ASPECTS OF SOIL CONTAMINATION WITH *FUSARIUM SOLANI* VAR. *COERULEUM* AND CERTAIN PATHOGENS OF POTATO TUBERS.

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CONTENTS

SUMMARY

1. An alternative method to that of tuber inoculation (McKee & Boyd, 1952) for detecting Fusarium solani var. coeruleum in soil was developed using the soil dilution plate technique with a selective medium. Based on the modified PCNB-peptone medium (Papavizas, 1967), the medium contained (a) 20 g/l sucrose and 2 g/l KNO_3 (instead of peptone) to promote the diagnostic blue colony colour of the fungus for macroscopic identification on culture plates, and (b) 70 ppm dodine acetate to increase the selectivity of the medium. When this medium was used, population estimates after log transformation of F. solani var. coeruleum in soils showed good correlation with infectivity indices obtained by the tuber inoculation method.

2. The influence of the seed tuber on soil contamination by F. solani var. coeruleum was investigated. In field experiments in 1972 and 1973 potato plant rhizosphere soil samples were examined using the soil dilution plate method with the selective medium. These experiments showed that high populations of the fungus were associated with the planting of infected seed tubers. Healthy seed, or healthy seed contaminated with the fungus were not associated with the development of high soil populations. In 1973 a build-up of populations during the growth period was shown to occur in the rhizosphere soil of plants grown from infected tubers. Populations of F. solani var. coeruleum in rhizosphere soil of plants grown from infected seed treated with benomyl in

1972 and tecnazene in 1973 and from tubers inoculated at planting time were found to be lower and to be later in developing than those of soil from plants grown from infected, untreated tubers. In 1973 benomyl treatment of infected tubers completely restricted the spread of the fungus from the seed tubers.

Good agreement was obtained between the results from the tuber inoculation method and the soil dilution plate method used with the selective medium. Population estimates, by the latter method, of F. solani var. coeruleum in soil samples taken from different sources, tuber surfaces, rhizosphere, rhizoplane, also showed good agreement.

3. Some evidence was found that, when plants are grown and harvested under normal agricultural practice, the amount of contamination with F. solani var. coeruleum found on progeny tubers is related to the proportion of infected tubers originally planted.

4. Examination of soil samples from field experiments using the tuber inoculation technique showed that contamination of the rhizosphere soil with Fusarium sulphureum (another causal agent of potato dry rot) also appears to be associated with the infection of the seed tuber with the fungus. Of two experiments conducted to determine the influence of the seed tuber on soil contamination with Phoma exigua var. foveata (the causal agent of gangrene), one experiment clearly showed that the infected mother tuber gave rise to high rhizosphere soil contamination.

5. Glasshouse experiments to determine the distribution of F. solani var. coeruleum in rhizosphere soil of plants grown from infected seed tubers and the effect on this distribution of Trichoderma viride applied to the seed tuber were inconclusive.

6. Soil samples from the surface of VTSC stocks were examined in 1972 and 1973 for the presence of F. solani var. coeruleum and P. exigua var. foveata. Generally, little contamination with F. solani var. coeruleum was found but some stocks in 1972 showed a high contamination with P. exigua var. foveata.

7. Soil samples from a field planted with potatoes in 1971 of which part had been planted with infected tubers, were tested in 1973. Low soil populations of F. solani var. coeruleum were detected from the vicinity of the area planted with infected tubers but not from a neighbouring area.

8. Glasshouse experiments showed that dispersal of the fungus in the soil by hyphal extension was unlikely. The presence of F. solani var. coeruleum in the casts of earthworms taken from the rhizosphere of plants grown from infected tubers in the field indicates that earthworms may be involved in the dispersal of the fungus in the rhizosphere soil.

1. INTRODUCTION

The major part of the introduction to this study of potato dry rot disease consists of a review of the relevant literature. This has been divided into several sections; Causal Organisms, Symptoms, Contamination, Infection, Susceptibility, Environment, Survival and Control. Subsequent sections concern the particular aspect of the disease to be investigated, some general techniques for assessing populations of soil-borne plant pathogens and the choice of the method used in this study for measuring populations of Fusarium solani var. coeruleum in soil.

1.1. Causal organisms.

The species recorded as causing dry rot of potato tubers are listed by Boyd (1972). The most common of these are Fusarium solani var. coeruleum (Sacc.) Booth and F. sulphureum Schl. (Gibberella cyanogea (Desm.) Sacc.): the less common include F. avenaceum ([Corda] Fr.) Sacc. (Mooi, 1950; McKee, 1952, 1954), F. arthrosporioides Sherb. (Pethybridge & Lafferty, 1917; McKee, 1952), F. tricinctum (Corda) Sacc. (McKee, 1952), F. sporotrichioides Sherb., F. oxysporum Schl. (Upstone, 1970a,b), F. trichothecioides Wollenw. and F. solani (Mart.) Sacc.. Fusarium solani var. coeruleum is more prevalent in the United Kingdom and Europe whereas F. sulphureum is found more frequently in certain parts of North America.

F. solani var. coeruleum was named by Fuckel in 1869

as F. violaceum but this name is pre-empted by F. violaceum Crouan (Booth, 1971). Until recently the fungus has been generally known by the name F. coeruleum (Lib.) Sacc.. Snyder & Hansen (1941) in their revision of Section Martiella proposed that F. coeruleum be made synonymous with F. javanicum Koord. var. radicicola Wollenw. under the new combination F. solani var. radicicola (Wollenw.) Snyder & Hansen. This proposal was rejected by Gordon (1952) who, in his treatment of the species present on cereal seed, preferred to give the fungus species rank because of its morphological differences (as stated by Wollenweber & Reinking, 1935) from other species in the Section Martiella and its different distribution from that of F. javanicum var. radicicola.

Booth (1971), in renaming the fungus, recognized the overall similarity with F. solani (Mart.) Sacc. but, because of its strong blue pigmentation, relatively infrequent production of microspores and its pathogenicity spectrum, regarded the fungus as distinct enough to be classed as a variety and not as a forma speciale. Sharma (1971), in a numerical taxonomic study of the Fusarium genus, using growth characteristics of a type generally used in bacterial identification procedures, interpreted her results as showing F. solani var. coeruleum to be a separate and distinct species.

Pethybridge & Lafferty (1917) conclusively established the fungus now known as F. solani var. coeruleum to be the causal agent of dry rot in the U.K., and also gave a

comprehensive review of the confused identifications contained in earlier work carried out in Europe and North America. Booth (1971) stated that although the fungus has been described on Arachis and Phaseolus and also as attacking Picea seedlings and causing a crown rot of lucerne in Italy, there is no evidence relating these strains to the strains causing dry rot of potato.

The fungus is found in all the potato growing regions of the world including the United Kingdom, Europe, U.S.S.R., North America, Australia, New Zealand, Malawi and Argentina.

Considering F. sulphureum Schl. worthy of species rank, Booth (1971) restored this former name to the fungus known for many years as F. sambucinum Fuckel f.6 Wollenw.

F. sulphureum is also an economically important storage rot of potatoes in North America and Europe. It has been isolated from storage rots of groundnuts in Gambia, has been found to attack vegetable marrow in Canada and in association with canker of hops in Tasmania and New Zealand. It has been isolated also from a wide variety of herbaceous plants (Booth, 1971). Only recently has the fungus been found associated with rots of potato tubers in the United Kingdom (Boyd & Tickle, 1972).

1.2. Symptoms.

The symptoms of dry rot on affected tubers depend on the Fusarium species involved.

The external evidence of infection with F. solani var.

coeruleum may be an area with an ill-defined edge and of a darker brown colour than the surrounding tuber tissue. On advanced infections the fungus may produce sporodochial pustules which are pink or white when exposed to light or blue if the infected tubers have been kept from the light. A very common symptom expressed in dry conditions becomes evident as the affected tissue shrinks and produces concentric wrinkles in the tuber skin around the focus of infection. Affected tissue may be light to dark brown in colour with a diffuse margin. Newly attacked tissue at the edge of a lesion may be a cream colour. Often cavities form in the tissue, usually on the radius of the tuber, beneath or near the site of the original infection.

When the whole tuber is affected, the consequent moisture loss makes the tuber small, wizened and hard. In more moist conditions, such as in the soil, bacteria can accompany dry rot infection so that the final result is a tuber with symptoms typical of soft rot but with the tissue remains possibly having a ginger colour similar to that of a normal dry rot infection.

Symptoms caused by F. avenaceum differ from those of F. solani var. coeruleum in the affected flesh being a brown-black colour with a more clearly defined margin. Occasionally red mycelium is found in the cavities caused by the fungus (Boyd, 1972).

The rot caused by F. sulphureum has probably been confused with gangrene (caused by Phoma exigua var. foveata) as, externally, the affected area is slightly sunken and,

internally, there is extensive cavitation. The infected tissue is brown in colour, dry and mealy in texture and usually shows a clearly defined edge at the junction of healthy and diseased flesh.

In the field, dry rot of seed tubers may be expressed by the blanking of crops or the production of weak or late-developing plants with a consequent decrease in yield. In North America, according to Cunningham & Reinking (1946), the loss in stand due to seed piece decay could vary from 10% to complete failure. Foister & Wilson (1943) also noted that in some instances with the cultivar Doon Star in 1941 and 1943, gappy crops were so bad that whole fields were ploughed up as complete losses.

In such cases, seed tubers have been severely affected by dry rot when planted. The same effect may result after planting from adverse temperature and soil conditions delaying plant growth sufficiently to allow the disease to affect most or all of the tuber. Although plants grown from an infected tuber may become established before the mother tuber disintegrates, the weak plants that develop probably contribute to a far greater yield loss than is appreciated by the average grower (Cunningham & Reinking, 1946). Nielsen, Haynes & Johnson (1971), when testing different methods of controlling seed piece decay caused by Fusarium rots, noted that yields of seed piece stocks heavily contaminated with Fusarium propagules were 30-50% less than yields of those of lightly contaminated stocks.

1.3. Contamination.

Pethybridge & Lafferty (1917) concluded from their work that F. solani var. coeruleum was a normal inhabitant of the soil. Soils taken from twelve Cheshire farms were shown by tuber inoculation to be contaminated with this fungus (Small, 1944). Small believed this to mean that the fungus was widely distributed in Cheshire soils. Further soil samples taken by Small from tubers imported from Scotland and N. Ireland showed the presence of the fungus, thus leading to the speculation that the fungus was present also in the field soil of these countries. This was conclusively shown to be the case for Scotland by Foister, Wilson & Boyd (1945a) who inoculated soil samples from a number of fields in Scotland into Doon Star tubers. Their results showed the fungus to be present in 17 out of the 20 fields sampled. In similar experiments in France, Lansade (1949) found that only in a few cases did field tests give positive results. Mooi (1950) found some soils in Holland to be heavily infested with the fungus.

Small (1944), as mentioned above, and Cunningham & Reinking (1946) showed that healthy potato tubers can carry soil contaminated with F. solani var. coeruleum. Small substantiated this by bruising washed and unwashed tubers and noting the resultant dry rot; washing the soil off tubers markedly reduced the dry rot percentage. Recent work by Nielsen & Johnson (1972) has given similar results.

The fungus thus contaminating soil carried on the seed

surface was found by Small (1945) to remain viable on the seed until planting time. Ayers & Robinson (1956) showed that infestations of potato soil with F. solani var. coeruleum and F. sulphureum can be caused by planting seed (chitted) heavily contaminated with spores of the fungus. Therefore, the planting of contaminated or diseased seed seems to lead to an increase in disease incidence.

The work of Boyd & Logan (1967a) and of Boyd & O'Donnell (1968) gave further information on the role the infected seed tuber plays in the contamination of the soil around the plant and in the contamination of the progeny tubers. In the former work, the incidence of disease in wounded progeny tubers and of natural infection in tubers not deliberately wounded grown from infected mother tubers was significantly higher than that of the progeny of contaminated or disinfected mother tubers. Although the soil from plants grown from severely rotted mother tubers gave a low disease incidence when inoculated into test tubers, that from less severely rotted mother tubers gave figures similar to those obtained by wounding the progeny tubers. With the latter work (Boyd & O'Donnell, 1968), soils from plots that had carried diseased seed and healthy contaminated seed both showed high infectivity. However, when the progeny tubers taken from infected seed were wounded there was a low disease rating and no disease occurred on the progeny tubers from healthy contaminated and from disinfected seed.

These experiments thus indicated that in most cases

the planting of diseased seed increased the degree of contamination with Fusarium solani var. coeruleum of soil around the plant and the progeny tubers. The situation regarding contaminated healthy mother tubers seems to be a little confusing. A low level of contamination both in the plant soil and on the progeny tuber surface may result from contaminated mother tubers but there may be a high contamination in the plant soil associated with a low contamination on the tuber surface.

Various workers have mentioned the increase and spread of Fusarium solani var. coeruleum in soil. Foister (1940) stated that contamination of tubers with the fungi that cause dry rot and gangrene occurs while the tubers are still in the soil. Lansade (1949) mentioned that the spread of contamination in soil from a diseased tuber to progeny tubers, although easily imaginable, was not proved by his experiments. According to Ayers (1972) the dry rot fungus proliferates during the growing season in the area of tuber production. Although this suggestion is most reasonable, it has not been substantiated by experimental work (G. W. Ayers, pers. comm.).

Contamination of storage sheds, containers and machinery by the fungus has been reported by many writers. Pethybridge & Bowers (1908) showed the fungus to be present in a viable state in the air and in dust on the walls and floors of seed potato stores. Storage bags (Cunningham & Reinking, 1946), sprouting trays (Pethybridge, 1917) and potato riddles (Boyd, 1971) have been found to be contamin-

ated with the fungus also.

These sources of contamination have been described as secondary sources because they are not supposed to be of such importance. The primary source of contamination, according to Pethybridge & Bowers (1908) must be the soil on the tubers from the field as rotting in storage occurred even if the barn was disinfected. Secondary sources become important in the case of disinfected seed, particularly when the seed is cut or bruised (Cunningham & Reinking, 1946; Foister, Wilson & Boyd, 1952). Small (1945) illustrated the occurrence of recontamination by finding F. solani var. coeruleum present in soil samples taken from tubers that had been disinfected four months earlier and found to be clean. Lansade (1949), however, did not agree with the above writers and regarded potato stores and equipment as the principal source of contamination. However, Boyd (1947) stated that contaminated boxes and stores play no part in infection as long as tubers are undamaged.

The important connection between amount of contamination on the tuber surface and degree of infection has been noted by several workers. Small (1946a) showed that with artificial contamination added to natural contamination, the dry rot incidence was increased. Schippers (1962) noted a curvilinear relationship between the amount of infection in the soil falling off a riddle from a sample of tubers and the number of infections, after wounding, developing on tubers from the same sample. Nielsen & Johnson (1972), using slices of tuber tissue, showed that the number of

infectious propagules adhering to tuber surfaces was related to the infection that developed on the seed pieces of that stock.

1.4. Infection.

Small (1944, 1945) showed that little, if any, infection occurred in ordinary field crops before lifting. This supported the observations of Pethybridge & Lafferty (1917) who did not see any dry rot on tubers still attached to the plant or at lifting time. Foister (1940), however, stated that the fungus can live in the soil and infect tubers while these are still in the soil though the proportion of tubers so infected is very small. This view is not generally held now.

Results of Foister & Wilson (1943) and Small (1945) indicated that most infection takes place soon after lifting. But later experiments conducted by Small (1946a) showed that symptom expression may develop at different times. Healthy tubers picked out of a diseased clamp and stored in clean boxes gave progressively higher counts of dry rot the longer the tubers were stored. It is possible that these tubers may already have been damaged.

Pethybridge & Bowers (1908) noted that the incidence of disease was associated with wounds on the tubers and showed that the disease could be contracted by wounded or cut tubers from infected tubers or pure cultures of the fungus, when kept under moist conditions. Moore (1945)

and Cunningham & Reinking (1946) found that F. solani var. coeruleum did not affect uninjured tubers. Surprisingly, Small (1944) reported that the mature, unwounded tubers could contract dry rot when placed in contact with contaminated soil.

Small (1946a) showed that the severity of the wound plays a significant part in the degree of infection, particularly with disinfected tubers: tubers dipped at lifting, clamped for 3-6 months and then severely bruised developed serious dry rot, whereas tubers dipped, clamped and lightly damaged were found to remain practically sound.

Boyd (1947) in a series of experiments clearly showed the fungus to be a wound pathogen. He noted that infection from tuber to tuber, without injuries, in a box could occur but was extremely rare.

Sites, other than wounds, where infection was found to occur were lesions of powdery scab (Boyd, 1947) and blight (Foister, Wilson & Boyd, 1952) and through injured tuber sprouts (Mooi, 1950).

The major source of injuries leading to infection was found to be the riddling process (Boyd, 1947; Foister, Wilson & Boyd, 1952). Figures from Boyd's (1947) experiments illustrated well the importance of riddling injury; infection after hand dressing 4%, infection after riddling 16%, infection after riddling twice 24%. Boyd showed also that the injuries associated with lifting may be a relatively minor factor in the penetration of the fungus. Further

damage could be incurred by rough handling of bags and this could double losses.

Another important source of injury not encountered in Europe but found in North America, is the cutting of tubers to produce seed pieces. Small (1944) showed that when the susceptible cultivar Ninetyfold was cut into seed pieces, infection with F. solani var. coeruleum was increased by a factor of seven.

The postulation made by Pethybridge & Bowers (1908) that the disease was spread by contact from diseased tubers to healthy unwounded tubers in storage has not been confirmed by later work. Small (1944) and Boyd (1947) found such tuber to tuber infection to be an exceedingly rare occurrence and probably caused by the local poisoning action of the rotting tuber.

Cunningham & Reinking (1946) stated that there is ample evidence that under Long Island conditions seed pieces do not become infected in the soil after planting.

Boyd (1947) noted that, while the rate or success of infection will depend on environmental conditions, the fungus could not penetrate the callus tissue formed 2-8 days after wounding. In contrast, Lansade (1949) stated that at least 1½ months are needed for the formation of a "cork" layer capable of resisting the fungal attack. The development of resistant tissue is much dependent on the environmental conditions.

McKee (1954) carried out histological studies on the

development of F. solani var. coeruleum in potato tuber tissue. Mycelium of the fungus grew through the intercellular spaces and contiguous host cells remained alive for some time. Moore (1924) demonstrated the production of a cytase capable of dissolving the middle lamella of cell walls of tuber tissue.

Regarding other plant parts, Lansade (1949) noted that the roots, stem and leaves of the plant do not provide sites for development of the disease.

1.5. Susceptibility.

Pethybridge & Lafferty (1917) showed that different cultivars of potatoes have differing susceptibilities to the disease. Weiss, Lauritzen & Brierley (1928) and Cunningham & Reinking (1946) associated the more resistant cultivars with a rapid rate of wound healing. Boyd (1952b,c) distinguished between mechanical resistance offered by the skin and the physiological resistance of the tuber flesh. To date, the nature of this latter resistance is not known (Boyd, 1972). Using a riddle abrasion method to test mechanical resistance and a standard spore suspension injection test, Boyd (1952c) tested the resistance of a large number of cultivars. The high susceptibility of the cultivar Doon Star was responsible for such high losses from dry rot that the seed acreage in Scotland dropped from 12,000 in 1942 to 2,000 three years later.

Pethybridge & Lafferty (1917) noted also that tuber

susceptibility increased with time after harvesting. Small (1945) confirmed these results though previously Weiss et al (1928) had stated that no marked difference in susceptibility or in rate of decay was found between mature recently lifted tubers and those kept up to the end of April. Mooi (1950) also found that the longer tubers were stored the more susceptible they became. Lansade (1949) found that, with the cultivar Bintje, susceptibility increased after lifting, diminished, then increased again. Boyd (1952b) clearly demonstrated the seasonal variations in tuber susceptibility, with high susceptibility in the immature tubers in the post-flowering period, minimum susceptibility at haulm death and a rising susceptibility from lifting time into the storage period. Boyd (1952b) also showed that the susceptibility of different cultivars increased at different rates. These experiments were carried out with storage temperatures of 15°C. More recently, Boyd & Logan (1967b) have shown that tubers can be rendered highly susceptible at any time after lifting by subjecting tubers to a period of storage at 3°C.

Lansade (1949) noted that some soils seemed to produce tubers more susceptible than others, while Mooi (1950) found that potatoes grown in heavy soils were generally more resistant than those from sandy soils. Boyd (1952b) did not find conclusive evidence to support Mooi's findings that physical and chemical soil factors influence susceptibility, but noted (1952d) that susceptibility may be affected by higher soil temperature occurring in years of low rainfall.

Boyd (1967) showed that by shortening the growth period, susceptibility to F. solani var. coeruleum may be reduced. Mooi(1950) found that early harvest may reduce resistance to the disease.

Lansade (1949) claimed that the greened tissues of tubers were very resistant but Foister & Wilson (1943) found little if any benefit was obtained by greening tubers. Boyd (1952a) established that large tubers were more susceptible than small ones and the heel end more susceptible than the rose end. Susceptibility of the immature tuber, shown not to be the result of skin thinness (Boyd, 1952b), is closely related to the temporarily high sucrose content of the immature tuber. However, the increase in reducing sugars during storage is not directly related to the increasing susceptibility of tubers at this time (Boyd, 1967).

Schippers (1962) noted in fertiliser trials that nitrogen application seemed to increase the amount of Fusarium disease. Boyd (1967) found that the application of a 12:12:18 NPK fertiliser at 6 cwt/acre, a recommended rate, decreased susceptibility to F. solani var. coeruleum but additional nitrogen increased tuber susceptibility to that of tubers grown in unfertilised plots.

1.6. Environment.

The soil temperature following planting may seriously affect emergence where seed pieces are used. Cunningham & Reinking (1946) observed such an effect on the rate of

seed piece decay (caused by F. solani var. coeruleum or F. sulphureum) in the soil, where seed pieces could be completely rotted before a root system could be established.

Mooi (1950) noted that more dry rot was found after dry growing seasons and suggested that this could have some connection with sprouting which starts early after dry summers. Ayers & Ramsay (1961) and Ayers (1972) also reported that the extent of propagation of the pathogen in soil is apparently dependent on weather conditions of the growing season. Epidemics of Fusarium rot were found to have been preceded by warm dry growing seasons.

Moore (1945) showed that 15°C was the optimum temperature for infection of, and growth of the fungus in, potato tubers and that rotting was more rapid under conditions of high humidity such as those of a clamp. Lansade (1949) found 17-18°C to be the optimum temperature for fungal growth in the tuber and in culture. According to Cunningham & Reinking (1946) the fungus can germinate at 3°C and to Moore (1924) just below 5°C. Mooi (1950) noted that more disease is found when tubers are stored at 5°C than at 3°C. Weiss, Lauritzen & Brierley (1928) found the effect of low humidity upon infection to be very small. At 6-7°C, infection was not much influenced by allowing a cut surface to dry 2-72 hours before inoculation. Weiss et al (1928) also noted that the direct effects of environment (temperature, humidity, rate of air exchange) on pathogenic growth are much overshadowed by the effect of temperature on the wound-healing processes (suberization and wound periderm

formation). However, Boyd (1947) found that bagging or clamping immediately after wounding increased the losses, presumably because the higher humidity favoured infection.

Lansade (1949) stated that storage in an airy place with a sufficiently low temperature can impede the development of Fusarium disease, though his results indicate that tubers are more susceptible at a lower temperature (1-2°C). Boyd (1952d), on the other hand, found that the higher storage temperatures (1-18°C) made the tubers more susceptible than lower temperatures (4°C).

Boyd (1972) noted that if mechanical damage at harvest is followed by a curing period of higher temperatures (15°C is the optimum for wound healing) then excessive storage rots are not incurred. Where wounding is followed by a low temperature in storage, severe losses may arise.

1.7. Survival.

Small (1945) scraped soil samples off tubers and kept them in sealed envelopes in the laboratory. He found that the fungus was still viable after 16 months and stated that the fungus almost certainly could survive from season to season in lofts, sacks, boxes, etc.

Results of Foister, Wilson & Boyd (1945a) showed that the fungus may be present in soil at least two years after the last potato crop. Soil samples stored in the laboratory retained their infectivity for ten years after removal from the field (Boyd, 1970).

1.8. Control.

Control of this disease can take any of four forms. Firstly, the plant can be made less susceptible; secondly, environmental conditions can be made inimical for disease development; thirdly, the contamination or fungal inoculum can be reduced or eliminated; and, fourthly, opportunity for fungal entry can be decreased.

Cultivars of potatoes vary in their susceptibility to the disease and the use of resistant cultivars can effectively reduce disease losses. Boyd (1972) reviewed a number of investigations into the nature of defence mechanisms but reported that none of the substances studied appeared to be directly related to resistance to the disease. Factors such as the length of the growing period and nitrogen applied (Boyd, 1967) could also be managed so as to reduce susceptibility.

During storage the tuber can be made less susceptible by the control of environmental conditions. Wound periderm formation can be encouraged and fungal invasion slowed by the adjusting of storage temperatures.

Small (1944) and Boyd (1960) showed that by washing contamination off the surface of tubers after harvesting, storage losses from the disease were reduced. Nielsen & Johnson (1972) related seed piece infection with propagule contamination and showed that this surface contamination could be greatly reduced when tubers were washed with brushing before cutting.

Nielsen, Haynes & Johnson (1971) illustrated the effect of temperature on the disease by showing that yields were increased by 38% when heavily contaminated seed was stored in a warm temperature (15-21°C) for five days before cutting and planting.

The use of organo-mercury treatments has given good control of the disease. Foister & Wilson (1943) found that dipping with an organo-mercury compound gave satisfactory control only when carried out at lifting time. Boyd (1947) confirmed these results and found a formalin dip to be less effective. By inoculating soil samples taken from tuber surfaces, Small (1945) showed that dipping in an organo-mercury preparation (Aretan) almost entirely killed the fungus on dipped tubers. Cunningham & Reinking (1946) reported that yellow oxide of mercury, semesan bel and dithane gave good control of the disease. Small (1946a) showed that Aretan controlled the disease in clamps as well as in boxes.

Thymol was an effective treatment (Foister, Wilson & Boyd, 1945b) but was found also to have secondary phytocidal effects. Lansade (1949) listed chemicals that are toxic to F. solani var. coeruleum but found most gave phytocidal effects. He noted that tecnazene (TCNB) had serious disadvantages but, on the other hand, Mooi (1950) obtained good control with this compound.

Leach (1971) found that dusts and dips containing benomyl, thiabendazole or zinc plus maneb effectively

controlled dry rot (F. sambucinum). In this case, however, wounded and inoculated tubers were used as they also were in experiments carried out by Murdoch & Wood (1972) who showed that benomyl and thiabendazole gave good control of rotting when applied to wounds inoculated later with F. solani. Nielsen, Haynes & Johnson (1971) applied dithane M-45, captan, polyram, difolatan and daconil to contaminated seed pieces. Treatment with these fungicides increased the yields from heavily contaminated stocks but not those from the lightly contaminated stocks.

Pethybridge & Bowers (1908) found that the reduction of contamination in the storage barn by disinfection delayed the onset and reduced the amount of disease in the following year. Foister (1940) also recommended the disinfection of storage sheds along with the disinfection of boxes.

Nadvodnyuk (1962) has noted the successful use of a conidial suspension of Trichoderma koningii applied to tubers before being stored. The antibiotic activity of the fungus was claimed to be responsible for a 50% reduction in infection.

The reduction of the number of injuries inflicted on tubers is the primary method of controlling disease incidence. Small (1945) found little disease developing on seed lifted, not bruised and stored undisturbed. However, Foister & Wilson (1943) found that the reduction in dry rot percentage between a seed lot fork-lifted, hand-dressed and transported in crates and a seed lot spinner-lifted, machine-dressed and transported in bags was only 27% (58%-31%).

Thus they suggested that normal precautions to avoid injury would not be sufficient and a supplementary control would be needed. The two most dangerous wounding operations are the riddling process and the cutting of tubers into seed pieces.

1.9. Introduction to the study.

The above review indicates that few workers have studied to any degree the development of the primary contamination of tubers, i.e., the contamination that develops in the soil before the tubers are lifted. Ayers & Robinson (1956) and Ayers (1972) have recognized that a population development takes place in the soil. Boyd & Logan (1967a) and Boyd & O'Donnell (1968) have shown some link between the state of the mother tuber at planting time and the amount of contamination of the progeny tubers. This seemed to be that infected seed and sometimes contaminated seed gave rise to progeny tuber contamination that was detected by wounding the progeny tubers or by inoculation of rhizosphere soil samples into test tubers. Severely infected tubers are not wittingly planted in normal agricultural practice, and although seed tubers with small lesions may pass pre-planting inspection, these should form but a small percentage of the total planted. Hence, it seemed logical to suspect the contaminated seed tuber of playing a significant role in the spread and build-up of primary contamination.

Thus, further experiments using infected, contaminated and healthy seed were warranted. Information gained from such investigations could possibly be applied to devise methods to avoid or at least limit the build-up of this primary contamination.

Methods used for assessing populations of soil-borne pathogens are many and varied; a few examples of these techniques are noted in the next section. Following this, the reasons for the choice of an alternative method to the tuber inoculation method for the detection of F. solani var. coeruleum populations in soil are discussed.

1.10. General introduction to methods for assessing populations of soil-borne pathogens.

Methods used in the assessment of fungal populations in the soil can be grouped into several main sections: direct examination methods; indirect techniques involving selective media or special methods achieving selectivity; baiting or trapping methods; and plant infection tests.

Direct examination is not possible for most fungal populations but with fungi with macroscopic fructifications such as Sclerotinia spp. and Armillaria mellea this can be achieved readily. Chinn, Ledingham & Sallans (1960) were able to separate the spores of Bipolaris sorokiniana from soil using a flotation method. The Jones & Mollison (1948) technique with a thin layer of soil-agar mixture in slide-microscope examinations, was used by Nash, Christou &

Snyder (1961) with some success in the examination of Fusarium propagules in the soil. The direct examination of soil fungi, without lengthy isolation and diagnostic procedures, is achieved by the use of fluorescent antisera (Parkinson, Gray & Williams, 1971). This method allows the identification of species or even of strains of organisms.

The use of a selective medium with a soil dilution procedure for the indirect examination of soil populations is a frequently practised technique. Antibiotics and fungicides, many included in a list by Tsao (1970), incorporated into a medium may limit rather than prevent the growth of other fungi not being assessed and the colonies of these fungi can interfere with identification of the pathogen. With some techniques, other agents have been included in the media to enhance the growth of the fungus or the production of a distinctive feature so that the fungus being assessed can be recognized readily, e.g., Nadakavukaren & Horner (1959) with Verticillium albo-atrum and Menzies & Dade (1959) with Streptomyces scabies. Weber, Menzies & Paden (1963) tagged a bacterium with a specific resistance factor. Parmeter & Hood (1961) designed a selective medium making use of the fact that a fungus can tolerate its own by-products more than other fungi. Additional methods offering some selectivity have been based on washing techniques, U.V. treatment (Parmeter & Hood, 1962) and steaming (Warcup, 1951).

The dilution end point technique was used with a plant

infection test by Maloy & Alexander (1958) for estimation of soil populations of Fusarium solani f. sp. phaseoli and Thielaviopsis basicola. Nash & Snyder (1962) used a similar technique and found, as did Maloy & Alexander, good agreement with plate count estimations.

Baiting or trapping methods have been used extensively and successfully with some fungi, e.g., carrot discs for T. basicola (Yarwood, 1946) and sterile buckwheat for Rhizoctonia solani (Papavizas & Davey, 1959). However, the more selective the bait becomes, the more the situation resembles a plant infection test which measures the disease potential rather than the population of the causal organism. Another disadvantage is that a bait may not distinguish between single and multiple colonisation.

Plant infection tests are frequently avoided as a method for fungal population measurement because they involve other factors such as pathogenicity, susceptibility, environmental effects and, often, a long wait before results are available. The use of short cuts, such as the use of seedlings, or early symptoms on plant parts, e.g., Plasmodiophora brassicae on root hairs of cruciferous plants (Samuel & Garrett, 1945), are frequently implemented.

1.11. The detection of Fusarium solani var. coeruleum in soils.

The tuber inoculation test for the detection of Fusarium solani var. coeruleum, as described by McKee & Boyd (1952),

measures the disease potential or infectivity of the soils examined. This method is highly sensitive to F. solani var. coeruleum propagules and is simple to operate.

However, it has a number of disadvantages when used for population measurement and comparison. For example: the small range of forty intervals, when using twenty tubers, is hardly sufficient for a comparison of populations; although sensitive to low populations, multiple infection must occur with samples of higher populations and the method cannot discriminate between such samples; great care must be taken to obtain tubers at a similar stage of infectivity and to avoid extraneous contamination of these tubers; a large storage space is required in controlled temperature incubators when examining large numbers of samples; a delay of ten weeks before results can be read (formerly six weeks as described by McKee & Boyd, 1952).

The first objection could be overcome by increasing the number of tubers used for inoculation and the second by diluting (or decreasing the size of) the soil inoculum. Both such solutions are impractical on account of the extra time, labour and space involved.

It was decided to seek a simpler, less cumbersome and, above all, a more rapid method for the detection of F. solani var. coeruleum. Despite the drawbacks of the soil dilution method enumerated by Jensen (1968) and Parkinson (1970), it was thought that the development of a selective medium to be used with the soil dilution plate procedure would be most

suitable for the investigation. Reasons for this decision were as follows:

(a) the method was simple to operate;

(b) no expensive equipment was involved;

(c) it was relatively easy to examine large numbers of samples;

(d) the results were quickly obtainable;

(e) the disadvantages and drawbacks of the method were well researched;

(f) a medium selective for Fusarium was already devised by Nash & Snyder (1962) containing PCNB (later modified by Papavizas, 1967);

(g) relatively little space was required.

