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## STUDIES ON TUBER STEM END ROT AND STEM ROT OF POTATO IN NORTH QUEENSLAND CAUSED BY *FUSARIUM SOLANI* AND *FUSARIUM OXYSPORUM*

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### SUMMARY

*Fusarium solani* and *F. oxysporum* were identified as the casual organisms of potato stem end rot in north Queensland. A selective medium containing dichloran was very effective for the isolation of these two species from soils and a soil dilution plate technique enabled estimates of populations to be made of the two fungi in soils with varied cropping histories.

The number of colonies on individual plates in the soil isolation technique followed the normal and Poisson distributions thus demonstrating that an assumption that there is a random distribution of propagules in the soil is justified.

Populations were usually zero or low in virgin soils but all of the cultivated soils examined gave high counts for both species. While populations of *F. solani* were always lower in potato soils than in soils cropped to maize or pasture, *F. oxysporum* populations were often higher. Isolates of both fungi which were pathogenic to potato tubers were obtained from soils which had never been cropped to potato.

In pot trials, soil dressing with benomyl controlled stem end rot. Pre-planting applications at a high dosage rate were effective but phytotoxic. Three post-planting applications at intervals of 4 weeks increased the degree of control, decreased phytotoxicity and enabled the total dosage of benomyl to be reduced below the level used in the pre-plant application.

In a rotation trial, a cropping sequence of soybeans (summer) alternated with potato (winter) reduced the amount of tuber rot after three summers.

A collection of soil isolates of both species from the trial area was pathogenic to potato tubers and stalks but *F. solani* was more aggressive than *F. oxysporum*.

### I. INTRODUCTION

In north Queensland, which is a tropical region, the main potato crop is grown on the Atherton and Evelyn Tablelands at elevations ranging from 650 to 1 000 m. In these tableland areas, a spring crop planted in late July and an autumn crop planted from late December onwards can be grown, while in frost-free districts or in years when frost seems unlikely to be a hazard, a third crop is often planted during the period April to June. Such an extended

planting season means that many crops come to maturity during the warm weather of early autumn, late spring and summer. It is in crops harvested when soil temperatures are high that tuber stem end rot causes heavy losses.

This paper describes the disease and discusses the isolation, proof of pathogenicity, identity of the causal fungi and a technique for estimating soil populations of these causal organisms under a variety of conditions. Investigations into the effects of fungicidal and cultural treatments are described.

## II. THE DISEASE

### Symptoms

The symptoms of tuber stem end rot and a related stem rot have previously been described in detail by Goss (1923, 1924 and 1936) and McLean and Walker (1941). These workers have stressed the difference between '*oxysporum*' wilt and '*eumartii*' wilt. In north Queensland, no such difference has been noted and for this reason the symptoms encountered in this region are described below.

On tubers, a sunken brown lesion is usually evident around the stolon scar. Sometimes this is so small that it escapes notice. Often, small cavities lined with mycelia and spores are present in an area of dry brown rotted tissue extending inwards from the external lesion. In many cases, the stem end breakdown is accompanied by a very noticeable necrosis of the vascular ring, extending for all or part of the length of the tuber as a discontinuous brown band with a water-soaking of the surrounding tissue.

Tuber stem end rot is often associated with a stem rot above ground level which develops after flowering. Diseased tubers may, however, be found on plants that do not develop stem rot and this fact has been commented on by previous authors including Goss (1923) who found that apparently healthy plants grown from inoculated seed produced a percentage of infected tubers.

On plants which are visibly affected with stem rot, the original seed tubers are usually rotting or completely disintegrated and the roots are obviously decayed. Dark brown lesions are evident on the bases of the stalks and the vascular systems may be discoloured to varying degrees. Infected plants become evident when the oldest leaves begin to yellow and die. The final stage is premature death of the plant. Aerial tubers and purpling of foliage which are described by Goss (1936) as typical symptoms of both '*eumartii*' wilt and '*oxysporum*' wilt in wet soils are not often seen.

Another symptom which may be encountered is a temporary wilt associated with a jelly-like breakdown of the seed tuber, some degree of root rot and a limited rot at the base of the stalk. When affected plants regain turgour, an extensive marginal necrosis of leaflets becomes noticeable.

Plants infected with either of these stem rots invariably yield a proportion of tubers infected with stem end rot.

### The casual organism

1. ISOLATION AND IDENTIFICATION. Isolations from diseased material were made onto potato dextrose agar (PDA) plus an antibiotic mixture, consisting of streptomycin sulphate, chloromycetin and chlorotet in equal proportions, at 50 mg  $l^{-1}$  of medium.

In all isolations from diseased material, two species of *Fusarium* which were tentatively identified as *Fusarium solani* (Mart.) Sacc. and *Fusarium oxysporum* Schlecht. were consistently obtained. The identifications were subsequently confirmed by Dr. C. Booth of the C.M.I.

