AUTECOLOGICAL STUDIES ON PENICILLIUM EXPANSUM

by

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To

My dear uncle, Mr. Sawali Khan Zardari

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Summary

This thesis reports an autecological study on an apple rotting isolate of Penicillium expansum. The study was concerned with the survival and growth of the fungus in the soil, in the rhizospheres of apple, broad bean, maize and tomato, and in plant litter.

P. expansum spores did not germinate in natural soil but could remain viable (68%) for up to one year at least. Spores germinated and developed to form conidia microcyclically in soil sterilized by autoclaving.

Spores germinated in natural soil after the addition of nutrients, in the form of malt extract broth, indicating that nutrient deficiency might be one factor determining spore dormancy. Evidence was also obtained to show that non-volatile diffusible inhibitors of spore germination were present in natural soil.

Heat treatment of natural soil at 80°C destroyed the inhibitory effect whereas treatment at 60°C had no effect although it destroyed most fungi and bacteria isolatable on Malt extract agar or Modified Hutchinson's agar. If nutrient deficiency were the inhibitory factor the release of nutrients from the micro-organisms killed by treatment at 60°C should have stimulated germination. Thus the activities of other micro-organisms in the soil, probably through the heat labile inhibitors they produced, were the main factors inhibiting germination. Some of the fungi which survived at 60°C for 6h were inhibitory to the growth of P. expansum in culture.

The experiments on the survival of spores and of spore germination and growth in the rhizospheres of apple, broad bean, maize and tomato showed that spores could germinate and the fungus could grow in the rhizospheres of all three plant species. P. expansum was observed to grow occasionally closely attached to the root surfaces of broad bean, maize and tomato. It was never observed to invade the internal root tissues of tomato. The other two species were not investigated for the colonization of the internal tissue.

The removal of the inhibition of spore germination in the rhizospheres

could have been due to the effect of root exudates either serving as nutrients or as inactivators of inhibitory factors.

The presence of P. expansum in the rhizospheres of tomato plants can affect plant growth to a limited extent. Inoculum in the rhizosphere slightly reduced stem height and total leaf area but had no effect on total dry matter, the number of leaves or the development of reproductive structures.

Colonization by P. expansum of root and leaf litter from apple in the soil in the presence of other soil micro-organisms was very low. However it could survive in apple root tissues if the apple root was colonized before addition to the soil.

CHAPTER I

GENERAL INTRODUCTION

Penicillium expansum Link ex. S.F. Gray is tne oldest described species of the genus Penicillium, being first described by Link (1809) in his "Observation in ordines plantarum naturales". Link first reported P. expansum to be the species which commonly rots apples in store, and work over the years has established that it is responsible for 80 to 90% of the losses of apple fruit in transit and in store (Brooks et al., 1920). Heald and Ruehle (1931) found that it was capable of rotting apples twice as fast as any other fungal species. More recently Swinburne (1970) reported that it was responsible for most losses in refrigerated gas storage plants. It has also been reported to rot other pomaceous fruits including pears (Lovisolo, 1957; Borecka, 1977) and quinces (Christoff & Christova, 1939; Horst, 1979).

However, P. expansum has a much wider host range than pomaceous (Rosaceae) fruits, having been isolated from fruits rotting either in transit or during storage of many other plant families including grapes (Vitaceae), passion fruits (Passifloraceae), pecans (Juglandaceae), avocados (Lauraceae), persimmons (Ebenaceae) and feijoas (Myrtaceae) (Thom, 1930; Sprague, 1953; Pruthi, Srivastava & Lal, 1958; Huang & Hanlin, 1975; Horst, 1979).

Thus it appears to be a weak parasite of a wide range of plant reproductive structures but it does show a certain degree of specificity. For example, in fruit stores where apple and citrus fruits are present together, it only rots apple fruits, being apparently unable to colonize citrus fruits to any extent. Two other species of Penicillium, P. digitatum and P. italicum are responsible for the soft rots of citrus fruits in store (Rapor & Thom, 1949; Horst, 1979).

There are also many reports that indicate that P. expansum may occur widely in soils. Thus it has been isolated from a wide range of soils throughout the world, extending from arctic regions to the subtropics, and from sea level to high alpine soils (Gilman, 1957; Domsch & Gams, 1972; Domsch, Gams &

Anderson, 1980). These soils include many different types and textures from sandy soils through loams to heavy clays (Waksman, 1916; Abbott, 1923; Apinis, 1964; Joffe, 1967), of different reactions from acidic to alkaline (Waksman, 1916; LeClerg, 1931; McLennan and Ducker, 1952; Widden, 1979), and of different salinities (LeClerg & Smith, 1928). It has been recovered from such soils at a range of depths from the surface down to 42" (LeClerg & Smith, 1928).

Its presence in soils is presumably in association with living or dead vegetation, but there is little evidence to indicate that such associations are specific. Thus it has been found under many different types of vegetation including tundra, grassland, and many forest types, and under a wide range of crops including potato, wheat, corn, clover and sugar beet (Cooke & Fournelle, 1960; Thornton, 1958; McLennan & Ducker, 1954; Widden, 1979; Waksman, 1916, 1917; LeClerg, 1931). In these situations it has been reported from both freshly fallen and decomposing leaf litters. Thus Kendrick (1963) isolated it from a litter layer composed of tightly packed, often fragmentary, flattened needles of Pinus sylvestris, and Brandsberg (1969) isolated it from the duff of Abies grandis and Pinus monticola.

Other reports indicate that it is present in soil in association with root systems (Domsch & Gams, 1972; Domsch, Gams & Anderson, 1980).

Apart from terrestrial habitats it has occasionally been isolated from aquatic environments (Cooke, 1968; Park, 1972).

The identification of P. expansum in most of these studies has been based on morphological characteristics only. Very few workers have used pathogenicity as a criterion.

According to Raper and Thom (1949) and Samson, Stolk and Hadlok (1976), P. expansum forms rapidly growing colonies on malt extract agar and Czapek agar, attaining a diameter of about 4-5 cm in 8-14 days when grown at about 25°C. The colonies are generally white at first but shading quickly through yellow to blue-green, near celandine green or sage green with the ripening of the conidia. Colonies smell strongly "mouldy", suggestive of rotten apples (Raper & Thom, 1949) or aromatic fruity, suggestive of apples (Samson, Stolk & Hadlok, 1976).

Exudates are absent, or may be present as colourless drops which are partially embedded in the conidial mass. The reverse of the colony is colourless or yellowish to yellow brown.

Conidiophores are commonly mononematous, but in fresh isolates they may be typically loosely synnematous, especially in the marginal areas. Very rarely are they distinctly synnematous in culture. Conidiophores are long, often up to 500 µm or more in length, with smooth walls or walls finely roughened; this latter feature is more pronounced on colonies grown on malt extract agar. Two to three branches develop close to the apex. Three to six more or less cylindrical metulae (10-15 x 2.2-3.0 µm) develop at the apex of each branch, each metula bearing five to eight phialides. The phialides are cylindrical with a short but distinct neck (8-12 x 2.0-3.5 µm) forming a chain of smooth-walled greenish elliptic to subglobose conidia (3.0-3.5 x 2.5-3.0 µm).

Raper and Thom (1949) grouped P. expansum together with P. crustosum in the P. expansum series of the section Asymmetrica, subsection Fasciculata. Penicillium crustosum differs from P. expansum in having shorter conidiophores with rough walls and conidia often forming a distinct crust on the colony.

Samson, Stolk and Hadlok (1976) rearranged the subsection Fasciculata as defined by Raper and Thom (1949) and indicated that both P. granulatum and P. verrucosum showed similarities with P. expansum. They are differentiated on the growth rates of colonies and on the lengths of their conidiophores. Penicillium granulatum grows to form a colony 2.5 to 3 cm in diameter within 7 to 14 days on Czapek agar and produces conidiophores which measure 100-200 x 3.5-4.5 µm. In contrast P. verrucosum forms a more restricted colony or spreads broadly on Czapek agar, producing conidiophores 100-400 x 3.0-4.0 µm.

Pitt (1979) also indicates relationships between P. crustosum, P. viridicatum and P. expansum on the basis of colony morphology and colour. Samson, Stolk and Hadlok (1976) considered P. crustosum to be the same as P. verrucosum Dierckxvar. cyclopium and P. viridicatum to be identical with P. verrucosum Dierckxvar. verrucosum. Pitt also indicatedthat certain microscopical features of P. expansum indicate close affinities with P. chrysogenum

because the conidiophores of P. chrysogenum are terverticillate and smooth-walled and thus very similar to those of P. expansum.

Thus the identification of P. expansum may present problems to researchers not specifically skilled in the identification of Penicillium species, so that isolates of Penicillium species obtained from the soils may have been wrongly identified as P. expansum. It would have helped if all workers had included a simple pathogenicity test on apple, just as McLennan and Ducker (1952) did, since only true P. expansum has the ability to cause a rapid soft rot in this fruit.

Thus although it is clear that P. expansum is a widespread soft rotting parasite of certain plant products, particularly apple, it is not clear how widespread it is, or how effective it is, as a colonizer of dead and decaying plant material or of plant products in soil. Without the pathogenicity test it is not possible to conclude that fungi identified as P. expansum from soil or soil litter, are in fact the fruit rotting species. There is also no evidence to indicate whether the isolations were derived from resting structures originally produced on rotting fruits which then arrived in the soil by chance dispersal, or from mycelia or resting structures produced while the fungus was actively colonizing plant litter in the soil. This study attempts to determine if a fruit rotting isolate of P. expansum can grow saprophytically in soil, either in the rhizosphere in association with living plant roots or in plant litter.

CHAPTER II

GENERAL MATERIALS AND METHODS

2.1. Fungus cultures

All cultures were obtained from the Commonwealth Mycological Institute.

Penicillium expansum Link ex S.F. Gray (CMI 39761) is a pathogen of apples and this isolate was originally obtained from apples in 1926. The pathogenicity of this isolate for apple was confirmed at the start of the work.

P. digitatum Saccardo (CMI 91956) is a pathogen of citrus fruits and this isolate was originally obtained from oranges in Scotland in 1926.

P. brevicompactum Dierckx (CMI 17456) of unknown origin. It is a common saprophytic soil-borne fungus occurring in decaying vegetation in many areas.

2.2. Maintenance of cultures

All cultures were maintained on malt extract agar slopes in 1 oz screw top bottles. Subculturing was generally by "mass transfer". All cultures were stored at 4 to 5°C in a refrigerator or cold room when not in use.

2.3. Media

All the media listed below were autoclaved at 120°C for 20 mins except for the selective malt extract medium, which was autoclaved at 120°C for 30 mins. Streptomycin was added to all media, except the Modified Hutchinson's Agar medium, to inhibit bacterial growth.

(i) Malt Extract Agar

Sucrose	20 g
Malt extract	20 g
Peptone	1 g
Streptomycin	0.5 g
Agar	25 g
Water (distilled)	1000 ml

(ii) Potato Dextrose Agar

Glucose	20 g
Potatoes (peeled)	20g
Streptomycin	0.5 g
Agar	17 g
Water (distilled)	1000 ml

(iii) Czapek Dox Agar

Sucrose	30 g
$NaNO_3$	3 g
K_2HPO_4	1 g
$MgSO_4\ 7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4\ 7H_2O$	0.01 g
Streptomycin	0.5 g
Agar	15 g
Water (distilled)	1000 ml

(iv) Peptone agar

Peptone	5 g
KH_2PO_4	1 g
$MgSO_4\ 7H_2O$	0.5 g
Streptomycin	0.5 g
Agar	20 g
Water (distilled)	1000 ml

(v) Lima Bean Agar

Ground lima beans	100 g
Streptomycin	0.5 g
Agar	17 g
Water (distilled)	1000 ml

The lima beans were soaked in 1000 ml distilled water for 30 minutes and then steamed for 30 minutes. The material was filtered through cheesecloth and the remaining liquid squeezed out. Distilled water was added to bring up to volume before adding the agar.

(vi) Selective Malt extract Agar medium

Sucrose	1000 g
Malt extract	20 g
Peptone	1 g
Streptomycin	0.5 g
Agar	25 g
Water (distilled)	1000 ml

(vii) Modified Hutchinson's Agar

Glucose	10 g
K_2HPO_4	0.5 g
$MgSO_4 \cdot 7H_2O$	0.2 g
$K NO_3$	0.05 g
Peptone	0.05 g
Agar	20 g
Water (distilled)	1000 ml

As this medium was used for the isolation and culturing of bacteria, streptomycin was not included.

2.4. Spore Suspensions

Spore suspensions were washed from 7 to 15 day old cultures grown on malt extract slopes at 23°C using sterile distilled water plus Tween 80 (two drops of Tween 80 to 500 ml distilled water). The numbers of spores were counted using a Bürker haemocytometer.

2.5. Collection and treatment of soil

The soil used in all experiments was collected at approximately 0-5" depth from the Department Experimental Gardens, Garscube. Prior to use it was dried at room temperature and sieved through a 2 to 4 mm mesh. The sieved soil was sterilized by autoclaving at 120°C for two hours in autoclave bags when required. Its mechanical and chemical analysis is described in Chapter III.

2.6. Sterilization of pots

The pots used in all experiments were first cleaned by washing with tap water and then sterilized by placing in 10% chloros (A.J. Beveridge Ltd.) solution for 24 hrs in a plastic container. After 24 hrs the pots were rewashed with tap water to remove the chloros solution and allowed to dry on the laboratory

bench before using.

2.7. Development of the selective medium

2.7.1. Preliminary experiments

In preliminary attempts to isolate P. expansum from soil the following media were used:- Malt extract agar, Potato-Dextrose Agar, Czapek-Dox Agar, Peptone Agar and Lima Bean Agar. The soil samples were collected from gardens in the vicinity of the Botany Department Glasgow and from the Departmental Experimental Gardens, Garscube. 5g of soil was added to 250 ml distilled sterilized water containing 3 drops of Tween 80 in a 500 ml flask and placed on a Griffin flask shaker for half-an-hour to disperse soil and fungal propagules. A dilution series 1:10, 1:100 and 1:1000 was prepared in distilled water containing Tween 80 (3 drops 250 ml^{-1}).

0.25 ml from each dilution was spread over the surface of plates of each medium and the plates then incubated at 23°C for 7 to 15 days.

On all media fast growing fungi rapidly overgrew the surface of the plates making it difficult to detect, let alone isolate, colonies of P. expansum or of any other Penicillium species that might have been present. Thus it was necessary to develop a selective medium which suppressed the growth of most fungi but supported the growth of Penicillium species, and particularly the growth of P. expansum.

2.7.2. The use of low water potential as the basis of a selective media

2.7.2.1. Introduction

Snow (1949) reported that Penicillium species can grow over a wider range of humidities than most fungi growing from 100% down to nearly 80% relative humidity. The optimum relative humidity for development and formation of conidia of P. expansum was between 95 and 97% with the minimum being within the

range 82 to 85% (Panasenko, 1967; Mislivec & Tuite, 1970; Galloway, 1935). P. expansum can thus grow at lower water potentials than many other fungi and so an attempt was made to develop a selective medium using low water potential as the selective factor. The reduction of water potential can be achieved by increasing the concentration of various salts or compounds in the medium, and in this work sucrose was used to produce media with different water potentials.

2.7.2.2. Methods:- Experiment I

Sucrose concentrations of 20, 30, 40, 60, 80 and 100 g l^{-1} were added to malt extract agar, potato-dextrose agar and Czapek-Dox agar.

An inoculum, prepared from one gram of soil containing 1.95×10^5 P. expansum spores dispersed in 250 ml sterilized distilled water containing Tween 80, was used to inoculate the plates. When the colonies were clearly visible to the naked eye, usually between 7 to 15 days after inoculation, they were counted.

2.7.2.2.1. Results and Discussion

The results are presented in Table 1. At all sucrose concentrations Penicillium species including P. expansum grew more rapidly than other fungi. Most Penicillium colonies were produced on the medium based on the malt extract agar. Media containing 160 g l^{-1} of sucrose appeared to support the fewest colonies of species from genera other than Penicillium and so this experiment indicated that higher concentrations of sugar might provide a selective medium for Penicillium.

2.7.2.3. Methods:- Experiment II

In this experiment malt extract media containing concentrations of sucrose up to 2100 g l^{-1} and glucose at concentrations up to 1250 g l^{-1} were tested. The inoculum was prepared and plates inoculated and incubated as described above.

Table 1. The effect of supplementing malt extract agar with increasing concentrations of sucrose on its value as a selective medium for isolating P. expansum from soil.

Sucrose/Litter		20g	30g	40g	60g	80g	160g
Mean number of colonies of all Penicillium species/plate.	Malt extract Agar	30.0	34.6	34.6	34.6	37.8	37.0
	Potato-Dextrose Agar	31.6	30.8	23.6	28.0	28.6	34.8
	Czapek-Dox Agar	30.2	32.4	27.0	32.6	36.4	35.2
Mean number of colonies of P. expansum/plate	Malt extract Agar	20.8	18.9	20.8	20.8	25.4	22.6
	Potato-Dextrose Agar	10.6	15.4	14.2	16.8	16.6	21.0
	Czapek-Dox Agar	14.2	21.0	17.0	23.0	22.2	18.8
Mean number of colonies of species of other fungal genera/plate.	Malt extract Agar	2.0	2.4	2.4	3.4	4.2	1.6
	Potato-Dextrose Agar	1.6	1.6	1.4	2.4	2.2	1.0
	Czapek-Dox Agar	0	0	0.4	0.2	0.2	0

Table 2. The effect of supplementing malt extract agar with increasing concentrations of sucrose on its value as a selective medium for isolating *P. expansum* from soil.

Sucrose/Litter	20g	600g	800g	1000g	1250g	1500g	1700g	1800g	2100g
Mean number of colonies of all *Penicillium* species/plate.	22.4	21.2	17.8	19.2	17.4	13.0	Colonies only. Restricted growth, few.	Colonies only. Restricted growth, few.	0
Mean number of colonies of *P. expansum*/plate.	15.2	13.0	11.2	10.6	11.6	7.8			0
Mean number of colonies of species from other fungal genera/plate.	4.6	1.2	0.8	0.2	0.4	0.2			0

Table 3. The effect of supplementing malt extract agar with increasing concentrations of glucose on its value as a selective medium for isolating P. expansum from soil.

Dextrose/litter	20g	600g	800g	1000g	1250g
Mean number of colonies of all Penicillium species/plate.	21.4	21.4	18.0	16.2	0
Mean number of colonies of P. expansum/plate.	14.4	13.6	11.6	9.4	0
Mean number of colonies of species from other fungal genera/plate.	6.2	0.8	0	0.2	0

2.7.2.3.1. Results and Discussion

The results given in Tables 2 and 3 show that the optimum concentrations of sucrose or glucose which allowed P. expansum to grow yet inhibited most other fungi was about 1000g l^{-1}. This gives a medium with a water potential of about -100 bar.

2.7.3. The use of the fungicide phenylmercuric acetate as the basis of a selective medium.

2.7.3.1. Introduction

Greenway, Cripps and Ward (1974) reported that P. expansum is resistant to the broad spectrum fungicide (phenylmercuric acetate) and will thus grow on media containing it. Therefore the possibility of developing a selective medium based on phenylmercuric acetate was considered.

2.7.3.2. Methods

In this experiment different amounts of phenylmercuric acetate (2.5, 5, 10, 20 and 40 μM l^{-1}) were added to malt extract agar after autoclaving at 120°C for 20 mins. In order to test the suitability of these media for the growth of P. expansum, P. expansum spores were added to the soil from which the inoculum was prepared. 10g of soil from the experimental gardens at Garscube were added to a 250ml flask containing 100ml sterile distilled water and 2 drops of Tween 80. Then 3.2 x 10^3 spores of P. expansum were added to the flask and dispersed by shaking. A dilution series was plated out and the plates were incubated at 23°C. P. expansum mycelial agar discs were also inoculated onto the malt extract media containing different concentrations of phenylmercuric acetate.

Table 4. Growth of P. expansum on malt extract medium containing phenylmercuric acetate.

Phenylmercuric acetate $\mu M\ l^{-1}$	COLONY DIAMETER (cm)					
	Replicates					
	1	2	3	4	5	Mean ± S.E.
0	7.45*	7.4	7.35	7.5		7.43 ± 0.032
2.5	3.9	3.8	4.15	4.2	4.5	4.11 ± 0.123
5.0	4.0	3.95	4.15	3.95	3.85	3.98 ± 0.049
10.0	1.45	1.15	1.1	1.55		1.31 ± 0.111
20.0	0.6	0.55	0.6			0.58 ± 0.016
40.0	0	0	0	0	0	0

* After 9 days

2.7.3.3. Results and Discussion

Very few P. expansum colonies had developed after 7 to 15 days, even on the medium containing the lowest concentration of phenylmercuric acetate. However, when mycelial discs were used as the inoculum P. expansum grew on concentrations up to 20 μM l^{-1} (Table 4). Thus the medium seemed to inhibit spore germination more than mycelial growth, and so phenylmercuric acetate does not appear to provide the basis for a selective medium.

Therefore malt extract agar containing 1000g l^{-1} sucrose was used as the selective medium in all further work.

2.7.4. The effect of the pH of the selective medium on the isolation of P. expansum and other fungi from soil.

2.7.4.1. Methods

P. expansum can grow over a wider range of pH than most other fungi. Therefore a series of the sucrose malt extract selective medium at different pHs from 3.5 to 9.5 were tested to see if the selectivity of the medium could be improved. These media were inoculated as before with a dilution series prepared from soil to which P. expansum had been added to give an inoculum of 4600 conidia g^{-1}.

2.7.4.2. Results and Discussion

The results given in Table 5 show that pH has no marked stimulatory effect on the growth of Penicillium colonies between pH 4.5 and 8.5. However the numbers of colonies of other fungi fell markedly between pH 5.4 and 4.5 and so the optimum pH for the selective medium appears to be about pH 5.0.

Table 5. The effect of pH on the value of the selective sucrose malt extract medium for isolating *P. expansum* from soil.

pH	All Penicillium spp. including *P. expansum*	*P. expansum*	Other Fungal spp.
9.5	36.0 ± 5.15	32.6 ± 4.8	1.4 ± 0.2
8.5	47.8 ± 3.11	44.8 ± 2.92	2.2 ± 0.5
7.0	46.0 ± 2.20	43.6 ± 2.25	3.8 ± 1.11
6.5	46.4 ± 1.96	44.0 ± 2.07	2.0 ± 0.31
5.4	50.8 ± 3.16	48.4 ± 3.05	4.0 ± 0.45
4.5	48.2 ± 3.24	45.6 ± 3.55	0.8 ± 0.37
3.5	29.75 ± 3.24	28.3 ± 4.02	0.6 ± 0.37

Mean number of colonies $\chi 10^4$ g^{-1} soil.

2.8. The development of methods for mixing spores into soil

During preliminary experiments difficulties were encountered in obtaining uniform mixing of spores into soil. This was shown by the extreme variability between replicate samples. In order to achieve uniform mixing a number of different methods were tried.

2.8.1. Mixing by hand

800g autoclaved soil were spread on sterilized (10% chloros) plastic sheet. A *P. expansum* spore suspension in 10ml distilled water, containing Tween 80 (4 drops l^{-1}), was added to the soil to give approximately 4300 spores g^{-1} soil. The soil was then thoroughly mixed by hand for ten minutes. Triflex plastic disposable gloves were worn during this operation. After mixing, three samples were taken and the numbers of spores in each determined by plating onto the selective medium using the dilution plate method. The plates were incubated at 23°C for 7 to 12 days and the numbers of *P. expansum* colonies which developed were counted.

2.8.2. Manual shaking

800g of sterilized soil was placed in a sterilized (10% chloros) plastic jar of volume 4800 cm^3. 10 ml of a spore suspension, to give approximately 4300 spores g^{-1} soil, were added to the soil in the jar. After closing the lid the jar was shaken manually for 30 mins, after which three samples were plated out as before. After incubation for 7 to 12 days the numbers of *P. expansum* colonies which developed were recorded.

2.8.3. Mechanical mixing

Either a concrete mixer (plate 1) or small scale drum mixer, built in the Botany Department mechanical workshop by J. Muckersie (plate 2), was used depending upon the volume of soil involved. Prior to use the mixer was thoroughly washed, and then sterilized using a 10% chloros solution. The soil

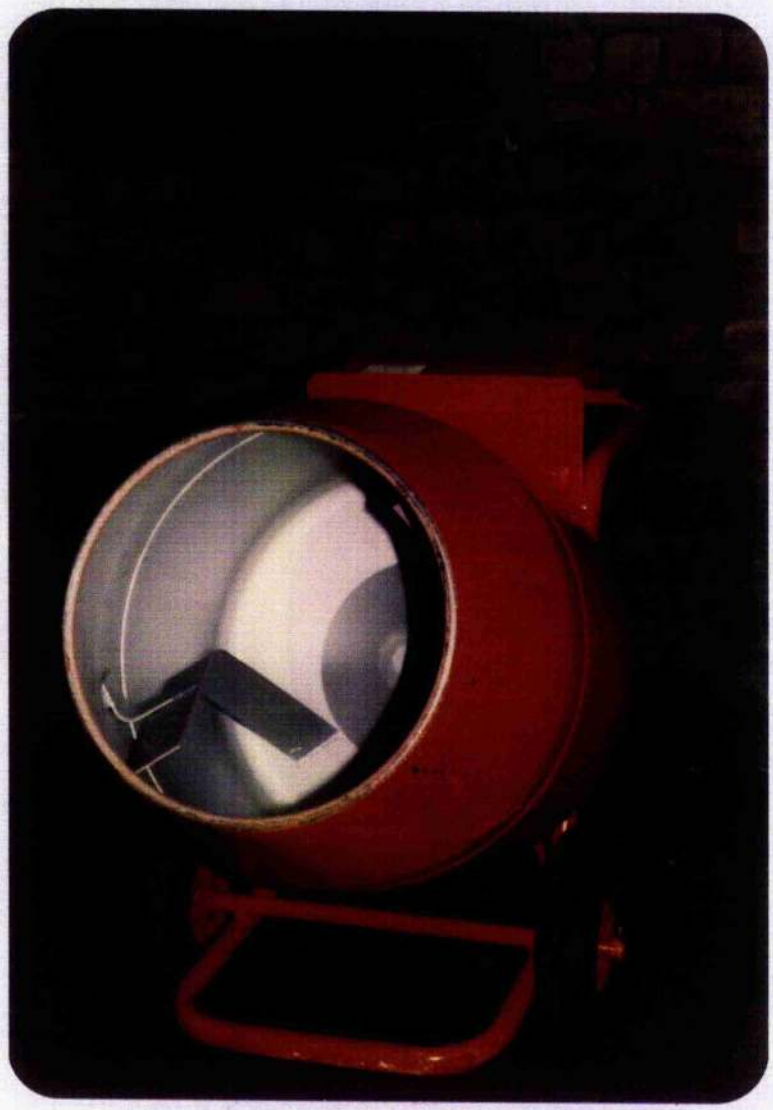


Plate 1. Concrete mixer.

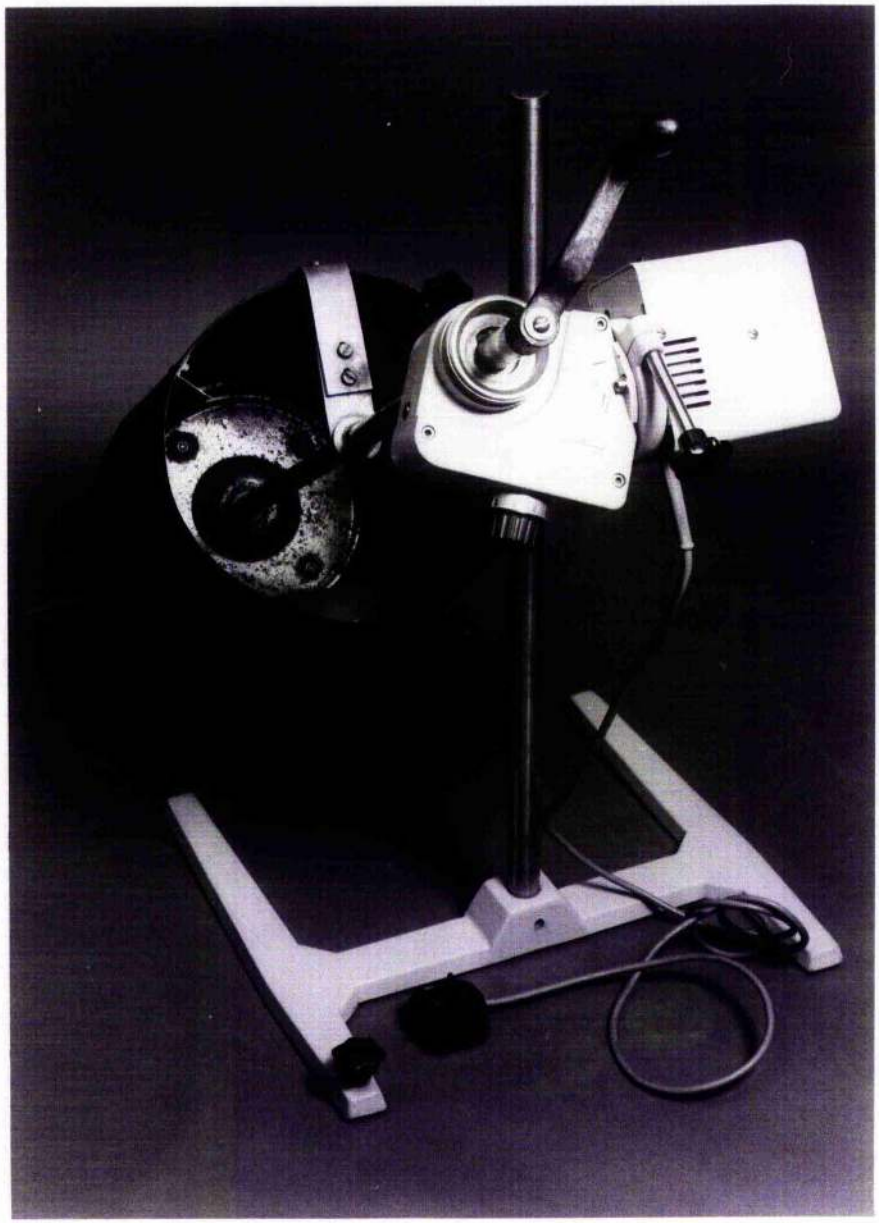


Plate 2. Small scale drum mixer.

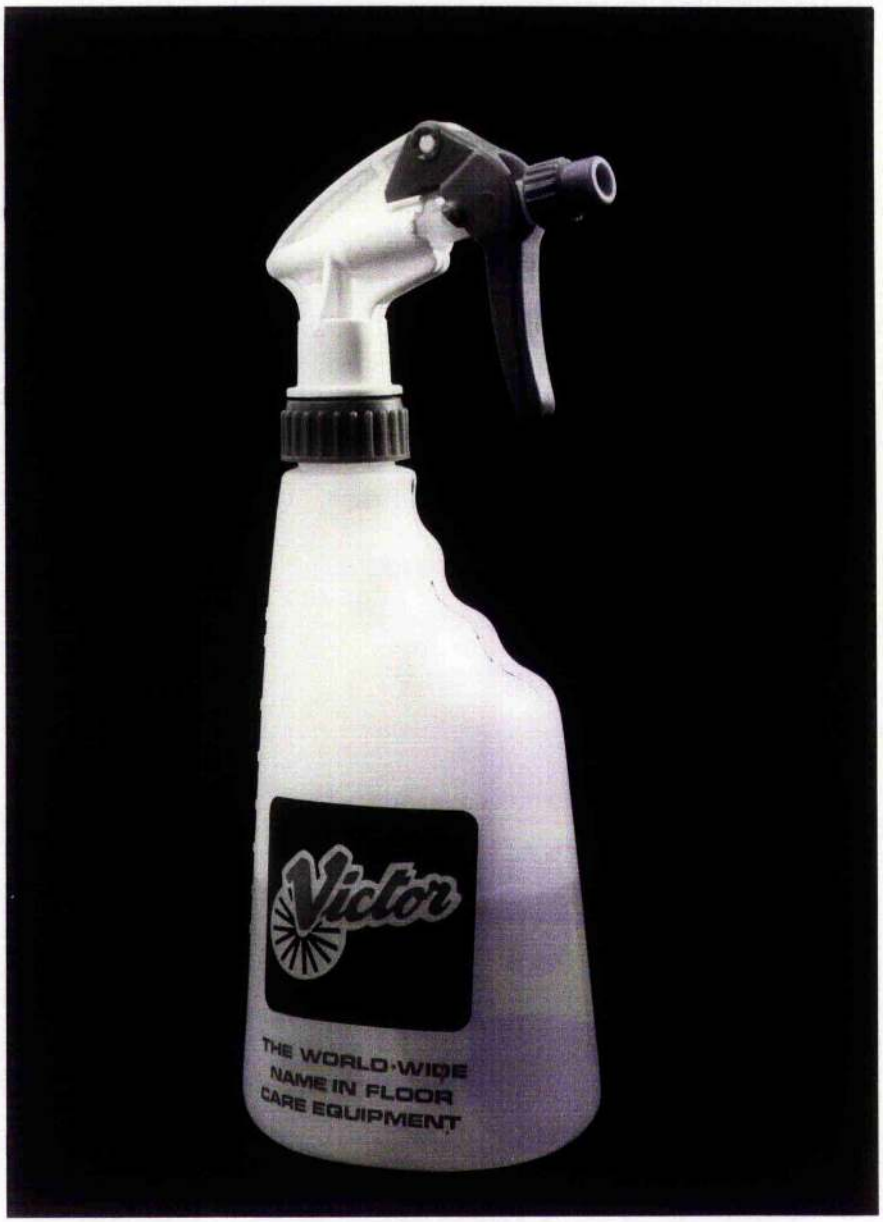


Plate 3. Spray gun

was placed in the mixer and the spore suspension added using a spray gun (plate 3). A fraction of the spore suspension was sprayed over the soil and the mixer operated for 15 mins. A further fraction was sprayed over and mixed for 15 mins as before. This procedure was repeated (usually 3 to 5 times) until all the spore suspension had been added. The mixer then continued in operation for a total of about 3 hours. Samples were plated using the dilution plate method, and after incubating as described previously the results were recorded.

2.8.4. Results

The results given in Tables 6A, B & C and Appendix Tables 1A, B & C show that the spores were mixed relatively evenly in soils by all methods, but the best mixing was achieved by the mechanical mixers. Mixing spores by hand or by manual shaking was also very laborious and time-consuming, and so the mechanical mixers were used for all experiments, the concrete mixer when a large bulk of soil was used and the small scale drum mixer when only small quantities were required.

Table 6A. Colonies of *P. expansum* g^{-1} soil after mixing by hand.

Samples	*P. expansum*	Mean ± S.E.	S.E. as a percentage of mean
1	3680		
2	2960	3120 ± 288.45	9.24
3	2720		

Table 6B. Colonies of *P. expansum* g^{-1} soil after mixing by shaking manually in plastic container.

Samples	*P. expansum*	Mean ± S.E.	S.E. as a percentage of mean
1	2400		
2	3840	3040 ± 423.33	13.92
3	2880		

Table 6C. Colonies of *P. expansum* g^{-1} (oven dry) soil after mixing with concrete mixer.

Samples	*P. expansum*	Mean ± S.E.	S.E. as a percentage of mean
1	1026		
2	1087		
3	933		
4	964		
5	810		
6	862	975.44 ± 38.077	3.9
7	943		
8	964		
9	1190		

CHAPTER III

THE SOIL AND ITS ANALYSIS

3.1. Collection of samples

Soil samples were collected from the same site at the Departmental Experimental Gardens, Garscube, as the soil used in the experimental work described in this thesis. They were dried at room temperature and thoroughly mixed to minimize the effect of local variations, then sieved through a 2mm mesh sieve. The air-dried, sieved soil was stored in a glass bottle closed with a tightly fitting lid until required for analysis.

3.2. Organic matter

Two different methods were used to determine the percentage of organic matter in the soil.

3.2.1. Ignition method

Organic matter consists of carbon compounds which when strongly heated are converted into CO_2 and water.

5g samples of oven-dry soil were heated in a muffle furnace at 700°C for 2 hours. The loss in weight after ignition represented the weight of organic matter in the soil samples. This was expressed as a percentage, calculated as follows:-

$$\% \text{ organic matter} = \frac{\text{wt. of oven-dry soil - wt. of ignited soil}}{\text{wt. of oven-dry soil}} \times 100$$

3.2.2. Walkley-Black method

Air-dried soil was ground and sieved through a 150μ mesh sieve. 0.1g of the sieved soil was placed in a 500ml conical flask and 10ml of 1N potassium dichromate ($K_2Cr_2O_7$) was added and mixed. 20ml concentrated H_2SO_4 was then

added and thoroughly mixed. Duplicate blank flasks without soil were also prepared as controls. All flasks were allowed to stand for 30 minutes.

An approximately 0.5N ferrous ammonium sulphate ($Fe(NH_4)_2\ (SO_4)_2\ 6H_2O$) solution was prepared and its exact normality was determined as described below. This standardised solution was then used to titrate the soil solution.

10ml $K_2Cr_2O_7$ were placed into a 500ml flask and then 10ml 85% H_3PO_4, 250ml 1M H_2SO_4 and 2ml of the barium diphenylamine sulphonate ($Ba(C_6H_5.NH.C_6H_4SO_3)_2$) indicator solution were added. The mixture was then titrated with the ferrous ammonium sulphate solution. The end point of the titration was taken when the colour changed from dark green through blue-grey to a sharp change, at the end point, to light green. The normality of the ferrous solution was then calculated.

After allowing the flasks containing the soil samples and the blank flasks to stand for 30 mins, they were diluted by adding 200ml water, 10ml 85% H_3PO_4 and 2ml of the barium diphenylamine sulphonate indicator. The samples were then titrated with the standardised ferrous ammonium sulphate solution.

Calculation:

$$\text{Volume } Cr_2O_7^{2-} \text{ reduced} = Vi\left(1 - \frac{T}{B}\right)$$

where Vi = initial volume of $Cr_2O_7^{2-}$

T = end point in sample titration

B = end point in blank titration

$$\% \text{ Carbon} = Vi\left(1-\frac{T}{B}\right) \times Cr_2O_7^{2-} \text{ Normality} \times \frac{12}{4000} \times \frac{1}{0.77} \times \frac{100}{\text{wt. of soil sample}}$$

where $\frac{12}{4000}$ = milliequivalent weight of carbon

0.77 = 77% recovery factor

% Organic matter = % Carbon x 1.72

where 1.72 = conversion factor.

The results are given in Table 7 and Appendix Table 2A.

3.3. Particle size analysis

The relative proportions of sand, silt and clay were determined by mechanical analysis. Before this analysis the soil samples were treated to remove organic matter since any organic matter may cause flocculation and hinder the separation of the fine particles into individual grains. The organic matter was removed from 10g air-dried soil in a 400ml beaker by adding approximately 50ml 6% H_2O_2, two drops of a silicon antifoaming agent and heating in a steam bath. When the reaction had stopped the suspension was cooled and a further 50ml of 6 % H_2O_2 were added. The heating was then continued until the oxidation of organic matter was complete as shown by the fact that the addition of further H_2O_2 produced no reaction. Distilled water was added to give approximately 2cm depth of suspension in the beaker. 10ml dispersing agent (50g sodium hexametaphosphate and 7g anhydrous sodium carbonate dissolved in 1 l water) were then added and the soil particles dispersed by 5 minutes treatment using an ultrasonic probe.

Determining the sand fractions (particles larger than 53µm diameter)

After dispersion the soil particles were washed, first through a 180µm mesh sieve and then through a 53µm mesh sieve. The coarse and medium sand fractions were retained by the 180µm sieve and the fine sand fraction by the 53µm sieve, while the silt and clay fractions passed through both. The sieves were placed in a 30°C oven for 24 hours and then the fractions were transferred into a porcelain basin and dried at 110°C.

$$\% \text{ Coarse + medium sand} = \frac{\text{Wt. of coarse + medium sand - wt. of basin}}{\text{Wt. of oven-dry original soil sample}} \times 100.$$

$$\% \text{ Fine sand} = \frac{\text{Wt. of fine sand + basin - wt. of basin}}{\text{Wt. of oven-dry original soil sample}} \times 100.$$

% Sand = Percentage of coarse medium sand + percentage of fine sand.

Determining the silt and clay fractions (particles less than approximately 60μm diameter

Silt and clay were measured by using the pipette method which was originally developed simultaneously by Jennings *et al* (1922) and Robinson (1922) and has since been adopted by the International Society of Soil Science (1929). The method is based on the different sedimentation rates of particles of different sizes in water at a given temperature; the bigger the particle the greater the settling velocity. The relationship between the radius of a particle and its settling velocity in a medium of given viscosity is stated by Stoke's law.

$$v = \frac{g(\sigma - \rho)d^2}{18\eta}$$

v = settling velocity in cms^{-1}

g = acceleration due to gravity (981 cms^{-2})

σ = density of settling particles (2.6 cm^{-3})

ρ = density of water (0.998 cm^{-3} at 20°C)

η = viscosity of water (0.01 g s^{-1} cm^{-1} at 20°C)

d = diameter of particles (equivalent settling diameter in cm).

The fraction passing through the two sieves was made up to 1000ml in a graduated cylinder. The cylinder was shaken thoroughly for 60 seconds to ensure that all the soil was in suspension before placing on a pipetting stand. It was calculated that after 62 seconds sedimentation at 21°C any particles larger than 60μm (i.e. any sand particles) would sediment below 20cm. Three 10ml samples were removed from a depth of 20cm shaking the cylinder thoroughly and allowing to sediment for the required time between each sampling. Samples were dispensed into basins and the water evaporated, first over a steam bath and finally by drying at 110°C. The basins were then cooled in a desiccator and reweighed.

$$\% \text{ Silt + clay} = \frac{\text{wt. of fraction - wt. of dispersant}}{\text{oven-dry wt. of original soil sample}} \times \frac{\text{volume of cylinder}}{\text{volume of sample}} \times 100.$$

The clay fraction

After removing the silt plus clay samples (i.e. all particles below 60μm in diameter) the cylinder was shaken thoroughly again and placed in a 20°C incubator for 479 mins to allow particles larger than 2μm diameter to sediment below a depth of 10cm. Without disturbing the sedimentation 10ml samples were removed from a depth of 10cm. The clay samples (particles less than 2μm in diameter) were dried and reweighed as before.

$$\% \text{ Clay} = \frac{\text{Wt. of fraction - wt. of dispersant}}{\text{Oven-dry wt. of original soil sample}} \times \frac{\text{Volume of cylinder}}{\text{Volume of sample}} \times 100$$

$$\text{Thus Silt} = \text{Silt plus clay - clay.}$$

Results

The results are given in Table 7 and Appendix Table 2B.

3.4. Cation exchange capacity

The cation exchange capacity gives the total quantity of negative charges in the soil, and indicates the ability of the soil to hold cations, in a form available to higher plants and micro-organisms. The cation exchange capacity consists of fixed charges on the clay due to imperfections in the crystal lattice and pH dependent charges on the edges of the clay particles and on the organic matter. A measure of the cation exchange capacity gives an indication of the potential fertility of a soil.

10g of the sieved, air-dried soil was mixed thoroughly with an equal volume of acid washed sand to assist percolation. The soil:sand mixture was placed in a glass column previously plugged at one end with glass wool. A small piece of

glass wool was also placed lightly on the top of the soil.

To ensure the removal of all exchangeable cations and the saturation of the negative charges with K^+ ions, the soil:sand mixture in the column was leached with 200ml 1N potassium acetate. After removal of excess K^+ ions, by leaching the column with 100ml 90% ethanol, the K^+ ions held on the negative sites were removed by leaching with 200ml 1N ammonium acetate at pH 7 and the leachate was collected in a 250ml flask. After all the ammonium acetate had drained through the leachate was made up to 250ml by adding distilled water.

The amount of K^+ in the leachate, in ppm, was measured by flame emission photometry using a flame photometer (Evans Electroselenium Ltd.).

The cation exchange capacity of the soil was calculated using the following method.

Using the measured K^+ concentration in ppm (1ppm = 1μg ml^{-1}), the amount of K^+ in mg.$100g^{-1}$ oven-dry soil was calculated. This was then converted to milliequivalents $100g^{-1}$ soil by dividing the amount of K^+ by the equivalent weight of K^+(39).

The results are given in Table 7 and Appendix Table 2C.

3.5. Exchangeable bases

The major exchangeable bases include many of the essential nutrients Ca^{++}, Mg^{++}, Na^+, and K^+. In an intensively leached soil or an acid soil most of these exchangeable bases will have been lost and the exchange sites will be occupied by equivalent amounts of H^+, Al^{+++} and Mn^{++} ions. The determination of the exchangeable bases indicates the actual fertility of the soil.

The method used was similar to that described for the determination of the cation exchange capacity except that the column of soil:sand mixture was leached directly with 1N ammonium acetate at pH 7 instead of with potassium acetate and ethanol.

The amounts of K^+ and Na^+ were determined in the leachate by flame emission photometry and the amounts of Ca^{++} and Mg^{++} using an atomic absorption spectro-

photometer (Perkin Elmer 370A). It was necessary to dilute the ammonium acetate leachate before analysing for Ca^{+}, Mg^{++}, and 1% Strontium chloride solution was added both to the leachate and to the standard samples to overcome interferences in the analytical method.

The amounts of each element in the soil in me.$100g^{-1}$ are given in Table 7 and Appendix Table 2C.

3.6. Available phosphorus and potassium

Some nutrients are found only in solution (e.g. NO_3^- and Cl^-) but most of them exist in a state of equilibrium with the exchangeable or readily soluble ions which are in turn in equilibrium with fixed or unavailable forms.

These unavailable nutrients act as a buffer which can maintain the levels of available and soluble nutrients. Plant and microbial growth is dependent on a continuing supply of readily available nutrients. Phosphorus and potassium are examples of nutrients which are required by organisms in high amounts.

The availability of phosphorus is complicated by the fact that it is retained in the soil in various forms including complexes or compounds of calcium, iron and aluminium (Fitts & Nelson, 1956).

These available nutrients were estimated by extracting the soil using 0.5M CH_3COOH as follows (this method is commonly used by the West of Scotland Advisory Service):-

5g samples of air-dried soil were placed into 4oz screw cap bottles and 50ml 0.5M CH_3COOH were added to each. The bottles were shaken on an end-over-end shaker for 1 hour and the extracts filtered into polythene storage bottles.

The concentration of K^+ in the CH_3COOH extract was determined by flame emission photometry as described earlier (page 27).

The amount of phosphorus in the CH_3COOH extract was determined using a method originally devised by John (1970). The reagents involved in the analysis were prepared as follows:-

(i) The mixed reagent:

151ml Analar concentrated H_2SO_4 was added slowly to 500ml distilled water. After cooling the diluted acid, 20g of ammonium molybdate [$(NH_4)_6 MO_7 O_{24} 4H_2O$], dissolved in 200ml distilled water, was added. 0.4g potassium antimony tartrate was then dissolved in 100ml of distilled water and added carefully to the acid molybdate solution. The solution was mixed well and made up to 1 litre and stored in a brown glass bottle. This reagent is stable for several months.

(ii) Complete reagent:

1.5g Analar ascorbic acid was dissolved in 100ml of the mixed reagent just before use. This reagent was prepared fresh as it does not keep for more than 24 hours.

(iii) Stock phosphorus solution (1000µg p ml^{-1}):

Potassium dihydrogen orthophosphate (KH_2PO_4) was dried at 100°C for 1 hour and then cooled in a desiccator. 1.0984g of the dried KH_2PO_4 were then dissolved in 200ml distilled water in a 250ml flask and 1 drop of concentrated H_2SO_4 was added. The solution was made up to 250ml by adding distilled water and then stored in refrigerator. When required for the analysis 0.5ml of this solution was diluted to 100ml with 2.5% CH_3COOH.

The analysis

1ml of the CH_3COOH soil extract was placed in a 50ml flask and 24ml of 2.5% CH_3COOH were added. The solution was then made up to 40ml with distilled water and 5ml of the complete reagent added. It was then made up to 50ml by adding further distilled water, and after 30 mins the optical density of the solution was determined in a Baush & Lomb spectronic 20 spectrophotometer at 880 nm.

A standard graph was prepared over the range from 0 to 25µg P and the concentration of phosphorus in the soil leachate in mg Kg^{-1} oven-dry soil was

determined by reference to this graph.

The results are given in Table 7 and Appendix Table 2C.

Table 7. Results of the soil analysis.

	Mean ± S.E.
Coarse + medium sand %	33.5 ± 1.27
Fine sand %	25.3 ± 0.29
Clay %	12.6 ± 0.36
Silt %	22.8 ± 1.06
Organic matter %	
(i) By loss on ignition	11.51 ± 0.48
(ii) Walkley-Black method	8.68 ± 0.59
Cation exchange capacity (me.100g^{-1} oven-dry soil)	26.182 ± 1.407
Exchangeable bases (me.100g^{-1} oven-dry soil)	
K	1.049 ± .011
Mg	2.002 ± 0.155
Na	0.735 ± 0.119
Ca	18.119 ± 0.399
Available (mg Kg^{-1} oven-dry soil)	
K	245.997 ± 17.636
P	220.266 ± 4.953

These results show that the soil is a well drained sandy loam of moderate fertility.

CHAPTER IV

SECTION I. SURVIVAL OF SPORES, SPORE GERMINATION AND SPORELING GROWTH OF PENICILLIUM EXPANSUM IN SOIL

4.1.1. Introduction

Soil fungi have been divided into two groups (Waksman, 1917); those which occur regularly in soil and complete their whole life cycle there (soil inhabitants) and those which are only transitory members (soil invaders). The transitory members have also been called allochthonous fungi by Saito (1955) and exochthonous fungi by Park (1957). Soil invaders are either parasitic organisms which arrive in the soil on the death or decay of their host or are saprophytes which normally colonize litter before it arrives in soil, e.g. epiphytic flora of aerial parts of plants.

Soil invaders do not possess the ability to develop or maintain themselves in an active condition in soil. They are in general unable to colonize dead organic materials although their propagules may survive in an active state for some time (Waid, 1960). It is likely that their spores or other propagules are maintained in a dormant condition by the widespread fungistatic effect of the soil (Dobbs & Hinson, 1953). Their hyphae may also survive for some period in dead plant and animal remains which were colonized while the organisms were alive or before the remains arrived in the soil, but they are eventually overcome by the antagonistic and competitive activities of soil inhabitants (Waid, 1960).

The subject of soil invaders has been reviewed by Garrett (1938, 1950, 1952, 1956), Burges (1958) and Waid (1960).

It is clear that P. expansum can grow on a wide range of plant reproductive structures as a weak parasite. However any isolations from soil do not demonstrate that it can grow and complete its life cycle in this environment and thus that it can be classified as a soil inhabitant. It is possible

that isolations from soil and plant litter arose from conidia which had developed on aerial plant parts and which then arrived in the soil by gravitational forces. P. expansum may thus be a soil invader rather than a soil inhabitant.

The following experiments were carried out to determine if spores of a pathogenic isolate of P. expansum (isolated from apple fruit) can survive and germinate in natural soil, and to compare the behaviour with that of another parasitic species and one saprophytic species of Penicillium.

4.1.2. Survival and germination of P. expansum spores in soil

4.1.2.1. Materials and methods

The method used for this investigation was originally devised by Old (1967).

Soil was collected from the Botany Experimental Gardens, Garscube and dried at room temperature before sieving through a 2mm mesh sieve. Half of the bulk was then sterilized as described in Chapter II. Spores of P. expansum were mixed as uniformly as possible into both sterilized and unsterilized soils using a small drum mixer (Plate 2) to give approximately 15×10^3 spores g^{-1} oven-dry weight of soil. Approximately 60g of the soil spore mixture were then placed into 9cm plastic petri-dishes which had previously been pierced, using a hot needle, to provide a total of 40 evenly spaced holes per dish, 20 in the lid and 20 in the base. 80 plates containing soil with spores (40 with sterilized soil and 40 with unsterilized soil) and 48 control plates containing soil without spores (24 with sterilized soil and 24 with unsterilized soil) were prepared for this experiment. The plates were buried individually in 17.8cm plastic pots containing either sterilized or unsterilized garden soil as appropriate. The pots were incubated in a dark room at 25°C and watered with 250ml tap water per pot once a week.

After various periods of incubation, five plates of each soil containing

spores and three plates of each soil without spores were retrieved and the numbers of viable propagules present were determined using the dilution plate method.

The oven-dry weights of the soils were determined at each sampling time and the mean soil moisture content calculated (Sterilized 38.58%, unsterilized 38.4%).

