THE SURVIVAL IN SOIL OF *ITERSONILIA PASTINACAE* CHANNON, THE CAUSE OF PARSNIP CANKER

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Summary

When parsnip roots, naturally infected with *Itersonilia pastinacae* Channon, were buried in the field, the fungus was still viable after 12 months. When the stem apical meristems of the roots were removed to promote their more rapid decomposition, the fungus did not survive burial longer than 7 months.

In laboratory experiments using pieces of fibre-glass mesh impregnated with *I. pastinacae*, the fungus remained viable after 12 months burial in sterile soil, whereas it was not viable in natural soil after 7 months. In natural soil marked lysis of the mycelium of the fungus occurred in 2 weeks, leaving intact the more resistant chlamydospores.

Ballistospores, the air-borne stage of *I. pastinacae*, could not be recovered 2 days after being added to natural soil, whereas the recovery rate from sterile soil was high after 5 days.

Isolates of *Bacillus subtilis* and *Streptomyces* spp. from natural soil were shown to be antagonistic to *I. pastinacae*.

I. INTRODUCTION

Parsnip canker, characterized by large, black, dry sunken lesions on the crown of parsnip roots, was shown by Wilkinson (1952) to be caused by *Itersonilia* sp. Channon (1963*a*) demonstrated that, in England, the disease was caused mostly by *Itersonilia pastinacae* Channon but sometimes by *Phoma* sp. A similar situation exists in Victoria (Smith, unpublished data) where the initial record of the pathogen (as *Itersonilia* sp.) was made by Stubbs (personal communication).

Little is known of the epidemiology of the disease. Wilkinson (1952) and Chupp and Sherf (1960) considered that crown infection followed the discharge of *I. pastinacae* ballistospores from leaf lesions. This was supported by the findings of Channon (1963*b*) who reduced the incidence of the disease by removing the infected parsnip foliage and by covering the parsnip crowns with soil or peat, the latter treatments presumably preventing the ballistospores from reaching the roots.

Although Cotton (1918) suggested a 2-year rotation and Chupp and Sherf (1960) "a long rotation" between parsnip crops, these recommendations have no experimental basis as the survival of *I. pastinacae* in soil has not been studied. The work reported in this paper is concerned with this aspect of the epidemiology of parsnip canker.

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II. Materials and Methods

Viability of *I. pastinacae* was determined by the method of Wilkinson (1952), in which the material under test was held with petroleum jelly to the inside of the lid of a Petri dish and suspended over 2% water agar. After incubation for 5–7 days at 20°C, the discharge of ballistospores of *I. pastinacae* could then be microscopically detected on the surface of the agar.

III. Results

(a) *Survival of* I. pastinacae *in Naturally Infected Parsnip Roots Buried in Soil*

Cankered roots were collected from a number of properties in the Melbourne market garden area. Small pieces, about 5 mm^3, were removed from the canker on each root to check for the presence of *I. pastinacae*, using the method described above.

One hundred parsnip roots of uniform size infected with *I. pastinacae* were enclosed individually in 4 by 1 in^2 sleeves of 1-mm Terylene mesh open at each end to allow for subsequent growth. The stem apical meristem tissues were gouged out from each of 50 roots to simulate severe cultivation damage that would normally induce rapid decomposition. The two groups were buried in the field, the crown of each root being approximately 6 in. below the surface of the soil. Disking of the soil after harvest usually covers unmarketable infected roots to approximately this depth.

Fifty pieces of sterile fibre-glass mesh (1 in^2) were placed on the surface of potato dextrose agar (PDA) which was then inoculated with a pathogenic isolate of *I. pastinacae* from a parsnip root (Legge 1952). After 12–14 days, the fungal mycelium had completely impregnated the fibre-glass pieces which were then stripped from the agar surface and washed three times in sterile water to remove nutrients. The fungus-impregnated pieces of fibre glass were placed in Terylene sleeves and buried in the same way as the infected roots. The experimental site, which had grown vegetables for many years, was watered regularly but not cropped during the period of the experiment.

At monthly intervals, for 12 months, four roots were removed at random from each group, together with four pieces of inoculated fibre glass. The roots were gently washed in tap water and 16 pieces, *c.* 5 mm^3, were excised from each root and tested for viable *I. pastinacae*, as described above. The pieces of fibre glass were also washed in tap water and divided into four before testing for viable fungus.

For the first 4 months of the experiment the pieces of root tissue were excised directly from the actual canker. After this time, however, advanced root decomposition in the mutilated roots made cankers difficult to locate. Subsequent samples were therefore taken at random from each root. In the intact roots, rapid decomposition did not usually occur until after the completion of flowering, i.e. after 5–6 months.

