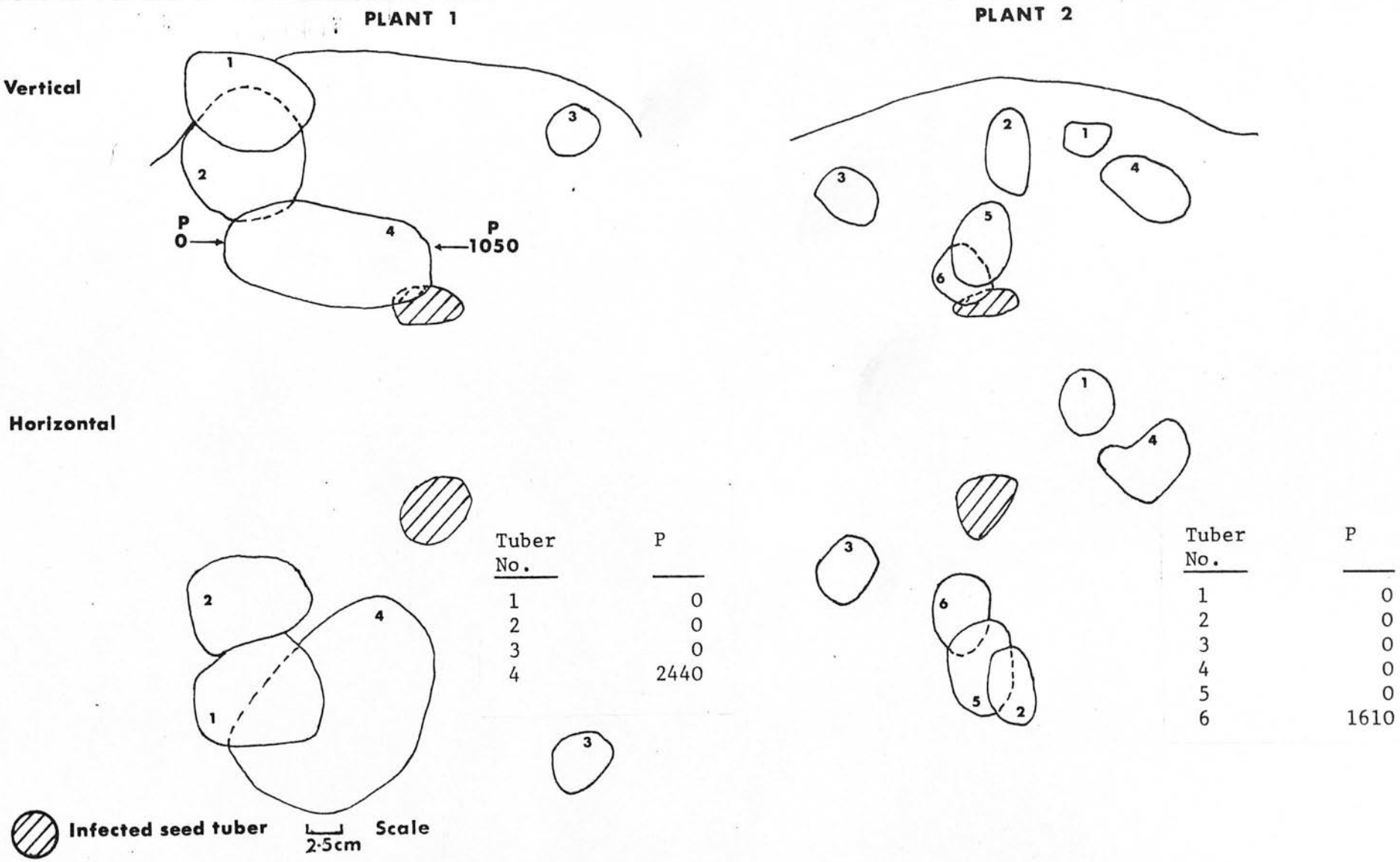




| Tuber No. | P |
|---|---|
| 1 | 0 |
| 2 | 0 |
| 3 | 0 |
| 4 | 0 |
| 5 | 0 |
| 6 | 0 |
| 7 | 0 |
| 8 | 1020 |
| 9 | 10 |
| 10 | 400 |

**Infected seed tuber**

Scale 2·5cm

Fig. 32.. Maps of vertical and horizontal tuber distribution, var. Pentland Crown, in relation to the number of *F. solani var. coeruleum* propagules (P) per g progeny tubersphere soil.




| Tuber No. | P |
|---|---|
| 1 | 0 |
| 2 | 0 |
| 3 | 0 |
| 4 | 2440 |

| Tuber No. | P |
|---|---|
| 1 | 0 |
| 2 | 0 |
| 3 | 0 |
| 4 | 0 |
| 5 | 0 |
| 6 | 1610 |

The distribution of Catriona and Pentland Crown tubers differed. No Pentland Crown progeny tubers were formed within *c.* 3cm of the seed whereas many Catriona tubers were formed within this distance, and these were generally the only ones highly contaminated. Although Pentland Crown tubers were not formed near the seed, tubers 4 and 6 from plants 1 and 2 respectively, were heavily contaminated (Fig. 32). Pentland Crown produced fewer tubers than Catriona.

Experiment 3

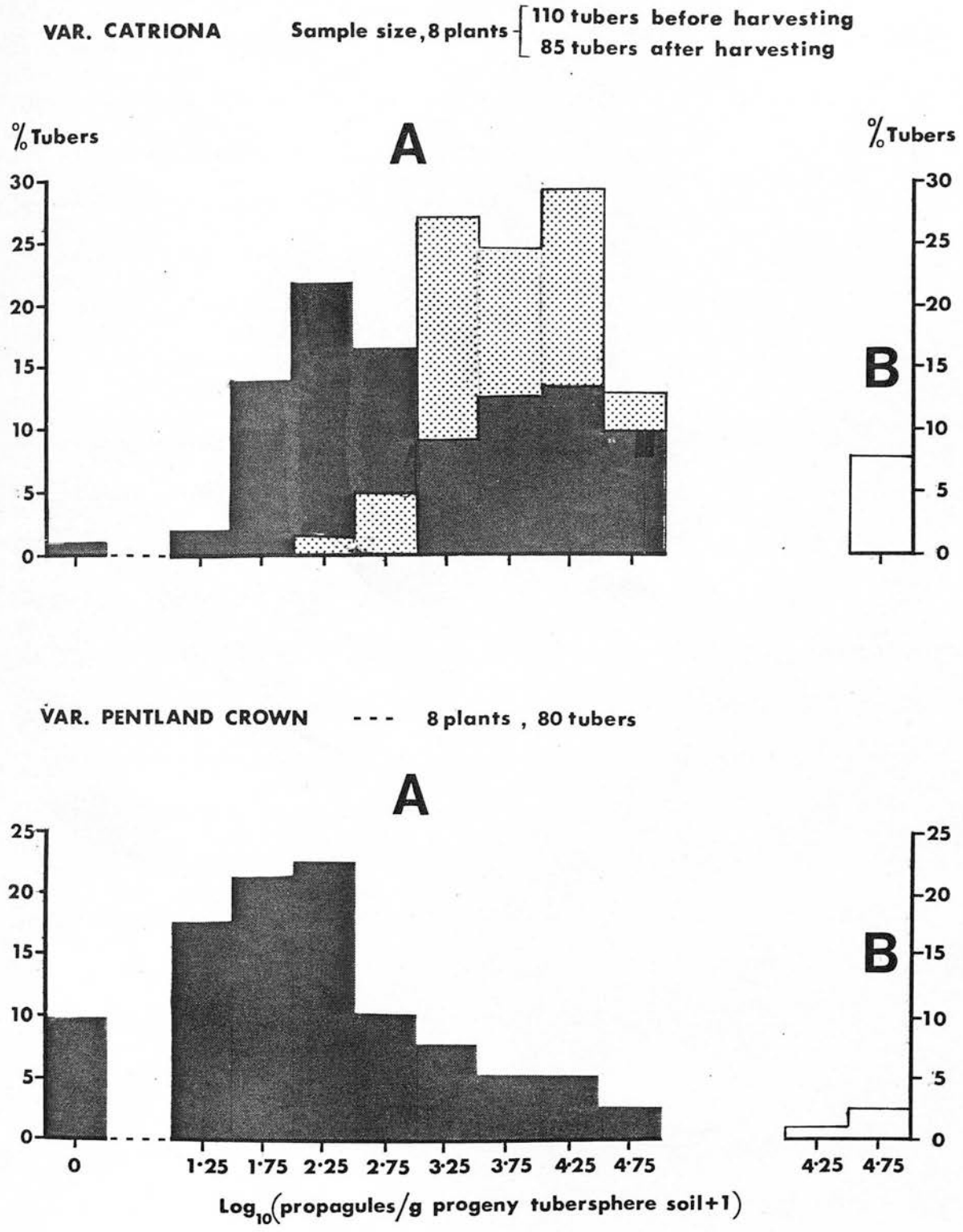
Results showing the percentage progeny tubers with a given level of propagule contamination and the number of progeny tuber contacts with infected seed are presented as frequency histograms in Fig. 33. Although the mode (2.25) was the same for both varieties, a greater proportion of Catriona than Pentland Crown progeny tubers were more heavily contaminated with *F. solani* var. *coeruleum* propagules. Also, more Catriona than Pentland Crown progeny tubers were formed in contact with the seed and these were generally the ones most heavily contaminated. Only 1% of the Catriona progeny showed no detectable contamination (ie <20 *F. solani* var. *coeruleum* propagules per g soil) whereas, for Pentland Crown this figure was 10%.

In the case of Catriona, harvesting the tubers markedly increased the overall levels of tuber contamination and no tubers remained uncontaminated.

## DISCUSSION

Basically, the incidence of dry rot depends on the susceptibility of tubers at the time of damage, and the amount of inoculum in tuber-sphere soil. Potato varieties differ in their resistance to *F. solani* var. *coeruleum* infection, and in the amount of inoculum they produce

Fig.33. Percentage progeny tubers with various levels of *F. solani* var. *coeruleum* propagule contamination before and after harvest (A) and the percentage progeny in contact with the seed before harvest and the levels of contamination (B).



from seed. The reason for the difference in propagule production is not clear, but may be related to the relative rates of substrate colonisation by the pathogen and secondary invaders. Propagule production would be hindered in Pentland Crown rather than Catriona because of its greater tissue resistance to growth of *F. solani* var. *coeruleum* (as shown by lesion size). Other potato varieties which show higher levels of resistance than Catriona (Section 5.2) might also be expected to produce fewer propagules: the relative levels of dry rot incidence for different varieties is strongly correlated with the rates at which their tuber tissues are colonised by the causal fungus (Wellving, 1976).

In discussing the reason for the decline in the importance of dry rot, Boyd (1972) suggested that the change in varieties grown was not wholly responsible: although the acreage of the very susceptible var. Doon Star had decreased, susceptible varieties were still grown. It is possible that Doon Star produced considerably more inoculum than the other susceptible varieties, thereby increasing the disease potential of the resulting crop and the contamination of field soils. Although infected Pentland Crown seed tubers produced less fungal inoculum than Catriona seed tubers, this was not consistently reflected in lower levels of tuber contamination before harvest; the position of tubers in relation to the seed influences the degree of progeny tuber contamination, seed—progeny tuber contacts being associated with high levels of progeny tuber contamination.

The distribution of progeny tubers in the ridge influences the transmission of some tuber diseases such as potato blight, *P. infestans* (Lacey, 1966) and skin spot, *Polyscytalum pustulans* (Bannon, 1972). Although tuber distribution is influenced by planting depth, plant spacing, the time and method of earthing up, and the number of stems produced per plant (Svensson, 1962; Kouwenhoven, 1970), it is mainly

a varietal characteristic. In the present work, it was found that the maximum vertical and lateral dispersion of progeny tubers was similar in both Catriona and Pentland Crown, but Catriona tended to form a higher proportion of tubers in contact with, or very close to the seed. In view of the varying gradient of fungal inoculum surrounding the seed, transmission of propagules would be expected to differ between varieties. However, although individual Pentland Crown progeny tubers were less contaminated than those of Catriona, it was not possible to ascribe this to differences in tuber distribution only, as Pentland Crown seed tubers produced less fungal inoculum than Catriona seed tubers.

Progeny tubers formed closest to the seed are the most highly contaminated, but in practice it is unlikely that use of long-stoloned varieties will reduce the incidence of dry rot as redistribution of propagules occurs in harvesting and infection takes place at or after harvest. Long-stoloned varieties may reduce the incidence of disease in cases where pathogens (eg *P. infestans*, *P. pustulans*) spread from the seed tuber to infect progeny tubers during the growing season. With dry rot it would be necessary to avoid disturbance of the highly contaminated soil surrounding the seed. Adjusting harvesting depth might achieve this but it would be then necessary to plant varieties which formed tubers near the ridge surface, increasing the risk of infection by *P. infestans* (Lacey, 1966) or greened potatoes.

# SECTION 5

# CONTROL OF POTATO DRY ROT

# SECTION 5

## CONTROL OF POTATO DRY ROT

Wherever potatoes are grown *F. solani* var. *coeruleum* propagules may be found contaminating soil in potato stores and on potato trays, boxes and potato handling machinery (Pethybridge & Bowers, 1908; Small, 1945; Lansade, 1949; Boyd, 1971). These propagules survive for long periods in field soil (Section 3.1) and thus fields which have cropped potatoes are still likely to be contaminated with *F. solani* var. *coeruleum*. It will be difficult, therefore, to maintain stocks free from dry rot.

The incidence of dry rot may be reduced by use of fungicide treatments and resistant varieties. These two aspects of control of dry rot (*F. solani* var. *coeruleum*) and powdery dry rot (*F. sulphureum*), are considered in Section 5 along with the possibilities of biological control.

The section is divided into two parts:-

5.1 Effect of fungicides and fungal antagonists on the transmission of *F. solani* var. *coeruleum* and *F. sulphureum* from infected and contaminated seed.

5.2 The susceptibility of seed-size tubers of 13 potato varieties to infection by *F. solani* var. *coeruleum* and *F. sulphureum* and the influence of temperature.

## 5.1 Effect of fungicides and fungal antagonists on the transmission of *F. solani* var. *coeruleum* and *F. sulphureum* from infected and contaminated seed

### INTRODUCTION

Control of dry rot in storage has been attempted using a range of chemicals applied in various ways to the harvested crop: organo-mercury dips (Foister, 1940; Foister & Wilson, 1950; Boyd, 1960); tecnazene dusts (Anon, 1948; Foister & Wilson, 1943); formaldehyde dips (Foister, 1940; Boyd, 1947) and fumigation (Moreau, 1973); and thiabendazole (TBZ) or benomyl dusts, dips, mists and fogs (Leach, 1971; Bommer & Patzold, 1972; Henriksen, 1975; Meijers, 1975b; Meredith, 1975). In addition, pre-plant treatment of seed tubers with benomyl dust or methoxyethylmercuric chloride (MEMC) may reduce the levels of *F. solani* var. *coeruleum* contamination of the resulting crop (Boyd & O'Donnell, 1968; Tickle, 1974) and the incidence of disease (Olofsson, 1976).

The fungi *Gliocladium virens* and *Trichoderma viride* are also active against *F. solani* var. *coeruleum*. Viridin, produced by *G. virens* (Webster & Lomas, 1964), previously thought to be *T. viride* (Brian & McGowan, 1945), prevented germination of macroconidia (Brian, Curtis, Hemming & McGowan, 1946), and *T. viride* reduced the infectivity of field soil which had been contaminated with *F. solani* var. *coeruleum* propagules (A.E.W. Boyd, unpublished).

The object of the work reported in Section 5.1 was to assess in the field over 3 seasons the effect of seed treatments, ie MEMC, TBZ, *G. virens* and *T. viride*, on the transmission of *F. solani* var. *coeruleum* and *F. sulphureum*. The seed tubers were infected separately with each fungus or were visually free from lesions but naturally contaminated with *F. solani* var. *coeruleum* propagules. Also examined over one

season (1976) was the possibility of treating seed infected with both *F. solani* var. *coeruleum* and *F. sulphureum*. TBZ was used rather than benomyl because it has been shown to be more effective in reducing sporulation of *F. solani* var. *coeruleum* (Murdoch & Wood, 1972).

As well as field experiments, investigations were conducted in the laboratory on the effect of *G. virens* and *T. viride* on soil populations of *F. solani* var. *coeruleum* and *F. sulphureum*.

## MATERIALS AND METHODS

### Field experiments

Fungicides shown in Table 20 were applied to *F. solani* var. *coeruleum* and/or *F. sulphureum* infected seed tubers, var. Catriona, and contaminated seed tubers visually free from dry rot, several days before planting.

Tubers for treatment were placed in sprouting trays. TBZ was applied as a dust from a 'Dron-Wal' blower or as a suspension from a single-nozzle pressure sprayer or a 'Turbair Scamp' ultra-low volume mister. Adequate cover of the tubers with TBZ was achieved by rotating the tubers at intervals during treatment. The formulation of TBZ used in 1974 (Tecto RPH, 1% a.i.) was not available for use in 1975 and 1976 and therefore Tecto RPH 60% a.i. was diluted with a carrier (Kaolin) to give a concentration of 2% a.i., which is the rate recommended for the control of latent diseases during storage. With MEMC, tubers were immersed for 1 min. in a solution of the fungicide.

The fungi *G. virens* (IMI 24039) and *T. viride* (IMI 45553) in sterilised soil were compacted around the seed at planting, 100g soil per tuber. The soil was prepared from air-dried, sieved (3.15mm mesh) Sphagnum moss peat and Sourhope soil (from Boghall Glen) mixed in a

Table 20. Fungicides used for seed treatment

| Experiment | Fungicide | Formulation | Application | |
|---|---|---|---|---|
| | | | Method | Rate |
| 1974 | TBZ | Tecto RPH w.p. 1% a.i. | Dust | 1g a.i./50.8 kg tubers |
| 1975, 1976 | | Tecto RPH w.p. 60% a.i. | (Dust | 2g a.i./100g Kaolin/50.8 kg tubers |
| 1975, 1976 | | | (Spray | ) 2g a.i./100 ml water/50.8 kg tubers |
| 1976 | | | (Mist | ) |
| 1974, 1975, 1976 | MEMC | Agallol w.p. 3% Hg | Dip. 1 min. | 0.15g Hg/l |

1:9 ratio, and autoclaved (15lbs/sq in. for 1h) twice at 3-day intervals. After autoclaving, the soil (pH 4.9) was dispensed into polythene bags and spore-mycelial suspensions ($4 \times 10^7$ spores/ml) from 4-wk old cultures of *G. virens* and *T. viride* on Malt Extract Agar (Rifai, 1969) added until the moisture content was 30% by weight. The soil-cultures were incubated at 25°C for 2-3 wk, checked for the presence of the added fungi by plating, and then used in the field experiments.