4.2.2. Results

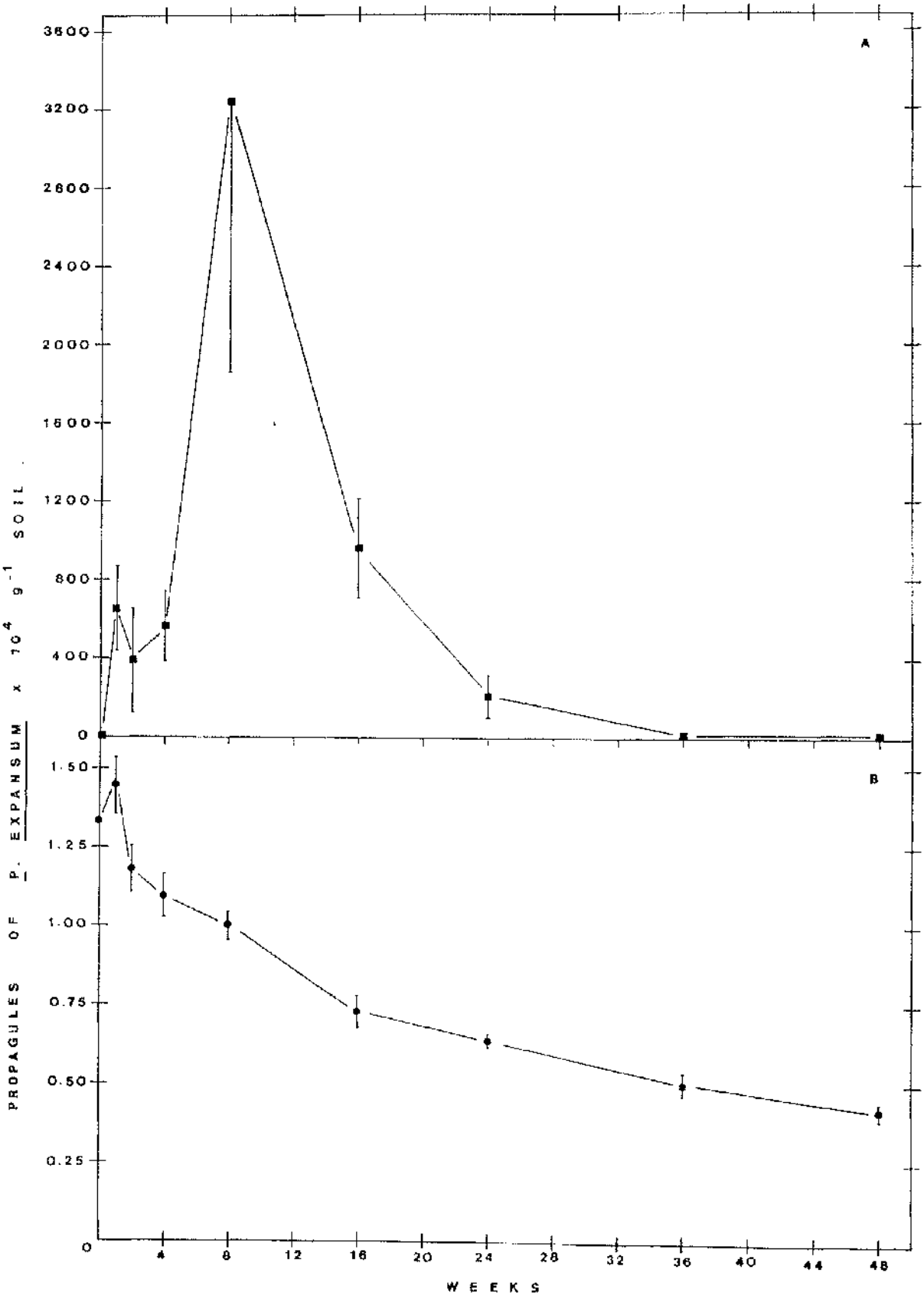
The results are given in fig. 1 and Appendix Table 3. An analysis of variance shows that the number of colonies of P. expansum developing on the dilution plates prepared from the sterilized soil was significantly higher than that on the plates prepared from unsterilized soil ($P < 0.001$). Thus fungal growth had occurred in the sterilized soil. The highest numbers of colonies were recorded about 8 weeks after inoculation but after that there was a gradual reduction with time ($P < 0.01$). In contrast there was no indication of growth in the unsterilized soil and the numbers of viable propagules declined over the period of the experiment. However at the last sampling time, at 48 weeks, the numbers were still approximately $^{2}/_{3}$ of the levels at inoculation.

P. expansum was never recovered from uninfested soils so all isolations from the infested soils must have originated from the inoculum of P. expansum.

The results show that P. expansum undergoes a substantial population increase in the sterilized soil but no such increase occurred in unsterilized soil, although the conidia can remain in a viable condition in the latter soil for a year or more.

Fig. 1

Survival and growth of P. expansum in sterilized (A) and unsterilized (B) soils.


A
B
3600
3200
2800
2400
2000
1600
1200
800
400
0
1.50
1.25
1.00
0.75
0.50
0.25
0
4
8
12
16
20
24
28
32
36
40
44
48
WEEKS
PROPAGULES OF P. EXPANSUM x 10^4 g^-1 SOIL

4.1.3. A comparative study of the behaviour of P. expansum, P. digitatum and P. brevicompactum spores in soil

4.1.3.1. Introduction

The last experiment indicated that P. expansum spores can germinate and sporeling growth can occur in sterilized soil but there was no indication of such growth in unsterile soil. The following experiments involved a microscopic examination of spore germination and sporeling growth in the two soils to determine more about the extent to which growth occurred. P. digitatum, another parasitic species, and P. brevicompactum, a saprophytic species, were included as comparisons.

4.1.3.2. Materials and methods

The method used was originally devised by Matturi and Stenton (1964).

Soil was collected, sieved and sterilized as described in Chapter II. Acid washed microscope slides were sterilized by flaming. Spore suspensions were prepared in sterilized distilled water from 7 day old stock cultures of P. expansum, P. digitatum and P. brevicompactum. One to two drops of the spore suspension were spread over about 6.25 cm^2 of the surface of the microscope slides and allowed to dry. The slides were then placed in petri-dishes with the spore smear uppermost and then covered with either sterilized soil or unsterilized soil. Ten slides of each species were buried in each treatment. After 16h, 45h, 3 days, 7 days and 12 days, two slides of each species were recovered from each treatment and dried for 15 minutes by gently heating on a hot plate. As much soil as possible was removed by tapping the edges of the slides onto the bench. One slide of each pair was stained with hot Phenolic rose bengal (2% water soluble stain in 5% Phenol) for 1 minute before washing in running tap water. A drop of water was then placed over the spores and a coverslip applied. The other slide was stained with a dilute solution of cotton-blue in lactophenol for five minutes before mounting

in plain lactophenol. In this experiment the slides which were stained with cotton-blue were used only for photomicrography, and all other records were based on observations made on the slides stained with rose bengal. However cotton-blue gave a better stain and, furthermore, as these preparations were mounted in lactophenol, they did not dry out and so could be stored before examination. In contrast, the rose bengal stained smears, being mounted in water, dried out rapidly and so had to be examined immediately. Thus lactophenol cotton-blue was used for all other experiments.

4.1.3.3. Results

The results are recorded in Table 8. None of the three species germinated in unsterilized soil although they all did so in sterilized soil.

In sterilized soil the percentage germination and germ-tube lengths continued to increase at least up to 45h after which it became impossible to continue measurements.

Soon after germination some germ-tubes apparently stopped growth, producing at their apex 1 or 2 phialides in P. expansum or 1 to 5 phialides in P. digitatum. These then produced conidia (Plates 4 & 7). Some germ-tubes grew longer and developed one or two lateral conidiophores with again 1 or 2 phialides in P. expansum and 1 to 5 phialides in P. digitatum (Plates 5, 6, 8 & 9). In P. brevicompactum phialides and conidia were never observed to develop directly at the end of germ-tubes, only on lateral conidiophores. However the conidia were commonly formed on 1 to 5 phialides as in P. expansum and P. digitatum (Plate 10). P. brevicompactum also occasionally developed long lateral conidiophores which then formed up to 3 metulae with several phialides on each (Plate 11). These structures were much more typical Penicillus structures than any formed by the other two species but they only appeared to develop during the first 3 days and later only the simpler structures were formed. Autolysis of the protoplasm of germ-tubes and hyphae was first observed at 3 days. Lysis of the protoplasm

Table 8. Behaviour of P. expansum, P. digitatum and P. brevicompactum spores in soils.

Culture	Soil	Time period				
		16h	45h	3 days	7 days	12 days
P. expansum	Sterilized	82.9* 36-108**	94.4* 547.0**	More conidia produced than after 45h. Germ-tubes and hyphae have started to lyse.	Conidia present. Greater lysis of germ-tubes and hyphae.	Many young conidia present. Hyphae completely disintegrated.
	Unsterilized	0	0	0	0	0
P. digitatum	Sterilized	68.9* 9-54**	72.8* 29-216**	More conidia produced than after 45h. Germ-tubes and hyphae have started to lyse.	Conidia present. Greater lysis of germ-tubes.	Many young conidia present. Germ-tubes lysed but outline of their walls still noticeable.
	Unsterilized	0	0	0	0	0
P. brevicompactum	Sterilized	43.3* 3.6-45**	64.6* 18-234**	More conidia produced than after 45h. Few spores still ungerminated. Germ-tubes and hyphae have started to lyse.	Conidia present. Greater lysis of germ-tubes and hyphae.	Many young conidia present. Hyphae completely lysed but outline of their walls still noticeable.
	Unsterilized	0	0	0	0	0

* Percentage spore germination. ** Germ-tube length (µm).

Plates 4 to 10

Photomicrographs of germinated conidia producing microcyclic conidiation in sterilized soil.

Plate 4. Germinated conidium of *P. expansum* with microcyclic conidiation at the tip of a germ-tube.

Plates 5 & 6. Germinated conidia of *P. expansum* with microcyclic conidiation on lateral conidiophore.

Plate 7. Germinated conidium of *P. digitatum* with microcyclic conidiation at the tip of a germ-tube.

Plates 8 & 9. Germinated conidia of *P. digitatum* with microcyclic conidiation on lateral conidiophore.

Plate 10. Germinated conidium of *P. brevicompactum* with microcyclic conidiation on a lateral conidiophore.

Plate 11. Germinated conidium of *P. brevicompactum* producing a conidiophore with matulae, phialides and conidia in sterilized soil.

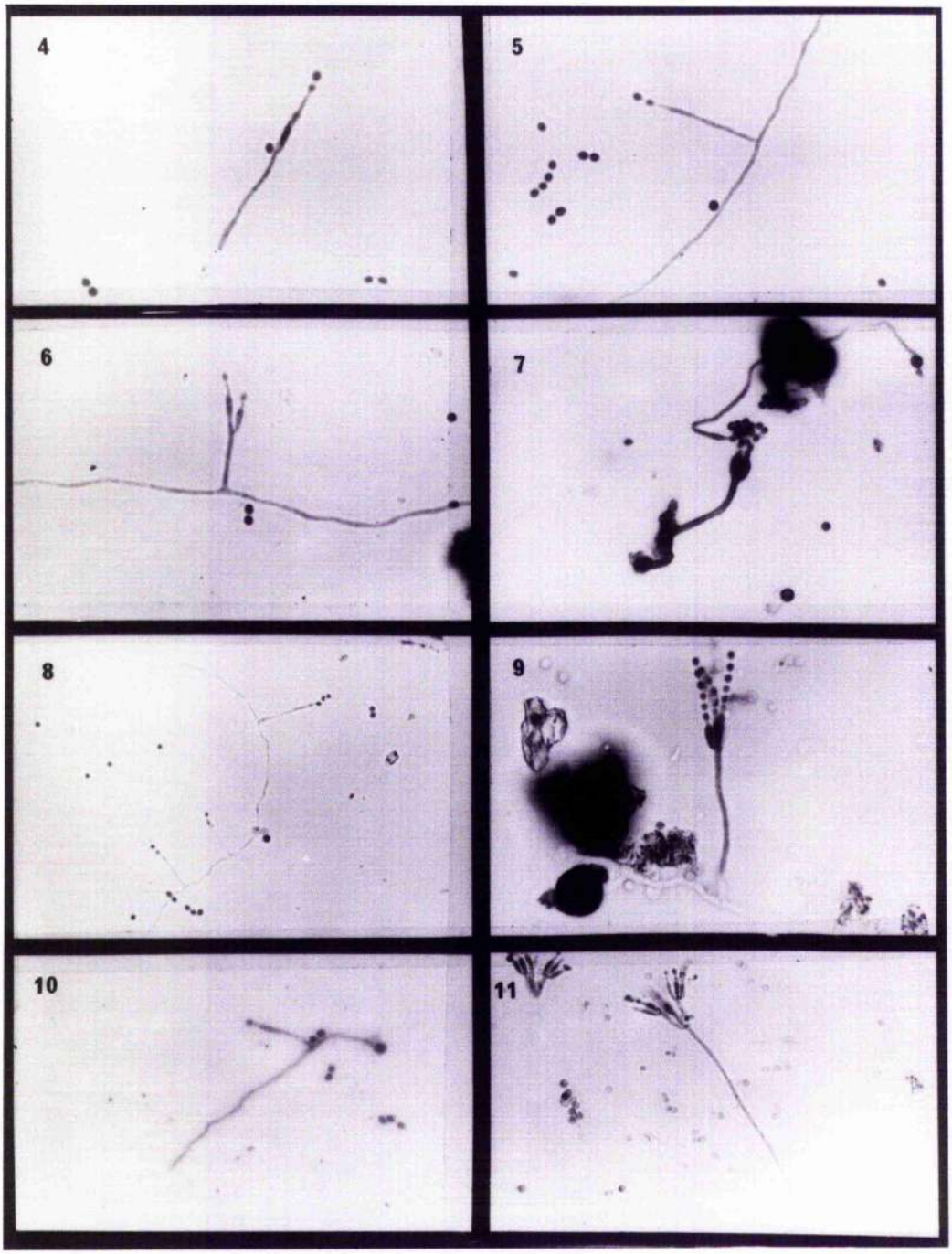

4
5
6
7
8
9
10
11

Plate 12.

Photomicrograph of sporeling *P. digitatum* in which the protoplasm has lysed after microcyclic conidiation in sterilized soil.

Plates 13 to 15.

Photomicrographs of new microcyclic conidia after disintegrating hyphae and mycelium in the sterilized soil.

Plate 13.

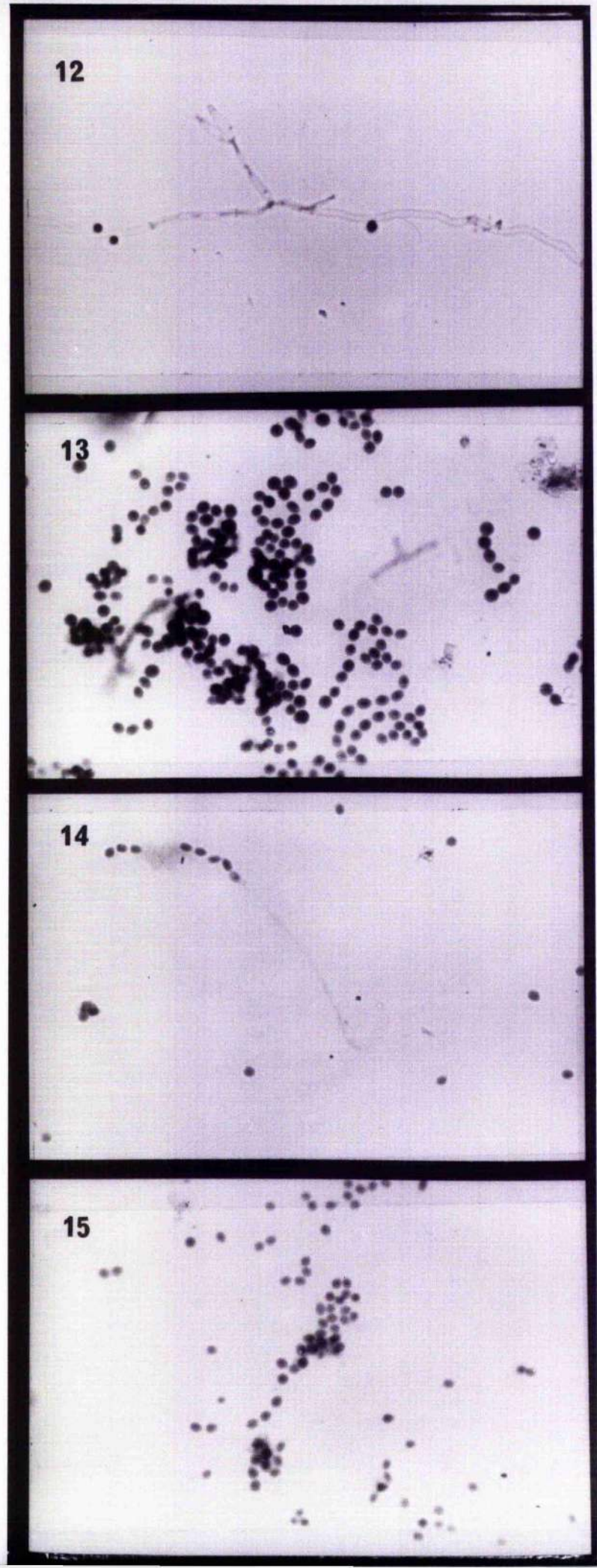
P. expansum microcyclic conidia.

Plate 14.

P. digitatum microcyclic conidia.

Plate 15.

P. brevicompactum microcyclic conidia.


12
13
14
15

and of much of the hyphal wall was well advanced by 7 days in P. expansum and P. brevicompactum. However at this stage, only the protoplasm of P. digitatum had lysed (Plate 12) and most of the hyphal wall was still intact. By 12 days the germ-tubes and hyphal walls of all 3 species had almost disintegrated and the newly formed conidia were scattered over the surface of the slides (Plates 13, 14 & 15).

The development of all species was thus similar to the microcyclic conidiation described for Aspergillus niger by Anderson and Smith (1971), for Penicillium digitatum by Zeidler and Margalith (1973) and for Penicillium urticae by Sekiguchi, Gaucher and Costerton (1975).

4.1.4. Discussion

These experiments clearly show that in non-sterile soils P. expansum spores are subject to the general fungistasis or mycostasis which is known to operate in most soils, and which results in the severe restriction in germination and growth of most fungi (Dobbs & Hinson, 1953). This subject has been reviewed by Lockwood (1964), Garrett (1970), Griffin (1972), Watson and Ford (1972) and Lockwood (1977).

The following discussion will be considered in two parts; firstly, factors affecting spore germination in unsterilized soil and, secondly, factors affecting sporeling growth in sterilized soil.

(i) Spore germination

There are two possible explanations for the failure of spores to germinate in unsterilized soil:

(a) Shortage of nutrients.

(b) Presence of inhibitors.

(a) Shortage of nutrients

Cochrane (1960) states that the relatively short-lived asexual spores of Ascomycetes and fungi Imperfecti require exogenous carbon, nitrogen and, occasionally, other compounds for germination. Failure of P. expansum spores to germinate in soil may thus be due to the absence of such nutrients.

The activities of micro-organisms may be the cause of these nutrient shortages since they would compete with the spores for the limited nutrients and thus could lead to levels of nutrients too low to support germination (Ko & Lockwood, 1967). Lingappa and Lockwood (1964) found that microbial activity increased very rapidly when the spores of certain fungi were incorporated into soil and this was shown to be due to substances released from spores. Thus the competition may be both for exogenous nutrients and for nutrients that diffused out of the spores (Lockwood, 1977). Marshall and Alexander (1960) have provided evidence which indicates that the inhibition of growth of Fusarium oxysporum in association with a bacterial isolate in sterilized soil was due to competition for carbon and nitrogen rather than to the production of an antibiotic.

It is commonly reported that sterilizing the soil by heat or chemicals can remove this general fungistasis. The sterilization process would kill micro-organisms and release soluble nutrients which would then support the germination of added spores. Ko and Lockwood (1967) observed that sterilizing natural soils by autoclaving increased the concentrations of soluble carbohydrates in the soil 27-fold and amino acids 37-fold. If nutrients had been released in such quantities in our experiments they could have been responsible for the germination and limited growth of P. expansum, P. brevicompactum and P. digitatum observed.

(b) Presence of inhibitors

An alternative or additional explanation for the failure of spores to germinate in soil is that micro-organisms or other factors produced diffusible inhibitory substances which inhibit spore germination. Lockwood (1959) suggested that diffusible toxins could be present in soils because if the soil and the fungus were separated by agar or cellophane barriers, inhibition still occurred. He also found that some actinomycetes, particularly Streptomyces spp. produced diffusible fungitoxic substances and suggested that they might play a role in natural fungistasis. Stevenson (1956) observed that specific morphological changes were produced in Helminthosporium sativum by an antibiotic produced by an actinomycete. Similar morphological changes were produced in soils inoculated with actinomycetes. Thus micro-organisms in the vicinity of spores in soil may produce antibiotics which inhibit spore germination (Lingappa & Lockwood, 1964).

Hora and Baker (1970) used a soil emanation agar method to differentiate between inhibitors diffusing in solution and inhibitors capable of diffusing as volatile gases. The method involved placing agar discs on sterilized glass slides which were then placed on soil in petri-dishes. Thus the agar discs themselves were separated from the soil by the glass slide so that any changes in the capacity of the agar to support fungal growth must have been due to the activity of volatile substances. The discs were incubated in the dishes for 24h at 28°-29°C before transferring to another petri-dish without soil. The discs were then inoculated with conidia of various test organisms and spore germination assessed. Agar discs which had not been incubated in the presence of soil were used as controls. They found marked reductions in the germination of spores of test organisms, particularly when the agar discs had been exposed to alkaline soils. This method, or variations of it, have been used by a number of workers (Hora & Baker, 1972a; Ko & Hora, 1972; Romine & Baker, 1973; Balis, 1976) and provided good evidence for the presence of volatile germination inhibitors in many types

of soil.

Some of the volatile compounds emitted by soil have been identified. They include alkanes such as methane, ethane, propane, butane and pentane; alkenes such as ethylene, propylene and butene; aldehydes such as formaldehyde, acetaldehyde, propionaldehyde; ketones such as acetone and 2-butanone, and inorganic gases such as carbon dioxide, ammonia and nitrous oxide (Pavlica et al., 1978). Of these compounds a number are potentially inhibitory to fungi. These include ammonia, ethylene, allyl alcohol and formaldehyde. There are also a number of other as yet unidentified compounds which appear to be equally inhibitory (Lockwood, 1977; Pavlica et al., 1978). However there is little work that provides good evidence that any of them are directly involved in fungistasis. Smith (1973) identified ethylene by gas chromatography in Australian soils whose pH was less than 7.0 and obtained evidence to indicate that the fungistasis in these soils was due to this gas. He observed that the passage of air over soils in petri-dishes removed the inhibition whereas it persisted if air containing 1 ppm ethylene was passed through. Ethylene occurs naturally in soil, normally ranging from less than 1 ppm to about 2 ppm, but may often accumulate to as high as 10 ppm (Smith & Restall, 1971; Smith & Cook, 1975; Primrose & Dilworth, 1976). In contrast, many workers have reported that ethylene does not appear to be very inhibitory to fungi in general. Several fungi required more than 1000 ppm ethylene before germination was inhibited (Hora & Baker, 1975; Lynch, 1975) and some were stimulated by it (Hora & Baker, 1975). Balis (1976) was also unable to confirm the inhibitory properties of ethylene but suggested it might be somehow involved in the induced formation of another fungistatic compound which has been partially characterized as allyl alcohol.

Ko et al. (1974) extracted a volatile fungistatic compound from an alkaline soil which proved to be ammonium chloride, and an ammonium chloride solution was shown to have the same effect on a group of test fungi and bacteria as the soil and soil extract. This result clearly indicates that

ammonia may be involved in fungistasis in alkaline soils. Pavlica et al. (1978) also obtained evidence to implicate ammonia as a major factor in soil fungistasis in alkaline soils, but also detected formaldehyde in some soils at concentrations high enough to inhibit spore germination. The inhibitory effects of formaldehyde on some fungi could be overcome by the addition of exogenous nitrogen and carbon.

The volatile fungistatic compounds are probably mostly produced by the soil microflora. Thus Hora and Baker (1972b) found that volatile inhibitors were produced when sterilized soil was recolonized by soil actinomycetes, fungi or bacteria. Formation of inhibitors was also evident when sterilized soils were re-colonized by various Trichoderma species. They also found that soil actinomycetes are amongst the more active of the soil microflora in their capacity to produce volatile inhibitors. Satyanarayana and Johri (1974) also showed that a number of Penicillium spp, in particular P. javanicum could produce volatile inhibitors which were active against spores of Alternaria solani, Rhizopus nigricans and Trichoderma viride, both in vivo and in vitro.

Thus it seems that the failure of P. expansum spores to germinate in natural soil may be due to the shortage of nutrients or the presence of inhibitors, or to a combination of both. Therefore the next experiments were carried out to investigate the roles of nutrient shortage and the presence of inhibitory factors produced by other micro-organisms on spore germination and sporeling growth of P. expansum.

(ii) Sporeling growth in sterilized soil

Earlier results showed that sporeling growth occurred in sterilized soil after spore germination. However the amount of growth was very limited, leading to microcyclic conidiation only, with very little vegetative growth.

The term 'microcyclic' conidiation has been used to describe an asexual

cycle possessing a greatly reduced vegetative phase (Anderson & Smith, 1971).

Microcyclic conidiation has been reported to occur in a number of fungi but the factors controlling this form of development are not clearly understood. However Anderson and Smith (1971) consider that microcyclic conidiation in Aspergillus niger is initiated by factors which inhibit the apical growth process associated with rapid vegetative growth. Rotem and Bashi (1969) also concluded that sporulation may be induced by factors which partially or completely inhibit vegetative growth, and they observed that the formation of secondary spores in Alternaria solani in vitro was induced by treatments which interfered with normal germination of the mother spores. These treatments included the application of toxins, shortage of nutrients, shock caused by high or low temperature, intermittent drying and exposure to solutions with a high osmotic potential.

Park and Robinson (1969) observed that when Geotrichum candidum is grown on cellophane over cultures of the same or other fungi, sporulation was induced in sporelings at a much earlier stage of development than normal. They presented evidence indicating that the induction of sporulation was due either to a diffusible fungal metabolite or to the depletion of a factor essential for the maintenance of vegetative growth. It was further stated that above critical nutrient levels of carbon and nitrogen the vegetative phase can be maintained even under conditions which would otherwise induce sporulation. The rapid reduction of exogenous nutrients, caused by the high percentage germination of conidia may be the factor stimulating the formation of microcyclic conidia. Timnick et al. (1952) observed that cultures of Melanconium fuligineum, growing on agar plates, inoculated at one point by mycelium or a few spores required 11 to 14 days for sporulation, with the production of approximately 1.2 billion spores per culture occurring by about 20 days. In contrast, when concentrated suspensions of spores were used to flood the agar plates, the formation of spores began within 24h and

approximately 2.5 billion spores were produced per culture within 4 days. In the latter case the first spores were produced on the germ-tubes and very young mycelium. He also found that when cultures were leached continuously in a stream of water they began to sporulate within 24h, while the sporulation of other cultures which were continuously supplied with fresh liquid medium, was delayed for three days or more although vegetative growth was abundant. Zeidler and Margalith (1973) examined the effects of the addition of certain amino acids to the growth medium on microcyclic conidiation in Penicillium digitatum and found that glutamic acid, aspartic acid, proline, arginine, serine and alanine accelerated it in varying degrees while other amino acids had either no effect, delayed it or inhibited it more or less completely. Morton (1961) examined the conditions required for conidiation by several Penicillium spp in submerged cultures and concluded that the most suitable condition was the absence or exhaustion of available nitrogen when an assimilable carbon supply still remained. However Jicinska (1968) observed that carbohydrate starvation can also induce conidiation in certain Penicillium species. Righelato et al. (1968) have shown that when Penicillium chrysogenum is grown in a chemostat culture that glucose is essential for conidiation and that conidiation occurred when the glucose feed rate was between 0.022g g^{-1} mycelial dry weight hr^{-1} and 0.056g g^{-1} mycelial dry weight hr^{-1} with maximum conidiation at a feed rate of 0.038g g^{-1} mycelial dry weight hr^{-1}. At a feed rate of 0.022g g^{-1} mycelial dry weight hr^{-1} or slightly below, the growth rate was zero with all the glucose being used for maintenance only, and above 0.056g g^{-1} mycelial dry weight hr^{-1} only vegetative growth occurred.

The temperature also may be the cause of microcyclic conidiation in the soil. Thus Anderson and Smith (1971, 1972) showed that an elevated temperature of 44°C induced conidial swelling and inhibited vegetative growth in Aspergillus niger. A temperature shift down to 30°C induced the swollen conidia to produce germ-tubes which developed to form conidiophores and conidiogenesis occurred. Cortat and Turian (1974) also observed that if

conidia of Neurospora crassa are shaken in liquid cultures at 46°C for 15h and then the temperature is reduced to 25°C, they germinate directly to form conidiophores.

SECTION 2. EXPERIMENTS ON FUNGISTASIS

4.2.1. Role of nutrient shortage

4.2.1.1. Nutrient requirements for the germination of P. expansum spores in vitro

4.2.1.1.1. Introduction

Before investigating if the failure of P. expansum spores to germinate in natural soils could be due to a shortage of nutrients, it is necessary to know the nutrient requirements for spore germination.

Many fungal spores will germinate in distilled water while others require a supply of nutrients for germination. Cochrane (1960) states that in general the relatively short-lived asexual spores of Ascomycetes and Fungi Imperfecti require exogenous organic carbon, nitrogen and occasionally other compounds for germination. Most workers report that Penicillium spp require both carbon and nitrogen to support a high percentage germination (Gottlieb & Tripathi, 1968; Fletcher & Morton, 1970; Martin & Nicolas, 1970). Fletcher and Morton (1970) observed that the main requirement for germination of Penicillium griseofulvum conidia was a suitable exogenous carbon source such as glucose, but that maximum germination required the addition of nitrate and phosphate in the medium. Pelser and Eckert (1977), working with P. digitatum, found that glucose was significantly more effective than other carbohydrates in

supporting germination and germ-tube growth. They however found that although nitrogen compounds were not required for germination, amino acids and vitamins together substantially increased germ-tube growth. In contrast Thom (1910) stated that P. digitatum required nitrogen for germination which could be provided by ammonium salts but not by sodium nitrate. He found that the failure to germinate in the presence of the nitrate ion was not due to its toxicity but to the inability of the fungus to use the ion as a source of nitrogen.

Brian (1933) observed that P. expansum would not germinate in water but required certain exogenous nutrients including glucose, nitrate and phosphate.

The following experiments were carried out to determine the effects of carbon and nitrogen sources on the germination of the isolate of P. expansum used in this investigation.

4.2.1.1.2. Materials and methods

All the glassware used in the experiments was washed by steeping in chromic acid solution for 24h before rinsing in sterilized distilled water.

Spores of P. expansum were collected from 7 day old cultures on malt extract slopes using a sterilized camel hair brush to minimise nutrient transfer. They were then suspended in 10ml sterilized distilled water plus Tween 80 (one drop 100 ml^{-1} water). The spore suspension was filtered through four layers of sterilized muslin to remove any mycelial fragments.

3ml of the filtered suspension was then placed into a sterilized centrifuge tube and centrifuged at low speed for 15 minutes. The supernatant was discarded and the spore pellet resuspended in sterilized distilled water plus Tween 80 using a Whirlimixer before centrifuging again. This process was repeated until the spores had been washed 5 times to ensure that little carry-over of nutrients from the medium occurred.

0.2ml of the washed and unwashed spore suspensions were each added to 5ml of different concentrations of the nutrient solutions (glucose or sodium nitrate).

Five acid washed sterilized slides were prepared from each nutrient solution by placing one drop on the surface. Each slide was placed on a sterile glass support in a petri-dish. At the bottom of the dish was a piece of filter paper saturated with sterile distilled water. The plates were incubated for 24h at 23°C in an incubator. After 24h one drop of cotton-blue was added to each of the slides and mixed with the spores. A clean coverslip was placed over the specimen and the slide was examined microscopically.

4.2.1.1.3. Results

(i) The effect of glucose on germination

The results are given in fig. 2 & 3 and Appendix Tables 4A & B.

In the sterile distilled water controls only 5% of the washed or unwashed spores germinated, but increasing concentrations of glucose lead to higher germination of both washed and unwashed spores. The washed and unwashed spores responded in a similar manner in all treatments (fig. 2).

Germ-tube length increased with increasing concentration of glucose reaching a maximum with 2.5mg glucose ml^{-1} and remaining constant up to at least 20mg glucose ml^{-1}, the highest level incorporated. The growth for washed spores was slightly but generally not significantly lower than that of unwashed spores (fig. 3).

(ii) The effect of sodium nitrate on spore germination

The results of this experiment are given in fig. 4 & 5 and Appendix Tables 5A & B.

They show that sodium nitrate is not required for germination.

Fig. 2

Percentage germination of P. expansum spores at different concentrations of glucose, after 24h incubation at 23°C.

--------- washed spores

———————— unwashed spores

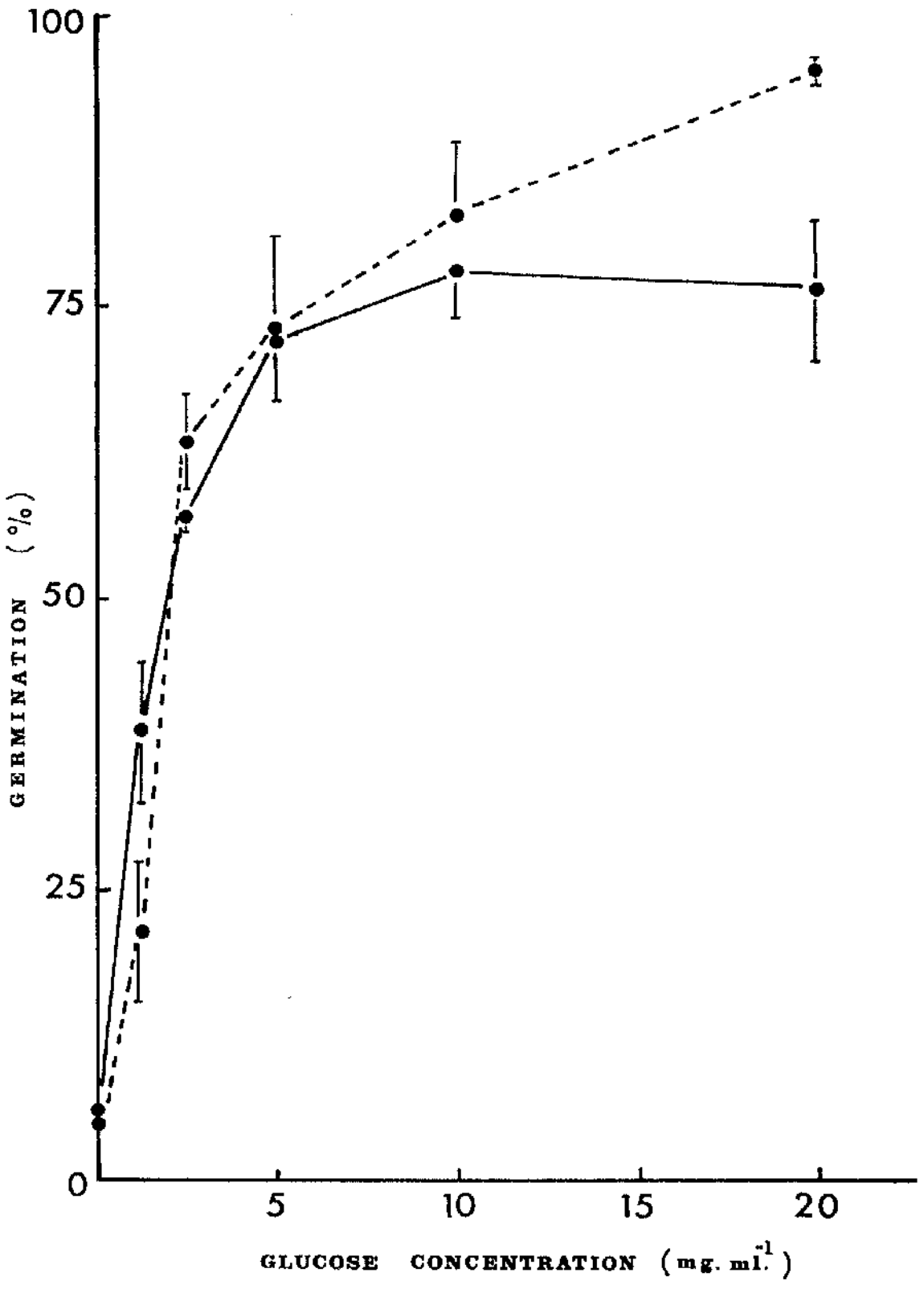

100
75
50
25
0
GERMINATION (%)
5
10
15
20
GLUCOSE CONCENTRATION (mg. ml.-1)

Fig. 3

Length of germ-tubes of P. expansum spores at different concentrations of glucose, after 24h incubation at 23°C.

----- washed spores

——— unwashed spores

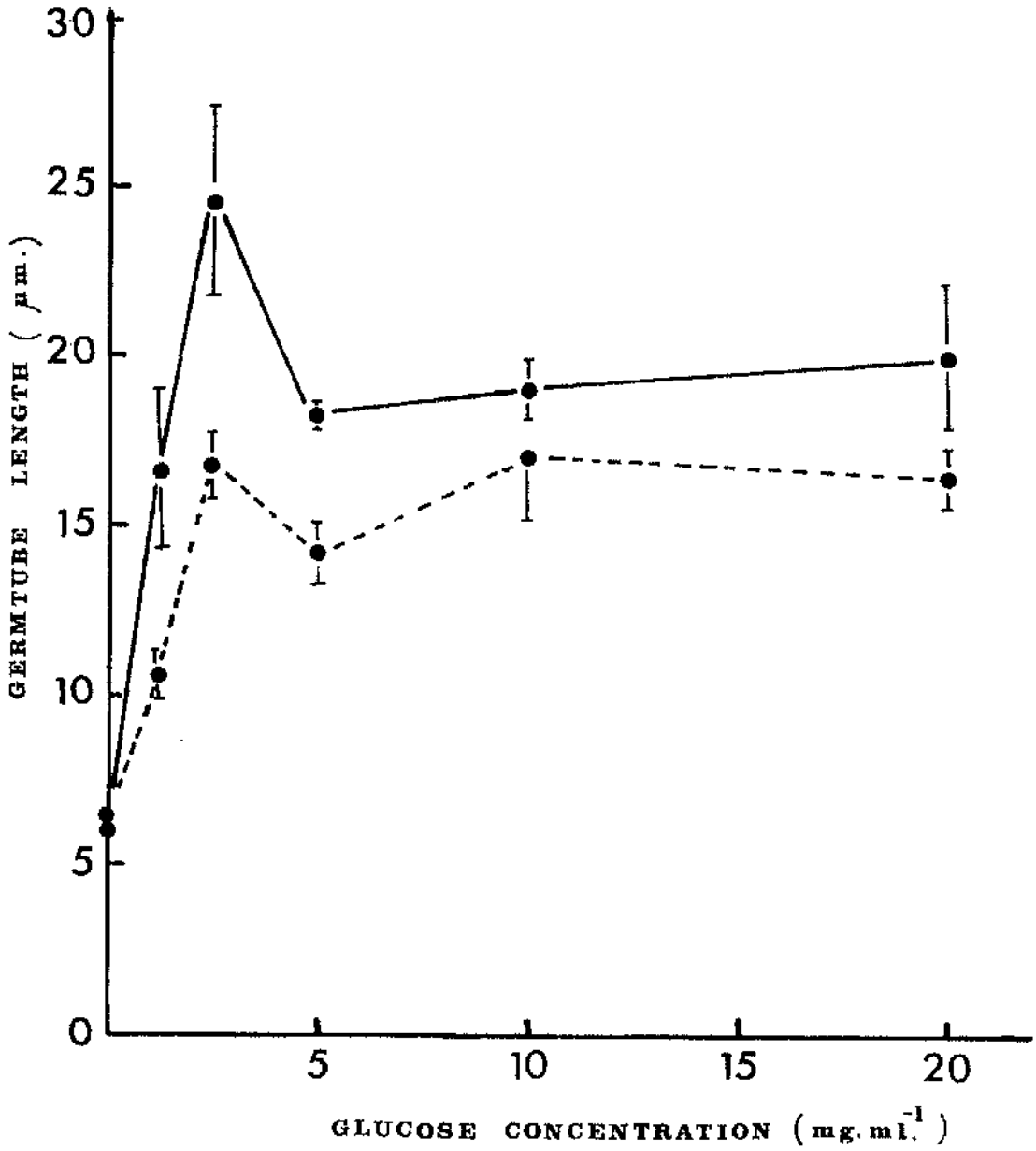

30
25
20
15
10
5
0
GERMTUBE LENGTH (µm.)
5
10
15
20
GLUCOSE CONCENTRATION (mg. ml.-1)

Fig. 4

Percentage germination of P. expansum spores at different concentrations of sodium nitrate, after 24h incubation at 23°C.

----- washed spores

——— unwashed spores

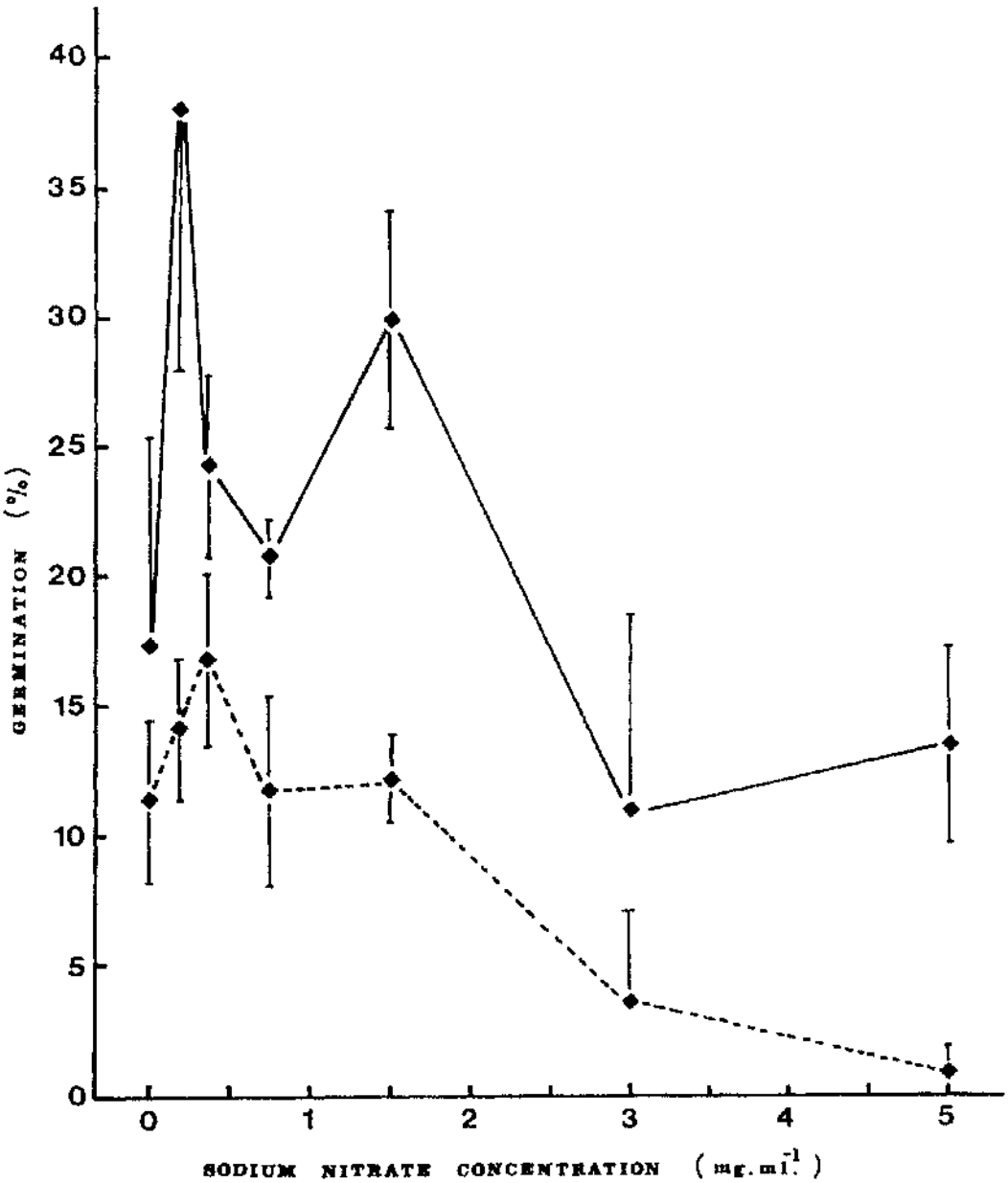

40
35
30
25
20
15
10
5
0
GERMINATION (%)
0
1
2
3
4
5
SODIUM NITRATE CONCENTRATION (mg.ml⁻¹)

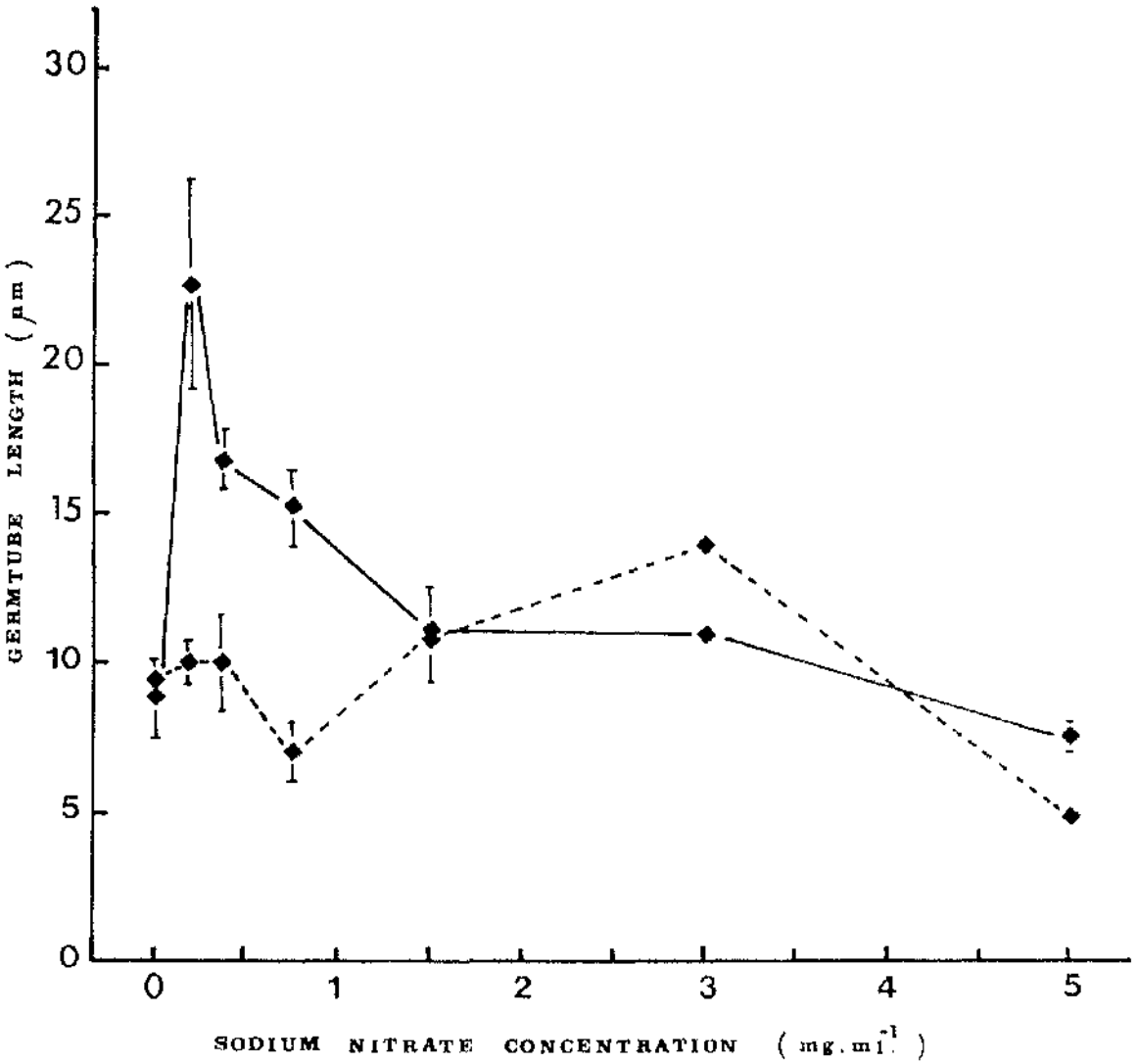
Fig. 5

Length of germ-tubes of P. expansum spores at different concentrations of sodium nitrate, after 24h incubation at 23°C.

Value at 300mg $NaNO_3$ ml^{-1} and above except for 500mg of unwashed spores based on too few samples to be able to calculate S.E.

----- washed spores

——— unwashed spores


30
25
20
15
10
5
0
GERMTUBE LENGTH (μm)
0
1
2
3
4
5
SODIUM NITRATE CONCENTRATION (mg.ml⁻¹)

Low concentrations stimulate germination and germ-tube growth, but high concentrations have no effect or are inhibitory.

4.2.1.1.4. Discussion

Brian (1933) found that P. expansum spores seldom germinated in water. This investigation also showed that washed and unwashed spores displayed low percentage germinations in water (6-17%). Brian (1933) also found that phosphate and nitrate together with sugars were required for germination. In contrast this investigation showed that glucose alone supported a high percentage germination of both washed and unwashed spores. Nitrogen, supplied as $NaNO_3$, stimulated germination at low concentrations but inhibited it at high concentrations. These results are similar to those of Pelser and Eckert (1977). They found that glucose was significantly more effective than other carbohydrates in supporting spore germination and germ-tube growth in P. digitatum. They also showed that nitrogen compounds were not required for germination, but amino acids and vitamins together substantially increased germ-tube growth.

4.2.1.2. The effect of added nutrients on spore germination and germ-tube growth of P. expansum in soil

4.2.1.2.1. Introduction

In the last section it was reported that P. expansum spores did not germinate in unsterilized soil and it was considered that one factor might be the shortage of nutrients in such soils. The last experiment showed that P. expansum has a requirement for glucose only to germinate in vitro.

According to Hsu and Lockwood (1971) natural soils contain little or no detectable carbohydrates and fungal spores incubated directly on such soils fail to germinate. This inhibition of spore germination can often be annulled by the addition of nutrients (Lockwood, 1964).

Dobbs and Hinson (1953) observed that the spores of Penicillium frequentans would germinate in soil if the soil was moistened with dilute solutions of glucose plus inorganic salts. However if a solution of asparagine or asparagine plus a mineral salt solution were used the spores failed to germinate. Toussoun et al. (1960) showed that the germination of F. solani f.sp. phaseoli in soil was accelerated by the application of glucose. Jackson (1960) reported that the germination of Penicillium citrinum conidia on agar discs over soil increased with increasing concentrations of certain monosaccharides in the agar discs. Disaccharides and trisaccharides had less effect, while mineral salts and amino acids failed to overcome mycostasis. Dobbs et al. (1960) reported that glucose by itself or when incorporated with mineral salts gave similar results. They also reported that different soils varied widely in the degree to which the addition of glucose could overcome mycostasis. Thus beech wood soil supported some germination with the addition of a 0.1% (w/v) of glucose but a garden soil required a 4% (w/v) solution before there was any effect.

Ayanrus and Green (1974) observed that sclerotia of Macrophomina phaseolina germinated abnormally on the surfaces of soils but germinated normally if the soil was amended with certain sugars, amino acids, organic acids and their hyphae then colonized the soil surface.

In the following experiments a similar approach, the addition of nutrients to the soil, has been adopted to determine if a shortage of nutrients could be responsible for the failure of P. expansum to germinate in natural soil.

4.2.1.2.2. Materials and methods

Seven sterile 15.2cm pots were each filled with 400g of sieved soil, which had been previously air-dried to approximately 5% moisture content. Each pot was then saturated with 500ml of a malt-extract medium at one or other of the following concentrations: $^{1}/_{8}$ or $^{1}/_{2}$ normal strength, normal

strength or 2x, 3x or 4x normal strength. The amended soils were left for about 2-3h in the pots to allow excess medium to drain away and then emptied out onto a sterilized polythene bag and dried at room temperature using a fan to assist drying. After drying to about 20% moisture content the soils were placed in plastic petri-dishes. A spore smear slide of P. expansum was then buried in each dish and after incubating for 24h at 23°C the slides were removed. They were examined, after staining with lactophenol cotton-blue. This experiment was repeated.

4.2.1.2.3. Results

The results of both experiments are given in figs. 6, 7, 8 & 9 and Appendix Tables 6A, B, C & D.

The percentage germination and the lengths of germ-tubes produced increased in both experiments in response to increasing nutrient levels (fig. 6 & 7). Many germ-tubes developed microcyclic conidia (Plate 16) and the percentage doing so increased in both experiments up to about 3x normal strength, but above this concentration more vegetative mycelium was produced with fewer conidiophores (fig. 8).

Germ-tubes developing microcyclic conidia were never observed to branch and only a single phialide with a short chain of conidia was formed at their tips (Plate 16). The percentage of the germ-tubes with or without phialides in which the protoplasm was undergoing lysis was very variable and there was no obvious relationship with nutrient levels (fig. 9).

4.2.1.2.4. Discussion

It is evident from both experiments that the addition of nutrients, in the form of malt-extract broth, to soil removes fungistasis and allows the spores of P. expansum to germinate. However its addition at 3 to 4 times normal concentration is required to remove fungistasis completely. After

Fig. 6

Percentage of P. expansum spores germinating in malt-extract broth supplemented soil after 24h incubation at 23°C.