Where the stem apical meristem had been removed from naturally infected parsnip roots buried in soil, *I. pastinacae* was gradually eliminated and could not

be recovered after 7 months (Fig. 1). The fungus also survived in soil for the same period on pieces of fibre glass. However, the fungus was still viable after burial for 12 months in intact roots, although the percentage of infected root pieces had fallen to a low level after 8 months.

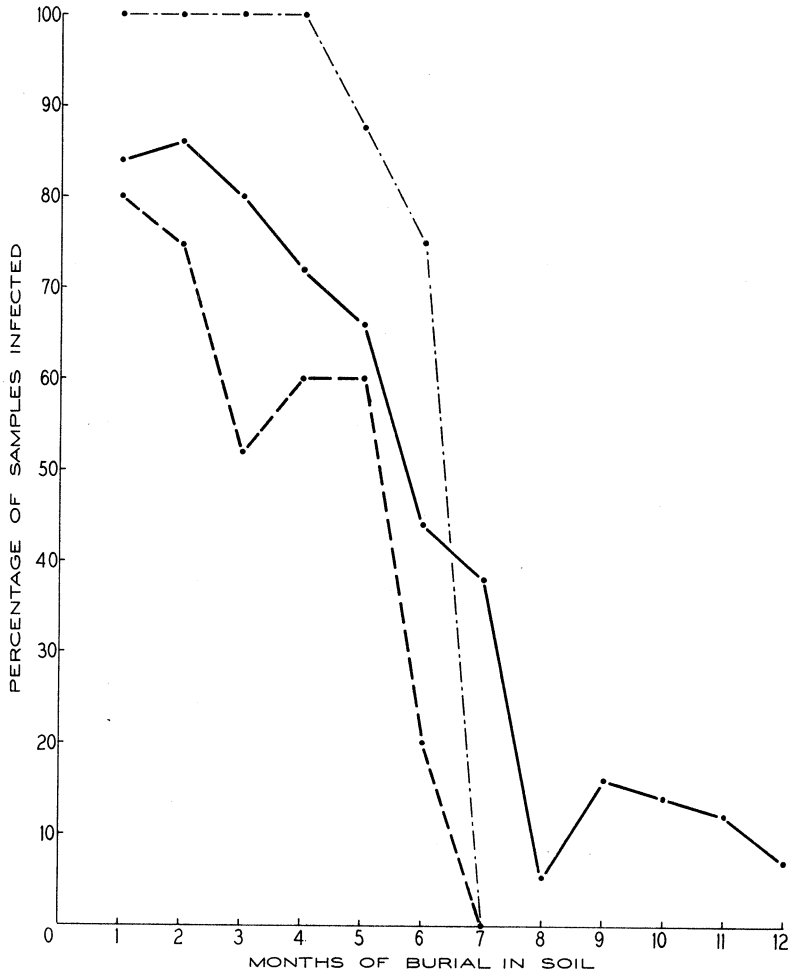

Fig. 1.—*In vivo* survival of *I. pastinacae* in naturally infected intact parsnip roots (●——●) and in naturally infected debudded parsnip roots (● - - - ●). Survival of *I. pastinacae* on fibre glass also shown (●-•-•-●).

(*b*) *Survival of* I. pastinacae *in the Soil in the Absence of Parsnip Tissue*

Soil used in these experiments was obtained from a vegetable farm at Braeside, Vic. The soil, which was typical of the area where parsnips are grown, was classed as a grey sandy loam with moderate organic matter. Soil moisture at field capacity was 27% and the pH was 6·2.

After sieving, 500-g samples of soil were placed in plugged Erlenmeyer flasks and the soil moisture adjusted to 80% of field capacity with sterile water. Five flasks were autoclaved three times on consecutive days while the other five were left untreated.

Fifty pieces of fibre-glass mesh, impregnated with *I. pastinacae* and prepared as described above, were buried in the soil in each flask and incubated for 12 months at room temperature, soil moisture adjustments being made every 2 weeks.

Each month, four pieces of fibre glass were removed from each flask, washed in sterile water to remove soil, and tested for fungal viability. Three pieces were suspended over water agar, as described above. The remaining piece was ground up in a Servall Omnimixer and diluted 1 : 500 with sterile water. Five 1-ml aliquots from this suspension were dispersed in Petri dishes in 10 ml of Martin's peptone dextrose agar (Martin 1950) containing 0·03% quintozene (MPDA-Q). After incubation for 10 days at 20°C, the numbers of *I. pastinacae* colonies per plate were counted.