*Fusarium solani* predominated in the isolates obtained from tuber stem end rot lesions (16 affected per 27 samples tested). Sometimes both species were obtained (9 out of 27) and occasionally only *F. oxysporum* was recovered (2 out of 27). Isolations from plants affected with either stem rot or temporary wilt were fewer in number. In the case of stem rot, *F. oxysporum* was recovered more frequently from stem lesions (5 out of 9) while *F. solani* was more prevalent in rotting roots. *F. oxysporum* was isolated from the rotting seed pieces (2 out of 2), roots and stems of plants affected with the temporary wilt syndrome.

In no case was either fungus isolated from necrotic vascular tissue in tubers and stalks.

2. PATHOGENICITY TESTS. The soil mix used contained equal volumes of peat moss, fine sand and loamy soil. After sterilization by heat a complete fertilizer was incorporated together with lime to raise the pH to a level of approximately 6. Inoculum for infection studies was grown on steamed and autoclaved wheat grain. Between 70 and 85 g of grain inoculum, 21 days old was incorporated into the top 10 cm of the potting mix in each 9 litre plastic pot. Potato seed pieces were surface-sterilized in a solution of a proprietary organic mercurial and planted one in each pot.

Both stem end rot and stem rot were produced with either *F. solani* or *F. oxysporum*. In one such test, six pots were inoculated with *F. solani*, 6 with *F. oxysporum* and 12 were uninoculated controls. With *F. oxysporum*, 79% of the resulting plants produced tubers with stem end rot and 80% of the plants had stem rot. The figures for *F. solani* were 67% and 100% respectively while, in the uninoculated controls, they were 21% and 33%.

The presence of natural infections in uninoculated controls has been noted previously (McLean and Walker 1941). While *F. solani* alone was recovered from plants in soil inoculated with this fungus, it was sometimes isolated along with *F. oxysporum* from plants in soil inoculated with *F. oxysporum*. This was presumably due to seed-borne infection.

It was found that there was a high incidence of stem end rot on plants affected with stem rot. However, stem end rot was also found on tubers from plants not showing any stem rot symptoms, thus confirming field observations. This was illustrated in a glasshouse trial involving 116 plants, half of which were grown in soil inoculated with *F. solani* and half with *F. oxysporum*. Of the plants from the *F. oxysporum* pots, 58% had tubers with stem end rot and 34% had stem rot. With *F. solani*, the figures were 86% and 76% respectively.

### III. DISTRIBUTION OF THE PATHOGENS IN DISTRICT SOILS

#### Methods

1. SOIL SAMPLING. Samples were collected from a number of district sites with different cropping histories (table 2). At each site, 12 to 20 random samples were taken with a soil auger or spade, generally to the depth of the top soil, with a maximum depth of 15 cm. The random samples were combined and a small sub-sample obtained for each site. With the exception of sites 2 and 7 (Alluvial soils), all soils were of volcanic origin and derived from basalt. Many of the fields sampled had never grown potatoes.

2. PLATE COUNTS. *Selective medium*. *Fusarium* populations were established by a plate count method using a modified Park's medium (Park 1963) in which 20 g agar was used instead of 15 g l<sup>-1</sup> and dichloran (Allisan R) was added at

either 0.25 or 0.5 g  $l^{-1}$  to inhibit *Rhizopus* spp. which were prevalent in some soils. The lower rate was used if fast growing fungi were not prevalent. Where bacterial contamination was high neomycin sulphate (12 ml of 1% solution  $l^{-1}$ ) was added to the medium after autoclaving.

In the preparation of the medium, the agar was suspended in 500 ml distilled water and the remaining ingredients added to a further 500 ml distilled water. Because combination before autoclaving resulted in softening of the agar gel these solutions were autoclaved separately (103 kPa for 20 min) and combined before dispensing at the rate of 10 ml per plate (10 cm petri dish). The plates were allowed to dry for 2 to 3 days before they were inoculated with soil suspension.

On this medium after 4 to 5 days incubation in diffuse light, colonies of *F. solani* attained a maximum diameter of 25 mm. They were mainly white in colour with central yellow spore masses and sparse aerial mycelia but some variants induced a blue pigmentation in the medium and others were smaller with less aerial mycelia.

Colonies of *F. oxysporum*, though they ranged in size from that of a pin head to 15 mm diameter and had variable amounts of aerial mycelia, could always be distinguished by a characteristic pinkish orange colour.

*Soil dilutions.* A soil suspension was prepared by adding 50 mg of a freshly pulverized sample of the test soil to a 100 ml flask containing 50 ml of 1% sodium carboxy methyl cellulose (or 0.1% agar) plus antibiotics (5 mg streptomycin sulphate and 2.5 mg chlorotet) and a few grains of sterilized fine sand. The suspension was shaken for 30 min on a mechanical flask shaker

TABLE 1

FREQUENCY TABLES OF COLONY COUNTS OF *Fusarium solani* AND *F. oxysporum* DERIVED FROM 180 SOIL DILUTION PLATES (8 SOIL CORES BULKED)