(a) 1st experiment

(b) 2nd experiment

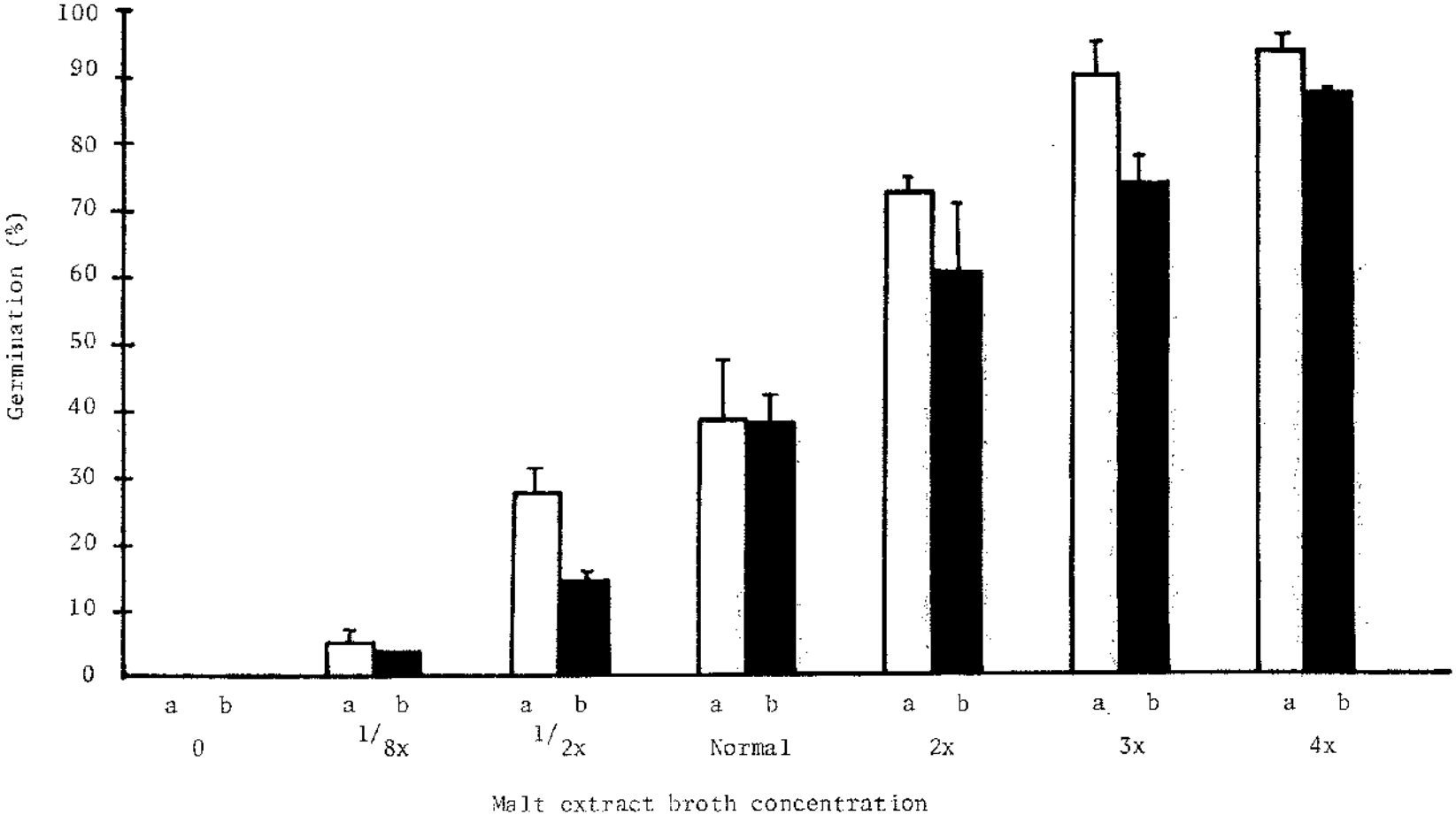
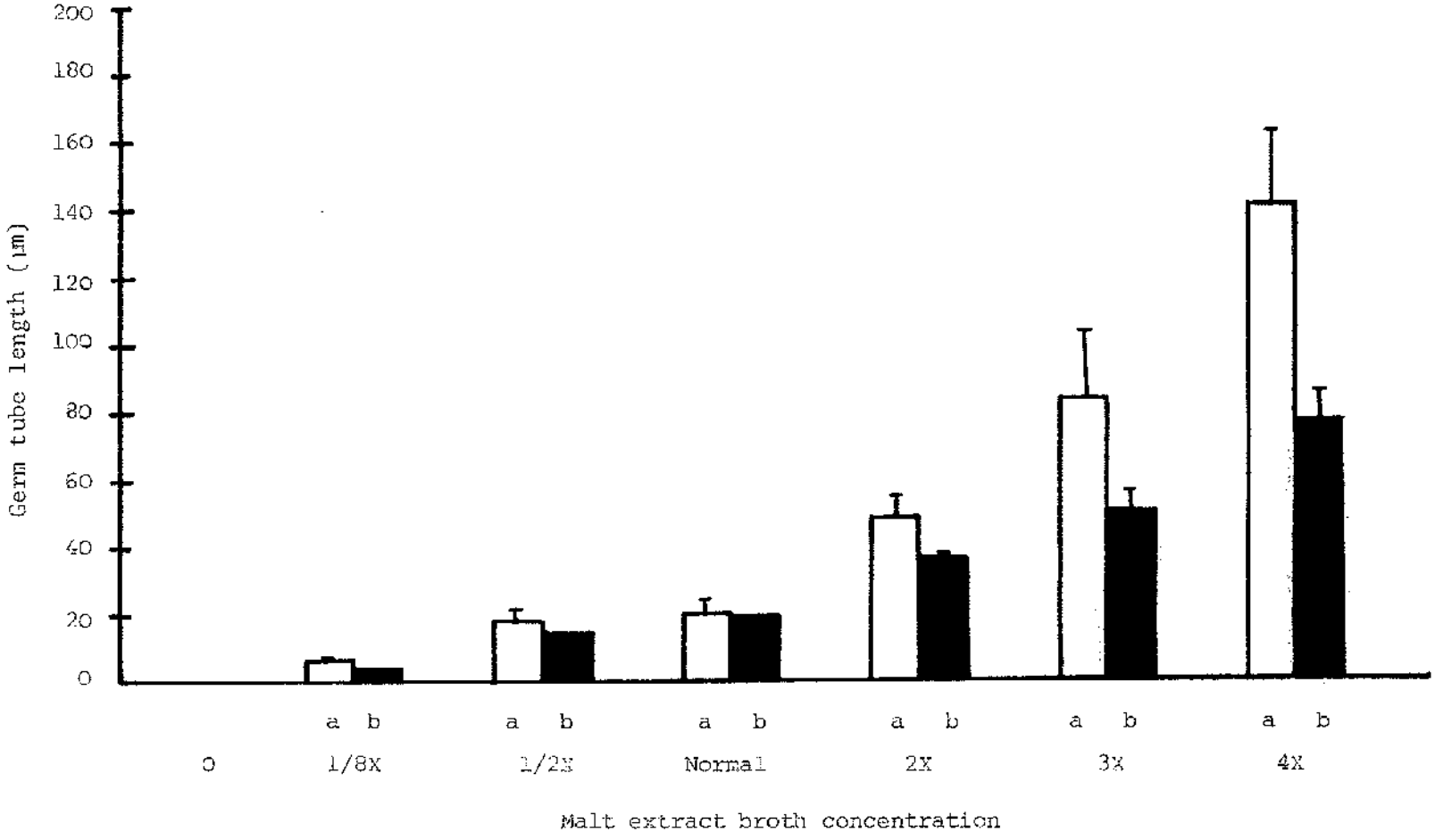

Germination (%)
100
90
80
70
60
50
40
30
20
10
0
a b
0
a b
1/8x
a b
1/2x
a b
Normal
a b
2x
a b
3x
a b
4x
Malt extract broth concentration

Fig. 7

Germ-tube length of P. expansum after 24h incubation at 23°C in malt-extract broth supplemented soil.

(a) 1st experiment

(b) 2nd experiment


Germ tube length (µm)
200
180
160
140
120
100
80
60
40
20
0
a b
a b
a b
a b
a b
a b
0
1/8X
1/2X
Normal
2X
3X
4X
Malt extract broth concentration

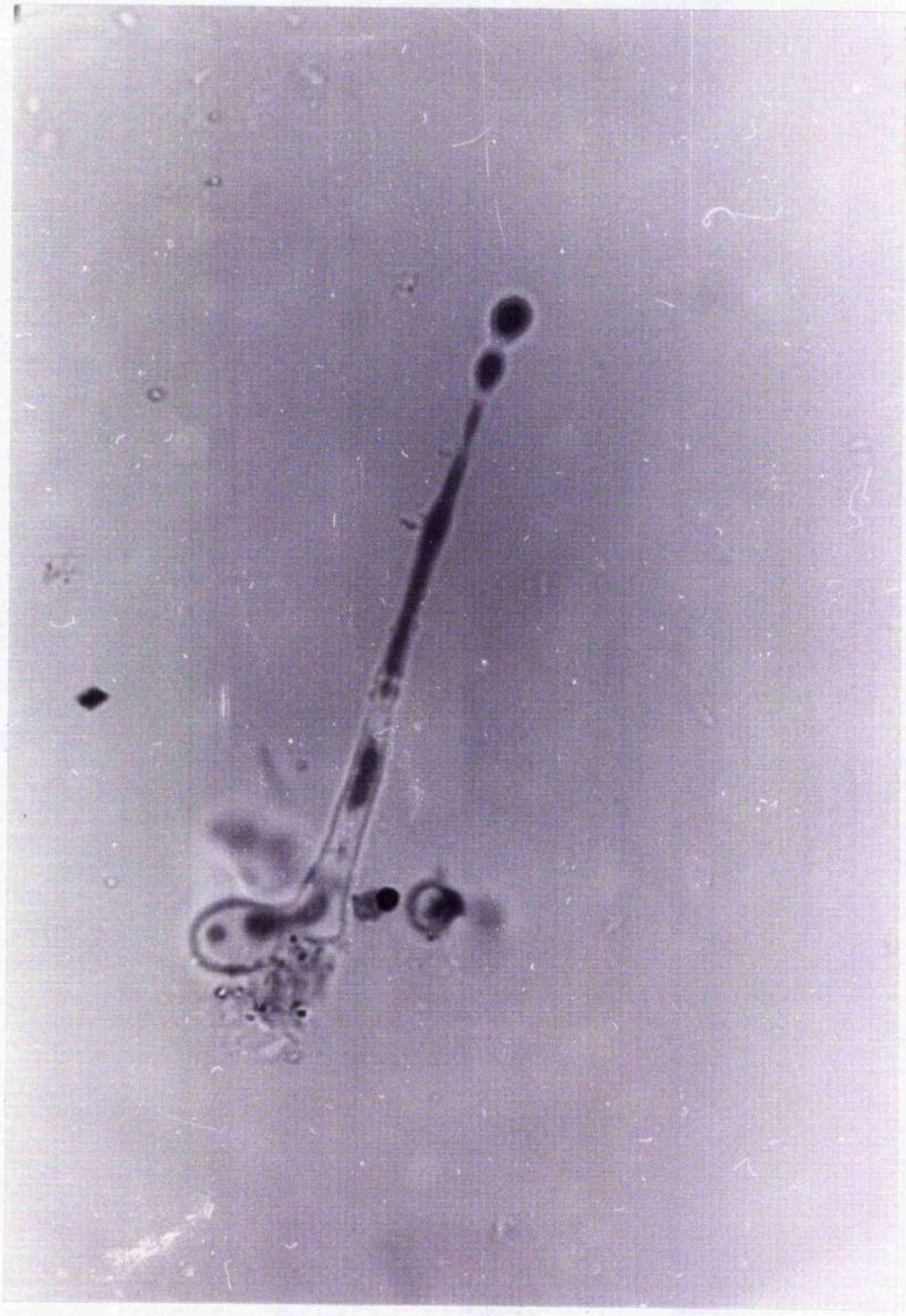


Plate 16. Photomicrograph of germinated conidium of P. expansum with microcyclic conidia at the tip of a germ-tube in malt-extract supplemented soil.

Fig. 8

Proportion of germ-tubes of P. expansum spores producing microcyclic conidia after 24h incubation at 23°C in malt-extract broth supplemented soil.

(a) 1st experiment

(b) 2nd experiment

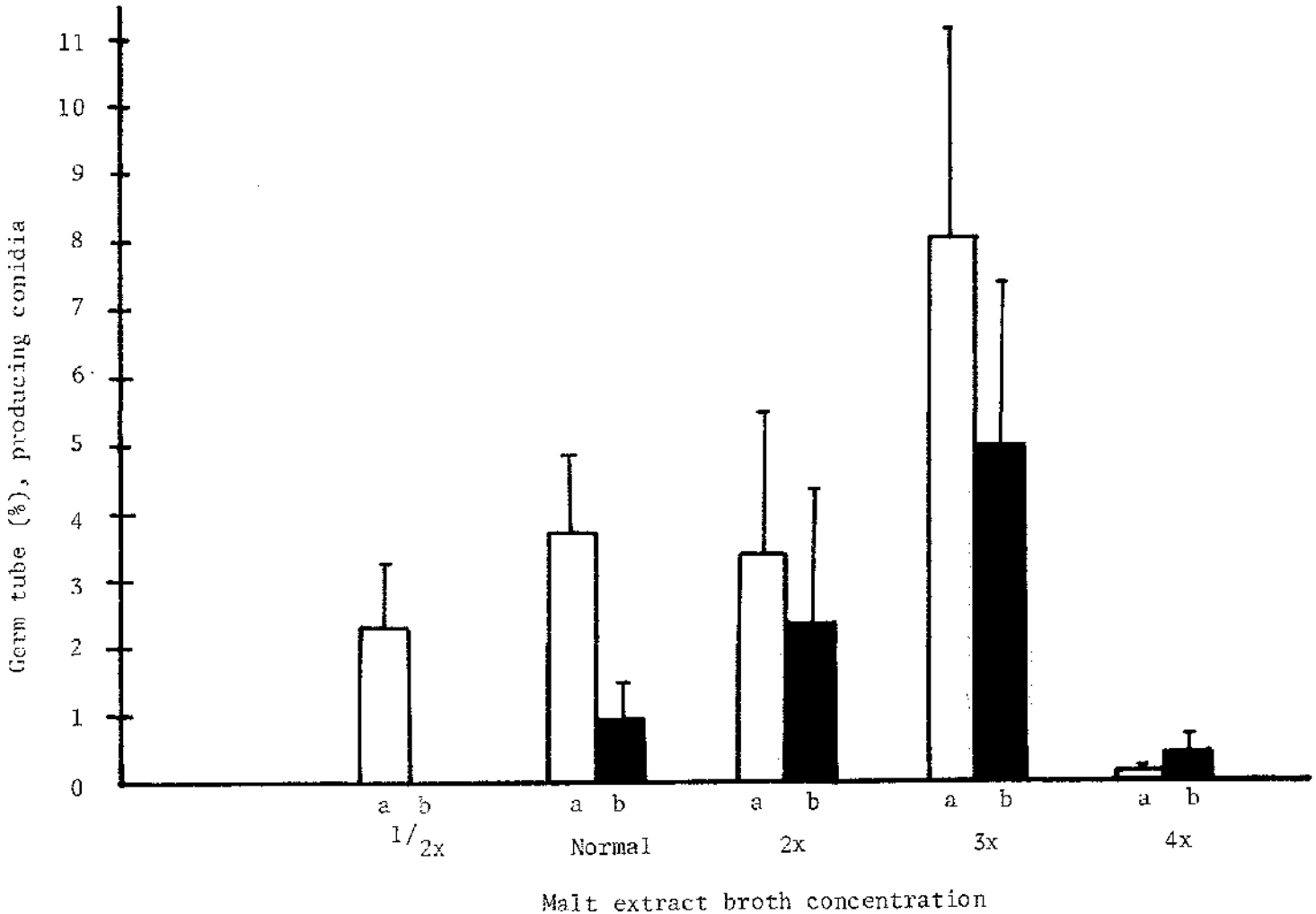
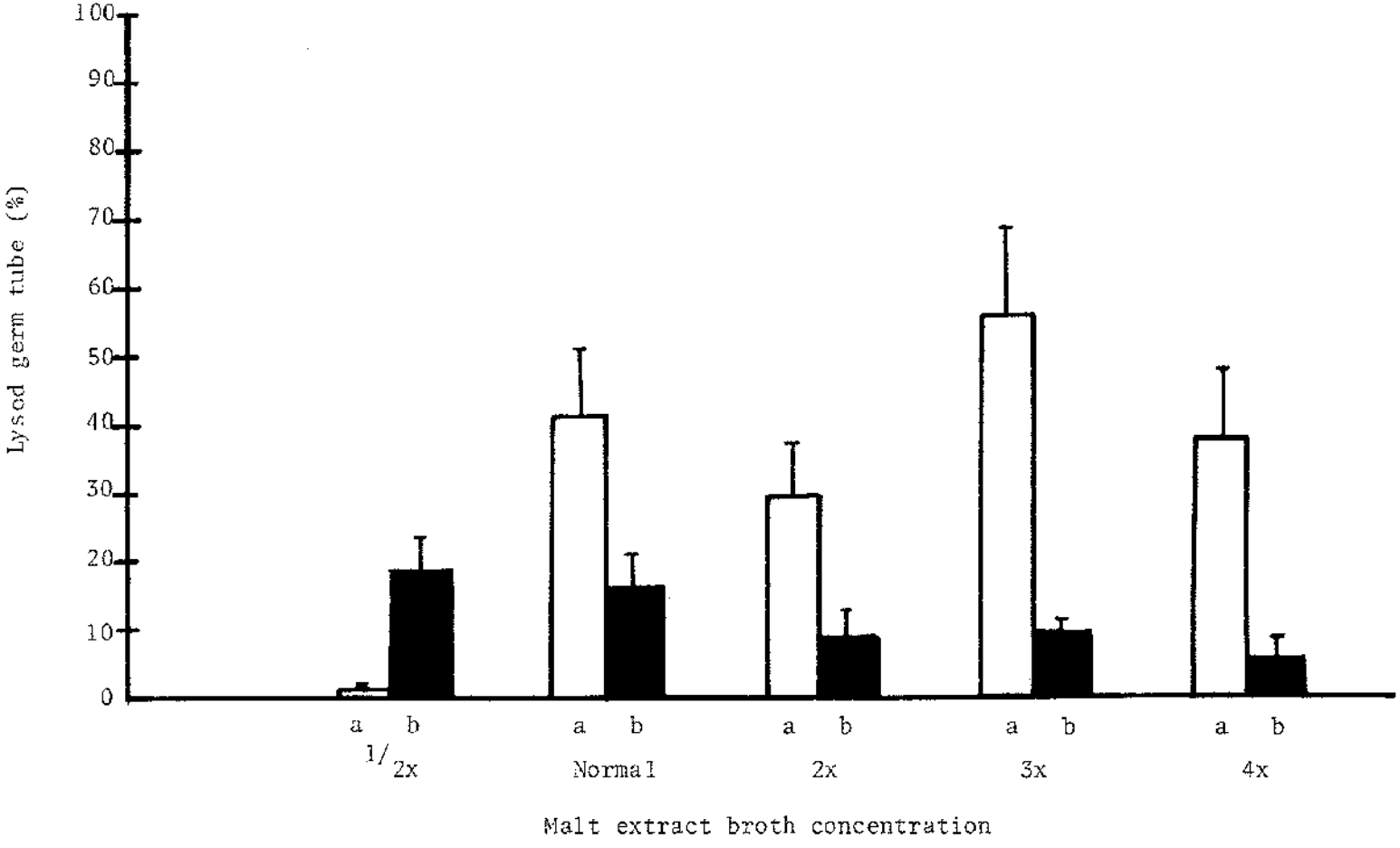

Germ tube (%), producing conidia
11
10
9
8
7
6
5
4
3
2
1
0
a b
1/2x
a b
Normal
a b
2x
a b
3x
a b
4x
Malt extract broth concentration

Fig. 9

Proportion of lysed germ-tubes of P. expansum spores after 24h incubation at 23°C in malt-extract broth supplemented soil.

(a) 1st experiment

(b) 2nd experiment


Lysed germ tube (%)
100
90
80
70
60
50
40
30
20
10
0
a b
1/2x
a b
Normal
a b
2x
a b
3x
a b
4x
Malt extract broth concentration

germination a proportion produced single phialides with a chain of conidia at the tip of unbranched germ-tubes.

Boosalis (1960) made similar observations in work with Helmintho-sporium sativum. He found that the addition of potato dextrose broth stimulated conidial germination up to 75% and that approximately 50% of the germinated spores produced unbranched germ-tubes bearing single viable conidia.

4.2.2. The effect of diffusible inhibitors in soil on spore germination and germ-tube growth of P. expansum

4.2.2.1. Introduction

A second possible explanation for the inhibition of spore germination in the garden soil was the presence in the soil of diffusible inhibitors. The following experiment employs a method originally devised by Hora and Baker (1970) to investigate the presence of such inhibitors in the soil.

4.2.2.2. Materials and methods

Soil was collected, sieved and half the volume sterilized as described in Chapter II. The moisture content and pH of both the sterilized and unsterilized fractions was 25% and 6.1 respectively.

'A' size Geneco-biovessels were filled with 100g unsterilized soil. Boiled, sterilized cellophane was then placed on the top of the soil and agar discs (3% Oxoid purified agar + 1% peptone (Dix, 1972)) 2-3mm thick and 12mm diam. were placed on top of the cellophane. Geneco-biovessels containing 100g sterilized soil served in one set of controls and in another set agar discs were placed on cellophane in contact with 1% Oxoid purified agar in petri-dishes.

To determine if the inhibitors were volatile some agar discs were placed on sterilized glass slides in the Geneco-biovessels instead of on

cellophane. As a further control agar discs were placed on microscope slides in petri-dishes without soil.

All containers were incubated for 24h at 23°C and after 24h one agar disc from each Geneco-biovessel was removed and placed on a sterilized glass slide in a petri-dish whose internal atmosphere was maintained at a high humidity. These discs were called pre-activated discs. Other discs were left in the Geneco-biovessels in contact with soil. All agar discs were then inoculated with one drop of spore suspension (79.16×10^4 spores ml^{-1}) obtained from 7 day old cultures of P. expansum. After 12h incubation at 23°C the discs were removed, stained with cotton-blue and examined under the microscope. At least 400 spores selected at random were counted to assess percentage germination. The lengths of 50 germ-tubes were also measured.

4.2.2.3. Results

The results are given in fig. 10 & 11 and Appendix Tables 7A & B.

Only 9% of the spores germinated on agar discs placed on cellophane in contact with unsterilized soil. In contrast, 96-99% germinated on the agar discs on cellophane in contact with sterilized soil or in the 1% agar controls (fig. 10). There was, however, no significant difference between the percentage germination on pre-activated agar discs (removed after 24h in contact with soil before inoculation) and that on agar discs which remained in contact with the soil. Similarly there was no significant differences between pre-activated discs and other discs in the control treatments.

Germ-tube growth on agar discs in treatments containing sterilized soil or 1% agar was also greater than on discs in contact with unsterilized soil (fig. 11). There was no marked difference between germ-tube growth on pre-activated agar discs and on agar discs which were left in contact with unsterilized soil, but in contrast there was a marked difference between

Figs. 10 & 11

Effect of diffusible inhibitors from soil on the percentage germination and germ-tube growth of P. expansum spores. Agar discs inoculated with a spore suspension and incubated for 12h at 23°C.

p Agar discs were incubated 24h in contact with the soil for 24h, then removed, inoculated and incubated in petri-dishes (pre-activated discs).

c Agar discs were incubated in contact with the soil before and after inoculation.

U unsterilized soil.

S sterilized soil (control).

A 1% agar (control).

Fig. 10

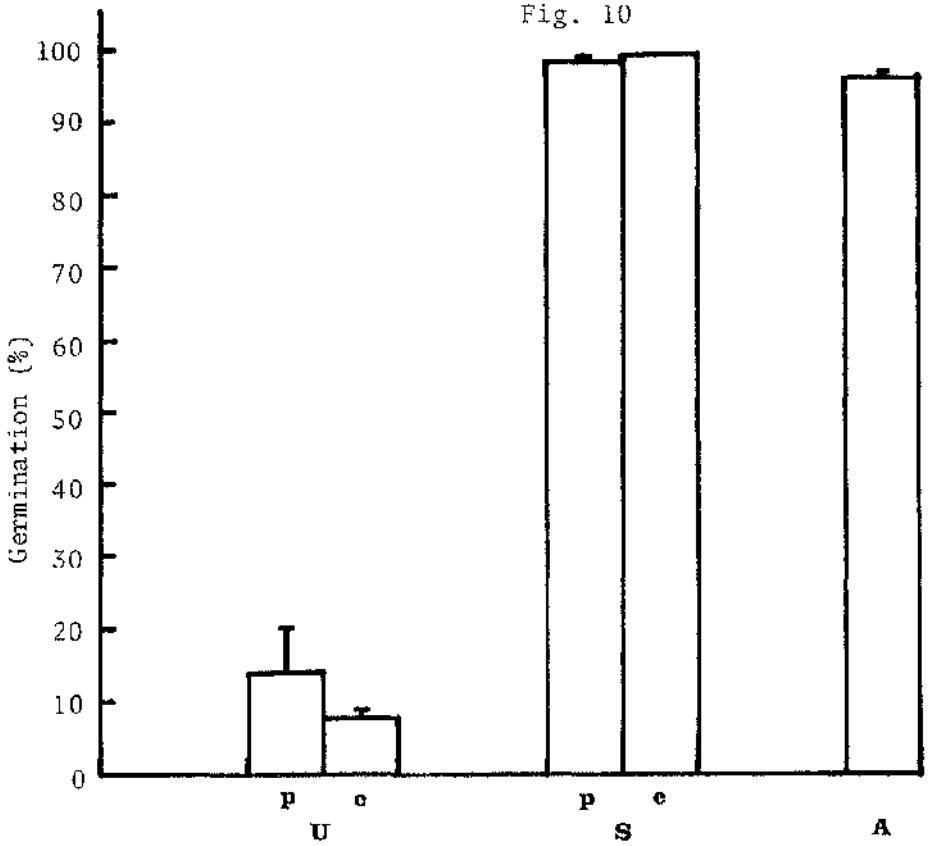
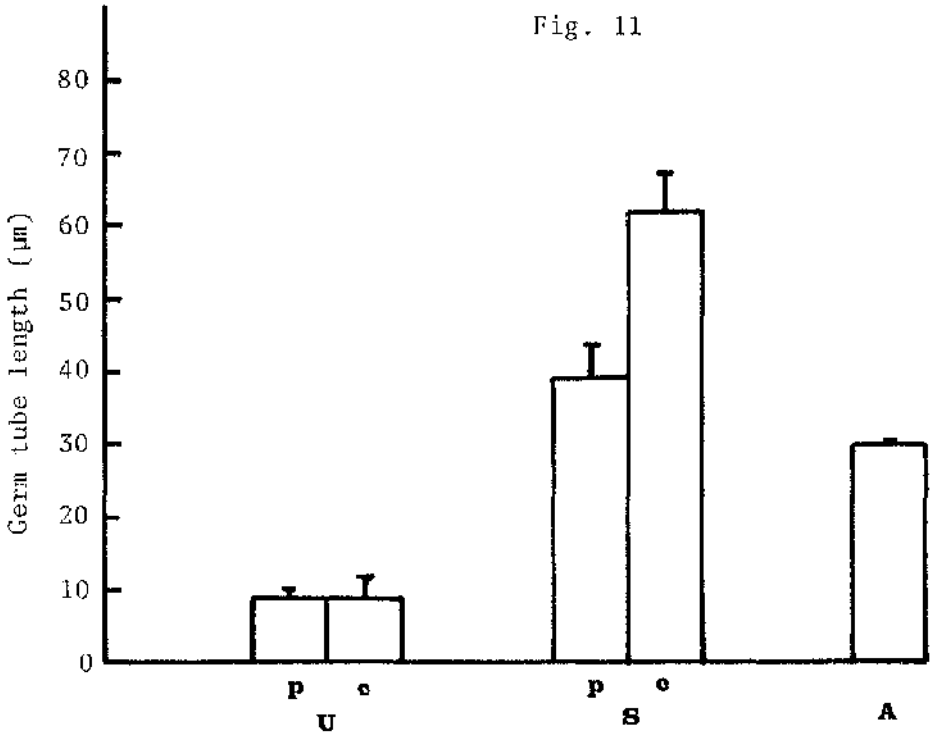

Germination (%)
100
90
80
70
60
50
40
30
20
10
0
p
c
U
p
c
S
A

Fig. 11


Germ tube length (µm)
80
70
60
50
40
30
20
10
0
p
c
U
p
c
S
A

growth on discs in the sterilized soil treatments. In these treatments the germ-tube length was greater on discs which were left in contact with the soil than on the pre-activated discs which were removed after 24h in contact with the soil. Germ-tube growth on agar discs in contact with sterilized soil was also higher than that on the discs in contact with 1% agar (fig. 11).

The volatility of the diffusible inhibitors

The results, given in figs. 12 & 13 and Appendix Tables 8A & B, show that the discs in all treatments supported a high percentage (97-99%) of spore germination and there were no significant differences between any of them.

There were also no significant differences in germ-tube growth between any of the treatments except in the case where the agar discs were left in contact with the sterilized soil where greater growth was found (fig. 13).

4.2.2.4. Discussion

The results provide no evidence for volatile inhibitors affecting P. expansum spore germination and germ-tube growth in soil. However non-volatile or relatively non-volatile inhibitory compounds capable of diffusing through agar were present, which could markedly reduce germination and germ-tube growth (fig. 10 & 11). Dix (1972), using a similar method, which used filter paper instead of cellophane to separate the agar discs from the soil, found fungistatic effects on the speed of germination, the final percentage germination and the growth rate of germ-tubes of P. expansum.

The diffusible substances are probably produced by micro-organisms since if the soil is sterilized by autoclaving it loses its activity. However such treatments do not conclusively prove this because autoclaving may affect nutrient levels in the soil which may then reduce or remove the effect of toxic substances in the soil, thus allowing the spores to germinate

Fig. 12 & 13

The volatility of the inhibitors of spore germination and germ-tube growth. Agar discs inoculated with a spore suspension and incubated for 12h at 23°C.

p Agar discs were incubated for 24h in contact with soil before inoculation (pre-activated discs).

c Agar discs were incubated in contact with soil before and after inoculation.

U Unsterilized soil.

S Sterilized soil (control).

W Without soil (control).

Fig. 12

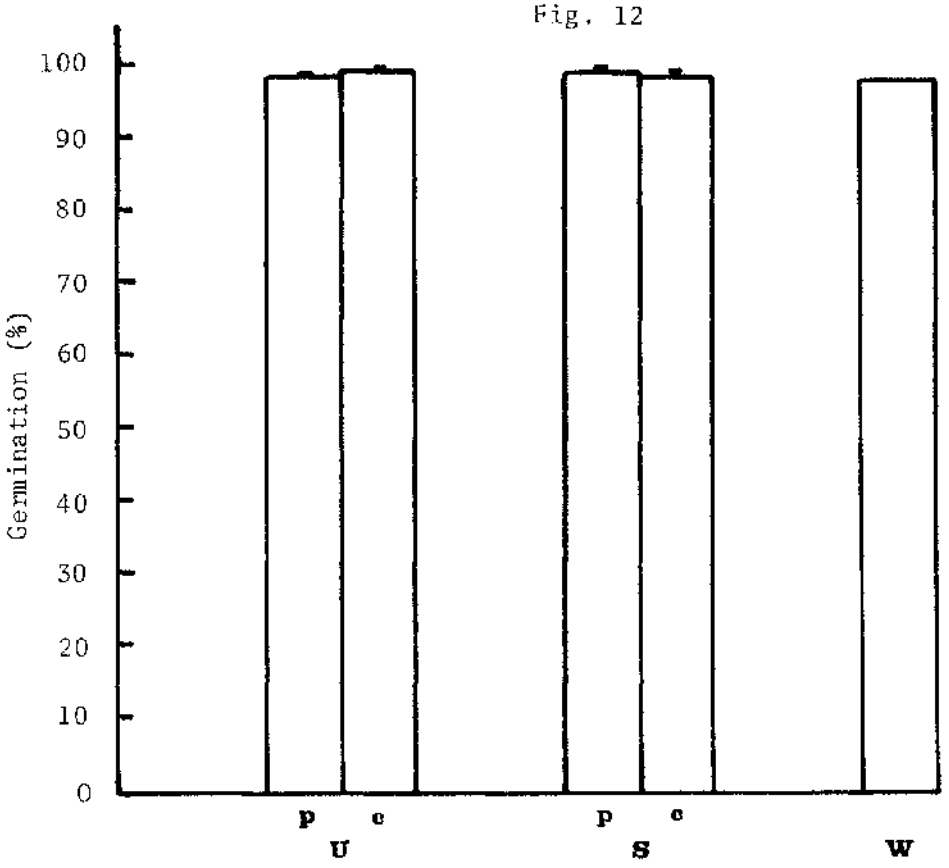
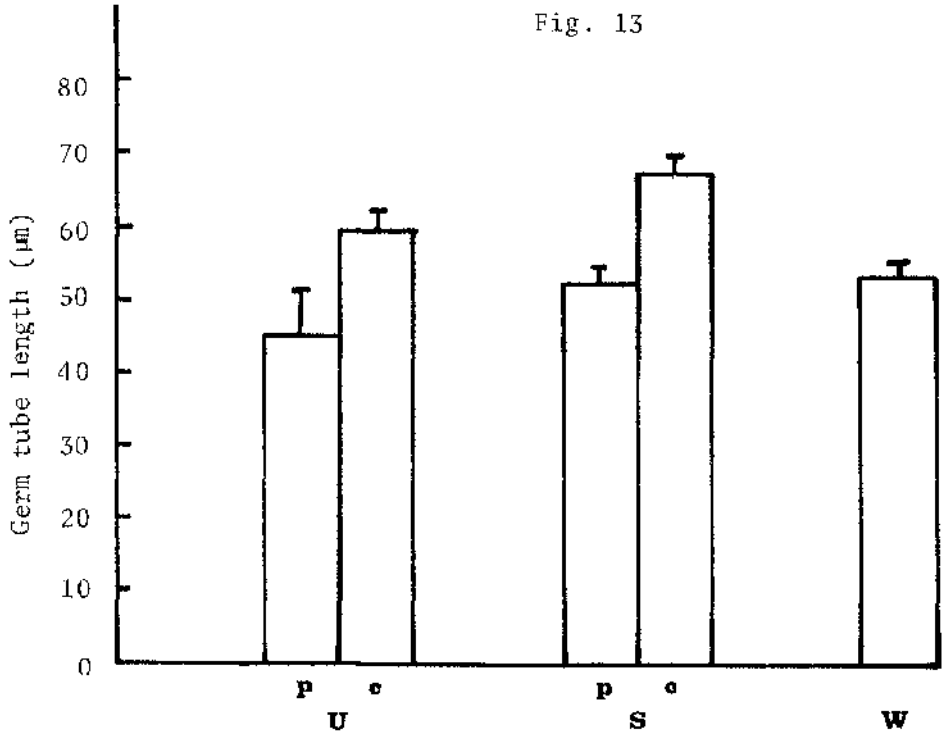

Germination (%)
100
90
80
70
60
50
40
30
20
10
0
p
e
U
p
e
S
W

Fig. 13


Germ tube length (μm)
80
70
60
50
40
30
20
10
0
p
e
U
p
e
S
W

and grow. According to Ko and Lockwood (1967) reduced germination on agar discs in contact with soil may also result from dilution of nutrients due to their diffusion from the disc into soil. However in these experiments, when agar discs were placed on cellophane in contact with 1% purified agar to allow such diffusion to occur, the percentage germination remained high. Such results indicate that the effect cannot be attributed to depletion of nutrients from the discs. In any case these results indicate that germination inducers may diffuse into the discs from the soil since germ-tube growth on discs placed on cellophane in contact with sterilized soil was greater than on discs on 1% agar. Further support for the diffusion of growth stimulators from sterile soil is given by the observation that germ-tube growth was lower on the pre-activated discs, which were incubated in petri-dishes after inoculation, than on discs left in contact with the sterilized soil after inoculation.

Thus a major cause of the inhibition of germination and germ-tube growth in natural soil appears to be the presence in the soil of relatively non-volatile diffusible inhibitors. Such factors will clearly interact with nutrients which other work reported earlier in this chapter showed to be important.

4.2.3. The effect of quantitative and qualitative changes in the soil microflora on inhibition of spore germination

4.2.3.1. Introduction

Two methods were used to change the soil microflora.

Quantitative changes were brought about by diluting natural soil with autoclaved soil. This method would reduce the populations of all micro-organisms in proportion to dilution.

Qualitative changes were brought about by heating the soil to different temperatures in order to selectively destroy particular groups of micro-

organisms. These treatments are likely to reduce the populations of surviving species and also to bring about quantitative changes as well as qualitative changes.

The effects of the changes on spore germination and germ-tube growth were investigated.

4.2.3.2. Effects of quantitative changes in the soil microflora on spore germination and germ-tube growth

4.2.3.2.1. Materials and methods

Unsterilized soil was mixed with autoclaved soil to give the following percentage compositions of unsterilized soil: 100%, 75%, 50%, 25% and 0%. Plastic petri-dishes were filled with each soil mixture. Spore smear slides of P. expansum were prepared as described before and buried in each soil mixture. After 24h incubation at 23°C the slides were removed, stained and examined. 25 random fields were examined on each slide and the percentage germination and mean germ-tube length determined.

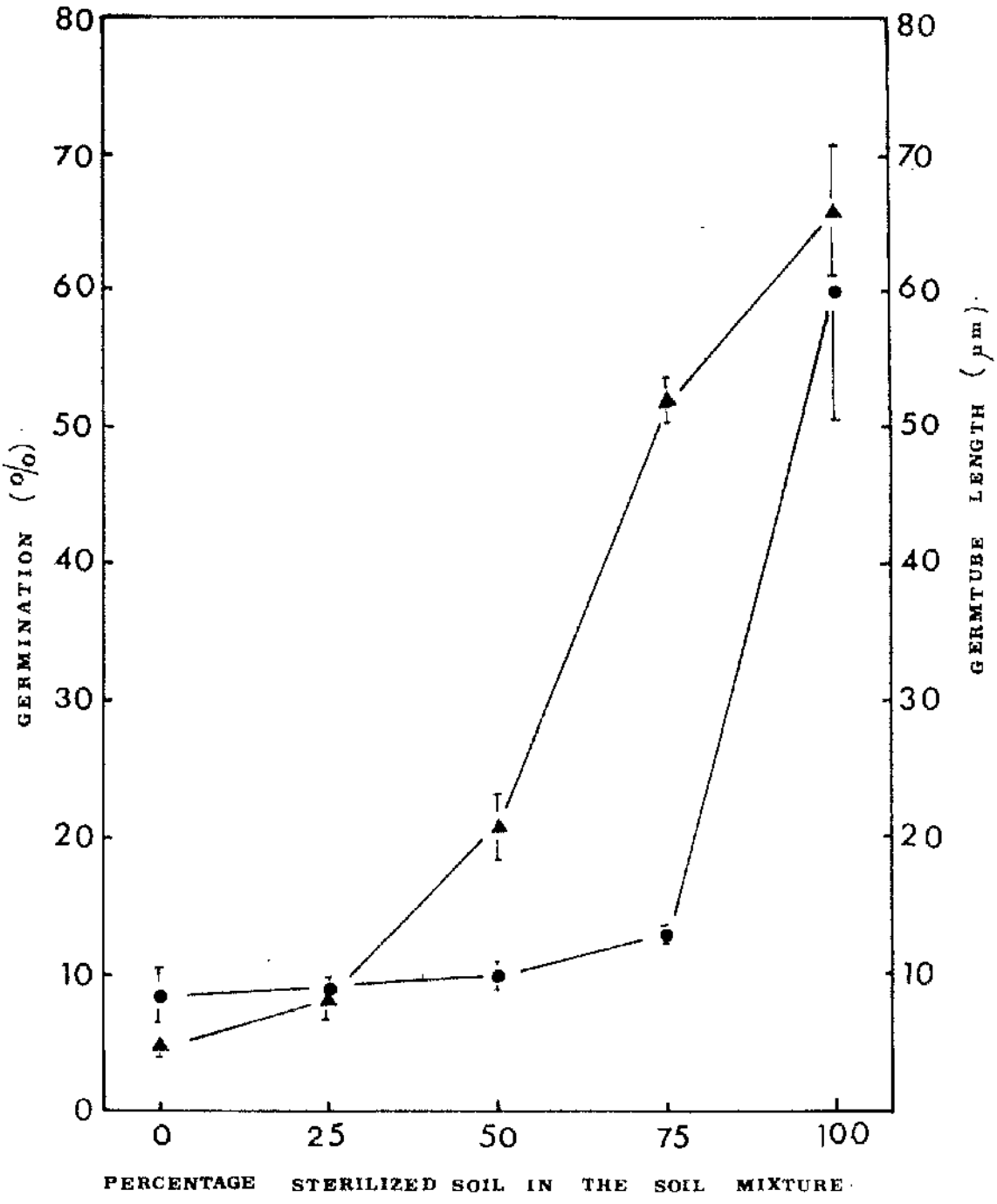
4.2.3.2.2. Results

The results are given in fig. 14 and Appendix Tables 9A & B.

A low percentage (4%) of the spores germinated in the unsterilized soil and the percentage germination progressively increased as the proportion of sterilized soil increased in the mixture. However little effect was observed on germ-tube growth until the unsterilized soil had been diluted with sterilized soil by at least 75%.

Fig. 14

Percentage germination (▲) and germ-tubo growth (●) in sterilized : unsterilized soil mixtures after 24h incubation at 23°C.


80
70
60
50
40
30
20
10
0
GERMINATION (%)
GERMTUBE LENGTH (µm)
0
25
50
75
100
PERCENTAGE STERILIZED SOIL IN THE SOIL MIXTURE

4.2.3.3. The effect of qualitative changes in the soil microflora on spore germination and germ-tube growth

4.2.3.3.1. Materials and methods

(i) Soil treatment

500g of sieved (2mm mesh) soil were placed in 500 cm^3 conical flasks which were closed with a loosely fitting rubber bung. The soils were then heated for 6h in a water bath at various temperatures (60°C, 70°C and 80°C) or for 2h in an autoclave at 120°C. Un-heated soil was used as a control.

(ii) Assessment of changes in the soil microflora

After treating the soils at the different temperatures, samples were taken to determine the survival of the microflora using the dilution plate method. Samples were plated onto the malt-extract medium for fungi and onto a modified Hutchinson's agar medium for bacteria.

(iii) Effects on spore germination of P. expansum

After treatment the soils were allowed to cool before spore smears on microscope slides were buried in them in plastic petri-dishes as described previously. Untreated soil was used in the controls. After 24h incubation at 23°C the slides were removed, stained and examined.

This experiment was repeated.

4.2.3.3.2. Results

(i) Assessment of qualitative changes to the soil microflora

The results, given in Table 9, show that the number of micro-organisms decreased with increasing temperature of treatment. 99.8% of all fungal

Table 9. Micro-organisms g^{-1} oven-dry soil after treatment at various temperatures.

	Experiment 1				Experiment 2			
Treatment	Fungi	% reduction in relation to control	Bacteria	% reduction in relation to control	Fungi	% reduction in relation to control	Bacteria	% reduction in relation to control
Control Un-treated	451.9×10^3	0	181.7×10^6	0	512.0×10^3	0	106.69×10^6	0
60°C	0.7×10^3	99.8	3.5×10^6	98.07	0.9×10^3	99.82	9.97×10^6	90.65
70°C	0.1×10^3	99.97	1.6×10^6	99.11	0.1×10^3	99.98	1.76×10^6	98.35
80°C	0.1×10^3	99.97	0.1×10^6	99.97	0.04×10^3	99.99	0.376×10^6	99.6
Autoclaved	0	100	0	100	0	100	0	100

propagules were destroyed by heating at 60°C. The surviving organisms, however, appear to be able to survive at 80°C but all were destroyed by autoclaving. Heating at 60°C destroyed at least 90% of the bacteria and most of the survivors were eliminated by heating at 80°C. Autoclaving destroyed all bacteria.

(ii) Effect of qualitative changes in the soil microflora on spore germination and sporeling growth

The percentage germination and the lengths of the germ-tubes produced are given in fig. 15 & 16 and Appendix Tables 10A & B.

Heating the soils to 60°C had no effect on the inhibitory factors since none of the spores germinated in these soils. However temperatures of 70°C and above markedly reduced the inhibition. In neither experiment did autoclaving stimulate germination more than did the treatment at 80°C. However in both of these experiments the percentage germination in the autoclaved soil was much lower than that found in most other experiments. The reasons for this are not known.

Germ-tube growth was also much greater in both experiments in soils treated at 70°C or higher (fig. 16). Observations were not continued over a long enough period to determine effects on conidiation.

4.2.3.4. Discussion

Penicillium expansum spores germinated in natural soil diluted with sterilized soil. Chacko and Lockwood (1966) also overcame fungistasis in natural soil by progressively diluting the soil with sterilized soil. The sterilized soil may provide additional nutrients and uncolonized niches, or produce its effect by diluting the populations of the micro-organisms and the inhibitors which may be involved in the inhibition of spore germination.

Although approximately 99% of fungi and 98% of bacteria were killed when the natural soil was treated at 60°C for 6h, this treatment did not

Fig. 15

Percentage germination of P. expansum spores in natural soil treated at different temperatures. The spores were incubated at 23°C and germination was assessed after 24h.

(a) 1st experiment.

(b) 2nd experiment.

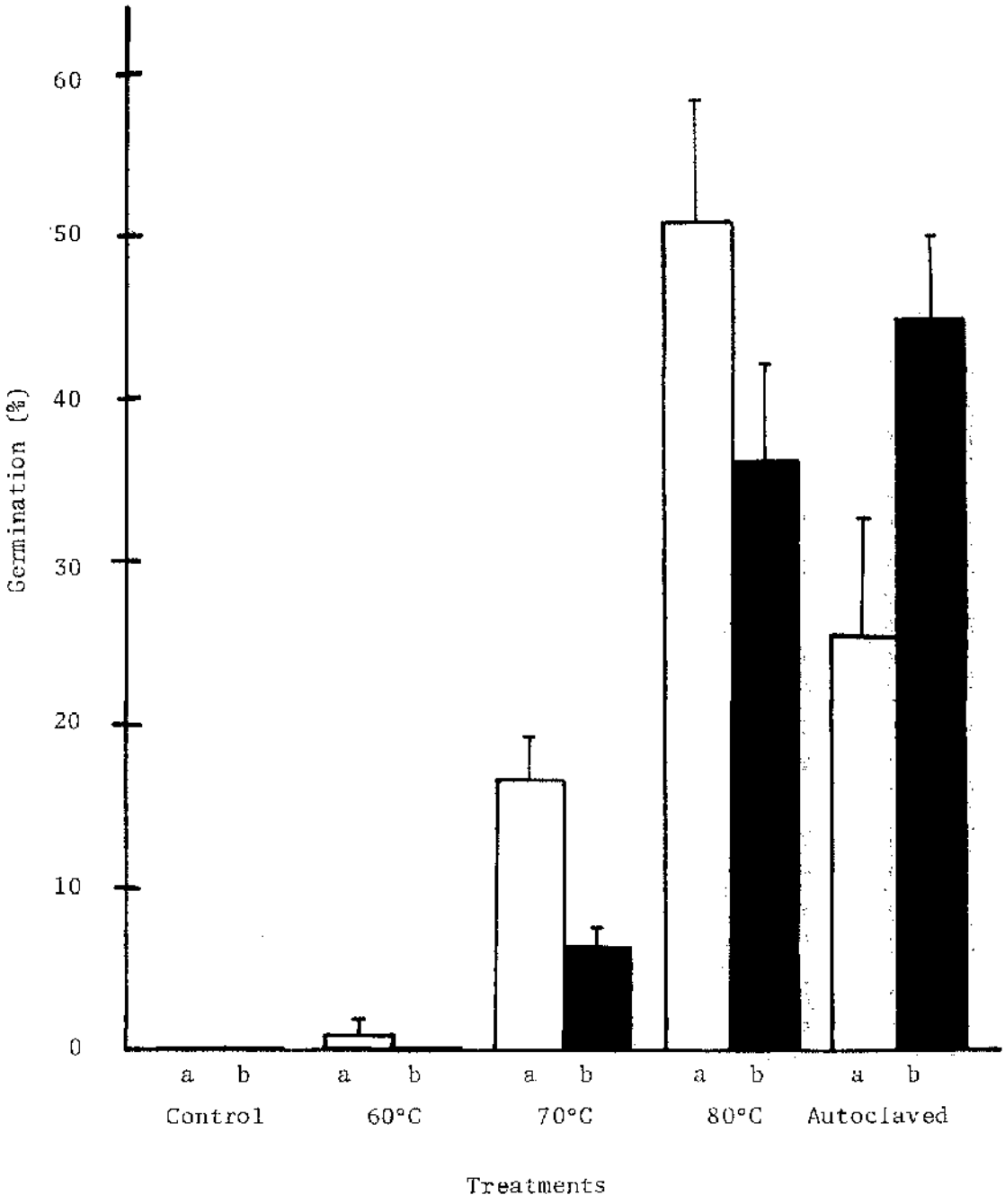
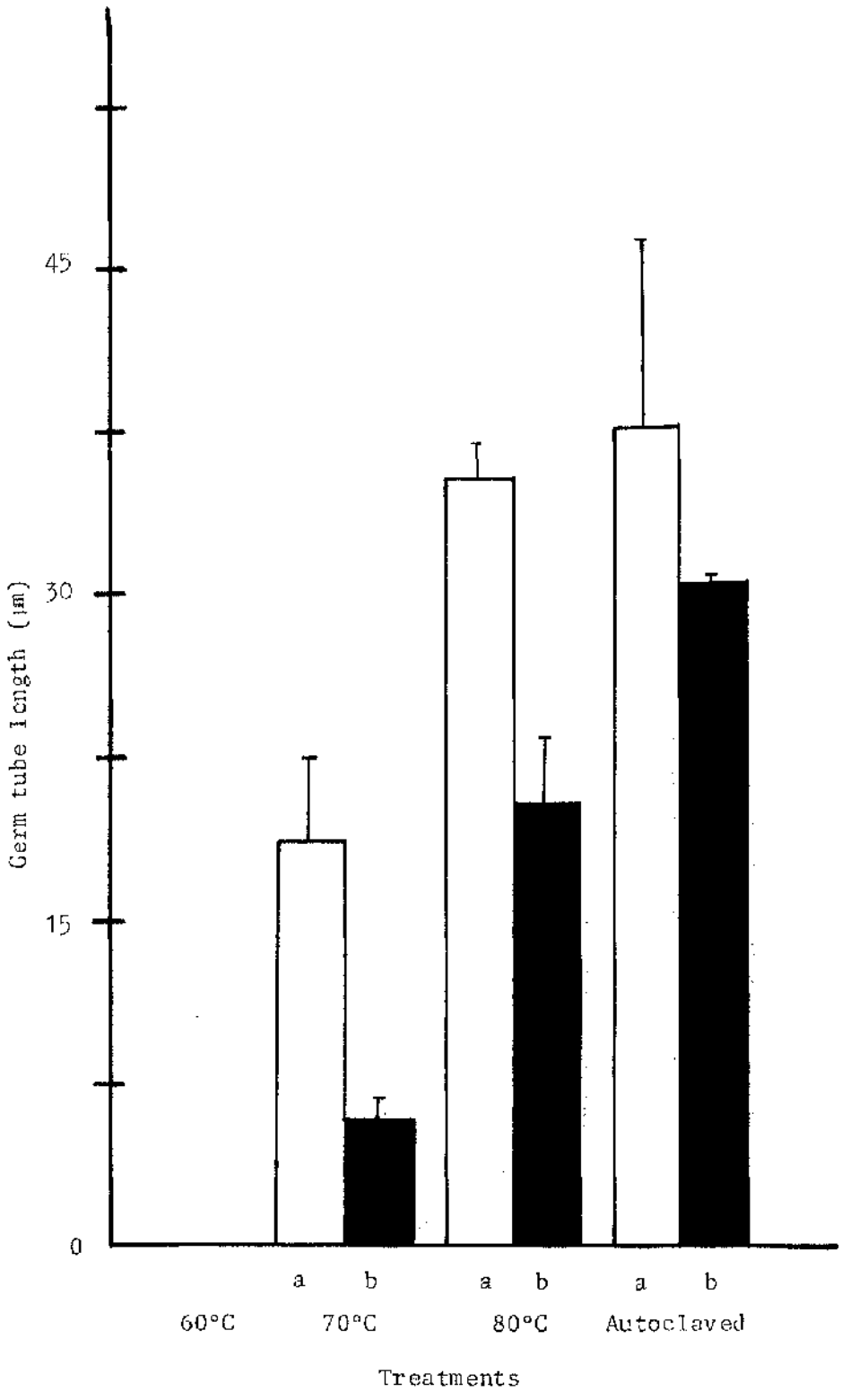

60
50
40
30
20
10
0
Germination (%)
a b
Control
a b
60°C
a b
70°C
a b
80°C
a b
Autoclaved
Treatments

Fig. 16

Germ-tube length (µm) of P. expansum spores after 24h incubation at 23°C in natural soil treated at different temperatures.

(a) 1st experiment.

(b) 2nd experiment.


45
30
15
0
Germ tube length (µm)
a
b
a
b
a
b
60°C
70°C
80°C
Autoclaved
Treatments

remove the inhibition of spore germination. Thus the removal of inhibition by the higher temperature treatments cannot be due to the release of nutrients from killed micro-organisms because most of the nutrients would have been released by the treatment at 60°C. The inhibition is thus due either to the activities of soil micro-organisms which are only destroyed by temperatures of 70°C or above, or to heat labile inhibitory substances which are not totally destroyed until such temperatures are reached. The loss of inhibition which occurs when sterilized and unsterilized soils are mixed probably results from the dilution of these microbes or inhibitors.

Mishra and Pandey (1978) also overcome fungistasis when the soil microflora was more or less completely destroyed by treating at 80°C or above.

4.2.4. Investigations of some fungi isolated from heat treated soils for their antagonistic activity to P. expansum

4.2.4.1. Introduction

It was observed in the last experiment that P. expansum spores did not germinate in natural soil treated at 60°C. Such effects may be due to the antagonistic effects of micro-organisms which survive treatment at 60°C.

Catovic (1964) found that of 23 genera of fungi isolated from maple rhizospheres, 7 were antagonistic to Verticillium species in vitro and their culture filtrates were also inhibitory to the growth of Verticillium spp. Mathur (1965) observed the antagonistic effects of 13 soil saprophytes against 8 soil-borne pathogens on Czapek's medium. He found that Trichoderma lignorum and Cunninghamella echinulata inhibited all pathogens by their rapid growth, the former also producing antagonistic metabolites. Macrophomina phaseoli and Fusarium udum were inhibited by 11 saprophytes, Rhizoctonia solani by 9 and Fusarium orthoceras f.sp. ciceri by 8, and he

also observed that certain Aspergillus and Penicillium species showed specific inhibitory action.

Recently Sivasithamparam and Parker (1980) tested one hundred and thirty-two isolates of fungi from the rhizosphere of wheat for their interaction with Gaeumannomyces graminis var. tritici on nutrient rich and nutrient poor agar media. They observed ten different types of interaction. A window type of lysis of the pathogen, not described before, was found in which lysis occurs in the older parts near to the centre of the colony. This was observed only when it was paired with certain isolates on the nutrient poor medium. They also found that Gaeumannomyces graminis was able to inhibit the growth of certain soil fungi on the nutrient poor medium.

The following experiments investigate the antagonistic effects of some of the fungi, which survive treatment at 60°C, on the growth of P. expansum in vitro. The method used was originally devised by Sivasithamparam and Parker (1980).

4.2.4.2. Materials and methods

Fungi surviving heat treatments at 60°C were isolated and single spore cultures were prepared. Stock cultures were maintained on malt-extract agar slopes. Cultures were grown on potato dextrose agar plates for the production of the inoculum. Agar discs, 5mm diameter, infested with mycelium of the test fungi and of P. expansum were placed at opposite sides of 9cm petri-dishes containing potato dextrose agar. The plates were incubated at 23°C for 7-15 days and examined for inhibition.