Each field plot consisted of two adjacent drills, 0.7m apart, planted with 10 tubers per drill, 0.4m apart. The experimental layout was a design of four randomised blocks. Planting dates are shown in Table 21.

In 1974, three categories of seed, *F. solani* var. *coeruleum* and *F. sulphureum* infected seed tubers and healthy contaminated tubers were planted in randomised blocks in the same experiment. In 1975 and 1976 the different categories of seed were planted in separate randomised block experiments. This was to avoid cross contamination at harvest of soils contaminated with different *Fusarium* spp. and soils of potentially high and low propagule numbers. Tubers were harvested by single-row elevator digger, which it was not practicable to sterilise between plots, and stored in new paper sacks. Harvest dates are shown in Table 21).

Table 21. Location of trials, planting and lifting dates

| Season | Trial | Location | Planted | Lifted |
|---|---|---|---|---|
| 1974 | Infected and contaminated tubers | Lodge Field, Langhill Farm | 24.4.74 | 10.10.74 |
| 1975 | Infected and contaminated tubers | Anchordales, Boghall Farm | 13.5.75 | 6.11.75 |
| 1976 | Infected tubers | March Park, Boghall Farm | 4.5.76 | 20.10.76 |
| | Contaminated tubers | Bush plots, Bush | 2.6.76 | 20.10.76 |

Assessment of transmission To estimate the efficiency of the treatments in reducing transmission of the pathogens, soil samples were collected before harvest from around seed and progeny tubers as described on p 80 and the fungal population (p 22 ) and/or soil infectivity determined (p 23 ). After harvest, transmission was assessed by determining the fungal population in tubersphere soil (p 39 ) and the progeny tuber contamination index, PTCI (p 39 ). When determining the fungal population a $10^{-2}$ soil dilution was used except on soils from infected tubers in 1976 when a $10^{-3}$ dilution was used.

In 1976, harvested tubers from selected treatments were damaged by passing them over a hand riddle as described on p 39 . The incidence of dry rot was recorded after 3 months incubation in paper bags in a farm store. Also in 1976, the contamination of field soil from plots planted with seed tubers infected with both *F. solani* var. *coeruleum* and *F. sulphureum* was assessed as follows.

After harvest five soil samples, each 0.3m apart, were taken with a soil corer (20 x 1.5cm) from the centre 1.5m length of each drill. Soil from each plot (ie two drills) was bulked, air-dried, sieved (0.85mm mesh) and the fungal population determined. A $10^{-2}$ soil dilution and 10 plates of PAB selective medium were used for each soil.

Laboratory experiments

Experiments were conducted to determine the effect of *G. virens* and *T. viride* on the survival of *F. solani* var. *coeruleum* and *F. sulphureum* propagules. Two air-dried soils were used: an autoclaved Sphagnum moss peat soil (pH 4.9) described previously, and a non-autoclaved soil (pH 5.9) from the Macmerry soil series, shown to be free of *F. solani* var. *coeruleum* and *F. sulphureum* propagules (ie <10 per g soil). Spore-mycelial suspensions ($4 \times 10^7$ spores/ml) of *G. virens* and *T. viride* were added to peat soil or Macmerry soil contained in polythene bags (wall thickness 0.35mm) until the moisture content was 15% by weight. The soils were incubated at 25°C for 2-3 wk and then checked for the presence of the added fungi by plating. Suspensions of *F. solani* var. *coeruleum* and *F. sulphureum* macroconidia prepared as described on p 53 were each added to four replicate lots of peat soil or Macmerry soil containing *G. virens*, *T. viride* or water only until the moisture content of the soil was 30% by weight.

The populations of *F. solani* var. *coeruleum* and *F. sulphureum* were determined (on the PAB medium) initially and after 6 months incubation at 20°C. The experiment was repeated (Experiment 2).

## RESULTS

### Field experiments

a. Transmission of *F. solani* var. *coeruleum* from infected seed in relation to seed treatment.

Season 1974 (Table 22). Only TBZ dust significantly reduced the *F. solani* var. *coeruleum* population and infectivity of progeny tubersphere soil before harvest and the effect was still evident after harvest. MEMC also appeared to reduce transmission of *F. solani* var. *coeruleum* to the progeny but this was apparent only after harvest; unlike TBZ reduction in transmission was not associated with reduced propagule numbers in soil surrounding the seed. *G. virens* did not affect propagule production or transmission.

Season 1975 (Table 23). No seed treatment significantly reduced the contamination of progeny tubersphere soil before harvest. However, TBZ spray and *T. viride* significantly reduced the number of *F. solani* var. *coeruleum* propagules in soil surrounding the infected seed.

Season 1976 (Table 24). MEMC, TBZ dust, spray and mist significantly reduced the transmission of *F. solani* var. *coeruleum* propagules to the progeny before harvest, but only in the case of the TBZ treatments was the reduction also associated with fewer propagules in soil surrounding the seed. After harvest, however, only progeny grown from TBZ spray and mist treated seed showed significant reductions in propagule transmission. Although the progeny tuber contamination index (PTCI) showed no differences between treatments the incidence of dry rot after riddling was significantly reduced in those tubers grown from MEMC, TBZ spray and *T. viride* treated seed.

Table 22. Transmission of *F. solani* var. *coeruleum* from infected seed in relation to seed treatment in 1974

| Seed treatment | [a]Contamination of: | | | | | |
|---|---|---|---|---|---|---|
| | Seed tuber soil | | Progeny tubersphere soil | | | |
| | Before harvest | | Before harvest | | After harvest | |
| | Propagules | Infectivity | Propagules | Infectivity | Propagules | PTCI |
| Untreated | 3.88 | 73.7 | 3.63 | 84.5 | 4.11 | 72.0 |
| MEMC | 3.84 | 70.1 | 3.60 | 71.6 | 3.68** | 59.7 |
| TBZ dust (1% a.i.) | 2.91 | 54.2 | 1.37** | 39.8** | 3.22** | 47.5** |
| *G. Virens* | 3.46 | 69.1 | 3.50 | 83.8 | 4.20 | 73.3 |
| S.E.D. | 0.393 | 8.28 | 0.650 | 12.73 | 0.197 | 6.68 |

[a] Transformed data from four replicates: propagules to $\log_{10}$ (propagules/g soil +1); infectivity and PTCI to arcsin.

** Significantly different from untreated at P = 0.01.

Table 23. Transmission of *F. solani* var. *coeruleum* from infected seed in relation to seed treatment in 1975

| Seed treatment | $Log_{10}$ (propagules/g seed tuber or progeny tubersphere soil +1) before harvest | |
|---|---|---|
| | Seed | Progeny |
| Untreated | 4.20 | 3.84 |
| MEMC | 4.28 | 4.17 |
| TBZ dust (2% a.i.) | 3.89 | 3.83 |
| TBZ spray (2% a.i.) | 3.63** | 3.79 |
| *T. viride* | 3.72* | 4.08 |
| Sterilised soil | 4.38 | 4.06 |
| S.E.D. | 0.164 | 0.259 |

*, ** Significantly different from untreated at P = 0.05 and 0.01 respectively.

Table 24. Transmission of *F. solani* var. *coeruleum* from infected seed in relation to seed treatment in 1976

| Seed treatment | $Log_{10}$ (propagules/g seed tuber or progeny tubersphere soil +1) | | | PTCI (arcsin) | % Rots (arcsin) |
|---|---|---|---|---|---|
| | Before harvest | | After harvest | | |
| | Seed | Progeny | Progeny | | |
| Untreated | 4.49 | 4.42 | 4.46 | 66.0 | 66.4 |
| MEMC | 4.55 | 4.01* | 4.32 | 58.4 | 52.4* |
| TBZ dust (2% a.i.) | 4.27 | 3.93* | 4.27 | 53.9 | - |
| TBZ spray (2% a.i.) | 3.73*** | 3.70*** | 4.14** | 52.6 | 44.8** |
| TBZ mist (2% a.i.) | 4.11** | 4.02* | 4.26* | 66.6 | - |
| *G. virens* | 4.59 | 4.31 | 4.37 | 62.2 | - |
| *T. viride* | 4.54 | 4.20 | 4.41 | 60.4 | 52.1** |
| Sterilised soil | 4.63 | 4.14 | 4.43 | 65.8 | - |
| S.E.D. | 0.125 | 0.187 | 0.095 | 6.50 | 4.33 |

*, **, *** Significantly different from untreated at P = 0.05, 0.01 and 0.001 respectively.

b. Transmission of *F. sulphureum* from infected seed in relation to seed treatment.

Season 1974 (Table 25). MEMC, TBZ dust and *G. virens* treatment of infected seed significantly reduced the infectivity of seed tuber soil, but only the latter two treatments significantly reduced the infectivity of progeny tubersphere soil. The soils were stored at 4°C and then re-examined in 1976 when the soil infectivity and the number of *F. sulphureum* propagules were determined. Soil infectivities were lower than found in 1974 and only in seed tuber soil did infectivities follow the pattern as found previously. Infectivities from progeny tubersphere soils showed no differences between treatments. The number of propagules showed the same pattern between treatments as did soil infectivities.

Season 1975 (Table 26). No treatment significantly affected transmission of *F. sulphureum* to the progeny but MEMC and TBZ spray significantly reduced the number of propagules in soil surrounding the seed.

Season 1976 (Table 27). No seed treatment significantly reduced transmission of *F. sulphureum* or the number of propagules in seed tuber soil.

c. Transmission of *F. solani* var. *coeruleum* and *F. sulphureum* from seed infected with both fungi (Tables 28 and 29).

Treating infected seed with TBZ spray reduced significantly the number of *F. solani* var. *coeruleum* and *F. sulphureum* propagules in seed tuber soil but had no effect on the number of propagules in progeny tubersphere soil before harvest. However, after harvest, progeny grown from TBZ treated seed were significantly less contaminated with *F. solani* var. *coeruleum* propagules than progeny grown from untreated seed. Also

Table 25. Transmission of *F. sulphureum* from infected seed in relation to seed treatment in 1974

| Seed treatment | [a]Contamination before harvest: | | | | | |
|---|---|---|---|---|---|---|
| | Seed tuber soil | | | Progeny tubersphere soil | | |
| | Propagules 1976 | Infectivity 1974 | Infectivity 1976 | Propagules 1976 | Infectivity 1974 | Infectivity 1976 |
| Untreated | 2.16 | 73.0 | 28.7 | 1.93 | 68.1 | 32.2 |
| MEMC | 0.83 | 44.1** | 8.9 | 1.08 | 51.4 | 20.3 |
| TBZ dust (1% a.i.) | 0.73 | 45.0** | 4.5 | 1.54 | 38.5** | 29.9 |
| *G. virens* | 0.52 | 41.4** | 5.5 | 2.14 | 37.3** | 22.8 |
| S.E.D. | 0.717 | 7.56 | 10.03 | 0.799 | 8.56 | 20.34 |

[a] Transformed data from four replicates: propagules to $\log_{10}$ (propagules/g soil +1) and infectivity to arcsin.

** Significantly different from untreated at P = 0.01.

Table 26. Transmission of *F. sulphureum* from infected seed in relation to seed treatment in 1975

| Seed treatment | $Log_{10}$ (propagules/g seed tuber or progeny tubersphere soil +1) before harvest | |
|---|---|---|
| | Seed | Progeny |
| Untreated | 4.07 | 3.38 |
| MEMC | 3.88* | 2.84 |
| TBZ dust (2% a.i.) | 3.95 | 3.63 |
| TBZ spray (2% a.i.) | 3.68** | 2.84 |
| *T. viride* | 4.09 | 3.71 |
| Sterilised soil | 4.02 | 3.36 |
| S.E.D. | 0.084 | 0.048 |

*, ** Significantly different from untreated at P = 0.05 and 0.01 respectively.

Table 27. Transmission of *F. sulphureum* in relation to seed treatment in 1976

| Seed treatment | $Log_{10}$ (propagules/g seed tuber or progeny tubersphere soil +1) | | | PTCI (arcsin) | % Rots (arcsin) |
|---|---|---|---|---|---|
| | Before harvest | | After harvest | | |
| | Seed | Progeny | Progeny | | |
| Untreated | 4.99 | 4.83 | 4.73 | 78.1 | 32.3 |
| MEMC | 4.78 | 4.99 | 4.67 | 67.3 | 23.4 |
| TBZ dust (2% a.i.) | 4.98 | 4.92 | 4.70 | 61.5 | - |
| TBZ spray (2% a.i.) | 4.85 | 5.21 | 4.65 | 66.5 | 25.2 |
| TBZ mist (2% a.i.) | 4.95 | 5.07 | 4.84 | 57.2 | - |
| *G. virens* | 5.01 | 5.13 | 4.89 | 71.3 | - |
| *T. viride* | 5.13 | 5.40 | 4.99 | 64.2 | - |
| Sterilised soil | 4.91 | 4.71 | 4.79 | 65.6 | - |
| S.E.D. | 0.198 | 0.301 | 0.131 | 9.68 | 2.58 |

Table 23. The effect of TBZ on the transmission of *F. solani* var. *coeruleum* from seed infected with both *F. solani* var. *coeruleum* and *F. sulphureum* in 1976

| Seed treatment | $Log_{10}$ (propagules/g seed tuber, progeny tubersphere or field soil +1) | | | |
|---|---|---|---|---|
| | Before harvest | | After harvest | |
| | Seed | Progeny | Progeny | Field |
| Untreated | 4.02 | 3.61 | 3.88 | 2.51 |
| TBZ spray (2% a.i.) | 3.56* | 2.52 | 3.32** | 1.59** |
| S.E.D. | 0.138 | 0.859 | 0.120 | 0.233 |

*, ** Significantly different from untreated at P = 0.05 and P = 0.01 respectively.

Table 29. The effect of TBZ on the transmission of *F. sulphureum* from seed infected with both *F. solani* var. *coeruleum* and *F. sulphureum* in 1976

| Seed treatment | $Log_{10}$ (propagules/g seed tuber, progeny tubersphere or field soil +1) | | | |
|---|---|---|---|---|
| | Before harvest | | After harvest | |
| | Seed | Progeny | Seed | Field |
| Untreated | 4.85 | 5.01 | 4.31 | 2.74 |
| TBZ spray (2% a.i.) | 4.32** | 4.41 | 4.23 | 2.42 |
| S.E.D. | 0.083 | 0.488 | 0.218 | 0.339 |

** Significantly different from untreated at P = 0.01.

Table 30. Transmission of *F. solani* var. *coeruleum* from contaminated seed in relation to seed treatment

| Seed treatment | Progeny tuber contamination after harvest[a] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1974 | | 1975 | | 1976 | | |
| | Propagules | PTCI | Propagules | PTCI | Propagules | PTCI | % Rots |
| Untreated | 2.83 | 26.7 | 2.12 | 7.5 | 1.73 | 5.6 | 12.8 |
| MEMC | 3.09 | 26.8 | 0.93 | 6.8 | 2.11 | 17.5 | 15.4 |
| TBZ dust (1% a.i.) | 2.11 | 19.2 | - | - | - | - | - |
| TBZ dust (2% a.i.) | - | - | 0.73 | 3.2 | 0.78* | 10.4 | 11.2 |
| TBZ spray (2% a.i. | - | - | 0.85 | 3.2 | 1.71 | 1.6 | 9.8 |
| TBZ mist (2% a.i.) | - | - | - | - | 1.77 | 5.9 | 7.9 |
| *G. virens* | 3.90 | 41.5 | - | - | 1.88 | 3.2 | 12.4 |
| *T. viride* | - | - | 1.14 | 11.5 | 1.67 | 7.2 | 17.3 |
| Sterilised soil | - | - | 1.59 | 9.2 | 1.82 | 5.5 | 10.2 |
| S.E.D. | 0.681 | 8.5 | 0.646 | 6.69 | 1.096 | 8.77 | 2.45 |

[a] Transformed data from four replicates: propagules to $\log_{10}$ (propagules/g soil +1), PTCI and % rots to arcsin.
* Significantly different from untreated at P = 0.05.

significantly fewer propagules were left in field soil. In contrast TBZ did not affect *F. sulphureum* propagule numbers, in progeny tubersphere or field soil.

Much higher propagule numbers were found for *F. sulphureum* than *F. solani* var. *coeruleum*.

d. Transmission of *F. solani* var. *coeruleum* from contaminated seed (Table 30).

In 1974 and 1975, TBZ dust or spray treatment of contaminated seed reduced levels of progeny tuber contamination, but not significantly. Although the dust significantly reduced the number of propagules in progeny tubersphere soil in 1976, the effect was not shown in the PTCI or percentage rots data. Neither MEMC, *G. virens* or *T. viride* reduced consistently progeny tuber contamination.

In an attempt to explain the action of TBZ in reducing progeny tuber contamination, samples of soil were collected from around untreated and TBZ dusted seed tubers before harvest in 1975. TBZ reduced the number of seed tubers giving rise to high levels of soil contamination (Table 31).

Table 31. Percentage contaminated seed tubers giving various propagule levels in field soil in 1976

| Seed treatment | Propagules/g soil | | |
|---|---|---|---|
| | 0 | 3-33 | >300 |
| Untreated | 62.5 | 31.3 | 6.2 |
| TBZ dust (2% a.i.) | 76.6 | 21.9 | 1.5 |

Laboratory experiments

*G. virens* and *T. viride* were ineffective in non-autoclaved soil at reducing the populations of *F. solani* var. *coeruleum* and *F. sulphureum*

(Tables 32 and 33). In initially autoclaved soil, results were inconsistent (Tables 34 and 35). Although *T. viride* significantly reduced the population of *F. solani* var. *coeruleum* and *F. sulphureum* in Experiment 1, no effect was found in the repeat experiment (Expt 2). *G. virens* was not effective in either experiment.

Storage of non-autoclaved soil at 15°C for 6 months did not affect the survival of *F. solani* var. *coeruleum* or *F. sulphureum* despite the moisture content of the soil declining from 30% to *c.* 19%. In autoclaved soil, however, the fungal populations were reduced markedly in all treatments; *F. sulphureum* appeared to be less affected than *F. solani* var. *coeruleum*. Both *G. virens* and *T. viride* were isolated successfully from their respective soils. In autoclaved soil of the controls, *Penicillium* spp. were isolated.