No. of Colonies per Plate (r)	Observed Plates with r <i>F. solani</i>	Expected Plates (Normal Dist.)	Expected Plates (Poisson Dist.)	Observed Plates with r <i>F. oxysporum</i>	Expected Plates (Normal Dist.)	Expected Plates (Poisson Dist.)
0 .. ..	20	20	13	0	0	0
1 .. ..	32	27	34	0	1	0
2 .. ..	36	31	45	2	2	1
3 .. ..	41	29	39	2	4	3
4 .. ..	22	23	26	6	7	7
5 .. ..	16	15	14	15	11	12
6 .. ..	8	8	6	17	15	18
7 .. ..	5	4	2	25	20	22
8 .. ..	0	1	1	19	22	24
9 .. ..	..	..	..	27	23	24
10 .. ..	..	$\chi^2 = 6.81$ 6 N.S.	$\chi^2 = 8.69$ 7 N.S.	23	21	21
11 .. ..	..	..	..	12	18	17
12 .. ..	..	..	..	8	14	12
13 .. ..	..	..	..	10	9	8
14 .. ..	..	..	..	6	6	5
15 .. ..	..	..	..	4	3	3
16 .. ..	..	..	..	1	2	2
17 .. ..	..	..	..	1	1	1
18 .. ..	..	..	..	0	0	0
19 .. ..	..	..	..	2	0	0
					$\chi^2 = 11.04$ 11 N.S.	$\chi^2 = 7.14$ 12 N.S.

then dispensed at the rate of 1 ml (1 mg soil) per plate to the modified Park's medium. At least 15 plates were poured for each soil. The plates were carefully agitated to spread the suspension over the medium and were then incubated on benches at room temperature.

The accuracy of the plating technique in reflecting the distribution of propagules in the soil was tested by the method of Nash and Snyder (1962).

The frequency distribution of colonies of *F. solani* and *F. oxysporum* was determined in two series of dilution plates with soil from two sources. Counts of colonies occurring in individual plates were made.

In both cases, the data conformed to either a normal or Poisson distribution. The data in table 1, from the second sample, were derived by combining eight cores of soils and preparing 180 dilution plates from a subsample of this bulked sample.

3. PATHOGENICITY TESTS. The pathogenicity of isolates of both *F. solani* and *F. oxysporum* obtained from some potato soils, from maize and pasture soils which had never been cropped to potatoes and from virgin soils was assessed by wound inoculations into whole potato tubers.

TABLE 2

ESTIMATED POPULATIONS OF *Fusarium solani* AND *Fusarium oxysporum* IN A SELECTION OF DISTRICT SOILS

Site	Cropping History	Propagules g <sup>-1</sup> Soil	
		<i>F. solani</i>	<i>F. oxysporum</i>
1 .. .. .	Potatoes .. .. .	600	1 080
2 .. .. .	Potatoes .. .. .	880	1 320
3 .. .. .	Potatoes .. .. .	400	3 480
4 .. .. .	Potatoes .. .. .	520	560
5 .. .. .	Potatoes .. .. .	360	4 400
6 .. .. .	Potatoes .. .. .	600	2 800
7 .. .. .	Potatoes .. .. .	440	7 720
8 .. .. .	Potatoes, maize .. .. .	880	3 840
9 .. .. .	Maize .. .. .	1 600	2 400
10 .. .. .	Maize .. .. .	1 640	7 920
11 .. .. .	Maize .. .. .	2 560	3 760
12 .. .. .	Maize .. .. .	1 640	6 480
13 .. .. .	Maize .. .. .	2 640	3 240
14 .. .. .	Maize .. .. .	1 520	1 560
15 .. .. .	Maize .. .. .	3 440	2 680
16 .. .. .	Maize .. .. .	1 920	2 400
17 .. .. .	Maize .. .. .	2 760	6 480
18 .. .. .	Maize .. .. .	2 120	2 840
19 .. .. .	Maize, peanuts .. .. .	2 880	2 440
20 .. .. .	Maize, peanuts .. .. .	1 880	1 240
21 .. .. .	Maize, peanuts .. .. .	3 080	1 600
22 .. .. .	Maize, peanuts .. .. .	3 800	2 080
23 .. .. .	Maize, peanuts .. .. .	1 200	7 760
24 .. .. .	Pasture .. .. .	2 800	400
25 .. .. .	Pasture .. .. .	880	9 600
26 .. .. .	Pasture .. .. .	1 040	7 360
27 .. .. .	Pasture .. .. .	1 920	3 120
28 .. .. .	Pasture .. .. .	320	7 920
29 .. .. .	Virgin .. .. .	0	0
30 .. .. .	Virgin .. .. .	160	0
31 .. .. .	Virgin .. .. .	0	4 600

TABLE 3  
GROWTH\* OF *F. solani* AND *F. oxysporum* ON PLATES AMENDED WITH DIFFERENT FUNGICIDES