TABLE 1

LONGEVITY OF *I. PASTINACAE* IN FIBRE-GLASS PIECES IN SOIL IN ABSENCE OF HOST TISSUE

Fungal viability detected by agar suspension method

Period of Incubation in Soil (months)	Mean No. of Fibre-glass Pieces Infected with *I. pastinacae*		Period of Incubation in Soil (months)	Mean No. of Fibre-glass Pieces Infected with *I. pastinacae*	
	Sterile Soil	Unsterile Soil		Sterile Soil	Unsterile Soil
1	3	3	7	3	0
2	3	3	8	3	0
3	3	3	9	3	0
4	3	3	10	2·2	0
5	3	3	11	1·6	0
6	3	1·6	12	1·4	0

In this experiment the fungus did not survive burial for 7 months in unsterile soil, whereas it remained viable for 12 months in sterile soil (Table 1). The suspension-over-agar method determined only the presence or absence of viable fungus in the fibre glass, but the dilution plate method gave a quantitative measure of fungal viability (Fig. 2). This later method demonstrated that, although *I. pastinacae* survived in unsterile soil for 6 months, colony counts indicated a rapid reduction of inoculum during the first 2 months.

In sterile soil, *I. pastinacae* remained viable for 12 months and colony counts were high at the end of this period (Fig. 2).

Microscopic examination of pieces of fibre glass incubated in sterile soil for 6 months did not reveal any visible change in fungal structure. However, in unsterile soil hyphal strands between individual glass fibres were completely lysed after 2 weeks, but chlamydospores remained intact (Plate 1, Fig. 1). Chlamydospores were detected each month until the sixth month, but not thereafter.

Chlamydospores incubated for 6–7 days at 100% relative humidity, following lysis of the mycelium in natural soil, were found to germinate directly to produce a short germ tube and a terminal ballistospore (Plate 1, Fig. 2).