Table 32. Population of *F. solani* var. *coeruleum* /$\overline{\text{Log}_{10}}$ (propagules/g soil +1)$\overline{/}$ in non-autoclaved Macmerry field soil contaminated with *G. virens* and *T. viride*

| Fungal antagonist | Sampling intervals (months) | | | | % Soil moisture after 6 months | |
|---|---|---|---|---|---|---|
| | 0 | | 6 | | | |
| | Expt 1 | Expt 2 | Expt 1 | Expt 2 | Expt 1 | Expt 2 |
| None | 3.70 | 3.58 | 3.64 | 3.69 | 17 | 20 |
| *G. virens* | 3.70 | 3.63 | 3.68 | 3.46 | 20 | 17 |
| *T. viride* | 3.65 | 3.62 | 3.65 | 3.69 | 19 | 20 |
| S.E.D. | 0.038 | 0.068 | 0.028 | 0.098 | 3.8 | 2.0 |

Table 33. Population of *F. sulphureum* /$\overline{\text{Log}_{10}}$ (propagules/g soil+1)$\overline{7}$ in non-autoclaved Macmerry field soil contaminated with *G. virens* and *T. viride*

| Fungal antagonist | Sampling interval (months) | | | | % Soil moisture after 6 months | |
|---|---|---|---|---|---|---|
| | 0 | | 6 | | | |
| | Expt 1 | Expt 2 | Expt 1 | Expt 2 | Expt 1 | Expt 2 |
| None | 3.54 | 3.64 | 3.33 | 3.44 | 20 | 19 |
| *G. virens* | 3.59 | 3.61 | 3.50 | 3.50 | 18 | 19 |
| *T. viride* | 3.57 | 3.60 | 3.48 | 3.42 | 15 | 18 |
| S.E.D. | 0.096 | 0.044 | 0.034 | 0.042 | 3.4 | 2.3 |

Table 34. Population of *F. solani* var. *coeruleum* $\overline{/\overline{\text{Log}_{10}}}$ (propagules/g soil +1)$\overline{\overline{/}}$ in initially autoclaved Sphagnum moss peat soil contaminated with *G. virens* and *T. viride*

| Fungal antagonist | Sampling interval (months) | | | | % Soil moisture after 6 months | |
|---|---|---|---|---|---|---|
| | 0 | | 6 | | | |
| | Expt 1 | Expt 2 | Expt 1 | Expt 2 | Expt 1 | Expt 2 |
| None | 3.52 | 3.66 | 0 | 0.38 | 30 | 18 |
| *G. virens* | 3.59 | 3.68 | 0.77 | 0.76 | 23* | 20 |
| *T. viride* | 3.14*** | 3.60 | 0 | 0.38 | 22* | 17 |
| S.E.D. | 0.045 | 0.050 | 0.361 | 0.688 | 2.0 | 1.9 |

*, *** Significantly different from control at P = .05 and 0.001 respectively.

Table 35. Population of *F. sulphureum* /$\overline{\text{Log}_{10}}$ (propagules/g soil +1)$\overline{/}$ in initially autoclaved Sphagnum moss peat soil contaminated with *G. virens* and *T. viride*

| Fungal antagonist | Sampling interval (months) | | | | % Soil moisture after 6 months | |
|---|---|---|---|---|---|---|
| | 0 | | 6 | | | |
| | Expt 1 | Expt 2 | Expt 1 | Expt 2 | Expt 1 | Expt 2 |
| None | 3.72 | 3.71 | 2.91 | 1.13 | 29 | 15 |
| *G. virens* | 3.67 | 3.72 | 2.58 | 2.87 | 22 | 19 |
| *T. viride* | 2.79*** | 3.75 | 0*** | 1.74 | 22 | 18 |
| S.E.D. | 0.115 | 0.041 | 0.224 | 0.839 | 3.0 | 2.4 |

*** Significantly different from control at P = 0.001.

DISCUSSION

Over 3 seasons, TBZ was the only seed treatment which consistently reduced the number of *F. solani* var. *coeruleum* propagules in soil around *F. solani* var. *coeruleum* infected seed tubers because it inhibited sporulation of the fungus on the surface of the seed tuber (Murdoch & Wood, 1972). This is supported by laboratory experiments where it was observed that although the number of pustules on the surface of untreated and TBZ treated seed tubers were similar, TBZ inhibited macroconidia production (unpublished). Therefore many of the propagules which remain in soil around TBZ treated seed are probably formed within the infected seed tuber and are released as the tuber decomposes.

A reduction in propagule production was generally associated with less contamination of the progeny. An exception to this occurred in 1975, and may be linked with a failure of many seed tuber eyes to produce sprouts. This would have affected tuber distribution and therefore also the level of progeny tuber contamination before harvest (Sections 4.1 & 4.2). Harvesting would have minimised any effects due to tuber distribution but this procedure was not carried out due to insufficient plant numbers. In 1976, the PTCI data showed no significant reductions due to TBZ. This is in contrast to data of the fungal population in tubersphere soil and indicates that the PTCI is too insensitive at high fungal populations to detect treatment differences.

TBZ did not show the same consistency in its effect against *F. sulphureum* as it did against *F. solani* var. *coeruleum*. This may be related to the discovery, made in this work, that in the field *F. sulphureum* sporulates on stems growing from infected tubers. In addition, in the laboratory the fungus was shown to sporulate on potato shoot and stem segments inoculated with *F. sulphureum*

macroconidia (Fig. 34). Stems and shoots inoculated with *F. solani* var. *coeruleum* showed slight discolouration but no sporulation. On stems it is unlikely that *F. sulphureum* is affected by fungitoxic concentrations of TBZ applied to seed tubers and thus spores washed into the soil may increase inoculum levels. Because inhibitory concentrations of TBZ may be present in the stem-base (Bolkan & Milne, 1975), however, it is improbable that *F. sulphureum* moves systemically from seed tuber to stem.

Since the action of TBZ is believed to be against surface sporulation, the inconsistency shown against *F. sulphureum* may be also related to the observation that *F. sulphureum* does not sporulate profusely on the surface of untreated tubers.

The effect of MEMC on transmission of *F. solani* var. *coeruleum* was variable. When reductions in the levels of progeny contamination were obtained this was not associated with reduced numbers of propagules in seed tuber soil. In contrast MEMC sometimes reduced the number of *F. sulphureum* propagules in seed tuber soil but did not affect the contamination of progeny tubers. The reasons for the differential effect of MEMC against the two pathogens is not known.

The success achieved with *G. virens* against *F. sulphureum* in 1974 probably stemmed from the production of two antibiotics, gliotoxin and viridin (Brian, 1944; Brian & McGowan, 1945). It is not clear why no action was found against *F. solani* var. *coeruleum* but it is possible that *F. sulphureum* is more sensitive to these antibiotics than has been reported for *F. solani* var. *coeruleum* (Brian *et al*, 1946). In field soil the activity of *G. virens* will depend on its being able to maintain adequate concentrations of gliotoxin and viridin, despite leaching

Fig. 34. Stem segments inoculated with (a) water, (b) *F. solani* var. *coeruleum* macroconidia and (c) *F. sulphureum* macroconidia showing after 7 days incubation at 20°C pustulation by *F. sulphureum* only


and dilution in soil-water and inactivation because of unsuitable pH (Brian *et al*, 1946). Failure to achieve any level of control in 1976 may be related to the higher pH of field soil that year, (ie 6.3) than in 1974 (ie 5.5). The higher pH might also inhibit *G. virens* from colonising new substrates (Wright, 1956). In the field, *G. virens* probably inhibited propagule production since in laboratory experiments in autoclaved soil (pH 4.9) it failed to reduce the population of *F. sulphureum* macroconidia.

In the field, *T. viride* reduced the population of *F. solani* var. *coeruleum* in seed tuber soil in 1975 but not in 1976. As with *G. virens* activity of *T. viride* is dependent on low pH (Weindling & Fawcett, 1936; Aytoun, 1953). However, soil pH was similar in 1975 (pH 6.1) and 1976 (pH 6.3). The activity of *T. viride* against *F. solani* var. *coeruleum* may be due to non-volatile inhibitors (eg Trichodermin), volatile antibiotics (eg acetaldehyde) or mycoparasitic capabilities (Weindling, 1932; Aytoun, 1953; Dennis & Webster, 1971a; 1971b; 1971c). The latter two suggestions are unlikely: the isolate of *T. viride* used did not possess a coconut smell (Bisby, 1939; Rifai, 1969) characteristic of isolates producing volatile inhibitors (Dennis & Webster, 1971b) and high nutrient status and temperatures >25°C, necessary for intense mycoparasitism, are unlikely to be met in the field for most of the season (Griffin, 1972).

Results of the laboratory experiments were inconclusive. *G. virens* showed no effect on populations of *F. solani* var. *coeruleum* and *F. sulphureum* propagules, and *T. viride* in initially autoclaved soil, although markedly reducing these populations in Experiment 1 failed to have an effect in a repeat experiment. After storage of the soil for 6 months at 15°C, populations of *F. solani* var. *coeruleum*

and *F. sulphureum* were lower than those in field soil, irrespective of whether *G. virens* or *T. viride* was present. The reasons for this are unknown and further experiments are needed to determine if reduced survival was an effect of: pH (the lower pH of autoclaved soil preventing formation of chlamydospores); the formation of toxic products after autoclaving; antagonism by *G. virens* and *T. viride*, but because of possible contamination of controls by fungi also antagonistic to *F. solani* var. *coeruleum*, a failure to find differences between treatments.

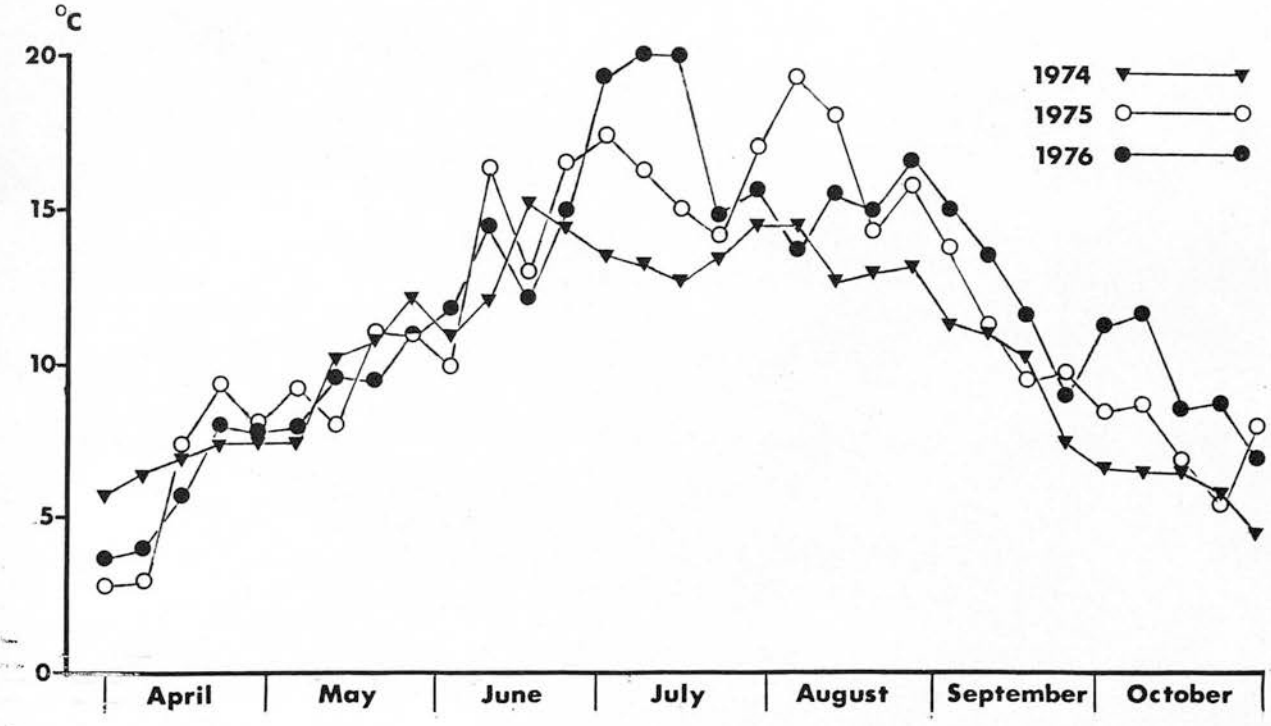
In the laboratory experiments, soils were stored in polythene bags of wall thickness 0.35mm. Since the bags were inadequate in preventing moisture loss over long periods, they cannot be recommended for use in future work.

It was not possible to prevent transmission of *F. solani* var. *coeruleum* by planting seed tubers visually free from lesions but contaminated with *F. solani* var. *coeruleum* propagules. This supports other evidence presented in this thesis (Section 3.2) and elsewhere (Tickle, 1974). Generally, the level of progeny tuber contamination was fairly low, except in 1974 when cross contamination occurred at harvesting from plots in the same experiment planted with infected seed tubers which were lifted by the same elevator digger. In later experiments contaminated seed tubers were planted in separate experiments to infected seed tubers. TBZ treatment of contaminated seed was the only treatment which consistently reduced propagule numbers in progeny tubersphere soil. Similarly seed treatments (eg MEMC, benomyl) have been shown to reduce the incidence of dry rot (Boyd & Logan, 1967; Tickle, 1974). The method by which fungicides reduce transmission from contaminated seed is uncertain but is probably

related to the finding that despite careful examination of the seed before planting, infected tubers may be planted, as shown by relatively high propagule numbers in soil surrounding some seed tubers. Infection may have occurred through damage inflicted at planting, in which case TBZ would have reduced inoculum levels before damage and thus the number of infected seed tubers. A similar conclusion was reached by Leach & Nielsen (1975) in the USA, who showed that treatment of whole seed with fungicides, before cutting into seed-pieces reduced the incidence of rotting. They did not, however, study the transmission of propagules from treated seed. Since TBZ does not move systemically from seed to progeny the efficiency of treatment of contaminated seed depends on the relative amounts of inoculum from seed and field soil which may be contaminated from previous potato crops. In 1976, field soil was found to be contaminated before planting and this may account partly for the lack of effect shown by TBZ spray and mist.

Besides treatment effects, the season also affected propagule production. Inoculum levels were higher for *F. solani* var. *coeruleum* than *F. sulphureum* in 1974 and 1975, but in 1976 the positions were reversed: ten times as many *F. sulphureum* propagules were detected as found previously. Ayers & Ramsay (1961) suggest that warm dry summers favour propagation of *F. sulphureum*. An attempt was made to relate seasonal differences in propagule production to meteorological data obtained *c.* 1 mile from the experimental sites. No records of moisture deficit were available but rainfall was similar over the growing periods. Soil temperature, however, showed marked differences between seasons: July 1976 was 4-6°C warmer than July 1974 and 1975, and the latter part of the growing season was warmer also (Fig. 35). If soil temperature is important it may act in a number of ways. It

Fig.35. Soil temperature 20 cm below the soil surface.



may increase the growth rate of *F. sulphureum* so that a powdery dry rot lesion is formed rather than the wet type rot formed at lower temperatures (Section 5.2). In the former case many spores are produced in cavities within the tuber whereas at lower temperatures cavitation does not occur. Secondly, it may stimulate propagule production on the surface of the tuber which is rarely seen on *F. sulphureum* infected tubers. This is unlikely, as TBZ, which is known to affect surface sporulation, showed no action against *F. sulphureum* in 1976. Thirdly, high temperatures may increase sporulation on potato stems, thereby adding to inoculum produced by the seed.

*F. sulphureum* differed also from *F. solani* var. *coeruleum* in another respect. When both fungi were inoculated into the same tuber the population of *F. sulphureum* was similar to that from tubers inoculated with *F. sulphureum* only. In contrast the population of *F. solani* var. *coeruleum* was reduced markedly in tubers inoculated with *F. solani* var. *coeruleum* and *F. sulphureum*. More recent unpublished work has supported these preliminary observations. Inhibition of *F. solani* var. *coeruleum* by *F. sulphureum* on the soil dilution plates is unlikely to be responsible for these results, since increasing the soil dilution so that only a few colonies of each fungus were present, did not affect the general conclusion.

Inhibition of propagule production of *F. solani* var. *coeruleum* by *F. sulphureum* may be related to the higher growth rate of *F. sulphureum*, and it is possible that it utilises nutrients at the expense of growth of *F. solani* var. *coeruleum*. Further work is required on the interaction between *F. sulphureum* and *F. solani* var. *coeruleum* and the effect of environmental factors on propagule production.

## 5.2 The susceptibility of seed-size tubers of 13 potato varieties to infection by *F. solani* var. *coeruleum* and *F. sulphureum* and the influence of temperature

### INTRODUCTION

Information is available on the susceptibility of potato varieties in the UK to *F. solani* var. *coeruleum* infection (Boyd, 1952c; Jellis, 1975) but no comparable data are available for *F. sulphureum*. However, Ayers (1956; 1962) in Canada and Wellving (1976) in Sweden have shown that the ranking order of varietal resistance to *F. solani* var. *coeruleum* and that to *F. sulphureum* are different.

Temperature is undoubtedly an important regulating factor in the development of *F. solani* var. *coeruleum* in stored potatoes. To reduce the incidence of dry rot it is often advised to store newly-lifted potatoes at a relatively high temperature (*c.* 15°C) since wound healing occurs faster at higher than at lower temperatures (Anon, undated; Henriksen, 1975). Many potato stores, however, operate at much lower temperatures and under such conditions the incidence of dry rot caused by *F. solani* var. *coeruleum* is increased (Boyd, 1952d; Boyd, 1974). No comparable data are available for *F. sulphureum*. Data on susceptibility are often gained at one temperature only (*c.* 15°C) and therefore their application to commercial storage conditions is limited.

The experiments reported deal with the susceptibility of a number of common potato varieties to infection by *F. solani* var. *coeruleum* and *F. sulphureum* at two times during the season and at two temperatures. The inoculation method of Boyd (1952a) which assesses biochemical resistance of the tuber tissue rather than resistance to mechanical damage was used. Boyd (1967) showed that premature removal of the haulm reduces the susceptibility of tubers to infection by *F. solani*

var. *coeruleum*. Since early haulm destruction is an established practice for the specialist seed producer for regulating tuber size, reducing blight incidence and reducing the incidence of aphid transmitted viruses (Anon, 1978), this procedure was adopted in the experiments. An experiment *in vitro* was also carried out to compare the development of *F. solani* var. *coeruleum* and *F. sulphureum* at 4°C and 15°C.

## MATERIALS AND METHODS

### Varietal susceptibility and the influence of temperature

Over two seasons, 1975-76 and 1976-77 the susceptibility of 10 and 13 potato varieties respectively was assessed. Each season, stocks of undisinfected, certified seed were grown at a common source to eliminate differences in susceptibility which may be caused by planting at different locations (Boyd, 1952b). When it was judged, after sample lifting, that the numbers of seed-size tubers were at a maximum for each variety the haulm was cut down. Approximately 4wk later the tubers were harvested, surface sterilised in 0.8% formalin for 1 min. and when dry, stored in paper sacks in a farm store.