Fungicide	ppm active ingredient											
	1		5		10		100		500		1000	
	F.s.	F.o.†	F.s.	F.o.	F.s.	F.o.	F.s.	F.o.	F.s.	F.o.	F.s.	F.o.
Bayer 6059 <sup>1</sup> .. .. .	54	100	29	38	23	9	0	0	0	0	0	0
Bayer 5768 <sup>2</sup> .. .. .	48	62	28	39	26	56	18	6	14	0	9	0
Nabam .. .. .	65	100	59	84	54	79	0	0	0	0	0	0
Carboxin .. .. .	77	100	77	91	70	83	70	57	70	40	70	27
Benomyl .. .. .	40	76	30	0	26	0	20	0	10	0	0	0
Thiabendazole .. .. .	66	100	33	100	30	61	6	0	0	0	0	0
Bas 2201 F <sup>3</sup> .. .. .	100	100	100	100	100	100	30	40	13	6	10	0
Captan .. .. .	100	100	100	100	100	100	33	30	16	17	13	15
Captafol .. .. .	34	39	14	24	10	20	10	12	10	10	10	10
Fenamiosulf .. .. .	100	90	100	88	100	86	100	83	100	67	86	52
Quintozene .. .. .	94	100	91	93	84	87	63	69	63	63	63	56
Chloroneb .. .. .	100	96	100	96	100	87	76	77	76	54	76	43
Pennwalt TD 5056 <sup>4</sup> .. .. .	38	77	23	0	16	0	5	0	0	0	0	0
Quintozene 10% plus Truban 5% .. .. .	94	100	87	93	80	89	64	62	35	47	35	36
Terrazole <sup>5</sup> .. .. .	92	100	92	100	92	100	75	68	19	50	17	36

\* Percentage compared with unamended plates; mean of 5 replications.

† F.s. = *F. solani*, F.o. = *F. oxysporum*.

<sup>1</sup> 2-(2-furyl) benzimidazole.

<sup>2</sup> N N' dipropyl-N,N'-bis-(fluorodichloromethyl thio sulfamide).

<sup>3</sup> Active ingredient not known.

<sup>4</sup> 2-methylsulfonyl-6-nitrobenzothiazole.

<sup>5</sup> 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole.

## Results

The figures for estimated numbers of propagules per gram of soil (table 2) have been deduced by assuming that one colony represents one propagule.

In the pathogenicity tests, all isolates, whether from potato, maize, pasture or virgin soil, were pathogenic to potato tubers. The lesions produced in all cases were areas of cortical soft rot from which the fungus could be re-isolated.

## IV. FUNGICIDE TESTS

### In vitro

Fifteen fungicides were screened against both *F. solani* and *F. oxysporum* using the following techniques—

A range of dilutions (0 p.p.m. to 1 000 p.p.m. active ingredient) of various commercial formulations was prepared in PDA and poured into petri dishes, five for each concentration (10 ml per plate) and plates were inoculated with a small cube from a culture of the test organism. Measurements of the diameters of the resulting colonies were made when the majority of the 0 p.p.m. plates were completely covered, that is, from 3 to 5 days.

Mean colony diameter for each concentration was expressed as a percentage of that for the 0 p.p.m. concentration (table 3).

### Pot trials

Nine-litre plastic pots were used with the soil mix and inoculum prepared as previously described. Pre-planting fungicide test materials were incorporated into the mix before inoculation. In all cases, sterilized seed potatoes cv. Sebago were planted one to each pot.

EXPERIMENT 1. As a result of the *in vitro* tests, five fungicides (table 4) were selected for screening at various concentrations in the potting mix. Plot size was a single pot and there were four replications. Assessment was made by counting the number of rotted tubers in each pot after the plants had matured and died off (table 4).

TABLE 4  
PERCENTAGE OF ROTTED TUBERS ON PLANTS GROWN IN SOIL AMENDED WITH VARIOUS FUNGICIDES AND INOCULATED WITH *F. solani* OR *F. oxysporum*

Pot Experiment 1				
Treatment	Concentration p.p.m. active ingredient	Mean percentage rotted tubers		
		F.s.*	F.o.	
Benomyl (50 W) .. .. .	110	10	18	
Benomyl (50 W) .. .. .	550	0	0	
Captafol (80 W) .. .. .	176	67	68	
Captafol (80 W) .. .. .	880	74	85	
Thiabendazole (90 W) .. .. .	198	Plants dead due to phytotoxicity		
Thiabendazole (90 W) .. .. .	990	Plants dead due to phytotoxicity		
Penwalt TD 5056 (50 W) .. .. .	110	88	58	
Penwalt TD 5056 (50 W) .. .. .	550	77	76	
Bayer 6059 (50 W) .. .. .	110	Plants dead due to phytotoxicity		
Bayer 6059 (50 W) .. .. .	550	Plants dead due to phytotoxicity		
Nil. Soil inoculated .. .. .	..	47	82	
Nil. Soil not inoculated .. .. .	..	14	1.75	

\* F.s. = *F. solani*, F.o. = *F. oxysporum*.

EXPERIMENT 2. Benomyl and thiabendazole were subsequently tested in another pot trial aimed at investigating further the phytotoxicity of thiabendazole and quantifying the relative effects of the two fungicides on soil populations of *Fusarium solani*. For the latter purpose, the plate count technique previously described was employed.

Seven replications of single pot plots were used.

Results are given in table 5.