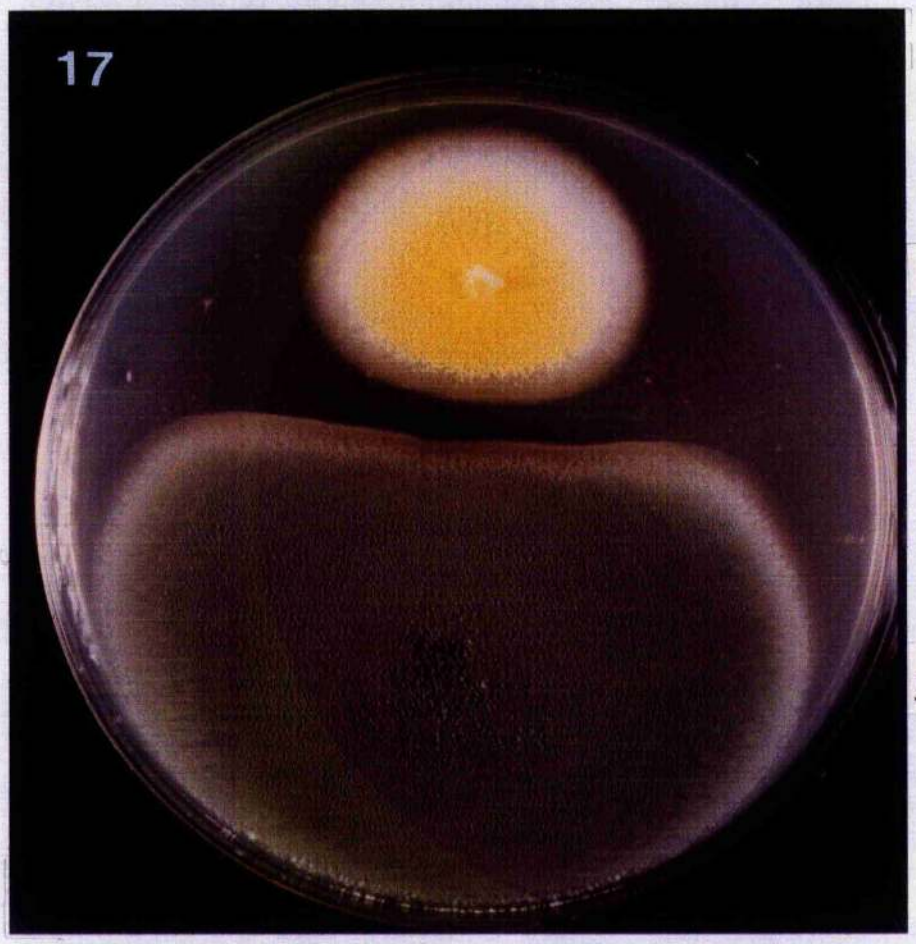
4.2.4.3. Results and Discussion

Plates 17, 18, 19 & 20 show that some of the fungi which survive in soil after treatment at 60°C for 6h could inhibit the growth of P. expansum on potato dextrose agar. These fungi have been identified as ascosporic species of Penicillium (Plate 17) and Aspergillus (Plates 18, 19 & 20).

Many workers have reported similar results and these were described in the introduction to this chapter and in an earlier discussion.

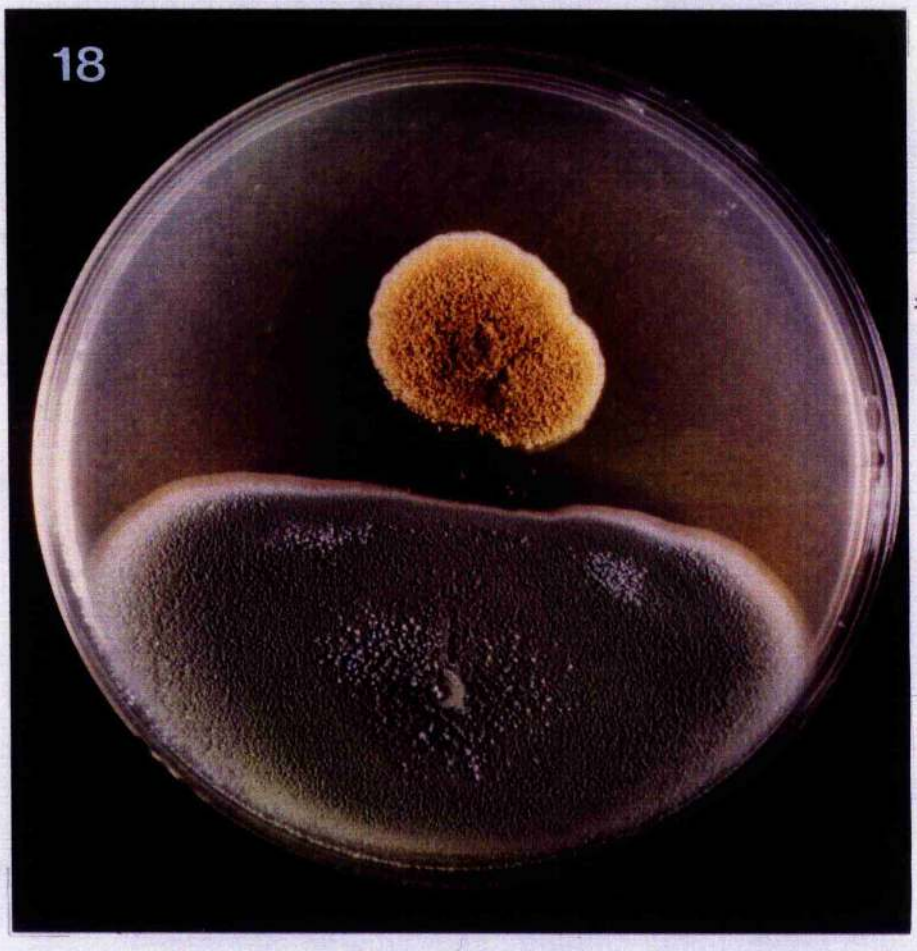
Plates 17 to 20

The antagonistic effects of an ascosporic *Penicillium* (Plate 17) and of an ascosporic *Aspergillus* (Plates 18, 19 & 20) on *P. expansum* in culture.



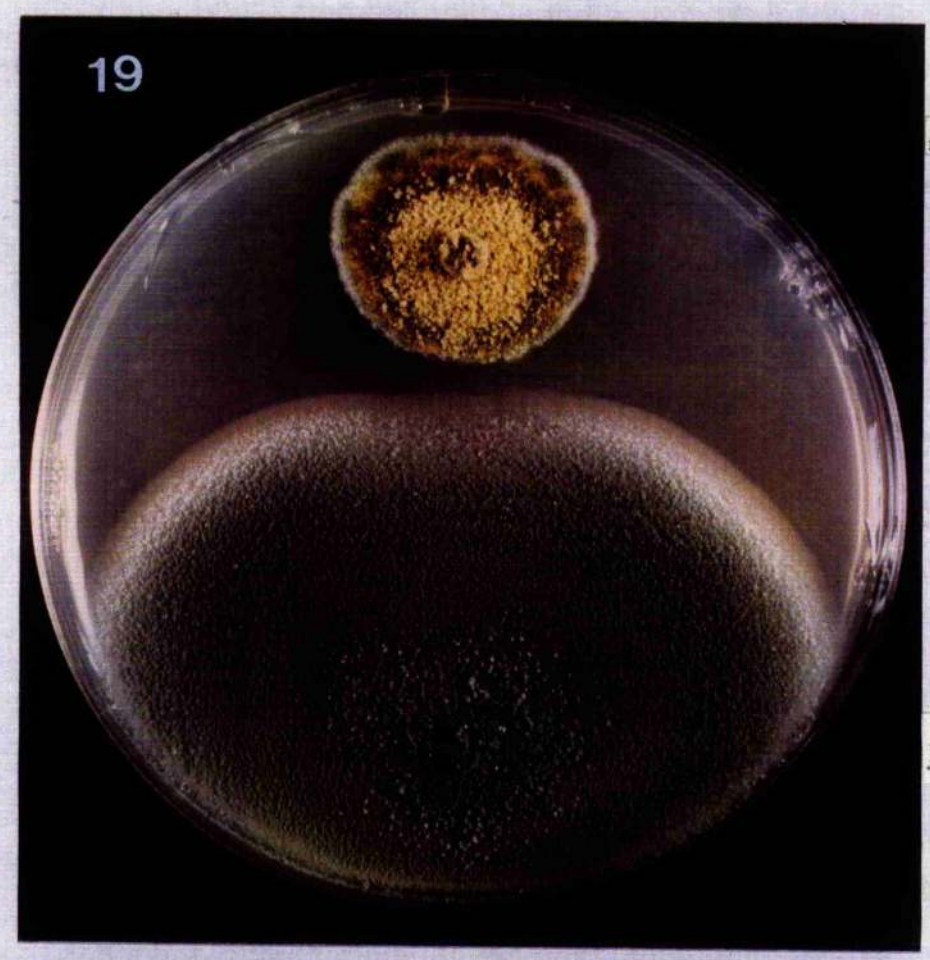
P.chrysogenum

P.expansum



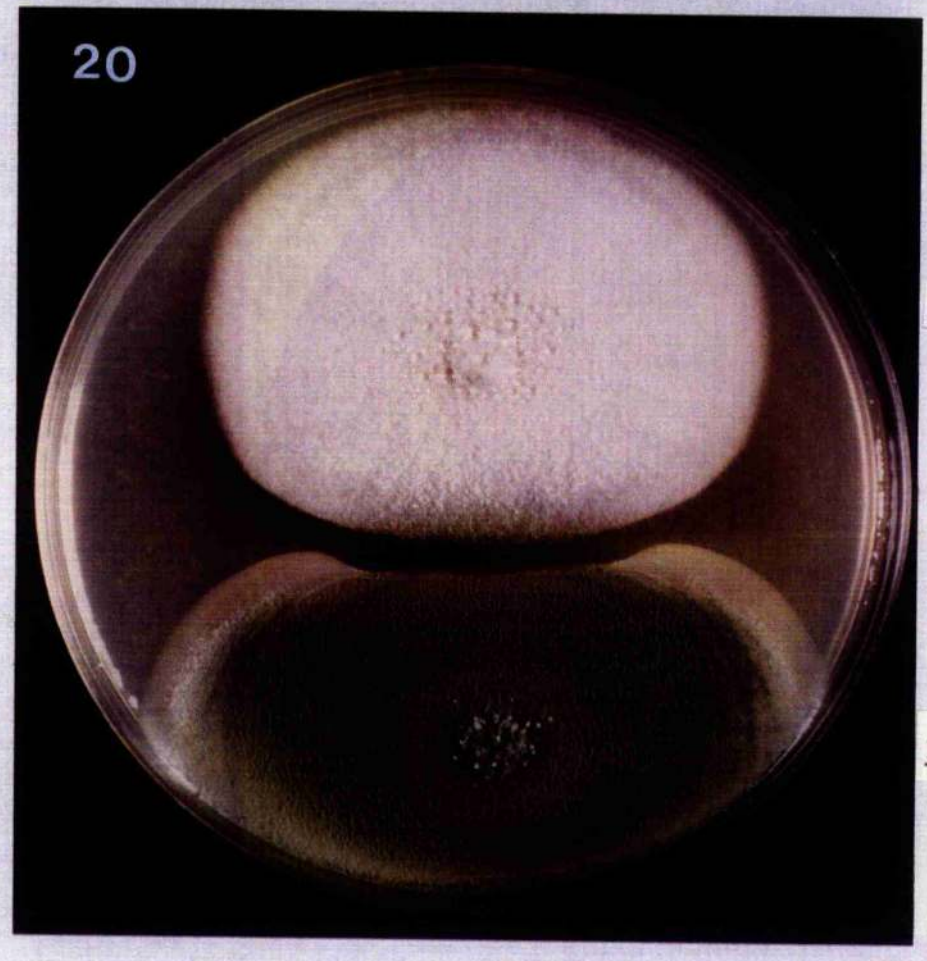
Aspergillus Sp

P.expansum



Aspergillus Sp.

P.expansum



Aspergillus sp.

P.expansum

CHAPTER V

SURVIVAL AND GROWTH OF PENICILLIUM EXPANSUM IN THE RHIZOSPHERE AND ON THE RHIZOPLANE

5.1. INTRODUCTION

Hiltner (1904) first observed that micro-organisms were more abundant in the soil surrounding plant roots than in the soil away from the roots. He called this region of soil the rhizosphere. Many workers, including Starkey (1931) have confirmed and extended these observations, reporting much greater numbers of organisms in the rhizosphere, particularly on the root surface, and a marked reduction in numbers with increasing distance from the root. All groups of micro-organisms include representatives which are selectively stimulated by roots, some which are not affected and some which are even inhibited. Papavizas and Davey (1961), in studies on blue lupin (Lupinus angustifolius) roots, showed that the rhizosphere effect was most pronounced to a distance of 3mm away from the root although the effect was still evident at a distance of 18mm. Thus they found the highest numbers of bacteria (159 x 10^7 g^{-1}), streptomycetes (467 x 10^6 g^{-1}) and fungi (355 x 10^4 g^{-1}) in the soil closely adhering to the root surface compared with numbers of 273 x 10^6, 91 x 10^6, and 91 x 10^4 g^{-1} respectively in the soil 80mm away from the root surface. Clark (1949) considered that the stimulus originated on the root surface and suggested a new term, the 'rhizoplane', regarding the rhizosphere merely as an extension of the rhizoplane. The distribution of micro-organisms on the rhizoplane of certain plants has been studied in considerable detail by Harley and Waid (1955a), Ishizawa et al. (1957), Peterson (1958), Dix (1964), and more recently by Campbell and Rovira (1973), Rovira and Campbell (1974), and Rovira et al. (1974). These studies have employed serial washing of roots or variations of this technique and various forms of microscopy including scanning electron microscopy. Most

investigators now recognise three distinct regions, the root surface or 'rhizoplane', the rhizosphere and the soil beyond the influence of the root.

The general topic of the rhizosphere has been reviewed many times and the following accounts show how ideas and perspectives have changed over the years (Starkey, 1929; Waksman, 1932; Katznelson et al., 1948; Clark, 1949; Harley & Waid, 1955b ; Garret, 1956; Krasil'nikov, 1958; Starkey, 1958; Lochhead, 1959; Sadasivan & Subramanian, 1960; Timonin, 1964; Katznelson, 1965; Rovira, 1965a; Brown, 1975; Bowen and Rovira, 1976).

This account will be concerned mainly with mycological aspects of the rhizoplane and rhizosphere and organisms other than fungi will only be considered as they affect fungal development.

The fungal flora of the root surface and rhizosphere has been investigated by numerous workers. The stimulatory effect of the root has been shown to vary according to a number of factors including the distance from the root and with the fungal species involved. Papavizas and Davey (1961), working with blue lupin (L. angustifolius), reported that Cylindrocarpon radicicola was only found on the rhizoplane while Paecilomyces marquandaii, which appeared to be the most abundant fungus on the rhizoplane, fell off sharply to negligible levels between 12-15mm from the surface. Aspergillus ustus, although dominant on the root surface, was present in high numbers even in the soil some distance from the root, while Fusarium oxysporum and Trichoderma viride were not markedly affected by the roots at all. They also found that the genus Penicillium comprised more than 50% of all fungi isolated but that the effects of the roots on this genus varied with the species.

Parkinson and Thomas (1969) carried out qualitative studies of the fungi in the rhizosphere of dwarf bean plants (Phaseolus vulgaris) using the soil washing technique, and observed small but consistent differences between the rhizosphere and the non-rhizosphere mycofloras. Thus they found that Fusarium spp, Mucor spp, Cylindrocarpon radicicola, Varicosporium elodae, Humicola grisea and certain sterile forms were more abundant in the rhizosphere than in the non-rhizosphere soil. Trichoderma viride was less

frequently isolated from the rhizosphere than from non-rhizosphere soil. Odunfa and Oso (1979) also found higher populations of fungi in the rhizosphere of cowpea plants (Vigna unguiculata) and that more species were present than in non-rhizosphere soil. They found that the most frequently isolated genera in the rhizosphere were Aspergillus, Fusarium, Trichoderma and Penicillium. Fusarium spp were the most abundant rhizoplane fungi while other common species included Trichoderma, Macrophomina and Curvularia.

Numerous studies indicate that the numbers of micro-organisms in the root zone are influenced by many factors, including the plant species, the age of the plant and the conditions under which the plants are growing, including such environmental factors as temperature, light, soil moisture content, pH and fertility.

Karimbaeva and Sizova (1976) found both quantitative and qualitative differences between the mycofloras of the rhizospheres of various trees including pine, fir, oak and birch. The number of fungal propagules per gram of dry soil was greater in conifer rhizospheres than in those of the deciduous trees but there were fewer species present. Recently El-Hissy et al. (1980) also reported that the number of genera and species of fungi in the rhizosphere was considerably influenced by the plant species and the age of the plant. They investigated the fungi associated with the rhizospheres of a number of plants of different ages all growing in the same soil type. The commonest fungus found in the rhizosphere of Helianthus annus after 45 days, was Stachybotrys atra and the same fungus was commonest after 90 days, but by 150 days Aspergillus niger was the predominant species. However in Chrysanthemum coronarium, Cladosporium herbarum was the commonest fungus isolated after 45 days growth, but by 90 days this had changed to Aspergillus sydowii and by 150 days to Penicillium funiculosum.

Even different cultivars of a single plant species may exert markedly different effects. Thus Rao (1962) compared the fungal populations of the rhizospheres of eight varieties of groundnut (Arachis hypogaea) grown in a red lateritic soil amended with farmyard manure. He observed a marked

increase in the fungal population in the rhizospheres over that present in non-rhizosphere soil of cultivars TMV_2, TMV_4, Pollachi Red and EC 1698, a smaller increase in the rhizospheres of Spanish improved RS_1, with very little change in the rhizospheres of TMV_3 and Pondicherry 8. Subba-Rao and Bailey (1961) found differences in the frequencies of different fungi in the rhizospheres and on the rhizoplane between five varieties of tomato (Lycopersicon esculentum), some susceptible and some resistant to Verticillium albo-atrum. They observed that fungi were more frequent in the rhizospheres of the susceptible varieties, Bonny Best and John Baer than in those of the resistant varieties Loran Blood and Moscow. However the rhizosphere and rhizoplane flora of the wilt resistant variety Geneva 11 did not differ appreciably from that of the two susceptible varieties. Of the fungi isolated from the rhizoplane, species of Fusarium were dominant on the varieties Bonny Best, John Baer and Geneva 11, and Trichoderma viride was dominant on the varieties Loran Blood and Moscow, but species of Mucor, Penicillium and Aspergillus occurred on all varieties in varying degrees.

Although many workers have shown that, for particular plant species, the mycoflora of the rhizosphere changes as the plants develop, there have been few detailed studies of the successional changes involved.

One of the first studies was that of Stenton (1958) who investigated fungal colonization of the first 4cm of the tap root of pea (Pisum sativum) over a period of 87 days using the root washing technique followed by plating out the root after cutting into sections 2mm long. He isolated over 40 different fungi but only species of the genera Cylindrocarpon, Pythium, Fusarium, Gliocladium and Mortierella were abundant. He also found that most sections from young roots yielded only one species but the percentage with two different species increased as the roots aged. Colonization was patchy at first, areas of occupied and vacant surface alternating. Lateral spread was rapid but occupied areas were not readily invaded by a second organism.

Peterson (1958), in studies on the rhizospheres of wheat (Triticum

aestivum), found that the total number of fungi increased with plant age, and Riviere (1959) in a similar study found that the rhizosphere population of wheat (T. aestivum) reached a maximum level at the stage of tillering. Peterson (1958) also examined the species composition of the rhizosphere in relation to plant development and found that Penicillium spp and certain species of the Mucorales were relatively more abundant on the root surface in the seedling stages than at later stages of growth. In later studies, which included barley (Hordeum vulgare) and flax (Linum usitatissimum) as well as wheat (T. aestivum), Peterson (1959) reported that Pythium spp predominated in the early stages of growth but declined as the plants of each species developed, being replaced by Phoma spp and Fusarium spp between the fifth and twentieth day of growth.

Parkinson and Thomas (1969) investigated qualitative changes in the rhizospheres of dwarf bean plants (P. vulgaris) as the plant aged. They found that Fusarium spp increased in frequency until pod maturity but thereafter decreased, while Mucor spp and Cylindrocarpon radicicola became more common.

Rao (1962) found the highest rhizosphere soil ratios for groundnut plants (A. hypogaea) after the plants had reached the maximum stage of vegetative growth and started to flower. He then found that this ratio gradually decreased until the plants were about three months old, when the rhizosphere population showed a small increase. This later increase was probably due to senescence and the death of roots. Similar results were obtained in a study of the groundnut (A. hypogaea) by Joffe (1969).

The changes responsible for these effects are not clearly understood. However the major factors are probably changes in the physiology of the host. These changes may affect the fungal population directly through changes in the amounts and composition of the root exudates and other nutrients, or indirectly through the effects of such nutrients on other micro-organisms including bacteria and actinomycetes which are present in the rhizosphere.

Many workers have reported that infection by parasites can affect the composition of the rhizosphere microflora. Thus Agnihothrudu (1961) studied fungal, bacterial and actinomycete populations in the rhizospheres of healthy and diseased tea plants (Camellia sinensis) in relation to root infections by Ustulina zonata. He found significantly higher numbers of fungi and bacteria but not of actinomycetes in the rhizospheres of diseased plants than in those of healthy plants. He also isolated 50 species of fungi from the rhizospheres of diseased plants but only 32 from those of healthy plants. Timonin (1966) has reported similar differences between the rhizosphere populations of healthy and diseased Lodgepole pine seedlings (Pinus contorta var. latifolia), as also have Vishwanath et al. (1969) for a disease of unknown cause of coffee (Coffea arabica) known as coffee decline disease.

All these infections are of the root systems but similar effects have been shown to result from infections of the aerial parts of plants by fungi (Chandra Kumar & Balasubramanian, 1981) or viruses (Singh, 1972). In contrast, Mishra and Kamal (1970) observed a reduction in fungal populations in the rhizospheres of cotton (Gossypium herbaceum), chillies (Capsicum spp) and tomato plants (Lycopersicon esculentum) which were infected by viruses.

Many environmental factors may affect both the rhizosphere and rhizoplane mycofloras. Thus soil moisture content can have marked effects. Taylor and Parkinson (1964) investigated the fungus flora of the roots of dwarf bean (P. vulgaris) seedlings grown in soils at different soil moisture levels. They found that species of Penicillium, particularly P. lilacinum, were dominant at low moisture levels of around 30% moisture holding capacity but decreased as the soil moisture content increased. However the incidence of Fusarium spp and Cylindrocarpon radicicola, which are considered to be normal root inhabitants, was relatively low at 30% moisture holding capacity but increased as the moisture content increased. Peterson et al. (1965) examined the microflora of the rhizosphere of wheat (T. aestivum) in fertile

soils of different moisture content and found that the distribution of fungi on roots was similar with moisture holding capacities between 30% to 60% but that there was some restriction at moisture holding capacities of around 90%. Species of Mortierella, Rhizopus, Chaetomium, Curvularia and Helminthosporium were not isolated from roots in soils at high soil moisture levels while the incidence of species of Fusarium and Phoma was lowest at these levels.

The environment around the aerial parts of the plant may also affect the populations of micro-organisms in the root region. Thus Harley and Waid (1955b) found that high light intensities favoured colonization of the root surface of beech by Trichoderma viride while low light intensities favoured the growth of Rhizoctonia. Srivastava (1971) also recorded higher microbial populations in the rhizospheres of wheat (T. aestivum) and barley (H. vulgare) plants which had been exposed to continuous light than of plants which had been subjected to continuous darkness. The microfloras present around the roots of plants subject to 12h alternating light and dark were at intermediate levels. He also recorded P. expansum, with low frequency, from wheat plants grown with 12h alternating light and dark periods.

Little work has been done upon the effect of temperature on the rhizosphere microflora. However Rouatt et al. (1963) studied the number and kinds of micro-organisms in the root zones of wheat (T. aestivum) and soybeans (Glycine max) grown under three different temperature regimes (12.8-15.6°C, 21.1-23.9°C and 29.4-32.2°C) in the greenhouse. He found that the numbers of bacteria in the rhizosphere and on the rhizoplane of wheat increased as the temperature decreased whereas they increased with increasing temperature in the rhizosphere and rhizoplane of soybean and in the root free soil of both plants. He also isolated more fungi, including species of Mucor, Rhizopus, Rhizoctonia and Gliocladium from roots of soybeans grown at the highest temperature whereas species of Fusarium and Cylindrocarpon were more common at the low temperatures. With wheat the most striking feature was the predominance of non-sporing dark pigmented species at the high

temperatures and non-sporing hyaline types at the low temperatures.

Treating soils with inorganic nutrients has marked effects on the rhizosphere and rhizoplane microflora. Thus Vuurde (1978) studied the colonization of the seminal roots of seedlings and the nodal roots of tillering plants of spring wheat (T. aestivum) cultivated in growth chambers in soils to which low or high levels of a complete inorganic (NPK) fertilizer had been added. He observed much higher numbers of bacteria and actinomycetes in the rhizoplanes of both seminal and nodal roots of plants grown with the high levels of NPK than with the low levels of NPK. In contrast he observed no effects of NPK on the fungal populations of the rhizoplane but unfortunately did not look for effects on the rhizosphere mycoflora.

The application of nutrients as foliar sprays also affects the rhizosphere mycoflora. Thus Venkata Ram (1960) studied the effects of spraying tea plants (Camellia sinensis) with solutions of $(NH_4)_2\ SO_4$, $MgSO_4$, KCl, $NaNO_3$, $Na_2\ HPO_4$, and Urea. The soil was protected from the nutrients during spraying, with a plastic cover. Four applications were made at 2-day intervals and four days after the last spray he found significant reductions in the total microbial population of the rhizosphere in all treatments. However the general effects were variable. Thus KCl and Na_2HPO_4 significantly increased the numbers of fungi, $NaNO_3$ had no effect and $(NH4)_2SO_4$, $MgSO_4$ and urea significantly reduced fungal populations. Kandasamy and Rangaswami (1967) also observed the effects of foliar sprays containing $(NH4)_2SO_4$, Na_2HPO_4, KCl or urea on 15, 30, 45, and 75 day old sorghum plants (Sorghum vulgare) and found marked but variable changes in the rhizosphere mycoflora. The numbers of fungi generally increased when the plants were young but population changes varied with older plants, sometimes increasing sometimes not. Those changes appeared to vary according to the cultivar of sorghum used. For example, Penicillium spp were present in greater numbers in the rhizospheres of the cultivar CO.4 than in those of the cv K.1 after treatment with either urea or KCl.

Sullia (1968) observed the effects of foliar sprays of the two plant hormones, gibberellic acid and indole acetic acid, on the rhizosphere populations of two leguminous weeds, Cassia tora and Crotalaria medicaginea. He observed no significant differences in fungal species between the rhizospheres of treated and untreated plants, although higher populations of these fungi were present in the rhizospheres of the sprayed plants. Different concentrations of hormones, of between 50 and 200 ppm for gibberellic acid and between 100 and 200 ppm for indole acetic acid stimulated populations equally in the rhizospheres of C. tora but solutions of 100 ppm of each hormone gave maximum stimulation in the rhizosphere of C. medicaginea. He suggested that the increase in the rhizosphere mycoflora after foliar treatment with the hormones might be due to enhanced sporulation of the fungi in the rhizosphere as a consequence of the exudation of stimulatory factors from the roots. Gupta (1971) carried out similar studies on the effects of gibberellic acid on the rhizosphere mycoflora of Withania somnifera, Datura alba and Ocimum sanctum and found slight increases in some treatments only. Recently Singh (1981) reported that foliar applications of maleichydrazide produced an increase in the numbers of fungi on the rhizoplanes of both flax (Linum usitatissimum) and mustard (Brassica campestris), but that the numbers of fungi increased significantly only in the rhizosphere of the flax, not in the mustard.

Factors determining the population size and activities of fungi in the rhizosphere

The factors which are considered to be mainly and directly responsible for the increased fungal populations in the rhizospheres are root exudates and sloughed off root cells from the root cap and outer layers. However changes in oxygen or carbon dioxide concentrations, in soil pH, soil moisture content and mineral nutrients may also be involved (Rovira, 1965a). The supply of nutrients from the growing root may also stimulate the activities of other micro-organisms including bacteria and actinomycetes,

and these organisms may affect, usually in an inhibitory way, the fungal populations in the rhizosphere.

Root exudation and its controlling factors have been reviewed by Rovira (1965b, 1969), Hale et al. (1971, 1978) and Hale and Moore (1979), and the following account is to a large extent based on these reviews.

The elongating region of the root, 1 to 3 cm from the apex, is considered to be the major site of exudation but other parts also provide significant amounts of nutrients, mostly however in the form of sloughed off and dead cells rather than by exudation. Root hairs also exudate materials in droplets which often accumulate at their tips (Rovira, 1969; Brown, 1975).

Investigations into the nature of root exudates show that a wide range of substances can be excreted. Many workers have, however, examined root exudation under various artificial conditions, for example of plants growing under aseptic conditions in nutrient solution or in sterile quartz sand. Such conditions are necessary, otherwise contaminating micro-organisms would rapidly metabolise and change the amounts and composition of the exudates. Thus it is not really possible to investigate root exudation in natural soils under natural conditions, and it must be borne in mind that it is likely that the types of compound found in sterile conditions and their amounts may not be the same as that which occurs naturally, since micro-organisms may affect exudation by altering the permeability of root cells and may modify root growth.

The compounds identified include carbohydrates, for example mono-saccharides, disaccharides, trisaccharides and oligosaccharides, and much high molecular weight mucilaginous materials. The mucilaginous material is probably the bulk of the exudate. Other compounds produced in varying amounts include amino acids, organic acids, growth factors such as biotin, thiamine, pantothenate and niacin, nucleotides, flavonones, glycosides, saponins, hydrocyanic acid and proteins, many of which may have enzyme activity (Rovira, 1965b, 1969; Vancura and Hanzlikova, 1972; Rovira & Harris, 1961). Although most of these compounds are likely to stimulate

growth, some in certain instances may be inhibitory or even toxic. Thus Claudius and Mehrotra (1973) identified Glycine and phenylalanine in the exudates of 21 day old lentil plants (Lens culinaris) and found these compounds to inhibit the germination of the spores of Fusarium oxysporum. This observation may partly explain the lower wilt incidence in lentil plants which are more than 3 weeks old.

Many workers have reported that the pattern of exudation of organic compounds from roots varies according to many factors including the plant species involved (Rovira, 1959; Rovira & Harris, 1961; Vancura, 1964; Ayres & Thornton, 1968; Vancura & Hanzlikova, 1972) and even the cultivar (Kraft, 1974; Keeling, 1974). The age of the plant is also important (Rovira, 1956; Vancura & Hovadik, 1965; Balasubramanian & Rangaswami, 1969; Smith, 1970; Hamlen et al., 1972), and its physiological condition as it may be affected by such environmental factors as temperature (Rovira, 1959; Husain & McKeen, 1963; Nezgovarov et al., 1970), and light (Rovira, 1959; Srivastava, 1971). The rooting medium, through its nutritional status, water content and oxygen concentration (Katznelson et al., 1954; Vancura, 1964; Ayers & Thornton, 1968; Bowen, 1969; Rittenhouse & Hale, 1971; Shay & Hale, 1973), is particularly important since it can have a profound effect on plant growth. Root damage, whether caused by chemical or physical factors, may also affect the quantity of exudates (Clayton & Lamberton, 1964; Ayers & Thornton, 1968), as well as foliar applications of chemicals such as $NaNO_3$, Na_2HPO_4 and urea (Agnihotri, 1964; Balasubramanian & Rangaswami, 1969). Other organisms, particularly micro-organisms present in the rhizosphere, are known to have a marked effect on the exudation of the roots (Rovira, 1969).

The occurrence of P. expansum in the rhizosphere

A number of workers have reported the isolation of P. expansum from the rhizospheres of plants. Thus Thrower (1954) isolated it from the rhizospheres of some Australian heathland plants and found that the stimulatory effect of the roots varied between species. It was highest in the rhizospheres of Dillwynia floribunda at a population 46.5 times greater than in the soil, and lowest in Epacris impressa. D. floribunda is a member of the Leguminoseae and this may explain its greater stimulatory activity. Dix (1964) isolated P. expansum from the rhizospheres and from the rhizoplanes of French bean plants (Phaseolus vulgaris) grown in a beech wood soil, and Joffe (1969) found it associated with the roots of groundnuts (A. hypogaea). It has also been isolated from the rhizospheres of ash (Fraxinus excelsior), maize (Zea mays), clover (Trifolium subterraneum), wheat (T. aestivum), poplars (Populus spp), apple (Malus spp), and various steppe grasses (Srivastava, 1971; Domsch & Gams, 1972; Domsch, Gams & Anderson, 1980).

However it is not known if all the isolates of P. expansum were parasitic isolates capable of rotting apple or other fruits, or if they were purely saprophytic strains. The present study was undertaken to determine if a pathogenic isolate of P. expansum could survive and grow in the rhizospheres of plants. The species of plant selected for these studies were maize, apple, broad bean and tomato. As mentioned above, P. expansum has been reported to occur in the rhizospheres of maize, bean and apple plants. It has also been isolated from seeds of tomato (Domsch, Gams & Anderson, 1980).

5.2. COLONIZATION OF THE RHIZOSPHERES AND RHIZOPLANES OF MAIZE, BROAD BEAN, TOMATO AND APPLE

5.2.1. Materials and methods

5.2.1.1. Preparation of soil

Soil was collected and sieved as described in Chapter II. Sieved peat was mixed with the soil (25% peat, 75% soil) and half of the soil peat mixture was sterilized by autoclaving. The sterilized soils were used as a control to compare the growth and survival of P. expansum in association with plant roots in the presence of the normal soil flora with that in its absence. P. expansum spores were mixed into half the bulk of each soil by hand as described in Chapter II. 800g of soil were placed in 15.24 cm sterilized pots for the maize (Zea mays) and apple (Malus pumila) experiments and 300g were placed in 7.62 cm sterilized pots for the tomato (Lycopersicon esculentum) and broad bean (Vicia faba) experiments. 100 pots were filled with soil, fifty with unsterilized and fifty with sterilized soil for each experiment. Twenty-five pots of each soil type contained spores while the other twenty-five pots were without spores to follow changes in the natural populations of P. expansum and to check for contamination.

5.2.1.2. Production and maintenance of plants

Tomato cv Moneymaker plants were raised from seed. The seeds were sterilized in 10% chloros solution and sown in autoclaved Levington compost in a seed tray. When the seedlings had reached the 2 to 3 leaf stage, they were planted singly into the pots of prepared soils.

Apple seeds were collected from fruits of the cv Golden Delicious. The seeds were sterilized in 10% chloros and sown in trays of moist peralite. The trays were incubated in a cold room to vernalize the seed before transferring the seed into Levington compost in 8.89 cm pots. The pots were

placed in a greenhouse and when the plants had reached the 2 to 3 leaf stage, they were transplanted singly into the pots of prepared soils.

Maize and broad bean plants were grown directly from seed, by sowing singly into each pot of prepared soils.

All plants were subsequently placed on the bench in a heated greenhouse and watered whenever required. Because of problems with apple mildew (Podosphaera leucotricha) the apple plants were sprayed with 1% Mildan (May and Baker) solution as required.

5.2.1.3. Estimation of the numbers of viable propagules of P. expansum in the soil

Populations of P. expansum in the rhizospheres were estimated at different times after sowing, or planting, in the infested or control soils. Plants were removed from the pots and shaken to remove loosely adhering soil particles from their roots. The roots were then cut off using sterilized scissors and shaken vigorously in previously weighed 250ml flasks with 100ml of sterilized distilled water plus 0.1% Tween 80. A dilution series was prepared from each suspension and 0.25ml of each dilution was plated onto the selective medium. The plates were incubated at 23°C and the numbers of colonies which developed were counted.

After removing the samples for plating, the oven-dry weight of the soil in the flasks was determined to calculate the numbers of viable propagules g^{-1} oven-dry soil.

The occurrence of P. expansum in the soil, approximately 2.3cm away from the roots, was also determined using the dilution plating technique so that rhizosphere : soil ratios could be determined.

5.2.1.4. Methods for assessing the extent of colonization of the root surface and of the internal root tissues

Two methods were used, one involving root washing only, while the other involved surface sterilization.

(i) Root washing technique

The method used was originally devised by Harley and Waid (1955a) and is based on the assumption that vigorous washing will remove loosely adhering propagules and mycelium from the root surface, and so any isolations must be derived from tightly adhering mycelium or from hyphae present within the tissue.

At each harvest, 3cm long root pieces of approximately equal diameters were collected from plants grown either in P. expansum infested or in uninfested soils. After preliminary washing in running tap water, ten root pieces were transferred into screw-capped 1oz bottles containing 10ml of sterile distilled water plus Tween 80 (one drop in 100ml water). The bottles were shaken for a 2min period on a Griffin flask shaker. The water was then removed, using a sterile syringe, and replaced with a further 10ml of sterile distilled water plus Tween 80 before shaking for a further 2min. The process was repeated 20 to 30 times and after the last washing 1ml of the washing water was taken and tested for the presence of P. expansum by plating onto the differential medium. The washed root pieces were cut into 2mm lengths under sterile conditions before plating onto the selective medium (10 lengths/ petri dish). The dishes were incubated at 23°C and after 10 to 15 days the number of root pieces giving rise to colonies of P. expansum and other fungi were counted.

(ii) Surface sterilization technique

Root treatment with chloros to kill any surface contaminants, without killing any internal hyphae, would provide a further method for differentiating

between surface and internal tissue colonization.

This experiment was carried out to determine the length of treatment with chloros which is required to remove propagules of P. expansum from the surface of roots.

Roots were harvested from tomato plants grown in sterile soil free from P. expansum. They were washed under running tap water to remove soil and then cut into 5cm lengths. In order to determine the time of sterilization required to remove surface spores the roots were contaminated by mixing P. expansum spores into sterilized soil. The roots were recovered on the same day, rewashed with tap water to remove adhering soil particles, and then treated with 1% or 10% chloros solution for various times to remove surface contamination. After removal from the chloros solution the roots were washed with sterile distilled water 3 times before being cut into 2mm lengths and inoculated onto the selective medium. After 10-15 days incubation the numbers of root pieces colonized by P. expansum, Penicillium spp, other than P. expansum, and other fungi were counted.

Root lengths washed with sterilized distilled water, but without chloros treatment, were used as controls.

(iia) Results

The results of two experiments using 1% or 10% chloros are given in fig. 17 and 18.

The percentage number of root lengths contaminated with P. expansum decreased with increasing time in both concentrations of chloros, but the reduction was more rapid in the 10% chloros which reduced contamination to 4% after 4mins, and completely removed it by 12 mins treatment (fig. 18). However 30 mins treatment with 1% chloros failed to remove all contamination (fig. 17). Thus sterilization in 10% chloros concentration for at least 10 mins is required to remove P. expansum from the surface of roots.

Fig. 17

Colonization of roots (2mm lengths) after surface sterilization in 1% chloros solution for different times. Fifty 2mm root lengths tested. Colonization given as % ± S.E.

(a) P. expansum

(b) Penicillium spp other than P. expansum

(c) Fungi other than Penicillium spp.

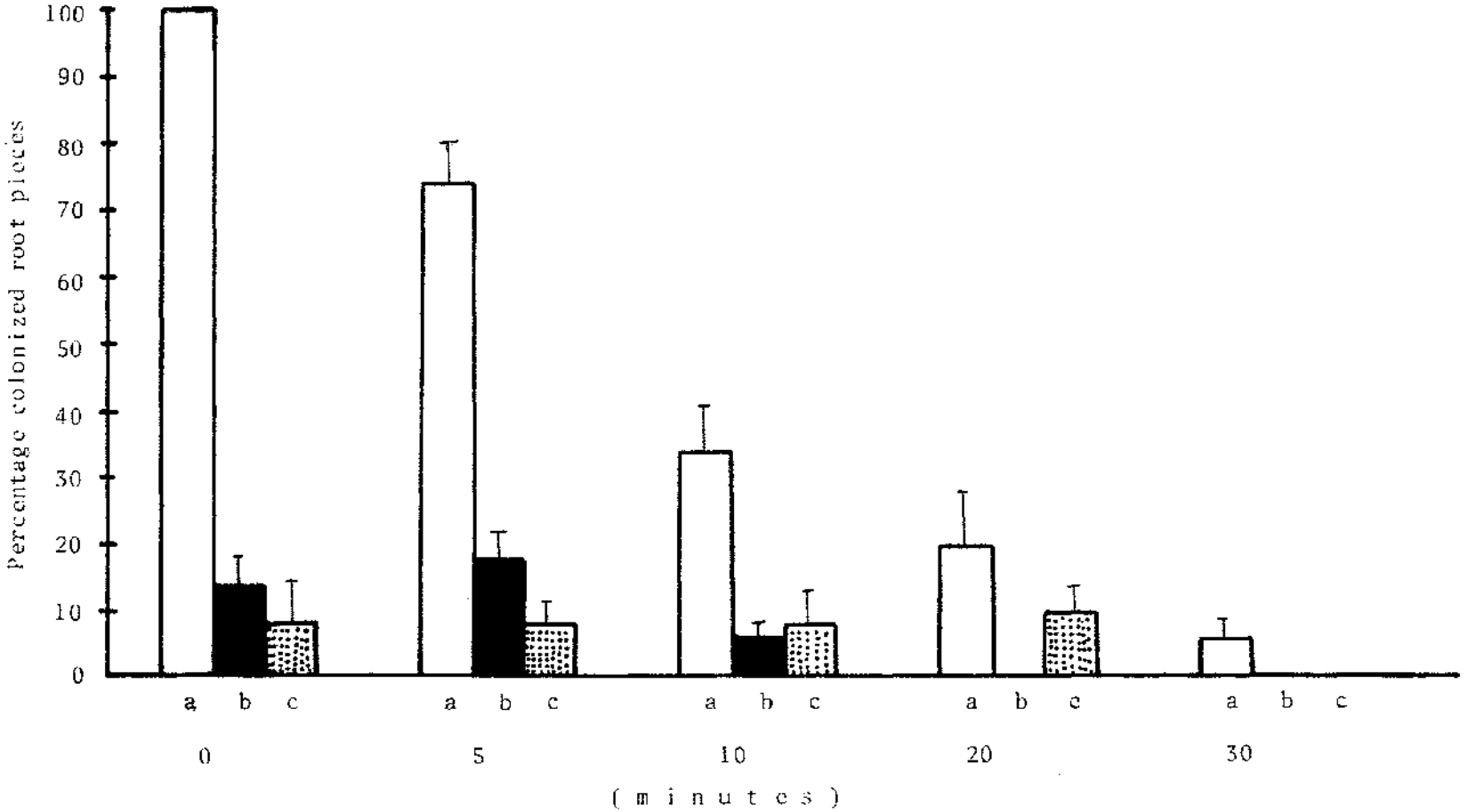

Percentage colonized root pieces
100
90
80
70
60
50
40
30
20
10
0
a b c
a b c
a b c
a b c
a b c
0
5
10
20
30
(m i n u t e s)

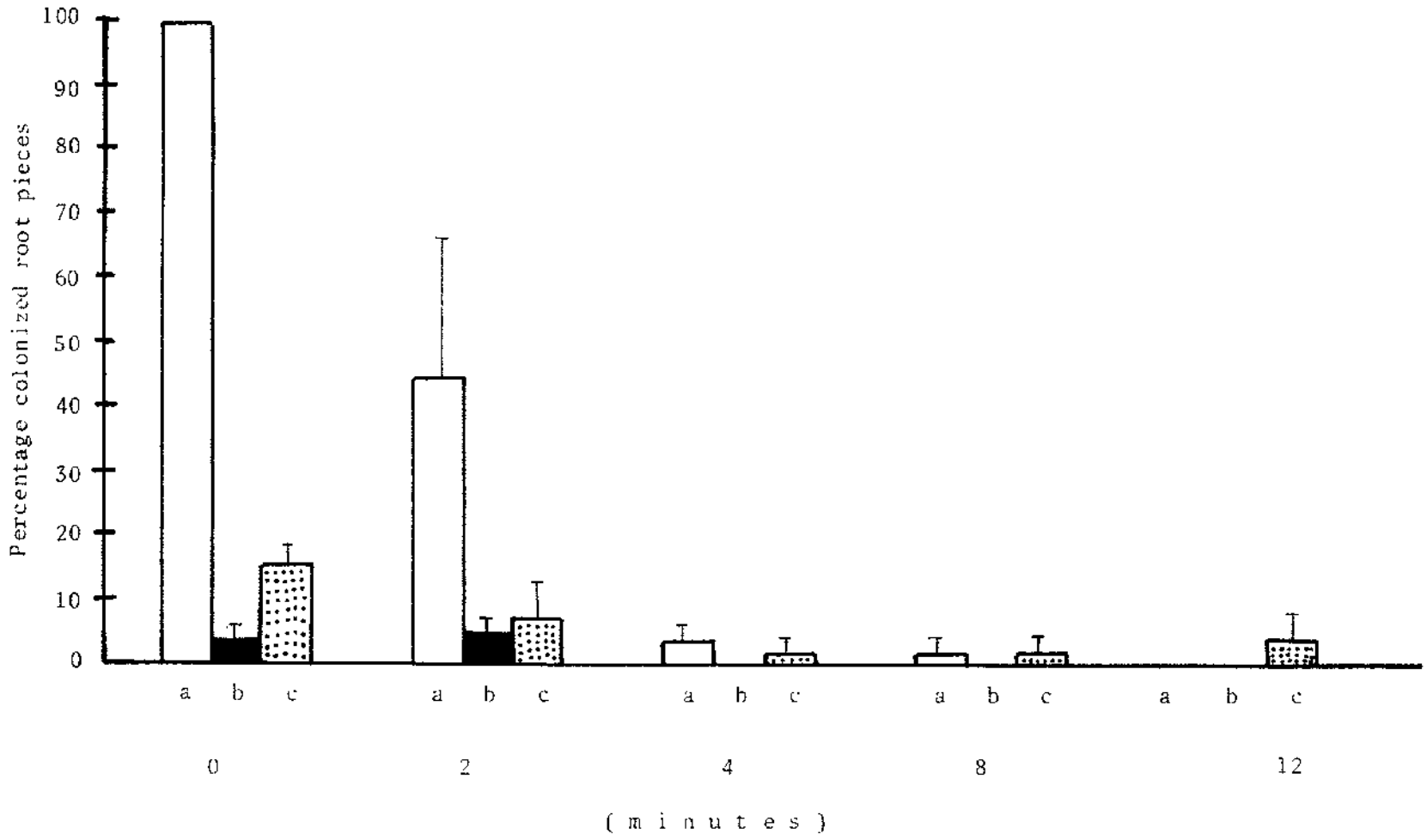
Fig. 18

Colonization of roots (2mm lengths) after surface sterilization in 10% chloros solution for different times. Fifty 2mm root lengths tested. Colonization given as % ± S.E.

(a) P. expansum

(b) Penicillium spp other than P. expansum

(c) Fungi other than Penicillium spp.


Percentage colonized root pieces
100
90
80
70
60
50
40
30
20
10
0
a b c
a b c
a b c
a b c
a b c
0
2
4
8
12
(m i n u t e s)

5.2.2. Results

The results of the experiments will be described in two parts, firstly for the sterilized soils and secondly for the unsterilized soils.

5.2.2.1. Sterilized soil treatments

5.2.2.1.1. Rhizosphere colonization

The results are given in figs. 19, 20 and 21, Tables 10 and 11, Appendix Tables 11 to 13.

The mean numbers of viable propagules of P. expansum recovered from the rhizospheres of maize, broad bean and tomato were consistently higher at all harvests than the numbers recovered from non-rhizosphere soils (figs. 19, 20 and 21) although differences were only significant for maize ($P < 0.001$) and broad bean ($P < 0.01$). The numbers of viable propagules reached a maximum in the rhizospheres of all plants at about 16-30 days after sowing (maize and broad bean) or planting (tomato) and then decreased rapidly over the next 30-60 days, levelling out at slightly above the numbers in non-rhizosphere soils. The maximum levels attained, expressed as a multiple of the numbers of propagules added to the soil, were:- maize 20,000x, broad bean 180x and tomato 600x.

The numbers of viable propagules in the non-rhizosphere soils also increased (maize 2000x, broad bean 20x and tomato 100x) and then remained more or less constant for the duration of the experiments (figs. 19, 20 and 21).

P. expansum was not recovered at any harvest from soils to which its spores were not added at the beginning of the experiment (Table 10).

The numbers of viable propagules of Penicillium spp other than P. expansum contaminating both the P. expansum infested and control soils were also higher in the rhizospheres of the three plants at all harvests than in the non-rhizosphere soils (Tables 10 and 11) but the differences were only significant

Figs. 19 to 21

Survival and growth of P. expansum in the rhizosphere (———) and non-rhizosphere (-----) soils of maize, broad bean and tomato grown in sterilized soil. 500 P. expansum spores g^{-1} soil were incorporated into the soils at the start of experiments.

Fig. 19 Maize

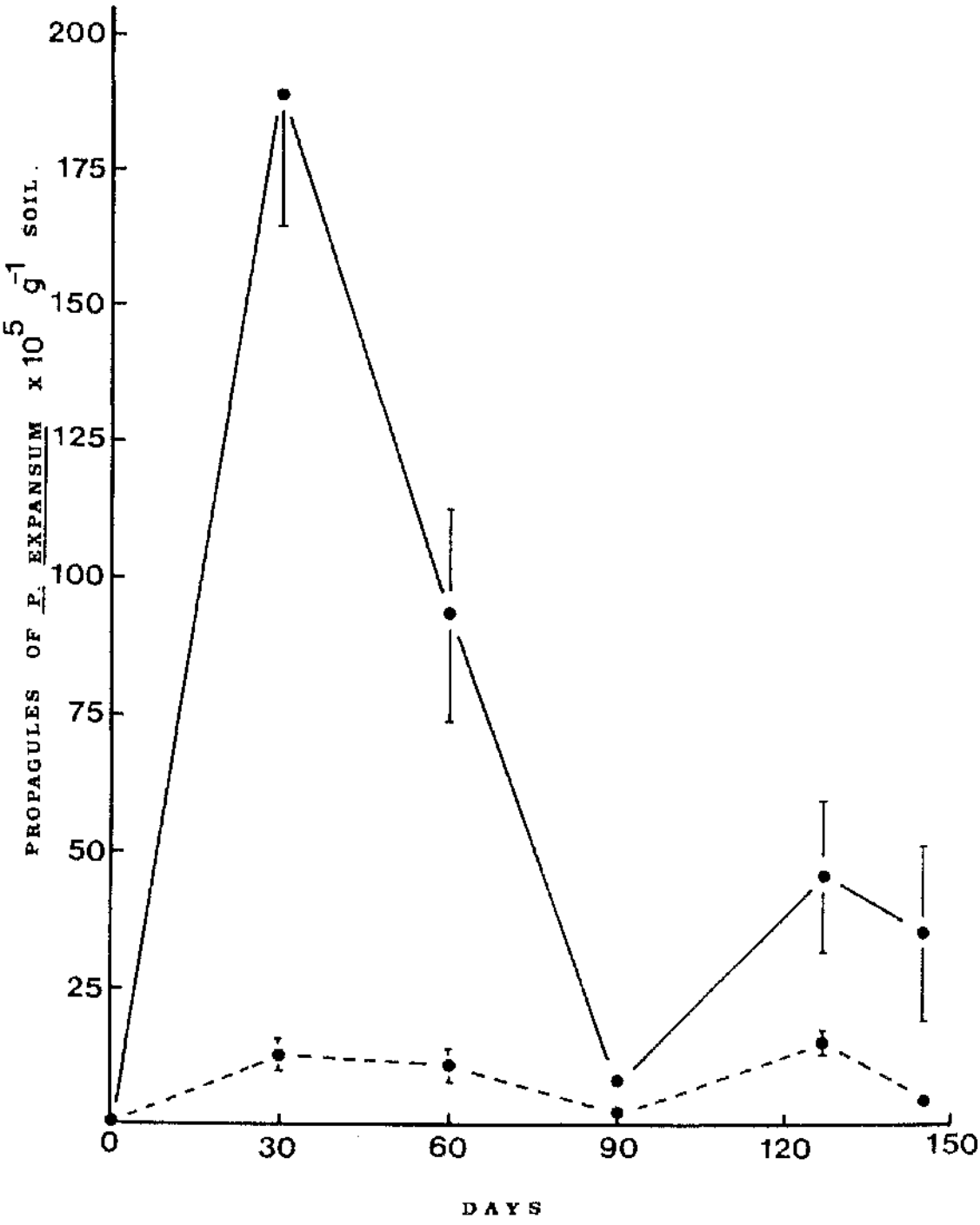

200
175
150
125
100
75
50
25
0
30
60
90
120
150
PROPAGULES OF P. EXPANSUM x 10^5 g^{-1} SOIL
DAYS

Fig. 20 Broad bean

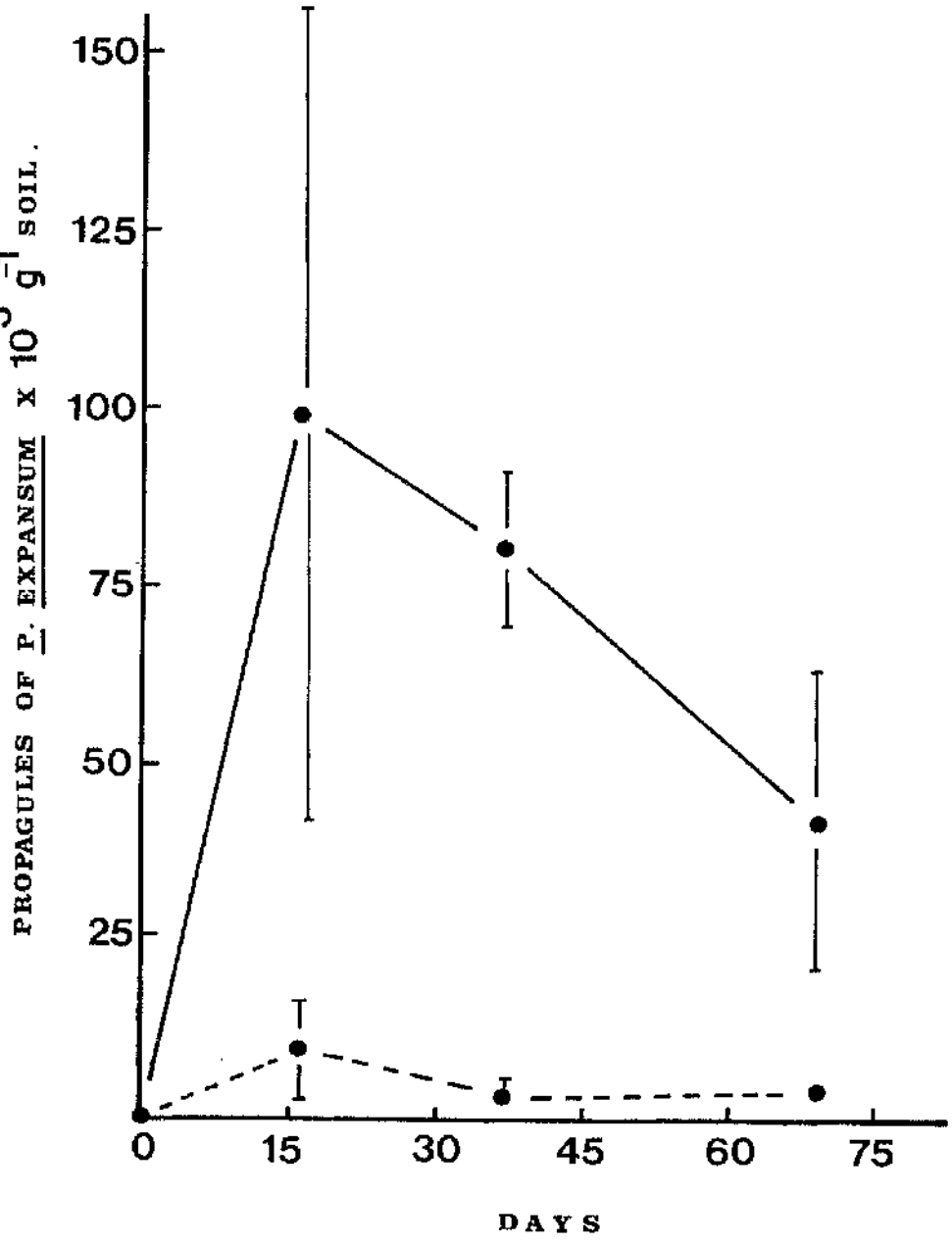


Fig. 21 Tomato

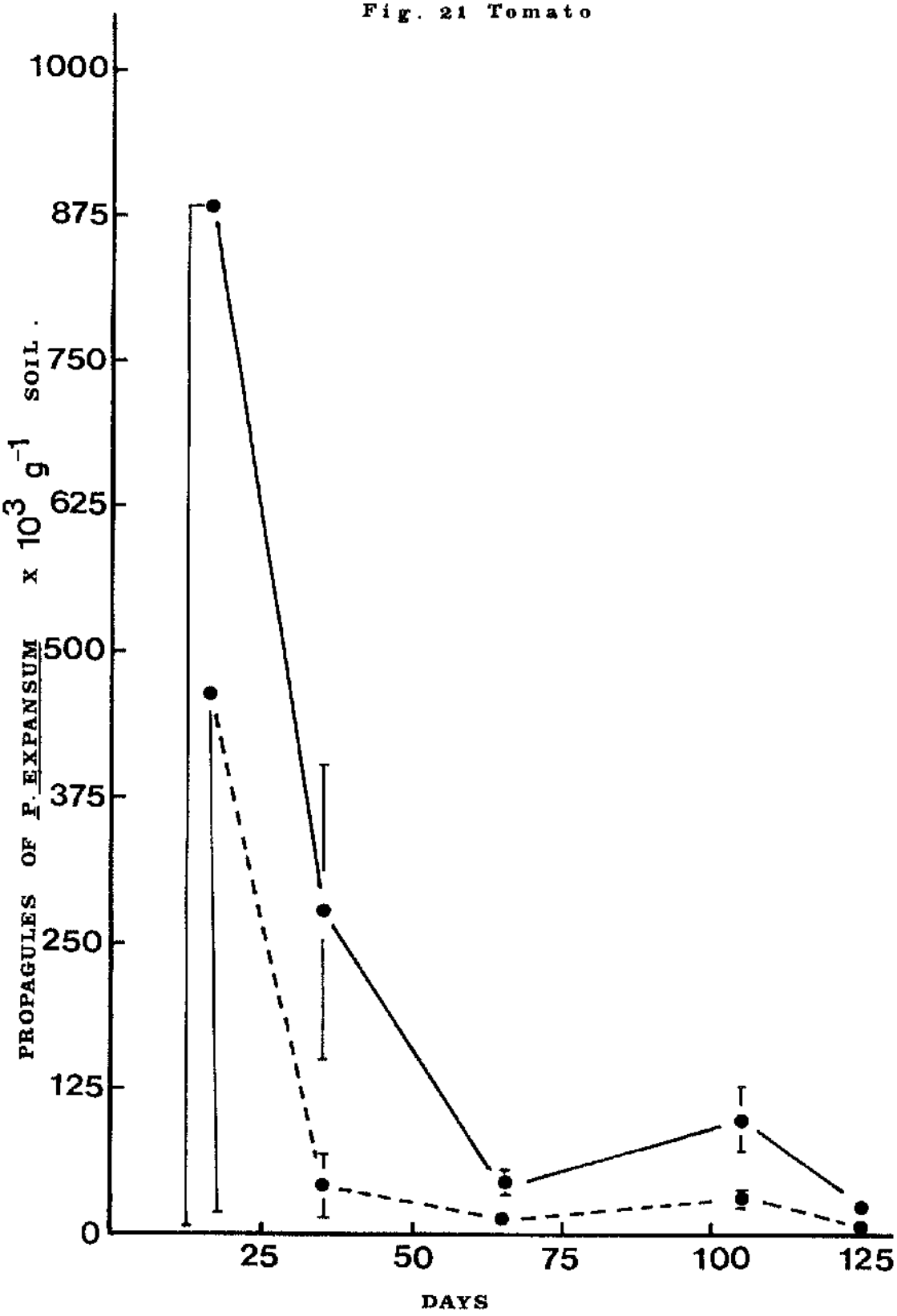


Table 10. Viable propagules of *P. expansum* and other *Penicillium* species in uninfested sterilized soils.