SURVIVAL IN SOIL OF *ITERSONILIA PASTINACAE*

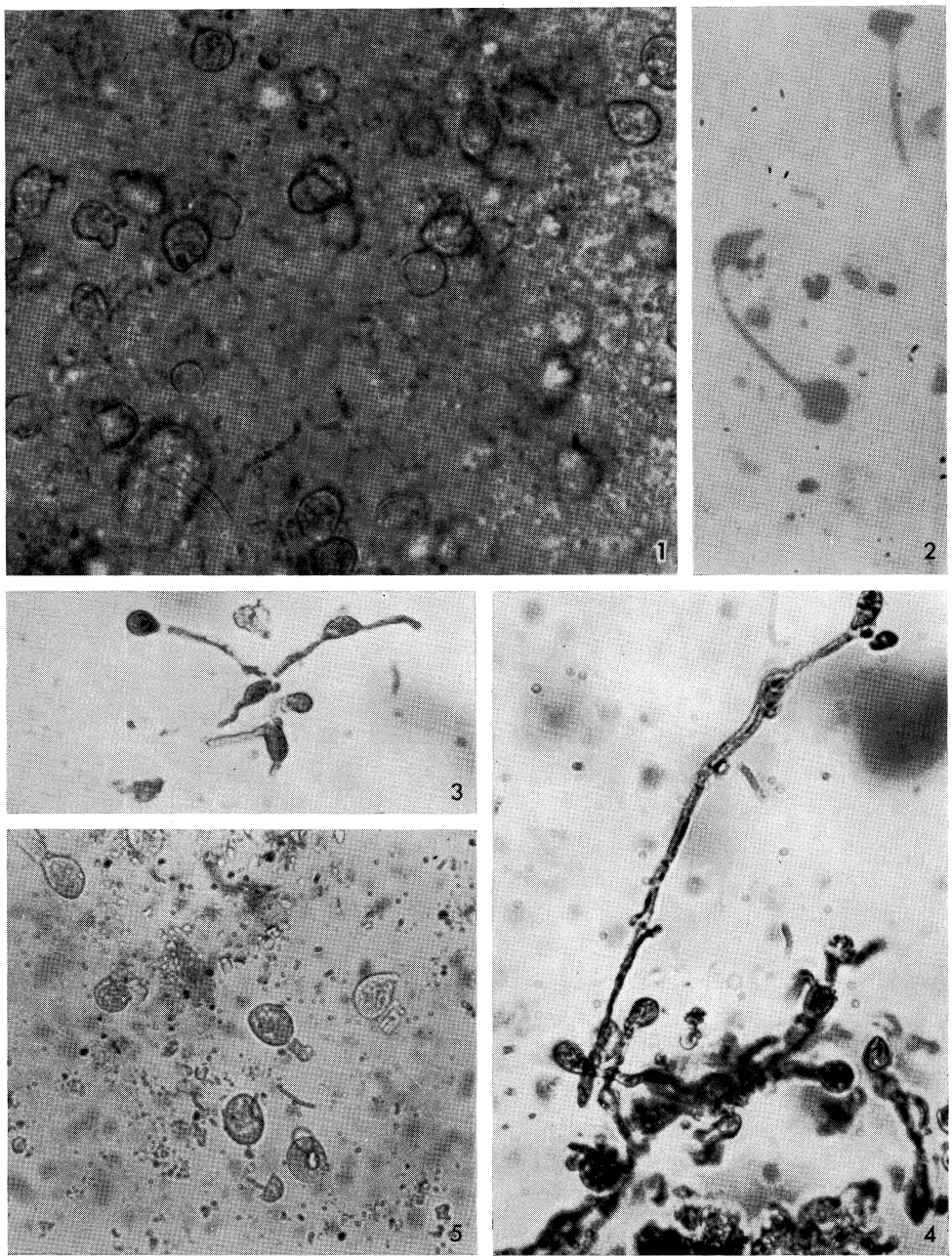

Fig. 1.—Chlamydospores of *I. pastinacae* remaining after incubation for 2 weeks in natural soil. Note complete lysis of mycelium and ballistospores.

Fig. 2.—Germination of chlamydospore of *I. pastinacae* to form a single ballistospore.

Figs. 3–5.—Ballistospores of *I. pastinacae* recovered by agar strips (direct assay method) after 6 hr (Fig. 3) and 48 hr (Fig. 4) in sterile soil and after 6 hr in natural soil (Fig. 5).

(c) *Survival of Ballistospores of* I. pastinacae *in Soil*

(i) *Soil Dilution Method*

Aliquots (10 ml) of a washed ballistospore suspension, containing $1{\cdot}2\times10^6$ spores per millilitre, were added to each of 10 plugged Erlenmeyer flasks, half of which contained 100 g of sterile soil and half unsterilized soil, as used in the previous experiment. The moisture content of the soil in each flask was adjusted to 80% of field capacity with sterile water. Immediately, and then on each of the five following days, 10 g of soil was removed from each flask and diluted 1 : 1000 with sterile water.

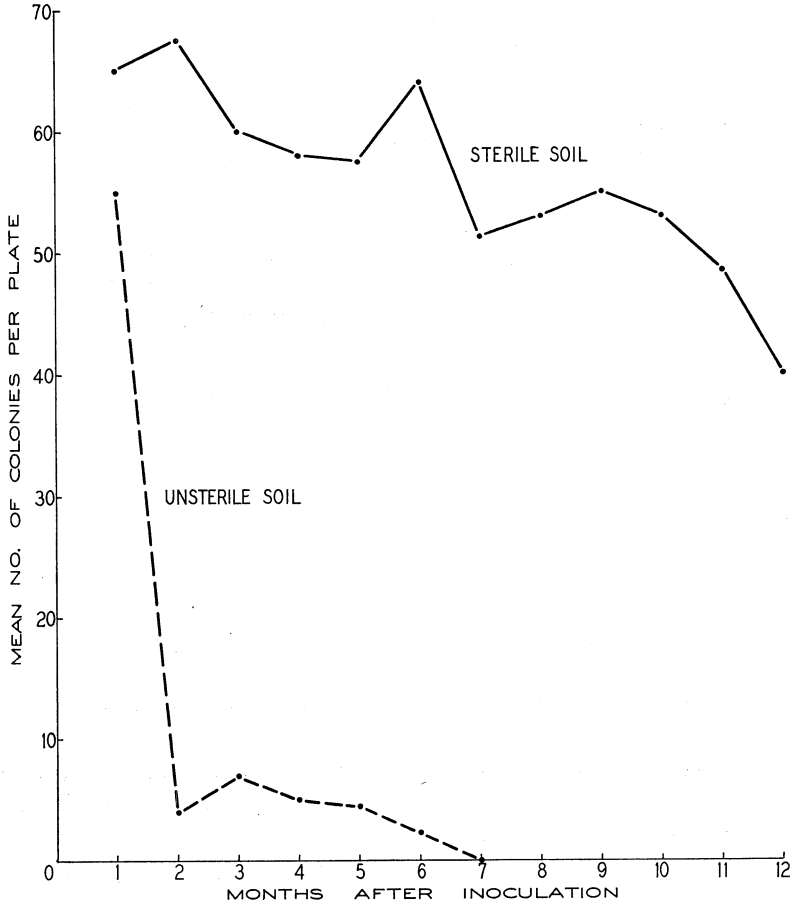

Fig. 2.—Survival of *I. pastinacae* in sterile and unsterile soil in absence of parsnip tissue. Fungus added to soil on pieces of fibre-glass mesh. Recovery assessed by soil dilution plate method.

Five 1-ml aliquots were then dispersed in 10 ml of MPDA-Q in Petri dishes. Two experiments were conducted and counts of *I. pastinacae* colonies were made after 10 days. These counts indicated that relatively high numbers of ballistospores survived in sterile soil for the duration of the experiment. There were 75% less ballistospores recovered from natural soil after 1 day and none was recovered after 2 days (Fig. 3).