TABLE 5

PERCENTAGE OF ROTTED TUBERS AND ESTIMATED POPULATIONS OF *Fusarium solani* IN POTS OF INOCULATED SOIL AMENDED WITH BENOMYL OR THIABENDAZOLE

Pot Experiment 2			
Treatment	Concentration p.p.m. active ingredient	Mean % rotted tubers	No. <i>Fusarium</i> propagules g <sup>-1</sup> soil (Mean of 4 pots)
Benomyl .. .. .	110	10.4	1,800
Benomyl .. .. .	550	0	837
Thiabendazole .. .. .	198	Plants dead due to phytotoxicity	2,725
Thiabendazole .. .. .	990	Plants dead due to phytotoxicity	75
Nil inoculated .. .. .	..	82	86,125
Nil not inoculated .. .. .	..	0	226

TABLE 6

INCIDENCE OF ROT IN TUBERS ON PLANTS GROWN IN POTS INOCULATED WITH *F. solani* AND AMENDED IN VARIOUS WAYS WITH BENOMYL

Pot Experiment 3		
Treatment	Mean number tubers per pot	Percentages rotted tubers
Benomyl seed soak 1.8 kg 450 l <sup>-1</sup> (24 hours' immersion) .. .. .	12.6	98
Benomyl pre-plant (dry) 330 p.p.m. .. .. .	15.2	37
Benomyl seed soak + pre-plant (dry) .. .. .	10.25	17
Benomyl pre-plant (drench) 210 p.p.m. + post-drench 140 p.p.m. (4 wk) .. .. .	10.8	11
Benomyl post-plant drench 140 p.p.m. (4 wk) .. .. .	10	15
Benomyl pre-plant (drench) 210 p.p.m. + post-plant drench 140 p.p.m. (7 wk) .. .. .	10.8	16
Benomyl post-plant drench 140 p.p.m. (7 wk) .. .. .	14.4	47
Benomyl pre-plant (drench) 210 p.p.m. + post-plant drench 140 p.p.m. (8 wk) .. .. .	12	14
Benomyl post-plant drench 140 p.p.m. (8 wk) .. .. .	10	29
Benomyl pre-plant (drench) 210 p.p.m. + post-plant drench 140 p.p.m. (10 wk) .. .. .	9.2	11
Benomyl post-plant drench 140 p.p.m. (10 wk) .. .. .	14.5	46
Benomyl pre-plant (drench) 210 p.p.m. + post-plant drench 35 p.p.m. (4, 8, 12 wk) .. .. .	10.75	19
Benomyl post-plant drench 35 p.p.m. (4, 8, 12 wk) .. .. .	11.8	10
Benomyl pre-plant (drench) 210 p.p.m. + post-plant drench 70 p.p.m. (4, 8, 12 wk) .. .. .	10.8	2
Benomyl post-plant drench 70 p.p.m. (4, 8, 12 wk) .. .. .	12.6	6
Nil .. .. .	14.8	60



EXPERIMENT 3. In this trial, 15 treatments and combination of treatments of benomyl, involving seed soak, pre-plant dry and drench applications, were compared with an untreated control in pots of soil inoculated with *F. solani*. There were five replications of single pot plots. Results were assessed as before and are presented in table 6.

## V. ROTATION EXPERIMENT

### Disease incidence and *Fusarium* populations

A trial was established at Walkamin Research Station with the object of studying the effect of a limited crop diversification on tuber rot incidence and *Fusarium* populations in the soil. It was initiated late in the winter of 1967 when the disease organisms were established in the soil by planting with potato seed affected by stem end rot. Subsequent potato crops were grown from certified seed, presumed free of stem end rot.

The nine treatments (table 7) were laid out in three randomized blocks. The potato plots comprised 10 rows, 90 cm apart and 20 m long. All other crops were planted at the recommended row spacings and sowing rates.

Counts of stem-rot-affected plants, rotted tubers (after storage for 1 week) and nematode-infested tubers were made. Yield measurements were also recorded. No consistent difference which could be related to treatments were noted in the 1968 and 1969 potato crops. The 1970 results are given in table 7.

For estimates of *Fusarium* populations, eight random soil samples, using a 5.5 cm diameter auger to a depth of 10 cm, were taken from each plot after each potato crop was harvested and again when the summer crops were approaching maturity, after the green manure crops had been ploughed in. The eight samples were bulked and a sub-sample was taken from which dilution plates were poured. The counts for individual plots were then used to calculate a mean for each treatment. Results are shown in table 8.

### Pathogenicity of soil isolates

During the course of the rotation trial, the pathogenicity of a range of soil isolates of *F. solani* and *F. oxysporum* was checked by inoculation of cubes of PDA cultures onto slices of potato tuber tissue or potato stem segments and, on one occasion, of drops of spore suspension onto the surfaces of uninjured tubers. The use of tuber slices ensured that the inoculations could be adequately replicated without using a large number of tubers for wound inoculations. Stem segments and uninjured tubers were inoculated to compare the relative susceptibility of these substrates with that of wounded tuber tissue.

Isolates of *Fusarium sporotrichioides* Sherb. and *F. semitectum* Berk. and Rob. (both identified by C.M.I.) which were invariably recovered in small numbers from all of the trial soil samples were included in some tests.