(A) Maize

		30 days	60 days	90 days	127 days	145 days
		Number x 10^3 g^{-1} oven-dry soil				
P. expansum	Rhizosphere	0*	0	0	0	0
	Non-rhizosphere	0	0	0	0	0
Other *Penicillium* species	Rhizosphere	11.88 ± 1.12	5.83 ± 5.83	2.72 ± 0.74	1.08 ± 0.55	59.62 ± 24.38
	Non-rhizosphere	0	0	2.95 ± 0.046	0.76 ± 0.15	8.15 ± 6.82

(B) Broad bean

		16 days	37 days	69 days
		Number x 10^3 g^{-1} oven-dry soil		
P. expansum	Rhizosphere	0*	0	0
	Non-rhizosphere	0	0	0
Other *Penicillium* species	Rhizosphere	0	21.54 ± 0.59	654.18 ± 642.28
	Non-rhizosphere	0	1.67 ± 1.34	3.16 ± 2.17

(C) Tomato

		16 days	35 days	65 days	105 days	125 days
		Number x 10^3 g^{-1} oven-dry soil				
P. expansum	Rhizosphere	0*	0	0	0	0
	Non-rhizosphere	0	0	0	0	0
Other *Penicillium* species	Rhizosphere	239.59 ± 237.58	48.26 ± 46.52	0	19.81 ± 1.52	4.57 ± 3.43
	Non-rhizosphere	0.72 ± 0.198	0	0.061 ± 0.061	3.32 ± 2.88	0.115 ± 0

* Figures are means from two replicates ± 1 S.E.

Table 11. Viable propagules of Penicillium spp other than P. expansum in P. expansum infested sterilized soil.

(A) Maize

	Number x 10^3 g^{-1} oven-dry soil				
	30 days	60 days	90 days	127 days	145 days
Rhizosphere	39.8* ± 1.98	24.86 ± 8.09	19.0 ± 12.0	12.05 ± 6.26	10.59 ± 5.47
Non-rhizosphere	0	0	0.46 ± 0.25	12.3 ± 6.09	4.9 ± 0.99

(B) Broad bean

	Number x 10^3 g^{-1} oven-dry soil		
	16 days	37 days	69 days
Rhizosphere	108.58* ± 93.93	94.25 ± 89.16	148.24 ± 142.55
Non-rhizosphere	0.16 ± 0.08	2.35 ± 1.15	2.97 ± 2.48

(C) Tomato

	Number x 10^3 g^{-1} oven-dry soil				
	16 days	35 days	65 days	105 days	125 days
Rhizosphere	32.67* ± 25.13	9.19 ± 5.05	6.11 ± 0.93	6.12 ± 2.22	6.59 ± 3.17
Non-rhizosphere	4.42 ± 4.10	0.41 ± 0.25	0.46 ± 0.25	0.56 ± 0.14	1.95 ± 0.86

* Figures are means from 3 replicates ± 1 S.E.

for maize in the P. expansum infested soil ($P < 0.001$).

5.2.2.1.2. Colonization of the internal root tissues and/or the root surfaces

(i) Root washing technique

The results are given in figs. 22, 23, 24, 25, 26 and 27.

P. expansum colonized the root surface and/or internal tissues of all three plants grown in the sterilized soils but the percentage colonization was variable both between and within species (figs. 22, 23 & 24). Maximum colonization of maize roots was reached about 60 days after sowing, of broad bean about 37 days after sowing, and of tomato about 35 days after transplanting. After attaining the maximum levels, colonization decreased very slightly in maize (fig. 22) but quite markedly in broad bean and tomato (figs. 23 & 24).

Root surfaces and/or internal tissues were also colonized by other species of Penicillium and by species of other fungal genera in both the P. expansum infested and the uninfested soils (figs. 22,23,24,25,26 & 27). These fungi were almost certainly contaminants arriving in the soils during the setting up and during the course of the experiment.

(ii) Surface sterilization technique

Only the roots of maize were examined by the surface sterilization technique. The results, given in Table 12, show that P. expansum colonized the internal tissues of maize roots in sterilized soil although it was present to the greatest extent on or just under the outer surface of the roots.

Figs. 22 to 24

Colonization of maize, broad bean and tomato roots in *P. expansum* infested sterilized (S) and unsterilized (U) soils. Fifty 2mm root lengths tested. Colonization given as % ± S.E.

(a) *P. expansum*

(b) *Penicillium* spp other than *P. expansum*

(c) Fungi other than *Penicillium* spp

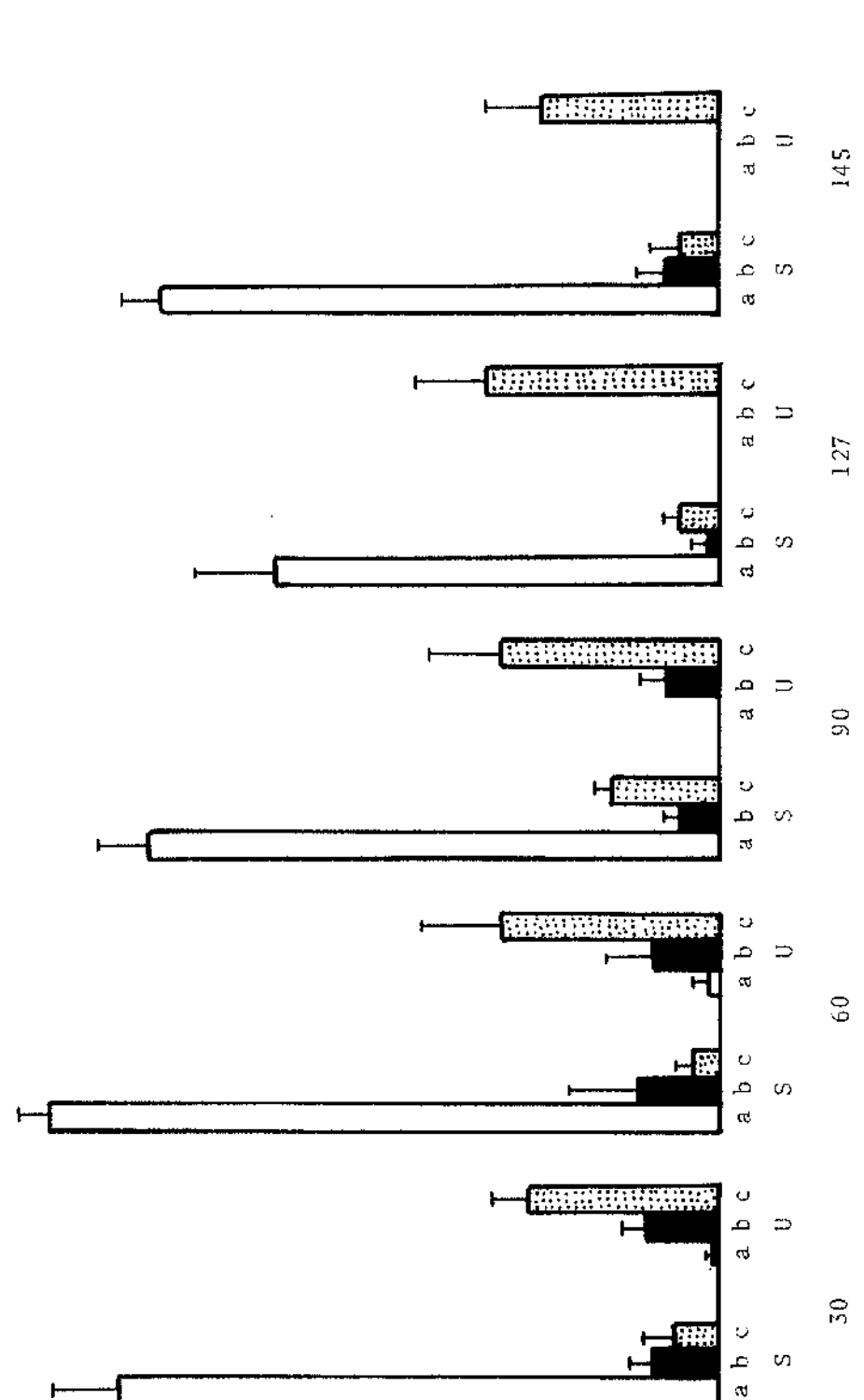

Percentage colonized root pieces
100
90
80
70
60
50
40
30
20
10
0
a b c
S
a b c
U
30
a b c
S
a b c
U
60
a b c
S
a b c
U
90
a b c
S
a b c
U
127
a b c
S
a b c
U
145
(D A Y S)

Fig. 22 Maize

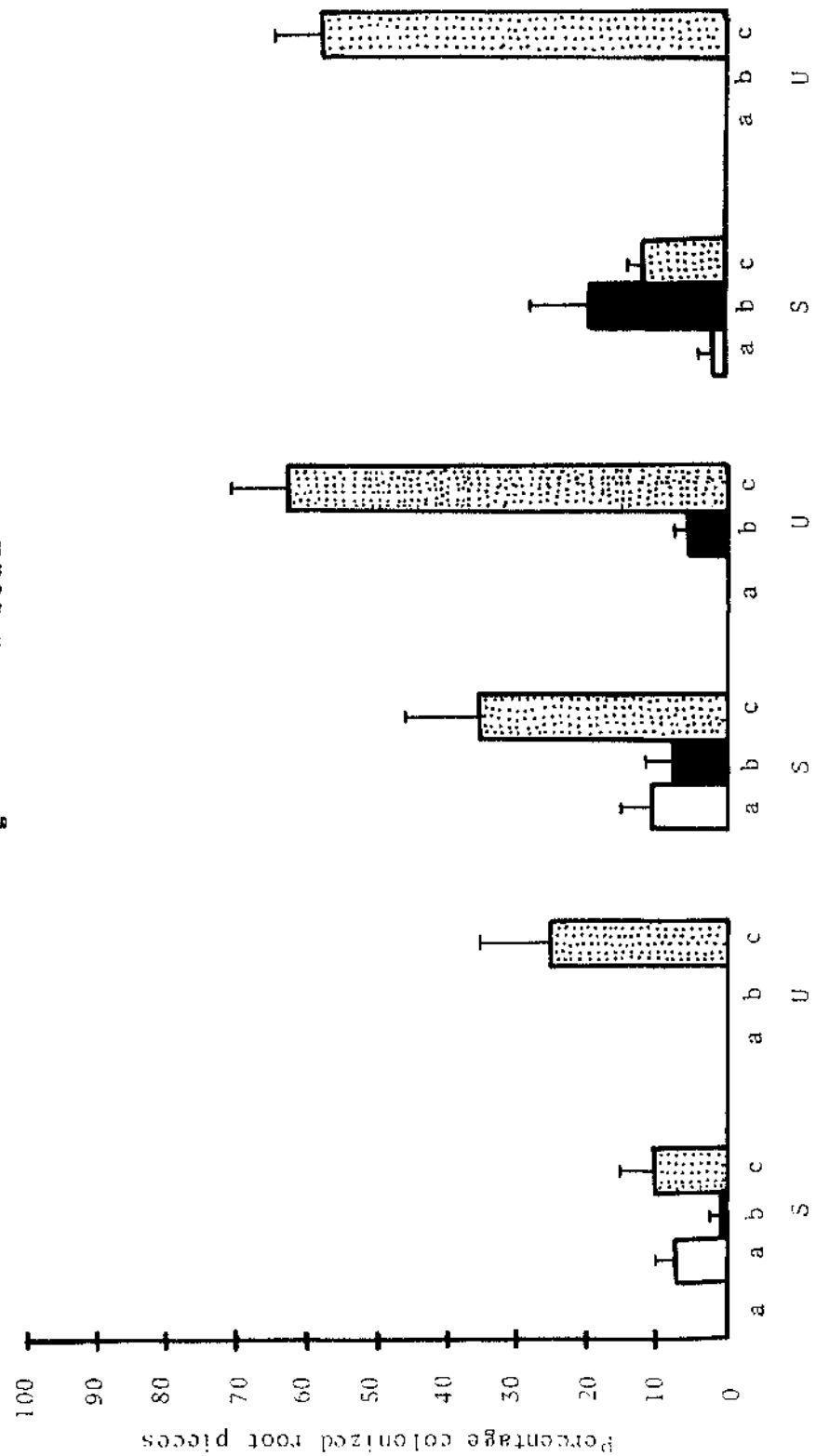
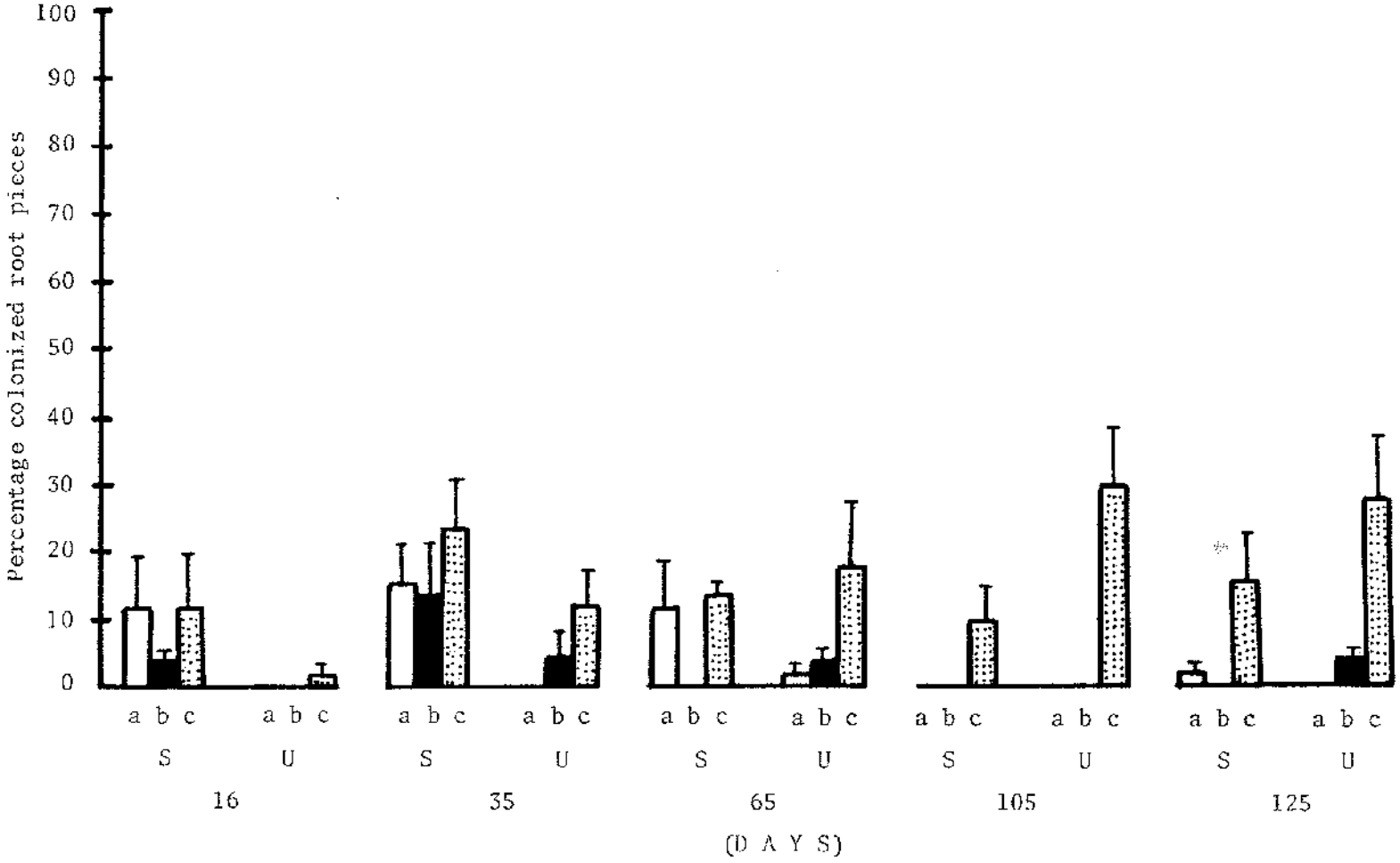


Fig. 23 Broad bean

Fig. 24 Tomato



Figs. 25 to 27

Colonization of maize, broad bean, and tomato roots in *P. expansum* uninfested sterilized (S) and unsterilized (U) soils. Fifty 2mm root lengths tested. Colonization given as % ± S.E.

(a) *P. expansum*

(b) *Penicillium* spp other than *P. expansum*

(c) Fungi other than *Penicillium* spp

Fig. 25 Maize

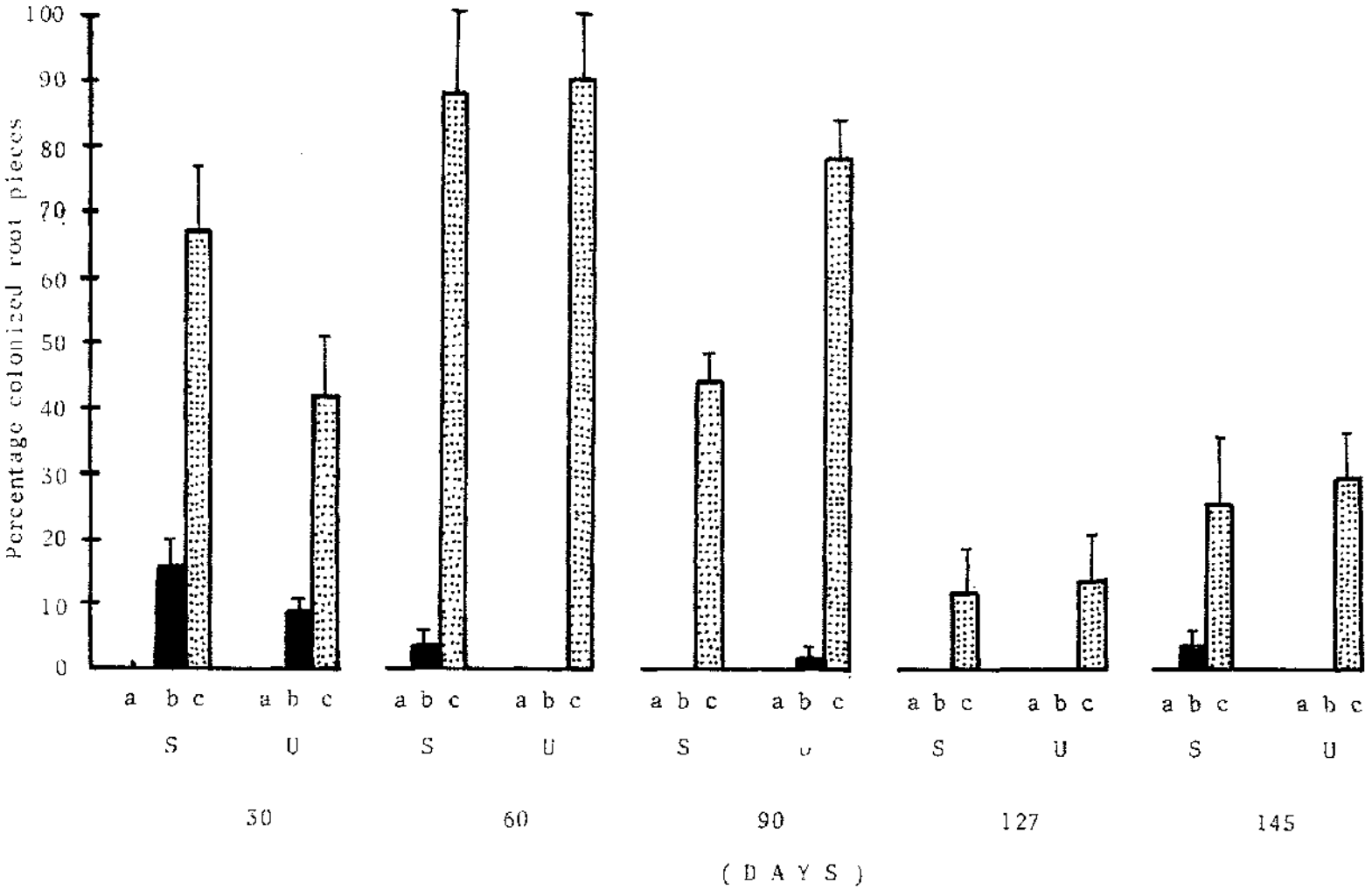

Percentage colonized root pieces
100
90
80
70
60
50
40
30
20
10
0
a b c a b c
S U
30
60
90
127
145
(D A Y S)

Fig. 26 Broad bean

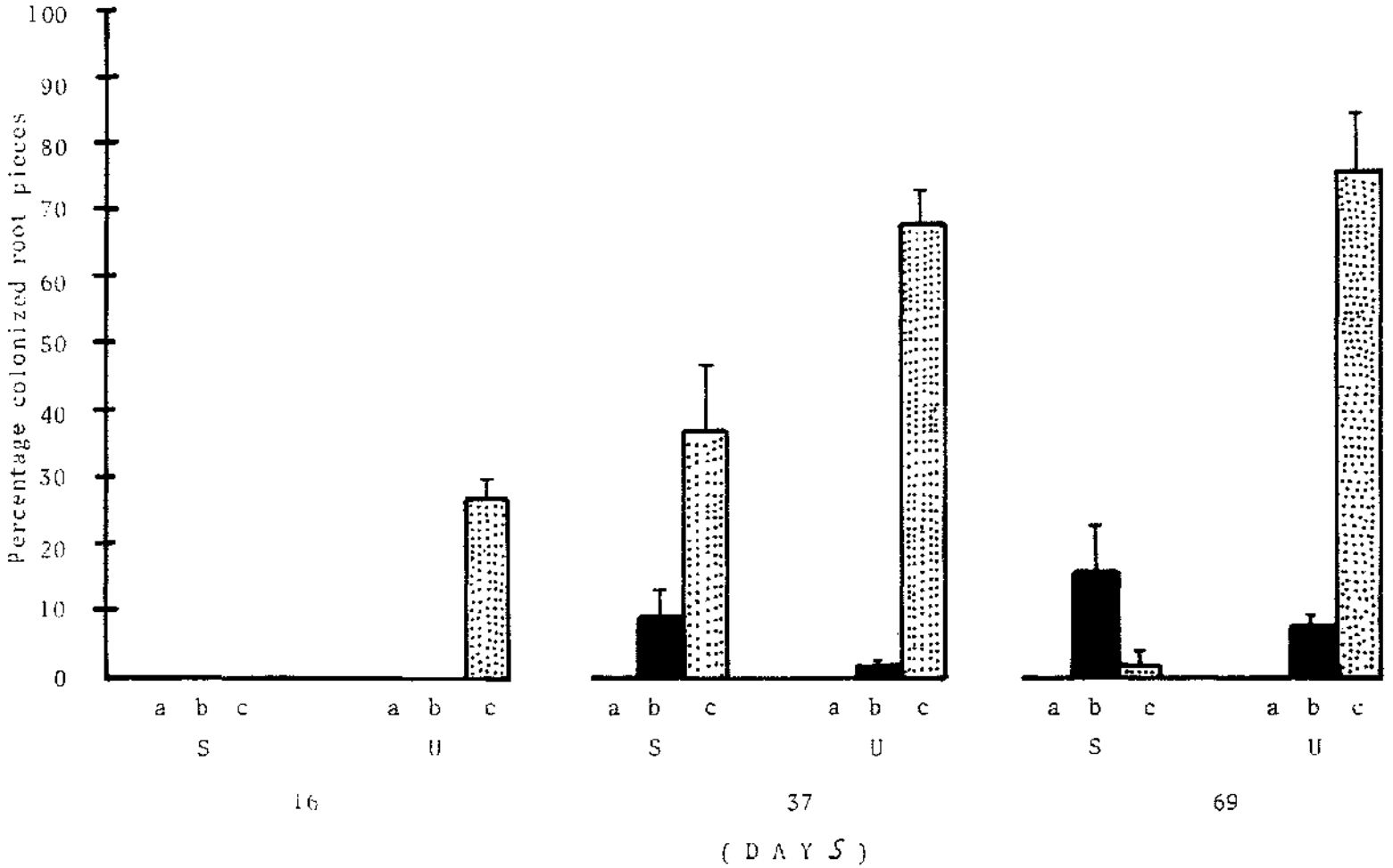


Fig. 27 Tomato

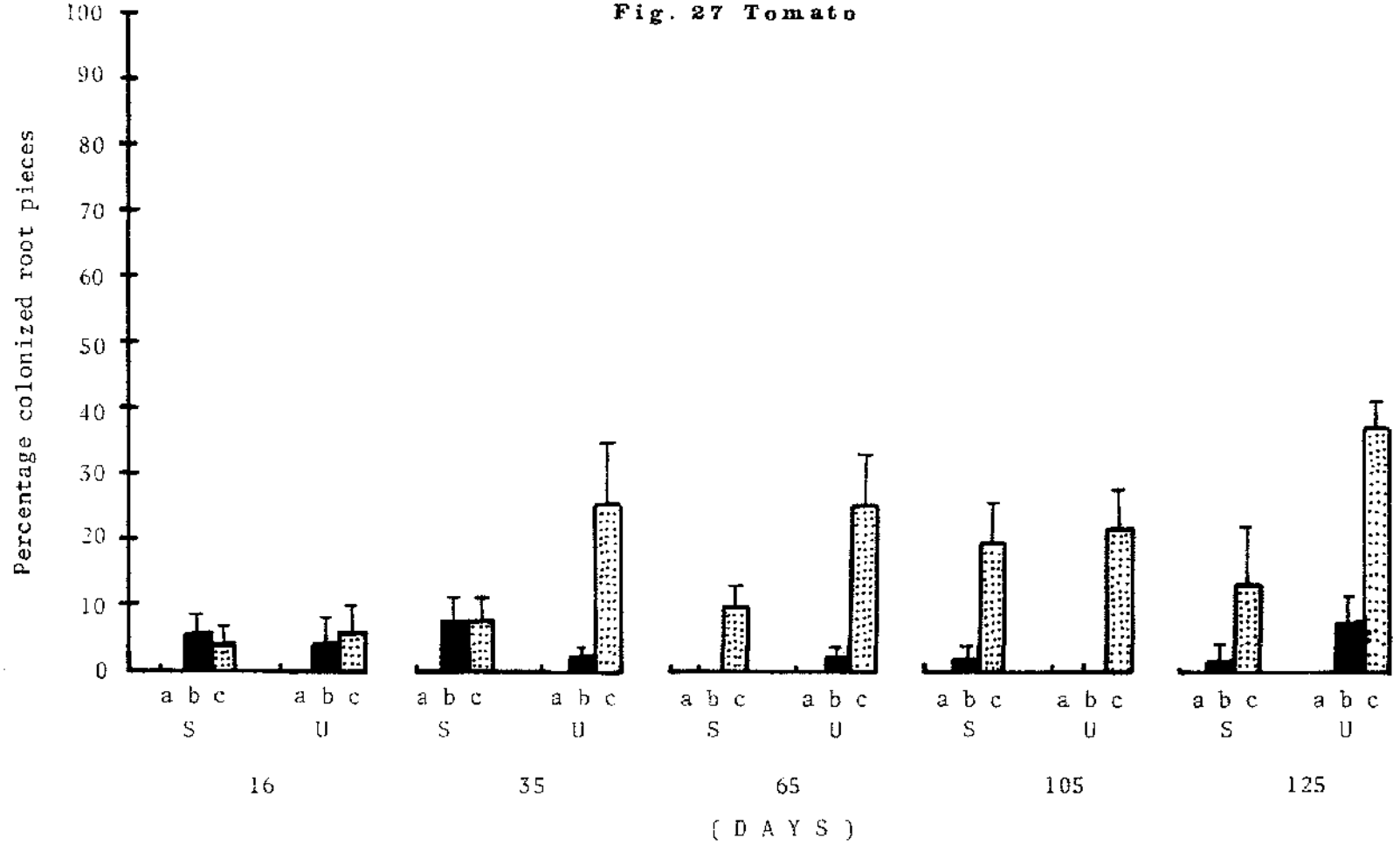


Table 12. Colonization of internal tissues of roots of maize grown in P. expansum infested sterilized soil.

	control	10* min	15 min
P. expansum	64** ± 10.7	30 ± 5.5	10 ± 5.5
Other Penicillium species	2 ± 2	0	0
Other fungi	6 ± 2.4	3.3 ± 2.3	0

* Time period of root surface sterilization in 10% chloros.

** Number ± S.E. of 2mm root lengths colonized given as percentage of 50 pieces tested.

5.2.2.2. Unsterilized soil treatments

5.2.2.2.1. Rhizosphere colonization

The results are given in figs. 28, 29, 30, 31, 32, 33, 34, 35, Table 13 and Appendix Tables 14 to 17.

The numbers of viable propagules of *P. expansum* recovered from the rhizospheres of maize, broad bean, tomato and apple grown in unsterilized soils were consistently higher than the numbers recovered from non-rhizosphere soils (figs. 28,29,30 & 31). These differences were significant for broad bean ($P < 0.05$), tomato ($P < 0.05$) and apple ($P < 0.05$) but not for maize. The maximum numbers of propagules were found between 30 and 67 days after sowing (maize, broad bean) or planting (tomato and apple) and then rapidly decreased to levels slightly above the non-rhizosphere soils. In contrast, in non-rhizosphere soils of maize and tomato, the numbers of viable propagules remained more or less the same for the duration of the experiment, but with broad bean and apple the results were more variable with an indication of a slight decrease during the course of the experiments (figs. 29 & 31).

P. expansum was not recovered at any harvest from soils to which its spores were not added at the beginning of the experiment (Table 13).

Higher numbers of propagules of *Penicillium* spp other than *P. expansum* were found in the rhizosphere soils than in the non-rhizosphere soils of all plants in all treatments at all harvests (figs. 32,33,34,35 & Table 13). These differences were significant in both *P. expansum* infested soils (maize $P < 0.05$, broad bean $P < 0.01$ and apple $P < 0.01$) and uninfested soils (maize $P < 0.05$, broad bean $P < 0.001$, and apple $P < 0.001$).

Figs. 28 to 31

Survival and growth of *P. expansum* in the rhizosphere (———) and non-rhizosphere (-----) soils of maize, broad bean, tomato and apple grown in unsterilized soil.

Fig. 28 Maize

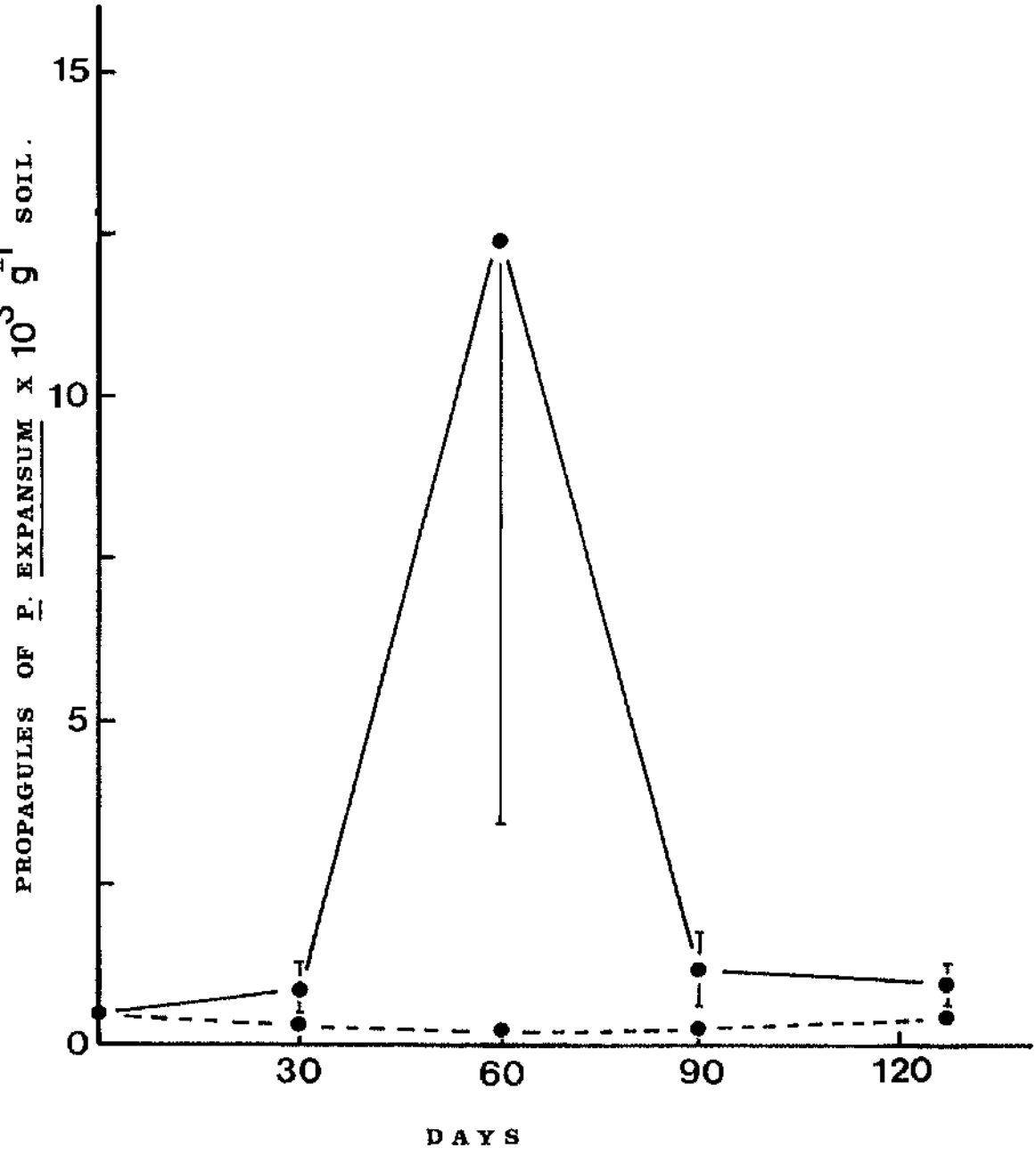

15
10
5
0
PROPAGULES OF P. EXPANSUM x 10^3 g^{-1} SOIL.
30
60
90
120
DAYS

Fig. 29 Broad bean

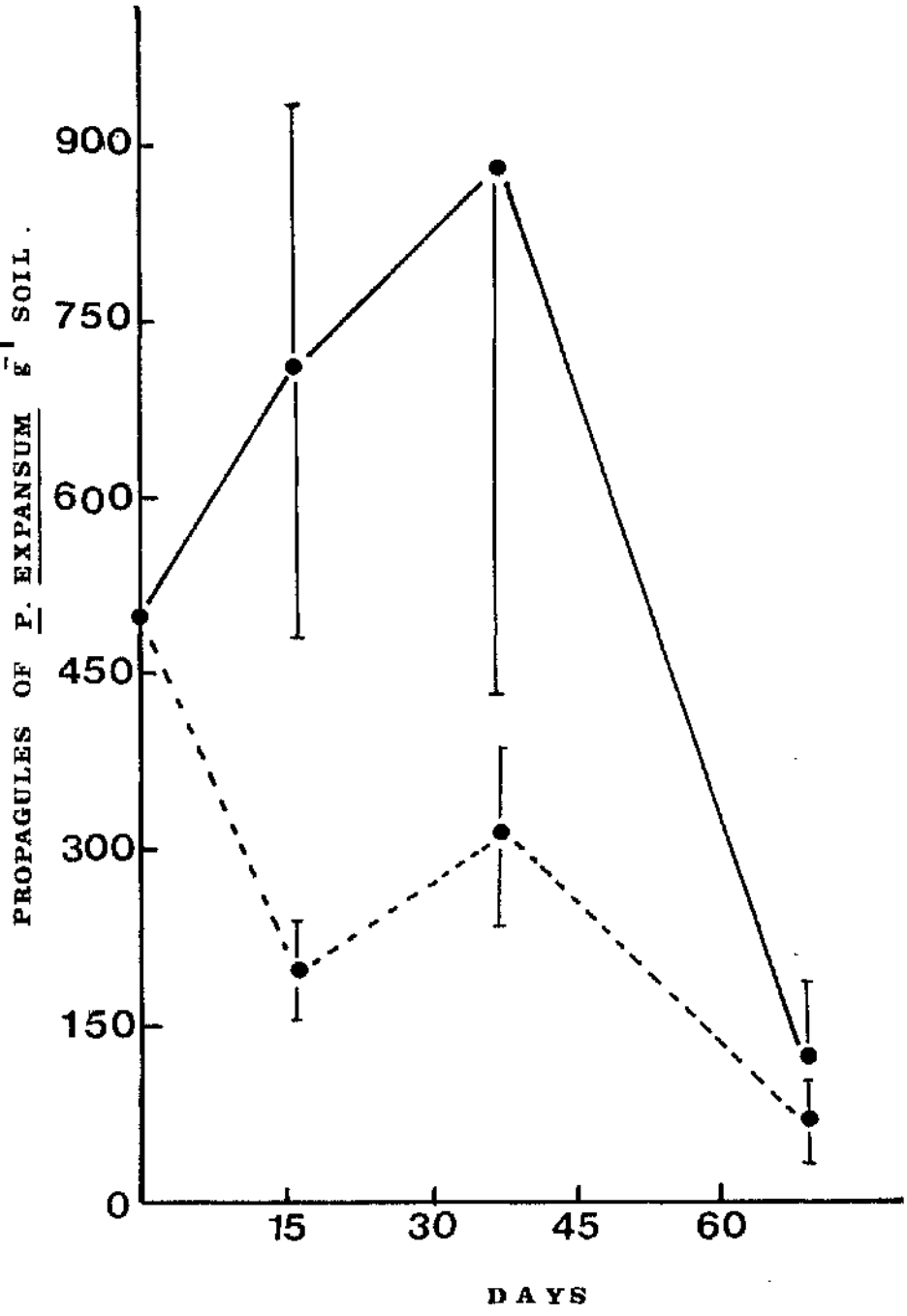


Fig. 30 Tomato

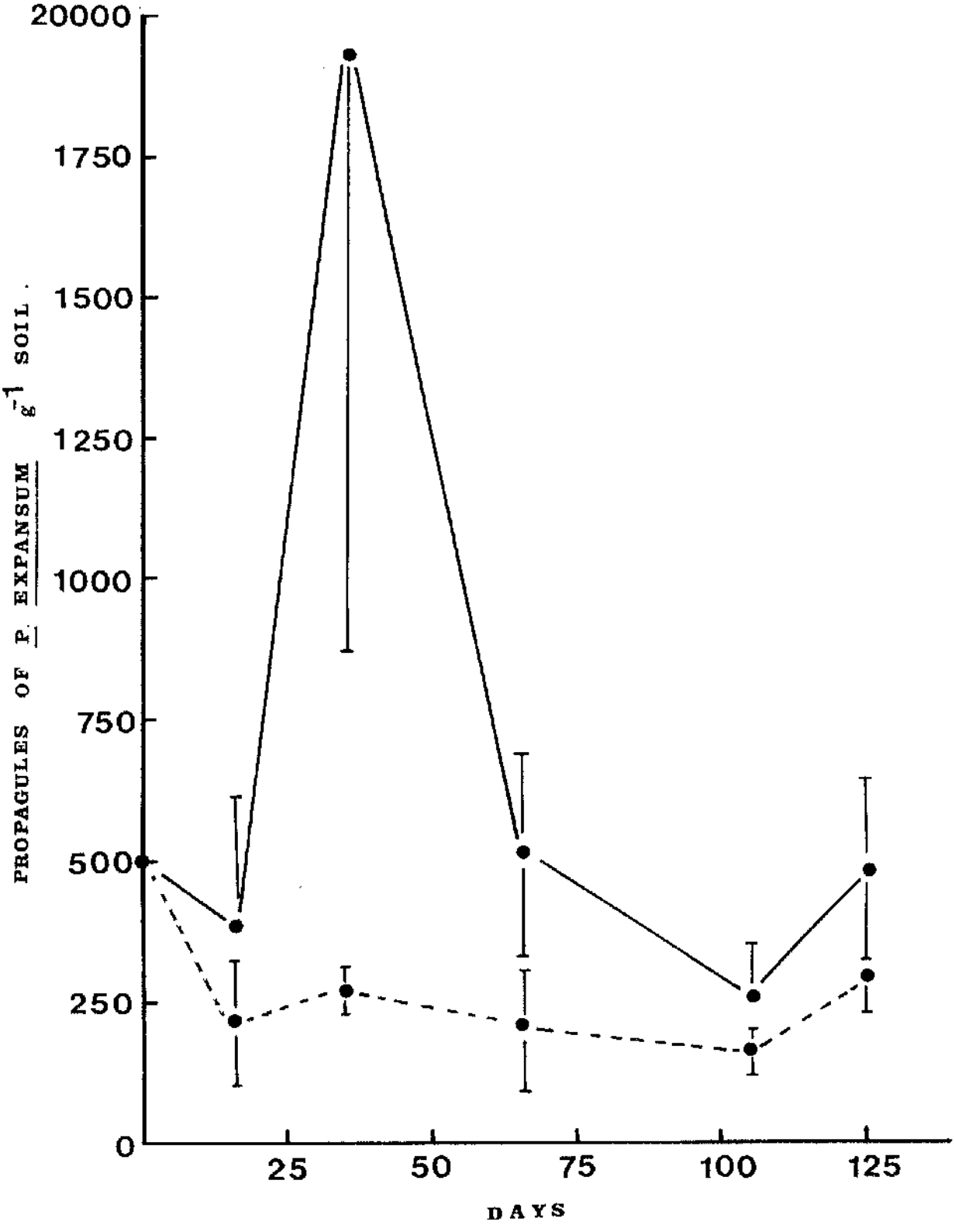


Fig. 31 Apple

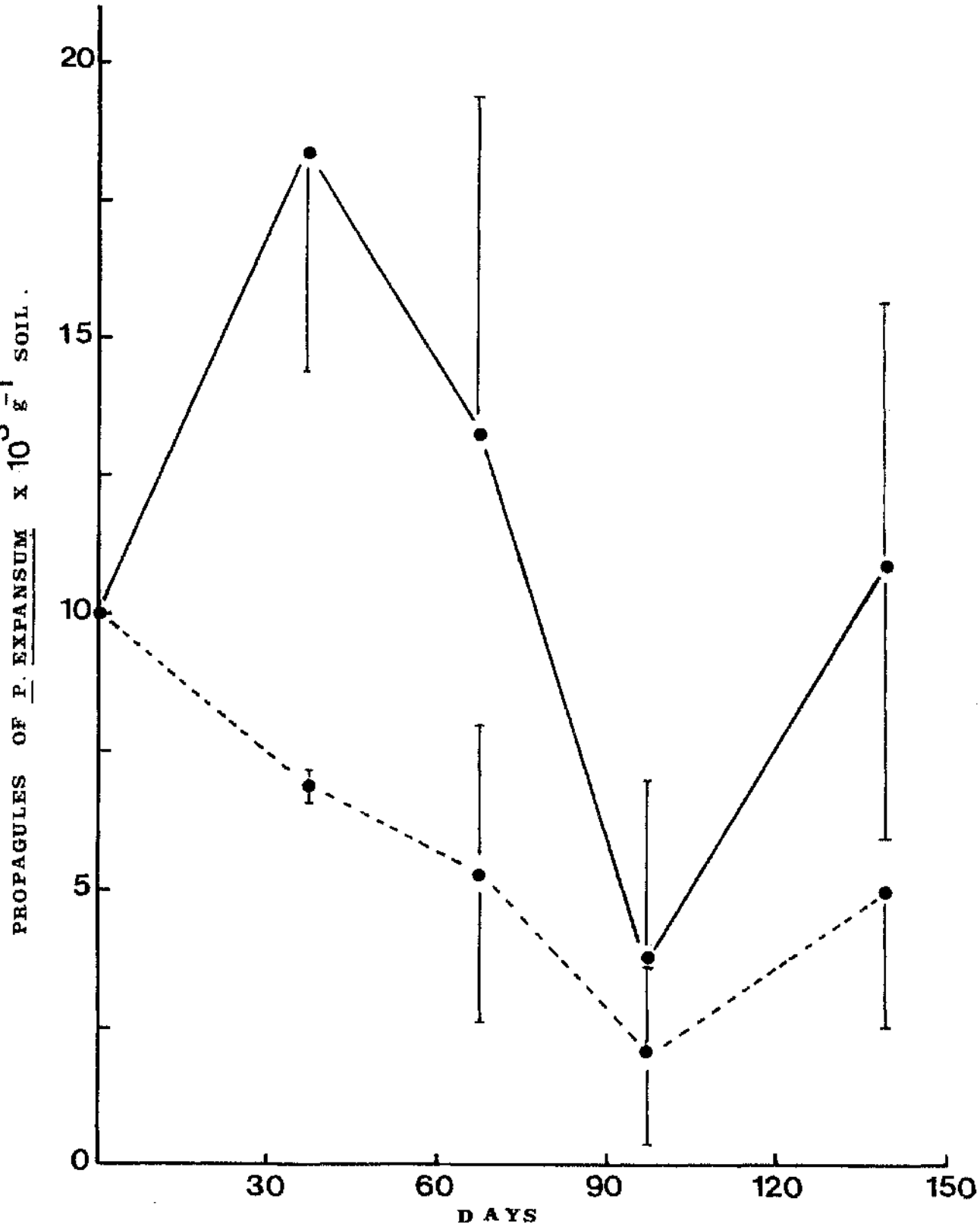

20
15
10
5
0
30
60
90
120
150
DAYS
PROPAGULES OF P. EXPANSUM x 10^3 g^{-1} SOIL.

Table 13. Viable propagules of *P. expansum* and of other *Penicillium* spp in uninfested unsterilized soil.

(A) Maize

		30 days	60 days	90 days	127 days	145 days
		Number x 10^3 g^{-1} oven-dry soil				
P. expansum	Rhizosphere	0*	0	0	0	0
	Non-rhizosphere	0	0	0	0	0
Other *Penicillium* spp	Rhizosphere	5.35 ± 3.74	5.93 ± 0.52	8.03 ± 1.2	11.0 ± 1.19	13.33 ± 3.69
	Non-rhizosphere	3.46 ± 1.91	5.18 ± 0.88	6.94 ± 0.72	6.63 ± 0.12	7.06 ± 2.06

(B) Broad bean

		16 days	37 days	69 days
		Number x 10^3 g^{-1} oven-dry soil		
P. expansum	Rhizosphere	0*	0	0
	Non-rhizosphere	0	0	0
Other *Penicillium* spp	Rhizosphere	13.72 ± 1.41	16.84 ± 0.61	17.41 ± 1.41
	Non-rhizosphere	6.27 ± 0.27	5.11 ± 0.65	4.94 ± 1.72

Table 13 (continued)

(C) Tomato

		16 days	35 days	65 days	105 days	125 days
		Number of viable propagules x 10^3 g^{-1} oven-dry soil				
P. expansum	Rhizosphere	0*	0	0	0	0
	Non-rhizosphere	0	0	0	0	0
Other *Penicillium* spp	Rhizosphere	15.83 ± 3.83	11.23 ± 0.37	9.54 ± 1.92	5.01 ± 5.02	8.64 ± 0.042
	Non-rhizosphere	9.96 ± 0.18	8.72 ± 0.001	8.1 ± 1.2	9.54 ± 1.66	4.62 ± 2.53

(D) Apple

		32 days	67 days	97 days	139 days
		Number of viable propagules x 10^3 g^{-1} oven-dry soil			
P. expansum	Rhizosphere	0*	0	0	0
	Non-rhizosphere	0	0	0	0
Other *Penicillium* spp	Rhizosphere	25.36 ± 1.84	17.45 ± 1.86	7.82 ± 1.92	5.55 ± 0.25
	Non-rhizosphere	2.00 ± 0.24	1.97 ± 0.25	4.08 ± 0.83	1.14 ± 0.077

* Figures are mean from two replicates ± 1 S.E.

Figs. 32 to 35

Survival and growth of *Penicillium* species other than *P. expansum* in the rhizosphere (———) and non-rhizosphere (-----) soils of maize, broad bean, tomato and apple grown in *P. expansum* infested unsterilized soil.