The succeeding account of experimental work commences with the experiments leading to the development of a selective medium for use with the soil dilution plate method.

EXPERIMENTAL WORK

2. Development of medium.

A number of experiments were undertaken in the development and testing of a suitable culture medium. These experiments and their results fall into the following categories.

2.1. Production of colour in colonies of F. solani var. coeruleum on a selective medium.

2.2-2.3. Variation in results of soil dilution plate method and relation to tuber inoculation results.

2.4. Examination of further technique modifications.

2.5-2.7. Increasing selectivity of medium and relation to tuber inoculation results.

2.1. Production of colour in colonies of F. solani var. coeruleum on a selective medium.

2.1.1. Production of blue pigmentation in colonies.

Initial work with the fungus showed the typical blue colouration of colonies to occur only irregularly on Potato Dextrose Agar (PDA) and not at all on Potato Sucrose Agar (PSA).

A series of experiments to obtain the regular production of the bright blue colony colour, the character necessary for the rapid identification of the fungus on culture plates, was undertaken in which the pH of and concentration of various constituents of the medium were varied.

Booth (1971) noted that pigmentation in Fusarium species was markedly affected by the pH of the medium. Experiments with PSA media of different pH values did not produce colonies of the desired colour. Similarly unsuccessful results were achieved when four different nitrogen sources were incorporated into a basic medium containing sucrose (10 g/l), KH_2PO_4 (1 g/l) and $MgSO_4$ (0.5 g/l). These were peptone (used by Nash & Snyder, 1962), $NaNO_3$ (as in the Czapek-Dox medium), asparagine (used by Brown, 1925) and a salts mixture (as in the modified Raulin-Thom medium (Sebek, 1952)).

Further experiments showed colony colour to be affected by the C:N ratio and by the carbon and nitrogen content of the medium.

Moore (1924) stated that with F. solani var. coeruleum colour production appeared when the C:N ratio was high, being blue with KNO_3 and purple or russet with asparagine. Brown (1925) noted with a Fusarium sp. that colour formation occurred when the C:N ratio was sufficiently high and that there appeared to be an optimum concentration, above and below which colour formation diminished. Nord, Fiore & Weiss (1948) recorded that with F. lycopersici increasing amounts of pigment were produced with increasing concentration of glucose. Mahadevan (1960) found a similar reaction with all the carbohydrates he used under conditions of alternate light and dark culture. Carlile (1956) working with F. oxysporum found two groups of pigments present, the

carotenoids produced by exposure to light and the naphthoquinones regulated mainly by the C:N ratio of the medium. This latter group were pH indicators.

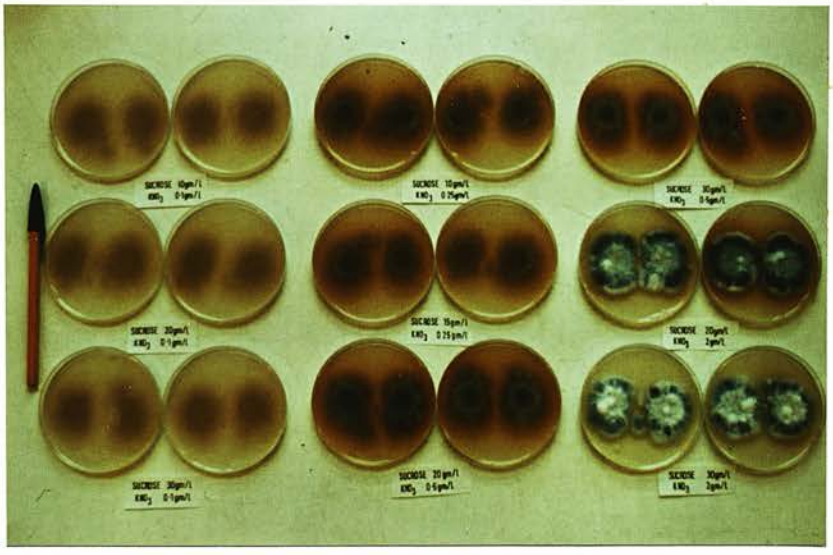
In a series of experiments using a basic medium of agar (20 g/l), KH_2PO_4 (1 g/l), $MgSO_4$ (0.5 g/l) and PCNB (0.75 g/l), sucrose concentrations of 10, 20 and 30 g/l were varied with KNO_3 at concentrations of 2, 4 and 8 g/l. Plates were inoculated at the centres with a drop of F. solani var. coeruleum macrospore suspension.

Blue pigment production was most satisfactory at two pairs of concentrations, 20 and 30 g/l sucrose to 2 g/l KNO_3 (C:N ratios of 29.6:1 and 44.5:1 respectively). The 29.6:1 ratio was considered the better of the two, as the 44.5:1 had a red pigment diffusing into the medium around the colonies. The remainder of the C:N ratios gave cultures with white, pale blue or blue streaky colouration. Plate 1 shows the variation in colour achieved with one of this series of experiments. In this case, results are a little atypical in that the red pigmentation in the medium is more prominent in the 29.6:1 ratio than in the 44.5:1 ratio.

Further experiments confirmed that the 20 g sucrose: 2 g KNO_3 C:N ratio of 29.6:1 gave optimum blue colour formation. Working from this carbon and nitrogen content, alteration of the carbon and nitrogen levels gave the following results.

At a constant nitrogen level, the increase in carbon

PLATE 1. The effect of different amounts of sucrose and KNO_3 in a medium on the colour of F. solani var. coeruleum colonies.



Sucrose (g/l)	10	10	30
KNO_3 (g/l)	0.1	0.25	0.5
Sucrose (g/l)	20	15	20
KNO_3 (g/l)	0.1	0.25	2
Sucrose (g/l)	30	20	30
KNO_3 (g/l)	0.1	0.5	2

content caused a strong red diffusate to appear in the medium surrounding the colony which remained a blue-purple colour, whereas the decrease in carbon content caused a decrease in the blue area of the colonies and an increase in the white area.

At a constant carbon level, the increase in nitrogen content caused an increase in the white area of the colonies, whereas the decrease in nitrogen caused a decrease of the blue to nil with the production of a red-wine pigment only (Appendix, Table A.1).

If the carbon content was too low, even though a C:N ratio of 30:1 was present, colour production was negligible or a pale purple-gray.

The production of wine and purple pigments on PDA and PSA media was changed successfully to the production of blue pigments by the addition of KNO_3, as shown in Plate 2.

Early difficulties in producing the characteristic blue colour are thus explained by the deficiency of nitrogen in the media. The occasional appearance of a single blue colony among many wine-coloured cultures with series of both single spore isolates and mass inoculum isolates on PDA from a batch of medium can be explained, it is suggested, by a maldistribution of nitrogen in a series of plates giving an adequate nitrogen content in a small percentage of plates only.

It should be mentioned at this point, while discussing the carbon concentration of the medium, that at high carbon

PLATE 2. The effect of addition of KNO_3 to Potato Sucrose Agar (PSA) on colour of F. solani var. coeruleum colonies.

Media from left to right:

PSA; PSA + 0.5 g/l KNO_3; PSA + 1 g/l KNO_3; PSA + 2 g/l KNO_3; PSA + 5 g/l KNO_3; Czapek-Dox agar.

concentrations macrospores were usually malformed, with bulbous black segments in the spores and with expanded ends. Fat globules appeared to be common under such conditions. This agrees with remarks of Butt & Beevers (1966) stating that "yeasts and mycelial fungi possess a marked capacity of fat synthesis when living on a medium rich in sugars as the sole source of carbon" and also that fat accumulation in fungi is dependent on the C:N ratio. These authors noted that there seems to be a competition between nitrogen utilisation and fat synthesis for some common intermediate. Spore shape was normal when the blue pigment was present.

2.1.2. Effect of various compounds in selective media on fungal germination, growth and pigment production.

As it was hoped to incorporate the amounts of carbon and nitrogen appropriate for blue colony colour production into a medium selective for Fusarium spp. (such as that developed by Papavizas, 1967), experiments were carried out investigating the effects of the compounds used in such a medium on the germination and growth of F. solani var. coeruleum. The results of some of these experiments are reported below.

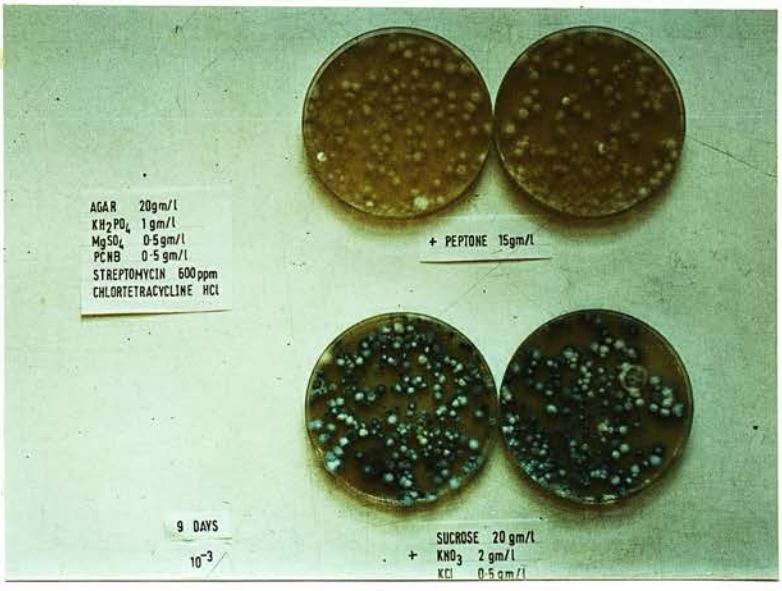
PCNB (pentachloronitrobenzene in a 50% active formulation) at 0.25 g/l and 0.5 g/l, Streptomycin at 300 and 600 mg/l, Neomycin at 12 mg/l and Chlortetracycline HCl at 50 mg/l were added separately to Czapek-Dox medium. Average diameters of colonies after five days were reduced

from 6.23 cm for Czapek-Dox medium, to, respectively 3.61, 3.53, 3.57, 5.00, 5.82 and 5.53 cm.

Little difference was found between colony numbers on media containing PCNB at 0.5 g/l plus streptomycin at 600 mg/l and PCNB at 0.25 g/l plus streptomycin at 300 mg/l when plates were spread with suspensions of inoculated sterile and unsterile soils. These suspensions were prepared from sterile and unsterile soil to which had been added macrospores of F. solani var. coeruleum. As the spore form in field soil was thought to be chlamydospores and so that these test soils might simulate, to some extent, naturally contaminated soils, the inoculated test soils were stored for a month before use to allow the macrospores to change to chlamydospores. Suspensions were prepared by stirring a small amount of soil with sterile dilute water agar solution in a Waring Blendor for one minute. Five ml amounts of suspension were pipetted into further containers of sterile dilute water agar to give dilutions of 10^{-2} and 10^{-3}. (N.B. In following work, the term "dilution of 10^{-2}", etc., is used to mean a concentration of 1 g soil per 100 ml soil suspension, etc..)

The C and N content found suitable for blue colony colour production was substituted for the peptone in Papavizas' Modified PCNB-peptone medium to determine if the same blue pigmentation was produced in the selective medium. Blue colour production was found to occur, as seen in Plate 3. No colony colour was produced on plates of Papavizas' medium in its original form (i.e., including

PLATE 3. The effect of changing carbon and nitrogen source of a selective medium (after Papavizas, 1967) on colour of F. solani var. coeruleum colonies.



Upper plates: C and N source 15 g/l peptone

Lower plates: C and N source sucrose 20 g/l
KNO_3 2 g/l.

peptone as the C and N source).

Where PCNB (0.375 g/l, as in Papavizas' medium), oxgall, streptomycin or chlortetracycline HCl were withheld from the medium separately, no increase was found in colony counts of F. solani var. coeruleum on plates prepared with sterile or unsterile inoculated soils or riddle soils. (The latter soils consist of soil samples scraped from potato riddles in commercial use collected during an investigation into the contamination of potato riddles with tuber pathogens. The soils had been shown to be contaminated with F. solani var. coeruleum using the tuber inoculation method and thus represented naturally contaminated soils with high fungal components.) When PCNB was not included in the medium, the density of colonies was such that the plates were completely covered, thus inhibiting the growth or masking the colonies of F. solani var. coeruleum. The lack of any other of these compounds did not decrease the counts from the various soils.

When three media with different rates of PCNB (0.375, 0.75 and 1.125 g/l) were prepared and used with inoculated and riddle soils, no real differences in the counts of F. solani var. coeruleum or the suppression of other fungi were noted.

Thus, it was found that the diagnostic blue colony colour could be produced in a selective medium and the fungus detected in this modification of Papavizas' medium in numbers equivalent to those detected by the original medium.

The constituents of the modification of Papavizas' (1967) medium, now referred to as the PCNB-sucrose medium are listed below. Contents of brackets refer to different constituents and different amounts used by Papavizas (1967).

agar	20 g/l
sucrose	20 g/l)
KNO_3	2 g/l) (15 g/l peptone)
KH_2PO_4	1 g/l
$MgSO_4$	0.5 g/l
KCl	0.5 g/l (Nil)
oxgall	0.5 g/l
PCNB	0.75 g/l @ 50% a.i. formulation
streptomycin	600 mg/l (100 ppm)
chlortetracycline HCl	50 mg/l

2.2. Variation in results of soil dilution plate method.

2.2.1. Variation in colony counts.

In early experiments considerable differences between the numbers of colonies of F. solani var. coeruleum per culture plate of PCNB-sucrose medium were found when diluting soil samples of inoculated or riddle soils. This phenomenon could readily be explained if the numbers of viable F. solani var. coeruleum propagules in the diluted soil sample capable of forming colonies conformed to a Poisson distribution. Such would be the case under ideal conditions (Egdell et al, 1960). This was checked with a number of dilutions by calculating the ratio of the variance to the mean, i.e., the index or coefficient of dispersion. With a Poisson distribution this ratio should be close to one. Figures from these experiments, within

the limits of the small numbers of plates taken per sample, were satisfactory to assume a Poisson distribution (Prof. R. M. Cormack, Univ. of St. Andrews, pers. comm.). The plate counts involved in these experiments were low.

To allow the application of statistical procedures used with the Normal distribution to the results of experiments investigating sources of errors, plate count figures were transformed with the $\sqrt{x + \frac{1}{2}}$ or $\log_{10}(x + 1)$ transformations.

2.2.2. Errors in the soil dilution plate technique.

An analysis of data from early dilution experiments with inoculated and riddle soils indicated that the variance of the mean of a series of population estimates from successive samplings from a well-mixed soil sample was caused primarily by the variance within the separate samples and not between the samplings. This indicates that the samples were homogeneous with respect to propagules of F. solani var. coeruleum and satisfactorily prepared for examination.

The term "population estimate" is the multiple of the reciprocal of the dilution and the mean number of colonies per plate (usually of 10 plates). The word "estimate" is employed as an indication that the relevant data are not necessarily the true soil populations of the fungus, but figures obtained from the methods chosen as feasible approximations towards measuring the true populations (see remainder of section 2). In later experiments

where the population estimates from different soils of different moisture contents are compared, a further multiple, the moisture factor, is included in the calculation. Soils were oven-dried to determine this factor.

The use of 0.15% water agar as a diluent instead of sterile water did not appear to make any significant decrease in the variance within samples. This variance could be caused by the action of the medium and of the plate environment on the viable F. solani var. coeruleum propagules or by the action of other fungi present which could inhibit the germination or growth of these propagules.

2.2.3. Tests of technique of sampling from the blendor.

Population estimates of F. solani var. coeruleum propagules in an inoculated sterile soil and a riddle soil were calculated from a series of 11 and 9 different dilutions respectively (i.e., plates containing 0.1×10^{-3} g of soil per plate, plates containing 0.2×10^{-3} g of soil per plate, etc., with the means multiplied by the different factors in each case). Such a series of estimates immediately shows any great variation owing to faulty technique or other causes such as masking or inhibition due to other colonies. The extent of the dilution series in this experiment was limited by the low or high numbers of colonies on culture plates. Results are shown in Figs 2.2.3a,b rather than presented in a table.

Population estimates with the sterile inoculated soil were stable and the only variation noticeable was the

FIG. 2.2.3a,b. Population estimates of F. solani var. coeruleum in two soils in relation to dilution.

FIG. 2.2.3a. Inoculated soil.

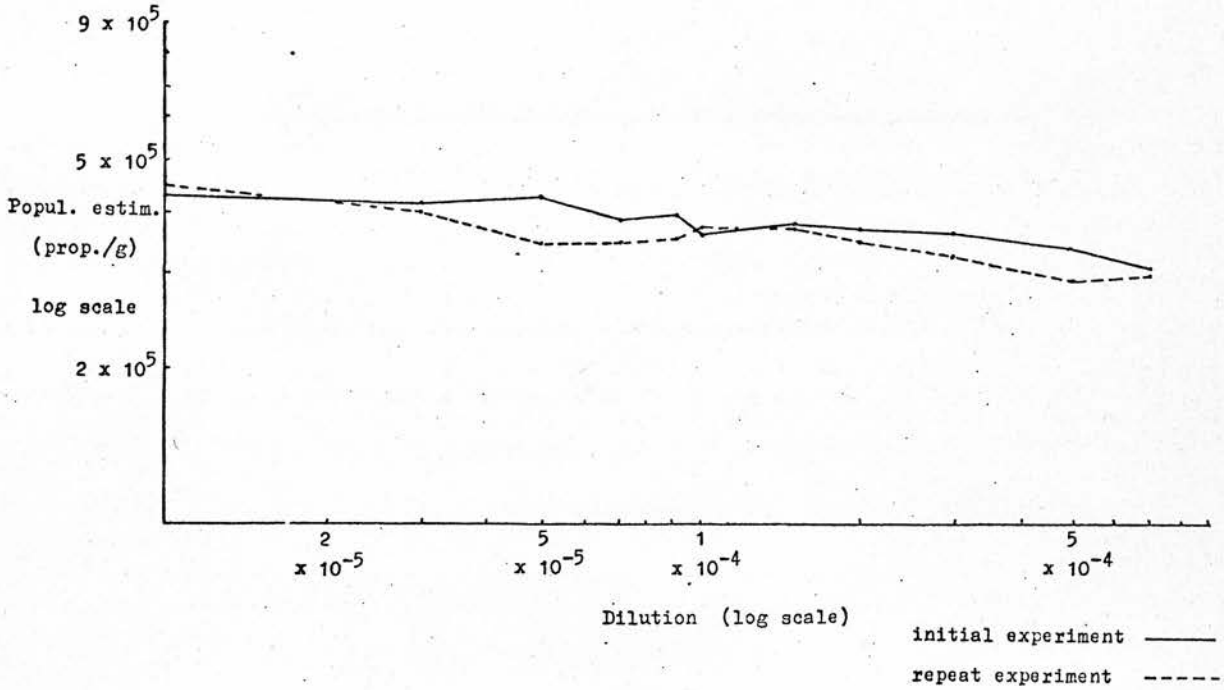
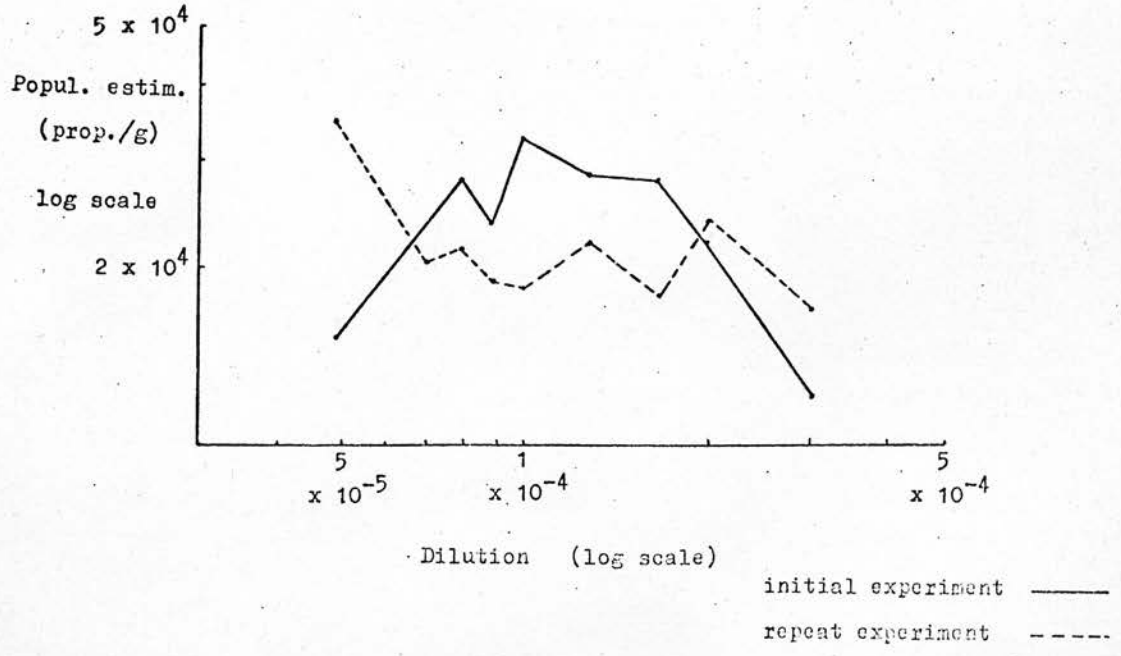


FIG. 2.2.3b. Riddle soil.



higher population figures from the higher dilutions. With more colonies per plate at the lower dilutions, it is to be expected that masking and inhibition may decrease the population estimates. The results of this experiment indicated that the laboratory technique in operating the method was satisfactory. The riddle soil estimates presented a similar picture but with more variation than the inoculated soil. This variation can seem considerable when viewed on a linear scale but when results are plotted after log transformation the variation does not seem unreasonable.

2.3. Relation of plate counts and tuber inoculation results.

A preliminary experiment was carried out with ten riddle soils comparing results obtained by using the tuber inoculation method (20 Catriona tubers per sample, 2 inoculations in each; technique fully described in section 3.1) and the dilution plate method with the PCNB-sucrose medium (C:N ratio modified to allow the distinctive colour development of F. solani var. coeruleum; 10 plates per sample, 2 x 10^{-4} dilution). Results were disappointing with little relation between figures being evident.

A larger experiment comparing figures from 31 riddle soil samples was then set up, the results of which are presented in Table 2.3a. The results of the 40 tuber inoculations per sampe in this and future tables are expressed as percentage figures. The use of this

TABLE 2.3a. F. solani var. coeruleum colony counts arranged in order of successful tuber inoculations.

% Dry rot in tubers	Mean plate count	% Dry rot in tubers	Mean plate count
2.5	0	50.0	0.4
5.0	0	52.5	0.6
7.5	0	57.5	0
10.0	0	65.0	0.2
10.0	0	72.5	0.8
15.0	0	75.0	0.4
17.5	0	87.5	0.1
20.0	0.5	92.5	4.1
20.0	0.1	95.0	2.2
25.0	0.2	95.0	0.1
27.5	0.1	97.5	0.3
35.0	0	97.5	1.0
35.0	0.1	100.0	0.2
37.5	0.1	100.0	1.7
42.5	0.1	100.0	2.8
50.0	0		

TABLE 2.3b. Four ranges of % dry rot in tubers with corresponding plate counts and mean plate counts.

% Dry rot in tubers	Plate counts	Mean plate count
0 - 24	0.1, 0.5, 0, 0, 0, 0, 0, 0, 0	0.07
25 - 49	0.1, 0.1, 0.1,), 0.1, 0.2	0.10
50 - 74	0.8, 0.2, 0, 0.6, 0, 0.4	0.33
75 - 100	2.8, 1.7, 0.2, 1.0, 0.3, 0.1, 2.2, 4.1, 0.1, 0.4	1.29

infectivity index based on a percentage scale, rather than 0-40, was thought to aid immediate comprehension of the data presented.

Although not immediately obvious, a poor correlation did exist between the results of the methods. This was shown by reducing the number of divisions in the tuber inoculation scale to four (0-24%, 25-49%, 50-74%, 75-100%) and taking the mean of the corresponding plate counts as in Table 2.3b.

The assumption that there is a close correlation between the infectivity of a soil sample and the propagule population of that sample is supported, for this fungus, by data presented by McKee & Boyd (1952). There should be then a close correlation between the results of the tuber inoculation and dilution plate methods, though population estimations would probably require transformation.

The small correlation seen in the above figures, while showing the plate method using this medium to be relatively insensitive, suggested that the method had the basic potential for population measurement and that improvements might allow a more favourable correlation with the tuber inoculation method to be made.

As it was, the dilution method was not sensitive enough, the range of populations not large enough and the results were not related sufficiently to infectivity figures. Reasons for this could be that the medium allowed too many fungi to grow, thus masking or inhibiting the growth of

F. solani var. coeruleum, or that the populations in the soil were so small as not to be detected regularly when 2/10,000 g of soil is put on a plate. It was thought that the latter reason was primarily responsible for the poor results. To remedy this fault more soil must be screened on each plate. This was not possible with the medium as it was constituted because a lower dilution than 2×10^{-4} gave complete masking of F. solani var. coeruleum colonies by the growth of colonies of other fungi. In order that more soil could be placed on each culture plate, the medium would have to be more selective. Experiments leading to the development of such a medium are described in section 2.5.

In another experiment, comparison of propagule population estimates from the dilution plate method using the PCNB-sucrose medium with results from the tuber inoculation method again showed the former method to be relatively insensitive. This experiment is described and results given in detail in section 3.1.

2.4. Examination of further technique modifications.

Various technique modifications were attempted, most of which proved to be unsuccessful or not to give higher population estimates than the standard method already in use. This standard method was as follows: the sieving and drying of the soil sample, the use of a MSE Waring Blendor to agitate the soil sample for one minute, a dilution series of 2 or 3 steps from an original 1:20 suspension

in the blendor sufficient to give a final dilution of 2×10^{-4}.

2.4.1. Various modifications described by Bouhot & Rouxel (1971) were tested. The medium they described, incorporating V8 juice, $CaCO_3$ and TCNB was found to be unsuitable. Using the medium in the standard way (i.e., pipetting 1 ml dilute soil suspension on to the solid agar plate), there was poor colony growth and colour development together with unsatisfactory control of bacteria. When the medium was used as suggested for soil plates (Warcup, 1950), colour development was still poor and colony recognition made more difficult by the masking of sub-surface colonies by surface-growing colonies.

Grinding the soil sample with ball-bearings in a container as suggested by Bouhot & Rouxel (1971) did not increase population estimates of the samples tested. PCNB was replaced by TCNB in the PCNB-sucrose medium at the rate used by these authors. TCNB did not appear to be superior to PCNB with this medium or with the samples tested. Acidifying the medium, in this case the PCNB-sucrose standard, to pH 5.2 as described by these authors, was also unsuccessful in giving higher population estimates.

2.4.2. Abawi & Lorbeer (1971) mixed soil suspensions by shaking the soil sample in a dilute water agar solution for 30 minutes. Population estimates were lower than those from the standard method using a Waring Blendor.

2.4.3. Experiments conducted using a dilute water agar as diluent instead of sterile water, where a wetting agent (Tween 80) was added to the suspension in the blendor and where the mixing time was extended beyond one minute, did not provide population estimates above those of the standard method.

2.4.4. If the propagules of F. solani var. coeruleum were associated in the main with heavy mineral particles, as are the hyphae of some fungi, a soil washing technique could give high population estimates. When a washing procedure was tested, the fungus was found to be present in the wash water and the sediment. Hence the method was unsatisfactory.

2.4.5. A preliminary experiment was carried out to investigate the feasibility of concentrating the F. solani var. coeruleum propagules by selective sieving, using "Millipore" filters. Results were not promising as it appeared that the F. solani var. coeruleum propagules and many other spores of smaller dimensions lodged in the interstices of the filters.

2.4.6. Instead of the inhibition of the growth of other fungi in the soil, the opposite approach of the selective stimulation of Fusarium species was considered. Choline, found by Strange, Smith & Major (1972) to be a factor stimulating invasion of wheat heads by F. graminearum, was incorporated into the medium but with no success.

2.5. The testing of various chemical compounds to increase the selectivity of the medium.

A number of chemical compounds were screened to test for selective action when incorporated in the PCNB-sucrose medium. In most cases the compounds were tested at concentrations of 1, 10 and 100 ppm. Four soils were used in the tests: two riddle soils which had a large fungal component of many species (thus providing a severe test for selective action) and a sizeable population of F. solani var. coeruleum; one field soil with a lower population of F. solani var. coeruleum to present the more normal situation; and a sterile soil inoculated with F. solani var. coeruleum to check any inhibitory action of the compound on the germination, the growth of colonies and colour development of the fungus. Ten plates in most cases were used for each of the four soils at each concentration of each compound. The growth rate and colour development were checked also from colonies grown from 5 cm diameter agar plugs taken from F. solani var. coeruleum cultures (one colony on each of five plates for each concentration).

It was hoped to detect selective action by the inhibition of the background flora of the sample (i.e., fungi other than F. solani var. coeruleum) and to determine the action of the compound, when such action occurred, on F. solani var. coeruleum from the number of F. solani var. coeruleum colonies developing on all plates from all four soils, more particularly those from the inoculated soil. If one compound was successful in preventing the growth of a species or group of species, possibly this could be

combined with another compound with a similar action upon another section of the background flora, thus producing a more comprehensive inhibitory action on the background flora.

The compounds tested (listed in Table 2.5) were selected so as to contain representatives from most of the large fungicide groups and to this were added antibiotics and two compounds suggested by Dr. D. C. Graham (Dept. Agric. & Fish., Edinburgh, pers. comm.).

With all compounds except one, any inhibiting action on the background flora, impeding either germination of spores or the growth of colonies, was exerted also on F. solani var. coeruleum. The compound dodine acetate, as in Melprex, gave remarkable results at 100 ppm level. Although the numbers of F. solani var. coeruleum colonies were reduced, virtually no other fungus appeared on the plates. Colour was blue-purple and the colonies were convoluted. At 10 ppm, the colonies seemed to be more definite than those of the control.

Tests conducted earlier had shown that TCNB was not superior to PCNB with the samples used and that increased rates of PCNB did not afford higher population figures.

Further tests were carried out using benomyl at 2 and 4 ppm, thiram at 25 and 50 ppm, and dodine acetate at 25, 50 and 75 ppm. Those with benomyl and thiram proved unsuccessful. Once again, at the higher concentrations, dodine acetate inhibited the development of

TABLE 2.5. Compounds tested for selectivity towards F. solani var. coeruleum.

Compound	Chemical description	Proprietary name
Chloronitrobenzenes		
Quintozene	pentachloronitrobenzene (PCNB)	Bras-sicol
Tecnazene	1,2,4,5-tetrachloro-3-nitrobenzene (TCNB)	Fusarex
Dithiocarbamates		
Thiram	bis(dimethyldithiocarbamoyl)disulphide	
Maneb	manganese ethylene-1,2-bisdithiocarbamate	
Cufraneb	Zinc, manganese, copper, iron dithiocarbamate complex	Blitzblight
Dinitro- compounds		
Dinocap	2-(1-methyl-n-heptyl)-4,6-dinitrophenyl crotonate	Crotothane
Mercurial		
Corrosive sublimate	Mercuric chloride	Mersil
Benzamidazole		
Benomyl	methyl N-(1-(butylcarbamoyl)-2-benzimidazole) carbamate	Benlate
Morpholine		
Tridemorph	2,6-dimethyl-4-tridecylmorpholine	Calixin
Miscellaneous		
Dodine acetate	n-dodecylguanidine	Melprex
Captan	N-(trichlormethylthio)-cyclo-hex-4-ene-1,2-dicarboxymide	
Organotin		Tubotin
Sulphur		Plenisan
Chloraniformethan	1-(3,4-dichloroanilo)-1-formylamine-2,2,2-trichloroethane	Milfaron
Griseofulvin		Fulcin 125
Nystatin		
Fungizone	deoxycholate complex of Amphotericin B	
Furaspor	5-nitrofurfuryl methyl ether	
Phenylethyl ether		

the background flora while allowing a reduced number of F. solani var. coeruleum colonies to grow. At the lowest rate (25 ppm), although the background flora seemed unaffected, the Fusarium colonies present seemed to be larger and more vigorous than those of the control.

These results indicated that further investigation of the use of dodine acetate for improving the selective action of the PCNB-sucrose medium was worthwhile.

An alternative method of testing compounds for antagonistic activity, usually against bacteria, was tried. This was to use "Multo-disks" with each arm of the "disk" impregnated with a different compound or with one or two compounds at different rates. The range of discs available impregnated with suitable compounds was not large and those that were tested, e.g., Nystatin, against six organisms from a local soil were unsuccessful in inhibiting the growth of the fungi tested. Theoretically, using the Multo-disk principle with different compounds spotted on the arms at different rates against all soil fungi not inhibited by the PCNB-sucrose medium, would perhaps provide a more complete screening for selective action.