In the case of tuber slices, blemish-free tubers were washed well and surface sterilized in mercuric chloride (1:1 000 solution plus surfactant) for 5 min. Suitably sized slices were pared from the tubers with a sterile knife and placed in a petri dish on PDA medium plus the triple antibiotic mixture mentioned previously or on filter paper dampened with a sterile water solution of the same antibiotic mixture. As many as five slices were incubated in each dish and two petri dishes were allowed for each isolate.

TABLE 7  
TUBER ROT, STEM ROT AND YIELD FIGURES, 1970 POTATO CROP IN ROTATION EXPERIMENT AT WALKAMIN

Treatment						% Tuber Rot†		% Stem Rot		Weight† of marketable potatoes at harvest (kg per 8 rows) Treatment Means	% Tubers nematode infested
1968		1969		1970		Transformed* Mean	Equiv. Mean	Transformed* Mean	Equiv. Mean		
Summer	Winter	Summer	Winter	Summer	Winter						
Bare fallow ..	Potato ..	Bare fallow ..	Potato ..	Bare fallow ..	Potato ..	0.453	19.19	0.473	20.76	181.74	0.1
Peanuts ..	Potato ..	Peanuts ..	Potato ..	Peanuts ..	Potato ..	0.593	31.21	0.388	14.32	213.49	0
Lablab bean (GM) <sup>1</sup>	Potato ..	Lablab bean (GM)	Potato ..	Lablab bean (GM)	Potato ..	0.722	43.65	0.180	3.20	215.00	0
Maize ..	Potato ..	Maize ..	Potato ..	Maize ..	Potato ..	0.550	27.32	0.303	8.91	198.82	0
Cowpea (S) <sup>2</sup> ..	Potato ..	Cowpea (S) ..	Potato ..	Cowpea (S) ..	Potato ..	0.266	6.91	0.167	2.77	160.72	77
Cowpea (GM) ..	Potato ..	Cowpea (GM) ..	Potato ..	Cowpea (GM) ..	Potato ..	0.447	18.67	0.100	1.00	168.28	50
Soybean (S) ..	Potato ..	Soybean (S) ..	Potato ..	Soybean (S) ..	Potato ..	0.424	16.95	0.260	6.63	209.11	2
Peanuts ..	Oats ..	Maize ..	Potato ..	Peanuts ..	Potato ..	0.731	44.58	0.292	8.29	248.12	0
Peanuts ..	Oats ..	Maize ..	Oats ..	Soybean (S) ..	Potato ..	0.438	17.97	0.249	6.09	194.74	0.3
				Necessary differences for } 5%		0.199		0.281		37.75	
				significance } 1%		0.274		0.387		52.01	

\* The inverse sine transformation used.

† F values in analyses of variance significant at the 1% level.

<sup>1</sup> GM = Green manure crop.

<sup>2</sup> S = Seed crop.

TABLE 8  
POPULATIONS OF *Fusarium* spp. AFTER EACH POTATO CROP AND SUMMER CROP IN ROTATION EXPERIMENT AT WALKAMIN

Treatment						Estimated propagules g <sup>-1</sup> soil					
						1967		1968			
1968		1969		1970		Fs* Fo*		Fs		Fo	
Summer	Winter	Summer	Winter	Summer	Winter	P†	P	P	S‡	P	S
Bare fallow ..	Potato ..	Bare fallow ..	Potato ..	Bare fallow ..	Potato ..	1,640	1,420	1,460	2,460	1,820	2,340
Peanuts .. ..	Potato ..	Peanuts .. ..	Potato ..	Peanuts .. ..	Potato ..	1,720	1,520	2,200	2,900	2,660	2,380
Lablab bean (GM)	Potato ..	Lablab bean (GM)	Potato ..	Lablab bean (GM)	Potato ..	1,640	1,380	2,480	3,160	3,180	2,220
Maize .. .. .	Potato ..	Maize .. .. .	Potato ..	Maize .. .. .	Potato ..	1,460	940	1,760	3,020	1,280	2,980
Cowpea (S) ..	Potato ..	Cowpea (S) ..	Potato ..	Cowpea (S) ..	Potato ..	1,640	1,440	1,680	3,000	1,520	6,500
Cowpea (GM) ..	Potato ..	Cowpea (GM) ..	Potato ..	Cowpea (GM) ..	Potato ..	2,080	2,320	1,480	3,820	1,500	8,360
Soybean (S) ..	Potato ..	Soybean .. ..	Potato ..	Soybean .. ..	Potato ..	2,040	1,220	700	2,960	400	2,300
Peanuts .. ..	Oats .. ..	Maize .. .. .	Potato ..	Peanuts .. ..	Potato ..	1,860	1,460	..	4,700	..	3,480
Peanuts .. ..	Oats .. ..	Maize .. .. .	Oats .. ..	Soybean .. ..	Potato ..	1,660	1,960	..	3,320	..	2,980

\* Fs = *F. solani*, Fo = *F. oxysporum*.

† P = After potato crop.

‡ S = At summer crop maturity.