Fig. 32 Maize

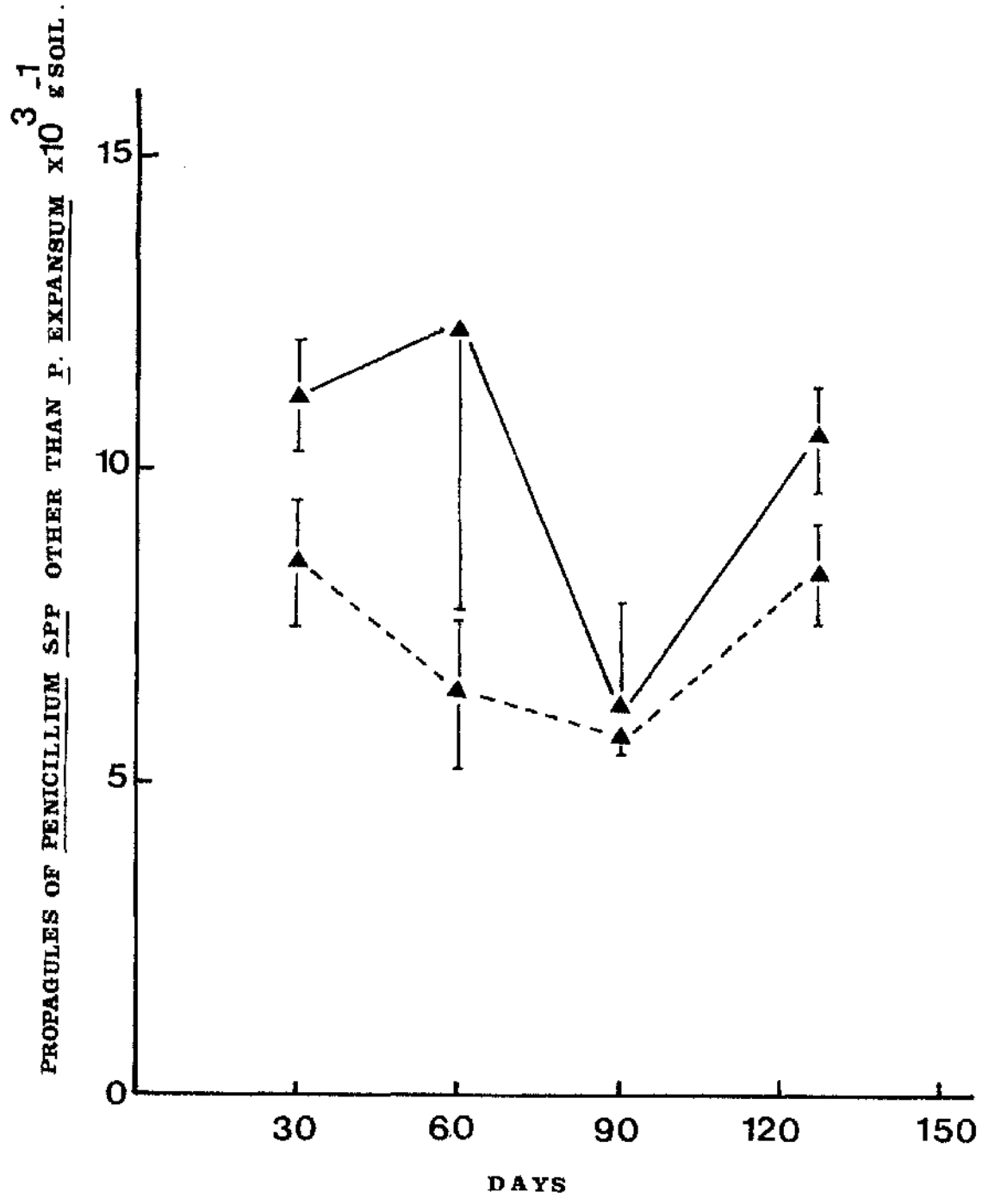

PROPAGULES OF PENICILLIUM SPP OTHER THAN P. EXPANSUM x10^3 g^-1 SOIL.
15
10
5
0
30
60
90
120
150
DAYS

Fig. 33 Broad bean

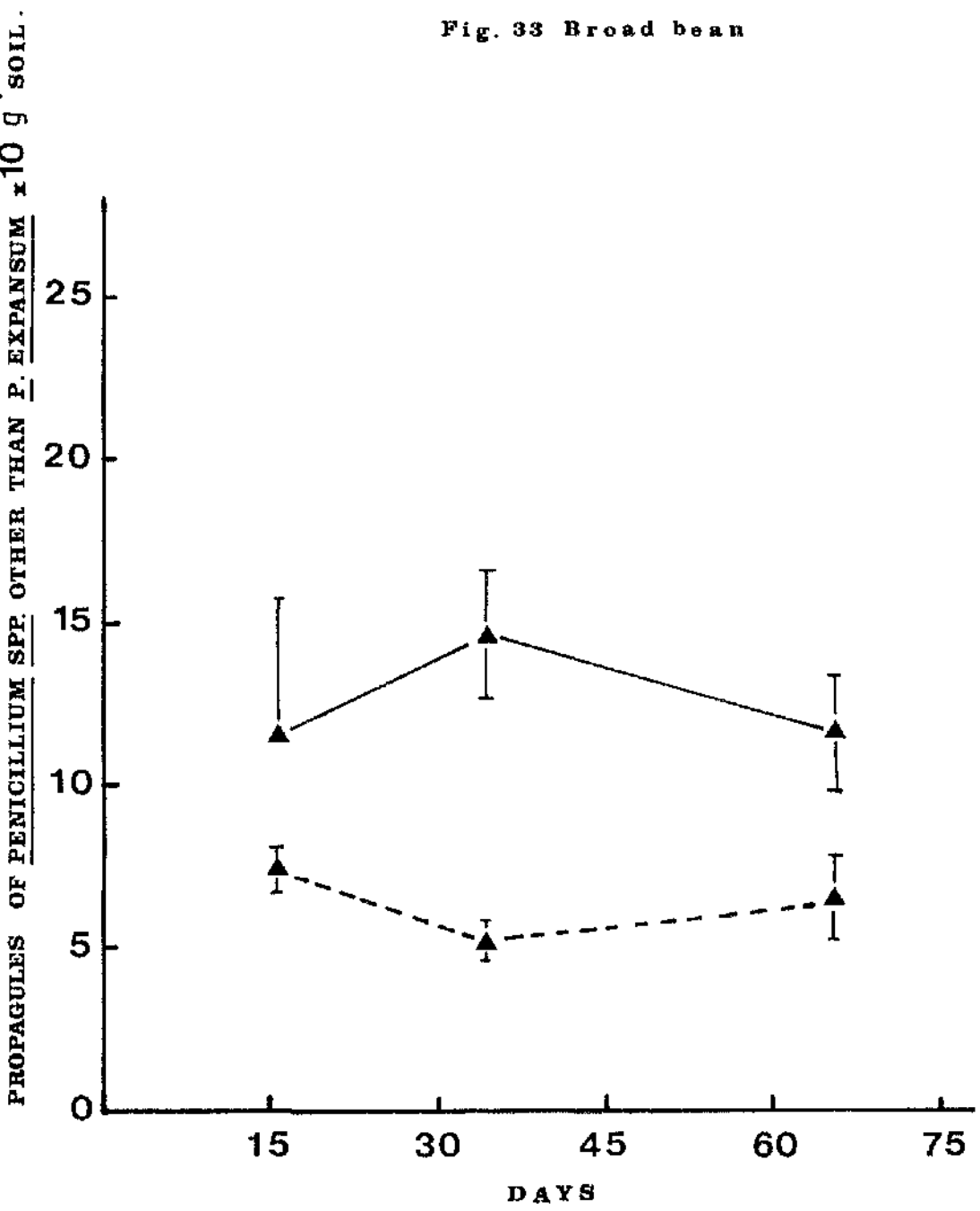


Fig. 34 Tomato

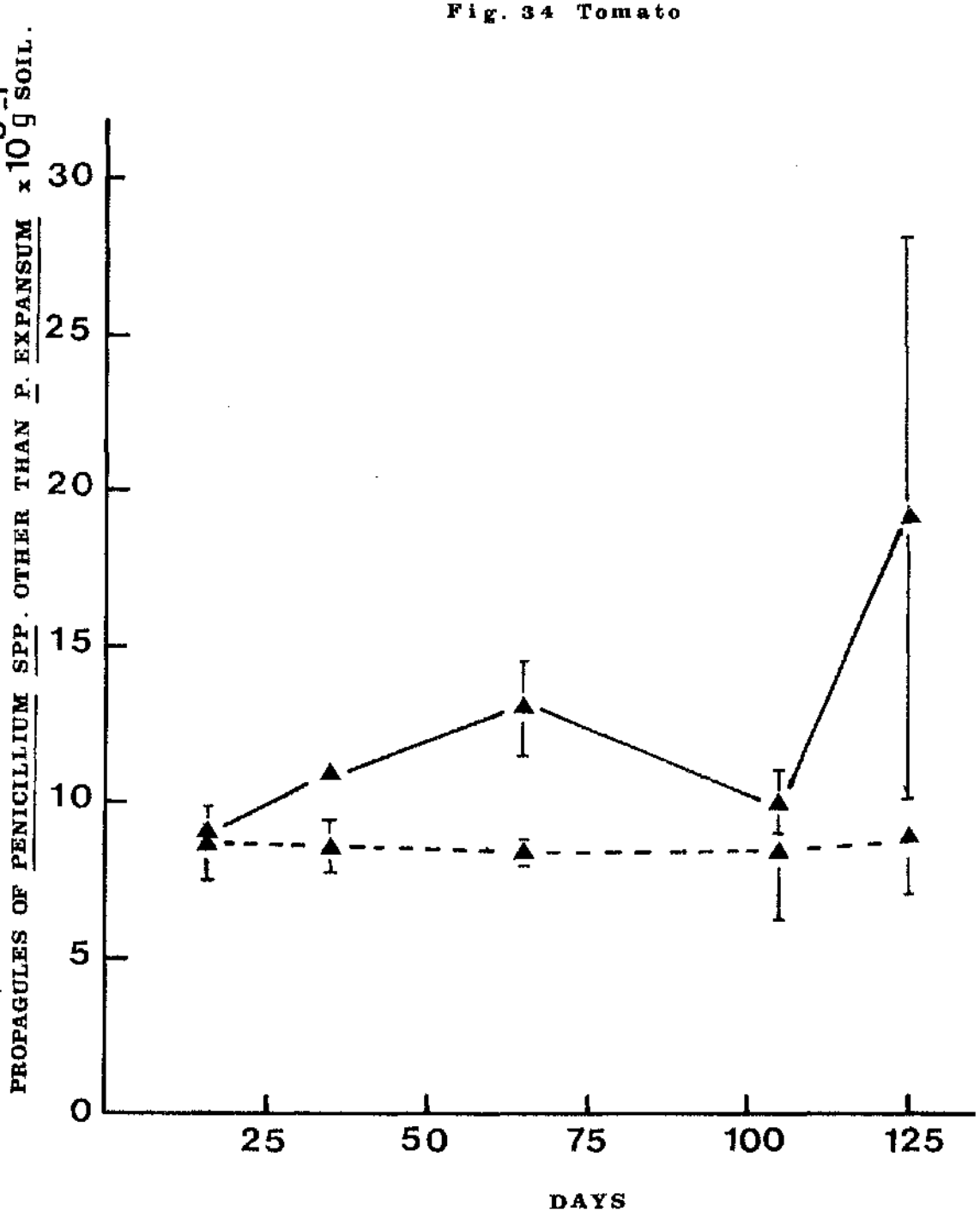
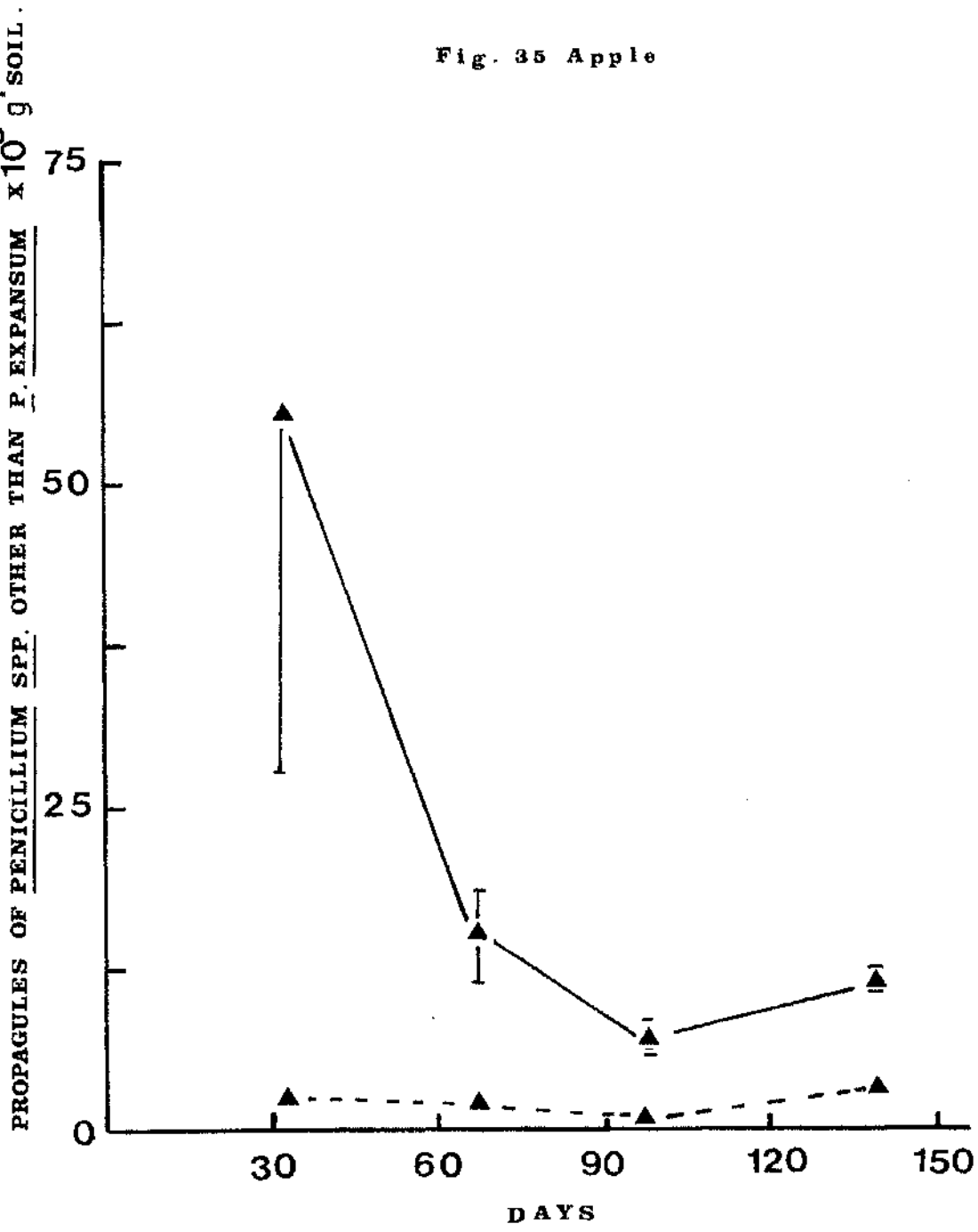


Fig. 35 Apple


PROPAGULES OF PENICILLIUM SPP. OTHER THAN P. EXPANSUM x10³ g⁻¹ SOIL.
75
50
25
0
30
60
90
120
150
DAYS

5.2.2.2.2. Colonization of the internal root tissues and/or the root surfaces

(i) Root washing technique

The results are given in figs. 22, 23, 24, 25, 26 and 27.

P. expansum did not colonize the root surfaces or internal tissues of either maize, broad bean or tomato, or did so to a very limited extent only (figs. 22,23 & 24).

Other fungal species, including various Penicillium spp, colonized the root surfaces of all three plants to some extent in both P. expansum infested and in uninfested soils (figs. 22,23,24,25,26 & 27). Maximum colonization by Penicillium spp other than P. expansum was found in maize 30 days after sowing after which numbers declined (figs. 22 & 25). In tomato, some colonization was evident 16-35 days after transplanting and the levels then remained more or less unchanged until the end of experiment (125 days) (figs. 24 & 27). In contrast, little if any colonization of bean roots was found (figs. 23 & 26).

Colonization by fungi of genera other than Penicillium in both P. expansum infested and uninfested soils was higher for all three plants than by all Penicillium spp including P. expansum (figs. 22,23,24,25,26 & 27). Colonization of the root surfaces and/or internal root tissues of broad bean and tomato gradually increased throughout the course of the experiment, but in maize it reached a maximum 30 days after sowing and then remained constant for the rest of the experiments in both infested and in uninfested soils.

5.2.3. Discussion

Populations of P. expansum markedly increased in the rhizospheres of all plants grown in both sterilized and unsterilized soils. However the fungus only colonized the roots and root surfaces of plants to any significant extent in sterilized soils. P. expansum was not recovered at any harvest

from soils to which spores had not been added at the beginning of the experiments so the observed increases in the infested soils must have originated from the applied inoculum and the results were probably not affected by any chance contamination which occurred during the course of the experiments. Contamination is clearly a danger with the type of experiment described and did occur with some species of Penicillium other than P. expansum and with other fungi. Populations of contaminating species of Penicillium were always higher in the rhizosphere soils than in the non-rhizosphere soils in both P. expansum infested and in uninfested soils. They, together with species of other genera of fungi, also colonized the roots and root surfaces of these plants to a significant extent in most soils.

One feature of this series of experiments was the considerable variation in numbers of viable propagules of P. expansum recovered between replicates at all harvests in both sterilized and unsterilized soils. This variability may have been due to uneven mixing of the spores into the soils. In these experiments the spores were mixed into the soil manually. During growth in the test soils, plant roots come into contact with a variable number of spores. Some of these will be stimulated to germinate and by microcyclic conidiation may rapidly produce high numbers of conidia. Therefore the variability between replicates may have been due to two factors. Firstly, uneven mixing of spores into the soil. Secondly, the fact that during the early stages of plant growth very few spores will be contacted by plant roots but those that are will be induced to produce high numbers of conidia by microcyclic conidiation.

In order to achieve greater uniformity of spore distribution in the test soils, various methods of mixing spores into large bulks of the soil were investigated (Chapter II). The experiment was then repeated with tomato plants using the improved methods for mixing spores into the soil.

5.3. COLONIZATION OF THE RHIZOSPHERE AND RHIZOPLANE OF TOMATO (EXPERIMENT II)

5.3.1. Materials and methods

When this experiment was first set up insufficient inoculum was mixed into the unsterilized soils. Thus half of the experiment had to be scrapped. Because of difficulties in obtaining and preparing a large bulk of soil, sterilized soil treatments were continued and the unsterilized soil treatments were set up as a separate experiment 4 weeks later.

5.3.1.1. Preparation of soil

Soil was dried at room temperature and sieved through a 2mm sieve. A mixture of this soil with peat (2 peat : 3 soil by volume) was prepared and then incubated for about two weeks on a greenhouse bench under a polythene sheet to allow the peat to become colonized by micro-organisms from the soil. The sterilized soils were autoclaved immediately after mixing in the peat. *P. expansum* spores were mixed into both sterilized and unsterilized soils using a concrete mixer as described in Chapter II. 2Kg of the soil-peat mixtures plus spores were placed in each of a series of 17.78cm pots (100 pots of sterilized and 100 pots unsterilized soil). A further series of pots were filled with 2Kg of the soil-peat mixtures without spores (35 pots sterilized and 35 pots unsterilized soil).

5.3.1.2. Production and maintenance of plants

Tomato plants of the cv Moneymaker were grown as described earlier and transplanted into the pots when they had reached the 2 to 4 leaf stage. Ten plants were transplanted into each of the 5 pots from each treatment, which were intended for sampling two weeks after transplanting, to ensure that as much of the soil was permeated by roots as possible. For a similar reason five plants were transplanted into each of the 5 pots which were intended for sampling from each treatment four weeks after transplanting.

Single plants were transplanted into all pots intended for sampling at stages later than four weeks after transplanting. The pots were placed on the bench in a heated greenhouse, 35 pots containing the soil spore mixtures of each treatment but without plants, were included as controls. They were treated in exactly the same way as the other pots.

All pots were watered with 500ml of a phostrogen nutrient solution (1.0g l^{-1} tap water) once a week during the first and second weeks and twice a week thereafter. The plants were sprayed with Ambush (25% w/v permethrin) to control aphids and white fly as necessary. As they developed, the plants were tied to strings and side shoots were removed when they appeared to ensure erect growth.

5.3.1.3. Estimation of the numbers of viable propagules of P. expansum in the soils

The numbers of viable propagules of P. expansum in the rhizosphere and in the non-rhizosphere soils was estimated by dilution plating method as described earlier. For the first and second harvests, when the amount of root tissue was low, rhizosphere populations were estimated by shaking the roots in 250ml flasks containing 100ml sterilized distilled water plus 0.1% Tween 80, but for all later harvests the roots were shaken in 250ml sterilized distilled water plus 0.1% Tween 80 in 500ml flasks.

5.3.1.4. Colonization of the internal root tissues and/or the root surfaces

The two methods, washing the root and root surface sterilization, used were exactly the same as described in the previous experiment, except that the roots were washed 10 times instead of 20-30 times. Washing 10 times was found to be sufficient to remove loosely adhering micro-organisms.

The roots were sterilized in 10% chloros solution for 5 or 10 mins.

5.3.2. Results

5.3.2.1. Sterilized soil treatments

5.3.2.1.1. Rhizosphere colonization

The results are given in fig. 36, Tables 14 and 15 and Appendix Tables 18A to 18C. They show that the numbers of viable propagules of P. expansum increased in both rhizosphere and control soils (pots without plants), with the greater increase occurring in the rhizosphere ($P < 0.001$) (fig. 36). The highest numbers of propagules were isolated from rhizosphere soils 4 weeks after transplanting, but from then on numbers steadily declined until the end of the experiment. The maximum levels attained, expressed as a multiple of the numbers added to the soil, were 286 times. The same trend was found in control soils with maximum numbers (85.2x) being recovered 4 weeks after transplanting followed by a steady reduction (fig. 36).

P. expansum was not recovered at any harvest from the rhizospheres of plants grown in soils which had not been infested with this species although species of Penicillium other than P. expansum and of other genera were (Table 14).

Populations of fungal contaminants including Penicillium spp other than P. expansum and species of other genera were also significantly higher in rhizosphere soils than in control soils ($P < 0.001$) (Table 15), but in contrast to P. expansum their populations increased steadily throughout the course of the experiment.

5.3.2.1.2. Colonization of the internal root tissues and/or the root surfaces

(i) Root washing technique

The results are given in figs. 37 and 38 and Appendix Table 19.

P. expansum was found to be capable of colonizing roots in this

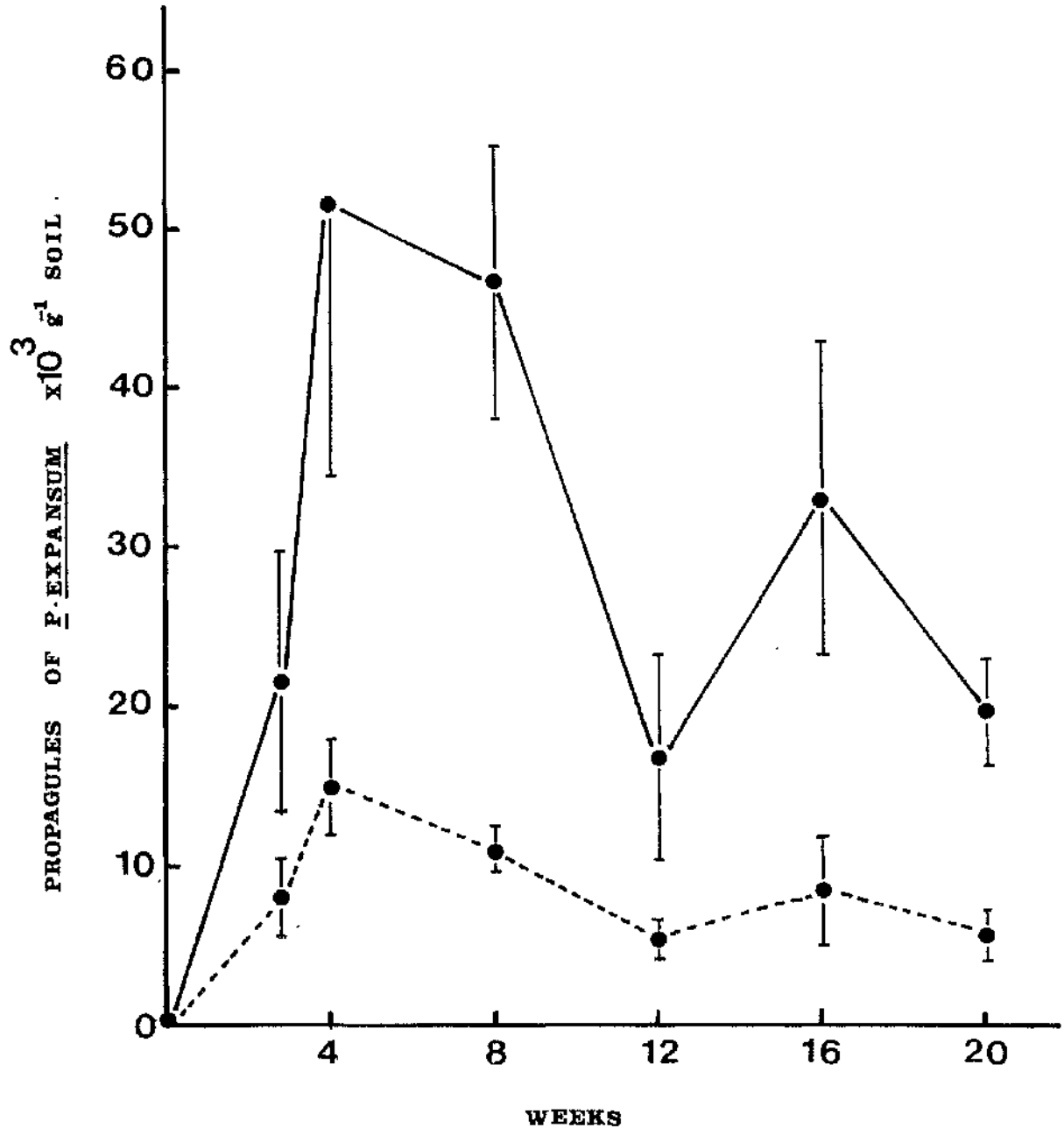


Fig. 36. Survival and growth of P. expansum in the rhizospheres of tomato grown in sterilized soil (——) and in control soils without plants (----). 175 P. expansum spores g^{-1} of oven-dry soil were incorporated into the soil at the start of the experiment.

Table 14. Viable propagules of P. expansum, Penicillium species other than P. expansum and fungi other than Penicillium in rhizosphere of tomato grown in P. expansum uninfested sterilized soil.

	2	4	8	12	16
			(weeks)		
	Number x 10^3 g^{-1} oven-dry soil				
P. expansum	0*	0	0	0	0
Other Penicillium species	29.07 ± 9.74	38.02 ± 11.9	12.21 ± 8.4	135.0 ± 61.52	132.84 ± 12.2
Other fungi	0.36 ± 0.18	3.76 ± 1.48	3.4 ± 1.6	124.93 ± 50.97	141.76 ± 22.43

* Figures are means of 3 replicates ± 1 S.E.

Table 15. Viable propagules of Penicillium species other than P. expansum and fungi other than Penicillium in P. expansum infested sterilized soil.

Penicillium species other than P. expansum

	0	2	4	8	12	16	20
				(weeks)			
				Number x 10^3 g^{-1} oven-dry soil			
Rhizosphere	0.08** ± 0.05	32.07* ± 9.86	21.32 ± 8.96	1.76 ± 0.35	10.13 ± 1.51	167.8 ± 51.94	102.06 ± 34.16
Control	0.08 ± 0.05	0.27 ± 0.09	0.32 ± 0.07	2.75 ± 2.36	0.61 ± 0.28	7.19 ± 4.27	15.25 ± 10.48

Fungi other than Penicillium species

	0	2	4	8	12	16	20
				(weeks)			
				Number x 10^3 g^{-1} oven-dry soil			
Rhizosphere	0.08** ± 0.04	2.13* ± 0.53	50.97 ± 41.98	61.65 ± 38.33	21.30 ± 4.14	196.89 ± 28.69	707.22 ± 224.56
Control	0.08 ± 0.04	0.45 ± 0.2	0.39 ± 0.15	0.45 ± 0.14	0.22 ± 0.04	10.76 ± 8.48	5.60 ± 1.56

* Figures are mean rrom 5 replicates ± 1 S.E.
** Figures are mean from 7 replicates ± 1 S.E.

experiment but percentage colonization was very low (fig. 37) compared to that which occurred with maize, broad bean, and tomato as reported in the last experiment (figs. 22,23 & 24). Percentage colonization was found to be at a similar level at all harvests between 2 and 13 weeks.

Roots were also colonized by other fungi including species of Penicillium other than P. expansum and by species of other genera which contaminated the soils during the course of the experiments (figs. 37 & 38), but percentage colonization was very low compared to that found in the previous experiments (figs. 22,23,24,25,26 & 27). Colonization of the roots by the various species of Penicillium reached a maximum at two weeks and then remained fairly constant. However the percentage colonization of roots by species of other genera increased with time (figs. 37 & 38).

The results of isolations from soils not infested with P. expansum are given in fig. 38 and show that although the roots were colonized by a variety of fungi, P. expansum was not one of them. The number of isolations of Penicillium spp other than P. expansum and of fungi of other genera was similar to those isolated from roots in P. expansum infested soils (fig. 37).

(ii) Surface sterilization technique

Results given in Table 16 show that P. expansum did not colonize the internal tissues of tomato roots although low numbers of P. expansum were found closely attached to the root surface at all harvests (fig. 38).

Other Penicillium spp and species of other genera were isolated from the internal tissues at very low levels.

Fig. 37

Colonization of tomato roots in *P. expansum* infested sterilized soil. 3 replicates, in each 50, 2mm root lengths were tested. Colonization given as % ± S.E.

(a) *P. expansum*

(b) *Penicillium* spp other than *P. expansum*

(c) Fungi other than *Penicillium* spp.

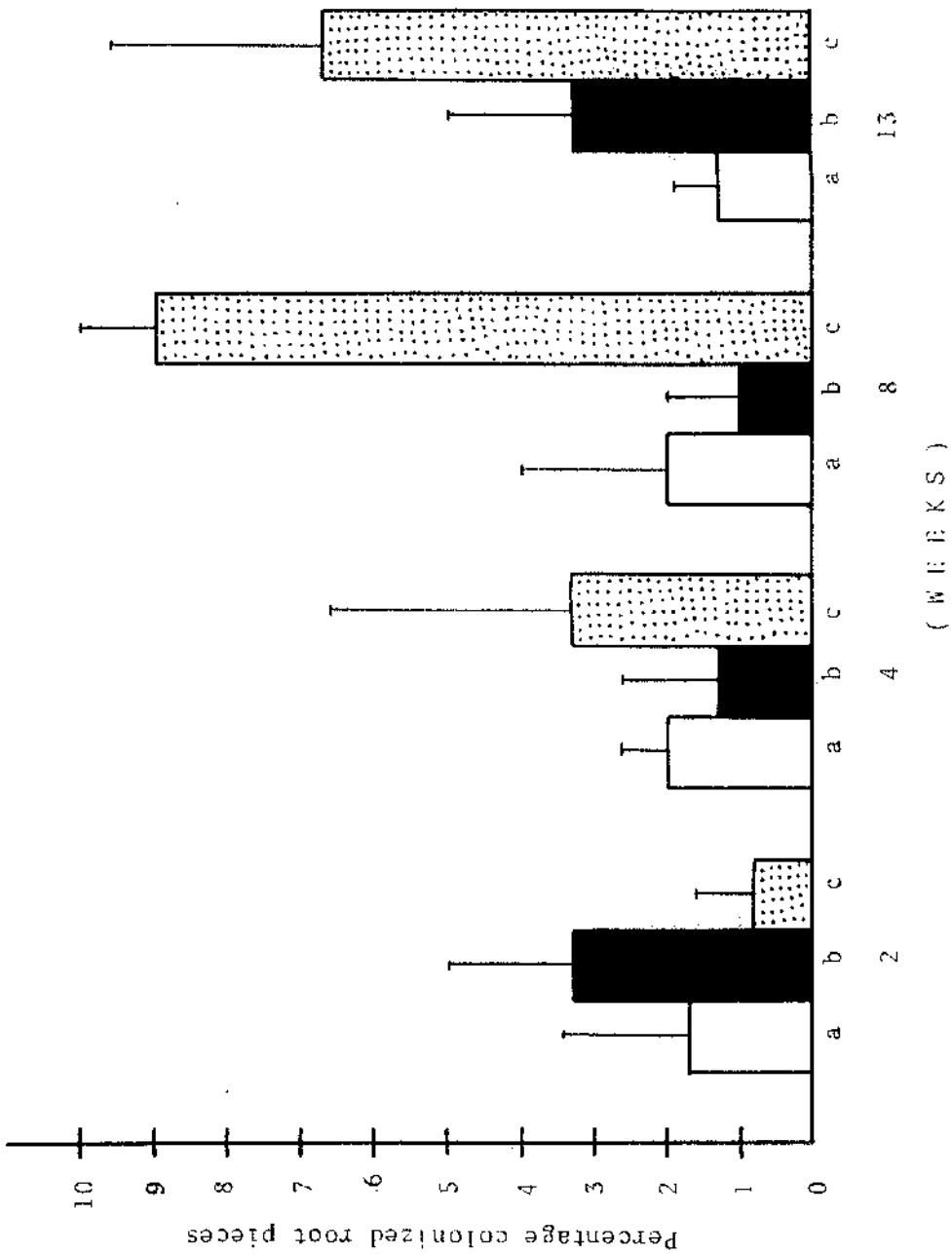

Percentage colonized root pieces
0
1
2
3
4
5
6
7
8
9
10
a
b
c
2
4
8
13
(W E E K S)

Fig. 38

Colonization of tomato roots in *P. expansum* uninfested sterilized soil. 3 replicates, in each 50, 2mm root lengths were tested. Colonization given as % ± S.E.

(a) *P. expansum*

(b) *Penicillium* spp other than *P. expansum*

(c) Fungi other than *Penicillium* spp.

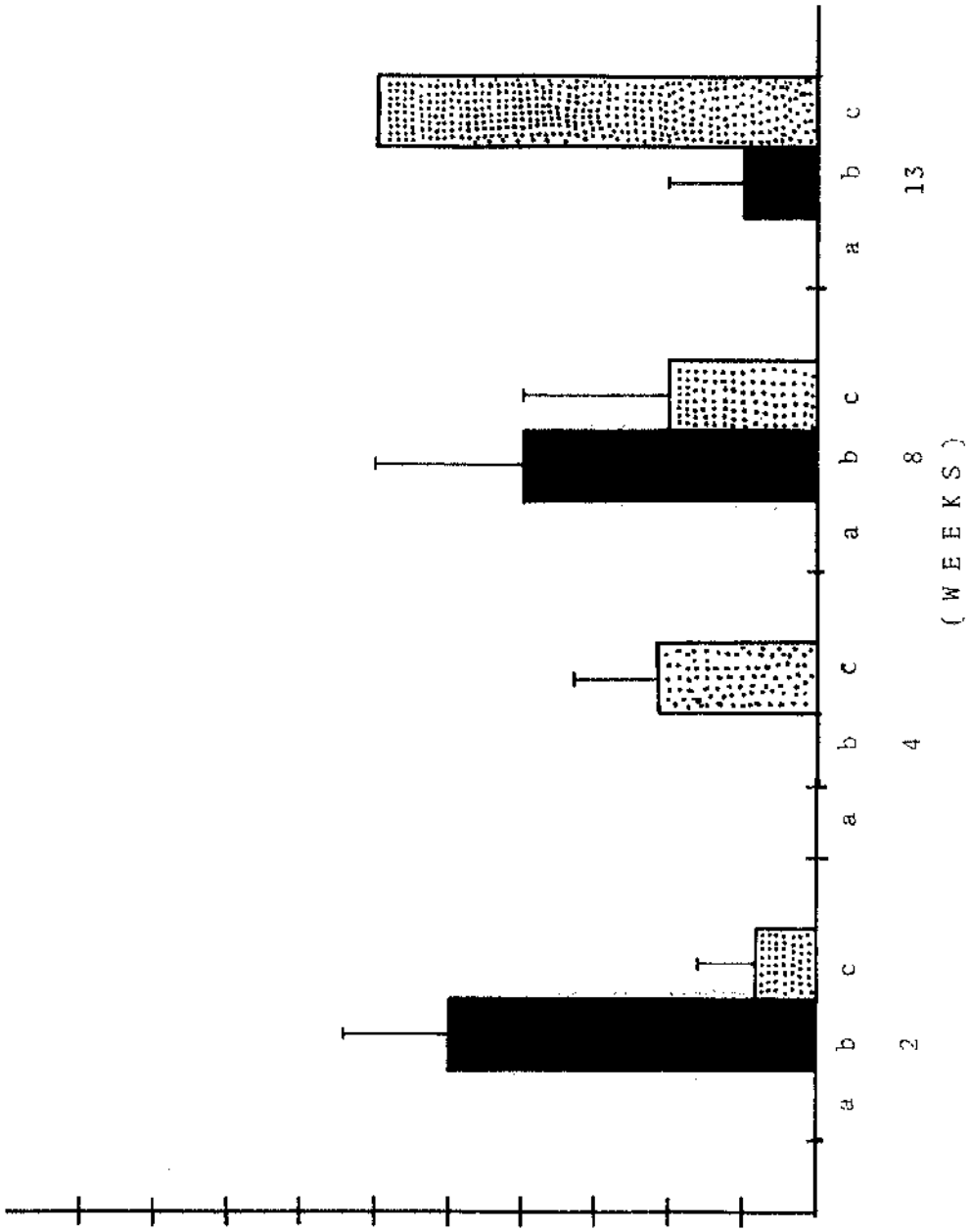

Percentage colonized root pieces
0
1
2
3
4
5
6
7
8
9
10
a b c
2
a b c
4
a b c
8
a b c
13
(W E E K S)

Table 16. Colonization of internal tissues of roots of tomato plants after 8 weeks transplanting in P. expansum infested sterilized soil.

	Control				10 minutes*			
	Replicates			Mean ± S.E.	Replicates			Mean ± S.E.
	1	2	3		1	2	3	
P. expansum	4**	0	2	2.0 ± 1.15	0	0	0	0
Other Penicillium species	4	0	0	1.3 ± 1.3	2.5	0	0	0.8 ± 0.8
Other fungi	0	10	0	3.3 ± 3.3	0	2.5	0	0.8 ± 0.8

* Time period of root surface sterilization in 10% chloros.

** Number of 2mm root lengths colonized given as percentage of 50 pieces tested.

5.3.2.2. Unsterilized soil treatments

5.3.2.2.1. Rhizosphere colonization

Results of isolations from the rhizospheres of unsterilized soil are given in figs. 39, 40 & 41 and Table 17 and Appendix Tables 20A to 20 C.

They show that populations of P. expansum reached significantly higher levels in the rhizosphere than in control soils ($P < 0.001$) (fig. 39). Maximum populations were reached 4 weeks after transplanting and then the numbers of viable propagules declined slowly over the duration of the experiment. The maximum level attained was approximately double the population attained in non-rhizosphere soil.

In the control non-rhizosphere soil the numbers of viable propagules of P. expansum had increased slightly by 4 weeks but then slowly declined more or less to their initial levels by the end of the experiment (fig. 39).

P. expansum was never isolated from the rhizospheres of plants grown in uninfested soils but other Penicillium spp and species of other genera were (Table 17).

The numbers of propagules of other Penicillium spp and species of other fungal genera were also significantly higher in the rhizospheres than in control soils ($P < 0.001$) (figs. 40 & 41). The maximum numbers of viable propagules of Penicillium spp were attained 4 weeks after planting and then decreased slowly over the next 16 weeks (fig. 40). Maximum numbers of viable propagules of species of other fungal genera were found about 12 weeks after planting and then their numbers declined over the course of the experiment (fig. 41).

In control soil populations of Penicillium spp other than P. expansum increased, reaching a maximum after 4 weeks, then remained more or less the same, but the number of propagules of other fungal species remained constant throughout the experiment (figs. 40 & 41).

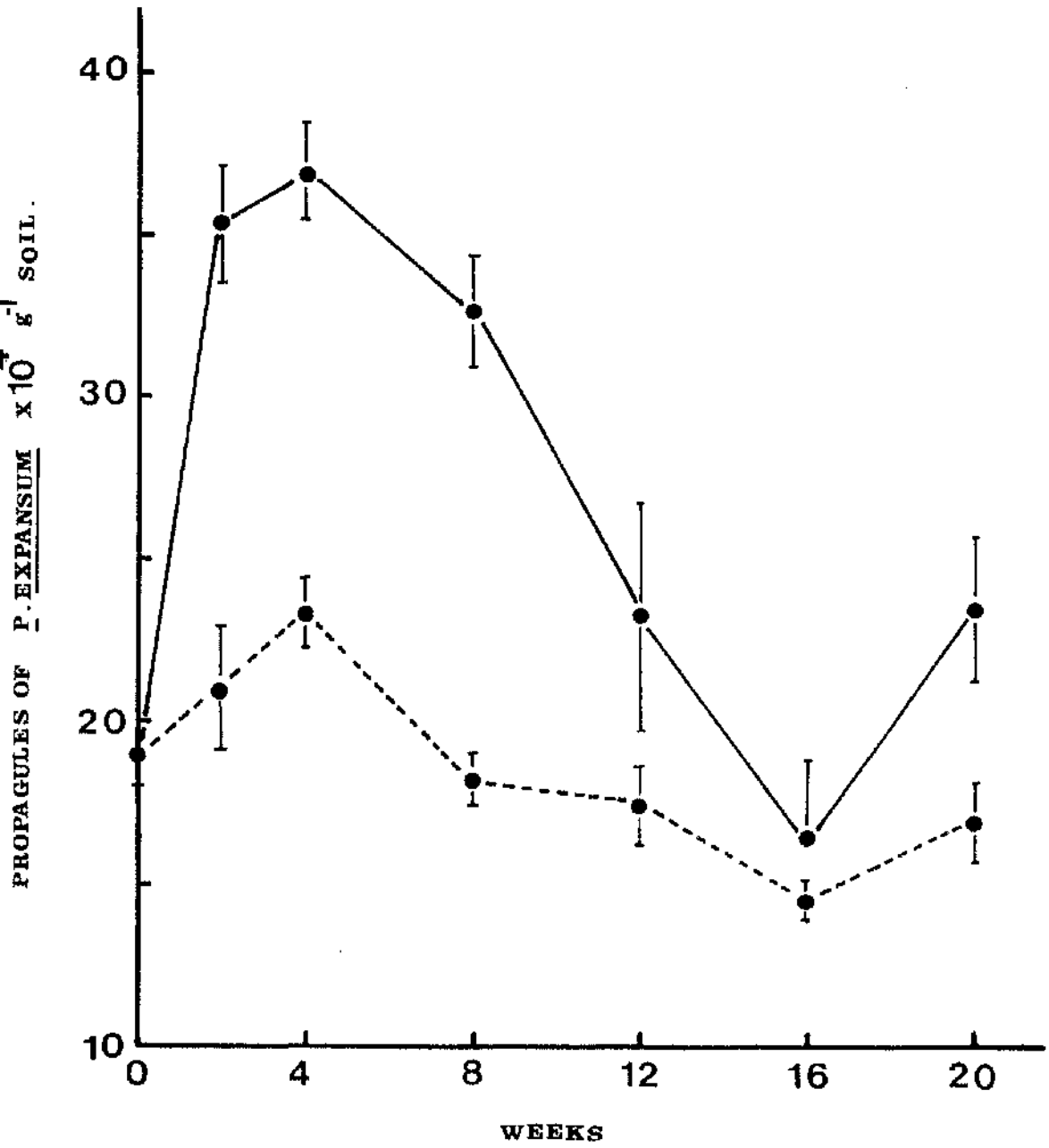


Fig. 39. Survival and growth of P. expansum in the rhizospheres of tomato grown in unsterilized soil (——) and in control soils without plants (----).

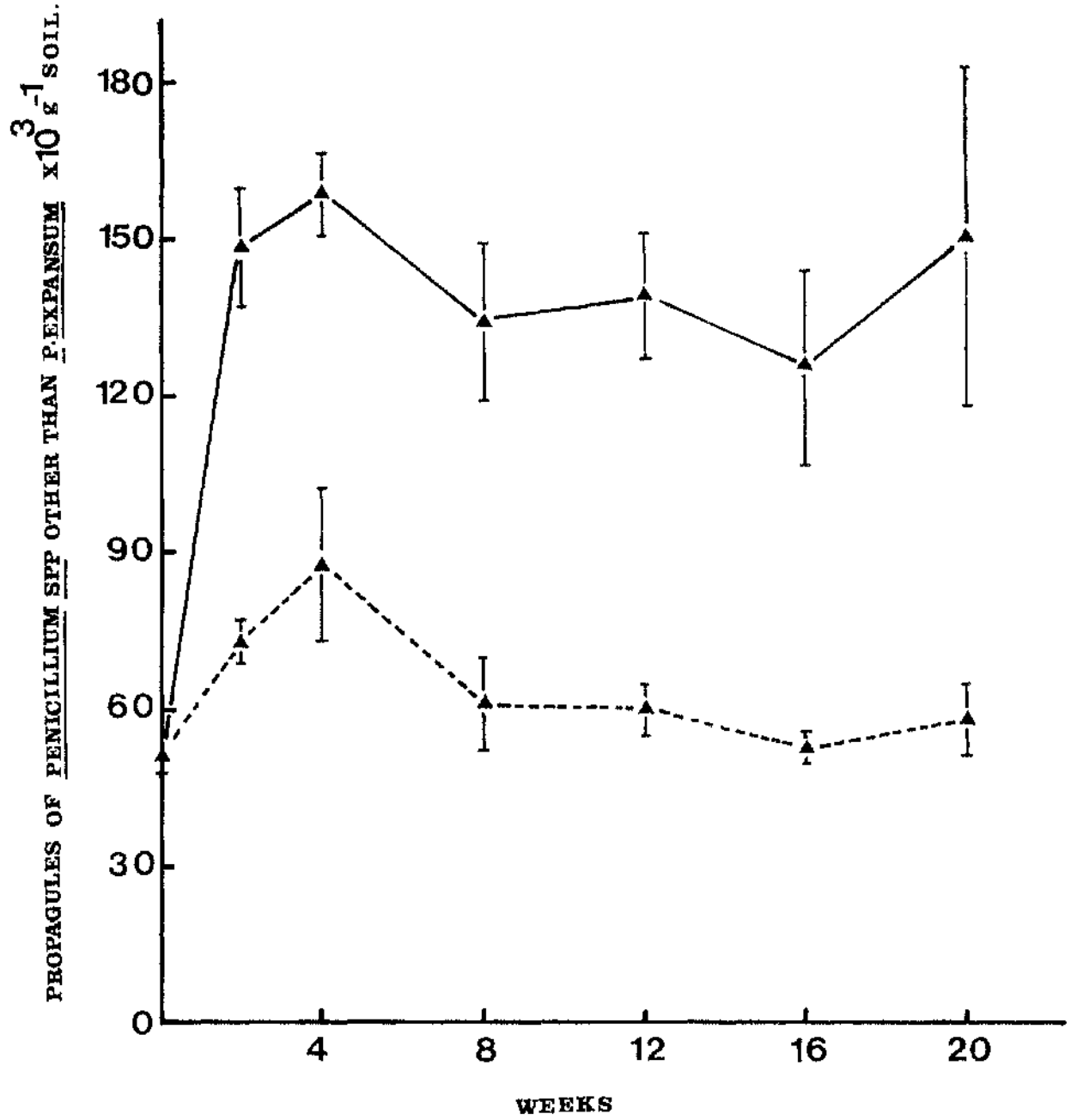


Fig. 40. Survival and growth of Penicillium species other than P. expansum in the rhizospheres of tomato grown in P. expansum infested unsterilized soil (·—·—) and in control soils without plants (----).

Fig. 41

Survival and growth of fungi other than Penicillium in rhizospheres of tomato grown in P. expansum infested unsterilized soil (——) and in control soils without plants (----).

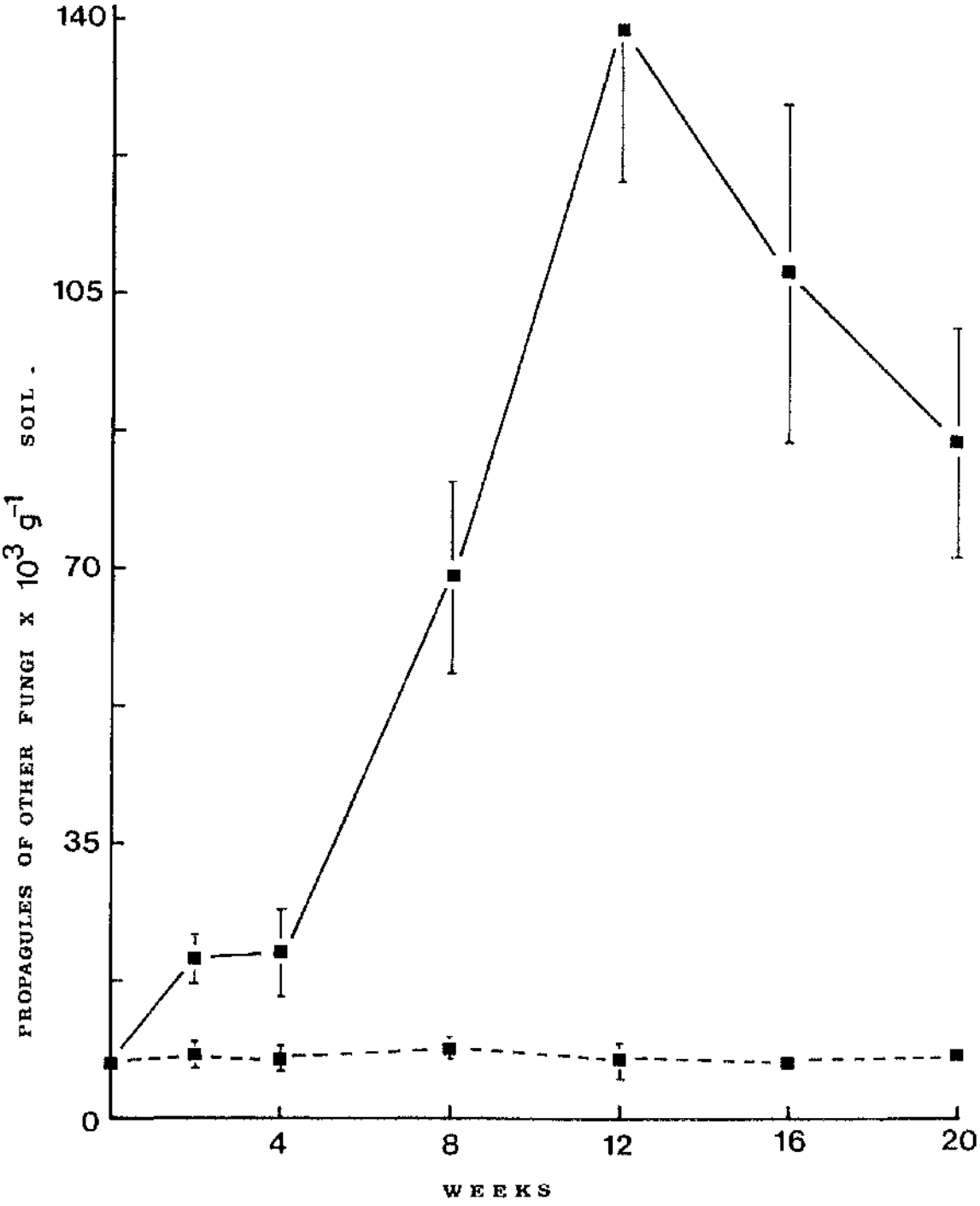

140
105
70
35
0
4
8
12
16
20
WEEKS
PROPAGULES OF OTHER FUNGI x 10^3 g^{-1} SOIL

Table 17. Viable propagules of *P. expansum*, *Penicillium* species other than *P. expansum* and fungi other than *Penicillium* in rhizospheres of tomato, grown in *P. expansum* uninfested unsterilized soil.

	2	4	12	20
		(weeks)		
	Number x 10^3 g^{-1} oven-dry soil			
P. expansum	0*	0	0	0
Other *Penicillium* species	161.84 ± 13.19	204.12 ± 35.59	222.34 ± 46.45	180.38 ± 58.19
Other fungi	26.41 ± 6.98	41.26 ± 16.65	96.10 ± 56.97	93.0 ± 37.51

* Figures are mean of 3 replicates ± 1 S.E.

5.3.2.2.2. Colonization of the internal root tissues and/or the root surfaces

(i) Root washing technique

The results are given in figs. 42 and 43 and Appendix Table 21.

In infested soils P. expansum was associated with the roots of plants at all harvests (fig. 42). The roots were colonized by other Penicillium spp and species of other fungal genera with the extent of colonization increasing steadily during the course of the experiment (figs. 42 & 43).

P. expansum was never isolated from the roots of plants grown in uninfested soil but the roots were colonized by other fungal species (fig. 43) and the patterns of colonization were similar to those found in the infested soils.

(ii) Surface sterilization technique

The results given in Table 18 show that even 5 mins treatment with 10% chloros removed all fungi, indicating that they were located on the root surface.

Fig. 42

Colonization of tomato roots in *P. expansum* infested unsterilized soil. 3 replicates, in each 50, 2mm root lengths were tested. Colonization given as % ± S.E.

(a) *P. expansum*

(b) *Penicillium* spp other than *P. expansum*

(c) Fungi other than *Penicillium* spp.