2.6. Effect of dodine acetate when added to the PCNB-sucrose medium at different concentrations.

As previous experiments had shown that dodine acetate when added to the PCNB-sucrose medium had little effect on the background flora at 25 ppm and markedly reduced F.

solani var. coeruleum population estimates at 100 ppm, only concentrations above 25 ppm and up to 100 ppm were tested. Three soil samples were used, an inoculated sterile soil (S11), a riddle soil (No. 24) and a field soil (1/5/3A). Four dilutions of riddle and field soil were made, 10^{-2}, 5×10^{-3}, 10^{-3} and 10^{-4}. The highest dilution was that at which F. solani var. coeruleum could still be detected without too great a variation in counts and the lowest that where colony crowding would not be too extreme. The inoculated soil was used at 10^{-3} dilution. Ten plates of each dilution at each dodine acetate concentration were made in most cases for the inoculated soil and for the 0 ppm dodine acetate controls and five plates per dilution per concentration for the remainder.

After counting the F. solani var. coeruleum colonies, means and population estimates were calculated and are presented in Table 2.6.

The figure of the inoculated soil population estimates (Fig. 2.6a) clearly shows the fall in the estimate with the increase in dodine acetate concentration in the medium.

The very low population estimates seen with 0 ppm dodine acetate at the two lowest dilutions of the riddle (Fig. 2.6b) and field (Fig. 2.6c) soil samples were caused by the masking and inhibition of F. solani var. coeruleum by the profusion of other colonies on the plates. In fact, overcrowding was evident on the plates of all except the highest dilutions. This lowering of the population

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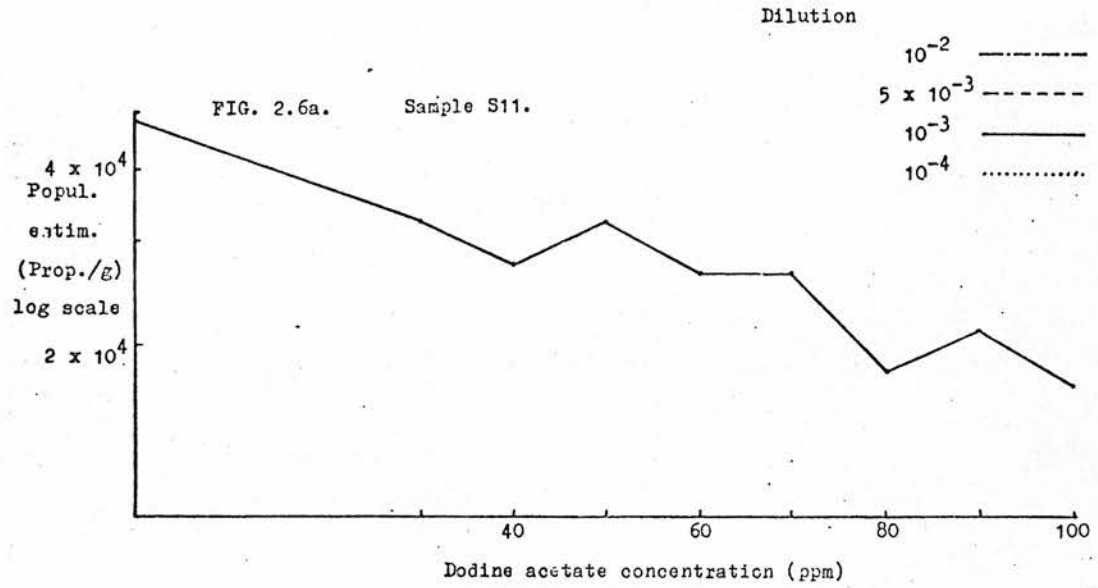
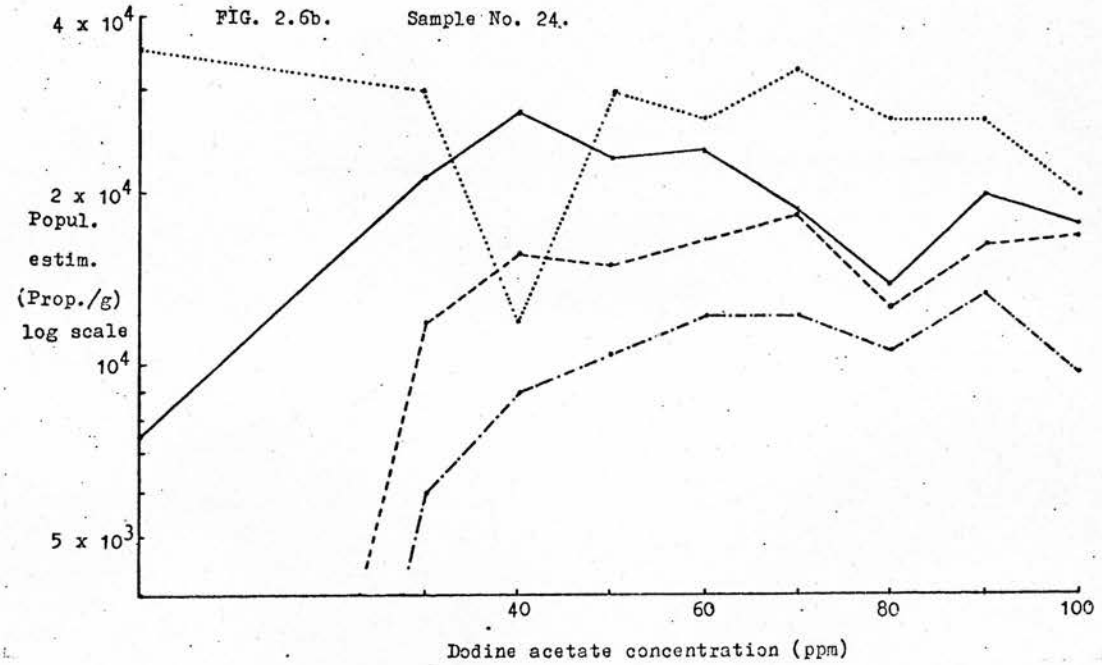
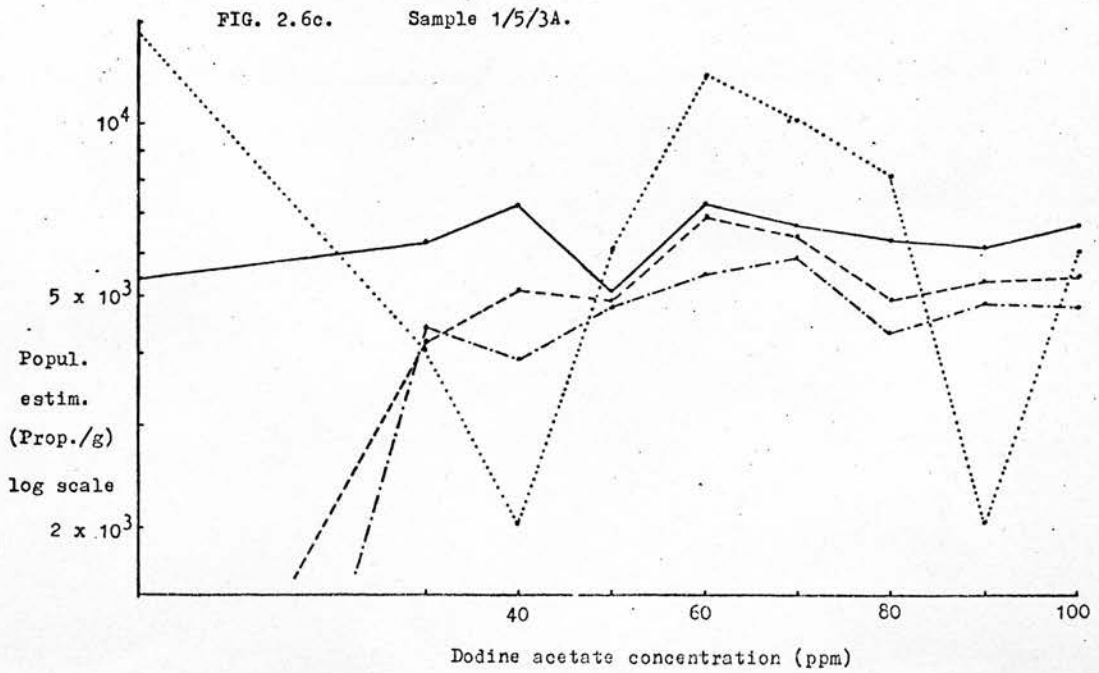
TABLE 2.6. Population estimates of *F. solani* var. *coeruleum* in three soils from different dilutions and different concentrations of dodine acetate (ppm) added to the PCNB-sucrose medium.

Soil sample	Dilution	Concentration of dodine acetate (ppm)								
		0	30	40	50	60	70	80	90	100
S 11	10^{-3}	48,100	32,800	27,500	32,900	26,600	26,600	18,000	21,000	16,800
No. 24	10^{-2}	60	6,020	9,000	10,420	12,200	12,280	10,730	13,650	9,930
	5×10^{-3}	120	11,800	15,740	15,000	16,560	18,460	12,800	16,340	17,040
	10^{-3}	7,600	21,400	27,800	23,000	23,800	18,700	14,000	20,000	18,300
	10^{-4}	35,000	30,000	12,000	30,000	27,000	33,000	27,000	27,000	20,000
1/5/3A	10^{-2}	80	4,440	3,940	4,780	5,400	5,810	4,300	4,820	4,730
	5×10^{-3}	580	4,040	5,120	4,920	6,860	6,280	4,880	5,260	5,400
	10^{-3}	5,300	6,200	7,200	5,000	7,200	6,600	6,200	6,000	6,600
	10^{-4}	14,000	4,000	2,000	6,000	12,000	10,000	8,000	2,000	6,000

FIG. 2.6a,b,c. F. solani var. coeruleum population estimates in relation to different concentrations of dodine acetate for three soils over a series of dilutions.







estimates because of inhibition caused by overcrowding may have occurred with large counts of F. solani var. coeruleum on plates of the lower dilutions with dodine acetate as well. With the lower dilutions, the strong effect of dodine acetate in removing much of the background flora and allowing colonies of F. solani var. coeruleum to grow and to be seen is remarkable. This effect is what was sought in the further development of selectivity. However, the action of dodine acetate against the germination and growth of F. solani var. coeruleum must be considered. Overall, it seemed that when large amounts of soil were added to a culture and problems of masking and inhibition emerged, dodine acetate in the medium at concentrations above 40 ppm vastly improved population estimations, but that at the higher dodine acetate concentrations this effect diminished and eventually gave lower estimates.

The great variation in the estimates provided by the highest dilution for the riddle and field soils was thought to be a factor of the dilution itself. When using such a high dilution with populations of these levels, very few colonies were produced per plate or, indeed, per five plates. With such a large multiplication factor to produce a population estimate, a matter of one or two colonies can cause a vast difference in the estimates. To solve this problem, more plates per dilution must be made. The low estimates achieved by the two highest dilutions at the 40 ppm dodine acetate figure is a little anomalous and cannot be satisfactorily explained. The depression

on nearly every curve (Figs. 2.6a,b,c) at the 80 ppm level was also anomalous. This situation was not repeated in later experiments.

In considering the use of this compound in a medium that is at least partially selective for F. solani var. coeruleum, two major questions can be asked: will this provide a medium with a significant advantage over the standard medium and, if the compound is used, what concentration is likely to give the best results?

Taking the second question first, it does appear, if the figures from the highest dilution are ignored, that population estimates rise to a maximum then fall off at the highest dodine acetate concentration tested. The maximum population estimate will be that concentration where the effect of reducing the inhibitory action of the background flora on the germination and growth of F. solani var. coeruleum begins to be exceeded by the direct adverse effect of dodine acetate on the germination and growth of F. solani var. coeruleum. In the samples examined, such a point will be between 60 and 90 ppm. Obviously, the optimum dodine acetate concentration will depend on the concentration of the background flora of the sample examined. The dodine acetate concentration could be adjusted to suit the nature of each sample but such a procedure would occupy far too much time and resources. A compromise level of 70 ppm dodine acetate was suggested as suitable.

With regard to the other question, if the population

estimates of the riddle and field soil samples at 70 ppm dodine acetate at the lowest dilution (12,280 and 5,810 propagules/g, respectively) are compared with the highest estimates of each soil at 0 ppm dodine acetate at 10^{-4} dilution (35,000 and 14,000, respectively), the 70 ppm low dilution figures have values, on the linear scale, rather less than half of the latter figures. The situation is similar for the inoculated soil. This seems a large reduction in the population estimates but, when the figures are transferred to a $\log_{10}$ scale, a more satisfactory picture emerges (4.09 and 3.76 compared with 4.54 and 4.15).

It should be noted that, at the 10^{-2} dilution, one hundred times more soil is screened than at the 10^{-4} dilution. Furthermore, when ten dilution plates are used, the smallest population estimate is 10 propagules/g at 10^{-2} but 1,000 propagules/g at 10^{-4} dilution and the interval between any two counts is 10 propagules at 10^{-2} but 1,000 at 10^{-4} dilution. The 10^{-4} dilution would give a more accurate estimation of a large population where the 10^{-2} dilution plate would be overcrowded. High population estimates at 10^{-2} dilution of a medium with 70 ppm dodine acetate may underestimate by a factor of 2 or 3, a factor that is reduced when population estimates are put on a $\log_{10}$ scale, but this is preferable to the possibility of most populations under 1,000 propagules per gram being undetected. At the time of the development of this medium, the detection of small populations of F. solani var.

coeruleum was thought to be of prime importance.

Thus, primarily because of the capability of this medium with dodine acetate added to detect small populations of F. solani var. coeruleum propagules, it was decided to conduct further tests at 50 ppm and 70 ppm to relate population estimates gained with the infectivity indices obtained with the tuber inoculation method.

Plate 4 shows F. solani var. coeruleum colonies on plates of PCNB-sucrose medium plus dodine acetate at 70 ppm at the 10^{-2} dilution.

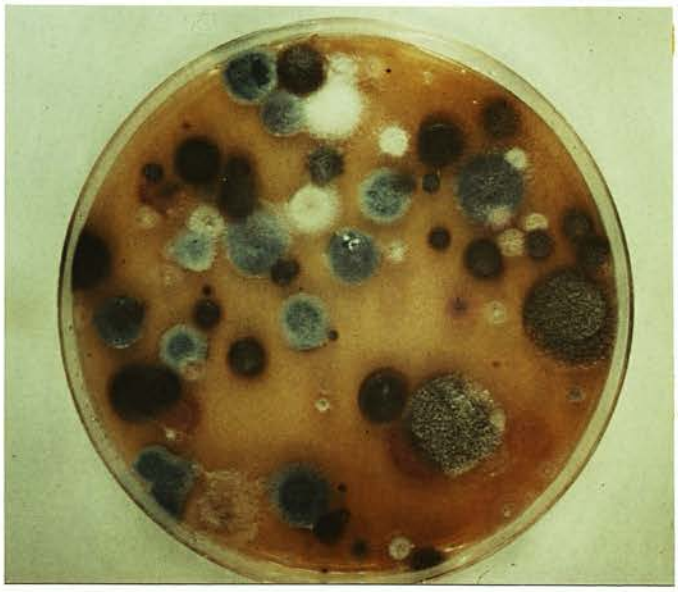
2.7. Relation of population estimates to infectivity indices using dodine acetate-modified medium with riddle and field soils.

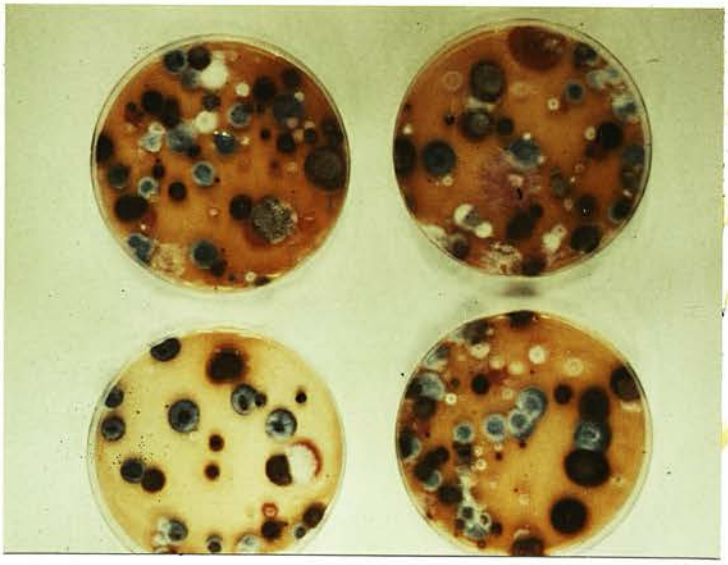
2.7.1. Riddle soils.

An initial experiment was set up to determine if a PCNB-sucrose medium containing dodine acetate would detect the presence of F. solani var. coeruleum propagules where the PCNB-sucrose medium without dodine acetate would not. Ten riddle soils, seven with infectivity indices less than 50% were tested with a 50 ppm dodine acetate medium at two dilutions, 10^{-2} and 5×10^{-3}.

The results (presented in the Appendix, Table A.2) showed that a PCNB-sucrose medium containing 50 ppm dodine acetate detected populations in seven of the ten soils whereas the PCNB-sucrose medium without dodine acetate detected F. solani var. coeruleum in only one of the

PLATE 4. Appearance of F. solani var. coeruleum colonies on PCNB-sucrose medium plus dodine acetate 70 ppm (PM70).





Plates taken from soils prepared from 10^{-2} dilution of rhizosphere soil samples from plants grown from infected seed tubers.

Blue colonies	F. solani var. coeruleum
Red colonies	F. culmorum
Brown colonies	Humicola sp.

ten samples. It is interesting to note that this one sample was the only sample with a population estimate of over 1,000 (on the dodine acetate medium) and theoretically was the only population large enough to be detected by the high dilution with the PCNB-sucrose medium. It was thought to be worthwhile conducting another experiment with a larger number of samples to determine if a relationship existed between the population estimates using the PCNB-sucrose medium plus dodine acetate and the results of the tuber inoculation method.

In another experiment, 34 riddle soil samples were examined by the dilution plate method using the PCNB-sucrose medium (dilution 10^{-4}) and the PCNB-sucrose medium containing dodine acetate at 70 ppm (dilutions 5×10^{-4} and 5×10^{-3}). (This latter medium is to be referred to as PM70.) Ten plates were used at each dilution. Results are shown in Table 2.7.1a. Moisture factors, not presented, used in the calculations of the population estimates varied from 1.01 to 1.20. Tuber inoculations into test Catriona tubers were carried out in March-April 1972 and the soil dilution carried out in November 1972. The results of the dilution plate method with PCNB-sucrose medium (dilution 2×10^{-4}) carried out in March 1972 are also included in Table 2.7.1a. These riddle soils were used as they were the largest group of soils recently tested by the tuber inoculation method and had a wide range of infectivity percentages.

TABLE 2.7.1a. Infectivity indices (%) and corresponding population estimates (propagules/g) of F. solani var. coeruleum from the plate counts of four different dilutions using two media.

Infectivity index (%)	Population estimate			
	PM70 5×10^{-3}	PM70 5×10^{-4}	P 10^{-4}	P 2×10^{-4}
5.0	63	0	0	0
7.5	21	0	0	0
10.0	0	21	0	0
10.0	231	0	0	0
15.0	82	206	1,030	0
15.0	0	0	0	*
17.5	44	0	0	0
20.0	371	206	0	2,575
20.0	200	222	1,110	555
20.0	1,082	832	1,030	*
25.0	624	832	0	1,040
27.5	374	208	0	520
35.0	42	210	0	0
35.0	286	408	0	510
37.5	268	0	0	515
42.5	3,424	214	0	535
50.0	318	424	0	2,120
50.0	0	0	0	0
52.5	4,223	7,828	1,030	3,090
57.5	82	0	0	0
65.0	2,843	4,738	5,150	1.030
72.5	1,186	1,664	0	4,160
75.0	390	2,884	4,120	2,060
87.5	806	1,060	0	525
90.0	21	0	1,050	*
92.5	*	*	*	20,100
95.0	58,298	11,240	6,180	11,330
95.0	16,315	25,956	11,330	515
97.5	18,437	28,840	11,330	1,545
97.5	7,670	11,832	8,160	5,020
97.5	7,390	11,408	7,440	*
100.0	5,068	4,738	1,030	8,755
100.0	17,469	39,964	17,510	8,755
100.0	*	*	*	14,140

P PCNB sucrose medium

PM70 PCNB sucrose medium plus 70 ppm dodine acetate

* Missing value

Results are listed according to increasing infectivity percentage. It can be seen that the PM70 medium used at a sample dilution of 5×10^{-3} has a detection success equivalent to that of the tuber inoculation method. The higher dilutions were not so successful.

In Figs. 2.7.1a and 2.7.1c the values of the infectivity indices are plotted against the population estimates of the 5×10^{-3} and 5×10^{-4} dilutions respectively. Correlation coefficients and the figures of the percentage variance accounted for (from calculating a linear regression with population estimate as the independent variable) are shown in Table 2.7.1b. The scatter of points in Figs. 2.7.1a and 2.7.1c shows the correlation coefficients and computer-plotted regression lines to be meaningless. Logarithmic transformation of the population estimates was carried out and the results plotted in Figs. 2.7.1b and 2.7.1d. These figures show that there is an association between the results of each method. (Infectivity indices were also transformed using the angular transformation but this did not markedly affect the correlation.) Figures of the infectivity indices against the population estimates from the other two dilutions are not presented as they are similar to Figs. 2.7.1a-d.

The degree of correlation achieved in this experiment, though reasonable, was not high. It was thought that this might have been caused by the methods not having been applied at the same time but separated by a period of several months. The infectivity indices of twelve samples

FIG. 2.7.1.a. Infectivity indices in relation to F. solani var. coeruleum population estimates from 5×10^{-3} dilution. Riddle soils.

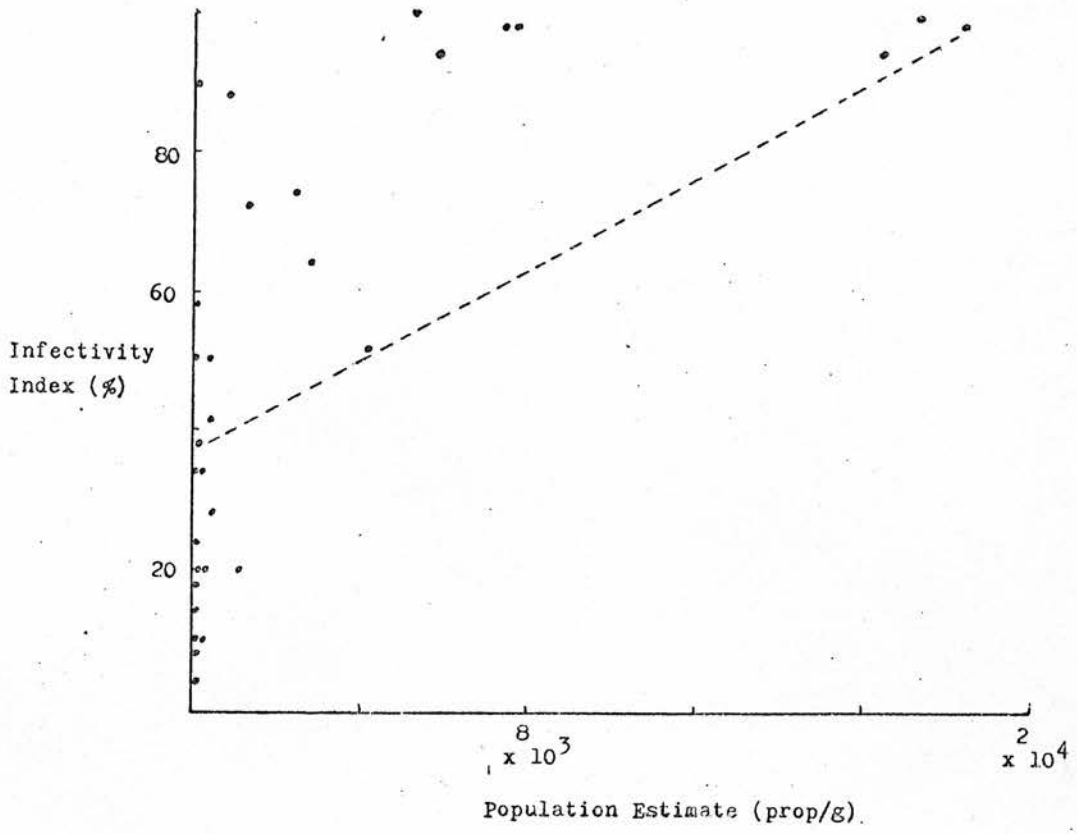


FIG. 2.7.1.b. Angular transformations of infectivity indices in relation to log transformations of F. solani var. coeruleum population estimates from 5×10^{-3} dilution. Riddle soils.

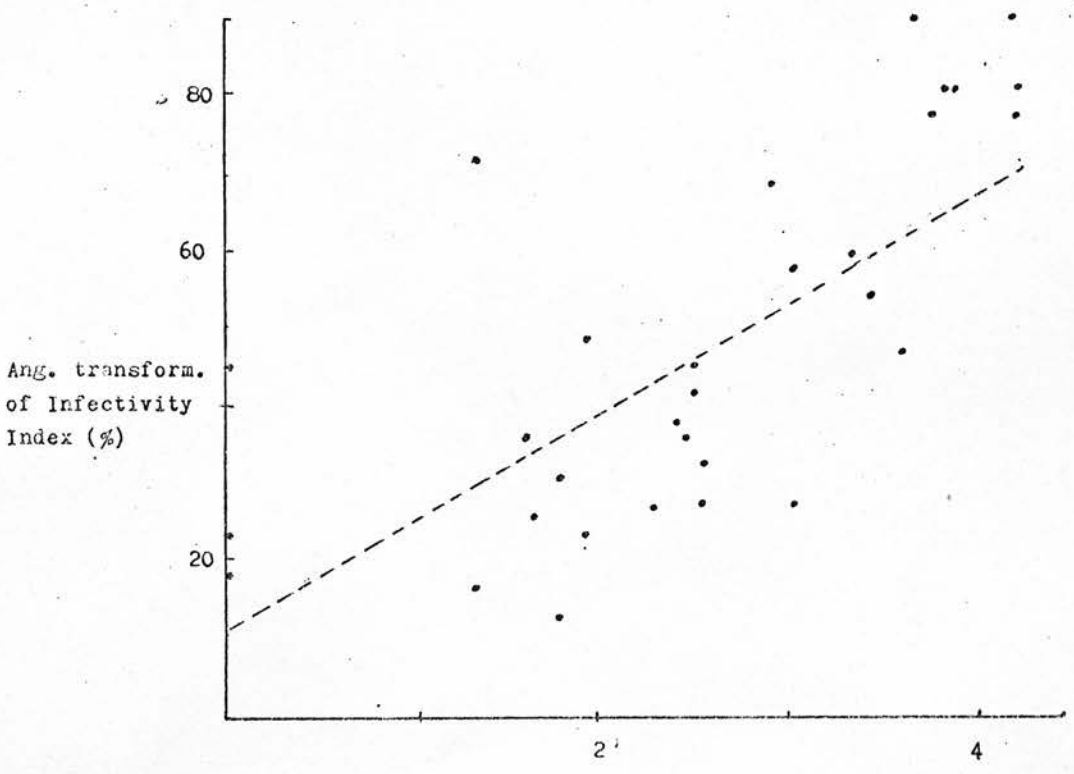


FIG. 2.7.1.c. Infectivity indices in relation to F. solani var. coeruleum population estimates from 5 x 10^{-4} dilution. Riddle soils.

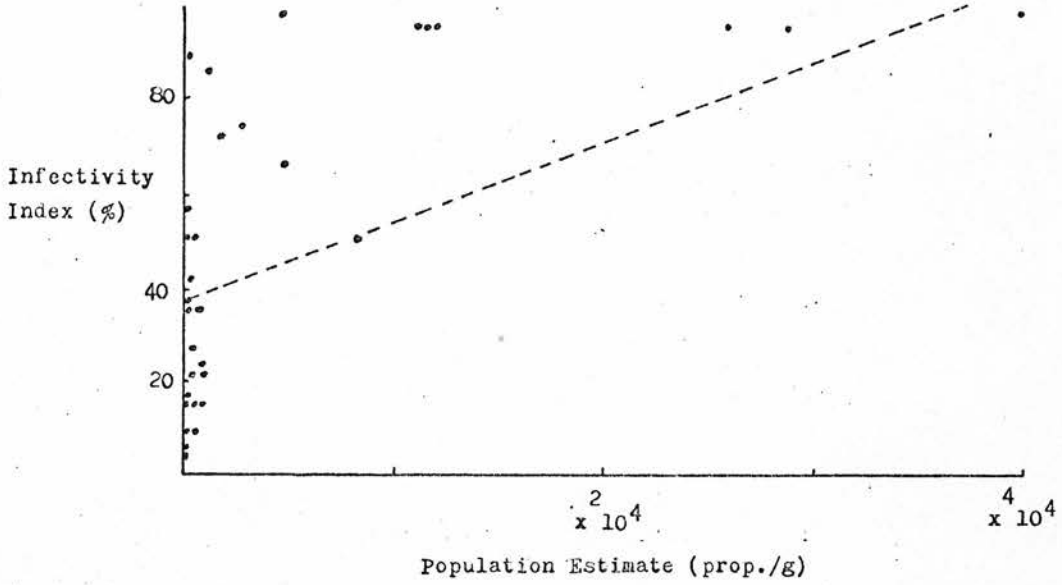


FIG. 2.7.1.d. Angular transformations of infectivity indices in relation to log transformations of F. solani var. coeruleum population estimates from 5 x 10^{-4} dilution. Riddle soils.

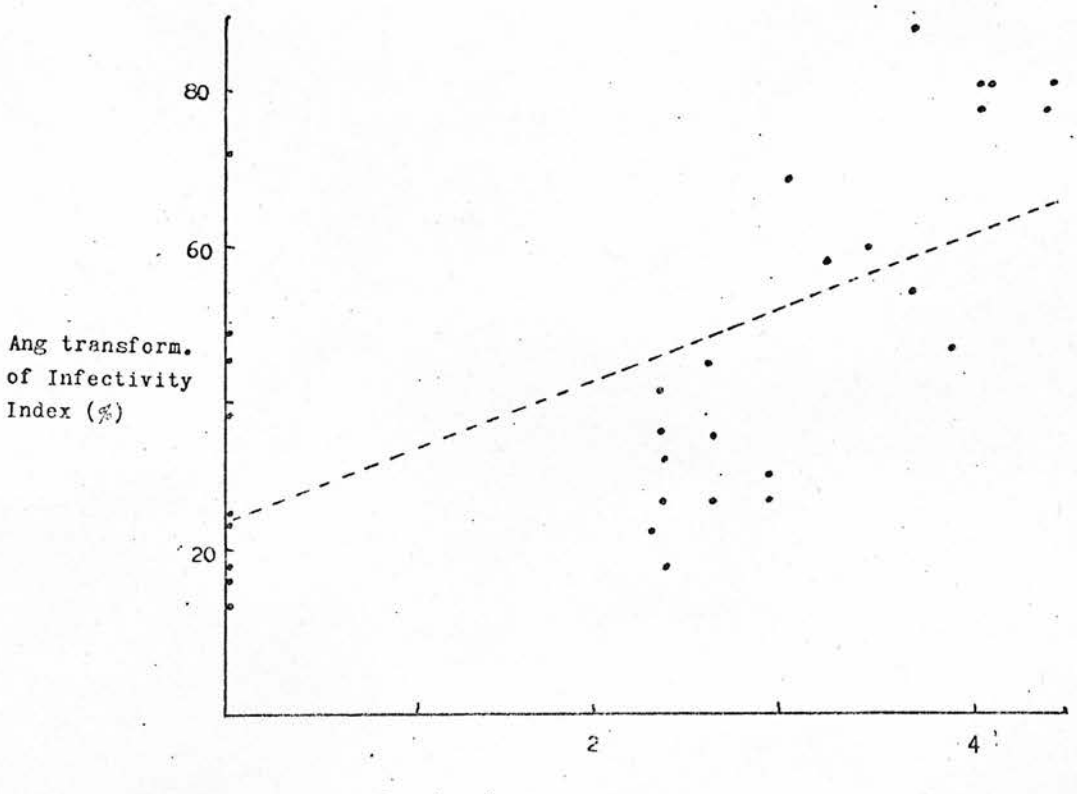


TABLE 2.7.1b. Data describing relationship between infectivity indices and population estimates from different dilutions.