In November, 40 tubers of each variety were inoculated with either *F. solani* var. *coeruleum* or *F. sulphureum* spores: 20 were incubated at 15°C for 6wk and 20 were incubated at 4°C for 6wk followed by 15°C for 4wk, or followed by no additional temperature treatment in the case of tubers from the 1976 crop inoculated with *F. sulphureum*. Inoculations were repeated in February.

The standard inoculation procedure was as follows. Each tuber was wounded (7mm diam. x 7mm deep), midway between the heel and rose ends and on opposite sides, using a sterilised glass rod (McKee & Boyd, 1952).

A 10µl drop of spore suspension containing *c.* 100 macroconidia of *F. solani* var. *coeruleum* or *F. sulphureum* was introduced into each wound using an automatic micro-pipette (Jencon's 2ml Repette). The inoculum was prepared from fungal isolates maintained in sterilised soil.

To maintain a relatively high humidity during incubation the tubers were placed on damp peat in small cardboard boxes (10 tubers per box) and these were placed in larger boxes lined with damp paper. Initially, the relative humidity was about 95%. After incubation the tubers were cut and the number of lesions out of a possible 40 recorded. Overall results are expressed as susceptibility indices using the very susceptible variety Catriona as a standard with an index of 100.

The effect of temperature on germination and growth rate *in vitro*

The percentage germination and growth rate of *F. solani* var. *coeruleum* and *F. sulphureum* were determined at 4°C and 15°C. Macroconidia were removed from 28 day-old cultures of *F. solani* var. *coeruleum* and *F. sulphureum*, washed 3 times in distilled water by centrifugation at 2500 rpm and sprayed onto Petri dishes containing Czapek Dox Agar (Oxoid). The spores were incubated at 4°C and 15°C and the percentage germination recorded at intervals. Four replicate lots of 100 spores were counted for each fungus and temperature level. Mycelial discs (0.4cm diam.) cut from the advancing mycelial front of 15 day-old cultures of *F. solani* var. *coeruleum* and *F. sulphureum* were used to inoculate Petri dishes containing Czapek Dox Agar. The fungi were incubated at 4°C and 15°C. Three replicate plates were used for each fungus and temperature level. At intervals colony growth was recorded by measuring the diameter of each colony along two lines at right angles.

## RESULTS

### Varietal susceptibility and the influence of temperature

The data for *F. solani* var. *coeruleum* and *F. sulphureum* infection are shown in Tables 36 and 37 respectively. Compared with Catriona most varieties lifted in 1975 showed a considerable degree of resistance to *F. solani* var. *coeruleum* infection when inoculated in November. In the following year infection levels were generally higher, but again all varieties showed some resistance compared with Catriona. The initial incubation of tubers at 4°C increased the susceptibility of most varieties to *F. solani* var. *coeruleum* infection. By February, in both years, all varieties with the exception of Pentland Ivory and Arran Banner (observed in 1976-77 only) were susceptible.

The results of the *F. sulphureum* inoculations showed that all varieties were susceptible to *F. sulphureum* infection at both inoculation dates and only Pentland Squire appeared to show any level of resistance. In some varieties infection with *F. sulphureum* tended to be reduced by incubation at 4°C.

Illustrations of *F. sulphureum* lesions in some varieties after 6wk incubation at either 15°C or 4°C are shown in Fig. 36. It is apparent that although the frequency of lesions may be occasionally reduced in some varieties by incubation at 4°C, tissue colonisation still proceeds rapidly at this temperature and a soft blackish lesion develops with some resemblance to frost damage and lesions caused by *F. solani* var. *coeruleum* (Fig. 37). This is in contrast to the mealy cavitated lesion which develops at higher temperatures and which may be confused with gangrene caused by *Phoma exigua* var. *foveata*.

Table 36. Tuber susceptibility to *F. solani* var. *coeruleum*

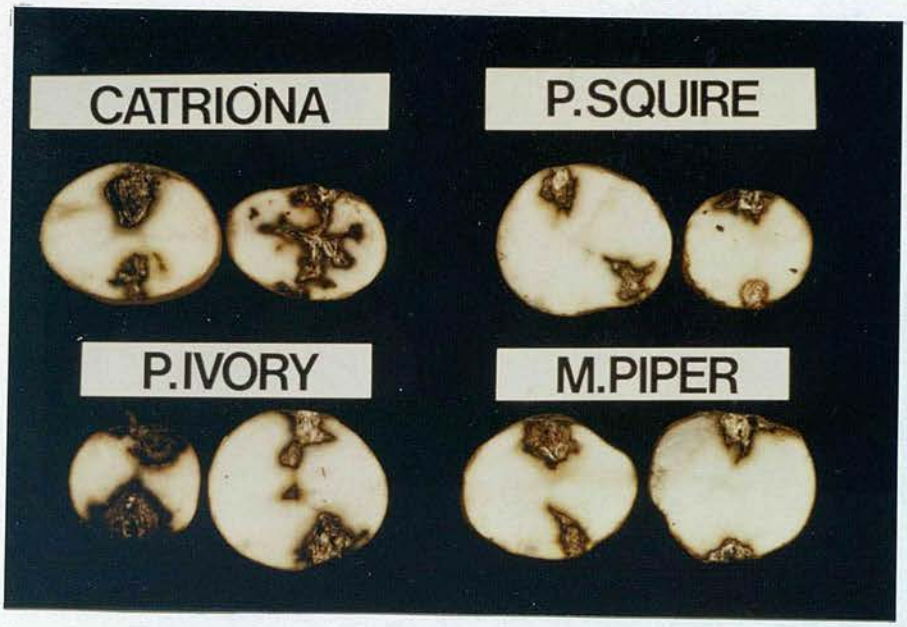
| Variety | Number of wounds with active rotting (max = 40) | | | | | | | | Susceptibility index Catriona = 100 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | November 1975 | | February 1976 | | November 1976 | | February 1977 | | Based on 1975-77 results | Based on 1976-77 results |
| | 15°C | 4°/15°C | 15°C | 4°/15°C | 15°C | 4°/15°C | 15°C | 4°/15°C | | |
| Catriona | 22 | 34 | 36 | 40 | 29 | 38 | 40 | 39 | 100 | 100 |
| Record | 15 | 18 | 34 | 37 | 6 | 20 | 33 | 39 | 73 | 67 |
| Desiree | 0 | 12 | 21 | 40 | 20 | 30 | 36 | 40 | 72 | 86 |
| Maris Piper | 2 | 16 | 40 | 40 | 10 | 17 | 36 | 40 | 72 | 71 |
| Pentland Squire | 0 | 7 | 32 | 40 | 17 | 26 | 34 | 33 | 68 | 75 |
| Pentland Crown | 3 | 9 | 39 | 36 | 15 | 20 | 33 | 28 | 66 | 66 |
| Ulster Sceptre | 8 | 7 | 38 | 39 | 4 | 17 | 31 | 40 | 66 | 63 |
| Pentland Hawk | 7 | 12 | 36 | 38 | 12 | 4 | 37 | 36 | 65 | 61 |
| Pentland Dell | 0 | 21 | 34 | 39 | 0 | 10 | 22 | 18 | 52 | 34 |
| Pentland Ivory | 0 | 0 | 10 | 14 | 1 | 3 | 6 | 18 | 19 | 19 |
| Stormont Enterprise | - | - | - | - | 5 | 31 | 40 | 40 | - | 79 |
| Ulster Concord | - | - | - | - | 3 | 24 | 34 | 39 | - | 68 |
| Arran Banner | - | - | - | - | 0 | 6 | 0 | 3 | - | 6 |

Table 37. Tuber susceptibility to *F. sulphureum*

| Variety | Number of wounds with active rotting (max = 40) | | | | | | | Susceptibility index Catriona = 100 | |
|---|---|---|---|---|---|---|---|---|---|
| | November 1975 | February 1976 | | November 1976 | | February 1977 | | Based on 1975-76 results | Based on 1976-77 results |
| | 15°C | 15°C | 4°/15°C | 15°C | 4°C | 15°C | 4°C | | |
| Catriona | 40 | 40 | 40 | 40 | 35 | 40 | 40 | 100 | 100 |
| Desiree | 38 | 36 | 40 | 34 | 39 | 37 | 40 | 96 | 97 |
| Pentland Dell | 38 | 40 | 38 | 35 | 38 | 38 | 37 | 96 | 95 |
| Pentland Hawk | 34 | 36 | 40 | 31 | 26 | 36 | 33 | 86 | 81 |
| Pentland Ivory | 36 | 38 | 37 | 36 | 26 | 28 | 36 | 86 | 81 |
| Ulster Sceptre | 40 | 37 | 31 | 28 | 26 | 34 | 37 | 85 | 81 |
| Record | 40 | 40 | 26 | 33 | 26 | 38 | 29 | 84 | 81 |
| Maris Piper | 29 | 40 | 35 | 22 | 28 | 31 | 29 | 78 | 71 |
| Arran Banner | - | - | - | 34 | 35 | 40 | 39 | - | 95 |
| Stormont Enterprise | - | - | - | 35 | 29 | 36 | 36 | - | 88 |
| Ulster Concord | - | - | - | 35 | 30 | 33 | 39 | - | 88 |
| Pentland Crown | 40 | 40 | - | 29 | 24 | 36 | 32 | - | 78 |
| Pentland Squire | 27 | - | - | 26 | 16 | 33 | 27 | - | 66 |

Fig. 36. Symptoms of infection by *F. sulphureum* in potato varieties incubated at different temperatures

36.1 15°C for 6wk




36.2 4°C for 6wk

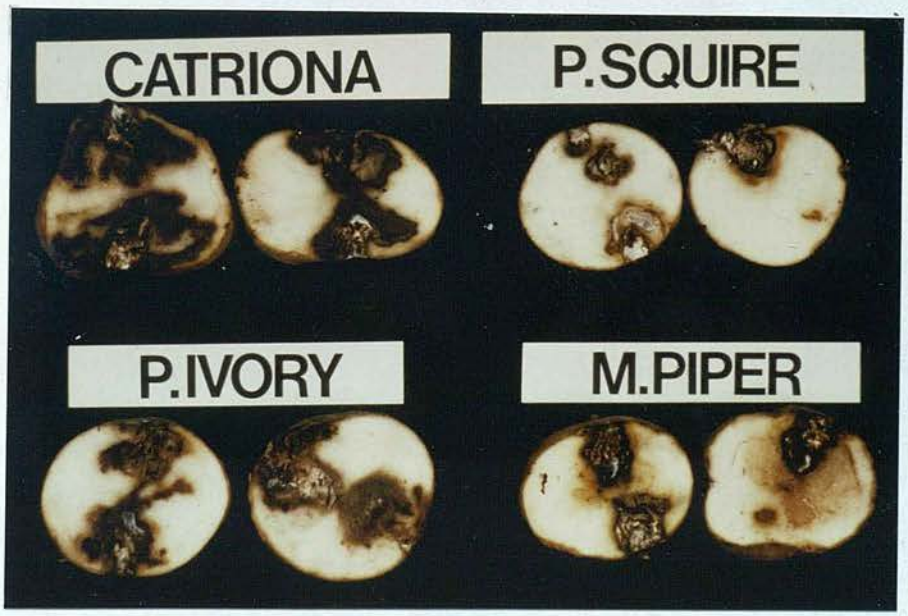
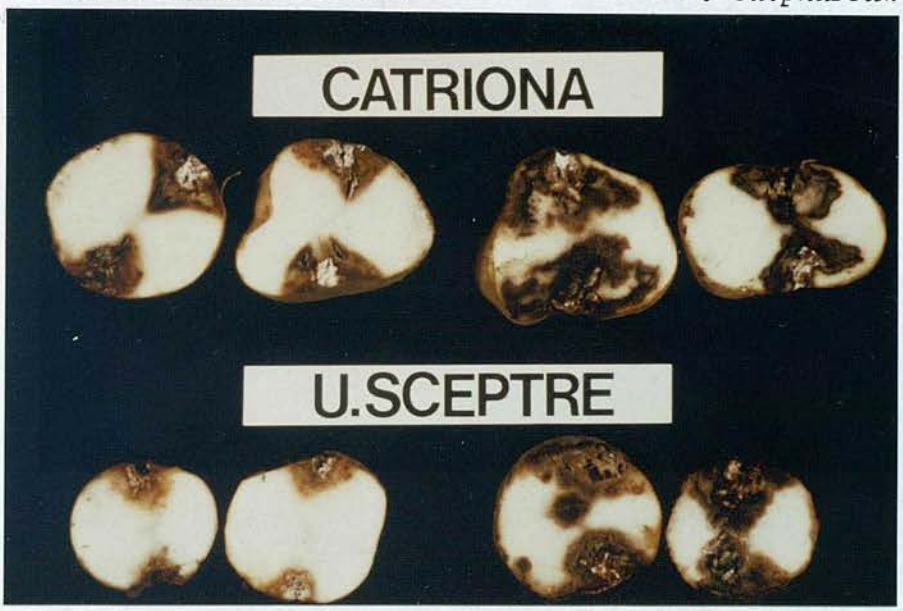


Fig. 37. Symptoms of infection by *F. solani* var. *coeruleum*[a] and *F. sulphureum* after incubation at 4°C for 6wk

*F. solani* var. *coeruleum* *F. sulphureum*




[a] Incubation at 4°C followed by incubation at 15°C for 2wk.

Effect of temperature on germination and growth rate *in vitro*

Germination of *F. solani* var. *coeruleum* and *F. sulphureum* macroconidia on Czapek Dox medium was most rapid at the higher temperature with most having germinated by 18h. (Table 38). At 4°C *F. solani* var. *coeruleum* macroconidia germinated faster than those of *F. sulphureum* and most had germinated by the time the experiment was terminated. In contrast 23% of the *F. sulphureum* macroconidia had not germinated by 66h.

Table 38. Percentage germination of *Fusarium* macroconidia after various incubation times at two temperatures

| Temperature (°C) | *F. solani* var. *coeruleum* | | | *F. sulphureum* | | |
|---|---|---|---|---|---|---|
| | Hours | | | Hours | | |
| | 18 | 40 | 66 | 18 | 40 | 66 |
| 4 | 58 | 75 | 96 | 4 | 6 | 77 |
| 15 | 99 | - | - | 99 | - | - |

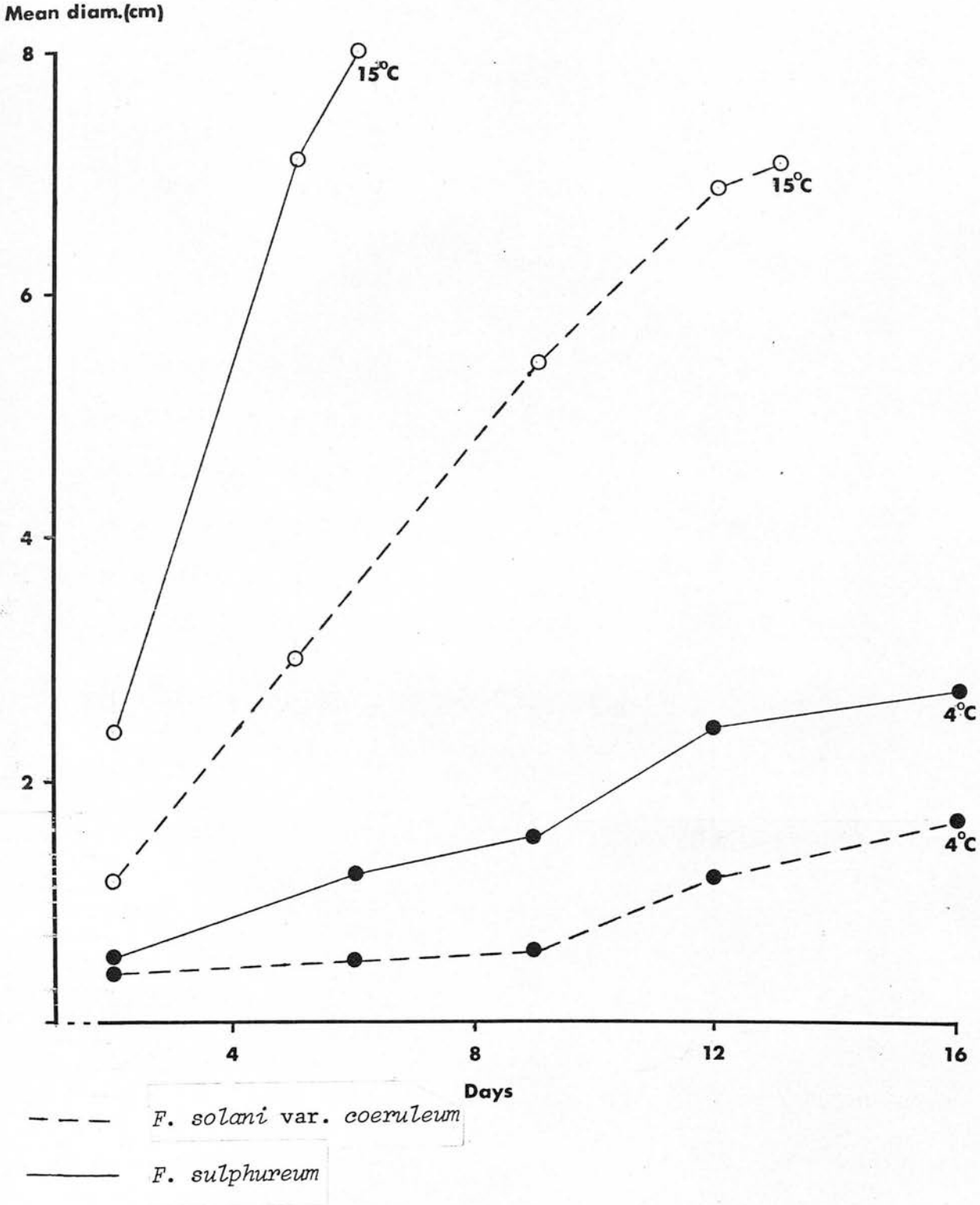
Mycelial growth of both fungi was more rapid at 15°C than 4°C and at both temperatures the isolate of *F. sulphureum* grew faster than did the isolate of *F. solani* var. *coeruleum* (Fig. 38).

DISCUSSION

The overall resistance of tubers to wound pathogens such as *F. solani* var. *coeruleum* and *F. sulphureum* is complicated but may be regarded as equivalent to resistance to mechanical damage (ie skin resistance), resistance to fungal growth (ie biochemical resistance) and an interaction of the two (Wellving, 1975; 1976).

The inoculation method used in the present study assesses biochemical resistance (Boyd 1952c). Resistance depends on the ability

Fig.38. Mycelial growth of *F. solani* var. *coeruleum* and *F. sulphureum* on Czapek Dox Agar at two incubation temperatures.



of a proportion of tubers within a variety to localise the fungal infection in an area which is surrounded by suberized tissue and wound periderm (Weiss, Lauritzen & Brierley, 1928). The pathogens remain viable but inactive (Boyd, 1952a; Schoene, 1967; Wellving, 1976; Langerfeld, 1977) since they do not cause active lesions unless the periderm is damaged later in the season. This is in contrast to the latent gangrene lesions mentioned by Todd & Adam (1967) which may become active without further damage (Langerfeld, 1977).