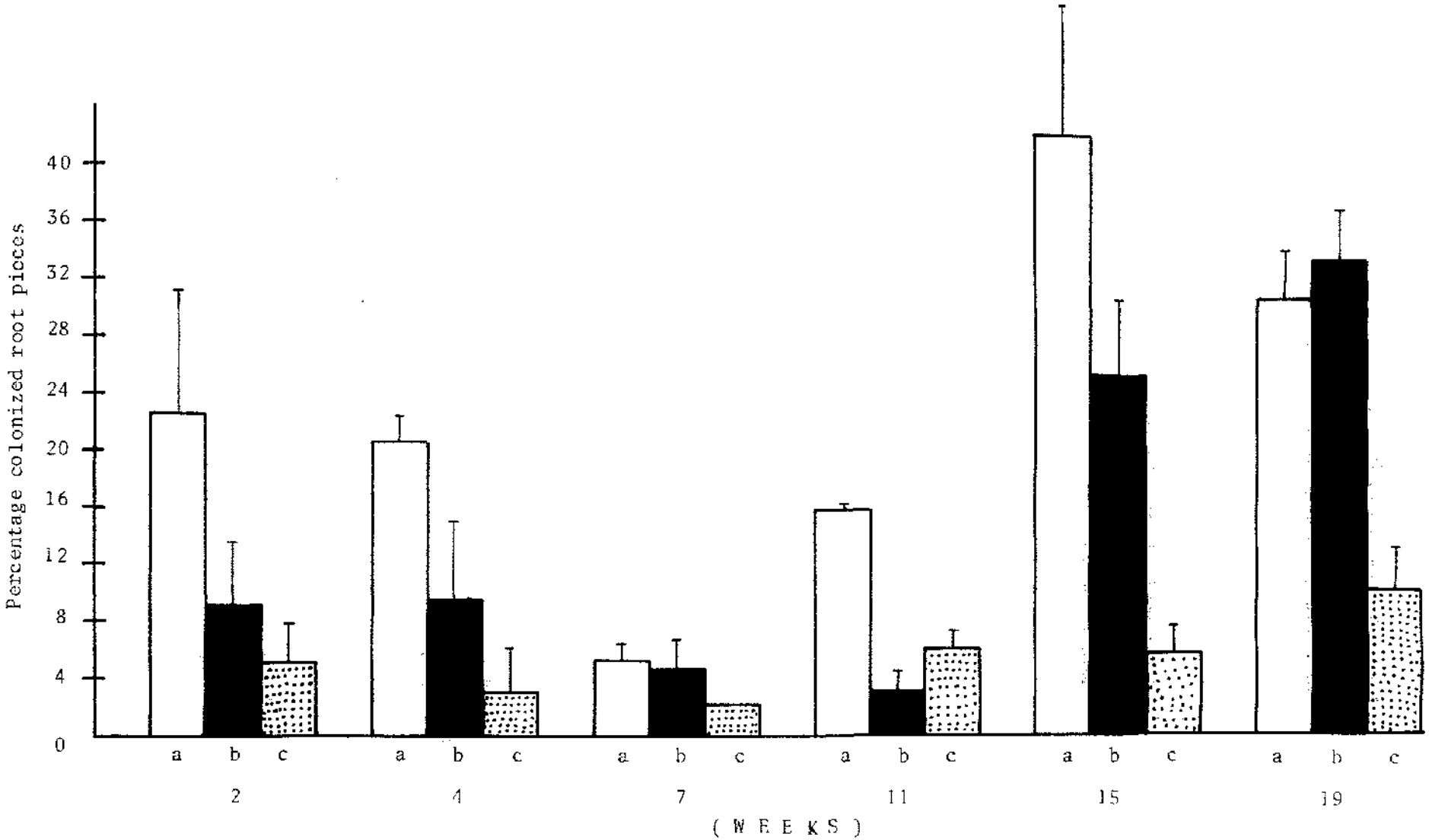

Percentage colonized root pieces
40
36
32
28
24
20
16
12
8
4
0
a b c
2
4
7
11
15
19
(W E E K S)

Fig. 43

Colonization of tomato roots in P. expansum uninfested unsterilized soil. 2 replicates, in each 50, 2mm root lengths were tested. Colonization given as % ± S.E.

(a) P. expansum

(b) Penicillium spp other than P. expansum

(c) Fungi other than Penicillium spp.

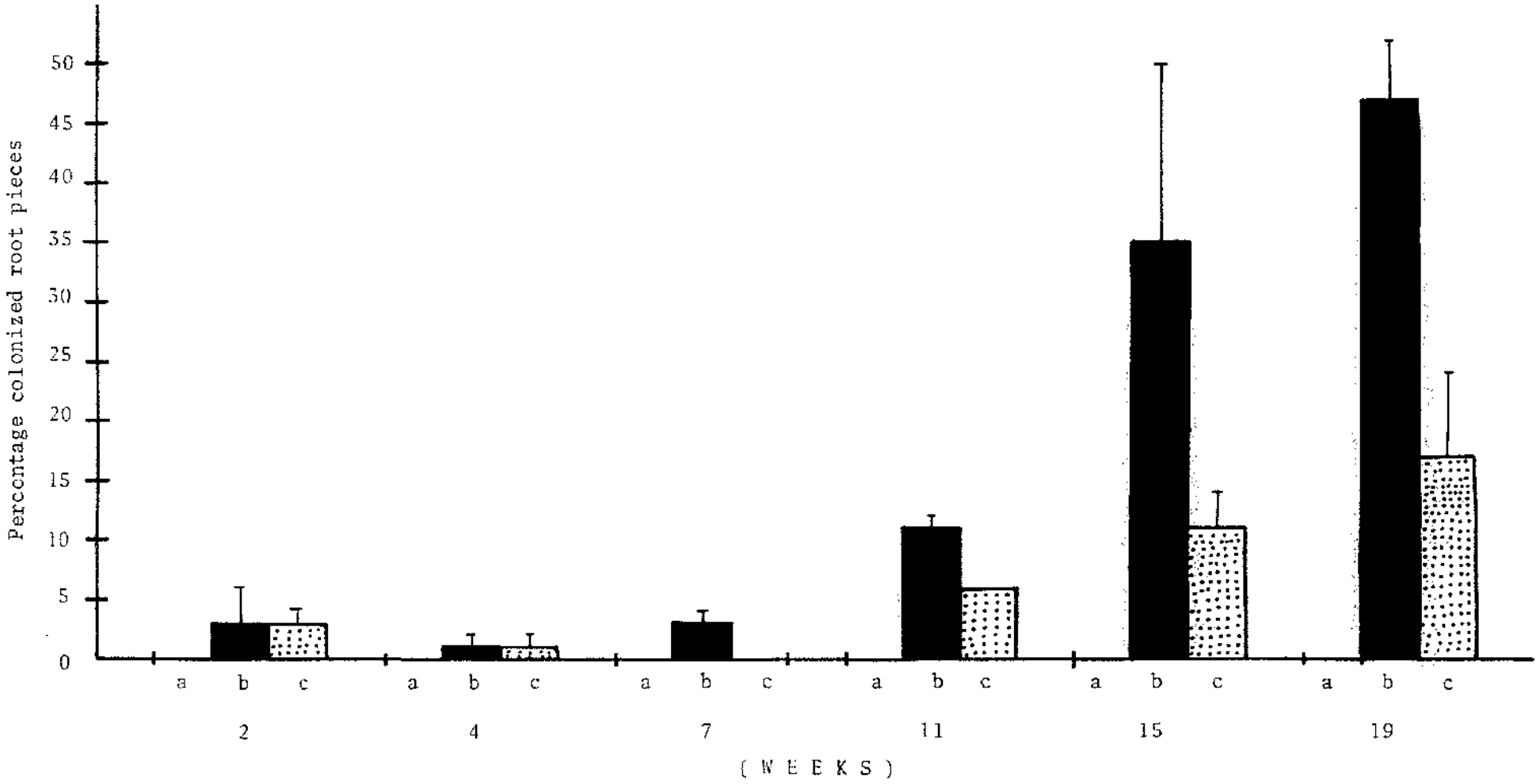

Percentage colonized root pieces
50
45
40
35
30
25
20
15
10
5
0
a b c a b c a b c a b c a b c a b c
2 4 7 11 15 19
(W E E K S)

Table 18. Colonization of internal tissues of roots of tomato plants 2 weeks after transplanting in *P. expansum* infested unsterilized soil.

	Control				5 mins*				10 mins			
	Replicates			Mean ± S.E.	Replicates			Mean±S.E.	Replicates			Mean±S.E.
	1	2	3		1	2	3		1	2	3	
P. expansum	12**	16	40	22.6 ± 8.7	0	0	0	0	0	0	0	0
Other *Penicillium* species	6	4	18	9.33 ± 4.4	0	0	0	0	0	0	0	0
Other fungi	8	0	8	5.3 ± 2.66	0	0	0	0	0	0	0	0

* Time period of root surface sterilization in 10% chloros.

** Number of 2mm root lengths colonized given as percentage of 50 pieces tested.

5.4. DISCUSSION

These experiments showed that the populations of P. expansum markedly increased in the rhizospheres of all test plants in unsterilized soil but, as with the experiments reported earlier, there was no evidence of growth in non-rhizosphere soils. The increase in the populations in the rhizosphere was probably due to root secretions and to cell debris sloughed off by the growing roots. These materials could act directly as nutrients or indirectly by inhibiting antagonistic organisms. As discussed earlier, the roots of plants are known to exude a range of potential nutrients into the soil in addition to the large amounts of materials which are added in the sloughed off debris from the root cap and outer root cortex. These nutrients are probably produced in sufficient amounts to overcome fungistasis and induce spore germination.

In the experiments described in Chapter IV it was shown that when spores of P. expansum were mixed into unsterilized soils together with nutrients they germinated to produce conidia by microcyclic development (Plate 16). This type of conidial formation may occur in the rhizosphere soil after spore germination. The numbers of viable propagules increased rapidly in the rhizosphere during the early stages of plant growth with greater increases in the rhizospheres of plants grown in sterilized soil than in unsterilized soil (Table 19). The greater increase in sterilized soil may be due to the relative absence of competitive and antagonistic micro-organisms from the rhizospheres in these soils and to the additional nutrients produced during sterilization. The increase to the maximum coincided with most root growth (fig. 46 & 53).

After the initial increase the numbers of viable propagules in the rhizospheres decreased fairly sharply in both sterilized and unsterilized soils. During most of this period very few new roots appear to develop and those that are already present will have continued to mature. Hamlen et al (1972) reported that the age and stage of plant development significantly influences

Table 19. The increase in populations of P. expansum in the rhizospheres in sterilized and unsterilized soils.

	Sterilized soil					Unsterilized soil					
Plants	Initial inoculum	Maximum population attained in non-rhizosphere soil (A)	Maximum population attained in rhizo-sphere soil (B)	Increase due to roots (B-A)	R:S* $\frac{B}{A}$	Initial inoculum	Maximum population attained in non-rhizosphere soil (A)	Maximum population attained in rhizo-sphere soil (B)	Increase due to roots (B-A)	R:S* $\frac{B}{A}$	Rhizosphere ratio sterilized: unsterilized
Maize	500	13.3×10^5	189.0×10^5	175.7×10^5	14.2	500	300	1100	800	3.6	17,181
Broad bean	500	10.0×10^3	98.0×10^3	88.0×10^3	9.8	500	300	800	500	2.6	122
Tomato	500	50.0×10^3	300.0×10^3	250.0×10^3	6.0	500	275	1000	725	3.6	300
	175	14.9×10^3	52.6×10^3	37.7×10^3	3.5	19.0×10^4	23.0×10^4	36.9×10^4	13.9×10^4	1.6	155
Apple	-	-	-	-		10.0×10^3	6.9×10^3	18.0×10^3	11.1×10^3	2.6	-

* R:S = Rhizosphere : soil ratio.

plant root exudates both qualitatively and quantitatively. Rovira (1959) found that exudation from tomato roots was greater during the first two weeks of growth than in the next two-week period. Vancura and Hovadik (1965) found that root exudation from tomato plants at the fruiting stage, assessed by comparing the intensity of spots on chromatograms of extracts produced by equal amounts of plant material, was generally lower than that occurring at earlier stages of growth. Arkadeva (1963) also observed differences in the carbohydrate composition of root exudates between 8 and 16 day old maize plants. He found that the concentration of glucose increased with age. Krasilnikov (1958) also reported that the numbers of micro-organisms in the rhizospheres of various plants during the fruiting period was about one third of the numbers present during the period of intensive vegetative growth.

The fairly rapid decrease in numbers of viable propagules in the rhizospheres, after attaining the maximum, contrasts with the more steady decline which occurred in unsterile soils in fallow (non-rhizosphere) soils. This result is consistent with that of the experiment described in Chapter IV which showed that 68% of the spores of P. expansum initially incorporated into unsterilized soil were still viable after 48 weeks. One possible explanation for the rapid loss of viability of spores formed by microcyclic conidiation in the rhizosphere is that such conidia are smaller than those formed in culture, and so may have insufficient food reserves to remain viable for a long period.

P. expansum does not appear to colonize the internal tissues of the roots of tomato plants in either sterilized or unsterilized soils (Tables 16 & 18), but in both cases did grow closely attached to the root surfaces (figs. 37 & 42). This result confirms that of Dix (1964).

Maize roots were the only roots whose internal tissues were colonized and thenonly in sterilized soil (Table 12).

The development of P. expansum on root surfaces was very variable, probably due to the variable number of spores contacted by the root (Bowen & Rovira, 1976).

CHAPTER VI

EFFECT OF PENICILLIUM EXPANSUM ON THE GROWTH OF TOMATO PLANTS IN SOIL

6.1. Introduction

Micro-organisms in the rhizosphere may have a marked influence on the growth of plants. The rhizosphere microflora is so intimately related to the root system, particularly the rhizoplane components which cover the root surface, that any active substances produced can have an immediate effect on root function and growth. Some of these substances stimulate plant growth in various ways while others inhibit. Thus Campbell in a review (1977) reported that up to 50% of the rhizosphere and rhizoplane organisms produced plant growth promoter substances in culture while as many more, not necessarily the same ones, produced growth inhibitors.

Stimulatory effects

Many workers have demonstrated the production of auxins, gibberellins, cytokinins and ethylene in soil. Thus Roberts and Roberts (1939) found that substances inducing an auxin-like response in *Avena* coleoptiles were produced in agar by 46% of all soil fungi examined, 77% of the bacteria and 66% of the actinomycetes. Brown (1972) also reported that many bacteria, especially those found in the rhizosphere and rhizoplane, produced growth regulators of both the gibberellin and the auxin type when grown in liquid culture. Brown and Burlingham (1968) showed that cultures of *Azotobacter chroococcum* produced both gibberellin (GA_3) and indolyl-3-acetic acid (IAA). Gibberellin-like substances have also been identified in cultures of *Bacillus megaterium*, *B. subtilis* and *Pseudomonas* species (Brown, 1974). These substances are probably responsible for the increased growth which occurs in plants derived from seed, or from seedlings treated with suspensions of these bacteria.

Lynch in a review (1983) reported that there was little evidence to indicate that the production of compounds with cytokinin-like activity by soil micro-organisms has any significance.

Many cases are known where ethylene produced by micro-organisms in soil is inhibitory of plant growth, but some cases are known where it is stimulatory (Smith, 1976).

Micro-organisms can help to solubilize mineral nutrients and increase their uptake by plants. Katznelson et al. (1962) isolated phosphate dissolving fungi from seed and plant roots and found that the most frequent fungi involved were species of Penicillium, Aspergillus and Rhizopus, but species of Candida, Oidiodendron and Pseudogymnoascus were also active. Bowen and Rovira (1966) observed a marked increase in phosphate uptake and translocation in tomato (Lycopersicon esculentum) and in subterranean clover (Trifolium subterraneum) when seedlings were grown in Hoagland & Aron's plant nutrient solution containing 0.1 ml of 1% soil suspension. In contrast, Subba-Rao et al. (1961) found that the presence of rhizoplane fungi around the roots of tomato plants (L. esculentum) grown in liquid culture could reduce the uptake of nutrients. They found that Fusarium sp. suppressed the uptake of phosphate, sulphate and bicarbonate ions and the uptake of glucose by the cvs Bonny Best and Geneva 11 and that Trichoderma viride suppressed the uptake of inorganic ions but increased the uptake of glucose by the cvs Moscow and Loran Blood, when these cultivars were grown aseptically. Benians and Barber (1974) also found that the uptake of phosphate by barley plants (Hordeum vulgare) decreased in the presence of soil micro-organisms when plants were grown in a basaltic loam soil. However, after the addition of small quantities of KH_2PO_4, the uptake of phosphate by plants was similar in the presence or absence of soil micro-organisms.

Subba-Rao et al. (1961) also showed that rhizosphere fungi not only affected the amounts of material taken up by plants but also affected their subsequent metabolism. When tomatoes (L. esculentum) were grown aseptically in nutrient solutions containing C^{14} labelled compounds, and the solutions

were infested with Fusarium species, the amounts of radioactivity entering amino acids was considerably reduced while that entering sugars was increased over that incorporated into plants grown in the absence of the fungus. In contrast, Trichoderma viride did not affect the amount of radioactivity entering amino acids but it did reduce that incorporated into sugars.

Inhibitory effects

Many workers have reported that micro-organisms in the rhizosphere can suppress the growth of plants. Thus Mirchink (1956) studied a large collection of fungi isolated from a turfy podsol soil from around Moscow and found many toxigenic species. He found that the most toxic and the most widespread fungi in these soils were species of Penicillium, Fusarium and Trichoderma. Species from all three genera strongly suppressed the germination and subsequent growth of wheat (Triticum aestivum). Wang (1962) extracted toxins from Penicillium miczynskii, P. purpurogenum, P. oxalium, Penicillium section polyverticillata, Aspergillus niger - lucchuensis and A. ochraceus which had been isolated from soils in China and found that they inhibited the germination of wheat (T. aestivum), vetch (Vicia sp.) and pea (Pisum sativum). He found that toxins penetrated the tissues of plants of all three species from solution, and after 7 days growth the toxins were detectable in their roots, stems and leaves. Absorption from soil was less than that from solution. Mirchink et al. (1962) also found that toxins of Penicillium species could penetrate wheat (T. aestivum), vetch (V. sp.) and pea (P. sativum) both from culture solutions and from soil. They also found that treatment of these plants with toxins had a pronounced effect on the total amino acid content of the aerial parts. The toxins of Penicillium purpurogenum had the most marked effect on the nitrogen content of all three species while those of P. cyclopium and P. martensii had marked effects on pea and vetch but almost no effect on wheat.

Catovic-catani and Peterson (1966) found that when culture filtrates of

Penicillium funiculosum were added to soil vermiculite mixtures in the proportions of 1 part culture filtrate : 1 part soil : 1 part vermiculite, seed germination and stem and root growth of radish (Raphanus sativus), tomato (L. esculentum) and egg plants (Solanum melongena) were markedly reduced. Such phytotoxic effects were greatest in filtrates from 25 day old cultures and was little affected by the C or N source. The toxic principle was nondialysable, but was filterable through bacterium proof filters, thermostable after heating to 100°C for 1 min and unchanged by storage at room temperature. They also observed that the application of either a fungal mat from a one month old culture or 100 ml of the culture liquid caused the death of two month old egg plants (S. melongena) or one year old maple saplings (Acer spp.). Death was more rapid when the mat and filtrate were applied together. Symptoms were more severe in sterile soil than in non-sterile soil.

Barnum (1924) found that P. expansum produces a toxin in culture which could be absorbed through the roots or through the cut ends of stems, and which induce wilting in vetch (Vicia gigantea), mint (Mentha sp.), mallow (Malva rotundifolia), cauliflower (Brassica oleracea) and alfalfa (Medicago sativa). He also found that this toxic principle was thermostable and non-volatile. This toxin is probably the phyto- and mycotoxin now known as patulin. Wilson and Nuovo (1973) reported that 60 isolates of P. expansum produced patulin in decaying apples. It is also produced by P. expansum in pear and stone fruits in similar quantities to those reported for apple (Buchanan et al., 1974). Sommer et al. (1974) found that P. expansum produced more patulin in potato dextrose broth than in apple over a wide range of temperatures.

Several reports indicate that patulin occurs in natural soil and that it can affect both micro-organisms and plants (Brian, 1957; Norstadt & McCalla, 1968, 1969, 1971).

Domsch and Gams (1972) and Domsch, Gams and Anderson (1980), in their reviews, reported the secretion of phenolic substances by apple roots and

the accumulation of P. expansum in the root region. They thus assumed a connection between the phytotoxicity of P. expansum and soil sickness. They also reported that in laboratory experiments, live cultures of P. expansum inhibited the growth of roots of oat (Avena sativa), wheat (Triticum aestivum), maize (Zea mays), several grasses, Lucerne (Medicago spp.), rape (Brassica napus) and peas (P. sativum). Pidoplichko et al. (1963) showed that P. expansum, when present in the rhizosphere, strongly inhibited shoot growth of maize plants (Z. mays) under laboratory conditions.

As was reported in Chapter V, P. expansum can survive and grow in the rhizospheres of several plants, and if it produces phytotoxins such as patulin in these regions it could well affect the growth of such plants.

In this chapter the effect of P. expansum in the rhizosphere on the growth of tomato plants (L. esculentum) was investigated.

6.2. Materials and Methods

6.2.1. Plant material

The tomato plants used in the rhizosphere study described in experiment II of Chapter V were used.

After each harvest their aerial parts were placed in polythene bags and the cut ends of their stems were dipped in distilled water to prevent wilting before leaf areas were measured.

6.2.2. Measurement of leaf area

A portable photoelectric leaf area meter (Lambda Instruments Corporation, Model LI-3000) was used.

6.2.3. Dry weight determinations

Roots, after using for the rhizosphere studies, were washed under tap water to remove soil particles and then wrapped in aluminium foil.

The stem and leaves, after leaf area measurements, were separately wrapped in aluminium foil. They were dried to constant weight at 80°C and their dry weights determined after cooling in a desiccator.

6.2.4. Other observations

The total number of flower buds, flowers and fruits of each plant were counted. The fresh weight of fruits was also determined. Determining the dry weight of fruits proved difficult and so fruit dry weights were not determined.

6.3. Results

6.3.1. Sterilized soil treatments

The results are given in figs. 44,45,46,48,49 & 50, Table 20 and Appendix Tables 22A to 22F.

Total means were compared using the student t-test.

6.3.1.1. Stem growth

There were no significant differences between plant heights in *P. expansum* infested and uninfested soils at any harvest except for the last one at 20 weeks ($P < 0.05$) (fig. 44). Dry weight measurements showed no significant differences at any harvest (fig. 45).

6.3.1.2. Root growth

Fig. 46 showed that root dry weights were similar for plants from both infested and uninfested soils at all harvests.

6.3.1.3. Leaf growth

No significant differences were found in total number of leaves (green + senescent), total leaf dry weights and total leaf area, between plants from infested and uninfested soils at any harvest (figs. 47,48 & 49).

However, at two harvests (8 and 16 weeks) the areas of individual leaves at the lower nodes of plants from infested soils were consistently smaller, and individual leaves at the upper nodes were consistently larger than those of leaves at similar positions on plants from uninfested soils (fig. 50).

6.3.1.4. Reproductive structures

Flower buds were present at the second harvest but no records of numbers were made until the third harvest, 8 weeks after transplanting. The numbers of flower buds, flowers and fruits produced and fruit fresh weight were not significantly different between treatments at any harvest (Table 20).

6.3.2. Unsterilized soil treatments

The results are given in figs. 51,52,53,54,55,56 & 57, Table 21 and Appendix Tables 23A to 23F.

6.3.2.1. Stem growth

Fig. 51 showed that the heights of plants grown in infested soils was slightly smaller than that in uninfested soils at all harvests, but significant differences were found only at the last harvest ($P < 0.01$). Stem dry weights were not significantly different at any harvest (fig. 52).

6.3.2.2. Root growth

Fig. 53 root dry weight measurements showed no significant differences between plants grown in infested and uninfested soils at any harvest.

Figs. 44 to 50

Growth of plants in sterilized soils.

——— Infested soil.

----- Uninfested soil.

Fig. 44 Stem height.

Fig. 45 Stem dry weight.

Fig. 46 Root dry weight.

Fig. 47 Total number of leaves.

Fig. 48 Leaf dry weight.

Fig. 49 Total green leaf area.

Fig. 50 Areas of individual leaves.

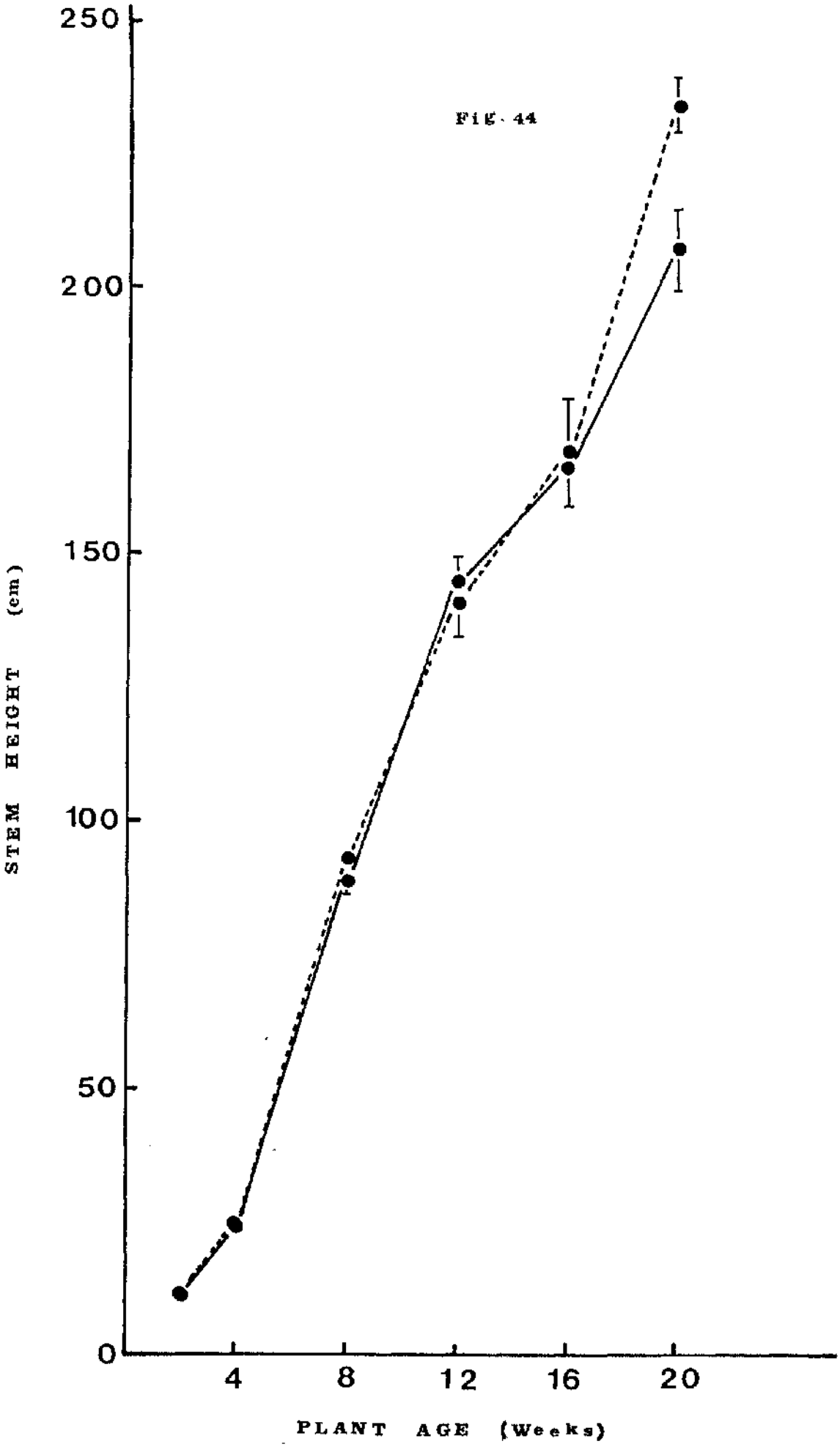
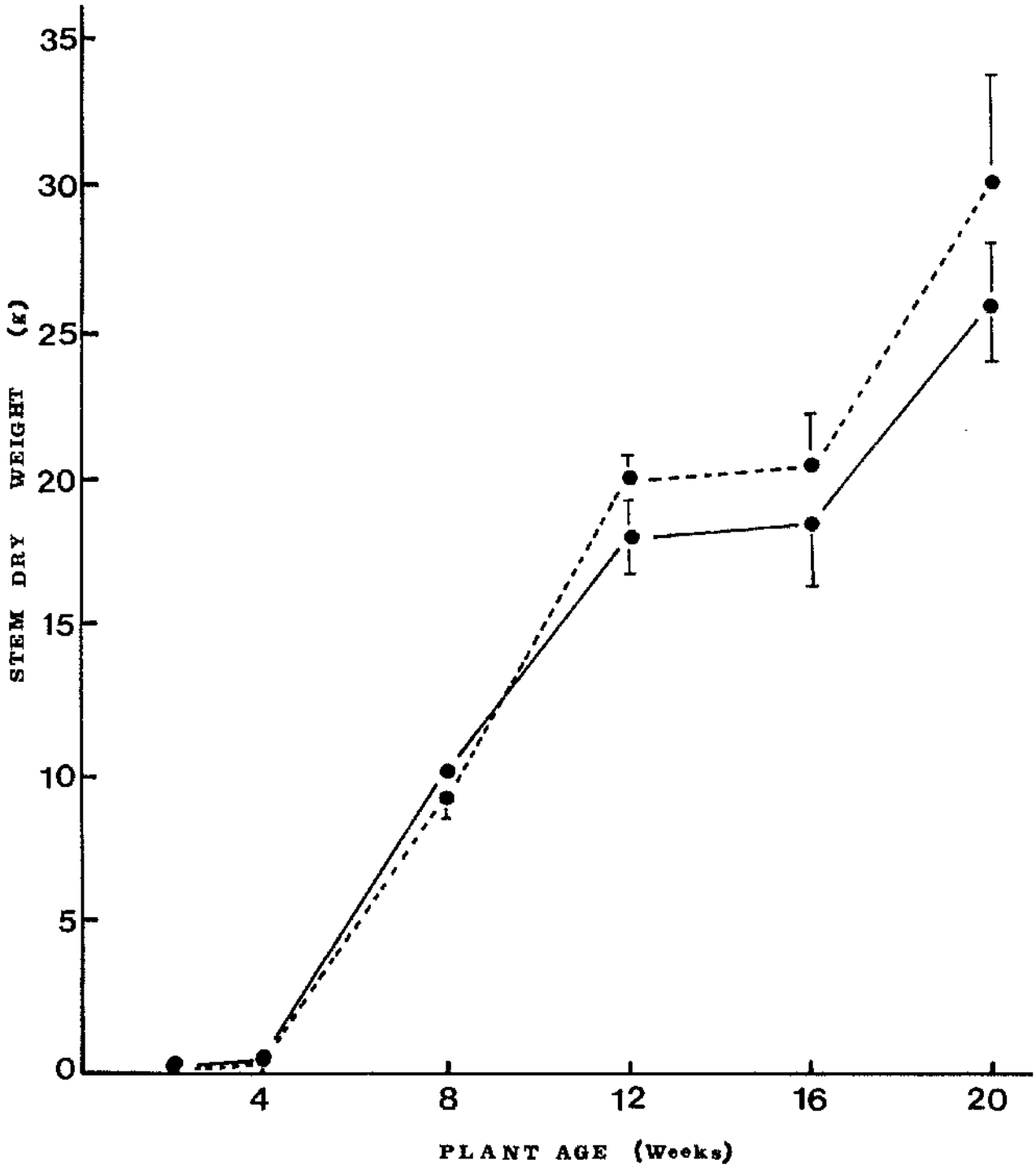


Fig. 44

Fig. 45



Fig·46

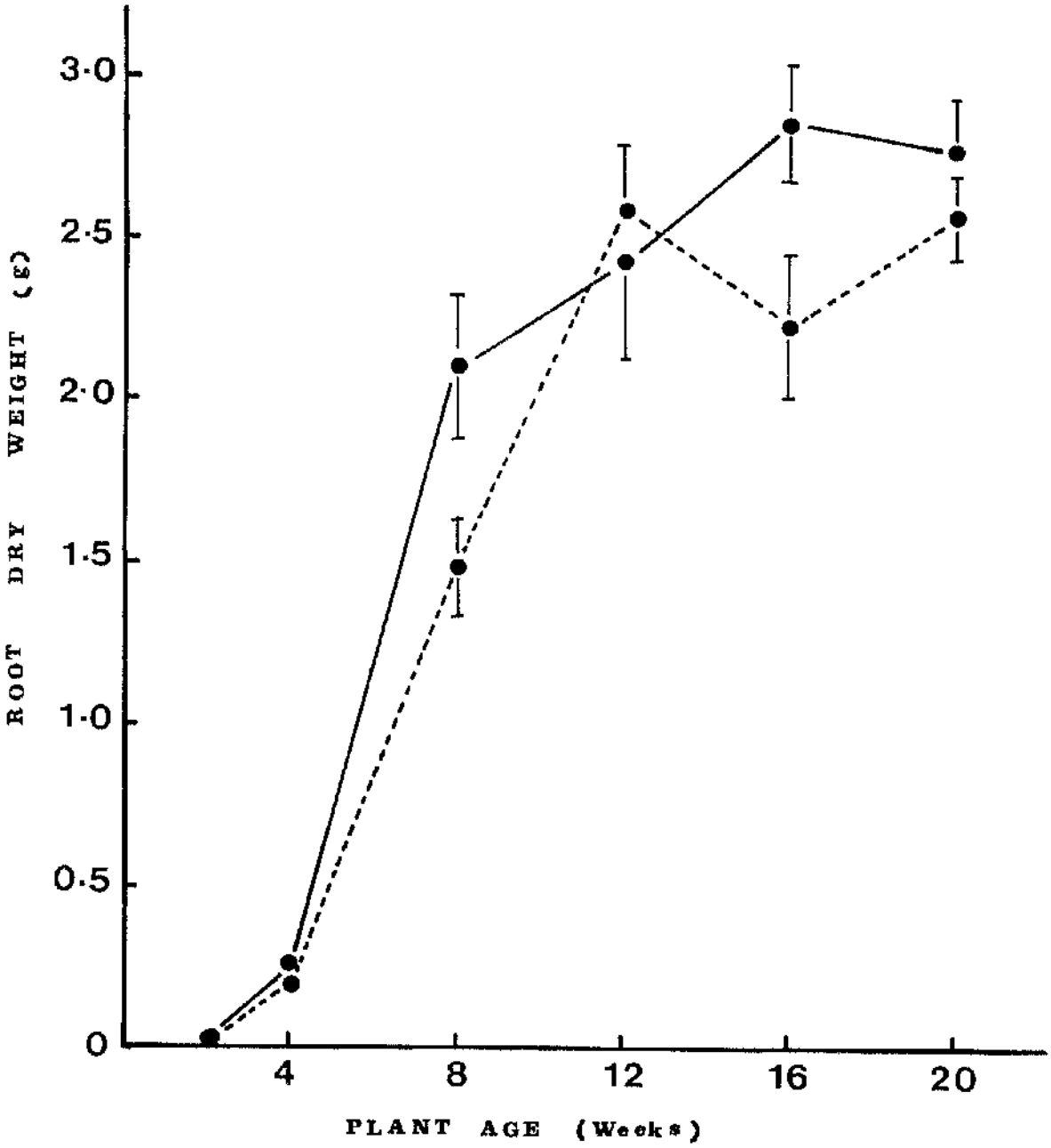


Fig. 47

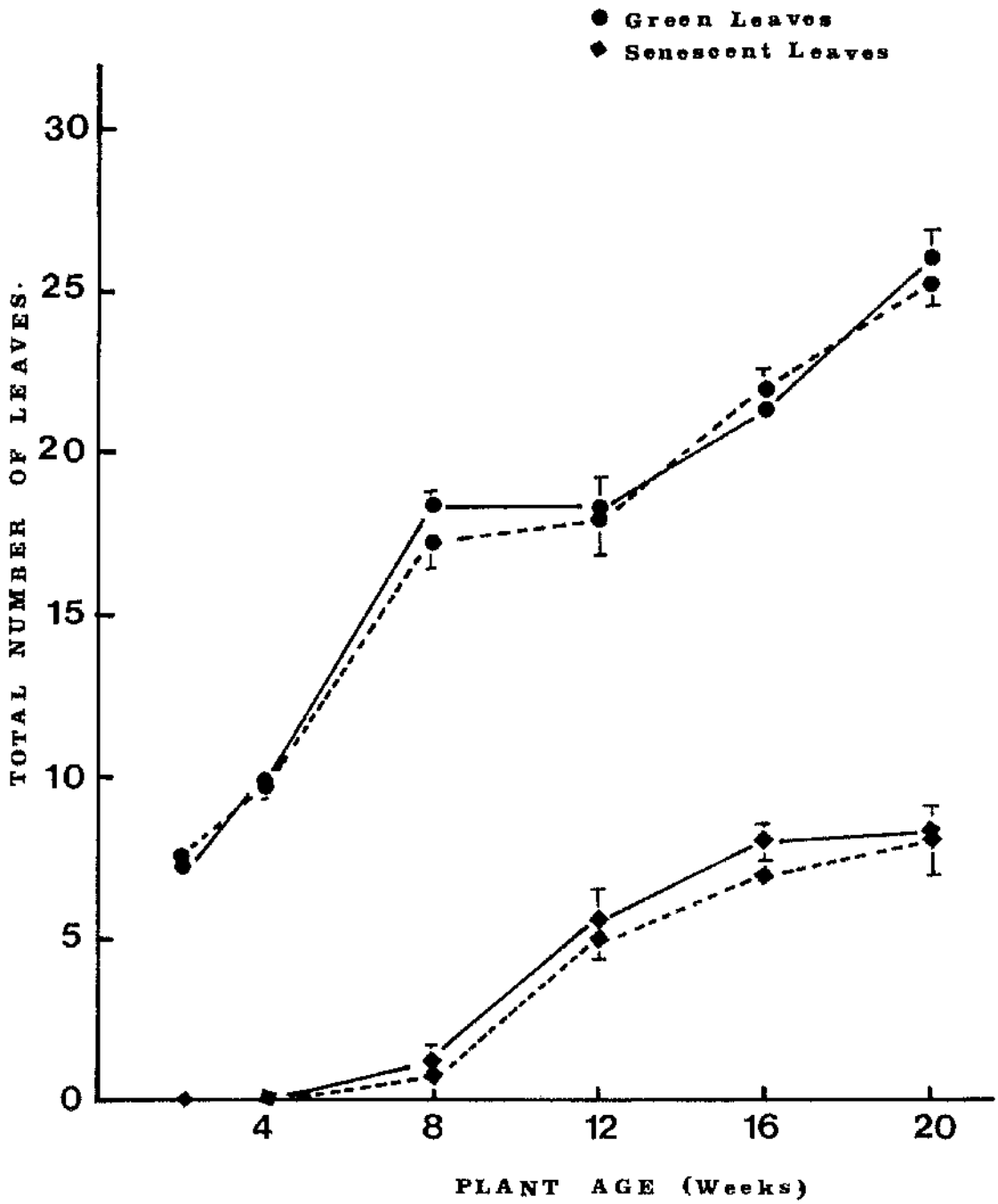

Green Leaves
Senescent Leaves
TOTAL NUMBER OF LEAVES.
0
5
10
15
20
25
30
4
8
12
16
20
PLANT AGE (Weeks)

Fig. 48

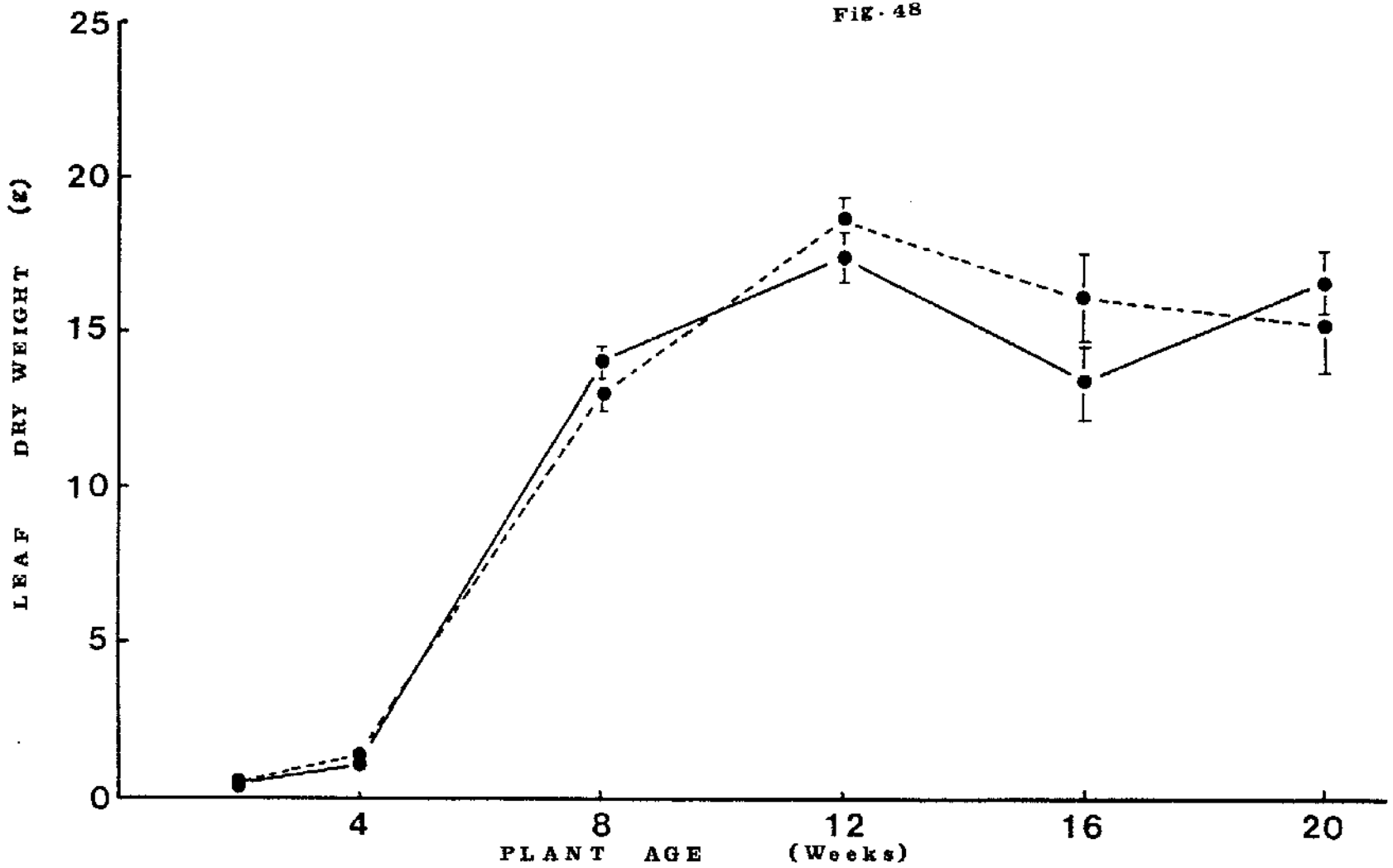


Fig. 49

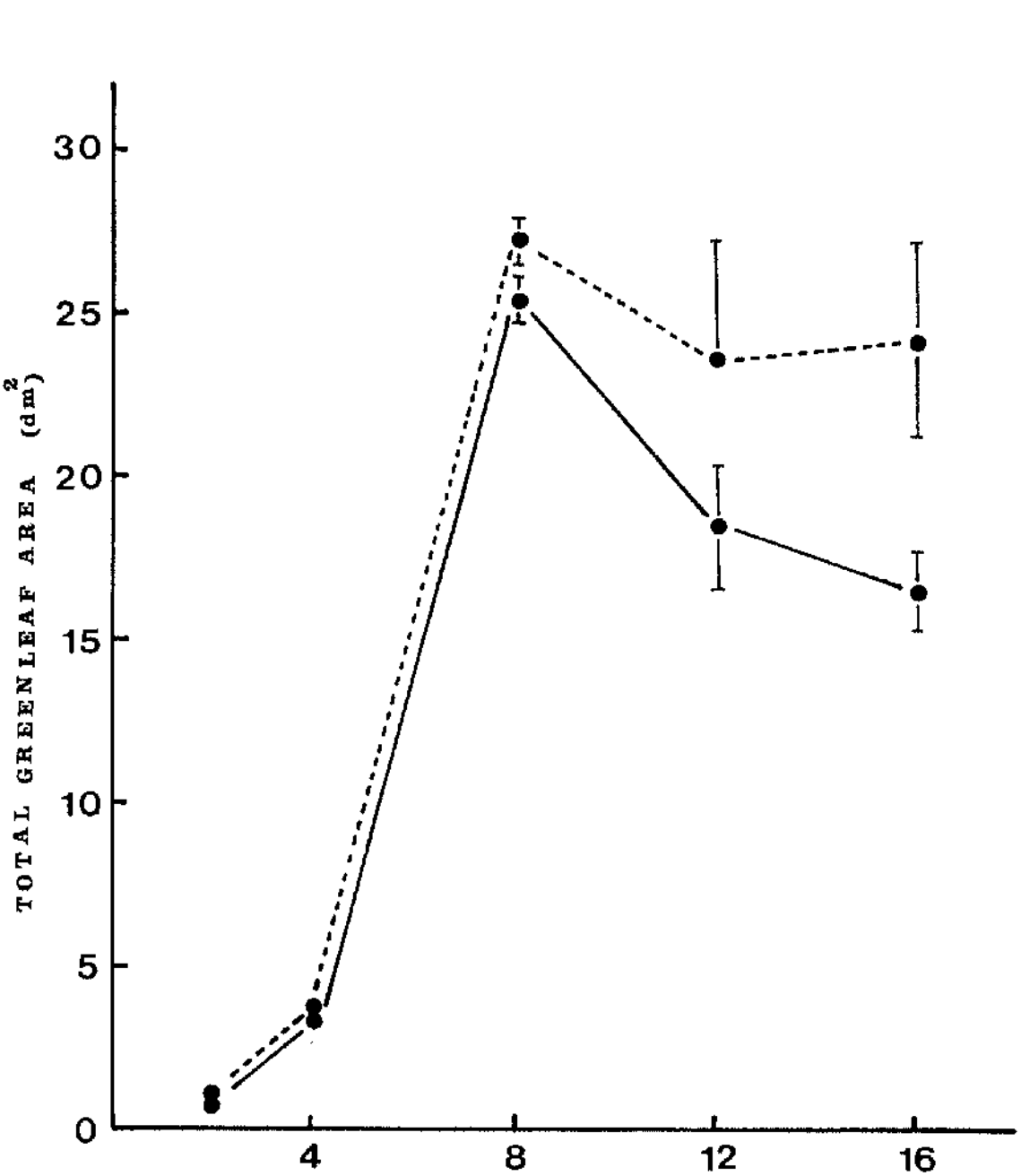

30
25
20
15
10
5
0
TOTAL GREENLEAF AREA (dm²)
4
8
12
16
PLANT AGE (Weeks)

Fig. 50

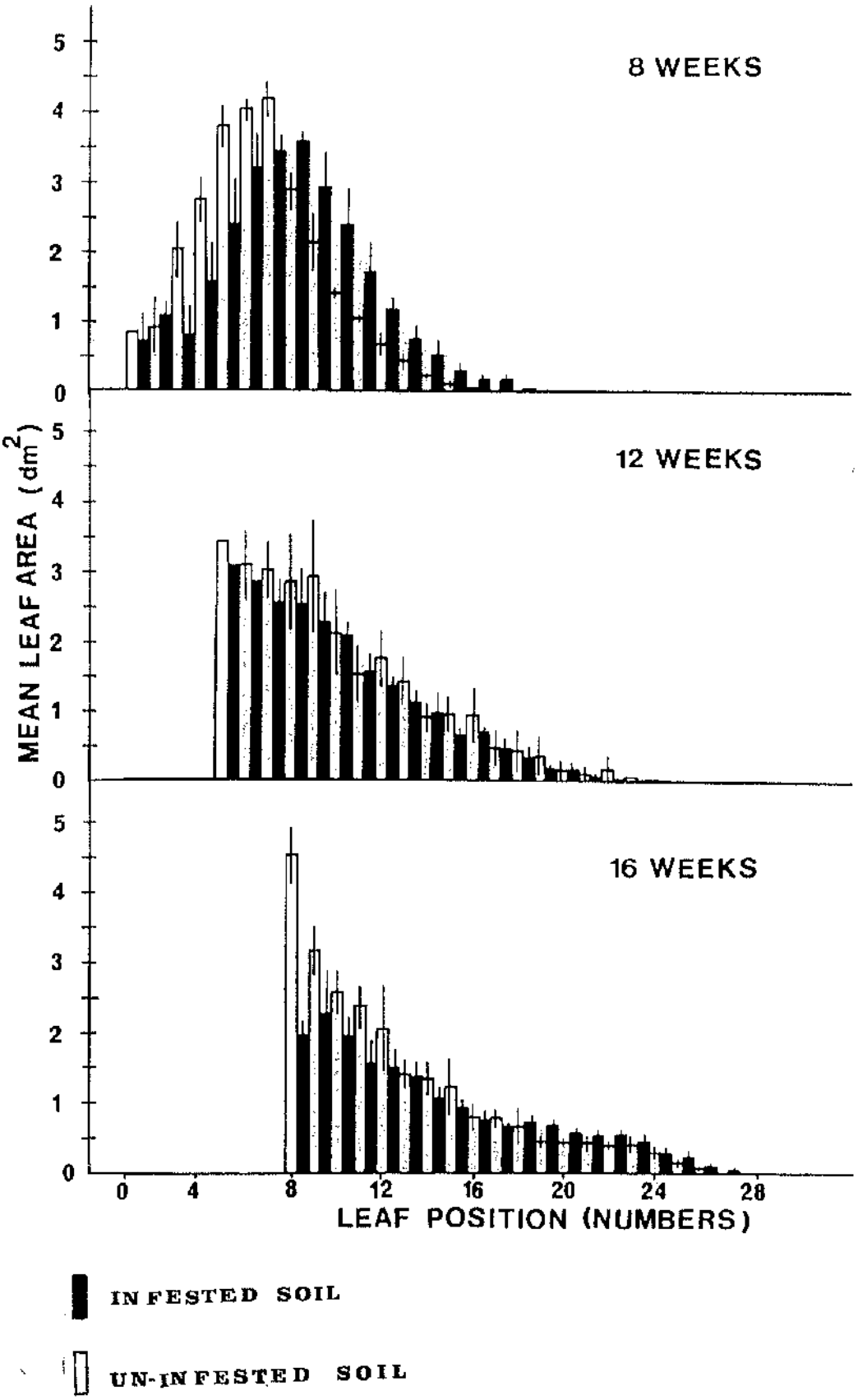

8 WEEKS
12 WEEKS
16 WEEKS
MEAN LEAF AREA (dm²)
LEAF POSITION (NUMBERS)
0
4
8
12
16
20
24
28
INFESTED SOIL
UN-INFESTED SOIL

Table 20. Data on reproductive structures of plants grown in P. expansum infested and uninfested sterilized soils.

Time Period trans-planting	Soil	No. of flower buds	No. of flowers	Total No. of fruits	No. of expanding fruits	Fruits fresh weight (g)
8 weeks	Infested	16.6 ± 2.227	9.6 ± 0.6	2.4 ± 0.678	-	1.936 ± 1.653
	Uninfested	21.0 ± 2.516 NS	9.667 ± 0.882 NS	2.0 ± 1.0 NS		0.23 ± 0.122 NS
12 weeks	Infested	1.33 ± 1.333	21.333 ± 0.882	11.333 ± 1.202	-	168.973 ± 12.518
	Uninfested	0 NS	15.333 ± 2.404 NS	18.0 ± 2.887 NS		135.9 ± 27.753 NS
16 weeks	Infested	3.6 ± 0.678	2.2 ± 0.86	41.8 ± 5.417	26.2 ± 3.426	591.74 ± 31.493
	Uninfested	4.667 ± 0.333 NS	1.333 ± 0.333 NS	31.667 ± 3.712 NS	23.333 ± 4.667 NS	657.2 ± 43.547 NS
20 weeks	Infested	1.0 ± 0.63	7.0 ± 1.673	33.8 ± 3.541	24.2 ± 3.023	451.68 ± 44.501
	Uninfested	2.0 ± 1.155 NS	8.33 ± 0.882 NS	51.667 ± 8.951 NS	40.0 ± 9.292 NS	442.267 ± 85.406 NS

All figures of infested soils are mean ± S.E. of 5 plants and uninfested soils are mean ± S.E. of 3 plants.

NS = not significant at P = 0.05.

- Fruits at these harvests were not differentiated into unexpanding and expanding.

6.3.2.3. Leaf growth

The total numbers of leaves (green + senescent) on plants from both infested and uninfested soils were similar at each harvest (fig. 54). Total leaf dry weights were also not significantly different between treatments at any harvest (fig. 55).

Total leaf areas were also similar on plants from both soils up to the 3rd harvest at 8 weeks, but after that, leaf areas on plants in infested soils were consistently smaller (significantly smaller at the 4th and 6th harvests) (fig. 56).

Leaf area measurements of individual leaves (fig. 57) showed that leaves at all nodes on plants grown in infested soils were generally smaller than leaves at similar positions on plants grown in uninfested soils, at all harvests.

6.3.2.4. Reproductive structures

The first flower buds were recorded at the 2nd harvest 4 weeks after transplanting plants, in both infested and uninfested soils.

No significant differences were found between treatments in numbers of flower buds, flowers or fruits produced or in fruit fresh weight at any harvest (Table 21).

Figs. 51 to 57

Growth of plants in unsterilized soils.

——— Infested soil.

----- Uninfested soil.

Fig. 51 Stem height.

Fig. 52 Stem dry weight.

Fig. 53 Root dry weight.

Fig. 54 Total number of leaves.

Fig. 55 Leaf dry weight.

Fig. 56 Total green leaf area.

Fig. 57 Areas of individual leaves.