Treatment		Medium & dilution			
		PM70 5×10^{-3}	PM70 5×10^{-4}	P 10^{-4}	P 2×10^{-4}
No transformation	a	0.6813	0.6379	0.6729	0.6297
	b	44.6	38.7	43.4	37.5
After angular & log transformation	a	0.6917	0.6340	0.6660	0.6297
	b	46.1	38.2	42.5	47.3

Media
- P — PCNB sucrose medium
- PM70 — PCNB sucrose medium plus 70 ppm dodine acetate

Data
- a — correlation coefficient
- b — percentage variance accounted for

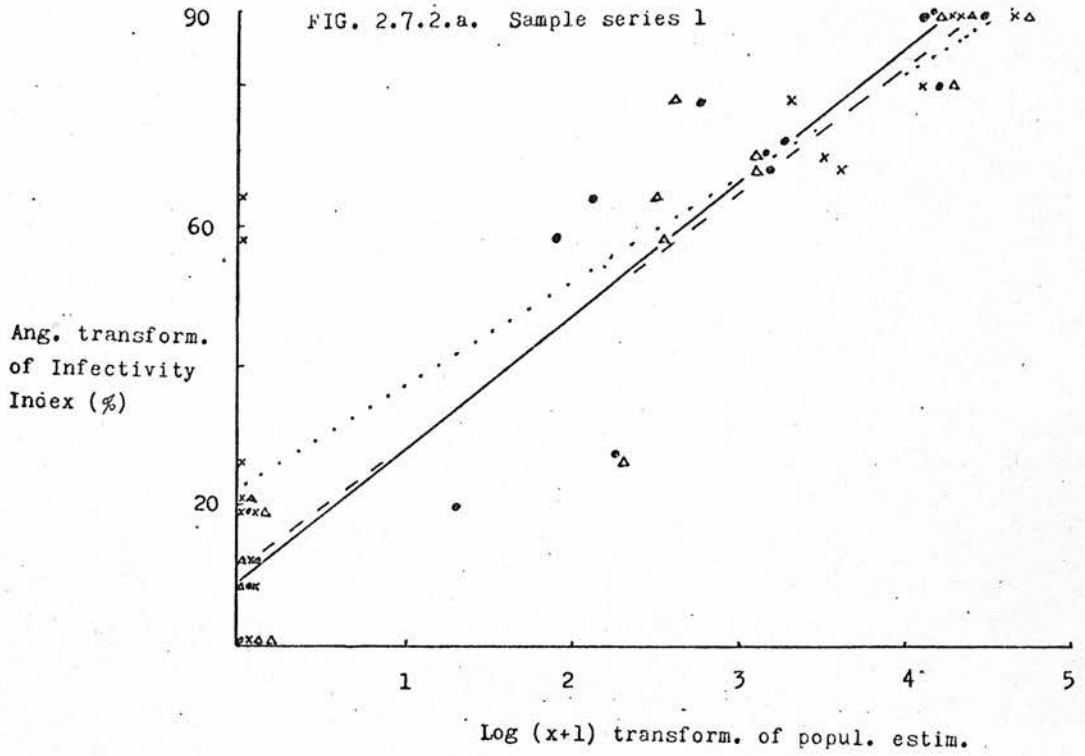
with anomalous results were checked by re-inoculation and several were found to have changed. When these figures were substituted into the correlation graph with the population estimates of the 5 x 10^{-3} dilution, a correlation coefficient of 0.750 and variance accounted for of 54.8% were obtained.

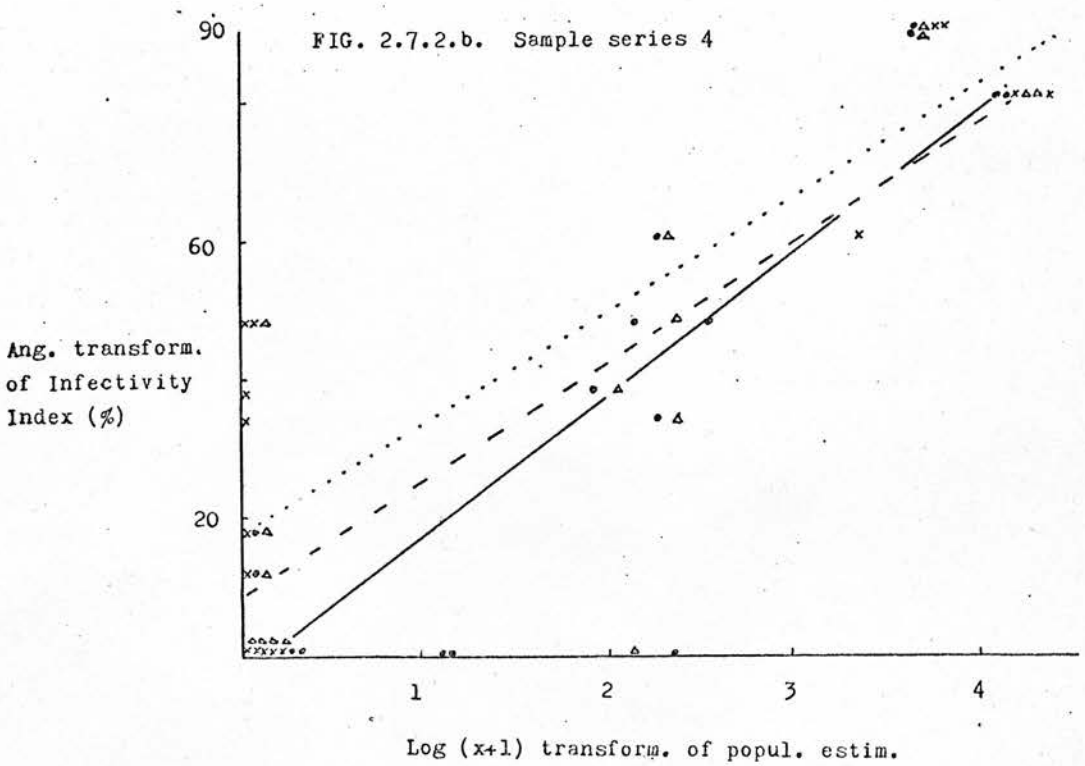
2.7.2. Field soils.

In a further experiment, to be described in detail later (section 3.2), seven series of infectivity indices and population estimates of a larger number of soil samples were compared to assess correlation. With these samples both methods were used at approximately the same time. Sixteen samples, each made up from a mixture of subsamples of three soils, each of these from a potato plant. The first three comparisons refer to soil samples from tuber surfaces and the remaining four to rhizosphere soil samples (Tables 3.2.1 and 3.2.2a,b).

Figures were drawn relating the population estimates (log transformation) of each of the three dilution rates (10^{-2}, 10^{-3} on PM70 medium and 10^{-4} on PCNB-sucrose medium) to the infectivity indices (angular transformation). Figs. 2.7.2a and 2.7.2b show the relationship of the transformed data for the methods for samples 1 and 4 (the remainder are not presented as they are essentially similar to these). The correlation coefficients and the % variances accounted for from linear regressions were calculated and are presented in Table 2.7.2.

FIG. 2.7.2. Angular transformations of infectivity indices in relation to log transformations of F. solani var. coeruleum population estimates from three dilutions. Two series of field soils.





Dilution 10^{-2} • •
Dilution 10^{-3} △ △
Dilution 10^{-4} × ×

Regress. lines from Ang. Inf. Ind. on transform. pop. est.
Dilution 10^{-2} ———
Dilution 10^{-3} – – – –
Dilution 10^{-4} .....

Table 2.7.2. Data describing relationship between infectivity indices and population estimates from different dilutions of seven series of soils.

Sample		Dilution		
		10^{-2}	10^{-3}	10^{-4}
1	a	0.9464	0.9443	0.8660
	b	88.8	88.4	73.2
2	a	0.9131	0.9218	0.8066
	b	82.2	83.9	62.6
3	a	0.9454	0.9614	0.9519
	b	88.6	91.9	89.9
4	a	0.8671	0.8521	0.8658
	b	73.4	70.7	73.2
5	a	0.9049	0.9552	0.7846
	b	80.6	90.6	58.8
6	a	0.9766	0.8936	0.7838
	b	95.1	78.4	58.7
7	a	0.9388	0.8766	0.7335
	b	87.3	75.2	50.5

a = correlation coefficient

b = percentage variance accounted for

The association between figures for the infectivity indices and the 10^{-2} dilution population estimates are very satisfactory as the graphs indicate. As expected, the 10^{-4} dilution shows a lower correlation coefficient, this being primarily because of the zero population estimates registered. As stated earlier, the high dilution itself prohibits the detection of lower populations of the fungal propagules.

In discussing the sensitivity of the tuber inoculation and soil dilution plate methods, it should be remembered that, with the former method using 20 tubers, 4-6 g of soil is inoculated and that, with the latter method with 10 plates, 0.1 g of soil is spread on the ten plates.

The upper limit of the 10^{-2} dilution population estimate with this series of samples closely matches the 100% level of the tuber inoculation method. This limit is imposed because of the physical restrictions upon the number of colonies it was possible to observe on one plate. Although the true population may be higher, as indicated by the higher dilutions of the plate method, the equivalent of a level of 100% infectivity is probably all that is required for this study.

These results indicated that the soil dilution plate method produces results similar to those of the tuber inoculation method. Thus it was considered reasonable to use the soil dilution method instead of the tuber inoculation method where large numbers of soil samples require testing for the presence of F. solani var. coeruleum.

In the following Experimental Work (sections 3-10), where treatment effects from one experiment are assessed using more than one method, the methods are described consecutively before the results are considered, also consecutively. This facilitates the comparison of results. Otherwise, experimental results follow the description of the method applied.

3. The influence of the seed tuber on soil contamination by F. solani var. coeruleum.

3.1. The influence of the seed tuber on soil contamination by F. solani var. coeruleum. 1971 experiment.

By taking soil samples from around plants and examining these samples for the presence of F. solani var. coeruleum, it was hoped to learn more about the effect of the seed tuber, infected or contaminated with the fungus, on soil contamination.

Five replicates of three treatments of Catriona tubers (2 x 16 per replicate) were planted, in a randomised block design, on Boghall farm in April 1971. The treatments were as follows:

A. Infected seed tubers (lesions approx. 2.5 cm diam.).

B. Healthy, naturally contaminated seed tubers.

C. Healthy seed tubers disinfected with an organo-mercury fungicide (Agallol, 1 lb/20 gals for 1 min.).

Soil and plant samples were taken in late September 1971 prior to the lifting of the whole experiment, from five plants randomly selected from each replicate. The samples were as follows:

(a). Soil from next to a tuber on the outside of the plant.

(b). That part of the stolons attached to the progeny tubers.

(c). Soil from the centre of the plant.

(d). Soil next to the mother tuber.

Progeny tubers from each replicate were harvested separately, by hand fork, and stored in new paper sacks in a farm store.

3.1.1. Evaluation of soil and tuber contamination using potato tubers.

3.1.1a. The level of surface contamination of the tubers with F. solani var. coeruleum was investigated by taking twenty progeny tubers from each replicate in December 1971 and applying standard wounds. Two wounds, approximately $\frac{1}{4}$ inch deep and $\frac{1}{4}$ inch in diameter, one on each side of the tuber were made using sterilised glass rods as described by McKee & Boyd (1952). Ten tubers were placed in a small cardboard box (previously sterilised with an alcohol spray), the bottom of which was covered with damp peat, then dampened with a fine water spray. These boxes were placed in larger cardboard boxes, the sides of which were lined with water-soaked newspaper. Each of these contained 12-16 small boxes. Ten tubers of each replicate were stored in

a temperature regime of 15°C for eight weeks and ten in a temperature regime of six weeks at 4°C followed by four weeks at 15°C. This was to allow the comparison of the dual temperature regime with the single temperature method.

In March 1972 the experiment was repeated but only twenty tubers per treatment per storage regime were wounded.

3.1.1b. Subsamples from each (c) sample (soil from centre of plant) from each treatment were combined and thoroughly mixed. These were then inoculated into forty Catriona test tubers (previously surface-sterilised with 2% formalin). The inoculation method of McKee & Boyd (1952) was used. This involves making a small wound, as in the previous experiment, and using the small spoon at the other end of the glass rod (described by the authors) to carry approximately 0.1 g of soil and to pour this into the wound. Two inoculations were made in each tuber. The tubers were packed and stored, twenty tubers per treatment per storage regime, as described in section 3.1.1a.

3.1.2. Evaluation of soil contamination using the soil dilution plate method with PCNB-sucrose medium.

Some of the samples collected were examined in March 1972 using the soil dilution plate method with the PCNB-sucrose medium. (At this time the PM70 medium had not been developed.) A full description of the procedure is given in section 3.2.2. As different amounts of soil were used for each (a), (b), (c) and (d) sample, a separate dilution series was calculated for each sample to achieve

a final dilution of 2×10^{-4}.

3.1.1. Results.

Soil samples from the trial area before planting were not tested so it is possible that contamination present in the field was responsible for the contamination detected in samples from the healthy, contaminated and healthy, disinfected treatments.

Figures from the soil inoculation tests (Table 3.1.1) indicate that the soil from around plants grown from infected seed is more contaminated with F. solani var. coeruleum propagules than that from plants grown from healthy seed. Figures from the wounding test (also in Table 3.1.1) do not present such a clear picture as the treatment differences are not so great. However, considering the results as a whole, the experiment does confirm earlier results of Boyd & Logan (1967a) and Boyd & O'Donnell (1968) that infected seed tubers do affect the degree of contamination with F. solani var. coeruleum propagules of soil around plants.

The superiority of the dual temperature storage in allowing infection to take place (Boyd & Logan, 1967b) is not clear from these results. This may have been caused by variation in the temperature of the incubators used.

3.1.2. Results.

In Table 3.1.2 it can be seen that, using the soil dilution plate method with the PCNB-sucrose agar, great variation between population estimates from different

TABLE 3.1.1. Dry rot infection (%) after inoculation of soils into test Catriona tubers and after wounding progeny tubers.

Seed planted	Soil inoculation into test Catrion tubers				Wounded progeny tubers			
	Dec 1971		Mar 1972		Dec 1971		Mar 1972	
	15°C	4-15°C	15°C	4-15°C	15°C	4-15°C	15°C	4-15°C
Infected with dry rot	70.0	100.0	100.0	72.5	26.0	49.0	37.5	45.0
Healthy, not disinfected	10.0	0	20.0	20.0	13.0	29.0	20.0	22.5
Healthy, disinfected	70.0	5.0	32.5	7.5	16.0	18.7	15.0	10.0

TABLE 3.1.2. Population estimates (propagules/g) of F. solani var. coeruleum in soil samples taken from plants grown from infected or contaminated seed as shown, using the soil dilution plate method on PCNB-sucrose medium. Sample origin, (a)-(d), as in text.

Replicate	Plant	A. Infected seed				Replicate	Plant	B. Healthy, not disinfected seed			
		Sample origin						Sample origin			
		(a)	(b)	(c)	(d)			(a)	(b)	(c)	(d)
1	1	0	0	0	0	1	1	0	0	0	0
	2	0	500	0	*		2	0	0	0	0
	3	0	500	1,000	25,500						
	4	0	8,000	0	*	2	1	0	0	0	0
							2	0	0	0	0
2	1	0	1,500	0	164,000						
	2	500	0	500	11,000	3	1	0	0	0	0
	3	0	0	1,000	0		2	0	0	0	0
	4	0	21,000	0	131,500						
						4	1	0	0	0	0
3	1	0	10,500	0	250,000 +		2	0	5,500	0	34,000
	2	0	2,500	1,500	8,000						
	3	0	7,000	0	3,500	5	1	0	0	0	500
	4	0	21,000	0	*		2	0	0	0	0

* No sample

samples (a, b, c and d) from the same plant was obtained. Despite this variation a comparison of population estimates of F. solani var. coeruleum in soil samples from plants grown from infected seed (A) and those from plants grown from healthy but contaminated seed (B) reveals consistent differences. Only two out of ten plants of treatment B showed evidence of contamination whereas eleven out of twelve plants of treatment A show contamination with F. solani var. coeruleum propagules. None of the treatment C samples were examined by the soil dilution plate method.

The variation of results may have been caused by uneven sampling and the unsatisfactory plating medium. Samples from origins (a), (c) and (d) were from one site only and (b) from a small number of sites. The samples could therefore be described as being not representative of the F. solani var. coeruleum propagule population around entire plants. The poor rate of detection achieved by the medium was caused in part by the small amount of soil placed on each of the ten plates which did not allow the detection of populations less than 500 prop./g.

3.2. The influence of the seed tuber on soil contamination by F. solani var. coeruleum. 1972 experiment.

The primary purpose of this experiment was the same as that of experiment 3.1. Also, soil samples were taken during the growth period to determine whether the soil population of F. solani var. coeruleum varies markedly through the season. A treatment of infected tubers given

a benomyl dust application was included to assess the effect of benomyl on the development of the fungal population.

The experimental plan was similar to that of experiment 3.1 with four replicates of 2 x 12 tubers planted on Easter Howgate farm.

The treatments of Catriona seed tubers were as follows:

A. Infected: inoculated tubers (lesions approx. 2.5 cm diam.).

B. Healthy, disinfected: tubers dipped in an organo-mercury compound (Agallol, 1 lb/20 gals for 1 min.) two days before planting.

C. Healthy, contaminated: tubers disinfected, then contaminated with F. solani var. coeruleum spores incorporated into a soil paste.

D. Infected: as in A with benomyl dust (Benlate (10% a.i.) applied at 10 lb/ton) applied before planting.

Soil samples were taken from the drills before planting and tested as described in section 3.1.1b. A low level of soil contamination with F. solani var. coeruleum (5-10% infectivity index) was found in some parts of the trial plot.

Samples were taken from three plants, randomly selected, in each replicate on four occasions during the growing season: 14 July, 9 Aug., 18 Sept., 6 Oct..

Each plant was carefully dug up and the soil, root system and tubers placed on a sheet of newspaper. The tubers were removed and put in a plastic bag. A sample

of the root system was taken and placed in a paper envelope. This was allowed to dry before being stored at 4°C. Whenever located, the mother tuber was removed and discarded. The root system was then shaken vigorously over the paper and most of the loosened soil poured into one or two plastic bags. In the laboratory the bags containing the tubers were slit to allow the soil on the tuber surfaces to dry. This soil was then brushed off with a bronze wire brush (sterilised between each use) and the soil stored in paper envelopes at 4°C. The rhizosphere soil samples were spread on newspaper and allowed to dry in the laboratory. They were then sieved (2 mm sieve), a sample placed in a screw-topped glass bottle and stored at 4°C.

Before examination by the tuber inoculation or the soil dilution plate methods, samples from the three plants of each replicate were mixed thoroughly before a subsample was taken.

The plants remaining after the fourth sampling were lifted and the tubers of each replicate stored separately in new ½ cwt sacks in a farm store. Some of these stored tubers were used to obtain tuber surface soil samples for examination in Dec. 1972 and Feb. 1973 and some for a wounding experiment in Oct. 1972 and Jan. 1973.

3.2.1. Evaluation of soil and tuber contamination using potato tubers.

3.2.1a. Progeny tubers from each replicate were wounded as in experiment 3.1.1a (twenty per replicate) in Oct. 1972

and Jan. 1973 and incubated in the $4^{\circ}/15^{\circ}C$ temperature regime.

3.2.1b. Aggregate soil samples, each representing a replicate plot of rhizosphere or tuber surface soil samples from each of the four samplings (except for tuber surface soil in the first sampling when not enough soil was available) were inoculated into surface sterilised Catriona test tubers during the 1972-3 winter (Jan. 1973 and Apr. 1973).

3.2.2. Evaluation of soil contamination using the soil dilution plate method with the PM70 and PCNB-sucrose media.

Examination of soil samples using the soil dilution plate method was carried out at three dilutions on two media, 10^{-2} and 10^{-3} on PM70 medium and 10^{-4} on PCNB-sucrose medium.

Tuber surface and rhizosphere samples.

A suspension of 5 g soil made up to 200 ml with 0.15% water agar solution was beaten for 1 minute in a MSE Atomix Blendor. Using a 5 ml pipette, 10 ml were pipetted into 15 ml sterile water, giving the 10^{-2} dilution, and 4 ml into 96 ml sterile water, giving the 10^{-3} dilution. Five ml of the latter suspension were pipetted into 45 ml sterile water to give the 10^{-4} dilution. Care was taken to charge and discharge the pipettes used several times to fill surface adsorption sites before actually taking the sample (Wieranga, 1958; Hornby, 1969). The blendor containing the soil suspension was held in the hand and the contents

swirled immediately before pipetting the samples, as was the 10^{-3} dilution before taking the sample for the 10^{-4} dilution.

Rhizoplane samples.

The dried root samples of the three plants of each replicate of each sampling were combined and added to a small weighed jar containing 30 ml sterile water. The roots were allowed to soak for 1 hour, then after being shaken vigorously in the water, removed, dried in an oven, allowed to return to ambient moisture levels and then weighed. The weight of the rhizoplane soil plus water plus jar was taken. From these weights were calculated the amount of water taken from the jar by the wet roots and thence the amount of soil in the remaining water. The weight of the soil and the volume of water in the jar allow the calculation of the amount of water to be added to achieve the first dilution of 10^{-2}. Five ml of the 10^{-2} dilution was added to 45 ml sterile water to give the 10^{-3} dilution and 5 ml of this added to a further 45 ml to give the 10^{-4} dilution.

The 10^{-2} and 10^{-3} dilutions were plated onto PM70 medium, 10 plates with 1 ml suspension on each. The plates had been prepared at least two days before and allowed to dry. This assisted the absorption of the suspension liquid when put on the surface of the plate. An Eppendorf 1000 automatic micro-pipette was used for this final pipetting. After pipetting five plates, the

plates were gently swirled around so that the surface of the plates was covered by the suspension. This was repeated for the second batch of five plates. The 10^{-4} dilution was plated on to PCNB-sucrose medium in a similar manner.

The plates were stored in a Gallenkamp cooled incubator at 20°C until the diluting water had dried on the plates. They were then stacked 20 plates high on the laboratory bench and counts of F. solani var. coeruleum and the red Fusarium colonies taken after 14 days. F. solani var. coeruleum counts were checked after a further 8-15 days.

When possible the moisture content of individual soil samples was measured. If only small amounts of soil were available, these were bulked and a combined soil moisture content taken. Soils were placed overnight in an incubator at 105°C and then reweighed.

3.2.1. Results.

3.2.1a. The progeny tuber wounding experiment showed clear differences in the degree of contamination of progeny tubers from the different treatments (Table 3.2.1a). Tubers from treatment A (infected mother tubers) showed high contamination, those from treatment D (infected plus benomyl) considerably less and those from treatments B (healthy, disinfected) and C(healthy contaminated) very little except for one case in treatment C. Percentage dry rot infection was greater from the second wounding

TABLE 3.2.1a. Dry rot infection (%) after wounding progeny tubers in Oct. 1972 & Jan. 1973.

Treatment	Replicate	% infection	
		Oct 1972	Jan 1973
A	1	16.7	62.5
	2	10.4	72.5
	3	27.1	90.0
	4	18.8	72.5
B	1	0	0
	2	0	7.5
	3	0	0
	4	2.1	20.0
C	1	0	12.5
	2	0	7.5
	3	0	10.0
	4	0	50.0
D	1	2.1	42.5
	2	0	55.0
	3	10.4	22.5
	4	2.1	20.0
Standard error		1.8	8.2
Significance rating		***	**

Significance ratings:

NS	$P > 0.05$
*	$0.05 > P > 0.01$
**	$0.01 > P > 0.001$
***	$0.001 > P$

(i.e., Jan. 1973). This is most likely to have been caused by an increase in tuber susceptibility or, on the other hand, by an increase in the amount of effective inoculum of F. solani var. coeruleum (either by an increase in the numbers of the fungus or by a decrease in the numbers of fungi antagonistic to it). Even though the $4^{o}/15^{o}C$ storage temperature regime increases the susceptibility of tubers to dry rot at the beginning of the storage season, it was thought that the tubers were still likely to have been less susceptible to dry rot in October than in January.

3.2.1b. The tuber inoculation method showed that the same kind of treatment differences in degree of contamination with F. solani var. coeruleum existed in soils from tuber surfaces and the rhizosphere (Table 3.2.1b). As with the wounding experiment, treatments A and D gave the highest percentage infection figures. Surprisingly, the plants of treatment B seem to have had more soil contamination than treatment C. A good association between the figures from soils from the two sources (i.e., tuber surface and rhizosphere) existed except for four pairs of samples in treatment B. No pattern over the time period of the four samplings seemed evident.

3.2.2. Results.

Table 3.2.2a contains the soil population estimates as shown by the soil dilution plate method for the four samplings for the soils from tuber surfaces, and Table 3.2.2b the $\log_{10}(x+1)$ transformed figures. This

TABLE 3.2.1b. Percentage dry rot lesions caused by F. solani var. coeruleum after inoculation of tuber surface and rhizosphere soil samples into test Catriona tubers.

Treatment	Replicate	Soil sample						
		Tuber surface			Rhizosphere			
		Sampling			Sampling			
		2	3	4	1	2	3	4
A	1	100.0	100.0	95.0	97.5	100.0	100.0	100.0
	2	97.5	100.0	100.0	100.0	97.5	100.0	97.5
	3	100.0	100.0	92.5	97.5	100.0	100.0	100.0
	4	100.0	100.0	100.0	100.0	97.5	100.0	100.0
B	1	72.5	0	2.5	5.0	100.0	5.0	0
	2	5.0	22.5	5.0	0	5.0	90.0	62.5
	3	2.5	2.5	2.5	0	10.0	2.5	2.5
	4	85.0	70.0	75.0	10.0	87.5	10.0	27.5
C	1	0	0	0	0	0	0	0
	2	12.5	2.5	20.0	0	7.5	12.5	17.5
	3	0	0	0	0	2.5	0	0
	4	10.0	2.5	0	37.5	12.5	0	2.5
D	1	80.0	62.5	67.5	55.0	65.0	77.5	77.5
	2	87.5	42.5	65.0	55.0	92.5	80.0	62.5
	3	95.0	42.5	72.5	30.0	97.5	77.5	92.5
	4	20.0	12.5	40.0	75.0	75.0	85.0	37.5
Standard error		15.7	10.9	11.1	5.1	14.3	10.2	10.0
Significance rating		*	***	***	***	**	***	***

N.B. Insufficient tuber surface soil from Sampling 1 for inoculation method.

TABLE 3.2.2a. Population estimates (propagules/g) of F. solani var. coeruleum in soils from tuber surfaces, taken at four sampling dates, as shown by the soil dilution plate method used at three dilutions, two of these on PM70 medium and the 10^{-4} on PCNB-sucrose medium.

Treatment	Replicate	SAMPLING 1			SAMPLING 2			SAMPLING 3			SAMPLING 4		
		10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
A	1	26,400	30,900	42,000	13,241	15,453	17,170	8,945	11,526	12,240	16,100	23,100	31,000
	2	4,200	4,800	4,000	15,978	18,382	13,130	7,430	16,200	30,000	10,940	11,600	19,000
	3	65,100	71,100	60,000	12,575	16,297	19,114	27,438	45,084	53,040	8,050	10,400	13,000
	4	5,030	8,000	3,000	29,425	49,026	43,645	15,606	23,562	21,420	12,760	18,200	21,000
B	1	0	0	0	81	40	0	0	0	0	20	0	0
	2	0	0	0	0	0	0	245	102	0	0	0	0
	3	20	0	0	0	0	0	10	0	0	10	0	0
	4	0	0	0	1,360	1,218	4,060	745	510	2,040	630	600	2,000
C	1	0	0	0	0	0	0	0	0	0	20	0	0
	2	0	0	0	20	0	0	0	0	0	60	0	0
	3	60	0	0	0	0	0	0	0	0	10	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0
D	1	80	0	0	121	303	0	418	102	0	1,420	1,400	2,000
	2	920	800	0	1,250	1,212	3,030	133	204	0	2,410	3,300	8,000
	3	1,930	1,500	5,000	579	407	2,034	3,417	5,610	9,180	1,240	1,700	4,000
	4	42,500	29,200	47,000	200	200	0	816	612	3,060	260	300	1,000

TABLE 3.2.2b. $Log_{10}(x + 1)$ transformations of population estimates of F. solani var. coeruleum in soils from tuber surfaces as shown in Table 3.2.2a.

Treatment	Replicate	SAMPLING 1			SAMPLING 2			SAMPLING 3			SAMPLING 4		
		10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
A	1	4.42	4.49	4.62	4.12	4.19	4.23	3.95	4.06	4.09	4.21	4.36	4.49
	2	3.62	3.68	3.60	4.20	4.26	4.12	3.87	4.21	4.48	4.04	4.07	4.28
	3	4.81	4.85	4.78	4.11	4.21	4.28	4.44	4.65	4.72	3.91	4.02	4.11
	4	3.70	3.90	3.48	4.47	4.69	4.64	4.19	4.37	4.33	4.11	4.26	4.32
B	1	0	0	0	1.91	1.61	0	0	0	0	1.32	0	0
	2	0	0	0	0	0	0	2.39	2.01	0	0	0	0
	3	1.32	0	0	0	0	0	1.04	0	0	1.04	0	0
	4	0	0	0	3.13	3.09	3.61	2.87	2.71	3.31	2.80	2.78	3.30
C	1	0	0	0	0	0	0	0	0	0	1.32	0	0
	2	0	0	0	1.32	0	0	0	0	0	1.78	0	0
	3	1.79	0	0	0	0	0	0	0	0	1.04	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0
D	1	1.91	0	0	2.09	2.48	0	2.62	2.01	0	3.15	3.15	3.30
	2	2.96	2.90	0	3.10	3.08	3.48	2.13	2.31	0	3.38	3.52	3.90
	3	3.29	3.18	3.70	2.76	2.61	3.31	3.53	3.75	3.96	3.09	3.23	3.60
	4	4.63	4.47	4.67	2.30	2.30	0	2.91	2.79	3.49	2.42	2.48	3.90

transformation was employed because it is a standard technique in the examination and analysis of biological population data. Also, figures resulting from this transformation show a more meaningful relationship between treatments regarding the relative infectivity of the soils.

The equivalent tables of results of the rhizosphere and rhizoplane samples and those of the tuber surface samples taken from tubers in storage are presented in the Appendix (Tables A.3-A.8).

Table 3.2.2c presents the mean (of the four aggregate samples) population estimates from the 10^{-2} dilution of the tuber surface, rhizosphere and rhizoplane samples and Table 3.2.2d the mean $\log_{10}$ (x+1) transformations of population estimates.

The aggregate soil samples from plants grown from infected seed tubers (treatment A) showed much higher population estimates than the samples from other treatments. As in experiments 3.2.1a and 3.2.1b, the samples from treatment D (infected seed plus benomyl) gave the next highest figures with the treatments B (healthy, disinfected seed) and C (healthy, contaminated seed) population estimates being, for the most part, zero. It was thought that the disinfection carried out on the seed tubers before the application of the contaminating soil paste (treatment C), may have affected the fungus in the paste. Subsequent results showed that this was not the case.

TABLE 3.2.2c. Mean population estimates of F. solani var. coeruleum in soils from tuber surfaces, the rhizosphere and rhizoplane, as shown by the soil dilution plate method at 10^{-2} dilution on PM70 medium.

Treatment	Tuber surface soil samples						Rhizosphere soil samples				Rhizoplane soil samples			
	Field				Storage		Field				Field			
	Sampling				Sampling		Sampling				Sampling			
	1	2	3	4	1	2	1	2	3	4	1	2	3	4
A	25,182	17,805	14,855	11,968	8,248	8,788	9,608	12,013	4,319	29972	4,627	5,286	6,634	37,924
B	4	360	250	165	22	135	3	431	24	47	0	684	20	121
C	15	4	0	22	2,330	12,560	73	14	0	5	199	0	76	30
D	11,358	538	1,196	1,332	1,310	1,155	199	279	295	425	112	288	720	8,264

TABLE 3.2.2d. Mean $\log_{10}(x + 1)$ transformation of population estimates of *F. solani* var. *coeruleum* in soils from tuber surfaces, the rhizosphere and rhizoplane, as shown by the soil dilution plate method at 10^{-2} dilution on PM70 medium.

Treatment	Tuber surface soil samples						Rhizosphere soil samples				Rhizoplane soil samples			
	Field				Storage		Field				Field			
	Sampling				Sampling		Sampling				Sampling			
	1	2	3	4	1	2	1	2	3	4	1	2	3	4
A	4.14	4.22	4.11	4.06	3.90	3.87	3.92	4.02	3.60	3.41	3.62	3.39	3.71	4.43
B	0.33	1.26	1.58	1.29	0.74	1.08	0.27	1.78	0.50	1.24	0.00	0.78	0.96	1.19
C	0.45	0.33	0.00	1.04	2.93	3.38	1.31	0.94	0.00	0.34	0.72	0.00	0.96	0.84
D	3.20	2.56	2.80	3.01	3.02	2.36	2.28	2.40	2.46	2.60	1.60	2.30	2.85	3.70

Standard errors of the differences of means for comparison of means of same treatment:

A 0.245
B 0.783
C 0.562
D 0.421

These figures do not apply to the means of tuber surface samples from storage.

This pattern was consistent over the results from the three methods of sampling (tuber surface, rhizosphere and rhizoplane) and over the four sampling times. However, the population estimates of F. solani var. coeruleum in soil samples from treatment C taken after storage showed some differences from the general pattern (Table 3.2.2c and Tables A.7-A.8). These figures fit in well with the observed infectivity indices of tubers wounded in Jan. 1973 (Table 3.2.1a).

The population estimates for the replicates of the aggregate samples are presented in the tables so that it can be clearly seen that in most cases the healthy, disinfected tubers (B) and healthy, contaminated seed tubers (C) induced little or no contamination of the plant soil samples. The anomalous figures seen in the population estimates from these treatments (as shown in Tables 3.2.2a,b) were thought to have been caused by late dry rot development in the mother tuber of one plant of the three making up the aggregate soil sample. Soil samples from single samples were checked and a sample from a single plant was found to have been giving rise to the discrepancy in most cases. That such population estimates could be linked with extraneous dry rot infection in the mother tubers was shown in the following year (1973), when all mother tubers were examined when samples were taken.

Differences in population estimates between aggregate samples within treatments A and D are thought to be acceptable and to be due to normal plant to plant variation.

Viewing these figures after $\log_{10}(x + 1)$ transformation showed that the variation, on a biological scale, is not large. There appears to be more variation among the estimates of treatment D than those of treatment A, caused, presumably, by the action of the fungicide.