(ii) *Direct Assay Method* (Lingappa and Lockwood 1963)

Thirty Petri dishes were filled to a depth of 6 mm with firmly compacted soil smoothed level and half were sterilized by autoclaving. Soil moisture was adjusted to 80% of field capacity with sterile water. Four to six drops of the ballistospore suspension, used above, were placed on the soil surface and the plates were incubated at 20°C. At intervals of 6, 24, and 48 hr after inoculation, the spores were stained and fixed *in situ* by drops of phenolic rose bengal (Waksman 1932) followed by drops of 3% water agar at 45–48°C applied to the inoculated sites. When

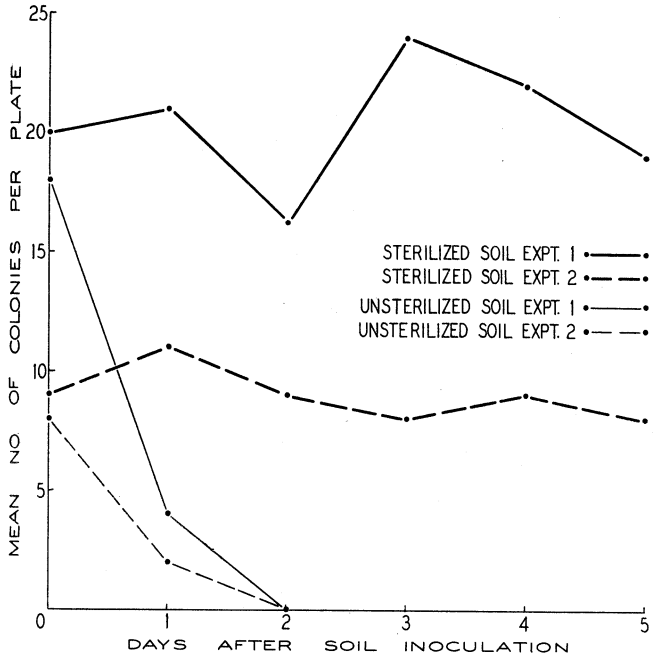

Fig. 3.—Recovery of ballistospores of *I. pastinacae* inoculated into sterile and unsterile soil. Recovery assessed by soil dilution plate method.

set, the agar was stripped from the soil surface, mounted in lactophenol, and examined microscopically for ballistospores. The numbers of ballistospores and germinated ballistospores per high-power field were counted, and germ tube lengths were measured. The results for 30 high-power field examinations from each site were averaged.

The results, summarized in Table 2, parallel those obtained by the soil dilution method. Ballistospores were recovered from sterile soil in significant numbers after 48 hr. Spore germination and hyphal growth were also vigorous at this time. Where ballistospores were inoculated onto unsterile soil, however, the rate of recovery was low even after 6 hr and none was recovered after 48 hr. Vegetative growth was also very limited. Ballistospores recovered from unsterile soil did not stain as deeply and were slightly larger (Plate 1, Fig. 5) than those obtained from sterile soil (Plate 1, Figs. 3 and 4).

(*d*) *Isolation from Soil of Organisms Antagonistic to* I. pastinacae

The results of the experiments described above have shown that *I. pastinacae* structures were lysed in natural soil. Attempts to demonstrate antagonism by soil microorganisms towards *I. pastinacae* by the direct methods of Herr (1959) and Williams and Kaufman (1962) were unsuccessful because the slow-growing *I. pastinacae* mycelium was rapidly overrun by faster-growing organisms so that no clear zones of inhibition were seen.

TABLE 2

RECOVERY OF BALLISTOSPORES OF *I. PASTINACAE* FROM SOIL BY A DIRECT ASSAY METHOD

Incubation Period (hr)	Type of Soil	Mean No. of Spores per High-power Field	Percentage Germination	Mean Germ Tube Length (μ)
6	Sterile	24	47	57
24	Sterile	27	69	109
48	Sterile	23	62	152
6	Unsterile	14	2	10
24	Unsterile	2	0	0
48	Unsterile	0	0	0

Soil microorganisms for testing for antagonism towards *I. pastinacae* were therefore randomly isolated as follows. A Potter tower (Potter 1952) was used to spray 0·5 ml of soil suspensions, diluted from 10^{-5} to 10^{-7} in sterile water, evenly over the exposed surfaces of Petri dishes containing the following media:

(1) 0·2% chitin agar (Lingappa and Lockwood 1962);

(2) 2·0% water agar (Lingappa and Lockwood 1962);

(3) Soil extract agar plus 0·1% yeast extract (Lockhead and Burton 1956);

(4) Ohio Agricultural Experiment Station (OAES) medium (Williams and Schmitthenner 1960);

(5) Nutrient agar (Park 1956): prior to inoculation onto this medium, 100-g samples of the soil were partially sterilized in sealed polyethylene containers by exposure to propylene oxide (1 ml per litre capacity of the container) for 24 hr. The soil was then incubated at room temperature for 14 days before the soil suspensions were prepared.