TABLE 8—*continued*  
 POPULATIONS OF *Fusarium* SPP. AFTER EACH POTATO CROP AND SUMMER CROP IN ROTATION EXPERIMENT AT WALKAMIN—*continued*

Treatment						Estimated propagules g <sup>-1</sup> soil							
						1969				1970			
						1968		1969		1970		Fs	
Summer	Winter	Summer	Winter	Summer	Winter	P	S	P	S	P	S	P	S
Bare fallow ..	Potato ..	Bare fallow ..	Potato ..	Bare fallow ..	Potato ..	1,840	2,340	1,700	2,540	1,820	2,060	2,100	2,060
Peanuts ..	Potato ..	Peanuts ..	Potato ..	Peanuts ..	Potato ..	1,640	2,320	1,840	2,640	1,880	1,960	2,940	1,540
Lablab bean (GM)	Potato ..	Lablab bean (GM)	Potato ..	Lablab bean (GM)	Potato ..	2,160	3,120	3,360	4,240	2,800	2,940	3,780	3,360
Maize ..	Potato ..	Maize ..	Potato ..	Maize ..	Potato ..	2,060	2,460	1,760	2,820	1,960	1,740	1,940	1,600
Cowpea (S)	Potato ..	Cowpea (S)	Potato ..	Cowpea (S)	Potato ..	2,060	2,600	2,340	4,160	2,880	2,200	3,440	2,080
Cowpea (GM)	Potato ..	Cowpea (GM)	Potato ..	Cowpea (GM)	Potato ..	2,300	2,560	2,660	3,280	2,900	2,540	4,160	3,560
Soybean (S)	Potato ..	Soybean ..	Potato ..	Soybean ..	Potato ..	2,360	2,060	2,160	2,220	2,240	1,760	1,920	1,320
Peanuts ..	Oats ..	Maize ..	Potato ..	Peanuts ..	Potato ..	1,940	2,020	1,780	2,940	2,040	1,400	2,620	1,580
Peanuts ..	Oats ..	Maize ..	Oats ..	Soybean ..	Potato ..	..	1,760	..	2,300	1,960	1,860	2,120	1,440

\* Fs = *F. solani*, Fo = *F. oxysporum*.

† P = After potato crop.

‡ S = At summer crop maturity.

TABLE 9

NUMBER OF LESIONS PRODUCED BY *Fusarium* SPP. FROM POTATO SOILS ON TUBER SLICES, STEM SEGMENTS AND UNINJURED TUBERS

Organism	Tuber Slices	Stem Segments	Uninjured Tubers
<i>F. solani</i> .. .. .	380 (384)*	60 <sup>1</sup> 57 <sup>2</sup> (128)	4 (56)
<i>F. oxysporum</i> .. .. .	226 (242)	3 <sup>1</sup> 6 <sup>2</sup> (16)	0 (28)
<i>F. sporotrichioides</i> .. .. .	63 (76)	..	..
<i>F. semitectum</i> .. .. .	38 (50)	..	..
Control .. .. .	0 (48)	0 (16)	0 (10)

\* Figures in parentheses represent the total number of inoculations made in each instance.

<sup>1</sup> Segments with an area of cortical soft rot around inoculation site.<sup>2</sup> Segments with small necrotic lesions around inoculation site.

For stem segments, sound potato haulms were pared of leaves, surface sterilized in mercuric chloride solution for 5 min, then washed well in sterile water and cut into segments about 4 cm long. The segments were plated out in petri dishes on filter paper soaked with either 10 ml sterile water or with 10 ml kinetin solution (1 mg  $l^{-1}$ ). No differences in behaviour, whether of the fungus or the potato tissue, could be attributed to the kinetin treatment which was used to prolong the life of the detached stem segments.

When uninjured tubers were used, they were washed well, surface-sterilized in mercuric chloride solution for 5 min and then washed again in sterile water.

The results of these tests appear in table 9.

## VI. PHYSIOLOGICAL MATURITY OF TUBER IN RELATION TO DISEASE REACTION

There were indications from the pathogenicity tests that the physiological age of the potato tuber influenced the size and type of the lesion resulting from inoculation. This was checked by inoculating tuber slices prepared from immature tubers, new ware potatoes and shot seed potatoes with drops of spore suspensions of *Fusarium solani* and *F. oxysporum* (concentration  $1.6 \times 10^5$  spores  $ml^{-1}$ ). Twenty-four tubers of each class were inoculated with *F. solani* and 12 with *F. oxysporum*.

Three reaction types were distinguished—

- R<sub>1</sub>. Brown blotched lesions up to 10 mm diameter, only slightly pitted if at all; mycelium faintly visible on lesion surface.
- R<sub>2</sub>. More extensive brown blotches, 8 to 15 mm diameter, with copious mycelial development and slight softening of infected tissues.
- R<sub>3</sub>. Lesions 20 to 25 mm diameter, deeply pitted and lined with mycelium; infected tissue softened.

All immature tubers inoculated with either fungus produced the R<sub>1</sub> reaction, new ware potatoes the R<sub>2</sub>, and shoot seed the R<sub>3</sub> except that with *F. oxysporum* there was no softening evident around the diseased tissue.