Population estimates for the aggregate samples from each sampling source (tuber surface, rhizosphere and rhizoplane) are generally in good agreement. Where differences occur this can be explained by the different nature of the sampling methods involved. With the small amounts of soil taken and the close proximity to the tubers, high population estimates would be expected from the tuber surface samples. Lower population estimates were obtained, as might be predicted, from rhizosphere samples which involve the entire plant root system. Rhizoplane samples were taken from root samples that were not necessarily generally representative of the plant. The major exception to the overall agreement is the results of A and D aggregate samples from the 4th rhizoplane sampling. These population estimates are much higher than the corresponding tuber surface and rhizosphere figures. This could mean that the dying roots at the end of the season were sites for saprophytic action of the fungus and that considerable build-up of soil contamination with F. solani var. coeruleum occurred at these sites.

The means of the transformed population estimates (Table 3.2.2d) of the treatment D (infected seed plus benomyl) were consistently lower than those of the

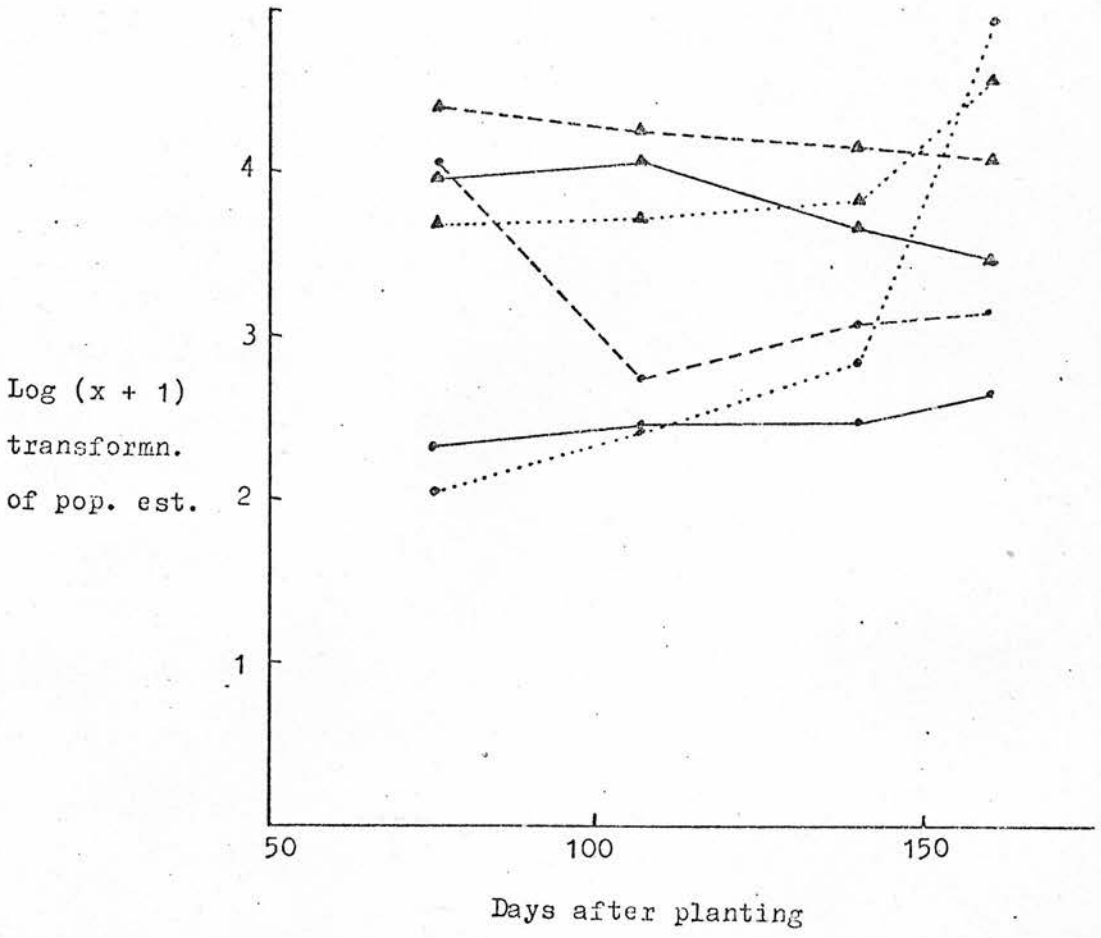
treatment A. Exceptions in treatment D were from the first sampling of the tuber surface and the fourth sampling of the rhizoplane. The former of these was caused by one anomalously high population estimate. Thus, the benomyl treatment had had some effect in reducing the F. solani var. coeruleum population development.

No evidence for an increase in soil populations around plants grown from infected seed (treatment A) in the later part of the growing season was shown by the results of the examination of tuber surface and rhizosphere samples. Some indications of a build-up of soil populations can be seen in the data from the rhizosphere and rhizoplane samples from treatment D and the rhizoplane samples from treatment A. These results are presented in Fig. 3.2.2 (figure drawn from transformed data).

Agreement between the three transformed population estimates from the three dilutions of each aggregate sample was generally good. The figures in Table 3.2.2b, showing that the higher dilutions produce higher estimates of high populations and that the use of low dilutions allows the detection of low populations, support the remarks made to this effect in section 2.6. In later experiments only the 10^{-2} dilution was used.

Excellent correlation between the results of the tuber inoculation method and the soil dilution plate technique was achieved. The correlation coefficients and percentage variances accounted for were presented

FIG. 3.2.2. Means of log(x 1) transformations of F. solani var. coeruleum population estimates of tuber surface, rhizosphere and rhizoplane soils for two treatments in relation to days after planting.



earlier in Table 2.7.2. In this table, samples 1-3 were tuber surface samples from the 2nd, 3rd and 4th sampling dates and 4-7 the rhizosphere samples, No. 4 sample being from the 1st sampling date.

Standard errors were calculated from a set of analyses of the transformed population estimates. These analyses are discussed in the Appendix under Table A.9.

Counts of red Fusarium colonies (mostly F. culmorum) are not presented.

3.3. The influence of the seed tuber on soil contamination by F. solani var. coeruleum. 1973 experiment.

This experiment was planned to verify the results of the previous year's experiment and to provide further data on the build-up of the F. solani var. coeruleum population. This was to be achieved by planting seed tubers inoculated with the fungus just prior to planting and by sampling earlier during the course of the season. Further information was to be obtained on the action of fungicides on the developing F. solani var. coeruleum populations. By examining more plants at each sampling date it was hoped that some of the anomalies of the previous year's results could be explained.

The experimental layout was similar to that of experiment 3.2 with four replicates of 2 x 16 plants each on Langhill farm.

Treatments of the Catriona seed tubers were as follows:

A. Infected: inoculated tubers (lesions approx. 2.5 cm diam.).

B. Infected: tubers inoculated two days before planting.

C. Healthy, contaminated: a mixture of heavily contaminated field soils from experiment 3.2 was crushed to a dust and sieved. The tubers were covered with this powder.

D. Healthy, disinfected: tubers dipped in an organo-mercury fungicide (Agallol, 1 lb/20 gals for 1 min.) before planting.

E. Infected: as in A with benomyl dust (Benlate (10% a.i.) at 10 lb/ton) applied before planting.

F. Infected: as in B and treated with benomyl dust as in E.

G. Contaminated: as in C with benomyl dust as in E.

H. Infected: as in B and treated with tecnazene dust (Fusarex dust applied at 10 lb/ton) applied before planting.

I. Contaminated: as in C with tecnazene dust as in H.

Soil samples were taken from the drills before planting and tested for F. solani var. coeruleum using the soil plate (PM70 medium) dilution method. F. solani var coeruleum was not detected in these samples.

The seed tubers were planted on 30 April, 1973. Five plants per replicate were lifted on each of the sampling dates shown in Table 3.3. The development of the plants

TABLES 3.3. Dates of sampling of soils and progeny tubers in experiment 3.3.

Planted 30 April 1973. 1-5 = sampling sequence. A-I = treatments.

Day	June			July				August					September			
	10-16	-23	-30	-7	-14	-21	-28	-4	-11	-18	-25	-1	-8	-15	-22	-29
Sunday																
Monday		1 BFG	1 I	1 H 2 ACE				3 BFG				4 HI				
Tuesday								3 DHI								
Wednesday	1 AE					2 H										5 ABC
Thursday		1 D			2 DI						4 ABE					5 DEFG
Friday	1 C			2 BFG			3 AEC				4 DFG					5 HI
Saturday																

Experiment 3.3.

Sampling intervals

Sampling	Days after planting	Days between A treatment sampling
1	44-63	
		19
2	63-79	
		25
3	88-92	
		27
4	115-119	
		34
5	149-151	

in some treatments, particularly the tecnazene treatments, was slow so the first two samplings of these treatments were delayed until the plants reached the same growth stage at which treatments A and E had been sampled.

Plants were lifted by hand fork and the soil and root system placed in the neck of a large plastic bag. Progeny tubers and the mother tuber were removed carefully and placed in two separate plastic bags (except for samplings 1 and 2 where tubers were absent or too small to supply enough soil for examination). The root system was shaken vigorously in the large plastic bag and then removed. This rhizosphere soil in the bag was thoroughly mixed. The soil and bag were weighed, then a sample tipped into a smaller plastic bag.

The rhizosphere soil samples were dried, sieved and sampled again in the laboratory and the soil on the surface of the tubers dried and brushed off as in experiment 3.2. Samples were stored as in experiment 3.2.

The mother tubers were cut open in the laboratory and assessed for infection with F. solani var. coeruleum. This was extremely difficult with the later samplings as many tubers either had soft rots or had decayed almost completely, leaving only the thickened epidermal layers intact. However, even with soft rotted tubers, the colour of the rots in which F. solani var. coeruleum had been present initially was distinctive in most cases. With some lesions and with all mother tubers of the last sampling where diagnosis was uncertain, some tuber

tissue remains were plated onto PM70 medium.

Moisture factors were taken as in experiment 3.2.

After the plants had been lifted at the fifth sampling date, the remainder of the plants were dug up and the tubers from each replicate stored in separate new paper bags in a farm store.

3.3.1. Evaluation of soil contamination using potato tubers.

Progeny tubers were wounded as in experiment 3.2.1a in December 1973.

3.3.2. Evaluation of soil contamination using the soil dilution plate method with PM70 medium.

Rhizosphere samples were examined as in experiment 3.2.2, except that only the 10^{-2} dilution was used and the five samples from each replicate were not mixed together but were tested separately.

Because of the pressure of time, the tuber surface samples of only the fifth sampling date were tested.

Tuber surface samples were taken also in Jan. 1974 from a limited number of tubers of the progeny tubers lifted in September and kept in a farm store. One sample per replicate was examined by the method described in experiment 3.2.2.

3.3.1. Results.

Results from the progeny tuber wounding method are presented in Table 3.3.1. Treatments D (healthy,

TABLE 3.3.1. Percentage of dry rot lesions on progeny seed (Catriona) which had been given standard wounds. Dec. 1973.

Treatment	Replicate				Mean
	1	2	3	4	
A	32.5	30.0	12.5	20.0	23.8
B	2.5	30.0	10.0	17.5	15.0
C	5.0	15.0	0	7.5	6.9
D	0	0	0	0	0
E	0	0	0	0	0
F	0	0	0	0	0
G	2.5	0	0	0	0.6
H	15.0	5.0	5.0	10.0	8.8
I	10.0	35.0	15.0	0	15.0

disinfected seed), E (infected with benomyl applied), F (inoculated at planting time, followed by benomyl treatment) and G (contaminated with benomyl applied) show zero or very low infectivity percentages. The results of treatments A (infected), B (inoculated at planting time) and C (healthy, contaminated) agree substantially with those of experiments 3.1 and 3.2. The tecnazene treatment applied before planting (treatments H and I) appears to limit the amount of soil contamination of progeny tubers but does not prohibit its development. In comparison, the contamination on the hand-lifted progeny of dry rot infected benomyl dusted seed is extremely low.

Analysis of the results of treatments A, B, C, H and I shows a significant difference between treatment A and treatments C and H at the 5% probability level.

3.3.2. Results.

The results from the soil dilution plate method are presented in Tables 3.3.2a-d. Tables 3.3.2a and 3.3.2b refer to the population estimates in the untransformed and transformed states from the rhizosphere samples taken on five occasions through the season. Table 3.3.2c shows the means of the population estimates and the means of the $\log_{10}(x+1)$ transformations of the population estimates of each treatment for the five rhizosphere samplings and for two tuber surface samplings (the 5th field sampling and the sampling after storage).

The full tables containing the F. solani var.

TABLE 3.3.2a. Population estimates (propagules/g) of F. solani var. coeruleum in rhizosphere soil samples taken at five sampling dates as shown by the soil dilution plate method on PM70 medium at 10^{-2} dilution. Mean of ten plates.

Replicate	Plant	Treatment A					Treatment B					Treatment C					Treatment D				
		Sampling					Sampling					Sampling					Sampling				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	1	113	1,555	1,048	65	357	0	20	22	1,466	196	30	115	113	32	21	0	0	565	0	0
	2	0	621	395	1,406	907	0	0	206	673	2,007	10	0	32	10	0	11	0	0	0	0
	3	820	161	21	1,232	2,022	0	101	54	0	1,082	21	11	0	53	0	0	0	0	150	10
	4	354	159	125	5,696	503	63	82	318	416	343	30	0	0	162	71	10	0	0	0	0
	5	799	104	357	625	1,990	0	20	168	218	1,597	0	0	11	0	1,988	0	0	0	0	0
2	1	32	124	706	3,434	125	0	0	21	233	422	0	0	0	0	0	0	0	0	0	0
	2	302	84	318	647	1,880	11	10	31	265	364	0	0	245	23	0	0	0	0	0	0
	3	330	281	437	978	583	0	113	20	201	483	20	11	21	0	458	0	0	0	0	0
	4	110	85	541	1,487	357	0	102	1,123	177	835	0	0	0	1,895	31	0	0	0	0	0
	5	4,610	65	162	912	336	21	0	10	206	0	0	0	0	0	260	0	0	231	0	0
3	1	270	653	1,548	639	1,914	31	10	10	54	205	0	10	52	21	0	0	0	0	0	0
	2	389	2,322	1,796	1,550	3,392	2,688	11	0	99	242	0	0	10	0	0	0	0	0	0	0
	3	125	1,049	1,477	185	2,776	11	62	0	244	52	0	11	10	0	42	0	258	0	10	0
	4	114	1,484	880	653	1,537	0	163	62	126	191	0	0	21	0	0	0	0	0	0	11
	5	177	39	1,239	313	609	614	31	62	126	2,115	0	0	10	5,995	0	0	0	0	0	0
4	1	124	273	218	1,787	483	95	31	0	770	57	0	0	0	494	0	0	0	0	0	0
	2	10,740	347	276	477	1,091	76	42	306	396	433	0	0	0	1,238	32	0	0	0	0	11
	3	159	281	32	917	281	52	31	803	986	33	31	0	28	1,319	160	0	0	0	0	11
	4	21	120	421	410	182	0	173	1,362	162	251	0	0	32	0	0	0	0	0	0	0
	5	250	0	168	76	150	363	82	0	1,277	203	20	43	884	0	0	11	0	0	0	0

TABLE 3.3.2a contd.

Replicate	Plant	Treatment E					Treatment F					Treatment G					Treatment H					Treatment I				
		Sampling					Sampling					Sampling					Sampling					Sampling				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	1	52	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	319	147	583	388	0	412	578	242	614
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	310	525	1,154	498	1,448	0	10	0	75	0
	3	0	0	10	0	339	0	0	0	0	0	0	0	0	0	0	0	51	30	74	20	0	0	10	225	283
	4	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	610	31	0	21	1,895	294	104	10	42	1,040
	5	0	0	0	0	0	22	0	0	0	0	0	0	0	0	655	32	40	0	158	1,689	11	133	31	2,214	1,685
2	1	0	0	0	0	0	0	0	0	0	0	0	0	21	0	84	147	53	147	746	545	10	489	31	2,935	52
	2	0	42	0	43	21	0	0	0	0	0	0	0	0	0	666	0	71	32	1,092	603	32	0	0	159	1,134
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–	214	336	76	666	32	0	93	95	374
	4	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	32	31	180	702	0	0	0	0	0	288
	5	40	0	0	0	0	0	0	0	0	0	0	0	10	0	11	–	10	942	137	512	0	144	886	0	371
3	1	0	10	0	0	0	0	0	0	0	196	0	0	0	0	0	–	52	43	368	659	42	0	0	0	494
	2	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	10	–	150	64	336	1,279	0	2,290	121
	3	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	80	216	229	1,331	343	11	185	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1,017	0	53	10	0	43	375	150
	5	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	80	51	382	95	0	82	622	1,045	665	0
4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	191	75	313	0	10	0	42	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	187	52	462	21	0	10	62	198	414
	3	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	280	2,184	32	138	1,156	0	10	0	32	0
	4	0	0	0	11	0	0	11	0	0	0	0	0	0	0	0	0	–	286	238	281	10	0	0	11	286
	5	0	0	20	0	0	0	0	11	0	0	0	0	63	0	0	0	364	578	179	11	0	72	64	0	332

– No sample taken

TABLE 3.3.2b. $Log_{10}(x + 1)$ transformations of population estimates of F. solani var. coeruleum in rhizosphere soils shown in Table 3.3.2a.

Replicate	Plant	Treatment A					Treatment B					Treatment C					Treatment D				
		Sampling					Sampling					Sampling					Sampling				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	1	2.06	3.19	3.02	1.82	2.55	0	1.32	1.36	3.17	2.29	1.49	2.06	2.06	1.52	1.34	0	0	2.75	0	0
	2	0	2.79	2.60	3.15	2.96	0	0	2.32	2.83	3.30	1.04	0	1.52	1.04	0	1.08	0	0	0	0
	3	2.91	2.21	1.34	3.09	3.31	0	2.01	1.74	0	3.03	1.34	1.08	0	1.73	0	0	0	0	2.16	1.04
	4	2.55	2.20	2.10	3.76	2.70	1.81	1.92	2.50	2.62	2.54	1.49	0	0	2.21	1.86	1.04	0	0	0	0
	5	2.90	2.02	2.55	2.80	3.30	0	1.32	2.23	2.34	3.20	0	0	1.08	0	3.30	0	0	0	0	0
2	1	1.52	2.10	2.85	3.54	2.10	0	0	1.34	2.37	2.63	0	0	0	0	0	0	0	0	0	0
	2	2.48	1.93	2.50	2.81	3.27	1.08	1.04	1.51	2.42	2.56	0	0	2.39	1.38	0	0	0	0	0	0
	3	2.52	2.45	2.64	2.99	2.77	0	2.06	1.32	2.31	2.68	1.32	1.08	1.34	0	2.66	0	0	0	0	0
	4	2.05	1.93	2.73	3.17	2.55	0	2.01	3.05	2.25	2.92	0	0	0	3.28	1.51	0	0	0	0	0
	5	3.66	1.82	2.21	2.96	2.53	1.34	0	1.04	2.32	0	0	0	0	0	2.42	0	0	2.37	0	0
3	1	2.43	2.82	3.19	2.81	3.28	1.51	1.04	1.04	1.74	2.31	0	1.04	1.72	1.34	0	0	0	0	0	0
	2	2.59	3.37	3.25	3.19	3.53	3.43	1.08	0	2.00	2.39	0	0	1.04	0	0	0	0	0	0	0
	3	2.10	3.02	3.17	2.27	3.44	1.08	1.80	0	2.39	1.72	0	1.08	1.04	0	1.63	0	2.41	0	1.04	0
	4	2.06	3.17	2.95	2.82	3.19	0	2.21	1.80	2.10	2.28	0	0	1.34	0	0	0	0	0	0	1.08
	5	2.25	1.60	3.09	2.50	2.79	2.79	1.51	1.80	2.10	3.33	0	0	1.04	3.78	0	0	0	0	0	0
4	1	2.10	2.44	2.34	3.25	2.68	1.98	1.51	0	2.89	1.76	0	0	0	2.69	0	0	0	0	0	0
	2	4.03	2.54	2.44	2.68	3.04	1.89	1.63	2.49	2.60	2.64	0	0	0	3.09	1.52	0	0	0	0	1.08
	3	2.20	2.45	1.52	2.96	2.45	1.72	1.51	2.91	2.99	1.53	1.51	0	1.46	3.12	2.26	0	0	0	0	1.08
	4	1.34	2.08	2.63	2.61	2.26	0	2.24	3.13	2.21	2.40	0	0	1.52	0	0	0	0	0	0	0
	5	2.40	0	2.23	1.89	2.18	2.56	1.92	0	3.11	2.31	1.32	1.64	2.95	0	0	1.08	0	0	0	0

TABLE 3.3.2b contd.

Replicate	Plant	Treatment E					Treatment F					Treatment G					Treatment H					Treatment I				
		Sampling					Sampling					Sampling					Sampling					Sampling				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	1	1.72	1.53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.51	2.17	2.77	2.59	0	2.62	2.76	2.39	2.51
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.49	2.72	3.06	2.70	3.16	0	1.04	0	1.88	0
	3	0	0	1.04	0	2.53	0	0	0	0	0	0	0	0	0	0	0	1.72	1.49	1.68	1.32	0	0	1.04	2.35	2.42
	4	0	0	0	0	0	0	0	1.08	0	0	0	0	0	0	0	2.79	1.51	0	1.34	3.28	2.47	2.02	1.04	1.63	3.02
	5	0	0	0	0	0	1.36	0	0	0	0	0	0	0	0	2.82	1.52	1.61	0	2.20	3.23	1.08	2.13	1.51	3.35	3.23
2	1	0	0	0	0	0	0	0	0	0	0	0	0	1.34	0	1.93	2.17	1.73	2.17	2.87	2.74	1.04	2.69	1.51	3.47	1.72
	2	0	1.63	0	1.64	1.34	0	0	0	0	0	0	0	0	0	2.82	0	1.86	1.52	3.04	2.78	1.52	0	0	2.20	3.05
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	2.33	2.53	1.89	2.82	1.52	0	1.97	2.00	2.57
	4	0	0	0	0	0	0	0	0	0	0	0	0	1.04	0	0	1.52	1.51	2.26	2.85	0	0	0	0	0	2.43
	5	1.61	0	0	0	0	0	0	0	0	0	0	0	1.04	0	1.08	-	1.04	2.97	2.14	2.71	0	2.16	2.95	0	2.57
3	1	0	1.04	0	0	0	0	0	0	0	2.29	0	0	0	0	0	—	1.72	1.64	2.57	2.82	1.62	0	0	0	2.69
	2	0	0	0	0	1.04	0	0	0	0	0	0	0	0	0	0	0	1.04	-	2.18	1.81	2.53	3.11	0	3.36	2.28
	3	0	0	0	1.36	0	0	0	0	0	0	0	0	0	0	0	1.91	2.34	2.36	3.12	2.54	1.08	2.27	0	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.01	0	1.73	1.04	0	1.64	2.58	2.18
	5	0	0	0	0	0	0	0	1.53	0	0	0	0	0	0	0	1.91	1.72	2.58	1.98	0	1.92	2.79	3.02	2.64	0
4	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.52	2.28	1.88	2.50	0	1.04	0	1.63	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.04	2.27	1.72	2.67	1.34	0	1.04	1.80	2.30	2.62
	3	0	0	0	0	0	0	0	1.08	0	0	0	0	0	0	0	2.45	3.34	1.52	2.14	3.06	0	1.04	0	1.52	0
	4	0	0	0	1.08	0	0	1.08	0	0	0	0	0	0	0	0	0	-	2.46	2.38	2.45	1.04	0	0	1.08	2.46
	5	0	0	1.32	0	0	0	0	1.08	0	0	0	0	1.81	0	0	0	2.56	2.76	2.26	1.08	0	1.66	2.81	0	2.52

- No sample taken

TABLE 3.3.2c. Means of population estimates and of $\log_{10}(x + 1)$ transformations of population estimates of F. solani var. coeruleum in soils from the rhizosphere, and tuber surfaces, as shown by the soil dilution plate method at 10^{-2} dilution on PM70 medium.

Treatment	Mean population estimate							Mean log(x + 1) transformation of population estimate						
	Rhizosphere samples					Tuber surface		Rhizosphere samples					Tuber surface	
	Sampling					5th sampling	*After storage	Sampling					5th sampling	*After storage
	1	2	3	4	5			1	2	3	4	5		
A	249**	490	608	1,170	1,070	7,520	3,320	2.13**	2.31	2.57	2.85	2.84	3.54	3.15
B	70***	54	229	229	556	3,940	230	0.96***	1.41	1.58	2.34	2.39	2.78	2.15
C	8	10	73	563	154	1,630	15	0.48	0.40	1.02	1.26	0.92	1.30	0.69
D	2	13	40	8	2	48	32	0.16	0.12	0.26	0.16	0.21	0.46	0.78
E	5	4	2	4	18	24	3	0.17	0.21	0.12	0.20	0.25	0.24	0.26
F	1	1	3	0	10	2	3	0.07	0.05	0.24	0	0.11	0.17	0.26
G	0	0	5	0	71	22	0	0	0	0.26	0	0.43	0.50	0
H	88	233	304	356	533	2,630	1,500	1.06	1.87	2.05	2.04	2.20	2.59	2.50
I	44	174	142	490	393	1,750	460	0.84	1.29	1.05	1.73	1.93	2.65	2.07

Standard errors of differences of means (transformed figures) for treatments A, B, H and I of rhizosphere samples only: when comparing means of same level of sampling 0.289;
when comparing means of same level of treatment 0.261.

* Means of 4 samples from replicates (plots)

** Two anomalous figures discarded } computer-assigned values inserted

*** One anomalous figure discarded

TABLE 3.3.2d. Means of (i) total F. solani var. coeruleum rhizosphere population estimates, and (ii) $\log_{10}(x + 1)$ transformations of total F. solani var. coeruleum rhizosphere population estimates.
Total population estimate (propagules/plant) = population estimate (propagules/g) x rhizosphere soil weight.

	Sampling				
	1	2	3	4	5
Treatment A					
Mean total F. solani var. coeruleum rhizosphere population estimate	-	627,750	2,889,850	3,568,400	3,464,350
Mean $\log_{10}(x+1)$ transformation of total F. solani var. coeruleum rhizosphere population estimate	-	5.394	6.221	6.309	6.337
Treatment B					
Mean total F. solani var. coeruleum rhizosphere population estimate	-	159,850	804,950	1,131,350	1,250,900
Mean $\log_{10}(x+1)$ transformation of total F. solani var. coeruleum rhizosphere population estimate	-	4.709	4.953	5.739	5.777
Treatment H					
Mean total F. solani var. coeruleum rhizosphere population estimate	53,500	166,500	1,088,368	1,097,315	1,711,700
Mean $\log_{10}(x+1)$ transformation of total F. solani var. coeruleum rhizosphere population estimate	3.851	4.835	5.459	5.719	5.656

coeruleum population estimates for the rhizosphere samples from each plant (Tables 3.3.2a,b) are presented in the text so that the number of zero population estimates from treatments C, D, E, F and G can be appreciated. This is not shown so clearly in the table of the means of the population estimates (Table 3.3.2c) for 20 plants because of the disproportionate effect of a few large population estimates.

Table 3.3.2d shows the mean total population estimates for three treatments; that is, the mean of the multiples of the population estimate (propagules/g) and the weight of rhizosphere soil (g/plant) present with the plants when they were lifted in the field. Three treatments only (treatments A, B and H) which had high population estimates and for which rhizosphere soil weight figures were available, were considered. As these mean total population estimates do not alter the general picture shown by the mean population estimates (propagules/g), the total population estimates for all plants sampled are not presented. (Soil weights from plants of treatments A, B and H are in the Appendix, Table A.10.)

The results shown in these tables agree substantially with those of the wounding experiment (Table 3.3.1).

Soil samples from plants grown from tubers that were disinfected (D) or which were infected (E), inoculated just prior to planting time (F) or contaminated but to which benomyl had been applied before planting (G), were

found to have little or no contamination. Where a lesion had been present on the seed tuber (A), more soil contamination with F. solani var. coeruleum developed than from tubers inoculated just prior to planting (B) and the latter was greater than that from seed tubers contaminated only (C).

Where tecnazene was applied (treatments H and I), a surprising amount of soil contamination developed. The population estimates show that this compound did have some effect on population development but did not inhibit the spread of the fungus as did benomyl.

An increase in the level of contamination with time over the five sampling dates is evident. Figures 3.3.2a and 3.3.2b present the mean F. solani var. coeruleum population estimates and the mean total population estimates (transformed data) of four and three treatments against sampling times. Consideration of the results of statistical analysis confirmed that there was a definite trend of population increase over the sampling period. The development of soil contamination in treatments B and H (inoculated, without and with tecnazene) seems to be at a similar rate to that of treatment A, but it occurs at a later stage in the growth of the plant and thus, at time of harvest, the soil populations of F. solani var. coeruleum had reached a much lower level.

Table 3.3.2c presents the mean soil population estimates, and the mean $\log_{10}(x+1)$ transformations, of the tuber surface samples taken at the fifth sampling time. Complete results

FIG. 3.3.2a. Means of log(x+1) transformations of F. solani var. coeruleum population estimates of rhizosphere soils for four treatments in relation to days after planting.

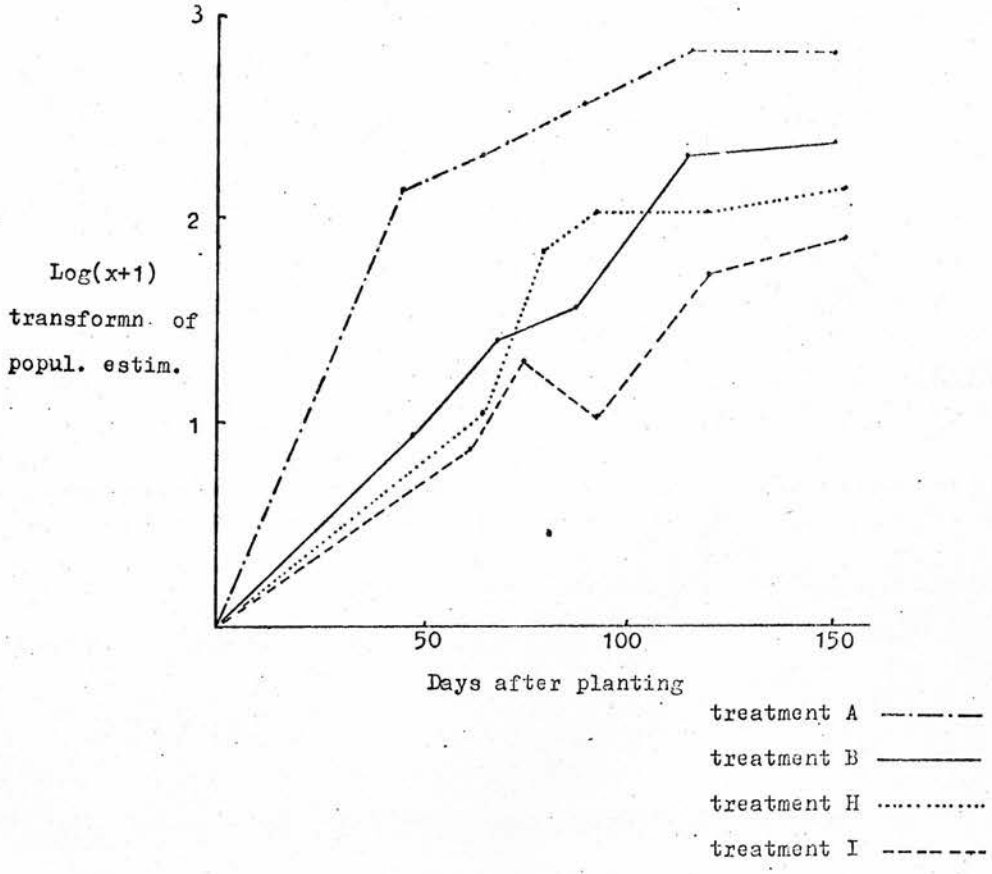
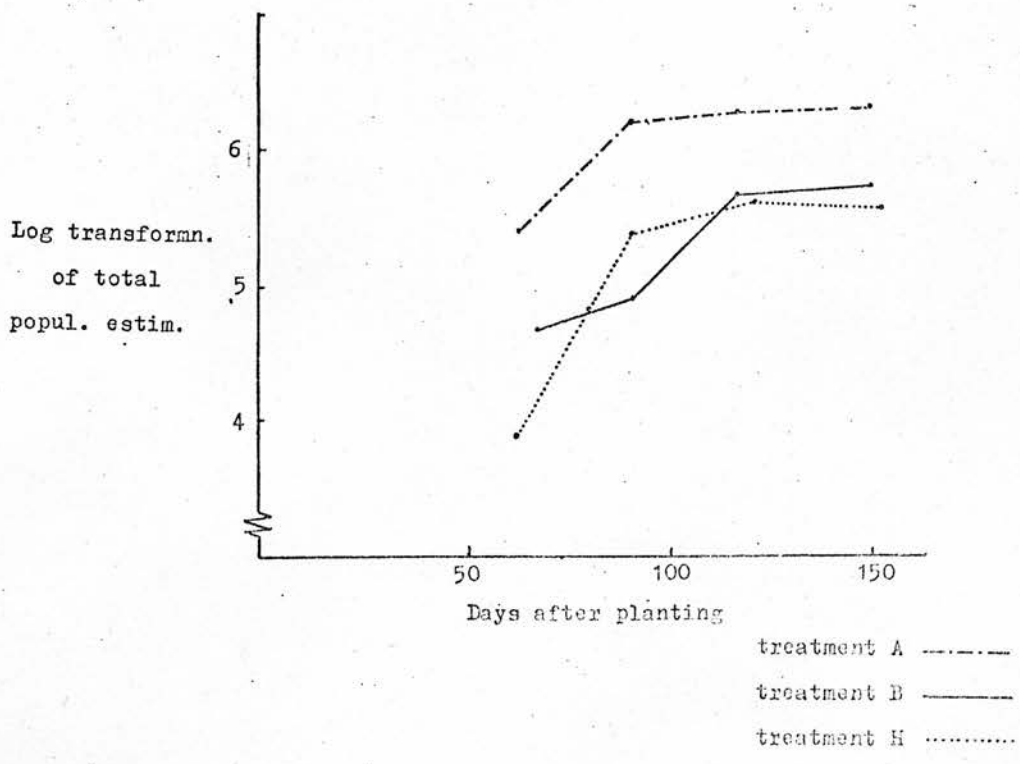


FIG. 3.3.2b. Means of log transformations of total F. solani var. coeruleum population estimates of rhizosphere soils for three treatments in relation to days after planting.



are given in the Appendix, Tables A.11 and A.12. The degree of contamination, over the nine treatments, is similar to that shown by the rhizosphere sample results.