After incubation for 5–10 days, organisms from each medium were subcultured onto PDA slopes. The following isolates were tested for antagonism towards *I. pastinacae*:

	No. Tested
Fungi	
Aspergillus niger van Tieghem	2
Botrytis cinerea Pers. ex Fr.	1
Cladosporium cladosporioides (Fresen.) de Vries	1
Cladosporium sphaerospermum Penz.	1
Epicoccum nigrum Link.	1
Epicoccum purpurascens Ehrenb. ex Schlecht	1
Hemicola fuscoata Traaen	1
Penicillium brevicompactum Diecks	2
Penicillium meleagrinum Biourge	1
Penicillium nigricans (Bainier) Thom.	3
Phoma sp.	1
	15
Actinomycetes	
Streptomyces spp.	16
Bacteria	
Bacillus megaterium De Bary	7
Bacillus subtilis Cohn emend. Prazmowski	15
Bacillus spp.	3
	25

The following modification of the agar plate test described by Vasudeva and Roy (1950) and Park (1956) was used for antagonism tests. Plates containing PDA were inoculated in the centre with a 4-mm disk of *I. pastinacae* growing on PDA. Inoculum of the test organism was then placed on either side and 1 in. away from the disk of *I. pastinacae*. After 10 days, measurements of the diameter of the *I. pastinacae* colony were made along the axis joining the three inoculation sites and at right angles to this line. The inhibition of the growth of *I. pastinacae* was then calculated as follows:

$$\text{Percentage inhibition} = \frac{\text{long axis} - \text{short axis}}{\text{long axis}} \times 100.$$

Of the organisms tested, 12 of the 25 bacteria, 7 of the 16 actinomycetes, but none of the 15 fungi inhibited the growth of *I. pastinacae*. The organisms showing antagonsim are listed in Table 3. The strongest inhibition was caused by some of the isolates of *B. subtilis*.

(*e*) *Demonstration of Inhibitors in Cell-free Filtrates of Organisms Antagonistic to* I. pastinacae

Organisms showing antagonism towards *I. pastinacae* in the agar plate tests were inoculated into 100-ml of a liquid medium (Vasudeva, Jain, and Nema 1952). After 9 days, each culture was sterilized by passage through a Millipore filtering apparatus of pore size 0·45 μ. Five 1-ml aliquots of each filtrate were sterilized by autoclaving and five were untreated. Each 1-ml sample was dispersed in a Petri

dish with 10 ml of melted PDA and the plates were inoculated in the centre with a 4-mm disk of *I. pastinacae* growing on PDA. The diameters of the fungal colonies were measured every 3–4 days for 24 days.

Incorporation of cell-free filtrates into PDA did not prevent growth of *I. pastinacae*, but did reduce the rate of growth.

With *B. subtilis*, *B. megaterium*, and *Streptomyces* sp. the inhibitory effect was markedly reduced by autoclaving the filtrate, but with one isolate of *B. subtilis* (isolate 58), autoclaving had less effect. Filtrates of the other organisms did not reduce the growth of *I. pastinacae*.

TABLE 3

DEGREE OF ANTAGONISM TOWARDS *I. PASTINACAE* CAUSED BY SPECIES OF BACTERIA AND ACTINOMYCETES

Antagonism tested by the agar plate method

Organism	Isolate No.	Isolation Method	Percentage Inhibition
B. subtilis	C15	Nutrient agar	66
	58		58
	19.8/10		51
	19.8/12		50
B. megaterium	30.7/10	Nutrient agar	54
	19.8/13		47
Bacillus sp.	19.8/2	Nutrient agar	37
	19.8/7	Soil extract agar	36
	19.8/9	Nutrient agar	41
	19.8/20	Nutrient agar	38
	30.7/5	Soil extract agar	33
	30.7/8	Soil extract agar	37
Streptomyces sp.	19.8/6	Chitin agar	42
	19.8/7		42
	C1		44
Actinomycete	19.8/3	Water agar	36
	19.8/19		40
	19.8/18		35
	19.8/14		39

(*f*) *Action of Antagonistic Filtrates on* I. pastinacae *Fungal Structures*

Aliquots (5 ml) of each cell-free filtrate of *B. subtilis*, *B. megaterium*, and *Streptomyces* sp., prepared as described above, were placed in 10 test tubes, of which five were autoclaved. A 3-mm disk of *I. pastinacae* growing on PDA was added to each filtrate for 14 days and then examined microscopically for lysis of *I. pastinacae* mycelium and spores.