## VII. DISCUSSION

Work published recently by Chambers (1973a) has shown that there is no correlation between the incidence of *Fusarium* spp. in Victorian 'pathogen tested' potatoes and the *Fusarium* populations of soils in which the tubers were grown. He established that injury was of prime importance for infection of plants and tubers by *Fusarium* spp. Chambers was dealing mainly with *F. oxysporum*, *Fusarium avenaceum* (Corda ex Fr.) Sacc. and *Fusarium culmorum* (W. G. Smith) Sacc.

The work presented here has shown that, under glasshouse conditions, typical tuber stem end rot and stem rot were induced by exposing potato plants to high levels of soil inoculum of either *F. solani* and *F. oxysporum*. In addition, soil isolates of both species, which were pathogenic to potato tubers, were obtained from a variety of sites including virgin soils and soils which had never been cropped to potato. When the relative pathogenicity of a range of soil isolates was assessed by such criteria as ability to rot tuber slices, stem segments and uninjured tubers *F. solani* was more aggressively pathogenic than *F. oxysporum*. The results from soil inoculations agree with those obtained by Goss (1923, 1924) and McLean and Walker (1941). Goss (1924), however, classed *F. oxysporum* as 'weakly pathogenic under all conditions' and, in addition, commented that it was 'pathogenic only under conditions extremely unfavourable for host plant and with severe inoculations'. On the other hand, he rated *F. solani* as an active pathogen under a wide range of conditions and stated that 100% infection was always obtained in green house experiments, except at extreme temperatures, with all methods of inoculation. McLean and Walker (1941) have also shown that *F. avenaceum* and *F. oxysporum* are both less pathogenic than *F. solani*.

*F. solani* is apparently much more common in some soils than Chambers (1973a, 1973b) found it to be in Victoria. Lim (1972) estimated that populations in intensively cultivated soils in Singapore ranged as high as 10 030 propagules g<sup>-1</sup>. Nash and Snyder (1962) found that counts of *F. solani* f. sp. *phaseoli* units in Salinas Valley bean field soils may be as high as 3 000 propagules g<sup>-1</sup>. On the Atherton Tableland, estimates ranged as high as 3 800 propagules g<sup>-1</sup> in cultivated soils. The highest populations were found in soils which had been cropped to maize. Lower counts were obtained from pasture land or soil cropped to potatoes. In Victoria, populations of *F. solani* reached a level of only 70 propagules g<sup>-1</sup> in old pasture soils and were no higher than 25 propagules g<sup>-1</sup> following a crop of millet and 2 propagules g<sup>-1</sup> following potatoes (Chambers 1973a, 1973b).

The results reported here agree with Chambers' finding that *F. oxysporum* is often more prevalent in old pasture land than in soils with a history of potato growing.

In glasshouse trials when used as a pre-plant soil dressing, the fungicide benomyl at a level of 110 p.p.m. active ingredient in the potting soil did not eliminate stem end rot but at 550 p.p.m. gave complete control. However, field treatment with this dosage of benomyl would be unrealistic for economic reasons. In the one trial in which populations of *F. solani* in treated soil were estimated, both low and high dosages of benomyl appreciably reduced propagule numbers of this pathogen. The higher dosage (550 p.p.m.) caused mild phytotoxicity when ever it was used, but two other benzimidazole fungicides caused severe phytotoxicity at both low and high dosages.

Wensley and Huang (1970) found in pot trials that benomyl reduced *Fusarium* wilt of muskmelons significantly when used at 80 and 160 p.p.m. active ingredient and these dosages were equally efficient as single applications or as multiple doses of 40 p.p.m. active ingredient at 10-day intervals. In the work

described here, it was found that the amount of benomyl applied for the control of *Fusarium solani* could be reduced by using post-planting applications and that three post-plant drenches, giving a total dosage of either 105 p.p.m. or 210 p.p.m. active ingredient in the potting soil, gave better control than a pre-plant application giving a level of 330 p.p.m. active ingredient.

In the rotation trial, potatoes alternated with cowpea gave a lower yield of marketable potatoes due to severe nematode infestation. The best cropping sequence when both yield and freedom from tuber rot were considered involved potatoes following soybeans, and this legume warrants further investigation as an alternative crop for potatoes. Soil populations of both *Fusarium solani* and *F. oxysporum* tended to be highest in the cowpea and lablab bean (*Lablab purpureus*) treatments. This was quite evident in 1970 and, with lablab bean, could be related to high tuber rot incidence. Severe nematode infestation excluded the possibility of establishing a similar relationship in the case of the cowpea treatments.

The results of the inoculation experiments indicated that uninjured tubers were highly resistant to infection and that stem tissue was much more resistant than was wounded tuber tissue. This is in accord with observational evidence in the field or from pot trials which have shown that tuber infection originates through the decaying stolon (except when the *Fusarium spp.* penetrate through a wound on the tuber surface) and stems are infected subsequent to either seed piece or root infection.

The evidence linking increase in susceptibility with increasing physiological age of the tuber suggests that fungus development is more rapid in senescing tissue. It certainly has been noted in the field that tuber infection increases rapidly after the plants mature and, in fact, as Goss (1924) originally found, the amount of stem end rot can often be reduced by digging the crop early.

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