Examination of the mother tuber when plants were lifted showed that the anomalous high population estimates of F. solani var. coeruleum obtained from some soil samples of treatments C and I (contaminated, without and with tecnazene) were from plants where the mother tuber had developed dry rot after planting. Also, the majority of the low population estimates were associated with some decay of the mother tuber, the nature of which was not determined when the mother tubers were examined. This unintended development of lesions may have occurred in previous experiments and have been responsible for the soil contamination detected in samples from the contaminated seed tuber treatment in experiments 3.1 and 3.2. The results of the inspection of mother tubers carried out at lifting are presented in the Appendix, Table A.13.

Table 3.3.2c shows the mean population estimates, and mean log(x + 1) transformations of population estimates, of tuber surface samples taken in January 1974 from tubers lifted in September 1973. Complete results are presented in the Appendix, Table A.14. The same relative treatment differences in contamination can be seen. This differs from the results of the previous year's experiments (see Table 3.2.2c and Appendix, Tables A.7, A.8).

A short note discussing the statistical analysis of results is included in the Appendix after Table A.14.

4. The effect of different proportions of F. solani var. coeruleum infected mother tubers in seed tuber populations on the proportion of contaminated progeny tubers.

To prove that the dry rot infected tuber plays an important part in causing the contamination of progeny tubers, this must be shown to be the case under normal agricultural practice. It should be possible to show that a seed stock containing a high proportion of infected tubers produces more contaminated progeny tubers (and thus more tubers with a disease potential) than a seed stock with a low proportion of infected tubers.

The cultivar Catriona was again used and treatments were as follows:

A.	20	infected	tubers	20	healthy	tubers	(not disinfected)
B.	8	"	"	32	"	"	"
C.	2	"	"	38	"	"	"
D.	0	"	"	40	"	"	"

With treatment A the infected tubers were planted alternately with the healthy; with treatment B every fifth tuber was infected; and with treatment C the 9th and 30th of each 40 tubers were infected. There were four replicates. Healthy tubers were not disinfected because of time limitations immediately before planting.

To achieve some similarity to normal agricultural practice, the trial was lifted by machine (a single row elevator). So that the machine did not transfer contamination, the treatments were lifted in the order of that

which was expected to have least contamination to that expected to have the highest (i.e., D to C to B to A). (It was considered impracticable to attempt to sterilise the machine between treatments.) To allow this, and so that the newly lifted tubers did not fall onto soil more contaminated than that in which they had been grown, the treatments were planted in a series of low to high infection. Thus no real randomisation was possible in the confines of the described limits and the general field plan. After being lifted by machine, tubers were place in hessian sacks, the replicates being bagged separately, and stored in a farm store until 12 December, 1973.

The degree of contamination of the tubers was assessed by wounding the tubers and counting the F. solani var. coeruleum rots that developed. The tubers were passed over a Cooch reciprocating wire riddle to produce the wounding. The wire riddle and rollers were sterilised the day before use, with 2% formalin and between treatments with 70% alcohol (but not between replicates). Treatments were riddled in the following order, D1-4, C1-4, B1-4 and A1-4. Following riddling, tubers were stored in a farm store until the end of January 1974, then given three weeks in covered sacks in a glasshouse at 15°C to accelerate the development of lesions. In February 1974 tubers were examined and the number of infected tubers noted and the weights of the healthy and of the diseased tubers taken.

4. Results.

Far more rots developed on the B, C and D treatments than had been expected (see Table 4). It would seem likely that much of the seed planted as healthy (which had not been disinfected) subsequently rotted with F. solani var. coeruleum. Also, there was extensive opportunity during all the operations for cross-infection.

However, the means of the percentage numbers of infected tubers of each treatment show a gradation increasing with the increasing number of infected mother tubers. Although not demonstrated as well as would have been wished, it is clear that under field conditions and using a mechanical lifter, the proportion of infected mother tubers planted is related to the proportion of contaminated progeny tubers.

TABLE 4. The effect of different proportions of F. solani var. coeruleum infected seed tubers in seed lots on the proportion of infected tubers among progeny tubers.

Treatment	Replicate	Total no. of tubers	No. of infected tubers	% No. inf./tot. tubers	Total wt of tubers (lbs)	Wt of infected tubers(lbs)	% Wt inf./tot. tubers
A	1	322	95	29.5	76.0	32.0	42.1
	2	330	83	25.2	104.25	32.25	30.1
	3	401	155	38.6	91.75	54.0	58.8
	4	375	109	29.1	109.25	45.75	41.6
Mean				30.6			43.2
B	1	374	111	29.6	91.5	36.25	39.6
	2	342	99	28.9	91.0	29.0	31.8
	3	371	82	22.1	89.25	26.5	29.7
	4	316	42	13.3	72.5	15.75	21.7
Mean				23.5			30.7
C	1	384	79	20.6	91.75	22.75	24.8
	2	424	83	19.6	88.5	32.5	36.7
	3	351	53	15.1	90.5	17.0	18.8
	4	354	90	25.4	80.75	29.0	35.9
Mean				20.2			29.0
D	1	442	92	20.8	115.75	35.25	30.5
	2	315	14	4.4	75.25	5.25	7.0
	3	415	65	15.7	119.5	28.25	23.6
	4	464	61	13.1	129.75	26.0	20.0
Mean				13.5			20.3

5. The influence of the seed tuber on soil contamination by Fusarium sulphureum.

In the course of potato tuber disease control work at this time, the fungus F. sulphureum was isolated from tubers showing symptoms partly resembling those caused by F. solani var. coeruleum and partly those caused by Phoma exigua var. foveata (Boyd & Tickle, 1972) (see Plate 5). An attempt was made to determine if populations of F. sulphureum developed from infected seed tubers in a similar manner to those of F. solani var. coeruleum.

Experimental layout was in the form of randomised blocks. Four replicates, each of 10 tubers, of three treatments were planted on 11 May, 1973. Treatments of the Catriona seed tubers were as follows:

A. Infected with F. sulphureum (lesions approx. 2.5 cm diam.).

B. Healthy, contaminated with a fine dust of an unsterile soil previously inoculated with F. sulphureum.

C. Untreated: undisinfected tubers.

Three samplings were made during the season; 17 July, 5 Sept., and 7 Oct.. Rhizosphere and tuber surface soil samples were taken and stored in the manner described in section 3.3. Mother tubers when possible were examined for infection with F. sulphureum or F. solani var. coeruleum. Soil samples from the tuber surfaces were taken on the second and third samplings (tubers were too small at first sampling). Samples were tested separately for the presence of F. sulphureum by the tuber inoculation

PLATE 5. Symptoms of gangrene, dry rot caused by F. sulphureum and dry rot caused by F. solani var. coeruleum.

From left to right: symptoms of gangrene, F. sulphureum dry rot, F. solani var. coeruleum dry rot on Catriona tubers. All symptoms caused by inoculation of field soil samples into tubers. Inoculations carried out at different dates.

technique as described in section 3.1.1.

5. Results.

When examining mother tubers it was not possible in most cases to ascertain the cause of decay when tubers were rotting. Therefore no data regarding the state of the mother tubers are presented except for a note concerning a few anomalous results which is included in Table 5.

It appears from the results shown in Table 5 that F. sulphureum infection of the original mother tuber affects the contamination of the soil samples associated with the plants in a manner apparently similar to that of F. solani var. coeruleum. However, the infectivity indices are a little lower than one might expect if F. sulphureum behaved similarly to F. solani var. coeruleum. This could be due to the smaller production of inoculum in the soil, or to a more restricted spread of the inoculum, or the conditions of incubation of the test tubers may not have been optimum for infection. It was noted when examining tubers infected with F. sulphureum, from the tuber inoculation tests of this experiment, that no sporodochial sporulation occurred on the surface of lesions, a feature which is common on lesions caused by F. solani var. coeruleum under similar circumstances.

TABLE 5. Dry rot lesions (%) caused by F. sulphureum after inoculation of tuber surface and rhizosphere soil samples into test Catriona tubers.

Replicate	Plant	Tuber Rubbings						Rhizosphere Samples								
		Sampling 2			Sampling 3			Sampling 1			Sampling 2			Sampling 3		
		Treatment			Treatment			Treatment			Treatment			Treatment		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	1	0	2.5*	0	27.5	0	0	17.5	0	0	10.0	2.5*	0	0	0*	0
	2	7.5	0	0	40.0	0	0	2.5	0	0	17.5	0	0	0	0	0
	3	5.0	0	0	27.5	0	0	7.5	0	0	5.0	0	0	20.0	2.5*	0
2	1	0	0	0	12.5	0	0	32.5	0	0	0	0	0	40.0	0	0
	2	92.5	0	0	35.0	0	0	45.0	0	0	30.0	0	0	0	2.5*	0
	3	70.0	0	0	15.0	0	0	7.5	0	0	32.5	0	0	20.0	0	0
3	1	7.5	0	0	62.5	0	0	0	0	0	7.5	0	0	55.0	0	0
	2	25.0	2.5	0	27.5	0	0	0	0	0	0	0	0	12.5	0	0
	3	12.5	0	0	2.5	0	0	17.5	0	0	20.0	0	0	2.5	0	0
4	1	12.5	0	0	7.5	0	0	0	0	0	30.0	0	0	2.5	0	**
	2	5.0	0	0	5.0	0	0	0	0	0	20.0	0	0	0	0	0
	3	10.0	0	0	2.5	0	0	10.0	0	0	42.5	0	0	12.5	0	0
Treatment Mean		20.5	0.4	0	22.1	0	0	11.6	0	0	17.9	0.2	0	13.7	0.4	0

* Culture plate test indicated that mother tuber infected by F. sulphureum.

** No sample.

6. The influence of the seed tuber on soil contamination by Phoma exigua var. foveata.

6.1. The influence of the seed tuber on soil contamination by Phoma exigua var. foveata. 1971 experiment.

This experiment was planned to investigate the relationship between mother tubers infected with gangrene and the production of soil contamination around progeny tubers.

Experimental layout was in the form of randomised blocks with five replicates. Three treatments of Redskin seed tubers were as follows:

A. Infected: tubers inoculated with P. exigua var. foveata (lesions approx. 2.5 cm diam.).

B. Healthy, contaminated: natural contamination.

C. Healthy, disinfected: tubers dipped in an organo-mercury solution (Agallol, 1 lb/20 gals for 1 min.).

To determine differences between treatments in the level of contamination with Phoma exigua var. foveata of the soil near to or on the progeny tubers, soil samples from these areas were inoculated into tubers (as in section 3.1.1b) and the progeny tubers were wounded (as in section 3.1.1a).

Samples of soil from the central part of five plants of each replicate were taken at lifting, mixed, then inoculated in Dec. 1971 and March 1972 into surface sterilised Redskin test tubers. Tubers from plots were stored

separately and samples of each treatment given standard wounds in Dec. 1971 and Mar. 1972. The inoculated and wounded tubers were incubated under two storage regimes of 15°C for 10-12 weeks or 4°C for 6 weeks followed by 4 weeks at 15°C.

6.1. Results.

Table 6.1 shows that the infected mother tubers did not produce increased soil contamination as assessed by tuber inoculation and progeny tuber wounding experiments. These inconclusive results, with a lack of discernable pattern among treatments, are similar to those reported by Boyd & Logan (1967a) and Boyd & O'Donnell (1968). That no consistent result appears evident over the figures from any one seed tuber treatment indicates that there was great variation in the amount of contamination produced by the plants within a treatment. Thus, inoculum initiation and build-up was not related to the state of the mother tuber. Soil contamination present in the field before planting (not checked in this experiment) may have had some influence on this experiment.

With all three treatments, in both December and March with the two techniques, the dual temperature storage regime produced higher infectivity indices.

6.2. The influence of the seed tuber on soil contamination by Phoma exigua var. foveata. 1972 experiment.

It was decided to repeat the previous experiment and

TABLE 6.1. Gangrene infection (%) after inoculation of soils into test Redskin tubers and after wounding progeny tubers.

Seed planted	Soil inoculation into test Redskin tubers				Wounded progeny tubers			
	Dec 1971		Mar 1972		Dec 1971		Mar 1972	
	15°C	4-15°C	15°C	4-15°C	15°C	4-15°C	15°C	4-15°C
Infected with gangrene	2.5	20.0	0	15.0	13.0	26.0	5.0	30.0
Healthy, not disinfected	12.5	17.5	15.0	77.5	29.0	40.0	5.0	37.5
Healthy, disinfected	10.0	40.0	2.5	7.5	23.0	25.0	17.5	30.0

to investigate the action of benomyl on any contamination due to infected seed tubers.

The experimental layout was in the form of randomised blocks with four replicates. Redskin tubers were used and treatments were as follows:

A. Infected: naturally infected tubers from stock with a high % infection (lesions approx. 2.5 cm diam.).

B. Healthy, contaminated: tubers disinfected, then contaminated with a soil paste containing mycelium of P. exigua var. foveata.

C. Healthy, disinfected: tubers dipped in an organo-mercury solution (Agallol, 1 lb/20 gals for 1 min.).

D. Infected: as in A with benomyl dust (Benlate (10% a.i.) applied at 10 lb/ton) applied before planting.

Soil samples were taken from the drills in the trial area before planting and tested for contamination using the tuber inoculation method. The results of this test showed that a very low level of contamination was present over the trial area used.

Differences between treatments in the levels of contamination were investigated as in experiment 6.1. Soil samples were taken at lifting times and inoculated into healthy Catriona tubers in November 1972. Tubers from the plots were either wounded immediately after lifting in October 1972 or stored separately and samples from each replicate wounded in March 1973. In October, 160 tubers per treatment were given standard wounds and in March, 64 tubers per treatment were wounded.

Inoculated and wounded tubers were incubated at 4°C for 6 weeks, followed by a further 4 weeks at 15°C.

6.2. Results.

In contrast to a similar experiment carried out the previous year (section 6.1), the infection of the mother tuber had a marked effect on the soil and progeny tuber contamination by P. exigua var. foveata (see Table 6.2). It is possible that the weather conditions of 1972 were suitable for the expression of this effect and that this was not the case for 1971. Wetter soil conditions in 1971 could have encouraged the rapid decay of mother tubers so that they played little part in the initiation or build-up of contamination.

The contamination of the mother tuber (C) does not seem to have affected the build-up of soil contamination. The higher figures of the results of the October wounding experiment may indicate that tuber susceptibility or tuber contamination decreased during storage.

There appeared to be no limitation of the activity of the fungus by the application of benomyl to the infected tubers.

TABLE 6.2. Gangrene infection (%) after inoculation of soils into test Catriona tubers and after wounding progeny tubers. (Means of four replicates.)

Seed planted	Soil inoculation into Catriona tubers	Wounded progeny tubers	
	Nov 1972	Oct 1972	Mar 1973
A. Infected with gangrene	26.2	25.0	15.2
B. Healthy, disinfected	6.9	11.6	6.4
C. Healthy, contaminated	6.9	17.0	5.9
D. Infected plus benomyl	59.4	23.8	13.8
Standard error	5.68	5.28	1.77
Significance rating	***	N.S.	**

7. The influence of the seed tuber on soil contamination by F. solani var. coeruleum and the effect of a Trichoderma viride spore paste treatment. Glasshouse experiment 1973.

An attempt was made under glasshouse conditions to investigate three topics:

(a) that the influence of the seed tuber on the development of soil contamination by F. solani var. coeruleum was the same under glasshouse conditions as in the field so that the results of other glasshouse experiments could legitimately be related to the field situation.

(b) that when infected mother tubers decay rapidly the build-up of inoculum is limited. It has been observed that more dry rot occurs after dry than wet seasons (Mooi, 1950; Ayers & Ramsay, 1961), and the rate of decay of the mother tuber is thought to be related to this. In this experiment to simulate this early decay mother tubers were removed as soon as the plants were established.

(c) the effect of the application of a spore paste of Trichoderma viride to mother tubers on the development of soil contamination with F. solani var. coeruleum.

Brian, Curtis, Hemming & McGowan (1946) showed that the antibiotic viridin, produced by pigment-forming strains of T. viride, severely reduced the germination of spores of F. solani var. coeruleum. Nadvodnyuk (1962) reported the application of a conidial suspension of T. koningii to tubers before storage to give a 50% reduction in

infection. Joffe (1966) reported a strong negative relation between the incidence of T. lignorum and F. solani in the soil of a citrus grove.

Four replicates of each of nine treatments were grown. Twelve tubers were infected with F. solani var. coeruleum by inoculation and divided into three treatments:

I.C. Infected tuber with no further treatment.

I.R. Infected tuber with mother tuber removed when plant established.

I.T. Infected tuber with a paste of soil inoculated with a concentrated spore suspension (approx. 9×10^6 spores/ml) of T. viride.

The same three treatments were applied to tubers contaminated with a fine soil containing chlamydospores of F. solani var. coeruleum (treatments C.C, C.R and C.T) and to disinfected healthy tubers (treatments S.C, S.R and S.T).

Plants were grown in unsterile field soil with a 13:13:20 NPK potato fertiliser applied at 14 g/ton soil, in 10 inch diameter plastic pots. Soil samples taken at planting time and tested on PM70 medium did not detect any contamination with F. solani var. coeruleum. All tubers were planted on 25 May 1973 except for the T. viride treatment which were planted one week later.

Tuber removal was carried out 23 days after planting. The four infected tubers were found to be almost totally rotted. This premature rotting was thought to have been

induced by the warm conditions of the glasshouse.

7.1. Examination of soil samples taken from near the mother tubers and from the progeny tuber surfaces.

Plants were dug up and tuber and soil samples taken early in October 1973. Pots were tipped on their sides and the rhizosphere soil and tubers carefully removed. Progeny tubers were taken to the laboratory and dried to allow the surface soil to be brushed off and collected. The mother tubers were removed, examined and some tissue placed on PM70 medium to detect F. solani var. coeruleum. The soil immediately surrounding the mother tuber was collected and a sample of the rhizosphere soil taken also. These samples were air-dried, sieved, stored at $4^{o}C$ and tested as described in section 3.2.2 on PM70 medium at 10^{-2} dilution. No moisture factors were used in the calculation of population estimates.

7.1. Results.

The population estimates from samples from tuber surfaces and from near mother tubers are presented in Table 7.1. Samples from all plants except one show low or zero population estimates. As the mother tubers that were removed after 23 days were totally rotted at that stage, it is likely that the other dry rot infected mother tubers were similarly decayed. Thus it is likely that no restriction in population development would have been achieved by the removal of the mother tubers. The

TABLE 7.1. Population estimates of F. solani var. coeruleum (propagules/g) in soils taken close to mother tubers and from progeny tuber surfaces of glasshouse-grown plants.

Mother tuber treatment	Replicate	Untreated			Mother tuber removed			Application of T. viride		
		(a)	(b)	(c)	(a)	(b)	Condition at time of removal	(a)	(b)	(c)
Infected with dry rot	1	20	50	+	0	200	Totally rotted	0	0	-
	2	10	0	+	60	970	Totally rotted	620	60	+
	3	540	40	+	70	0	Totally rotted	10	20	+
	4	10	0	+	10	230	Totally rotted	190	0	+
Contaminated with dry rot propagules	1	0	0	+	0	0	Healthy	0	0	-
	2	0	0	+	0	0	Healthy	0	0	-
	3	0	0	-	10	170	Totally rotted	870	1060	+
	4	0	0	-	0	0	Healthy	10	20	+
Surface sterilised	1	0	0	-	0	0	Healthy	30	20	+
	2	0	0	-	0	0*	*	0	0	-
	3	0	0	-	0	0	Healthy	0	0	-
	4	0	0	-	0	0	Healthy	0	*	-

(a): population estimate of F. solani var. coeruleum in soil near mother tuber.

(b): population estimate of F. solani var. coeruleum in soil from surface of progeny tubers.

(c): + positive identification of F. solani var. coeruleum from tuber remains using PM70 medium.

\- F. solani var. coeruleum not detected.

* : no plant developed.

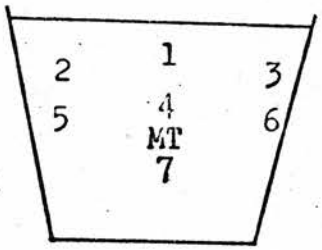
figures in Table 7.1 support this view. This premature rotting will probably have been the cause of the development of the small populations. To achieve any success with this experiment the total decay of the seed tubers must be delayed until later in the growth of the plant.

However, some soil contamination by the dry rot fungus had occurred by the time of tuber removal and, even under warm glasshouse conditions, did appear to be related to the infection of the mother tubers as shown to be the case for field experiments.

The results of the preliminary test of the T. viride soil paste were not encouraging. Six tubers treated with the paste were infected with F. solani var. coeruleum (three were unintended infections) and, of these, five yielded plants with progeny tubers contaminated with the fungus. Little weight can be attached to these results because of atypical soil conditions.

7.2. Distribution of F. solani var. coeruleum in pots.

Further samples were taken from two of the four replicates of treatments I.C, C.C, S.C, I.T, C.T and S.T. when the pot was tipped on its side one half of the pot contents along what had been its vertical axis was removed. Small soil samples were then taken from the positions shown in the diagram overleaf. Because of the shallow planting of some tubers, a position 4 sample could not always be taken.



Pot diagram showing soil sampling positions with respect to the mother tuber (MT).

All soil samples were examined as described in section 7.1.

7.2. Results.

The low soil populations provided an unsatisfactory picture with these results also (Table 7.2). Dispersal of the fungus in the "Infected, no further treatment" plants was not sufficient to contrast with the dispersal or lack of dispersal in the "Infected plus T. viride soil paste" treatment.

Soil samples were taken on two earlier occasions in the season from near the surface in four places from all the pots of experiment 7.1 with the intention of detecting any spread of the fungus. Apart from two of the many samples tested, no F. solani var. coeruleum was detected.

TABLE 7.2. Distribution and values of population estimates of F. solani var. coeruleum in soil samples from pot profiles, investigating the effect of Trichoderma viride on the development of soil contamination by F. solani var. coeruleum.

Primary treatment of mother tuber	Replicate	Secondary treatment of mother tuber: None	Secondary treatment of mother tuber: Covered with soil paste of T. viride spores
Infected with dry rot	1	0 0 0 / 0 10 MT 40 0	0 0 0 / 0 MT 160 0
	2	0 60 10 / 10 53 MT 94 0	0 10 0 / 0 MT 310 0
Contaminated with dry rot fungus propagules	1	0 0 0 / 0 0 MT 0 0	10 0 0 / 0 MT 10 0 *
	2	0 0 0 / 0 0 MT 0 0	0 580 0 / 0 MT 0 0 *
Surface sterilised	1	0 0 0 / 0 0.3 MT 0 0	0 0 0 / 0 MT 0 0
	2	0 0 0 / 0 0 MT 0 0	0 0 0 / 0 MT 0 0 **

* Mother tubers became infected

** No plant developed

8. Prevalence of F. solani var. coeruleum and P. exigua var. foveata on seed stocks.

Seed stocks from several growers were examined for the prevalence of surface contamination with the dry rot and gangrene causal organisms. Because of the great interest shown in seed grown from stem cutting material it was thought that an examination of such stocks might prove worthwhile. Virus tested stem cutting (VTSC) stocks of different grades were examined in 1972 and 1973.

8.1. 1972 Survey.

A preliminary survey was made in spring 1972 to determine the extent of contamination by the dry rot and gangrene fungi in seed stocks, particularly those from stem cuttings.

Samples were taken by brushing, with a sterilised wire brush, the soil off the surface of healthy tubers. Up to 10 g soil was collected. With the small stocks of VTSC first year, particularly those harvested in dry conditions, it was possible to collect only small amounts of soil.

Soil samples were inoculated into surface sterilised Catriona test tubers in March 1972 and incubated for 6 weeks at 4°C, then 4 weeks at 15°C. No lesions developed on the uninoculated, wounded controls.

8.1. Results.

Infectivity percentages are shown in Table 8.1.

It is clear that dry rot and gangrene propagules are

TABLE 8.1. Potential infectivity of soils from surface of seed tubers. Percentage dry rot and gangrene after inoculation into test Catriona tubers. March 1972.

Source	Stock	% Dry rot	% Gangrene	Source	Stock	% Dry rot	% Gangrene
	VTSC 1st Year				VTSC 4th Year		
A	P. Marble	0	2.5	B	King Edward	2.5	2.5
	Majestic	2.5	0		Kerr's Pink	2.5	2.5
	P. Ivory	2.5	0		P. Hawk	2.5	2.5
	A. Pilot	5.0	0		Redskin	2.5	17.5
	P. Dell	5.0	0		Majestic	7.5	77.5
	Craigs Alliance	7.5	2.5		Bintje	12.5	77.5
	King Edward	7.5	10.0	C	Sharpe's Express	2.5	25.0
	Roslin Castle	10.0	7.5		P. Crown	2.5	65.0
	Redskin	15.0	25.0		A. Consul	5.0	35.0
	VTSC 2nd Year				Commercial Stock		
	Pollock's Pink Early	2.5	97.5	D	P. Ivory	0	95.0
					P. Hawk	15.0	10.0
	VTSC 3rd Year			E	P. Dell	7.5	12.5
	P. Glory	10.0	87.5	F	King Edward	7.5	17.5
				G	King Edward	7.5	20.0
				H	Golden Wonder	20.0	7.5
					Majestic	37.5	10.0

A - H indicate different farms

VTSC virus tested stem cutting

present on the surface of seed tubers even in VTSC stocks. Levels of dry rot contamination are low but on some stocks the gangrene contamination is surprisingly high. Seed of VTSC stocks are treated with special care and planted in land that has been seven years out of potatoes, so this high degree of contamination with gangrene propagules was not expected.

8.2. 1973 Survey.

Because of the levels of tuber surface contamination found in 1972 on VTSC seed, soil sampling in 1973 was concentrated on further investigation of the contamination of VTSC stocks.

Soil samples were inoculated into test tubers as in experiment 8.1. The uninoculated wounded controls developed lesions of dry rot indicating that the test tubers had not been satisfactorily disinfected or had become re-contaminated. For this reason, dry rot % infectivities are not quoted. When considering potential gangrene infection, the infection of the control should be noted. Samples were examined also for the presence of F. solani var. coeruleum with the soil dilution plate method using PM70 medium.

8.2. Results.

Results are shown in Table 8.2. Only three samples had high F. solani var. coeruleum population estimates. Percentage infectivities of soils in relation to gangrene

TABLE 8.2. Potential infectivity of soils from the surface of seed tubers: dry rot (DR), number of propagules/g as shown by selective medium; gangrene (G), percentage lesions after inoculation into Catriona test tubers.

Source	Stock	DR	G
	VTSC 1st Year		
A	K. Edward	0	0
	P. Crown	0	0
B	Etoile du Leon	0	2.5
	G. Wonder	0	5.0
	K. Pink	0	5.0
	Majestic	0	5.0
	P. Crown (a)	0	2.5
	P. Crown (b)	0	0
	P. Ivory	0	2.5
	P. Meteor	0	2.5
	Redskin	0	5.0
	K. Edward	20	0
	VTSC 2nd Year		
A	P. Crown	0	2.5
B	A. Pilot	0	7.5
	C. Alliance	0	2.5
	Epicure	0	25.0
	H. Guard	0	5.0
	K. Edward	0	2.5
	P. Crown	0	10.0
	P. Javelin	0	2.5

A - E indicate different farms
VTSC virus tested stem cutting
FS foundation stock

Source	Stock	DR	G
	VTSC 2nd Year (cont.)		
B	P. Marble	0	2.5
	R. C. Royal	0	2.5
	S. Express	0	0
C	C. Alliance	0	0
	R. C. Royal	0	0
	Chancellor	20	0
	VTSC 3rd Year		
B	G. Wonder	0	0
	K. Pink	0	0
	P. Dell	0	5.0
	P. Hawk	0	2.5
	R. Castle	0	0
	Majestic	100	5.0
	Up-to-date	100	2.5
D	Bintje	0	5.0
	K. Pink	0	0
	P. Crown	0	0
	P. Dell	0	0
	P. Hawk	0	0
	Record	0	0
	Red K. E.	0	0
	Redskin	0	2.5

Source	Stock	DR	G
	VTSC 4th Year		
B	Majestic	0	15.0
	M. Peer	0	5.0
	P. Crown	0	2.5
	P. Glory	0	2.5
	P. Pink	0	2.5
	R. Kidney	0	5.0
	C. Alliance	100	5.0
D	K. Edward	0	5.0
	P. Dell	0	5.0
	Red K. E.	0	5.0
	Record	320	10.0
	Majestic	2700	2.5
	Redskin	5500	0
	VTSC (unspec)		
C	R. Kidney	0	0
	A. Comet	20	0
E	P. Crown	0	0
	P. Dell	20	0
	FS 3		
E	Majestic	0	0
	P. Crown	200	15.0
	M. Peer	21000	2.5
	Wounded only	-	0.8

were much lower than those of the previous year. Nevertheless, contamination with Phoma exigua var. foveata propagules was common among the samples examined, with 38 of the 61 samples being contaminated.

It was not possible to examine commercial stocks in numbers large enough for a reasonable comparison of levels of contamination to be made between VTSC and commercial stocks. Nevertheless, the present findings were thought to be of sufficient interest to be submitted.

The VTSC stocks, in most cases, had little or no contamination with F. solani var. coeruleum. On the other hand, some VTSC stocks examined in 1972 were highly contaminated with P. exigua var. foveata. This indicates that, at best, the VTSC scheme can reduce contamination of stocks by those fungi to a low level but does not eliminate them entirely. Thus a chemical treatment would be required at some stage of multiplication if the stocks were to be freed from contamination.

9. Survival of F. solani var. coeruleum in field soil.

To study the survival of the fungus in a field soil, an area of land was chosen which was known to be heavily contaminated with F. solani var. coeruleum and where populations were likely to be high enough to be readily detected. Soil samples were taken at intervals over a period of time.

The area of field used was that occupied by experiment 3.1 in 1971. This experiment, part of which was planted with infected tubers, supplied a small area with a suitably high degree of soil contamination with F. solani var. coeruleum.

9a. Soil samples from experiment 3.1 area were examined in 1972 using the PCNB-sucrose medium (PM70 medium not developed).

9b. In February 1973 a scatter of 8 samples was taken from the general area of experiment 3.1 and tested using the PM70 medium.

9c. Later in the same year a sampling plan of the area (20 yd x 4 yd) was followed at 4-6 week intervals. Points A-F were outside the original trial, points A, B, E and F being from vehicle tracks and not having grown potatoes. An area in the same field known to have been planted with potatoes (of a different cultivar) and of a size equivalent to the original sampling area was also sampled. Sampling dates were: (1) 21 June; (2) 25 July; (3) 4 September; (4) 15 October; (5) 4 December 1973.

The field had been sown with barley in 1972 and with grass for silage and grazing in 1973. PM70 medium was used for the examination of these samples.

The area occupied by one replicate of experiment 4 was also sampled in 1973-1974 with a view to continuing regular samplings. Results are not presented.

Samples were collected with a 1.5 cm diameter tube soil corer and 6 soil cores approximately 4 cm deep taken in an area approximately 30 cm x 30 cm around a set point. Samples were dried, sieved and stored at $4^{o}C$ before being tested. Twenty dilution plates at a dilution of 10^{-2} were made for each sample. No moisture factors were calculated with these samples.

9a. Results.

When soil samples from the area of experiment 3.1 were tested on PCNB-sucrose medium, only 6 of 32 samples yielded colonies of F. solani var. coeruleum. The population estimates were 100, 100, 200, 500, 500 and 1,000 propagules/g. Five of these six were from parts of the plot where infected tubers (treatment A) had been planted.

9b. Results.

Population estimates from the eight samples taken in February 1973 were 10, 25, 10, 20, 15, 30, 50 and 25 propagules/g. The presence of F. solani var. coeruleum propagules in all of the eight samples and at low population levels therefore shows the PM70 medium to be more successful than the PCNB-sucrose medium in testing field soils.

9c. Results.

Results of sampling from the contaminated area are presented in Table 9. Except for three instances, the population estimates in the contaminated plot are very low. An equivalent table of results from the control area is not presented as all the counts of the five samplings were zero. That the fungus was not found in this area well away from the contaminated plot but was found in the area contiguous to the latter (points A-F) seems to indicate that the area of contamination has increased. This could have been brought about by ploughing and associated agricultural practices.

Some relation between the area of high contamination and the area where infected tubers were planted was noted in 1972. Such a relation was not apparent for the results of the 1973 samplings. This may have been caused by a decline in the high populations in the soil or by the contamination becoming more evenly distributed as a consequence of ploughing.

FIG. 9. Distribution of sampling points.

A-F are points outside the area of the original trial.

Trial area 20 x 4 yds.