From the present study, it is apparent that the varietal ranking orders of resistance to *F. solani* var. *coeruleum* and *F. sulphureum* infections are markedly different. This supports evidence obtained in the USA (Weiss *et al*, 1928), Canada (Ayers, 1956; 1962) and Sweden (Wellving, 1976). However, whereas these authors found some varieties with a high level of resistance to *F. sulphureum* infection, in the present work no variety was found to reduce the incidence of infection to less than 50%. Even Arran Banner and Pentland Ivory, very highly resistant to *F. solani* var. *coeruleum* infection throughout the storage period, were susceptible to *F. sulphureum* infection.

Tuber maturity, with respect to length of storage, markedly affected susceptibility of varieties to *F. solani* var. *coeruleum* infection. Varieties which were judged to be resistant in November were susceptible by February with the exception of Arran Banner and Pentland Ivory. The practical aspect of these results is seen when supposedly resistant tubers are graded in the spring and extensive rotting occurs before planting or causes a high incidence of blanking in the field. The late season diminution in inter-varietal differences indicates that testing of varieties should be carried out both in November and February. Increased susceptibility of tubers to infection

by *F. solani* var. *coeruleum* with time of storage has also been observed by Pethybridge & Lafferty (1917), Moore (1924), Lansade (1949) and Boyd (1952b) but not by Weiss *et al* (1928). Although increasing susceptibility was associated with an increase in reducing sugars the two factors were not directly related (Boyd, 1967).

Resistance to *F. sulphureum* has been reported to increase with storage (Langerfeld, 1977) but this was not confirmed by the present results as infection levels were very high at both times of inoculation.

By using lower levels of inoculum it might be possible to detect differences in varietal or seasonal susceptibility to *F. sulphureum* infection. In previous work (Section 2.2) regression data from inoculum-density disease curves indicated a similar relationship between number of *F. solani* var. *coeruleum* or *F. sulphureum* propagules in soil and incidence of disease in the var. Catriona. Thus tubers became more susceptible to *F. sulphureum* infection with tuber storage. It would be useful to obtain inoculum-density disease curves for other potato varieties since they may prove helpful in discriminating between similar and dissimilar mechanisms of disease resistance.

In discussing the trend in recent years towards an increase and decrease in the levels of gangrene and dry rot respectively in farming practice, it is sometimes suggested that gangrene has been favoured by low temperatures which are now attained in bulk stores, whereas dry rot is favoured by higher temperatures (Booth, 1970; Wellving, 1976). In fact both *F. solani* var. *coeruleum* and *F. sulphureum* tolerate low temperatures with germination of macroconidia and slow mycelial growth occurring at $4^{o}C$ *in vitro*. The rate of germination

for *F. sulphureum* was found to be lower than for *F. solani* var. *coeruleum* confirming the work of Cunningham & Reinking (1946), but this does not appear directly to affect the pathogen's capacity to cause infection at 4°C. Weiss *et al* (1928) ascertained that the lowermost limit of infection by *F. solani* var. *coeruleum* and *F. sulphureum* was 0°C. Low temperature incubation after inoculation increased the susceptibility of tubers to infection by *F. solani* var. *coeruleum*, as also found by Boyd (1952d) and Langerfeld (1973), so that some varieties which were classified as resistant after incubation at 15°C were susceptible after initial incubation at 4°C. The results also suggest an interaction between varietal susceptibility and incubation temperature since the level of disease was more influenced by different incubation temperatures in Pentland Dell, Stormont Enterprise and Ulster Concord than in other varieties. Susceptibility tests should be therefore conducted at temperatures likely to be experienced in practice and tests made at 15°C only may no longer be appropriate.

Low incubation temperatures have been found to increase tuber susceptibility to *F. sulphureum* (Langerfeld, 1973; 1977) but this was not observed in the present experiments where disease incidence was generally very high. Instead low temperatures tended to reduce the susceptibility of some varieties and this was also shown by Langerfeld, (1973; 1977).

Temperature also affects growth of *F. solani* var. *coeruleum* and *F. sulphureum* in tuber tissue; both are favoured by higher temperatures. However, the optimum temperature for growth *in vitro* need not be the same as *in vivo*. The optimum temperature for growth of *F. solani* var. *coeruleum* on agar was 20°C but 15°C in tuber tissue (Moore, 1945). Under conditions of low temperature storage, lesions may take a

considerable time to develop (Boyd, 1952c; Wellving, 1976). The dual temperature regime used in these experiments enables a fairly rapid assessment of susceptibility to *F. solani* var. *coeruleum* to be made for conditions likely to be encountered during grading.

Tubers infected with *F. sulphureum* and incubated at 4°C developed symptoms within 6 wk without further incubation at 15°C. With *F. sulphureum*, lesions extend beyond the zone of infection (Langerfeld, 1973) and in some instances lesions were of greater size than after continuous incubation at 15°C for a comparable length of time. From a range of enzymes produced by *F. sulphureum in vitro* (ie pectinmethyl-esterases, polygalacturonases, cellulases and proteases) only the cellulases were active at 4°C (Roeb, Stegemann & Langerfeld, 1977). These were also active in *F. solani* var. *coeruleum* but it appears that the activity of *F. sulphureum* cellulase has a lower optimum temperature than that for *F. solani* var. *coeruleum* and it is produced more rapidly. Moreover, *F. sulphureum* tends to limit polygalacturonase synthesis in favour of cellulase synthesis. In addition protease activity is higher for *F. sulphureum* than *F. solani* var. *coeruleum* (Roeb *et al*, 1977). Whether differences in host symptoms under varying environmental conditions, or the increased pathogenicity and virulence of *F. sulphureum* as compared with *F. solani* var. *coeruleum* may be explained by differences in the pathogens enzyme production, differences in growth rate, or the observation that *F. solani* var. *coeruleum* grows intercellularly (McKee, 1954) and *F. sulphureum* intercellularly and intracellularly (Pett, 1977) requires further work.

In practice symptoms of *F. sulphureum* infection may be confused with those of *F. solani* var. *coeruleum*, *F. culmorum*, *F. sambucinum* and *P. exigua* var. *foveata*. It is interesting to note that symptoms described for *F. roseum* (Lk) var. *sambucinum* Snyder and Hansen (= *F. sambucinum* (Booth 1971)) at 30-70% r.h. and 80-100% r.h. (Mao &

Huguelet, 1971) are similar to those found for *F. sulphureum* at 15°C and 4°C respectively.

Much emphasis has been placed on wound healing (ie suberization and wound periderm formation (Priestley & Woffenden, 1923) in relation to the resistance of potato tubers to infection by *Fusarium* spp. This is because localised lesions are always surrounded by wound periderm and treatments which delay wound healing such as low temperatures (Priestley & Woffenden, 1923; Artschwager, 1927; Wigginton, 1974; Ali, Nelson & Freeman, 1975), antibiotics (Bonde & Malcolmson, 1956; Bonde & Hyland, 1960), methyl ester of naphthalenacetic acid (Cunningham, 1953) and *iso*-propylphenylcarbamate (McKee, 1955) increase susceptibility to *Fusarium* infection. Moreover, when ethanol and chloroform are applied to potato tubers the tubers lose their capacity to form wound periderm and become more susceptible to attack by *Fusarium* spp. which do not normally infect tubers (Wood, 1967).

In the UK, the Potato Marketing Board recommend farmers to cure tubers for a minimum of 2wk at *c.* 15°C (Anon, undated) in order to speed wound healing and reduce the incidence of tuber diseases. A wound barrier may be effectively formed within 7-14 days in a cut wound but 14-21 days in a crush wound (Anon, 1975b). Conditions which do not appear to be related to wound periderm formation do, however, affect the incidence of dry rot. For example, variations in r.h. above *c.* 70% have little effect on wound periderm formation (Wigginton, 1974) and yet tubers cured at 16°C, 75-80% r.h. develop markedly less dry rot than tubers cured at 95-100% r.h. and the level of dry rot was similar to that in tubers stored at 4°C or 8°C (Henriksen, 1975). For this reason Henriksen (1975) recommended that only 'dry' tubers should be cured.

Under conditions of low humidity it may be that germination of *F. solani* var. *coeruleum* propagules is inhibited, since in *in vitro* experiments Schneider (in Griffin, 1963) found that at 89% r.h., 20°C, the latent period for germination of macroconidia was 8wk. Increasing the latent period for germination in effect would be similar to delayed *F. solani* var. *coeruleum* inoculations of wounded tuber tissue, where incidence of dry rot decreases with time from wounding (McKee, 1954; Langerfeld, 1973) because of the formation of wound periderm or accumulation of substances toxic to growth of the fungus. It must be stated, however, that in practice the chlamydospore is probably the most abundant propagule type and the effect of humidity on its germination has not been studied. In addition, studies on the effect of humidity on wound periderm formation or incidence of dry rot, neglect the microenvironment of the wounded tissue where the humidity may be higher than that surrounding the tuber (Weiss *et al*, 1928). This makes subjective assessments of the effects of humidity difficult.

Differences in the rate of suberization and wound periderm formation between varieties have been reported (Priestley & Woffenden, 1923; Weiss *et al*, 1928) and are more clearly expressed at temperatures below 10°C than 15°C (Artschwager, 1927; Ali *et al*, 1975). In contrast Nielsen (1968) found no differences between varieties in wound periderm formation. Attempts have been made to relate the rate of wound periderm formation to tuber susceptibility (Weiss *et al*, 1928; Ali *et al*, 1975) but Ullrich (in Langerfeld, 1977) has shown that a variety with the lowest rate of periderm formation was most resistant and a variety which was susceptible had almost the highest rate of periderm formation. These results would seem to discount the significance of wound periderm formation in resistance to *F. solani* var. *coeruleum*. The importance of wound

periderm has also been questioned by McKee (1954), Radtke (1969), Kranz (1958) and Wellving (1976).

Wound healing is a complex process and factors which affect wound healing may alter any one of a number of physiological pathways such as the tricarboxcyclic acid cycle (Lange, Kahl & Rosenstock, 1971) or fatty acid synthesis (Willemot & Stumpf, 1967a; 1967b) which may also be involved in the synthesis of primary resistance compounds. Low temperatures for example not only inhibit wound periderm formation but the whole metabolism of the tuber.

Some substances present in the tuber before infection, eg chlorogenic acid (Bate-Smith, 1956), caffeic acid (Wellving, 1976) and some produced after infection, eg rishitin (Metlitskii & Ozeretskovskaya, 1970) inhibit the growth of either *F. solani* var. *coeruleum* or *F. solani* *in vitro*. In addition, Metlitskii (in Wellving, 1976) found that oxidised chlorogenic acid was considerably more inhibitory to germ tube growth of *F. solani* than chlorogenic acid. Wellving (1976) found that caffeic acid and chlorogenic acid were associated with resistance of potato tubers but the correlations between the levels of phenols and resistance were variable. In other studies neither solanine or chaconine (McKee, 1961), sugar content (Moore, 1924; Boyd, 1967; Radtke, 1969), osmotic pressure, proline content (Radtke, 1969) nor orthodihydric phenols (Griffin, 1964) were directly associated with resistance.

Clearly the mechanism of tuber resistance to *Fusarium* pathogens still requires clarification and cannot be solely accounted for by wound periderm formation.

The results of Boyd (1952c) suggest that for most varieties the laboratory tuber inoculation method, which does not take account of skin resistance, gives an adequate description of the susceptibility rating of varieties to infection by *F. solani* var. *coeruleum*. However, in farming practice where most damage occurs on the riddle (Foister, Wilson & Boyd, 1952) resistance to skin abrasions may decrease the ranking order of varietal susceptibility of some varieties such as Golden Wonder and Dunbar Standard (Boyd, 1952c). Although there appear to be varietal differences in susceptibility to damage due to inherited rheological (plasticity and elasticity of cells) characteristics (Blight & Hamilton, 1974) the problem remains that very big differences in damage levels occur between harvests, depending on the type of harvester (Foister *et al*, 1952) and soil and weather conditions. Dry sandy soil does not cushion the tubers as well as wet clay soil (Hesen & Kroesbergen, 1960) and leads to increased damage and dry rot (Ayers & Ramsay, 1961). Big differences also occur between riddles, a bare wire screen causing more damage than one coated with rubber or plastic (Foister *et al*, 1952).

Avoidance of superficial damage depends to a considerable extent on the tubers being fully mature when harvested (Howard, 1974). The effects of lifting and riddling immature tubers are often seen in early crops in a high incidence of dry rot, although early varieties left to mature tend to be more resistant to mechanical damage than late varieties (Hunnius & Fuchs, 1970). Since the extent of tuber damage may vary considerably, skin resistance is possibly not to be relied upon in practice. Nevertheless Umaerus & Umaerus (1976) consider that varietal differences in rheological characteristics due to genetic characteristics are so striking that selection for disease resistance

using methods to assess for mechanical damage should prove profitable.

Besides affecting crop loss, tuber resistance is a very important factor in the epidemiology of tuber-borne fungi. It affects the transmission aspect of epidemiology in two ways: (1) there is less chance of planting infected tubers (a primary source of fungal propagules), and (2) in the case of *F. solani* var. *coeruleum* the fungal inoculum produced by infected tubers of the moderately resistant Pentland Crown was less than that produced by the highly susceptible Catriona (Section 4.2). In both these ways contamination of progeny tubers and field soil is reduced. It is interesting to note that the decline in the importance of dry rot caused by *F. solani* var. *coeruleum* coincided in Scotland, with the reduction in acreage of the very susceptible variety Doon Star and the introduction of more resistant varieties. In view of this, the lack of biochemical resistance shown by a fairly common range of varieties to *F. sulphureum* infection at both 4°C and 15°C suggests a potentially dangerous situation if other factors do not interact to modify the disease potential. One of these factors might be skin resistance since the standard riddle abrasion test, which has been used to assess for skin resistance (Boyd, 1952c), shows that *F. sulphureum* is less able than *F. solani* var. *coeruleum* to cause infection in the var. Catriona despite being at a higher inoculum level (Table 39). The progeny tuber contamination index (PTCI) was higher for *F. sulphureum* than *F. solani* var. *coeruleum* indicating that *F. sulphureum* is able to infect through the type of wound (7mm diam. x 7mm depth) used in this test. Perhaps *F. sulphureum* is less able to establish itself in slightly damaged areas of the epidermis than *F. solani* var. *coeruleum*. Wellving (1976) has shown this to be the case for *P. exigua* var. *foveata* when compared with *F. solani* var. *coeruleum*.

Table 39. Relationship between contamination of progeny tubersphere soil with *F. solani* var. *coeruleum* and *F. sulphureum* and the incidence of dry rot after grading var. Catriona

| Seed treatments | *F. solani* var. *coeruleum* | | | *F. sulphureum* | | |
|---|---|---|---|---|---|---|
| | Progeny contamination | | % Dry rot after grading | Progeny contamination | | % Dry rot after grading |
| | Propagules per g soil | % PTCI | | Propagules per g soil | % PTCI | |
| Untreated | 4.46 | 66.0 | 66.4 | 4.73 | 78.1 | 32.2 |
| MEMC | 4.32 | 58.4 | 52.4 | 4.67 | 67.3 | 28.4 |
| TBZ spray (2% a.i.) | 4.14 | 52.6 | 44.8 | 4.65 | 66.5 | 25.2 |

Results are the means of the transformed data from four replicates: propagules/g soil to $\log_{10}$ (propagules/g soil +1); PTCI (%) and dry rot (%) to arcsin.

It appears that the cortex is less susceptible than the pith to infection by *Phoma* spp. (Kranz, 1958; 1959) and this also seems to be the case for *F. sulphureum*. With both pathogens this occasionally leads to the development of hidden rots where after infection fungal growth continues under the cortex, but growth is much more extensive for *F. sulphureum* than *P. exigua* var. *foveata*. However, experimental evidence (Lansade, 1949; Boyd 1952a) indicates that the pith is also more susceptible than the cortex to infection by *F. solani* var. *coeruleum* although no visual differences are apparent externally.

Resistance to *F. solani* var. *coeruleum* and *F. sulphureum* depends on many interrelated factors which may be modified by the environment to make otherwise resistant varieties (as determined by standard inoculation tests) susceptible. Growers are now encouraged to burn down the haulm and lift early in order to reduce the incidence of aphid transmitted viruses and gangrene. It remains to be seen what affect this will have on the field susceptibility of tubers to dry rot and hence the transmission of the causal organisms.

# SECTION 6

# CONCLUDING DISCUSSION

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Investigations on the ecology of plant pathogens in soil has been hindered because of inadequate methods of quantifying populations. One of the primary objectives of the present study was to develop a selective medium for isolation of the potato dry rot fungus, *F. solani* var. *coeruleum*, and the potato powdery dry rot fungus, *F. sulphureum*, and then to study factors which might affect their transmission. Such studies are of practical interest in relation to the production of seed tubers free from dry rot. Also important is an assessment of the potential risk of *F. sulphureum*, which was first discovered infecting potatoes in Great Britain as recently as 1971 (Boyd & Tickle, 1972).

The medium developed for the selective isolation of *F. solani* var. *coeruleum* and *F. sulphureum* from soil, designated the PAB medium, has as its main selective ingredients pentachloronitrobenzene and 2-aminobutane (Section 2.1). De Bokx & Mooi (1974) have indicated the desirability of a method for identifying stocks of seed potatoes contaminated with dry-rot fungi early in the storage season. Visual inspection is of little use since symptoms of the disease may not be evident at this time. The PAB medium/soil-dilution plate method should enable contaminated stocks to be identified by detection of *F. solani* var. *coeruleum* or *F. sulphureum* in progeny tubersphere soil before or after lifting. Experiments under standard conditions indicate that disease risk may be calculated from the density of inoculum in tubersphere soil (Section 2.3). In practice, however, the degree of damage and susceptibility of tubers to infection are possibly more important than the density of inoculum as factors affecting disease incidence.

For example, it is suspected that tubers lifted too soon after destruction of the haulm, and then riddled, or riddled late in the storage season will develop severe dry rot despite low inoculum densities.

In addition to soil isolation, methods are required to isolate potato pathogens, (eg *P. exigua* var. *foveata*, *F. solani* var. *coeruleum* and *F. sulphureum*) from tuber tissue. The PM70 medium developed by Tickle (1974) seems ideal, and in the region of 16 tissue segments may be plated per 9 cm Petri dish, whereas for a non-selective medium this figure is much less (Section 2.1).