Filtrates of *B. subtilis* produced moderate lysis of the mycelium, complete lysis of the ballistospores, and partial collapse of some of the walls of the

chlamydospores (Table 4). The filtrates of *B. megaterium* did not lyse any structure while *Streptomyces* sp. filtrates had a strong lytic effect on all types of fungal structures except the chlamydospores. Autoclaving the filtrates destroyed the lytic effect in all six isolates.

(*g*) *Lysis of* I. pastinacae *Structures by Antagonists Introduced into Sterile Soil*

The lytic effects of the same six antagonists were also investigated in soil. To 80-g lots of soil sterilized by autoclaving in 250-ml Erlenmeyer flasks were added 5-ml suspensions in sterile water of 10-day-old PDA cultures of the antagonists. After adjusting soil moisture to 80% of field capacity with sterile water, the flasks were incubated for 14 days at 20°C. Each flask was then inoculated with two pieces of fibre glass impregnated with *I. pastinacae* and incubated for a further 21 days.

TABLE 4

EFFECTS OF CELL-FREE FILTRATES OF ANTAGONISTS ON LYSIS OF *I. PASTINACAE* STRUCTURES 14 DAYS AFTER ADDITION OF FUNGAL DISKS

+++, strong lysis; ++, moderate lysis; +, slight lysis; 0, no lysis

Antagonist	Degree of Lysis of:		
	Mycelium	Ballistospores	Chlamydospores
B. subtilis (C15)	++	+++	0
B. subtilis (58)	++	+++	0+
B. megaterium (30.7/10)	0	0	0
B. megaterium (19.8/13)	0	0	0
Streptomyces sp. (C1)	+++	+++	0
Streptomyces sp. (19.8/7)	+++	+++	0

Microscopic examination of the fungal structures, after washing the pieces of fibre glass in water, showed that *B. subtilis* and *Streptomyces* sp. caused marked lysis of both mycelium and ballistospores whereas *B. megaterium* was not lytic towards *I. pastinacae* in this test (Table 5).

(*h*) *Inability of* I. pastinacae *to Invade Parsnip Roots in Natural Soil*

Twenty-four 200-g samples of air-dried soil were placed in 2-in. diameter plastic beakers. The soil in 12 samples was enriched with 2% by weight of cornmeal. Four-weeks-old unshaken cultures of *I. pastinacae* in potato dextrose broth were washed in running tap water for 15 min to remove nutrients. The mycelial mats were then macerated with a small volume of water in a Servall Omnimixer for 1 min to obtain a highly concentrated suspension of mycelium and chlamydospores. 30 ml of this suspension was then thoroughly mixed with the soil in each beaker. Soil moisture was adjusted to 80% of field capacity with tap water and polyethylene covers placed over the top of each beaker to conserve moisture.

Ten parsnip seedling roots approximately 5 cm long were buried in the soil in each beaker and incubated at 20°C. The seedlings were grown from seed treated with an air-stream mixture at 45·5°C for 30 min to eliminate seed-borne infection with *I. pastinacae* (Smith 1966). After 21 days the roots were removed and replaced by a second batch of roots of similar size.

TABLE 5

EFFECTS OF ANTAGONISTS INTRODUCED TO STERILE SOIL ON LYSIS OF STRUCTURES OF *I. PASTINACAE*

Inoculated flasks incubated at 20°C for 14 days. Each flask then inoculated with *I. pastinacae* and incubated for a further 21 days before degree of lysis assessed. +++, strong lysis; ++, moderate lysis; +, slight lysis; 0, no lysis

Antagonist	Degree of Lysis of:		
	Mycelium	Ballistospores	Chlamydospores
B. subtilis (C15)	++	+++	0
B. subtilis (58)	+++	+++	+
B. megaterium (30.7/10)	0	0	0
B. megaterium (19.8/13)	+	+	0
Streptomyces sp. (C1)	+++	+++	+
Streptomyces sp. (19.8/7)	+++	+++	+

Although the roots were living when placed in the soil, they did not resume growth and decomposition caused by organisms other than *I. pastinacae* commenced during the experiment. To detect infection by the latter, therefore, roots were washed and surface-sterilized in 0·5% sodium hypochlorite for 1 min, and then suspended over agar for ballistospore discharge. The results show that 8 out of the 120 roots placed in the untreated soil at the same time as the fungus and similarly 15 out of 120 roots placed in the enriched soil became infected, but infection was not detected in roots added to the same soils 21 days later.

IV. DISCUSSION

Chlamydospore germination of *I. pastinacae* clarifies the taxonomy of *Itersonilia* to some extent. Brady (1960) excluded *I. perplexans* Derx and *Tilletiopsis washingtonensis* Nyland from both *Entyloma calendulae* (Oudem.) de Bary and *E. dahliae* Syd. This study supports her conclusion that *Itersonilia* is a valid genus, as *I. pastinacae* chlamydospores do not germinate in the manner described for *Entyloma*.