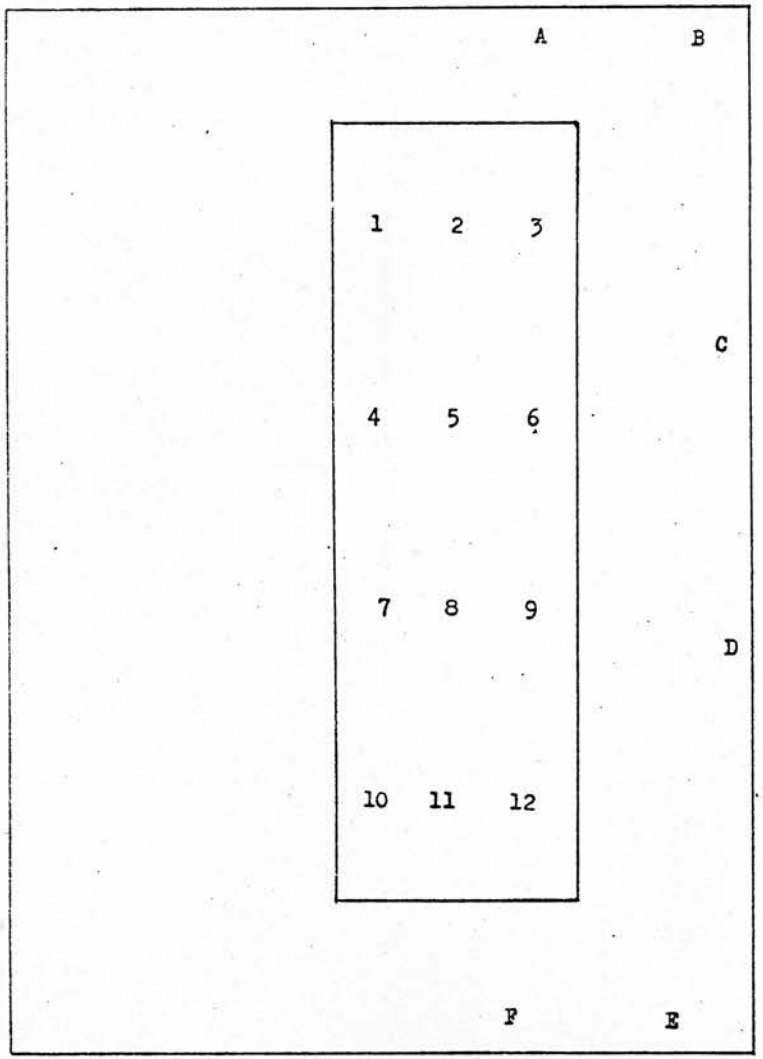


TABLE 9. Population estimates of F. solani var. coeruleum (propagules/g) in soil samples from the sampling points of Fig. 9.

Sampling point	Sampling				
	1	2	3	4	5
1	5	10	40	15	30
2	0	0	10	25	0
3	5	15	0	0	0
4	15	20	5	85	15
5	0	15	5	5	0
6	5	10	0	0	5
7	20	0	45	5	150
8	10	0	1315	15	20
9	25	90	10	15	175
10	20	0	5	0	5
11	20	0	0	5	0
12	30	0	5	0	5
A	15	20	10	10	0
B	0	5	30	0	35
C	0	5	25	0	15
D	10	0	10	0	20
E	15	5	20	10	15
F	5	10	0	5	5

10. The distribution and dispersal of propagules of F. solani var. coeruleum in the rhizosphere of potato plants.

Soil samples were taken at specific points within the rhizosphere of potato plants and examined to detect any distribution patterns that may occur in a plant and any changes of such patterns that may occur during the growth period.

Preliminary experiments on two dispersal mechanisms are reported.

10.1 Distribution patterns.

10.1.1. Distribution patterns from plant profile sampling.

All samples were taken from plants grown from Catriona tubers in a field at Langhill Farm. Samples were collected on three occasions: 27 June, 10 August and 6 October 1973. They were taken from a vertical section cut through the centre of each plant.

Plants were bisected along the ridge of the row and the plant and soil on one side of the row of each plant removed. Samples were taken from the soil face or plant profile of the remaining half thus exposed using six small soil corers (illustrated in Plate 6) 1 cm in diameter. These were sterilised with alcohol and flamed between samplings. Only the soil at the end of the corer was taken and pushed out using a sterile spatula inserted in

PLATE 6. Soil corer used in experiment 10.1.1.

Internal diameter 1 cm

the longitudinal gap of the corer. This was done to avoid taking any soil from the cut face of the profile which might have been contaminated with soil from other parts of the plant as the profile was cut.

The distribution of the sampling points is shown below (Fig. 10.1.1). Sampling points were 5-7 cm apart.

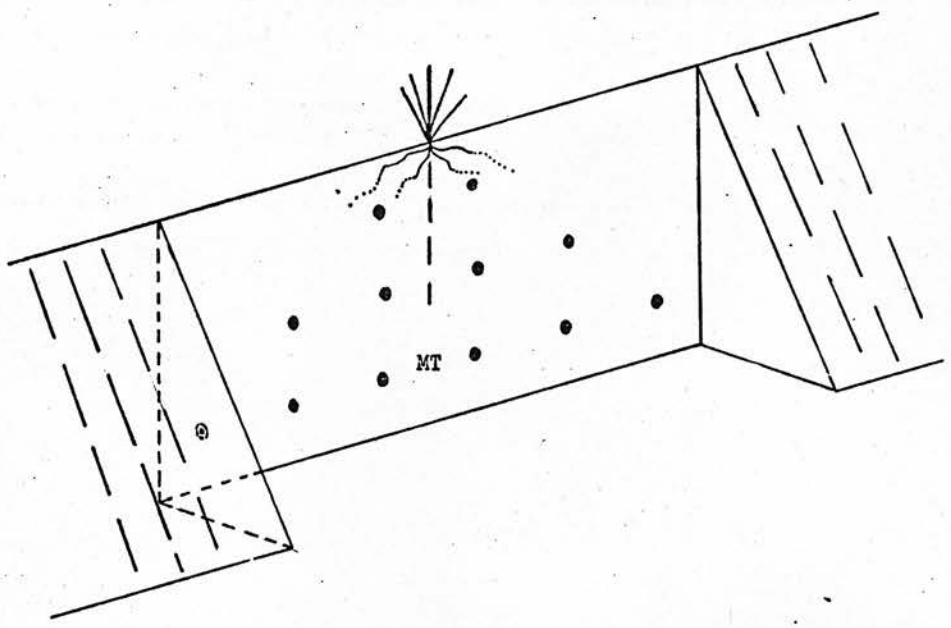


FIG. 10.1.1. Diagram of plant profile showing soil sampling positions with respect to mother tuber (MT).

At the first sampling three plants (Plants 1, 2 and 3) grown from tubers infected with F. solani var. coeruleum were sampled in the manner described above. After sampling, glass plates were placed next to the vertical face of the profiles and soil pushed against the outer

face of the glass to hold it in position. On the second and third samplings these glass plates were removed and samples taken from approximately the same points on each occasion. Three fresh profiles of plants grown from infected seed were cut at both the second (Plants 4, 5 and 6) and third (Plants 7, 8 and 9) samplings and samples taken. Samples were taken also at the third sampling from plants grown from seed that was healthy but contaminated at the time of planting (Plants 10, 11 and 12).

Soil samples were dried, stored and examined using the soil dilution plate method with PM70 medium at 10^{-2} dilution. A blendor jar of the same design as the Atomix but with a smaller base and cross section was used with samples of less than 1.5 g and this was found to give quite satisfactory results. No moisture factors were calculated.

10.1.1. Results.

Results (Table 10.1.1) are presented as a plan of the vertical face sampled with the appropriate population estimates written in at the approximate place of sampling in the profile.

Population estimates vary considerably from point to point in the plant profiles. This variation could possibly be caused by some parts of the plant being more favourable to the increase of fungal propagules than others, or it could be the result of some agency (e.g., soil fauna, root growth) moving soil containing high populations of the fungus from near the mother tuber to other parts of the

TABLE 10.1.1. Plan of the vertical soil face sampled with F. solani var. coeruleum population estimates (propagules/g) recorded at the approximate place of sampling.

Plant number	Profile with population estimates	Plant number	Profile with population estimates	Plant number	Profile with population estimates
	First sampling, 27.6.73.		Second sampling, 10.8.73.		Third sampling, 6.10.73.
1	0 0 0 MT 20 10 0 0 0 0 0 0 0 0	1	0 0 12290 MT 90 0 0 0 2290 110 0 0 10	1	0 0 15420 MT 23700 1810 0 0 90 3820 450 0 20
2	0 0 270 MT 0 110 0 0 0 0 0 0 0	2	110 540 20150 MT 18800 10 0 940 10 9120 0 0 0	2	1890 3560 640 MT 9120 30 0 150 7540 5090 20 20 150
3	110 50 30 MT 130 0 60 20 110 70 0 0 10	3	30 20 80 MT 440 20 10 0 30 40 40 10 190	3	30 10 40 MT 110 100 10 20 10 0 20 30 0
	Second sampling, 10.8.73.		Third sampling, 6.10.73.		Third sampling, 6.10.73.
4	10 20 9890 MT 20 0 0 0 0 0 0 0 0	7	10 0 2330 MT 200 0 - 0 0 170 0 0 1310	10	0 0 0 MT 0 0 0 0 70 20 0 0 0
5	0 0 7160 MT 500 4380 0 0 290 1310 0 0 0	8	0 0 1300 MT 1630 2190 10 0 0 8200 130 0 20	11	0 0 0 MT 0 0 0 0 0 0 0 0 0
6	0 0 10 MT 17300 0 10 0 0 0 0 0 0	9	0 0 590 MT 0 1140 0 10300 1800 900 0 0 790	12	20 0 0 MT 0 0 0 0 0 0 0 0 0

Plants 1-12 numbered as in text; plants 1-9 grown from infected tubers, plants 10-12 from healthy tubers.

plant. However, the general pattern from all samplings seems to be that high populations occur more frequently in the region of the infected mother tuber.

The three samplings from plants 1 and 2 show an interesting increase in F. solani var. coeruleum populations over the sampling period. Table 10.1.1 illustrates both the apparent numerical increase in fungal propagules during the growth period of these plants (more particularly with plant 1) and also the possible increase in spread of propagules to the upper and outer points on the plant profile away from the mother tuber (more particularly with plant 2).

It could be claimed that the insertion of the glass sheet in each plant may have created an environment for fungal population development different from that of the whole plant. However, that the change in F. solani var. coeruleum population levels is a natural and not an artificially induced event, is supported by the fact that plants 4, 5 and 6 at the second sampling and plants 7, 8 and 9 at the third sampling (which did not have repeated samplings and, hence, had no insertion of glass plates) show the same order of population estimates as those of plants 1 and 2 in their August and October samplings. Because so few plants were sampled, no definitive statements can be made.

Two of the three plants grown from healthy tubers showed no contamination with F. solani var. coeruleum.

With plant 10, it is possible that some late infection of the mother tuber took place.

10.1.2. Distribution patterns obtained by taking samples from separate tubers in plants.

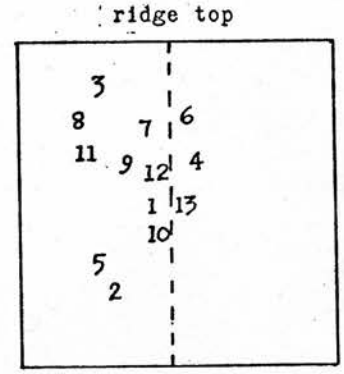
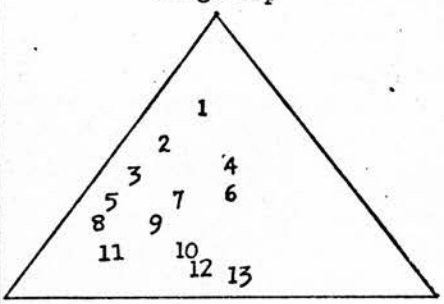
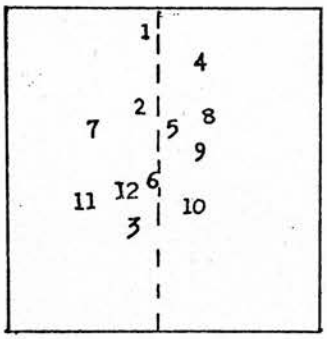
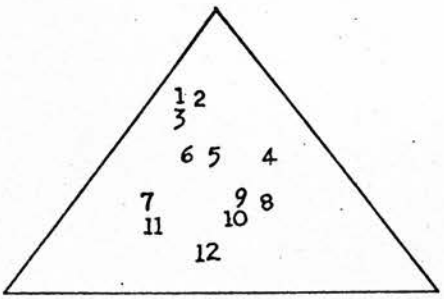
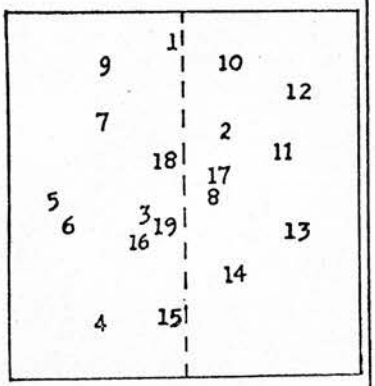
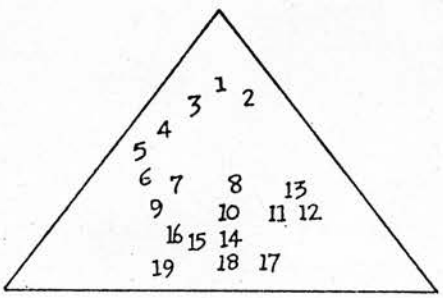
Plants were grown in the field as for experiment 10.1.1. Soil was removed carefully from the top of the row until a tuber was uncovered. A sample of soil on the surface of the tuber was taken and the position of the tuber within the row marked on a plan. This procedure was continued until the whole plant had been removed and samples taken. Four plants were sampled in this manner, two grown from infected seed (plants 13 and 15) and two from healthy though contaminated seed (plants 14 and 16). Soil samples weighed from 0.7-2.9 g and were treated as in experiment 10.1.1. Moisture factors were not used in the calculation of the population estimates.

10.1.2. Results.

The results in Fig. 10.1.2 show the F. solani var. coeruleum population distribution patterns for plants 13, 14 and 15 to be similar to those shown by the plant profiles. It is clear that the mother tuber of plant 14 developed dry rot after planting. None of the samples from plant 16 (not shown in Fig. 10.1.2) showed any contamination with F. solani var. coeruleum.

The contamination seems to be well distributed through the rhizosphere of the plants sampled. The population estimates of the samples from plant 15 indicated a substantially higher total population of F. solani var.

FIG. 10.1.2. Population estimates of F. solani var. coeruleum (propagules/g) from tuber surface soil samples showing sampling plan.

Sampling positions in horizontal plane	Sampling positions in vertical plane	Population estimates for sampling positions numbered as in plan
	Plant 13 	1 0 2 0 3 0 4 300 5 0 6 0 7 800 8 9100 9 730 10 0 11 70 12 10 13 1110
	Plant 14 	1 0 2 0 3 140 4 0 5 300 6 700 7 30 8 70 9 520 10 670 11 3760 12 1780
	Plant 15 	1 0 2 50 3 8060 4 660 5 90 6 8860 7 2440 8 7600 9 2460 10 3560 11 870 12 120 13 1220 14 13630 15 16110 16 5870 17 3010 18 1490 19 8940

coeruleum to be present than in plants 13 and 14. This could have been caused by a greater production of inoculum by the mother tuber or by a more effective dispersal from the infected tuber.

10.2. Dispersal.

The means of dispersal of the fungus from the source of inoculum, of which the chief is believed to be the infected seed, is not known. This dispersal could take place by hyphal extension, by mechanical transmission by water movement or plant growth, or by a member of the soil fauna acting as a vector. Two of these factors were investigated in preliminary experiments.

10.2.1. Dispersal by earthworms.

Earthworms were collected from three of the plants examined in experiment 10.1.2, two grown from infected seed (plants 13 and 15) and one from healthy (plant 16). Seven earthworms were found in the rhizosphere of the two "infected seed" plants and six from that of the "healthy seed" plant. The species were Allolabophora longa, A. caliginosa, A. rosea and A. chlorotica (identified by Dr. B. M. Gerard, Edinburgh School of Agric.). Their casts were collected in the laboratory, dried and plated onto PM70 medium at 10^{-2} dilution.

10.2.1. Results.

Population estimates of F. solani var. coeruleum in casts of earthworms from plants grown from infected

tubers were 10,220 and 4,080 propagules/g. The fungus was not detected in the casts of earthworms from the plant grown from the healthy mother tuber.

As the casts of worms from so few plants were examined, the results of this small experiment can but point the way for further research. However, one may speculate that it is possible for earthworms in the field to allow propagules of F. solani var. coeruleum to pass through their alimentary canal and remain viable. Thus it is possible that earthworms may play a role in the dispersal of F. solani var. coeruleum in the soil.

10.2.2. Dispersal by hyphal extension.

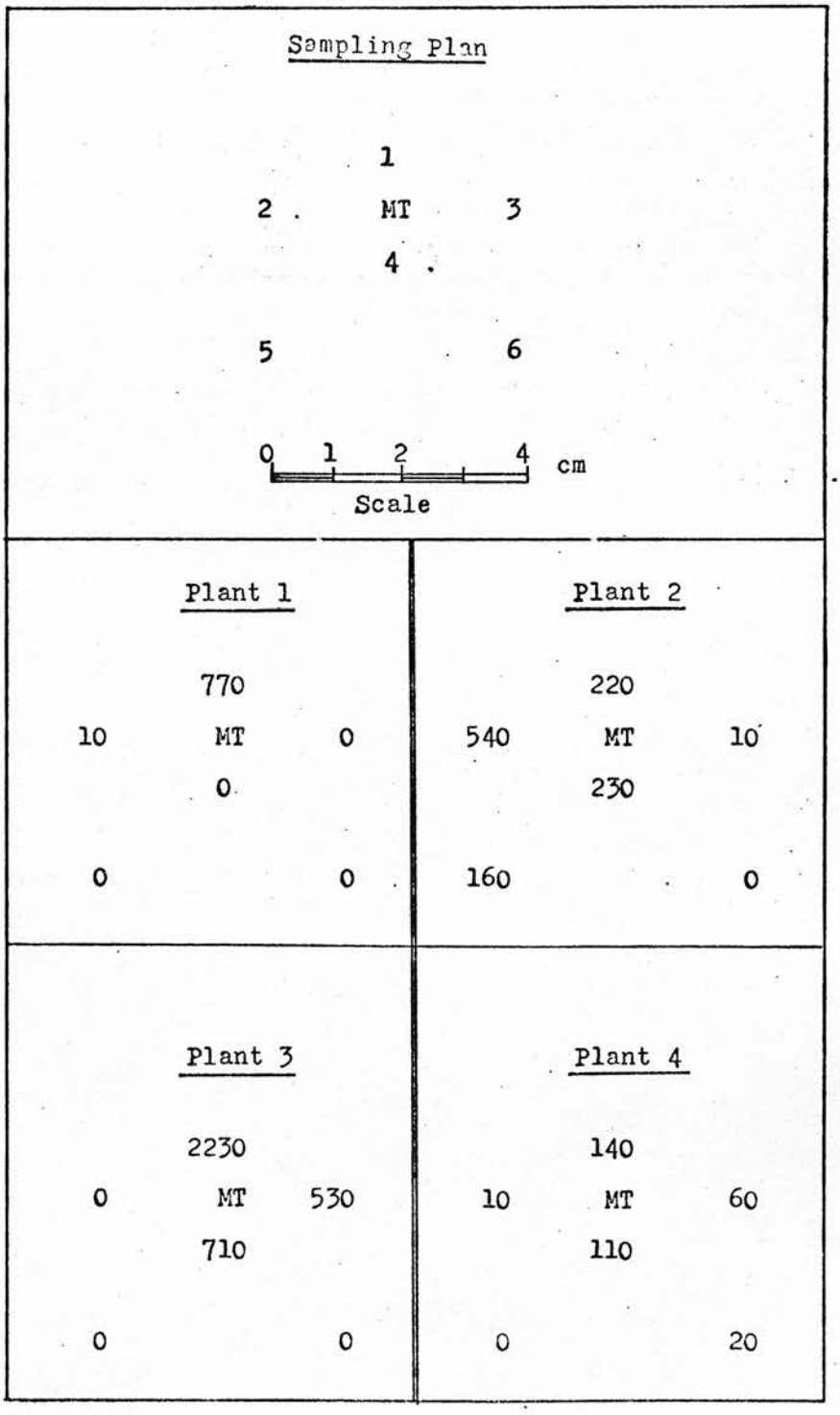
Four tubers with the eyes removed and infected with dry rot were planted in separate pots in the same manner as in experiment 7 and kept in the glasshouse. The eyes were removed so that plant growth would not provide a mechanism for propagule dispersal. The pots were covered with plastic to restrict water loss and thus were watered only lightly on three occasions between May and October.

Soil samples were taken as shown in the plan included with the results and tested as in experiment 7. Soil samples examined prior to planting did not show contamination with F. solani var. coeruleum.

10.2.2. Results.

The results of these preliminary investigations (Fig. 10.2.2) indicate that the fungus may play a passive role

FIG. 10.2.2. Population estimates of F. solani var. coeruleum (propagules/g) at sampling points close to infected mother tubers in pot experiments.



as regards its dispersal in the plant rhizosphere. Members of the soil fauna, such as earthworms, may act as vectors in the dispersal of the fungus. This factor was not taken into account at the initiation of this experiment.

11. DISCUSSION

11.1. Methods.

The measurement of populations of fungal propagules in the soil has been attempted by many workers. The complex form of the soil microflora, with its abundance of genera and species encompassing both heterogeneity, from Sclerotinia to Penicillium, and homogeneity, as in pathogenic strains of Fusarium, and the vast numbers of propagules in this soil microflora makes the measurement of the populations of a single species or variety a challenging task.

The assessment of soil contamination by F. solani var. coeruleum in earlier work (Boyd & Logan, 1967a; Boyd & O'Donnell, 1968) has been carried out using the potato tuber in a host-plant infection test. Although highly sensitive, the technique measures the disease potential or infectivity (McKee & Boyd, 1952) of the soil sample rather than the population of the pathogen. Thus the test is influenced by all the factors that influence plant infection (e.g., environmental conditions, host susceptibility, etc.), with the pathogen population being only one of these. This criticism is not so severe in the case of the tuber inoculation technique because of a number of reasons. As the pathogen is a wound pathogen the infection process is not so complex as with true parasites and therefore presumably is affected by fewer

factors. Also, the technique, as used by Boyd more recently (Boyd & Logan, 1967a) is easily standardised, with uniform environmental conditions and high susceptibility of the tubers. With many of the experiments carried out to date, the measurement of the disease potential rather than the pathogen intensity or population has sufficed.

However, in a more thorough investigation of soil contamination, estimations of the fungal populations rather than disease potentials are necessary. A major objection to the use of the tuber inoculation method in such a study is that, when large numbers of samples are examined involving a ten week incubation period, the method becomes rather cumbersome.

The soil dilution plate method was chosen as suitable for use in this study for a number of reasons some of which were discussed earlier. The method does give a population estimate, it is simple to operate and results are obtained quickly. The fungus involved was easily cultured on agar medium and relatively quick-growing. Groups of propagules are easily broken up to smaller units and these are fairly robust. Despite these advantages, some mention should be made also of its drawbacks. The soil dilution plate method used with a selective medium does not distinguish between pathogenic and non-pathogenic strains of the fungus. However, in this study, none of the latter were found. Jensen (1968) provided a useful summary of the physical difficulties involved in the use

of the method. Problems with the storage of soil samples, the preparation of soil suspensions, the nature of the diluting medium, the preparation of dilutions, the plating medium available, the period and temperature of incubation need to be resolved and standardisation achieved so that reliable results, among which comparisons can be drawn, are obtained. Two of these factors, the preparation of dilutions and the plating medium, were of particular interest in this study.

When the numbers of colonies from plates prepared from different dilutions of the same soil are compared with the degrees of dilution, frequently it is found that they are not proportional. For example, when a soil suspension is diluted in the ratio of 1:10, the number of colonies detected may decrease only in the ratio of 1:5 (Jensen, 1968; Meiklejohn, 1957; Ram Reddy, 1962). This is due primarily to the reduced numbers of microorganisms on a plate from a high dilution causing a lessening of the antagonistic and masking action of the fungi or bacteria upon each other, thus allowing more propagules to germinate and colonies to develop. Therefore in the calculation of population estimates it can be found that the higher the dilution used in the preparation of the soil suspension, the higher is the population estimate and this was indeed the case in the present study. However, the dilution of 10^{-2} was finally chosen for the later experiments in this investigation because it provided what appeared to be the most meaningful

results. The measurement of high populations was considered to be less important than the detection of low populations.

As discussed in an earlier section, the constituents of the PCNB-sucrose medium with the dodine acetate modification (PM70) can inhibit to some extent the germination and growth of spores of F. solani var. coeruleum. This inhibition was far less, however, than that exerted at the same dilutions by other fungi present on the plates when the fungicides were not incorporated in the medium. The inclusion of dodine acetate in the medium allowed a lower dilution of soil suspension to be examined so that smaller populations of the fungus could be detected using only low numbers of plates.

This study is concerned with the initiation and increase of soil populations of F. solani var. coeruleum that may contaminate the surface of progeny tubers, subsequently causing infection and rotting if the tubers are wounded. The soil dilution plate technique with the PM70 medium was shown to be able to detect soil populations of the fungus and the correlation between the population estimates (in a transformed state) obtained by this method and the infectivity indices of the tuber inoculation method indicate that the former was a suitable means of measuring soil populations of F. solani var. coeruleum for use in this investigation.

11.2. Influence of the seed tuber on soil contamination.

The evidence presented in earlier sections illustrates clearly the link between the infected mother tuber and the build-up of soil contamination by F. solani var. coeruleum. This primary contamination, found to be present on progeny tubers in the field before lifting time, develops only when the mother tuber becomes infected with the fungus. Seed tubers contaminated with propagules of F. solani var. coeruleum and which remain healthy after planting do not initiate a build-up of the fungus in the soil. One aspect of these results is that it is unlikely that any other plant parts, such as the roots, are infected by the fungus in the field, a finding which agrees with the results of earlier workers (Pethybridge & Lafferty, 1917; Lansade, 1949).

Healthy seed tubers may become infected some time after planting, e.g., when sprouting. As seen in the present study, anomalous treatment results were obtained from the healthy, contaminated seed tuber treatment when the seed tubers subsequently became infected. This post-planting infection may be offered as explanation for the results of experiment 3.1 and also may explain some results of Boyd & Logan (1967a) where high soil contamination was found in soils from plants grown from healthy, contaminated tubers.

Preliminary experiments with F. sulphureum indicate that a situation similar to that of F. solani var. coeruleum exists for this fungus. A build-up of soil

contamination developed only when the mother tuber was infected. It would be interesting to investigate the etiology of this disease further to see if it differs at all from that of dry rot caused by F. solani var. coeruleum, and if, for example, the existence of the perfect stage of F. sulphureum influences the development of the disease.

The effect of the infection of seed tubers with Phoma exigua var. foveata on subsequent soil contamination was demonstrated in the 1972 experiment (section 6.2). The lack of any clear pattern in the previous year's experiment may have been caused by existing soil contamination or possibly by an unexpectedly rapid decay of seed tubers. Although it appears that the infected mother tuber may provide a source of soil contamination by P. exigua var. foveata, the situation is a little different from that with dry rot disease. The mother tuber itself may not be the main source of contamination as P. exigua var. foveata can produce pycnospores from the extensive development of pycnidia on the stems of infected plants. The relative importance of the infected stems and the infected mother tuber in the production of soil contamination is not known. The importance of each source may depend on the weather conditions during plant growth and, if this were the case, it may be that weather conditions contributed to the difference in the results of the 1971 and 1972 experiments. Similar indefinite results were reported by Boyd & Logan (1967a) and Boyd & O'Donnell (1968).

Comparing dry rot of potatoes with diseases caused by other Fusarium species, several factors can be considered. F. solani var. coeruleum has an extremely limited host range and is generally restricted, as far as is known, to one plant organ of one plant species, i.e., the potato tuber. Rarely is the fungus able to infect this plant organ unless wounds provide a means of entry. Apart from these two factors, the increase of the soil-borne population of the fungus results from the infection of a subterranean plant organ as with most soil-borne Fusarium species.

There are relatively few crops where the seed, or propagating unit of the second generation, is grown in the soil adjacent to the first generation propagating unit, as is the case with the potato plant. Examples of these include groundnuts, some tropical root crops, and various ornamental bulbs and corm crops. It may be possible that some Fusarium wound pathogens attacking these crops act in a similar way to F. solani var. coeruleum on potatoes. Some aspects of the Narcissus basal rot disease caused by F. oxysporum f. sp. narcissi are strikingly similar to those of dry rot of the potato. Also, Booth (1971) noted that F. sulphureum, one of the causal organisms of dry rot of potato tubers, has been isolated from stored groundnuts. It is interesting to speculate on whether this fungus infects groundnut seed in the field and contaminates the pods. A number of Fusarium species have been found infecting groundnut pods (Jackson & Bell, 1970). With the gladiolus and probably with Narcissus basal rot, the

Fusarium species involved are not so restricted in their pathogenicity spectra as F. solani var. coeruleum appears to be. However, the latter is solely a wound parasite and one might imagine that it might have some method of perennation other than dependence on an injury to its only host.

In the field experiments carried out in the present investigation, a considerable variation in F. solani var. coeruleum population estimates between plants from the same treatment was shown to occur. Such a variation had been expected and, because of it, one sample from each of many plants had been examined rather than several samples from each plant. The use of the logarithmic transformation reduces the variation in the data obtained from the experiments.

Soil samples examined at different dates through the 1973 season (section 3.3) showed that an increase in contamination occurs during the development of the plant. Data indicate that the F. solani var. coeruleum population estimates of the soil contamination on the surface of progeny tubers on plants grown from infected seed tubers are at a level equivalent to a high infectivity soon after development. Any subsequent increase in population does not markedly increase the potential danger of the inoculum to these tubers.

Cook & Snyder (1965) have shown the multiplication of F. solani f. phaseoli to be influenced by the root and

hypocotyl exudates. It was hoped, in this study, to gain some information on the behaviour of fungal contamination on the surface of tubers during the storage period. Except for one case, no great changes were detected in the experiments conducted. Only in the 1972-1973 storage period was there a build-up of populations on the progeny seed grown from healthy but contaminated mother tubers. This did not occur in the 1973-1974 season. A laboratory study of the populations of F. solani var. coeruleum on the surface of tubers could be of great interest. The behaviour of chlamydospores and the factors affecting their germination and lysis, possibly in regard to the work of Cook & Snyder (1965), might lead to a mechanism of control.

Some information on the results of the application of fungicides to contaminated or infected seed tubers was acquired. An organo-mercury dip, although eliminating surface contamination, probably did not affect early stages of infection nor inhibit the subsequent development of those infections which produced high soil contamination. Benomyl applications appeared to destroy the surface contamination present on the mother tuber but not to halt the development of infection of the mother tuber. The fungicide seemed to prohibit surface sporulation and limit the development of soil contamination. A possible explanation of the difference in the degree of control of the build-up of soil contamination between 1972 and 1973 is that the weather conditions may have limited the

efficacy of the compound in the 1972 crop. A remarkable control of the fungus by benomyl was achieved in 1973. The application of tecnazene (as Fusarex) to contaminated tubers did not kill the fungus and seemed to increase the number of healthy contaminated tubers that developed infection after planting. Some limitation of the build-up of the populations from infected tubers was evident. This could be related to the slow plant development caused by the application of tecnazene before planting.

Thus it would appear that the spread of the fungus from the infected mother tuber can be prohibited by the use of an appropriate fungicide. However, the inoculum contained inside the remnants of an infected tuber appropriately treated to limit the spread of contamination during plant growth remains as a source of contamination which might be spread when plants are lifted by machine. The importance of this factor remains to be investigated.

Another method of controlling the development of F. solani var. coeruleum soil contamination utilising the antibiotic activity of Trichoderma species was tested. Nadvodnyuk (1962) found a conidial suspension of T. koningii applied before storage to give a 50% reduction in dry rot. Glasshouse experiments using a T. viride soil paste were inconclusive.

For the role of the infected mother tuber in the production of soil and progeny tuber contamination to be conclusively identified as a major mechanism for the

contamination of tubers grown in agricultural practice, the number of infected tubers planted under normal agricultural conditions requires to be shown to be related to the number of infected progeny tubers at the end of the storage season. The results of an experiment reported in this work gave some indications that this was the case but the results need verification. Some investigation into the spread of contamination from the soil about plants grown from infected mother tubers to "clean" tubers may produce worthwhile data.

Also, to relate the results of this study more closely to the conditions of normal agricultural practice, the population build-up using less susceptible cultivars could be investigated. The situation revealed using the highly susceptible cultivar Catriona is not really that of usual farming practice as the cultivar has been little planted in recent years.