Healthy seed tubers free from dry rot can be produced initially from virus-tested stem-cutting material, but as the bulking-up process continues through foundation stock to AA stock the tubers are continually open to re-contamination and re-infection. The most likely source of this contamination, providing adequate sanitary precautions are taken within the store, appears to be field soil since *F. solani* var. *coeruleum* has been shown to survive a 6 year rotation, albeit at low levels (Section 3.1). It would be very difficult to prove if *F. solani* var. *coeruleum* is a natural inhabitant of field soils, or is present only where potatoes have been grown, since isolation depends on the sensitivity of the method of detection. The latter would seem more probable, however, for reasons stated in Section 3.1.

Soil-borne inoculum in fields, stores or on potato handling equipment can lead to contamination of a 'clean' stock. The primary source of propagules is, however, the infected seed tuber, as compared with contaminated seed or field soil contaminated before planting (Section 3.2). Planting of infected seed is to be avoided since this increases the risk of disease in the progeny and the number of propagules available for survival through the rotation to contaminate 'clean' stocks.

Although infected seed is not planted intentionally, the procedure of grading out seed prior to planting and associated sprout removal is still occasionally carried out, despite the very high susceptibility of tubers to infection by *F. solani* var. *coeruleum* at this time. Another perhaps less obvious route of infection is through damage caused by the planter. The use of chitted seed might be expected to increase transmission of dry rot as sprouts may be easily detached from the tuber by rough handling: this requires further investigation.

The role of the infected seed tuber as the primary source of propagules seems quite clear. Removal of infected seed after plant establishment prevented further increase in the levels of soil contamination, indicating that no other food base present in soil was capable of supporting sporulation of the fungus (Section 4.1). The infected seed tuber is a possible source of food for other soil micro-organisms and complete decomposition of the seed tuber in soil by soft-rot bacteria may be observed in some years. Thus, it might be expected that competition with these organisms might affect propagule production, particularly since small seed tended to produce less inoculum than large seed. Inoculation of infected seed tubers with *E. carotovora* var. *carotovora*, however, appeared to have no effect on the population of *F. solani* var. *coeruleum*, possibly because the method did not produce a satisfactory level of soft-rotting (Section 4.1). Further investigations are therefore warranted. In contrast, inoculation of *F. solani* var. *coeruleum* and *F. sulphureum* together markedly reduced the *F. solani* var. *coeruleum* but not the *F. sulphureum* population, compared with populations from tubers infected with one fungus (Section 5.1). This effect has been confirmed in more recent investigations and preliminary experiments indicate that *F. sulphureum* may inhibit pustule formation by *F. solani* var. *coeruleum* on the surface of the infected seed tuber (not reported).

Potato varieties differ in their resistance to *F. solani* var. *coeruleum* infection and in the amount of inoculum produced by infected seed. The reason for the difference in propagule production is not clear but may be related to the relative rates of substrate colonisation by the pathogen and secondary invaders in the different varieties (Section 4.2).

It has often been recorded that dry rot is more serious after warm dry growing conditions. Several reasons have been suggested to explain this. Lansade (1949) considered that dry matter of the tuber was important, whereas Mooi (1950) thought this related to the warm dry growing season advancing the rate of tuber maturity and susceptibility. Additionally, the present work has suggested that these conditions are more favourable than cold wet conditions for inoculum production (Section 5.1).

Besides inoculum production, the position of the progeny in relation to the seed also affected transmission before harvest. Tubers formed nearest the seed were most contaminated whereas those formed further away were often uncontaminated. This has little practical significance as regards using long-stoloned varieties to reduce transmission since harvesting operations distributed inoculum to all tubers (Section 4.2).

*F. solani* var. *coeruleum* propagules are derived from pustules on the surface of the tuber or from pustules in cavities in the tuber which are exposed as the tuber decomposes. Contamination of field soil appears to occur quite quickly after planting and continues to increase throughout the growing season (Section 4.1). Although some progeny tubers were contaminated by growing in contact with the seed, the method by which *F. solani* var. *coeruleum* is able to spread through

soil is uncertain. The finding of few propagules above the seed tuber, but with many below and with the greatest lateral spread near the inoculum source, strongly suggests the passive movement of propagules in soil water (Section 4.1). However, the possible involvement of earthworms in dispersal (Tickle, 1974) is not discounted.

In Great Britain, the last 25 years has seen a decrease in the importance of dry rot (*F. solani* var. *coeruleum*) and an increase in the importance of gangrene. The reason for this change has not been fully explained, probably because of the lack of satisfactory methods for the study of factors which affect changes in population of these fungi. The change did, however, coincide with certain changes in farm practice (Section 1).

The possibility of change in the disease status of a pathogen has particular relevance to the present work since *F. sulphureum*, which was first reported infecting potatoes as recently as 1971, is more virulent than the indigenous dry rot fungus *F. solani* var. *coeruleum*. Of 13 varieties none was resistant to *F. sulphureum* infection, whereas most were resistant to *F. solani* var. *coeruleum* infection early in the storage season when grading, the process which causes the damage through which most infection occurs, is carried out (Section 5.2).

It is desirable, therefore, to study the ecology of *F. sulphureum* to determine whether conditions in Scotland are favourable for its transmission.

Despite laboratory inoculation tests indicating that varieties grown in Great Britain are more susceptible to *F. sulphureum* than *F. solani* var. *coeruleum* infection, results may not be applicable to

commercial practice where most damage is caused by the riddle. For example, in the var. Catriona levels of inoculum were greater for *F. sulphureum* than *F. solani* var. *coeruleum* but the incidence of powdery dry rot (*F. sulphureum*) was much less than the incidence of dry rot (*F. solani* var. *coeruleum*)(Section 5.2). Clearly, although resistance to damage (= skin resistance) has been shown to be an unimportant factor commercially in the resistance of most varieties to *F. solani* var. *coeruleum* infection, this requires closer examination in the case of *F. sulphureum*. Since skin resistance is affected by maturity of the tuber at lifting, haulm destruction dates and intervals to lifting require consideration. It is interesting to note that most cases of powdery dry rot have been found in early varieties (eg Arran Comet, Arran Pilot, Estima, Home Guard, Pentland Javelin, Ulster Sceptre, Vanessa and Wilja). However, some late varieties are also affected (eg Bintje, Desiree, Maris Piper, Pentland Crown, Pentland Dell and Redskin).

Other factors are also required for successful transmission. The capacity of infected seed to produce high numbers of propagules is important since this determines the numbers of propagules available for survival through the rotation. Survival through the rotation is essential if the pathogen is to re-contaminate 'clean' stocks. With both *F. solani* var. *coeruleum* and *F. sulphureum* the capacity to produce propagules appears to depend on environmental conditions and in particular soil temperature.

It seems as if *F. sulphureum* is less well adapted than *F. solani* var. *coeruleum* to produce propagules under soil conditions in most years in Scotland. However, it has the potential to produce very

large numbers of propagules, as was seen in 1976 when there were relatively high soil temperatures (Section 5.1).

Whereas the infected seed tuber is the primary source of *F. solani* var. *coeruleum* inoculum, *F. sulphureum* inoculum may also come from infected potato stems (Section 5.1). In this respect *F. sulphureum* is similar to *P. exigua* var. *foveata*. The relative importance of inoculum from the seed and stem remains to be determined, as does the effect of using different haulm desiccants which have been shown in *Phoma* to significantly affect pycnidia production (Logan, Copeland & Little, 1976).

Survival through the rotation is essential if *F. sulphureum* is to re-contaminate 'clean' stocks. In this respect, *F. sulphureum* may be less successful than *F. solani* var. *coeruleum* since over 2 years *F. sulphureum* declined in population more markedly than *F. solani* var. *coeruleum* (Section 3.1).

It therefore appears that *F. sulphureum* is in some respects less well adapted for soil-borne transmission than *F. solani* var. *coeruleum*. It is difficult, however, to assess the possible importance of *F. sulphureum*. In 1976-1977, of 134 samples from Scottish seed stocks investigated by the Potato Marketing Board because of unsatisfactory disease levels, 51%, 18% and 13% were probably affected by *P. exigua* var. *foveata*, *F. solani* var. *coeruleum* and *F. sulphureum* respectively. In 1977-1978, corresponding percentages were 64, 12 and 8 for 275 samples (M.A. Ali, personal communication). It may be many years before *F. sulphureum* spreads to a sufficient number of potato stocks and reaches inoculum levels high enough to affect its status markedly as a tuber pathogen.

Pre-plant treatment of seed tubers with thiabendazole (TBZ) will reduce transmission of *F. solani* var. *coeruleum* and *F. sulphureum* in so far as surface contamination of the tuber will be eliminated and therefore the risk of infection through damage caused prior to planting. The fungicide, however, has no eradicant action against established lesions but in the case of *F. solani* var. *coeruleum* some reduction in propagule transmission and hence incidence of disease in the succeeding crop may still be expected (Section 5.1). This is because TBZ reduces sporulation of the fungus on the surface of the tuber. However, as the mode of production of *F. sulphureum* propagules is different from that for *F. solani* var. *coeruleum* (ie little sporulation on the tuber surface but sporulation on stems) reduction in transmission of *F. sulphureum* was inconsistent.

Application of TBZ for the control of dry rot and powdery dry rot is most effective when carried out immediately after harvest. In the short term the increasing use of TBZ may reduce or eliminate tuber contamination with *F. solani* var. *coeruleum* and *F. sulphureum* and therefore the risk of planting infected tubers. In the long term, if field soil is no longer to be subjected to re-contamination, the populations of the pathogens in a field soil should decline. The use of fungicides to maintain seed stocks free from pathogens should therefore be encouraged, but the development of races of fungi, resistance to TBZ, for example, cannot be ruled out.

# BIBLIOGRAPHY

## BIBLIOGRAPHY

AGRAWAL, S.C., KHARE, M.N. & KUSHWAHA, L.S. (1973). A selective medium for isolation and quantitative estimation of *Fusarium* in soil. *Science and Culture 39*, 555-556.

ALI, S.A., NELSON, D.C. & FREEMAN, T.P. (1975). Suberization and periderm development in Norchief and Red Pontiac potatoes. *American Potato Journal 52*, 201-209.

ANON. (undated). *Potato storage*. 14 pp. London: Potato Marketing Board.

ANON. (1948). Chemical prevention of potato dry rot disease. *Nature, London 162*, 843.

ANON. (1969). 2-aminobutane. In *World Review of Pest Control 8*, No. 2, Supplement Guide to New Compounds.

ANON. (1974). Pre-harvest assessment of latent storage diseases. In *Sutton Bridge Experimental Station Annual Review, 1973*, pp. 8-14. Potato Marketing Board.

ANON. (1975a). Pre-harvest assessment of latent storage diseases. In *Sutton Bridge Experimental Station Annual Review, 1974*, pp. 19-27, Potato Marketing Board.

ANON. (1975b). Curing and storage regimes in relation to lifting the crop. In *Sutton Bridge Experimental Station Annual Review, 1974*, pp. 27-34. Potato Marketing Board.

ANON. (1976a). Pre-harvest assessment of latent storage diseases. In *Sutton Bridge Experimental Station Annual Review, 1975*, pp. 6-12. Potato Marketing Board.

ANON. (1976b). *Seed potatoes — the control of aphid-borne virus diseases*. Publication No. 24. The Scottish Agricultural Colleges.

ANON. (1977). Pre-harvest assessment of latent storage diseases. In *Sutton Bridge Experimental Station Annual Review, 1976*, pp. 5-7. Potato Marketing Board.

ANON. (1978). *Potato haulm destruction*. Publication No. 32. The Scottish Agricultural Colleges.

ARTSCHWAGER, E. (1927). Wound periderm formation in the potato as affected by temperature and humdity. *Journal of Agricultural Research 35*, 995-1000.

AYERS, G.W. (1956). The resistance of potato varieties to storage decay caused by *Fusarium sambucinum* f.6 and *Fusarium coeruleum*. *American Potato Journal 33*, 249-254.

AYERS, G.W. (1962). The susceptibility of potato varieties to storage rots caused by *Fusarium sambucinum* f.6 and *Fusarium coeruleum* and to wilt caused by *Verticillium albo-atrum* in 1961. *Canadian Plant Disease Survey 42*, 115-117.

AYERS, G.W. (1972). *Fusarium* decay in potatoes. *Canada Agriculture 17*, 38-39.

AYERS, G.W & RAMSAY, G.C. (1961). Epidemiology of *Fusarium* storage rot of potatoes in Prince Edward Island. *Canadian Plant Disease Survey 41*, 199-202.

AYERS, G.W. & ROBINSON, D.B. (1954). An inoculation technique for the study of dry rot of potatoes. *American Potato Journal 31*, 278-281.

AYERS, G.W. & ROBINSON, D.B. (1956). Control of *Fusarium* dry rot of potatoes by seed treatment. *American Potato Journal 33*, 1-5.

AYTOUN, R.S.C. (1953). The genus *Trichoderma:* its relationship with *Armillaria mellea* (Vahl ex Fries) Quel. and *Polyporus schweinitzii* Fr., together with preliminary observations on its ecology in woodland soils. *Transactions and Proceedings of the Botanical Society of Edinburgh 36*, Part 2, 99-114.

BADGER, E.H.M. & PANKHURST, E.S. (1960). Experiments on the accuracy of surface drop bacterial counts. *Journal of Applied Bacteriology 23*, 28-36.

BAILEY, N.T.J. (1959). *Statistical Methods in Biology*. London: The English Universities Press.

BAKER, R. (1968). Mechanisms of biological control of soil-borne pathogens. *Annual Review of Phytopathology 6*, 263-294.

BAKER, R., MAURER, C.L. & MAURER, R.A. (1967). Ecology of plant pathogens in soil. VII. Mathematical models and inoculum density. *Phytopathology 57*, 662-666.

BALLESTA, J.P.G. & ALEXANDER, M. (1972). Susceptibility of several Basidiomycetes to microbial lysis. *Transactions of the British Mycological Society, 58*, 481-487.

BANNON, E. (1972). A study of skin spot of potatoes in Ireland. PhD Thesis, National University of Ireland.

BATE-SMITH, E.C. (1956). Report from the Low Temperature Research Station. In *Report of the Food Investigation Board, London, 1955*, pp. 53-62.

BICK, I.R.C. & RHEE, C. (1966). Anthraquinone pigments from *Phoma foveata* Foister. *Biochemical Journal 98*, 112-116.

BISBY, G.R. (1939). *Trichoderma viride* Pers. ex Fr. and notes on *Hypocrea*. *Transactions of the British Mycological Society 23*, 149-168.

BLIGHT, D.P. & HAMILTON, A.J. (1974). Varietal susceptibility to damage in potatoes. *Potato Research 17*, 261-270.

BOLKAN, H.A. & MILNE, K.S. (1975). Systemic uptake of four fungicides by potato plants. *Plant Disease Reporter 59*, 214-218.

BOMMER, D. & PATZOLD, C. (1972). Versuche zur Bekämpfung fusarien-bedingter Langerfaulen bei Kartoffeln. *Landbauforschung Volkenrode 22*, 123-128.

BONDE, R. & HYLAND, F. (1960). Effects of antibiotic and fungicidal treatments on wound periderm formation, plant emergence and yields produced by cut seed. *American Potato Journal 37*, 279-288.

BONDE, R. & MALCOLMSON, J.F. (1956). Studies in the treatment of potato seed pieces with antibiotic substances in relation to bacterial and fungus decay. *Plant Disease Reporter 40*, 615-619.

BOOTH, C. (1971). *The genus Fusarium*. London: Commonwealth Mycological Institute.

BOOTH, R.H. (1970). Tuber diseases of seed potatoes. *Journal of the National Institute of Agricultural Botany 12*, Supplement, 72-76.

BOYD, A.E.W. (1947). Some recent results of potato dry rot research. *Annals of Applied Biology 34*, 634-636.

BOYD, A.E.W. (1952a). Dry-rot disease of the potato. IV. Laboratory methods used in assessing variations in tuber susceptibility. *Annals of Applied Biology 39*, 322-329.

BOYD, A.E.W. (1952b). Dry-rot disease of the potato V. Seasonal and local variations in tuber susceptibility. *Annals of Applied Biology 39*, 330-338.

BOYD, A.E.W. (1952c). Dry-rot disease of the potato. VI. Varietal differences in tuber susceptibility obtained by injection and riddle-abrasion methods. *Annals of Applied Biology 39*, 339-350.

BOYD, A.E.W. (1952d). Dry-rot disease of the potato. VII. The effect of storage temperature upon subsequent susceptibility of tubers. *Annals of Applied Biology 39*, 351-357.

BOYD, A.E.W. (1960). Fungicidal dipping and other treatments of seed potatoes in Scotland. *European Potato Journal 3*, 137-154.

BOYD, A.E.W. (1967). The effects of length of the growth period and of nutrition upon potato-tuber susceptibility to dry rot *(Fusarium caeruleum)*. *Annals of Appled Biology 60*, 231-240.

BOYD, A.E.W. (1970). Transmission of dry rot *(Fusarium caeruleum)* by seed tubers and by soil. *Proceeedings of the 4th Triennial Conference of the European Association for Potato Research, 1969*, pp. 192-193.

BOYD, A.E.W. (1971). Contamination by *Fusarium caeruleum* and *Phoma exigua* var. *foveata* of soils associated with potato riddles. (Report of the Pathology Section Meeting in Munich, Germany). In *Potato Research 14*, 337.

BOYD, A.E.W. (1972). Potato storage diseases. *Review of Plant Pathology 51*, 297-321.

BOYD, A.E.W. (1974). The effect of incubation temperature upon the reaction of potato tubers to *Fusarium solani* var. *coeruleum*. (Report of the Pathology Section Meeting in Dundee, Scotland). In *Potato Research 17*, 352.

BOYD, A.E.W. & LOGAN, J.W.H. (1967). The effect of planting seed tubers affected by dry rot (*Fusarium caeruleum*) and gangrene (*Phoma foveata*). *Experimental Work of the Edinburgh School of Agriculture 1966*, pp. 25-26.

BOYD, A.E.W. & O'DONNELL, B.H. (1968). The effect of planting seed tubers affected by dry rot (*Fusarium caeruleum*) and gangrene (*Phoma foveata*). *Experimental Work of the Edinburgh School of Agriculture 1967*, pp. 37-38.

BOYD, A.E.W. & TICKLE, J.H. (1972). Dry rot of potato tubers caused by *Fusarium sulphureum* Schlecht. *Plant Pathology 21*, 195.

BRIAN, P.W. (1944). Production of gliotoxin by *Trichoderma viride*. *Nature, London 154*, 667-668.

BRIAN, P.W., CURTIS, P.J., HEMMING, H.G. & McGOWAN, J.C. (1946). The production of viridin by pigment-forming strains of *Trichoderma viride*. *Annals of Applied Biology 33*, 190-200.

BRIAN, P.W. & McGOWAN, J.C. (1945). Viridin: a highly fungistatic substance produced by *Trichoderma viride*. *Nature, London 156*, 144-145.