Sowell and Korf (1960) considered *Tilletiopsis*, *Sporidiobolus*, and *Itersonilia* were synonyms. The germination process described above for *I. pastinacae*, however, does not agree with that described for either *Sporidiobolus* (Nyland 1948) or *Tilletiopsis* (Nyland 1950).

As Brady (1960) found *Itersonilia* and *Tilletiopsis* on lesions caused by *Entyloma*, it is possible that Stempell's (1935) "Myzel 11" isolate of *E. calendulae* contained also *Entyloma* instead of only *Itersonilia*, as suggested by Derx (1948), Nyland (1949) and Sowell and Korf (1960). Stempell (1935) described atypical *Entyloma* chlamydospore germination to produce a thin-walled secondary chlamydospore. The presence of chlamydospores of both *Itersonilia* and *Entyloma* in Stampell's culture would explain the two types of germination he observed as, in this study, *I. pastinacae* chlamydospores occasionally produced globose ballistospores, instead of the typical reniform-lunate ballistospores, particularly if viewed in the dorsal plane.

The results described now clarify some aspects of soil carry over of the fungus and their implications in the control of the disease. Infected parsnip roots obviously are an important source of carry over, in addition to infected seed (Smith 1966), unless the roots decompose rapidly and thereby expose the fungus to the lytic action of soil microorganisms. When infected roots were mutilated and placed in the soil, the survival time of the fungus was reduced to the same length as that of cultures of the organism added to soil on pieces of fibre glass. On the fibre glass the ballistospores and mycelium were rapidly lysed while the more resistant chlamydospores survived for 6 months. Chlamydospores have been found in infected parsnip roots (Smith, unpublished data) and they must be regarded as an important structure in the survival and epidemiology of the fungus.

However, infected roots must be exposed on the soil surface to act as a source of inoculum, otherwise any ballistospores discharged would be rapidly lysed. The location of the inoculum is important because *I. pastinacae* makes very limited vegetative growth through natural soil, even to colonize a suitable substrate such as parsnip root debris.

The rapid death of *I. pastinacae* propagules in natural soil is probably due to microbial antagonism, as lysis of the fungus does not occur in partially sterilized soil. Also microorganisms possessing antagonistic and lytic action towards *I. pastinacae* can be isolated from natural soil.

Although both living isolates added directly to sterile soil and cell-free culture filtrates of *B. subtilis* and *Streptomyces* sp. lysed *I. pastinacae* structures, the mechanisms of lysis was not determined. Lloyd and Lockwood (1966) suggest that, through competition between soil microorganisms, a state of nutrient starvation occurs which, coupled with antibiotic production by soil microorganisms, causes rapid autolysis of mycelium. It is possible that a similar pattern occurs with mycelium and ballistospores of *I. pastinacae*. Alternatively, cell-wall digesting enzymes produced by soil microorganisms cause heterolysis of fungal propagules (Mitchell and Alexander 1963). The lysis of *I. pastinacae* by cell-free culture filtrates supports this theory. Furthermore, the lytic effect of cell-free filtrates was destroyed by heat while the fungistatic effect of incorporating these filtrates into agar was, with one exception, also destroyed by heat. Enzymatic activity is destroyed by heat (Horikoshi and Iida 1958), although a range of heat-stable antibiotics have been reported (Foster and Woodruff 1946; Landy *et al.* 1948). It is possible that, with *I. pastinacae*, both types of mycolysis are implicated but the present research does not elucidate this problem. As concluded by Lloyd and Lockwood (1966), further work is required

to determine whether extracellular hydrolytic enzymes produced by soil microorganisms lyse cell walls of living fungi.

The results described have a practical value in demonstrating the need to alter cultural practices to achieve greater control of parsnip canker. In Melbourne vegetable farms, parsnips are grown in raised beds and *Itersonilia*-infected roots left in furrows after harvest are partially buried by subsequent ploughing. Removal and destruction of cankered roots at harvest would eliminate the source of long-term carry over of the fungus. Infected leaves and other parsnip debris would more readily decompose with rapid lysis of the fungus.

The rapid lysis of ballistospores in soil explains the control of parsnip canker obtained by covering the parsnip crowns with soil (Channon 1963*b*; Smith, unpublished data). Apparently soil acts as a very efficient biological barrier to ballistospores rather than a purely physical barrier as suggested by Channon (1963*b*).

V. Acknowledgments

The author wishes to thank Mr. M. P. Gellert for the photographs and graphs. Thanks are also due to the Commonwealth Mycological Institute, Kew, England, for the identification of the soil microorganisms.

VI. References

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