11.3. Prevalence and survival.

Experiments conducted in this study have shown that the primary contamination of progeny seed arises from seed tuber-borne infection. Thus, it is to be expected that the careful handling and treatment given to elite stocks should result in the reduction of contamination of dry rot propagules. The evidence gathered in the surveys of contamination of VTSC stocks support this. F. solani var. coeruleum was present at low levels in the stock examined in 1972 and, in most cases, was not detected in the stocks examined in 1973. However, to prove this hypothesis

satisfactorily, a large number of commercial stocks would have to be examined also to provide comparative figures. This was impossible in this study. The presence of F. solani var. coeruleum and Phoma exigua var. foveata in some stocks, and in the case of the latter occasionally at high levels, means that if these fungi are to be eliminated from the VTSC stocks some form of chemical treatment would have to be incorporated as part of the scheme.

F. solani var. coeruleum has been regarded as a soil-borne pathogen. Considering the situation when highly susceptible cultivars were used, it is probable that considerable infection and rotting from F. solani var. coeruleum occurred in the field after planting. High soil populations of the fungus would have resulted. When this took place it is possible that such soil-borne populations may have played a part in providing inoculum for the infection of mother tubers wounded at planting or in the direct contamination of progeny tubers. In such a situation the primary contamination of the field soil would still have been caused by the planting of infected seed. The results of experiments on the survival of soil populations of F. solani var. coeruleum indicate that it is unlikely that the above situation actually arose. Populations present in a field plot planted with infected tubers were relatively low when samples were examined two years after the potato crops were grown. It is difficult to estimate what further population decline might take place before the next crop and to gauge what contamination

of progeny tubers might result from such a low general population of the fungus. However, the continued planting of susceptible cultivars over a period of years could possibly build up a soil population which might pose a contamination hazard.

With the introduction of resistant cultivars, the presence of a high population of the fungus in the soil over whole fields is less likely to eventuate and the contamination arising from infected seed is suggested as providing the main source of contamination of progeny tubers.

Although the fungus can persist in the soil over a period of years it seems likely that soil populations decline to very low levels. However, the effect of the growth of other plants on the populations of F. solani var. coeruleum is not yet known and may be worthwhile investigating.

11.4. Distribution and dispersal of F. solani var. coeruleum within the plant/soil system.

As might be expected, experimental results indicate that the zone of highest contamination of the fungus is that surrounding the mother tuber. Nevertheless, the fungus was found to have spread through most of the soil surrounding the plant with only the extremities remaining uncontaminated. Experiments to determine the method of dispersal of the fungus about the plant were inconclusive. However, the finding of the fungus in the casts of earthworms taken from plants grown from infected tubers adds a

further dimension to this investigation.

Fusarium species have been reported as being found in the alimentary canals of earthworms (Taylor, 1917; Rathbun, 1918; Khambata & Bhat, 1957; Parle, 1963) and thus it is not surprising to find F. solani var. coeruleum in the casts of earthworms taken from soil known to contain high populations of the fungus. It is not known whether the fungus is dispersed by the earthworms or by some other means (e.g., plant growth) before being ingested by the earthworms. As it has been shown that a population increase occurs throughout the growing season in the rhizosphere of plants grown from infected tubers and as no alternative sites for the increase of the fungus are known, it seems reasonable to postulate that a vector might be responsible for the dispersal of the fungus.

There are a number of references in the literature to the dispersal of microorganisms by earthworms. Taylor (1917), Rathbun (1918) and Khambata & Bhat (1957) considered that earthworms disseminated the spores of such fungi as Fusarium spp. Hutchinson & Kamel (1956) found that several species of fungi spread through sterilised soil at a faster rate in the presence of earthworms than in their absence. Ghilarov (1963) stated that there is good evidence that earthworms are important in inoculating soil with micro-organisms and their casts foci for the dissemination of microorganisms. More recently Edwards & Lofty (1972) noted that earthworms must be important vectors of plant pathogens.

Observations made in the field also support this premise. On many occasions when lifting potato plants, it was found that earthworms were present in close association with rotting mother tubers. Many tubers that were initially infected with F. solani var. coeruleum were found later in the season to have had the infected tissue replaced by worm casts enclosed in the remains of the tuber skin. In dry soil conditions this association seemed to be more prevalent which agrees with observations of Dr. B. M. Gerard (Edinburgh School of Agric., pers. comm.). It has been reported that more dry rot is apparent after dry seasons and this may have some connection with the activity of earthworms. It is interesting, also, to note that in 1973 little spread of F. solani var. coeruleum propagules from infected mother tubers treated with benomyl occurred and that earthworms are known to be seriously affected by the presence of this fungicide. It is evident that this whole subject warrants further investigation.

The experiments reported in this study offer convincing evidence supporting the hypothesis that soil contamination with F. solani var. coeruleum and the subsequent contamination of progeny tubers results from infection of the seed tuber with the fungus. The importance of this seed-borne nature of the disease has

some significance in the long term plans made for a seed production system designed to improve the health of seed stocks. The development of soil contamination illustrated by potato dry rot disease may be of relevance when considering the multiplication of soil-borne inocula of other Fusarium diseases of root, bulb and corm crops. The role speculated upon for earthworms in the dispersal of the fungus underlines the fundamental point that the whole biological situation should be examined in any plant disease investigation.

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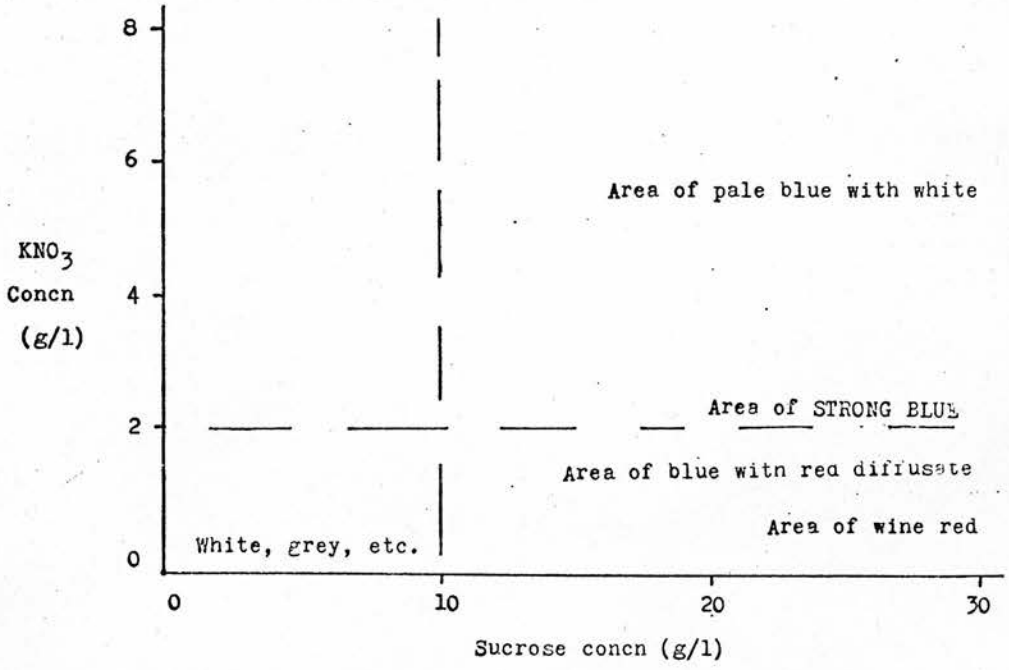
A P P E N D I X

TABLE A.1. Colours of *F. solani* var. *coeruleum* on media of different sucrose and KNO_3 concentrations.

Sucrose concentration (g/l)	KNO_3 concentration (g/l)	Description	Sucrose concentration (g/l)	KNO_3 concentration (g/l)	Description
0.5	0.025	white	20.0	4.0	pale blue, white rim
0.5	0.033	yellow-grey	20.0	4.9	" " " "
5.0	0.33	purple-grey	20.0	8.0	white with blue streaks
7.5	0.5	black-purple	30.0	0.1	pale mauve
10.0	0.1	pale mauve	30.0	0.5	wine
10.0	0.25	wine	30.0	2.0	STRONG BLUE, red diffusate
10.0	2.0	white with blue streaks	30.0	2.2	blue
10.0	4.0	" " " "	30.0	2.5	blue-white, blue rim
10.0	8.0	" " " "	30.0	3.0	" " " "
15.0	0.25	wine	30.0	3.2	white, blue rim
20.0	0.1	pale mauve	30.0	3.7	" " "
20.0	0.5	wine	30.0	4.0	" " "
20.0	1.5	blue with red diffusate	30.0	4.1	" " "
20.0	1.7	" " " "	30.0	6.0	white with blue streaks
20.0	2.0	STRONG BLUE	30.0	8.0	" " " "
20.0	3.0	white with little blue			



Diagrammatic representation of colony colour changes described in Table A.1.

TABLE A. 2. F. solani var. coeruleum colony counts arranged in order of successful tuber inoculations.

Infectivity Index (%)	Soil dilution mean plate count (10 plates)		
	P 2×10^{-4}	PM50 10^{-2}	PM50 5×10^{-3}
5.0	0	0.4	0
7.5	0	0	0
10.0	0	1.8	0.8
10.0	0	0	0
15.0	0	0.2	0.2
17.5	0	0.4	0.2
35.0	0	0	0.2
50.0	0	0	0
57.5	0	0.4	0.6
65.0	0.2	15.8	16.6

P PCNB sucrose medium

PM50 PCNB sucrose medium plus 50 ppm dodine acetate

TABLE A.3. Population estimates (propagules/g) of F. solani var. coeruleum in rhizosphere soil samples, taken at four sampling dates, as shown by the soil dilution plate method used at three dilutions, two of these on PM70 medium and the 10^{-4} on PCNB-sucrose medium.

Treatment	Replicate	SAMPLING 1			SAMPLING 2			SAMPLING 3			SAMPLING 4		
		10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
A	1	14,220	18,300	14,560	6,602	9,309	8,560	4,306	5,928	8,320	4,109	5,029	6,470
	2	5,660	5,970	6,180	19,440	28,296	32,400	1,976	1,560	2,080	1,691	2,461	3,210
	3	14,220	16,800	22,050	5,270	5,184	5,400	4,547	4,515	7,350	4,807	5,500	2,200
	4	4,330	5,030	5,350	16,740	28,512	34,560	6,448	5,512	9,360	1,281	1,995	1,050
B	1	0	0	0	1,221	1,210	3,630	0	0	0	0	0	0
	2	0	0	0	22	0	0	95	210	0	112	216	0
	3	11	0	0	0	0	0	0	0	0	64	318	1,060
	4	0	0	0	480	436	1,090	0	0	0	11	214	0
C	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	11	0	0	11	0	0	0	0	0	21	0	0
	3	208	124	0	21	0	0	0	0	0	0	0	0
	4	73	104	0	22	0	0	0	0	0	0	0	0
D	1	315	210	0	131	109	0	155	103	0	428	428	1,070
	2	125	0	0	425	436	1,090	227	206	0	498	530	0
	3	170	212	0	355	777	0	412	1,442	1,030	572	1,060	1,060
	4	165	206	2,060	206	206	0	385	0	1,040	201	424	0

TABLE A.4. $Log_{10}(x+1)$ transformations of population estimates of F. solani var. coeruleum in rhizosphere soil samples, taken at four sampling dates, as shown in Table A.3.

Treatment	Replicate	SAMPLING 1			SAMPLING 2			SAMPLING 3			SAMPLING 4		
		10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
A	1	4.15	4.26	4.16	3.82	3.97	3.93	3.63	3.77	3.92	3.81	3.70	3.81
	2	3.75	3.78	3.79	4.29	4.45	4.51	3.30	3.19	3.32	3.23	3.39	3.51
	3	4.15	4.23	4.34	3.72	3.71	3.73	3.66	3.65	3.87	3.68	3.74	3.34
	4	3.64	3.70	3.73	4.22	4.46	4.54	3.81	3.74	3.97	3.11	3.30	3.02
B	1	0	0	0	3.09	3.08	3.56	0	0	0	0	0	0
	2	0	0	0	1.36	0	0	1.98	2.32	0	2.05	2.34	0
	3	1.08	0	0	0	0	0	0	0	0	1.81	2.50	3.03
	4	0	0	0	2.68	2.64	3.04	0	0	0	1.08	2.33	0
C	1	0	0	0	0	0	0	0	0	0	0	0	0
	2	1.08	0	0	1.08	0	0	0	0	0	1.34	0	0
	3	2.32	2.10	0	1.34	0	0	0	0	0	0	0	0
	4	1.86	2.02	0	1.36	0	0	0	0	0	0	0	0
D	1	2.30	2.32	0	2.12	2.04	0	2.19	2.02	0	2.63	2.63	3.03
	2	2.10	0	0	2.63	2.64	3.04	2.36	2.32	0	2.70	2.73	0
	3	2.23	2.33	0	2.55	2.89	0	2.62	3.16	3.01	2.76	3.03	3.03
	4	2.27	2.32	3.31	2.32	2.32	0	2.59	0	3.02	2.30	2.63	0

TABLE A.5. Population estimates (propagules/g) of F. solani var. coeruleum in rhizoplane soil samples, taken at four sampling dates, as shown by the soil dilution plate method used at three dilutions. Media as in Table A.3.

Treatment	Replicate	SAMPLING 1			SAMPLING 2			SAMPLING 3			SAMPLING 4		
		10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
A	1	6,371	9,967	13,358	16,483	19,074	75,480	3,028	4,782	19,125	31,835	65,953	300,980
	2	1,969	1,938	1,020	949	1,836	7,140	15,800	8,563	11,424	21,836	67,670	82,820
	3	3,825	9,078	11,220	826	3,060	6,120	3,986	4,892	23,939	8,646	9,191	82,820
	4	6,344	6,222	8,160	2,887	7,344	10,200	3,723	6,499	25,689	89,377	247,683	277,621
B	1	0	0	0	2,734	2,550	8,160	0	0	0	0	0	0
	2	0	0	0	0	0	0	12	0	1,200	283	303	0
	3	0	0	0	0	0	0	8	0	0	0	0	0
	4	0	0	0	0	0	0	58	97	970	202	101	7,070
C	1	0	0	0	0	0	0	24	0	0	0	0	0
	2	0	0	0	0	0	0	281	208	0	101	202	0
	3	796	408	1,020	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	20	0	0
D	1	142	0	0	551	612	1,020	579	445	4,301	12,928	16,463	19,190
	2	235	0	0	61	306	0	738	2,007	4,301	6,926	50,568	169,709
	3	71	0	0	469	714	3,060	664	843	5,270	556	1,717	14,140
	4	0	0	0	71	204	0	899	1,422	4,228	12,726	9,494	326,230

TABLE A.6. $Log_{10}(x+1)$ transformations of population estimates of *F. solani* var. *coeruleum* in rhizoplane soil samples, taken at four sampling dates, as shown in Table A.5.

Treatment	Replicate	SAMPLING 1			SAMPLING 2			SAMPLING 3			SAMPLING 4		
		10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
A	1	3.80	4.00	4.13	4.22	4.28	4.88	3.48	3.68	4.28	4.50	4.82	5.48
	2	3.29	3.29	3.01	2.98	3.27	3.85	4.20	3.93	4.06	4.34	4.83	4.92
	3	3.58	3.96	4.05	2.92	3.49	3.79	3.60	3.69	4.38	3.94	3.96	4.92
	4	3.80	3.79	3.91	3.46	3.87	4.01	3.57	3.81	4.41	4.95	5.39	5.44
B	1	0	0	0	3.44	3.41	3.91	0	0	0	0	0	0
	2	0	0	0	0	0	0	1.08	0	3.08	2.45	2.48	0
	3	0	0	0	0	0	0	0.90	0	0	0	0	0
	4	0	0	0	0	0	0	1.76	1.99	2.99	2.31	2.01	3.85
C	1	0	0	0	0	0	0	1.38	0	0	0	0	0
	2	0	0	0	0	0	0	2.45	2.32	0	2.00	2.31	0
	3	2.90	2.61	3.01	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	1.30	0	0
D	1	2.15	0	0	2.74	2.79	3.01	2.76	2.65	3.65	4.11	4.22	4.28
	2	2.37	0	0	1.79	2.49	0	2.87	3.30	3.63	3.84	4.70	5.23
	3	1.85	0	0	2.67	2.85	3.49	2.82	2.93	3.72	2.74	3.23	4.15
	4	0	0	0	1.85	2.31	0	2.95	3.15	3.63	4.10	3.98	5.51

TABLE A.7. Population estimates (propagules/g) of F. solani var. coeruleum in soils from tuber surfaces, taken at two sampling dates, from tubers stored after lifting in a farm store. Media as in Table A.3.

Treatment	Replicate	SAMPLING 1			SAMPLING 2		
		10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
A	1	7,460	16,200	23,000	15,750	9,700	42,000
	2	10,610	20,600	29,000	11,300	17,400	30,000
	3	9,460	19,500	44,000	4,650	4,600	26,000
	4	5,460	13,200	86,000	3,450	2,700	9,000
B	1	10	100	0	40	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	80	300	0	500	900	3,000
C	1	670	1,700	4,000	2,700	1,900	15,000
	2	600	1,100	14,000	50	200	0
	3	160	300	2,000	6,090	6,300	11,000
	4	7,890	7,000	86,000	41,400	79,600	122,000
D	1	1,140	4,300	5,000	750	400	42,000
	2	1,780	1,800	0	2,100	2,700	5,000
	3	2,040	7,400	22,000	1,770	2,500	63,000
	4	280	400	2,000	0	0	0

TABLE A.8. $Log_{10}(x+1)$ transformations of population estimates of F. solani var. coeruleum in soils from tuber surfaces, taken at two sampling dates, from tubers stored after lifting in a farm store.

Treatment	Replicate	SAMPLING 1			SAMPLING 2		
		10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
A	1	3.87	4.21	4.36	4.20	3.99	4.62
	2	4.03	4.31	4.46	4.05	4.24	4.48
	3	3.98	4.29	4.64	3.68	3.66	4.42
	4	3.74	4.12	4.93	3.54	3.43	3.95
B	1	1.04	2.04	0	1.60	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	1.90	2.48	0	2.70	2.95	3.48
C	1	2.83	3.23	3.60	3.43	3.28	4.18
	2	2.78	3.04	4.17	1.71	2.30	0
	3	2.21	2.48	3.30	3.78	3.80	4.04
	4	3.90	3.85	4.93	4.62	4.90	5.09
D	1	3.06	3.63	3.70	2.88	2.60	4.62
	2	3.25	3.26	0	3.32	3.43	3.70
	3	3.31	3.87	4.34	3.25	3.40	4.80
	4	2.45	2.60	3.30	0	0	0

Comments on statistical analysis of results of experiment 3.2.2.

Analysis of experimental data was carried out to determine if significant differences existed between the means of population estimates from the samples from different sources (i.e., tuber surface, rhizosphere and rhizoplane), and between those from different sampling times. The $\log_{10}(x + 1)$ transformations of the population estimates were used for this analysis.

A partially hierarchical analysis (as suggested by the Statistics Dept. of the Rothamsted Expt. Station) with four strata was attempted:

(Replicates/Repl.Treatm./Repl.Tr.Samplings.Sources/
Repl.Tr.Sampl.Sources.Dilutions).

Apart from showing that treatment differences formed 69% of the sums of squares of the differences of means, the analysis revealed little useful information. This was mainly because of the variation introduced by the differences between treatment means and the variation in the results of the different dilutions. Consequently the treatment and dilution factors were taken out of the analysis and the remaining components examined in 12 separate analyses. Table A.9 shows the significance ratings of the variance ratios of the strata of the analyses.

TABLE A.9. Experiment 3.2.2: results of analyses.

Analysis	Strata			
	Repl.	S.	M.	SM.
T1D1	-	-	**	***
D2	-	-	**	**
D3	-	-	***	***
T2D1	-	-	-	-
D2	-	*	-	-
D3	-	-	-	-
T3D1	-	-	-	-
D2	-	-	-	-
D3	-	-	-	-
T4D1	-	*	-	*
D2	-	***	-	*
D3	-	**	*	-

T = treatment
D = dilution
R = replicate
S = sampling
M = source
- = not significant

T1D1-3 analyses show that there were significant differences between the means of population estimates of samples from different sources when examining soils of high populations. This is because means of tuber surface samples and the 4th rhizoplane sampling were high.

The means over the sampling dates show a trend to increasing value in T4D1-3, mainly evident in the means from the rhizoplane sampling (see Fig. 3.2.2a).

TABLE A.10. Weights (Kg) of rhizosphere soil from plants of treatments A, B and H (section 3.3.2).

Replicate	Plant	Treatment A				Treatment B				Treatment H				
		Sampling				Sampling				Sampling				
		2	3	4	5	2	3	4	5	1	2	3	4	5
1	1	1.6	4.0	4.0	2.0	4.2	3.2	1.4	1.8	2.5	1.1	3.9	2.1	3.5
	2	2.1	3.8	4.1	2.1	2.8	5.5	3.0	2.0	0.5	1.4	5.0	3.0	4.1
	3	3.0	7.0	3.4	2.0	2.8	5.6	1.1	4.1	0.6	0.8	2.2	1.5	5.0
	4	1.2	8.3	3.4	1.8	1.6	3.4	2.5	2.1	1.0	1.0	3.7	3.3	1.4
	5	4.0	3.2	2.7	4.4	3.1	5.0	4.7	1.4	1.3	0.6	3.8	3.7	2.9
2	1	1.6	3.0	2.8	2.0	1.2	3.8	3.0	1.7	0.1	-	3.9	2.5	1.6
	2	0.4	4.3	2.9	2.0	2.0	4.9	1.0	4.5	3.0	1.2	2.7	3.6	1.6
	3	3.2	1.4	2.9	2.0	2.0	1.6	2.3	2.7	2.0	0.9	1.4	2.5	2.8
	4	0.3	6.4	4.3	2.8	2.6	4.0	3.5	2.0	0.5	1.6	3.2	3.5	3.9
	5	1.7	6.7	3.7	3.0	3.6	5.1	3.4	3.2	-	1.0	4.5	3.0	4.4
3	1	1.0	4.4	1.0	3.7	2.0	4.8	2.9	2.5	-	0.5	4.0	3.7	4.8
	2	1.4	4.4	3.2	1.9	3.2	5.5	3.2	2.1	0.1	1.2	-	2.6	4.5
	3	1.0	4.4	2.0	1.9	2.6	2.2	2.0	3.8	0.5	1.0	2.1	1.5	3.3
	4	0.5	4.7	3.2	4.5	1.2	4.0	4.1	3.4	0.2	1.4	2.3	3.1	4.1
	5	1.6	3.7	3.8	4.0	2.0	4.9	1.3	1.6	0.2	1.1	3.0	4.1	2.2
4	1	1.0	5.6	2.0	4.9	3.2	4.1	2.0	2.8	1.2	2.0	3.7	-	5.0
	2	0.5	3.0	1.5	2.3	2.2	2.8	2.5	2.2	0.5	1.5	1.0	3.6	2.4
	3	1.6	6.6	1.5	3.2	2.4	2.8	4.1	4.2	0.1	0.2	4.1	4.1	5.0
	4	2.0	5.5	3.7	4.2	2.0	3.0	4.0	2.8	3.0	-	4.7	5.7	3.9
	5	2.0	5.4	4.6	4.4	2.2	3.6	4.0	4.8	2.5	1.0	3.0	3.4	2.3

- Missing value

TABLE A.11. Population estimates (propagules/g) of F. solani var. coeruleum in soils from tuber surfaces from the fifth sampling, as shown by the soil dilution plate method on PM70 medium at dilution 10^{-2}.

Replicate	Plant	Treatment								
		A	B	C	D	E	F	G	H	I
1	1	5,192	418	0	0	0	0	0	173	214
	2	5,222	500	204	0	0	0	10	1,081	31
	3	13,750	82	10	0	459	0	0	31	5,620
	4	928	887	0	0	0	0	0	8,456	1,877
	5	3,835	28,968	7,089	0	0	0	31	194	1,571
2	1	2,642	5,896	0	0	10	0	20	9,078	51
	2	38,760	10,608	0	0	0	0	194	31	1,408
	3	8,537	184	22,511	0	0	0	0	306	153
	4	9,537	4,253	112	20	0	0	0	31	82
	5	4,335	0	102	0	0	10	163	255	6,742
3	1	14,504	112	0	31	0	10	0	11,638	2,091
	2	7,130	663	0	0	10	0	0	20	408
	3	7,487	2,504	857	10	0	0	0	9,364	20
	4	25,296	632	41	0	0	0	0	714	2,111
	5	949	9,894	0	296	0	0	0	0	296
4	1	1,488	31	51	0	0	0	31	2,142	0
	2	989	7,630	82	612	0	20	0	449	6,987
	3	1,612	92	1,540	0	0	0	0	7,823	3,509
	4	153	10	0	0	0	0	0	714	316
	5	1,785	5,447	0	0	0	0	0	102	1,489

TABLE A.12. $Log_{10}(x+1)$ transformations of population estimates of F. solani var. coeruleum in tuber surface soils shown in Table A.11.

Replicate	Plant	Treatment								
		A	B	C	D	E	F	G	H	I
1	1	3.72	2.62	0	0	0	0	0	2.24	2.33
	2	3.72	2.70	2.31	0	0	0	1.05	3.03	1.51
	3	4.14	1.92	1.04	0	2.66	0	0	1.51	3.75
	4	2.97	2.95	0	0	0	0	0	3.93	3.27
	5	3.59	4.46	3.85	0	0	0	1.51	2.29	3.20
2	1	3.42	3.77	0	0	1.04	0	1.32	3.96	1.72
	2	3.59	4.03	0	0	0	0	2.29	1.51	3.15
	3	3.93	2.27	3.35	0	0	0	0	2.49	2.19
	4	3.98	3.63	2.05	1.32	0	0	0	1.50	1.92
	5	3.64	0	2.01	0	0	1.04	2.22	2.41	3.83
3	1	4.16	2.05	0	1.51	0	1.04	0	4.07	3.32
	2	3.85	2.82	0	0	1.04	0	0	1.32	2.61
	3	3.87	3.40	2.93	1.05	0	0	0	3.97	1.32
	4	4.40	2.80	1.62	0	0	0	0	2.86	3.32
	5	2.98	3.99	0	2.47	0	0	0	0	2.47
4	1	3.14	1.51	1.72	0	0	0	1.51	3.33	0
	2	2.99	3.68	1.92	2.79	0	1.32	0	2.65	3.84
	3	3.21	1.97	3.19	0	0	0	0	3.89	3.55
	4	2.19	1.04	0	0	0	0	0	2.85	2.50
	5	3.25	3.74	0	0	0	0	0	2.01	3.17

TABLE A.13. Condition of mother tuber, with respect to dry rot disease, at time of lifting.

(For explanation of symbols see following page.)

Replicate	Plant	Treatment B					Treatment C					Treatment D					Treatment F					Treatment G					Treatment H					Treatment I				
		Sampling					Sampling					Sampling					Sampling					Sampling					Sampling					Sampling				
		1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
1	1	I	I	I	I	ITr+	*	I	I	Sr	Sr+	H	H	I	Sr	Tr-	H	I	I	Sr	ITr	H	H	H	Sr	Tr-	I	I	I	*	ITr+	H	I	I	I	Tr+
	2	I	I	I	I	ITr	*	H	H	Tr	Sr+	H	H	H	Sr	Tr-	I	I	H	Sr	H-	I	H	H	I	Tr-	I	I	I	I	ITr	H	H	H	Sr	Tr-
	3	I	I	I	I	ITr+	*	H	H	I	Sr+	H	H	H	I	Tr-	I	I	I	I	Tr+	H	H	I	Tr	H-	I	I	I	I	ITr	H	H	Sr	Sr	Tr+
	4	I	I	I	I	ITr	*	H	H	I	Sr+	H	H	H	H	Sr-	I	I	I	H	ITr	I	I	H	Sr	Sr	I	I	I	I	ITr	I	H	I	Sr	Tr+
	5	I	I	I	I	ITr	*	H	Sr	Sr	Tr+	H	H	H	Sr	Tr-	I	I	I	I	Tr	H	H	H	Sr	lTr+	I	I	I	I	ITr	I	I	Sr	I	Tr+
2	1	I	I	I	I	ITr+	*	H	H	Tr	Tr+	H	H	H	Sr	Tr-	I	H	I	Tr	Tr-	H	I	H	Sr	Tr	I	I	I	I	ITr	I	I	Sr	*	Tr-
	2	I	I	I	I	ITr	*	H	I	Sr	Tr-	H	H	H	Sr	Sr-	H	H	I	Sr	Tr-	H	H	H	Sr	*	I	I	I	*	ITr	H	H	H	I	Tr+
	3	I	I	I	I	ITr-	*	H	Sr	Sr	Tr+	H	H	H	H	Sr-	H	I	I	H	Tr-	H	H	H	ISr	Sr	I	I	I	I	ITr	H	H	Sr	I	Sr+
	4	I	I	I	I	ITr-	*	H	H	ITr	Tr+	H	H	H	Sr	Sr+	I	I	I	H	I	I	H	H	H	Sr	I	I	I	I	ITr	H	H	H	Sr	Sr+
	5	I	I	I	I	*	*	H	H	I	Tr+	H	H	I	Sr	Sr	I	H	I	I	ITr	H	H	H	Tr	Sr+	I	I	I	I	ITr+	H	I	I	Sr	Sr+
3	1	I	I	I	I	ITr	*	H	H	I	Tr+	H	H	H	H	Tr-	I	I	I	I	ITr	H	H	H	ISr	Sr-	I	I	I	I	Tr+	H	I	H	Sr	Tr+
	2	I	I	I	I	ITr	*	H	H	H	Tr-	H	H	H	Sr	Sr-	H	H	I	H	ITr	H	H	H	H	Sr-	I	I	I	I	Tr+	I	I	H	*	Sr+
	3	I	I	I	I	ITr	*	H	H	Tr	Tr+	H	I	H	I?	Tr-	H	H	I	H	ITr	H	H	I	H	Sr-	I	I	I	I	*	H	I	H	Sr	Sr+
	4	I	I	I	I	ITr+	*	H	H	Tr	Sr-	H	H	H	Sr	Tr-	I	H	I	Sr	ITr	H	H	H	H	Sr-	I	I	I	I	ITr	I	H	Sr	ISr	Tr+
	5	I	I	I	I	*	*	H	Sr	Tr	Sr-	H	H	H	H	Tr+	I	I	I	Sr	ITr	H	H	H	H	Sr-	I	I	I	I	ITr	I	I	I	ISr	Tr+
4	1	I	I	H	I	ITr	*	H	H	I	Sr	H	H	H	Sr	Sr-	I	H	I	I	ITr	I	I	H	H	Tr-	I	I	I	I	Tr+	I	H	H	I	Tr
	2	I	I	I	I	ITr+	*	I	H	I	lTr	H	H	H	Sr	Sr+	I	I	I	I	ITr	H	H	H	Sr	Tr-	I	I	I	I	ITr	I	H	I	I	Tr+
	3	I	I	I	I	ITr	*	H	I	I	Tr+	H	H	H	Sr	Sr-	I	I	I	I	Tr-	I	H	H	Sr	Tr	I	I	I	I	*	H	I	H	Sr	Tr+
	4	I	I	*	I	ITr	*	H	I	Tr	Tr-	H	H	H	Tr	Sr-	H	I	H	Sr	Tr	H	H	H	H	Tr-	I	*	I	I	ITr	H	H	H	Sr	Tr
	5	I	I	I?	I	ITr-	*	I	I	H	Sr	H	H	H	Sr	Sr-	I	I	I	I	Tr-	H	H	H	Sr	Sr	I	I	I	*	ITr	H	I	Sr	Sr	Tr+

TABLE A.13. Condition of mother tuber, with respect to dry rot disease, at time of lifting.

Treatments A and E are not included in the table as the tubers had lesions at planting time.

I = macroscopic evidence of dry rot infection. This may have been an easily identified rot or the remains of a rot in an almost totally decayed or soft-rotted tuber.

H = no macroscopic evidence of dry rot infection. Mother tuber still whole.

Tr = mother tuber totally rotted or almost so.

Sr = mother tuber soft-rotted which may or may not have had F. solani var. coeruleum infection.

-, + following Tr or Sr = absence or presence of F. solani var. coeruleum in tuber remains as shown when sample of tuber remnants placed on PM70 medium. This test does not indicate infection of mother tuber as surface contamination of mother tuber would also be detected.

* = missing value.

TABLE A.14. Population estimates (and their $\log_{10}(x+1)$ transformations) of F. solani var. coeruleum from tuber surface samples taken in Jan. 1974 from tubers lifted in Sept. 1973.

Treatment	Population estimate				Log(x+1) transformation of population estimate			
	Replicate				Replicate			
	1	2	3	4	1	2	3	4
A	9,080	170	3,240	780	3.96	2.23	3.51	2.89
B	50	60	580	230	1.71	1.78	2.76	2.36
C	50	0	0	10	1.71	0	0	1.04
D	10	120	0	0	1.04	2.08	0	0
E	0	0	10	0	0	0	1.04	0
F	10	0	0	0	1.04	0	0	0
G	0	0	0	0	0	0	0	0
H	4,840	30	1,060	60	3.68	1.49	3.03	1.78
I	520	890	410	0	2.72	2.95	2.61	0

Comments on statistical analysis of results of experiment 3.3.2.

A partially hierarchical analysis was carried out on the results of experiment 3.3.2. Only the four treatments with high population estimates were considered (treatments A, B, H and I). The strata were: (Replicate/Repl.Treatm./Repl.Tr.Samplings/Repl.Tr.Sampl.Plants). The $\log_{10}(x+1)$ transformed figures were used for this analysis.

The main aim of the analysis was to determine if there were significant differences between the means of the transformed population estimates at the different sampling times. The variance ratios in the analysis of variance table showed that there were indeed very highly significant differences between the means of different sampling times and between the means of the four treatments considered.