BYRDE, R.J.W. (1967). Nonaromatic organics. In *Fungicides 3* (Ed. D.C. Torgesson), pp 331-578. New York and London: Academic Press.

CAMPBELL, W.P. & GRIFFITH, D.A. (1974). Development of endoconidial chlamydospores in *Fusarium culmorum*. *Transactions of the British Mycological Society 63*, 221-228.

CLOUGH, K.S. & PATRICK, Z.A. (1972). Naturally occurring perforations in chlamydospores of *Thielaviopsis basicola* in soil. *Canadian Journal of Botany 50*, 2251-2253.

COCHRANE, V.W., COCHRANE, J.C., COLLING, C.B. & SERAFIN, F.G. (1963). Spore germination and carbon metabolism in relation to germination. *American Journal of Botany 50*, 806-814.

CUNNINGHAM, H.S. (1953). A histological study of the influence of sprout inhibitors on *Fusarium* infection of potato tubers. *Phytopathology 43*, 95-98.

CUNNINGHAM, H.S. & REINKING, O.A. (1946). *Fusarium* seed piece decay of potato on Long Island and its control. *Bulletin of the New York State Agricultural Experiment Station*. No. 721.

DE BOKX, J.A. & MOOL, J.C. (1974). Methods of quality assessment of seed potatoes. *Potato Research 17*, 410-433.

DENNIS, C. & WEBSTER, J. (1971a). Antagonistic properties of species-groups of *Trichoderma*. I. Production of non-volatile antibiotics. *Transactions of the British Mycological Society 57*, 25-39.

DENNIS, C. & WEBSTER, J. (1971b). Antagonistic properties of species-groups of *Trichoderma*. II. Production of volatile antibiotics. *Transactions of the British Mycological Society 57*, 41-48.

DENNIS, C. & WEBSTER, J. (1971c). Antagonistic properties of species-groups of *Trichoderma*. III. Hyphal interaction. *Transactions of the British Mycological Society, 57*, 363-369.

DHINGRA, O.D., TENNE, F.D. & SINCLAIR, J.B. (1976). Method for the determination of competitive saprophytic colonization of soil fungi. *Transactions of the British Mycological Society 66*, 447-456.

DICKINSON, C.H. & PARKINSON, D. (1970). Effects of mechanical shaking and water tension on survival and distribution of fungal inoculum in glass microbead media. *Canadian Journal of Microbiology 16*, 449-552.

DIMOND, A.E. & HORSFALL, J.G. (1965). The theory of inoculum. In *Ecology of Soil-borne Plant Pathogens. Prelude to Biological Control* (Eds K.F. Baker & W.C. Snyder), pp 404-415. Berkeley: University of California Press.

DUNCAN, J.M. (1976). The use of bait plants to detect *Phytophthora fragarie* in soil. *Transactions of the British Mycological Society 66*, 85-89.

EDGINGTON, L.V., KHEW, K.L. & BARRON, G.L. (1971). Fungitoxic spectrum of benzimidazole compounds. *Phytopathology 61*, 42-44.

EDWARDS, C.A. & LOFTY, J.R. (1972). *Biology of Earthworms*. London: Chapman and Hall.

EGDELL, J.W., CUTHBERT, W.A., SCARLETT, C.A., THOMAS, S.B. & WESTMACOTT, M.H. (1960). Some studies of the colony count technique for soil bacteria. *Journal of Applied Bacteriology 23*, 69-86.

FISHER, R.A. (1970). *Statistical Methods for Research Workers*, 14th ed. Edinburgh: Oliver and Boyd.

FOISTER, C.E. (1940). Dry rot diseases of potatoes. *Scottish Journal of Agriculture 23*, 63-67.

FOISTER, C.E. & WILSON, A.R. (1943). Dry rot in seed potatoes. A summary of some recent experiments. *Journal of the Ministry of Agriculture 50*, 300-303.

FOISTER, C.E., WILSON, A.R. & BOYD, A.E.W. (1945). Potato dry rot and gangrene as soil-borne diseases. *Nature, London 155,* 793.

FOISTER, C.E., WILSON, A.R. & BOYD, A.E.W. (1952). Dry-rot disease of the potato. I. Effect of commercial handling methods on the incidence of the disease. *Annals of Applied Biology 39,* 29-37.

FOX, R.A., DASHWOOD, E.P. & WILSON, H.M. (1971). Gangrene of potato. *Report of the Scottish Horticultural Research Institute for 1970* pp. 33-34.

FOX, R.A. & DASHWOOD, E.P. (1977). Some effects of time of haulm destruction and harvest on the incidence of potato gangrene. In *Proceedings of a Symposium on Problems of Pest and Disease Control in Northern Britain held at the University of Dundee, Scotland* (Ed. R.A. Fox), pp. 44-46. Dundee: The Scottish Horticultural Research Institute.

GRAHAM, D.C. & HAMILTON, G.A. (1970). Control of potato gangrene and skin spot diseases by fumigation of tubers with sec-butylamine. *Nature, London 227,* 297-298.

GRIFFIN, D.M. (1963). Soil moisture and the ecology of soil fungi. *Biological Reviews 38,* 141-166.

GRIFFIN, D.M. (1972). *Ecology of Soil Fungi.* London: Chapman and Hall.

GRIFFIN, G.J. (1964). Effect of chilling on the phenol metabolism and *Fusarium* infection of cut potato tissue. *Phytopathology 54,* 1275-1277.

GRIFFITHS, D.A. (1973). Fine structure of the chlamydospore wall in *Fusarium oxysporum. Transactions of the British Mycological Society 61,* 1-6.

HARDIE, J.L. (1970). *Potato growers' guide to clonal selection.* Department of Agriculture and Fisheries for Scotland.

HARTER, L.L. (1938). A root rot of peas caused by *Fusarium coeruleum. Phytopathology 28,* 432-438.

HENRIKSEN, J.B. (1975). Prevention of gangrene and *Fusarium* dry rot by physical means and with thiabendazole. *Proceedings of the 8th British Insecticide & Fungicide Conference 2,* 603-608.

HEPPLE, S. (1960). The movement of fungal spores in soil. *Transactions of the British Mycological Society 43,* 73-79.

HESEN, J.C. & KROESBERGEN, E. (1960). Mechanical damage of potatoes. I. *European Potato Journal 3,* 30-46.

HIDE, G.A., GRIFFITH, R.L. & ADAMS, M.J. (1977). Methods of measuring the prevalence of *Phoma exigua* on potatoes and in soil. *Annals of Applied Biology 87,* 7-15.

HIRST, J.M. (1977). Potato health in retrospect and prospect. *56th Report of the Scottish Plant Breeding Station*, pp. 114-118.

HORNBY, D. (1969a). Gravimetrical and mycological investigations of soil suspensions in the soil dilution plate technique. *Journal of Applied Bacteriology 32*, 244-258.

HORNBY, D. (1969b). Quantitative estimation of soil-borne inoculum of the take-all fungus (*Ophiobolus graminis* (Sacc.) Sacc.). *Proceedings of the 5th British Insecticide & Fungicide Conference 1*, 65-70.

HOWARD, H.W. (1974). Factors influencing the quality of ware potatoes. 1. The genotype. *Potato Research 17*, 490-511.

HUNNIUS, W. & FUCHS, G. (1970). Zur Prüfung der Vollerntevertäglichkeit von Kartoffelsorten. *Potato Research 13*, 305-322.

HUTCHINSON, S.A. & KAMEL, M. (1956). The effect of earthworms on the dispersal of soil fungi. *Journal of Soil Science 7*, 213-218.

IVASHCHENKO, O.V. (1963). Vplȳv zhivlennya na stiĭkist' kartopli proti sukhoyi (fuzarioznoyi) gnili pid chas zberigannya. *Visnȳk si'ls'ko-hospodars'koyi 7*, 62-65.

JAMES, N. & SUTHERLAND, M.L. (1939). The accuracy of the plating method for estimating the numbers of soil bacteria, actinomyces, and fungi in the dilution plated. *Canadian Journal of Research 17*, Section C, 72-86.

JELLIS, J.G. (1975). Screening potato clones for resistance to dry rot (*F. solani* var. *coeruleum*). *Annals of Applied Biology 81*, 417-418.

JENSEN, V. (1968). The plate count technique. In *The Ecology of Soil Bacteria* (Eds T.R.G. Gray & D. Parkinson), pp 158-170. Liverpool: Liverpool University Press.

JOHNSON, L.F. & CURL, E.A. (1972). *Methods for Research on the Ecology of Soil-borne Plant Pathogens*. Minneapolis: Burgess Publishing Company.

JONES, P.C.T., MOLLISON, J.E. & QUENOUILLE, M.H. (1948). A technique for the quantitative estimation of soil micro-organisms. *Journal of General Microbiology 2*, 54-68.

KHAMBATA, S.R. & BHAT, J.V. (1957). A contribution to the study of the intestinal microflora of Indian earthworms. *Archiv für Mikrobiologie 28*, 69-80.

KO, W.H. & LOCKWOOD, J.L. (1970). Mechanism of lysis of fungal mycelia in soil. *Phytopathology 60*, 148-154.

KOUWENHOVEN, J.K. (1970). Yield, grading and distribution of potatoes in ridges in relation to planting depth and ridge size. *Potato Research 13*, 59-77.

KRANZ, J. (1958). Untersuchungen über die *Phoma*—Fäule der Kartoffelknolle unter besonderer Berücksichtigung des Wirt-Parasit-Verhältnisses. *Phytopathologische Zeitschrift 33*, 153-196.

KRANZ, J. (1959). Über sortenbedingte Anfälligkeit der Kartoffelknolle für *Fusarium caeruleum* (Lib.) Sacc. und *Phoma foveata* Foister und ihre Beeinflussungdurch den Anbauort. *Phytopathologische Zeitschrift 35*, 135-147.

LACEY, J. (1966). The distribution of healthy and blighted tubers in potato ridges. *European Potato Journal 9*, 86-98.

LANGE, H., KAHL, G. & ROSENSTOCK, G. (1971). Enzyme activities and substrate levels of carbohydrate metabolism in proliferating and suberin synthesizing tuber parenchyma cells of *Solanum tuberosum*. *Planta 91*, 18-31.

LANGERFELD, E. (1973). Einfluss der Temperatur auf den Befall von Kartoffelknollen durch Pilze der Gattung *Fusarium* Lk. *Potato Research 16*, 224-233.

LANGERFELD, E. (1977). Vergleichende Darstellung pilzlicher Lagerfäuleerreger an Kartoffelknollen. *Nachrichtenblatte des Deutschen Pflanzenschutzdienstes 29*, 20-24.

LANSADE, M. (1949). Recherches sur la Fusariose ou pourriture sèche de la pomme de terre, *Fusarium caeruleum* (Lib.) Sacc. *Bulletin technique d'information des ingénieurs des services agricoles 41*, 419-432.

LEACH, S.S. (1971). Post harvest treatments for the control of *Fusarium* dry rot development in potatoes. *Plant Disease Reporter 44*, 723-726.

LEACH, S.S. & NIELSEN, L.W. (1975). Elimination of Fusarial contamination on seed potatoes. *American Potato Journal 52*, 211-218.

LLOYD, A.B. & LOCKWOOD, J.L. (1966). Lysis of fungal hyphae in soil and its possible relation to autolysis. *Phytopathology 56*, 595-602.

LOGAN, C., COPELAND, R.B. & LITTLE, G. (1976). The effects of various chemical and physical haulm treatments on the incidence of potato gangrene. *Annals of Applied Biology 84*, 221-229.

LOGAN, C. & KHAN, A.A. (1969). Comparative studies of *Phoma* spp. associated with potato gangrene in Northern Ireland. *Transactions of the British Mycological Society 52*, 9-17.

LUCAS, R.L. (1977). The movement of nutrients through fungal mycelium. *Transactions of the British Mycological Society 69*, 1-9.

McKEE, R.K. (1952). Dry-rot disease of the potato. II. Fungi causing dry rot of seed potatoes in Britain. *Annals of Applied Biology 39*, 38-43.

McKEE, R.K. (1954). Dry-rot disease of the potato. VIII. A study of the pathogenicity of *Fusarium caeruleum* (Lib.) Sacc. and *Fusarium avenaceum* (Fr.) Sacc. *Annals of Applied Biology 41*, 417-434.

McKEE, R.K. (1955). Host-parasite relationships in the dry-rot disease of potatoes. *Annals of Applied Biology 43*, 147-148.

McKEE, R.K. (1961). Observations on the toxicity of solanine and related alkaloids to fungi. *Tagungsberichte. Deutsche Akademie der Landwirtschaftswissenschaften zu Berlin 27*, 277-289.

McKEE, R.K. & BOYD, A.E.W. (1952). Dry rot disease of the potato. III. A biological method of assessing soil infectivity. *Annals of Applied Biology 39*, 44-53.

MACKENZIE, M. (1968). Factors influencing the infection of potato tubers by *Phoma solanicola* f. *foveata*. *European Potato Journal 11*, 193.

McLENNAN, E. (1928). The growth of fungi in the soil. *Annals of Applied Biology 15*, 95-109.

MALOY, O.C. & ALEXANDER, M. (1958). The most "probable number" method for estimating populations of plant pathogenic organisms in the soil. *Phytopathology 48*, 126-128.

MAO, J.C., HUGUELET, J.E. (1971). Effect of temperature and relative humidity on the enzyme activity of *Fusarium roseum sambucinum* and concommitant symptom development in potato tubers. *American Potato Journal 48*, 307.

MAURER, C.L. & BAKER, R. (1964). Ecology of plant pathogens in soil. Influence of chitin and lignin amendments on development of bean root rot. *Phytopathology 54*, 1425-1426.

MAZUR, P. (1968). Survival of fungi after freezing and desiccation. In *The Fungi 3* (Eds G.C. Ainsworth & A.S. Sussman), pp. 325-394. New York and London: Academic Press.

MEIJERS, C.P. (1975a). Occurrence of dry rot after grading. *Abstracts of Conference Papers of the 6th Triennial Conference of the European Association for Potato Research, 1975*, pp. 113-114.

MEIJERS, C.P. (1975b). Experiences with thiabendazole in control of potato storage diseases in Holland. *Proceedings of the 8th British Insecticide & Fungicide Conference 2*, 597-602.

MEREDITH, D.S. (1975). Control of fungal diseases of seed potatoes with thiabendazole. *Proceedings of the 8th British Insecticide & Fungicide Conference 2*, 581-587.

METLITSKIĬ, L.V. & OZERETSKOVSKAYA, O.L. (1970). Fitontsidy i fitoaleksiny i ikh rol' v immunitete rasteniĭ. *Mikologija i Fitopathologija 4*, 146-156.

MISKA, J.P. & NELSON, G.A. (1975). Potato seed-piece decay: a bibliography, 1930-1975. *Canadian Plant Disease Survey 55*, 126-146.

MITCHELL, R. & ALEXANDER, M. (1963). Lysis of soil fungi by bacteria. *Canadian Journal of Microbiology 9*, 169-177.

MOORE, E.S. (1924). The physiology of *Fusarium caeruleum*. *Annals of Botany, London 38*, 137-161.

MOOI, J.C. (1950). Het fusarium-rot or droogrot bij aardappelen. *Landbouwkundig Tijdschrift 62*, 712-724.

MOORE, F.J. (1945). A comparison of *Fusarium avenaceum* and *Fusarium caeruleum* as causes of wastage in stored potato tubers. *Annals of Applied Biology 32*, 304-309.

MOREAU, C. (1973). Nouvelle technique d'utilisation du formol gazeux dans la lutter contre les champignons qui nuisent à la conservation des plants de pomme de terre. *Pomme de Terre Francaise 358*, 13-15.

MORROW, J.L. (1976). Commercial production of VTSC seed potatoes. *The Seed Potato 16*, 28-30.

MURDOCH, A.W. & WOOD, R.K.S. (1972). Control of *Fusarium solani* rot of potato tubers with fungicides. *Annals of Applied Biology 72*, 53-62.

NASH, S.M. & ALEXANDER, J.V. (1965). Comparative survival of *F. solani* f. *cucurbitae* and *F. solani* f. *phaseoli* in soil. *Phytopathology 55*, 963-966.

NASH, S.M., CHRISTOU, T. & SNYDER, W.C. (1961). Existence of *Fusarium solani* f. *phaseoli* as chlamydospores in soil. *Phytopathology 51*, 308-312.

NASH, S.M. & SNYDER, W.C. (1962). Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils *Phytopathology 52*, 567-572.

NIELSEN, L.W. & JOHNSON, J.T. (1972). Seed potato contamination with fusarial propagules and their removal by washing. *American Potato Journal 49*, 391-396.

NIELSEN, N.K. (1968). An investigation of the regenerative power of periderm in potato tubers after wounding. *Acta Agriculturae Scandinavica 18*, 113-120.

OLD, K.M. & ROBERTSON, W.M. (1969). Examination of conidia of *Cochliobolus sativus* recovered from natural soil using transmission and scanning electron microscopy. *Transactions of the British Mycological Society 53*, 217-221.

OLD, K.M. & SCHIPPERS, B. (1973). Electron microscopical studies of chlamydospores of *Fusarium solani* f. *cucurbitae* formed in natural soil. *Soil Biology and Biochemistry 6*, 153-160.

OLOFSSON, J. (1976). Viktiga sjukdomar i potatislager. *Växtskyddsnotiser 40*, 40-45.

PAHARIA, K.D. & KOMMEDAHL, T. (1954). A modified plating technique for the study of soil fungi. *Phytopathology 44*, 502.

PAPAVIZAS, G.C. (1967). Evaluation of various media and antimicrobial agents for isolation of *Fusarium* from soil. *Phytopathology 57*, 848-852.

PARK, D. (1957). Behaviour of soil fungi in the presence of bacterial antagonists. *Transactions of the British Mycological Society 40*, 283-291.

PARK, D. (1959). Some aspects of the biology of *Fusarium oxysporum* Schl. in soil. *Annals of Botany, N.S. 23*, 35-49.

PARK, D. (1965). Survival of micro-organisms in soil. In *Ecology of Soil-borne Plant Pathogens. Prelude to Biological Control* (Eds K.F. Baker & W.C. Snyder), pp. 82-97. Berkeley:University of California Press.

PÉROMBELON, M.C.M. (1974). The role of the seed tuber in the contamination by *Erwinia carotovora* of potato crops in Scotland. *Potato Research 17*, 187-199.

PETHYBRIDGE, G.H. & BOWERS, E.H. (1908). Dry rot of the potato tuber. *Economic Proceedings of the Royal Dublin Society 1*, 547-558.

PETHYBRIDGE, G.H. & LAFFERTY, H.A. (1917). Further observations on the cause of the common dry-rot of the potato tuber in the British Isles. *Scientific Proceedings of the Royal Dublin Society 15*, 193-222.

PETT, B. (1977). Symposium über pathogene Fusarien an der Kartoffelknolle in Rostock 1976. In *Potato Research 20*, 246-247.

PETT, B. & KLEINHEMPEL, D. (1976). Zum Problem der Mischfäule (*Pectobacterium carotovorum* var. *atrosepticum* (van Hall) Dowson and *Fusarium* spp.) an Kartoffelknollen. *Archiv für Phytopathologie und Pflanzenschutz 12*, 315-322.

POTGIETER, H.J. & ALEXANDER, M. (1966). Susceptibility and resistance of several fungi to microbial lysis. *Journal of Bacteriology 91*, 1526-1532.

PRIESTLEY, J.H. & WOFFENDEN, L.M. (1923). The healing of wounds in potato tubers and their propagation by cut sets. *Annals of Applied Biology 10*, 96-115.

RADTKE, W. (1969). Abwehrverhalten von Kartoffelgewebe gegenüber *Fusarium coeruleum* (Lib.) Sacc. unter besonderer Berücksichtigung des osmotischen Wertes. *Phytopathologische Zeitschrift 33*, 143-174.

RAO, A.S. (1959). A comparative study of competitive saprophytic ability in twelve root-infecting fungi by an agar plate method. *Transactions of the British Mycological Society 42*, 97-111.

RIFAI, M.A. (1969). A revision of the genus *Trichoderma*. *Mycological Papers*, No. 116.

ROEB, L., STEGEMANN, H. & LANGERFELD, E. (1977). Aktivitätsverteilung polysaccharid-und proteinabbauender Enzyme bei Kartoffelfäule-Erregern der Gattung *Fusarium*. *Potato Research 20*, 23-25.

SAITO, T. (1955). The significance of plate counts of soil fungi and the detection of their mycelia. *Ecological Review 69*, 69-74.

SCHIPPERS, P.A. (1962). Dry rot of the potato; preliminary publication. *European Potato Journal 5*, 132-146.

SCHNEIDER, E.F. & BARRAN, L.R. (1977). Cell walls of *Fusarium sulphureum*. II. Chemical composition of the conidial and chlamydospore walls. *Canadian Journal of Microbiology 23*, 763-769.

SCHNEIDER, E.F. & SEAMAN, W.L. (1974a). Development of conidial chlamydospores of *Fusarium sulphureum* in distilled water. *Canadian Journal of Microbiology 20*, 247-254.

SCHNEIDER, E.F. & SEAMAN, W.L. (1974b). Development of a cellular body during differentiation of conidial chlamydospores in *Fusarium*. *Canadian Journal of Microbiology 20*, 1205-1208.

SCHNEIDER, E.F. & SEAMAN, W.L. (1977). Ontogeny of lipid bodies in the endoplasmic reticulum of *Fusarium sulphureum*. *Canadian Journal of Microbiology 23*, 190-196.

SCHOENE, K. (1967). Untersuchungen zur Anfälligkeit von Kartoffelknollen gegenüber *Fusarium coeruleum* (Lib.) Sacc., dem Erreger der Weißfäule. *Phytopathologische Zeitschrift 60*, 201-236.

SCHÜTTE, K.H. (1956). Translocation in the fungi. *New Phytologist 55*, 164-182.

SINGH, R.S. (1951). Root rot of guar. *Science and Culture 17*, 131-134.

SINGH, R.S. & NENE, Y.L. (1965). Some observations on the use of malachite green and captan for determination of *Fusarium* populations in soil. *Plant Disease Reporter 49*, 114-118.

SKUJINS, J.V., POTGIETER, H.J. & ALEXANDER, M. (1965). Dissolution of fungal cell walls by a streptomycete chitinase and β-(1→3) glucanase. *Archives of Biochemistry and Biophysics 111*, 358-364.

SMALL, T. (1944). Dry rot of potato /*Fusarium caeruleum* (Lib.) Sacc./. Investigation on the sources and time of infection. *Annals of Applied Biology 31*, 290-295.

SMALL, T. (1945). The effect of disinfecting and bruising seed potatoes on the incidence of dry rot /*Fusarium caeruleum* (Lib.) Sacc./. *Annals of Applied Biology 32*, 310-318.

SMALL, T. (1946). Further studies on the effect of disinfecting and bruising seed potatoes on the incidence of dry rot /*Fusarium caeruleum* (Lib.) Sacc./. *Annals of Applied Biology 33,* 211-219.

STEVENSON, I.L. & BECKER, S.A.W.E. (1972). The fine structure of and development of chlamydospores of *Fusarium oxysporum*. *Canadian Journal of Microbiology 18,* 997-1002.

SVENSSON, B. (1962). Some factors affecting stolon and tuber formation in the potato plant. *European Potato Journal 5,* 28-39.

TICKLE, J.H. (1974). Aspects of soil contamination with *Fusarium solani* var. *coeruleum* and certain pathogens of potato tubers. PhD Thesis, University of Edinburgh.

TINLINE, R.D. (1977). Multiple infections of subcrown internodes of wheat (*Triticum aestivum*) by common root rot fungi. *Canadian Journal of Botany 55,* 30-34.

TODD, J.M. & ADAM, J.W. (1967). Potato gangrene: Some interconnected sources and factors. *Proceedings of the 4th British Insecticide & Fungicide Conference 1,* 276-284.

TSAO, P.H. (1970). Selective media for isolation of pathogenic fungi. *Annual Review of Phytopathology 8,* 157-186.

TSAO, P.H. & CANETTA, A.C. (1964). Comparative study of quantitative methods used for estimating the population of *Thielaviopsis basicola* in soil. *Phytopathology 54,* 633-635.

UMAERUS, V. & UMAERUS, M. (1976). Förädling för motstandskraft mot mekaniska skador i potatis. *Sveriges utsädesförenings tidskrift 86,* 41-64.

UPSTONE, M.E. (1970a). A potato tuber rot caused by *Fusarium sporotrichioides* Sherb. *Plant Pathology 19,* 150.

UPSTONE, M.E. (1970b). A corky rot of Jersey Royal potato tubers caused by *Fusarium oxysporum*. *Plant Pathology 19,* 165-167.

VAN ECK, W.H. (1976). Ultrastructure of forming and dormant chlamydospores of *Fusarium solani* in soil. *Canadian Journal of Microbiology 22,* 1634-1642.

VAN ECK, W.H. (1978). Lipid body content and persistence of chlamydospores of *Fusarium solani*. *Canadian Journal of Microbiology 24,* 65-69.

VAN ECK, W.H. & SCHIPPERS, B. (1976). Ultrastructure of developing chlamydospores of *Fusarium solani* f. *cucurbitae* in vitro. *Soil Biology and Biochemistry 8,* 1-6.

VIR, S. & GREWAL, J.S. (1973). Studies on the physiology of *Fusarium caeruleum* the casual organism of guar wilt. *Indian Phytopathology 26,* 72-75.

VUJICIC, R. & PARK, D. (1964). Behaviour of *Phytophthora erythrospetica* in soil. *Transactions of the British Mycological Society 47*, 455-458.

WAKSMAN, S.A. (1917). Is there any fungus flora of the soil? *Soil Science 3*, 565-589.

WAKSMAN, S.A. (1927). *Principles of Soil Microbiology*. Baltimore: Williams & Wilkins Co.

WARCUP, J.H. (1955). On the origin of colonies of fungi developing on soil dilution plates. *Transactions of the British Mycological Society 38*, 298-301.

WARCUP, J.H. (1960). Methods for isolation and estimation of activity of fungi in soil. In *The Ecology of Soil Fungi* (Eds. D. Parkinson & J.S. Waid), pp. 3-21. Liverpool: Liverpool University Press.

WASTIE, R.L. (1961). Factors affecting competitive saprophytic colonization of the agar plate by various root-infecting fungi. *Transactions of the British Mycological Society 44*, 145-159.

WEBSTER, J. & LOMAS, N. (1964). Does *Trichoderma viride* produce gliotoxin and viridin. *Transactions of the British Mycological Society 47*, 535-540.

WEINDLING, R. (1932). *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology 22*, 837-845.

WEINDLING, R. & FAWCETT, H.S. (1936). Experiments in the control of *Rhizoctonia* damping-off of citrus seedlings. *Hilgardia 10*, 1-16.

WEISS, F., LAURITZEN, J.I. & BRIERLEY, P. (1928). Factors in the inception and development of *Fusarium* rot in stored potatoes. *Technical Bulletin of the United States Department of Agriculture*, No. 62.

WELLVING, A. (1975). On methods for assessing resistance of potato to storage rots caused by *Phoma foveata* and *Fusarium coeruleum*. *Proceedings of the 6th Triennial Conference of the European Association for Potato Research*, pp. 32-33.

WELLVING, A. (1976). Studies on the resistance of potato to storage rots caused by *Phoma exigua* var. *foveata* and *Fusarium* species. Svalöv: The Swedish Seed Association.

WIGGINTON, M.J. (1974). Effects of temperature, oxygen tension and relative humidity on the wound-healing process in the potato tuber. *Potato Research 17*, 200-214.

WILLEMOT, C. & STUMPF, P.K. (1967a). Fat metabolism in higher plants XXXIV. Development of fatty acid synthetase as a function of protein synthesis in aging potato tuber slices. *Plant Physiology 42*, 391-397.

WILLEMOT, C. & STUMPF, P.K. (1967b). Fat metabolism in higher plants. XXXIII. Development of fatty acid synthetase during aging of stored slices. *Canadian Journal of Botany 45*, 579-584.

WOOD, R.K.S. (1967). *Physiological Plant Pathology*. Oxford and Edinburgh: Blackwell Scientific Publications.

WRIGHT, J.M. (1956). The production of antibiotics in soil. III. Production of gliotoxin in wheatstraw buried in soil. *Annals of Applied Biology 44*, 461-466.

YARWOOD, C.E. & SYLVESTER, E.S. (1959). The half-life concept of longevity of plant pathogens. *Plant Disease Reporter 43*, *125-128*.

# APPENDIX

# APPENDIX 1

## Frequency distribution of *F. solani* var. *coeruleum* and *F. sulphureum* colonies on soil dilution plates

The number of bacterial or fungal colonies on individual plates at any one dilution series should follow a Poisson distribution if the technique of dilution affords a random distribution of organisms which develop on the plates without mutual interference (Jones, Mollison & Quenouille, 1948; Badger & Pankhurst, 1960; Egdell, Cuthbert, Scarlett, Thomas & Westmacott, 1960; Nash & Snyder, 1962; Fisher, 1970). Agreement of colony counts with the Poisson distribution therefore affords a test of suitability of technique and medium. For true samples of a Poisson series, $\chi^2$ (the index of dispersion) calculated from

$$\chi^2 = \frac{\Sigma(x - \bar{x})^2}{\bar{x}}$$

$x$ = colony counts on parallel plates

$\bar{x}$ = mean of colony counts

will be distributed in a known manner indicated in $\chi^2$ tables (Fisher, 1970).

In the present work the number of *F. solani* var. *coeruleum* and *F. sulphureum* propagules were determined in 28 soil samples, using a $10^{-2}$ dilution and five plates of PAB medium for each sample. The index of dispersion was calculated for each soil sample and the distribution of the index of dispersion from 28 samples compared with the expected distribution, where $\chi^2$ values were for n = 4, ie number of parallel plates (=5) - 1.

Frequency distribution of the index of dispersion

| $\chi^2$ | Expected | Observed | |
|---|---|---|---|
| | | *F. solani* var. *coeruleum* | *F. sulphureum* |
| 0 | 0.28 | 0 | 0 |
| 0.297 | 0.28 | 0 | 0 |
| 0.429 | 0.84 | 2 | 2 |
| 0.711 | 1.40 | 0 | 1 |
| 1.064 | 2.80 | 3 | 3 |
| 1.649 | 2.80 | 5 | 4 |
| 2.195 | 5.60 | 6 | 7 |
| 3.357 | 5.60 | 4 | 4 |
| 4.878 | 2.80 | 3 | 3 |
| 5.989 | 2.80 | 2 | 2 |
| 7.779 | 1.40 | 1 | 1 |
| 9.488 | 0.84 | 1 | 1 |
| 11.668 | 0.28 | 1 | 0 |
| 13.277 | 0.28 | 0 | 0 |
| Total | 28 | 28 | 28 |

The observed series does not differ markedly from that expected for the Poisson distribution and therefore the soil dilution/PAB selective medium method is satisfactory for quantitative assessments of *F. solani* var. *coeruleum* and *F. sulphureum* populations in soil.

APPENDIX 2 (See Section 2.1)

(1) Recovery of *F. solani* var. *coeruleum* from moist and air-dried field soil on the PAB medium containing different amounts of antifoam emulsion.

| Silicone antifoam emulsion (ml) | Moist soil | | Air-dried soil | |
|---|---|---|---|---|
| | Mean number of colonies[a] | S.E. | Mean number of colonies[a] | S.E. |
| 0 | 32.2 | ±2.54 | 37.0 | ±2.72 |
| 0.1 | 31.2 | ±2.50 | 34.2 | ±2.62 |
| 0.5 | 38.6 | ±2.79 | 40.0 | ±2.83 |
| 1.0 | 38.8 | ±2.79 | 40.8 | ±2.86 |
| 5.0 | 38.4 | ±2.77 | 39.6 | ±2.81 |
| 10.0 | 35.4 | ±2.66 | 40.2 | ±2.84 |

(2) Recovery of *F. sulphureum* from moist and air-dried field soil on the PAB medium containing different amounts of antifoam emulsion.

| Silicone antifoam emulsion (ml) | Moist soil | | Air-dried soil | |
|---|---|---|---|---|
| | Mean number of colonies[b] | S.E. | Mean number of colonies[a] | S.E. |
| 0 | 27.0 | ±2.32 | 56.4 | ±3.36 |
| 0.1 | 23.6 | ±2.17 | 56.2 | ±3.35 |
| 0.5 | 23.2 | ±2.15 | 59.2 | ±3.44 |
| 1.0 | 27.8 | ±2.36 | 58.6 | ±3.42 |
| 5.0 | 26.6 | ±2.31 | 58.6 | ±3.42 |
| 10.0 | 26.4 | ±2.30 | 48.0 | ±3.10 |

[a] Mean of 5 replicate plates at a $10^{-2}$ dilution.

[b] Mean of 5 replicate plates at a $10^{-3}$ dilution.

S.E. Standard error, which because the data followed a Poisson distribution (Appendix 1) was calculated from the expression $\sqrt{\bar{x}/n}$ where $\bar{x}$ is the mean of a sample of n measurements (Bailey, 1959).

# APPENDIX 3

(1) Recovery on the PAB medium of *F. solani* var. *coeruleum* from soil suspensions which had been prepared from moist or air-dried field soil comminuted for different times.

| Time (min) | Moist soil | | Air-dried soil | |
|---|---|---|---|---|
| | Mean number of colonies[a] | S.E. | Mean number of colonies[a] | S.E. |
| 0.5 | 34.0 | ±2.61 | 41.8 | ±2.89 |
| 1.0 | 33.2 | ±2.58 | 42.2 | ±2.91 |
| 2.0 | 33.8 | ±2.60 | 46.4 | ±3.05 |
| 4.0 | 35.6 | ±2.67 | 48.0 | ±3.10 |
| 8.0 | 40.2 | ±2.84 | 49.4 | ±3.14 |

(2) Recovery on the PAB medium of *F. sulphureum* from soil suspension which had been prepared from moist or air-dried field soil comminuted for different times.

| Time (min) | Moist soil | | Air-dried soil | |
|---|---|---|---|---|
| | Mean number of colonies[b] | S.E. | Mean number of colonies[a] | S.E. |
| 0.5 | 89.2 | ±4.22 | 52.2 | ±3.23 |
| 1.0 | 92.6 | ±4.30 | 57.2 | ±3.38 |
| 2.0 | 90.4 | ±4.25 | 54.6 | ±3.30 |
| 4.0 | 81.8 | ±4.04 | 59.4 | ±3.45 |
| 8.0 | 92.4 | ±4.30 | 61.2 | ±3.50 |

[a] Mean of 5 replicate plates at a $10^{-2}$ dilution.

[b] Mean of 5 replicate plates at a $10^{-3}$ dilution.

S.E. Standard error, which because the data followed a Poisson distribution (Appendix 1) was calculated from the expression $\sqrt{\bar{x}/n}$ where $\bar{x}$ is the mean of a sample of n measurements (Bailey, 1959).

APPENDIX 4 (See Section 3.2)

Contamination of field soil (A) and progeny tubers (B, C and D) after harvest from seed stocks var. Catriona containing different proportions of tubers infected with *F. solani* var. *coeruleum*.

| Percentage infected tubers planted | 50 | | 10 | | 3 | | 0 | |
|---|---|---|---|---|---|---|---|---|
| Drill No.[a] | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Season | (A) Propagules per g field soil after harvest in Oct. | | | | | | | |
| 1974-1975 | 630 | 620 | 0 | 83 | 10 | 21 | 21 | 0 |
| 1975-1976 | 540 | 480 | 20 | 10 | 60 | 140 | 290 | 40 |
| 1976-1977 | 1420 | 370 | 400 | 100 | 240 | 30 | 10 | 10 |
| | (B) Propagules per g progeny tubersphere soil in Feb. after tuber storage. | | | | | | | |
| 1974-1975 | 39781 | 19910 | 15273 | 12235 | 7725 | 4645 | 2745 | 421 |
| 1975-1976 | 3215 | 5375 | 1670 | 315 | 135 | 60 | 2515 | 120 |
| 1976-1977 | 11207 | 10673 | 2627 | 2477 | 217 | 270 | 353 | 73 |
| | (C) Progeny tuber contamination index (%) in Feb. | | | | | | | |
| 1974-1975 | 74.6 | 65.9 | 48.1 | 33.2 | 17.5 | 10.6 | 20.4 | 5.6 |
| 1975-1976 | 13.1 | 37.5 | 10.0 | 6.3 | 0.8 | 0 | 11.9 | 0 |
| 1976-1977 | 85.4 | 75.4 | 36.7 | 38.3 | 5.4 | 2.9 | 5.8 | 1.7 |
| | (D) Dry rot (%) after riddling in Feb. | | | | | | | |
| 1974-1975 | 87.4 | 66.3 | 61.4 | 49.2 | 24.4 | 22.4 | 20.7 | 6.7 |
| 1975-1976 | 21.8 | 35.8 | 26.8 | 7.1 | 5.0 | 3.0 | 29.0 | 2.1 |
| 1976-1977 | 67.1 | 61.8 | 35.6 | 29.5 | 4.8 | 7.9 | 10.5 | 3.4 |

[a] Drills lifted in order of increasing percentage tubers planted with drill 1 lifted before drill 2.