Fig · 51

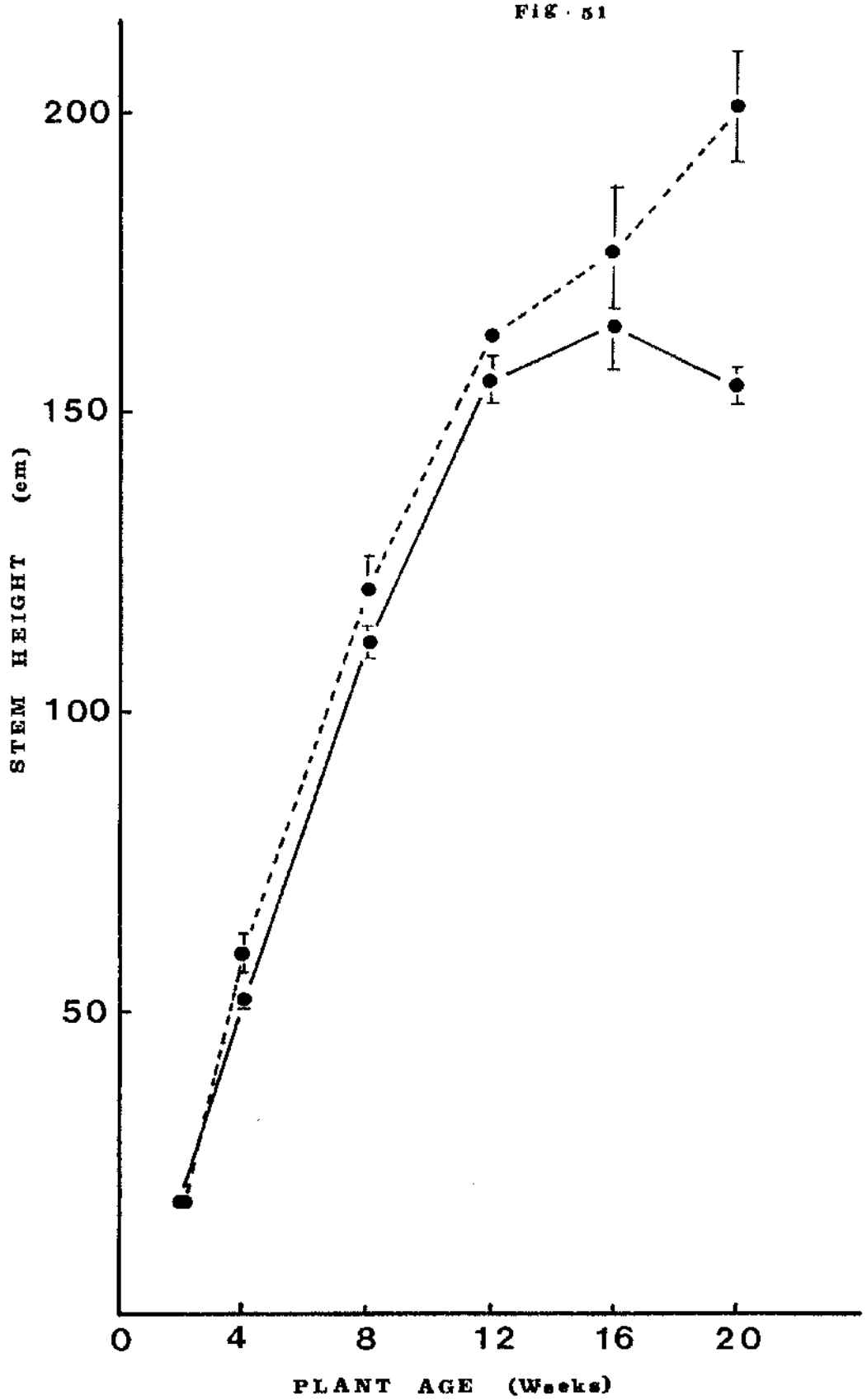


FIG · 52

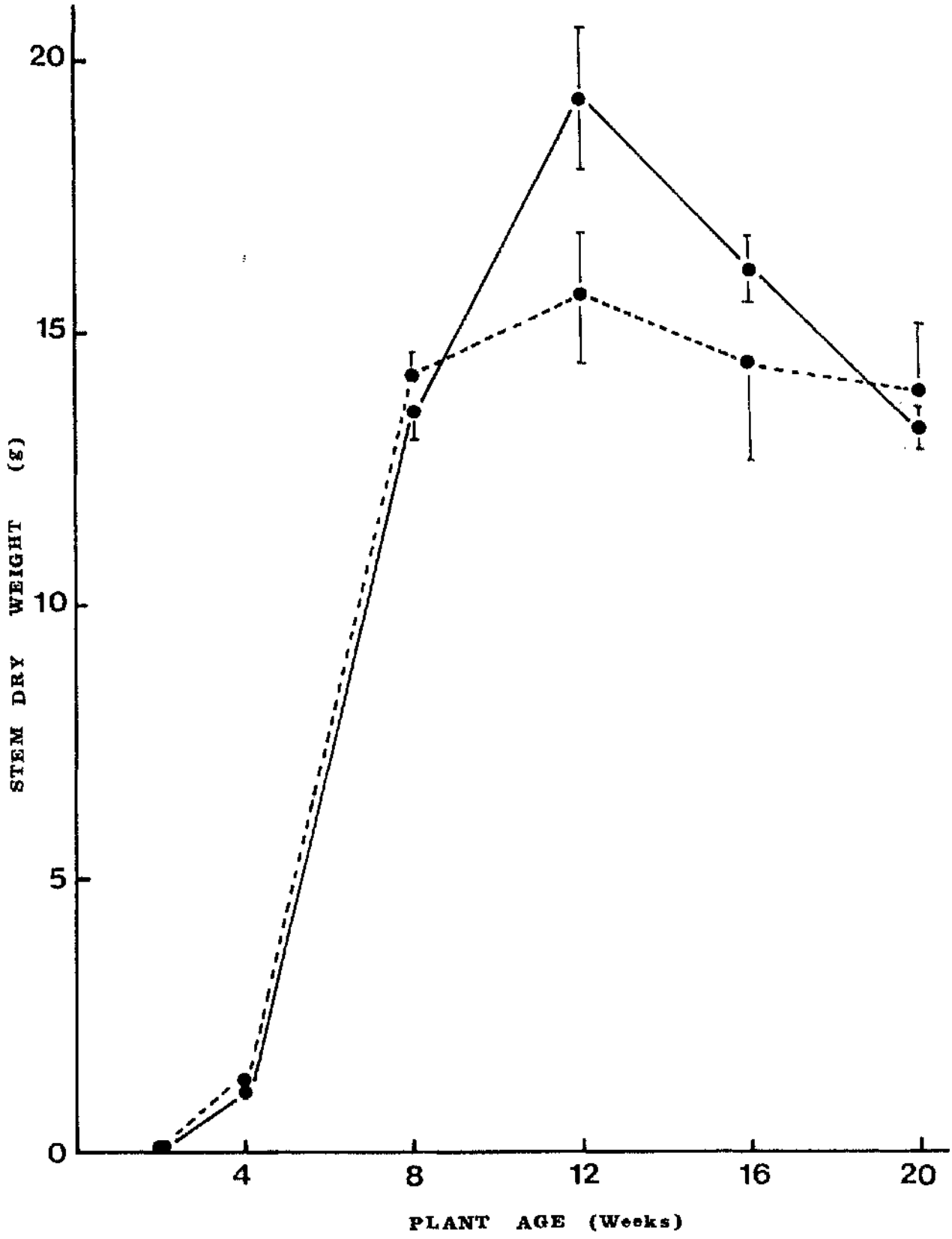


Fig. 53

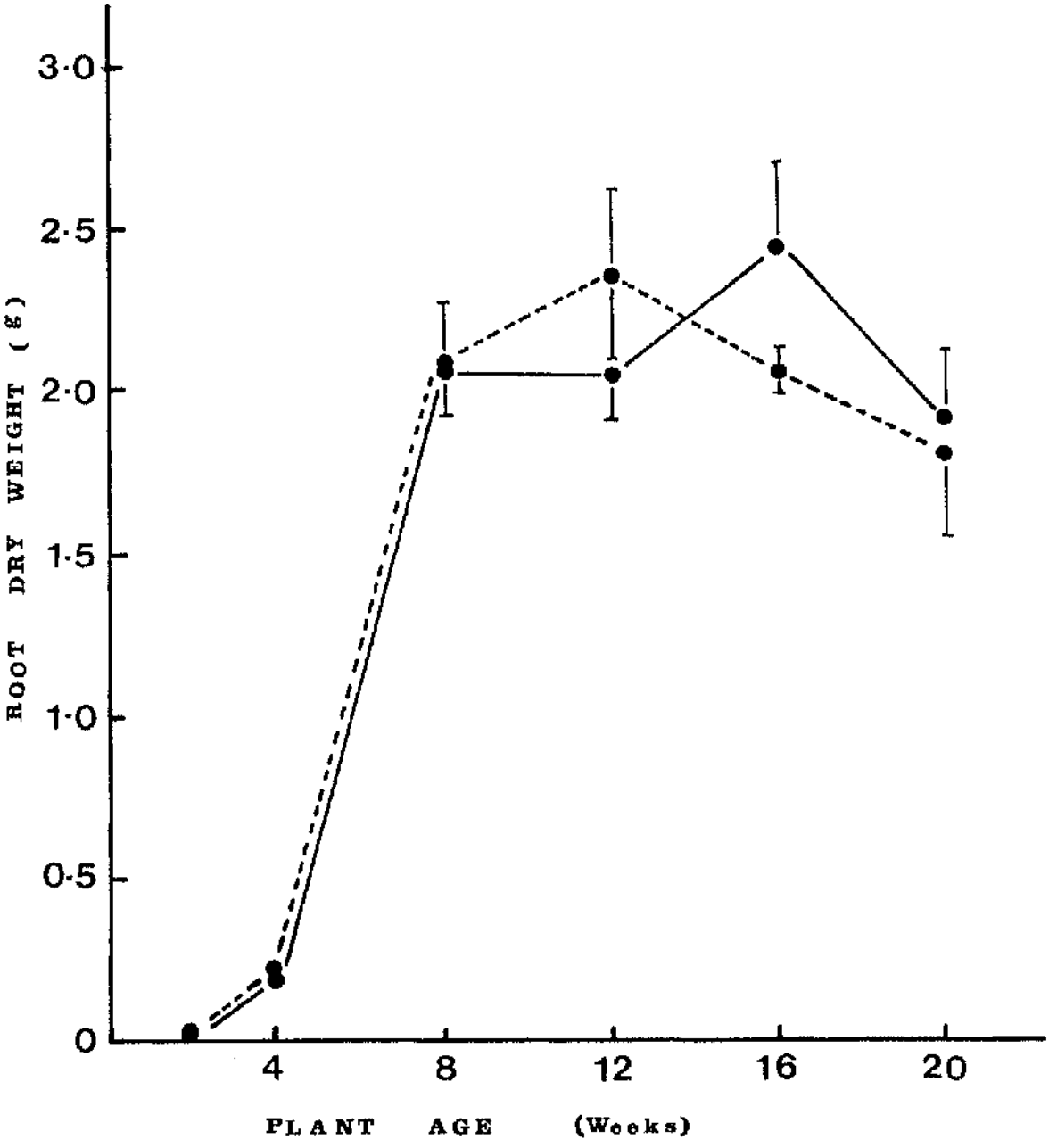
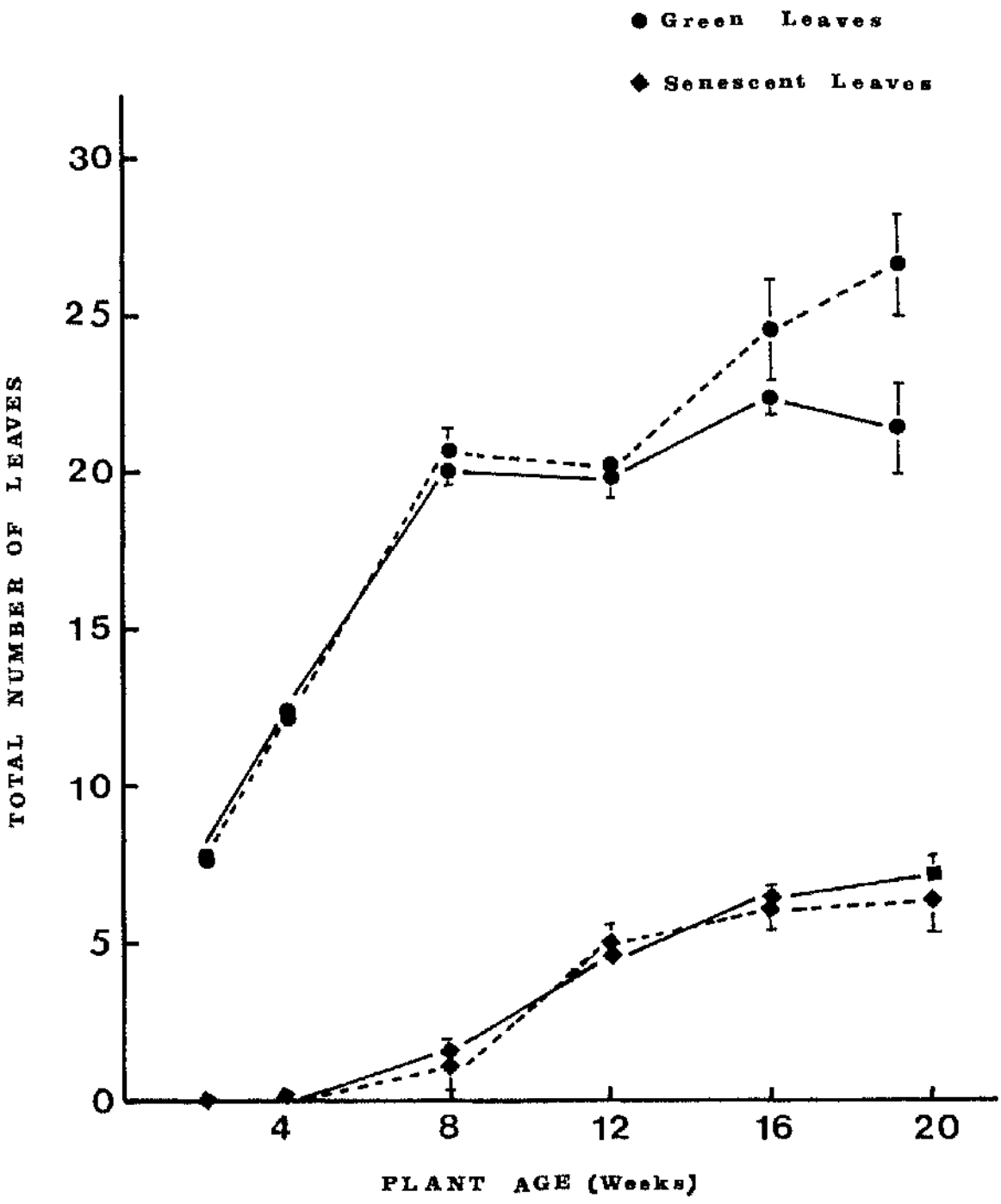


FIG · 54


● Green Leaves
◆ Senescent Leaves
TOTAL NUMBER OF LEAVES
30
25
20
15
10
5
0
4
8
12
16
20
PLANT AGE (Weeks)

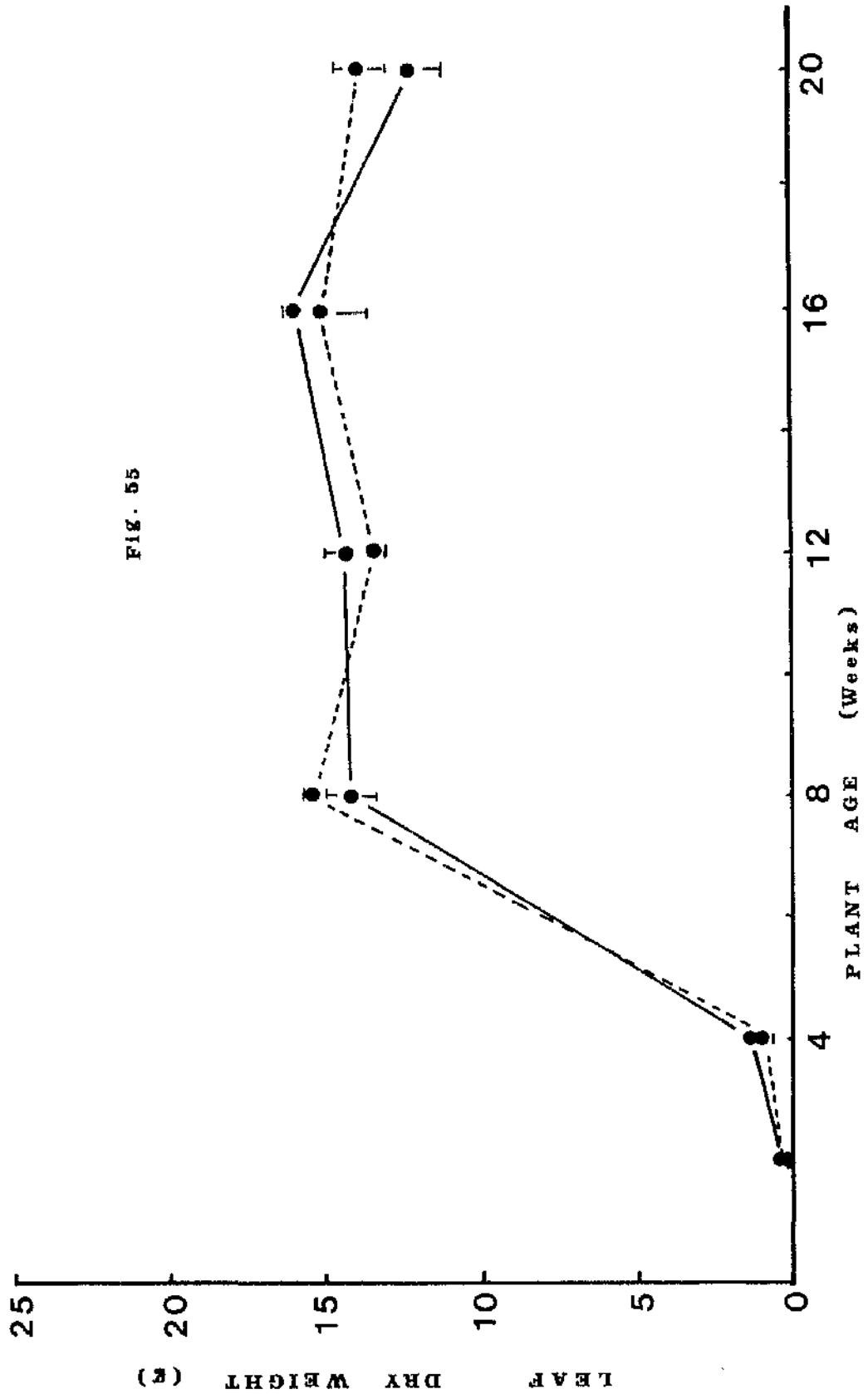


Fig. 55

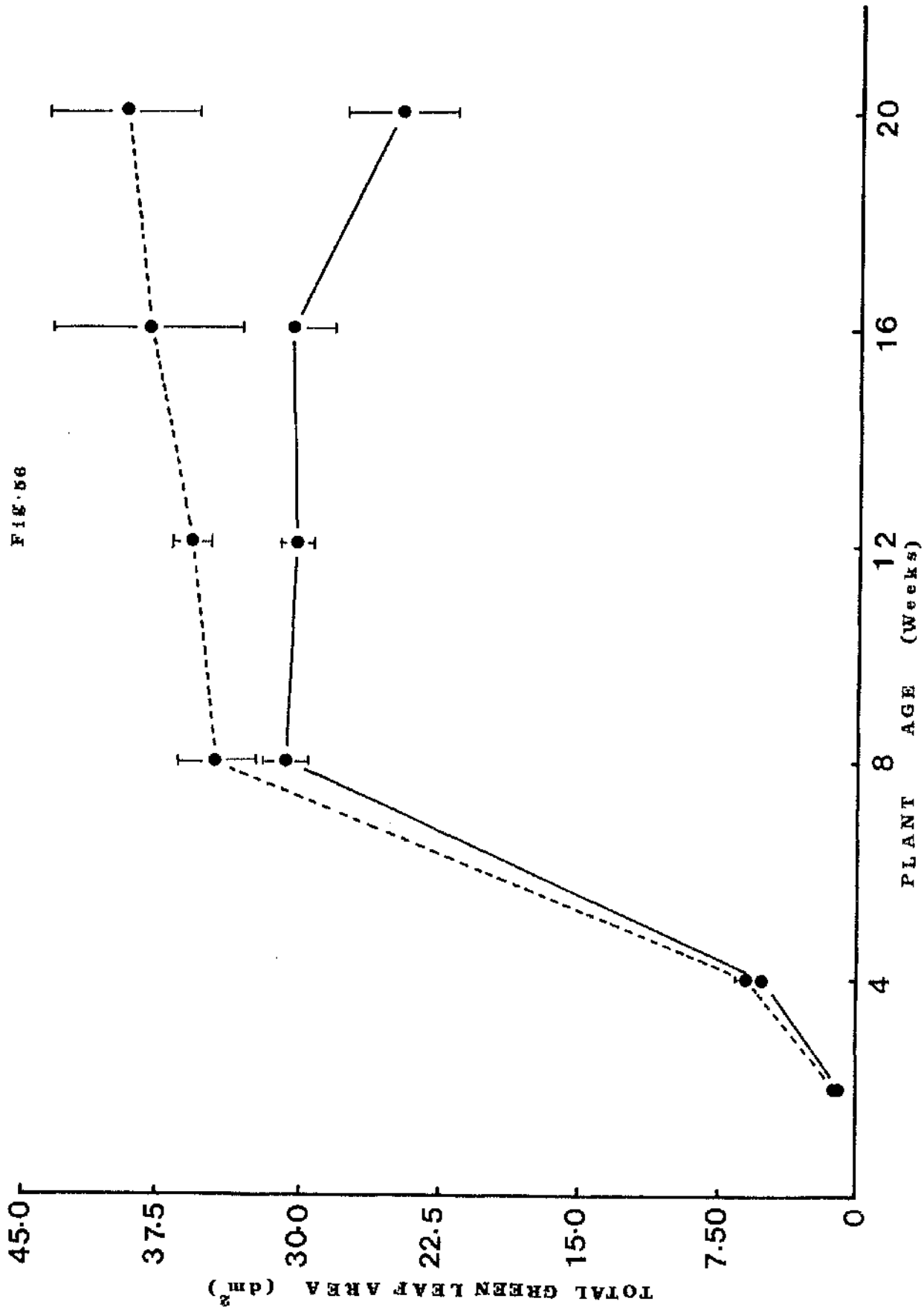

TOTAL GREEN LEAF AREA (dm²)
45·0
37·5
30·0
22·5
15·0
7·50
0
4
8
12
16
20
PLANT AGE (Weeks)

Fig. 56

Fig.57

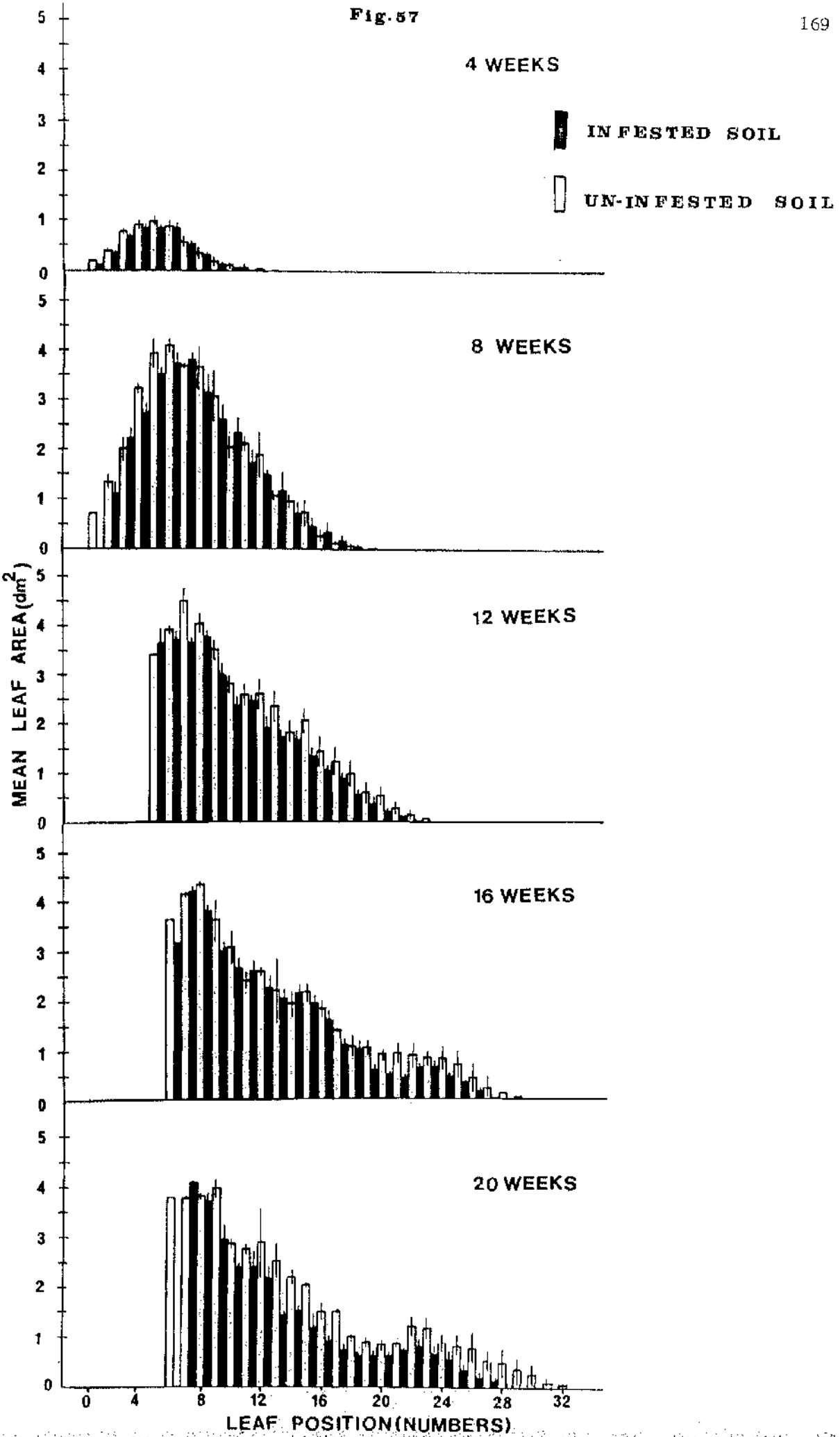

4 WEEKS
8 WEEKS
12 WEEKS
16 WEEKS
20 WEEKS
INFESTED SOIL
UN-INFESTED SOIL
MEAN LEAF AREA(dm²)
LEAF POSITION(NUMBERS)
0
4
8
12
16
20
24
28
32

Table 21. Data on reproductive structures of plants grown in P. expansum infested and uninfested unsterilized soils.

Time period transplanting	Soil	No. of flower buds	No. of flowers	Total No. of fruits	No. of expanding fruits	Fruits fresh weight (g)
4 weeks	Infested	3.45 ± 0.255	0	0	0	0
	Uninfested	3.5 ± 0.804 NS				
8 weeks	Infested	13.8 ± 1.908	13.4 ± 1.913	11.6 ± 2.462	6.4 ± 1.435	26.448 ± 8.925
	Uninfested	15.667 ± 1.764 NS	10.333 ± 1.333 NS	11.0 ± 0.577 NS	5.0 ± 0.577 NS	21.35 ± 4.384 NS
12 weeks	Infested	2.6 ± 0.245	3.8 ± 0.663	38.6 ± 2.96	10.4 ± 1.568	369.5 ± 14.361
	Uninfested	4.333 ± 1.333 NS	3.333 ± 1.453 NS	34.333 ± 0.667 NS	11.333 ± 1.202 NS	374.867 ± 40.004 NS
16 weeks	Infested	6.4 ± 1.123	0	38.8 ± 1.828	27.2 ± 1.855	727.92 ± 34.843
	Uninfested	7.0 ± 1.732 NS	1.333 ± 1.333 NS	34.667 ± 0.882 NS	19.333 ± 0.667 NS	629.2 ± 33.22 NS
20 weeks	Infested	3.2 ± 1.02	0	32.2 ± 1.934	24.4 ± 0.6	778.64 ± 33.348
	Uninfested	5.0 ± 1.0 NS	1.333 ± 1.333 NS	32.667 ± 4.409 NS	22.667 ± 0.882 NS	788.033 ± 22.877 NS

Figures of infested soil plants are mean ± S.E. of 5 plants and uninfested soil plants are mean ± S.E. of 3 plants.

NS = Not significant at P = 0.05.
S = Significant at P = 0.01.

6.3.4. Discussion

In terms of stem height and total dry matter content, tomato plants grown in P. expansum infested soils, whether sterilized or unsterilized, were not significantly different from plants grown in similar but uninfested soils. However, plants grown in infested unsterilized soils were slightly smaller at all harvests than those grown in uninfested soils.

The numbers of leaves, both green and senescent, of plants grown in sterilized or unsterilized infested soils were also not significantly different from those on plants in uninfested soils. However, total green leaf area was significantly smaller on plants grown in infested unsterilized soil than on plants grown in uninfested soils 12 weeks after transplanting. Total leaf area was slightly (but not significantly) lower on the plants grown in infested soils, both sterilized and unsterilized, than on plants from uninfested soils at all harvests. Thus the reduction in total green leaf area is not due to a reduction in the number of leaves produced but to an effect on leaf expansion.

Measurements of individual leaf areas showed that leaves at all nodes on plants in infested unsterilized soils, and at lower nodes on plants in infested sterilized soils, were smaller than those grown in uninfested soils. This observation also indicates that P. expansum affects leaf expansion.

It was observed in previous work (Chapter V) that P. expansum can grow and survive in the rhizospheres of tomato plants but does not colonize the internal tissues of the roots. Thus the small but consistent effects on the growth of leaves is probably due to the production of phytotoxic substances in the rhizospheres in both sterilized and unsterilized soils.

The small but consistent effect of P. expansum on stem growth found only in plants grown in unsterilized soils is thus different from that on leaf growth and probably results from an interaction between P. expansum and other components of the soil microflora.

In their review Domsch, Gams and Anderson (1980) assumed that the

accumulation of P. expansum in the rhizospheres of apple plants caused phytotoxicity and soil sickness. Pidoplichko et al. (1965) isolated P. expansum from the rhizosphere of maize plants and also found that it affected shoot growth of maize in laboratory conditions. Norsdat and McCalla (1968,1969, 1971) reported the presence of patulin in the soil and they also reported that it can inhibit both microorganism and plant growth. Thus P. expansum, when present in the rhizospheres of tomato plants, may produce such a toxin which could be in part responsible for the slight effects on plant growth observed in these experiments.

CHAPTER VII

COLONIZATION OF PLANT LITTER BY PENICILLIUM EXPANSUM IN SOIL

7.1. Introduction

The ability of a fungus to colonize organic matter in soil is a complex process determined by many factors. These include the nature of the organic matter and the physical, chemical and biological environment in which it is present.

The following account deals with the problems P. expansum would be likely to face if it does occur as growing mycelium in organic matter.

Chemical constituents of litter

The bulk of organic matter in soil is derived from the remains of plants (about 99%) with small amounts from other organisms, all of which had lived above or within soil (Krebs 1978; Hudson, 1980). There are many compounds in plant materials including soluble low molecular weight compounds such as simple sugars, amino acids and aliphatic acids etc. and many complex polymers such as protein, pectins, cellulose, hemicelluloses and lignins (Alexander, 1961). The low molecular weight compounds are generally readily utilized by most organisms but the ability of organisms to utilize the more complex materials depends upon their capacity to produce the enzymes involved in the degradation of the constituent polymers to their basic units.

Succession in litter decomposition

Colonization of organic material by fungi usually follows a fairly ordered succession (Garrett, 1963,1970,1981; Hudson, 1968; Visser & Parkinson, 1975).

The first fungal colonists of leaves or other aerial parts of plants

and of roots, during senescence, are often weak parasites such as Botrytis cinerea, Alternaria tenuis and Cladosporium herbarum. Hogg and Hudson (1966) found that the leaves of Fagus sylvatica are usually colonized first by members of the phylloplane flora rather than by soil fungi. The first colonists of roots are the root infecting fungi which then open the way for a sequence of saprophytic sugar fungi, and then the cellulose and lignin decomposers (Garrett, 1950,1951,1956).

Primary saprophytic sugar fungi may become established as the first part of a secondary succession, but in the absence of weak parasites they are the first colonists. They rapidly utilize the available sugar and other carbon compounds simpler than cellulose, but they generally have restricted abilities to decompose polymers. They are characterized by rapid spore germination followed by a high mycelial growth rate. These fungi are widely and abundantly distributed, mainly in the form of spores, throughout soil where they remain dormant until coming into contact with a fresh substrate (Garrett, 1951,1963). Zygomycotina, such as species of Mucor, Absidia and Rhizopus, and certain Oomycetes such as species of Pythium and various Fungi Imperfecti including species of Penicillium, belong to the "sugar fungi". Thus P. expansum could fit into this group.

The next stage of the succession generally involves colonization by cellulolytic fungi. Moore-Landecker (1972) reported that most cellulose decomposing fungi are Basidiomycetes but some members of the Fungi Imperfecti and Ascomycetes are also included. In contrast, Burges (1958) states that the cellulose decomposing fungi are mainly Ascomycetes and Fungi Imperfecti, although some of the faster growing Basidiomycetes are also involved.

P. expansum is known to produce cellulases in culture (Garber, Beraha & Shaeffer, 1965) and so has some of the potential to colonize plant litter.

The cellulolytic fungi in turn are succeeded by ligninolytic organisms including many Basidiomycetes which are well known for their capacity to decompose lignin (Kaplan & Hartenstein, 1980).

Fungal associations in litter decomposition

Many workers have described associations between fungi unable to degrade complex substrates such as cellulose and lignin with organisms that can. These fungi are often members of the Zygomycetes and the Oomycetes though several members of the Fungi Imperfecti have also been recorded (Hudson, 1968). Some of these fungi may parasitize the mycelia of other fungi but the majority probably exist by growing in close association with the polymer degraders sharing the enzymic breakdown products produced. These saprophytes have been termed "secondary sugar fungi" by Garrett (1963) because they do not occur among the pioneer colonizers of litter.

Non-nutritional factors affecting litter colonization

The rate of substrate colonization by any particular fungus depends on many factors such as its inoculum level, its competitive saprophytic ability and any associated antagonistic micro-organisms which are attempting to exploit the same substrate (Garrett, 1950,1956,1970,1981; Griffin, 1972).

For colonization to occur successfully, propagules must be either in direct contact with, or adjacent to the substrate, and the probability of such contact is high when the inoculum density is high (Dhingra, Tenne & Sinclair, 1976). There have been many studies of the competitive ability of fungi in the presence compared to the absence of particular antagonistic organisms. Thus Sneh, Katan & Henis (1972) found that incubating bean stem segments and chitin particles in Rhizoctonia-free soil, resulted in a significant suppression of colonization if Rhizoctonia solani was subsequently introduced. The suppression of colonization by R. solani was either due to its inability to compete for nutrients with the microflora established in the substrate units, or to its sensitivity to antifungal substances produced, or both. El-Abyad and Saleh (1973) have also shown that when the saprophytic colonization of roots by F. oxysporum f.sp. vasinfectum in

competition with certain common fungi tested at standard inoculum levels. The fungus was highly invasive but colonization was reduced when certain competitive fungi were inoculated two days before it was.

Clearly P. expansum has certain properties which could make it a successful litter decomposer. It was earlier shown (Chapter IV) that spores can survive in natural soil for a year or more. It can grow and survive over a wide range of temperature: -3 - 35°C (Panasenko, 1967), and pH 4.4 - 7.5 (Cochrane, 1958). The optimum temperature for the germination of conidia is within the range 23 to 30°C (Mislivec & Tuite, 1970). It has a high capacity for pectin decomposition (Garber, Beraha & Shaeffer, 1965; Domsch & Gams, 1969) and there are numerous records of its ability to degrade cellulose (Scales, 1915; Marsh, Bollenbacher et al., 1949; Garber, Beraha & Shaeffer, 1965).

The objective of the study reported in this chapter was to determine if P. expansum has the competitive ability to colonize dead plant tissue in soil. In the first series of experiments the ability of P. expansum to colonize apple root and leaf litter in the soil from a spore inoculum was examined. In the second series the ability of P. expansum to survive in the soil in apple root litter, which had been colonized before addition to the soil, was examined.

7.2. Colonization of apple roots in soil

7.2.1. Materials and Methods

Roots from 3 to 4 month old apple plants, grown in Levington compost in 15.2cm pots, were thoroughly washed under running tap water. Roots of approximately equal diameter were cut into short lengths of 2-3cm and washed again under running tap water to remove any remaining soil particles and debris. After surface sterilization in 10% chloros solution for ten minutes, the roots were finally washed three times in sterilized distilled water containing Tween 80 (100ml/2 drops Tween 80). Ten surface sterilized

root pieces were placed in each 90mm diameter plastic petri-dish and covered with either sterilized or unsterilized garden soil. These soils contained 1×10^5 spores g^{-1} oven-dry soil. The moisture content of both sterilized and unsterilized soils was approximately 20.2%. The dishes were sealed with Parafilm to reduce evaporation and then incubated at 23°C. After 4, 5, 7, 10, 15, 32 or 42 days the root pieces from three petri-dishes of each treatment were harvested to determine the extent of colonization. The pieces were first washed with running tap water to remove soil particles and then sterilized in chloros and washed as described above. They were then cut into 2mm lengths under sterile conditions. Ten of these lengths were plated in each petri-dish onto the selective medium and incubated at 23°C. After 10 to 14 days the numbers of root pieces giving rise to colonies of P. expansum or other fungi were recorded.

7.2.2. Results

The results of this experiment are given in fig.58 and Appendix Table 24 The percentage colonization of root segments by P. expansum in sterilized soil was very much higher than that in unsterilized soil. In the sterilized soil the percentage colonization increased rapidly up to 7 days and then remained more or less unchanged for the duration of the experiment. The apparent reduction on day 42 was not significant although it might have represented the beginnings of the elimination of P. expansum because it was correlated with an increase in colonization by other fungi which had contaminated the experiment.

The pattern of colonization by P. expansum in unsterilized soil was very different with very few of the pieces becoming colonized. Most of the pieces were colonized by fungi other than P. expansum. Surprisingly other Penicillium species, although readily isolated from these soils, were not found among the colonists.

Fig. 58

Percentage of 2mm root lengths colonized after incubation at 23°C in *P. expansum* infested soil.

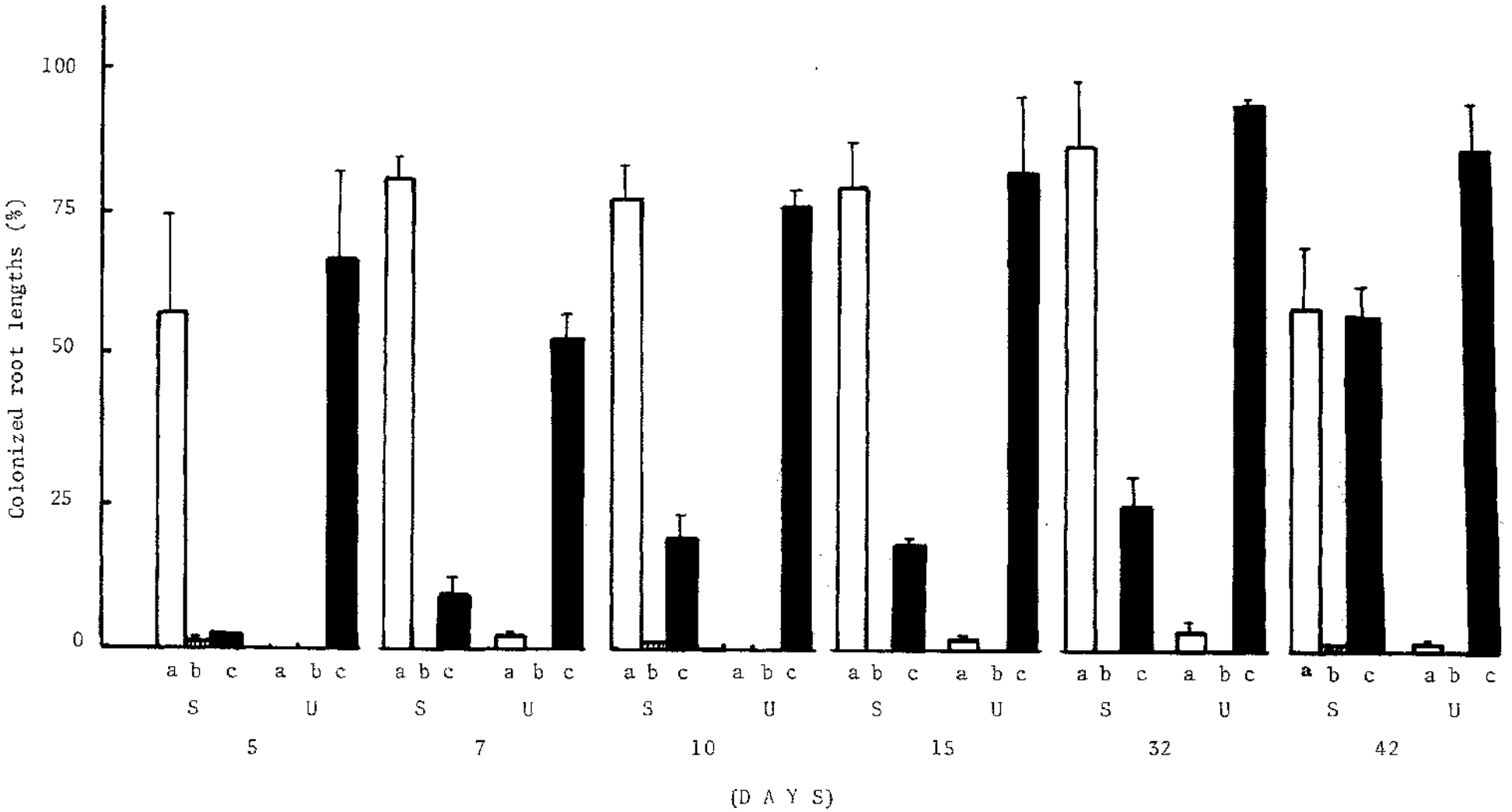
(a) *P. expansum*

(b) Other *Penicillium* species

(c) Fungi other than *Penicillium* species

(S) Sterilized soil

(U) Unsterilized soil.


Colonized root lengths (%)
100
75
50
25
0
a b c a b c
S U
5
7
10
15
32
42
(D A Y S)

7.3. Colonization of apple leaves in soil

7.3.1. Materials and methods

Green leaves were picked on 2nd October from apple trees growing in the Botany Department Gardens, Garscube. They were washed with distilled water and then wiped with cotton wool soaked in 90% ethanol before finally washing in distilled water. Two leaves were placed in each petri-dish and covered with either sterilized or unsterilized soil containing 9×10^3 P. expansum spores g^{-1} oven-dry soil. The moisture content of both soils was 23.3%. The dishes were then sealed with Parafilm to avoid water loss and incubated at 23°C. After different intervals the leaves were recovered, washed with running tap water to remove soil particles, and then sterilized in 1% chloros solution for 15-20 minutes before washing with three changes of sterilized distilled water containing Tween 80. Leaf discs were cut with a cork-borer (5mm diameter) and ten leaf discs per petri-dish were placed onto the selective medium. After incubation for 7 to 14 days at 23°C the number of leaf discs which gave rise to colonies by P. expansum and other fungi were recorded.

7.3.2. Results

The results in fig. 59 show that the percentage of apple leaf discs colonized by P. expansum in sterilized soil increased rapidly up to 4 days and then remained unchanged for the duration of the experiment. Colonization in unsterilized soil was significantly different with only a few leaf discs yielding P. expansum after 2 and 4 days. Most of the discs were colonized by fungi other than P. expansum. The number of colonized discs by other fungi was consistently higher than that of P. expansum in both sterilized and unsterilized soils throughout the experiment. The extent of the colonization of discs incubated in the sterile soil by fungi other than P. expansum indicates that the leaves had probably already begun

Fig. 59

Percentage of apple leaf discs colonized after incubation at 23°C in P. expansum infested soil. Fifty 5mm diameter leaf discs tested. Colonization given as % ± S.E.

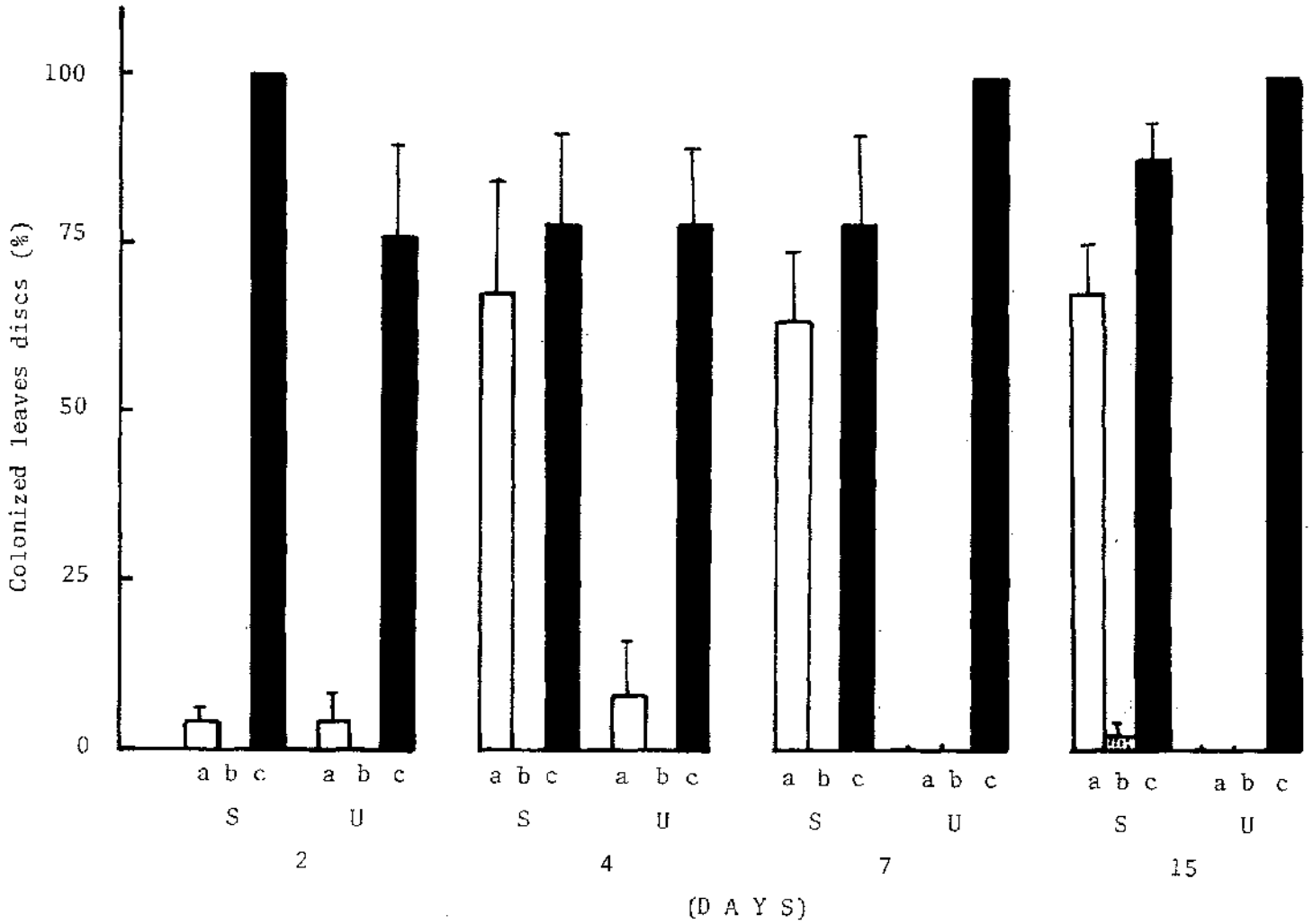
(a) P. expansum

(b) Other Penicillium species

(c) Fungi other than Penicillium species

(S) Sterilized soil

(U) Unsterilized soil.


Colonized leaves discs (%)
100
75
50
25
0
a b c a b c
S U
2
4
7
15
(D A Y S)

to be colonized when they were removed from the trees.

7.4. Survival of P. expansum in apple roots in soil

7.4.1. Materials and methods

Apple roots were collected and surface sterilized as described above. The root lengths (2-3 cm) were then dipped into a spore suspension of P. expansum and placed on sterilized moist filter paper in petri-dishes. Five pieces were placed in each petri-dish, taking care that they were separated from each other by a distance of at least 5mm. The dishes were incubated at 23°C and after 7 to 10 days, when the pieces had become colonized, they were buried in sterilized and unsterilized soils in petri-dishes (10 per dish). The moisture content of both soils was approximately 23%. The dishes were incubated at 23°C and after different intervals (4, 8, 16, 20 and 28 days), the pieces were collected and surface sterilized. The sterilized root lengths were then cut into 2mm long pieces under sterile conditions and plated onto petri-dishes containing the selective medium (10 pieces per dish). After incubation for 7 to 14 days at 23°C the numbers of root pieces colonized by P. expansum and other fungi were counted.

7.4.2. Results

The results are given in fig. 60.

The percentage colonization in both sterilized and unsterilized soils remained the same throughout the experiment. Only a few root pieces became colonized by other fungi and there was little indication that other fungi in the garden soil were very effective at eliminating P. expansum from the root lengths.

Fig. 60

Percentage colonized apple root lengths (2mm) after incubation for different times at 23°C in soil. Fifty 2mm root lengths tested. Colonization given as % ± S.E.

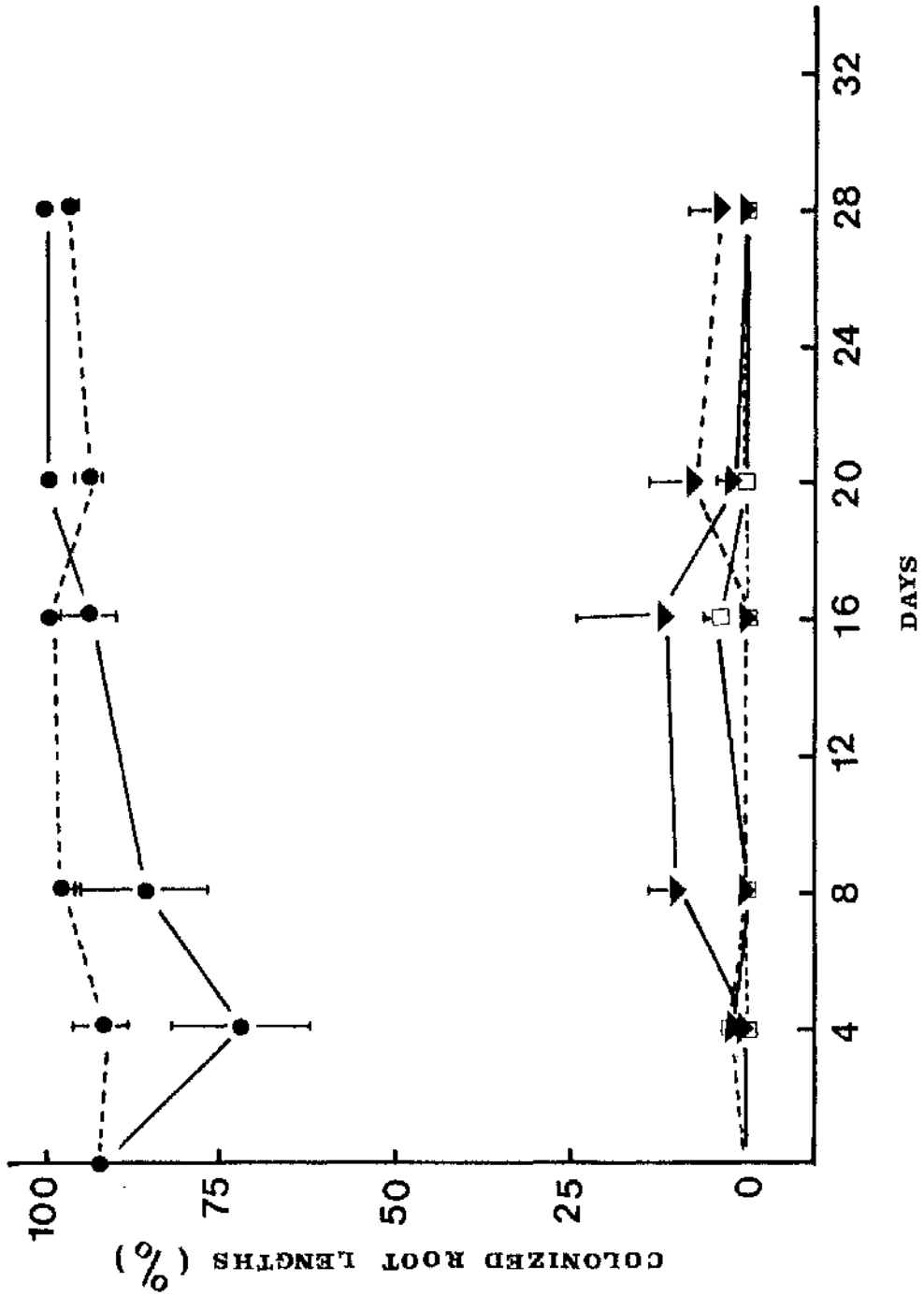
---- Unsterilized soil

——— Sterilized soil

● *P. expansum*

□ Other *Penicillium* species

▼ Fungi other than *Penicillium* species.


COLONIZED ROOT LENGTHS (%)
100
75
50
25
0
4
8
12
16
20
24
28
32
DAYS

7.5. Discussion

Root lengths which have been dipped in a suspension of P. expansum spores in culture do become colonized. Root and leaf litter also became colonized from spores in sterilized soil. Thus P. expansum has the potential to colonize some litter. However when roots or leaves were placed in unsterilized soil, even though it was infested with P. expansum spores, little colonization occurred. The failure of P. expansum to colonize the litter as well in unsterilized soil as in sterilized soil is probably due to its inability to compete with components of the soil microflora. The addition of nutrients to unsterilized soil has been shown to overcome the effects of fungistasis on P. expansum. Thus it is to be expected that the root and leaf litter would stimulate spore germination since the litter should provide sources of nutrients. It is likely then that the inhibition of root or leaf litter colonization operates after the stage of spore germination. Sneh, Katan & Henis (1972) reported that the colonization of bean stem segments and chitin particles by Rhizoctonia solani was suppressed if the two substrates were incubated in R. solani free soil before transferring into fresh soil artificially infested with mycelium of R. solani. Autoclaving the substrates, extracting them with ethanol, or sterilizing with gamma irradiation, however, allowed colonization by R. solani at levels similar to that which occurred if they had not been exposed to the suppressive soils. They suggested that decreased colonization by R. solani of the incubated substrates was probably due to competition with the established microflora for degradation products, or to the production of antifungal substances, or both. They also observed a significant reduction in the colonization of chitin particles by R. solani if the particles were pre-incubated in sterilized soils infested with known antibiotic producing micro-organisms, but not in soils infested with non-antibiotic producing micro-organisms. Experiments reported earlier, in Chapter IV indicated that the germination of P. expansum spores in unsteri-

lized soils increased if the numbers of soil micro-organisms was reduced in any way. It was also shown that some fungi are potentially antagonistic to the growth of P. expansum. Thus the failure of P. expansum to colonize the litter readily may be due to the antagonistic activities of elements of the soil microflora rather than to competition for nutrients.

However, P. expansum can survive in competition with the soil microflora if the root lengths are already colonized before introduction into the soil was shown it could.

Butler (1953) and Garrett (1970) studied the colonization of wheat straw in natural soil by Fusarium roseum (syn. F. culmorum), Curvularia ramosa, Cochliobolus sativus (syn. Helminthosporium sativum) and Gaeumannomyces graminis (syn. Ophiobolus graminis). Essentially the method used, known as the Cambridge method, was similar to the one used here. However, instead of mixing spores alone as inoculum into the soil, they mixed maizemeal-sand cultures of the different fungi into the soil in different proportions. They found at the lowest level of inoculum (2%), which is a much higher level of inoculum than the one used in this study, that only F. roseum and C. ramosa colonized wheat straw to any extent (55 & 88% respectively) while Co. sativus and G. graminis were more or less excluded (2 & 0% respectively). Because of the differences in inoculum levels between the experiments reported here and Butler and Garrett's experiments, it is difficult to compare the two, but the level of inoculum of P. expansum used was very much less than 2% and so the competitive saprophytic ability of P. expansum would appear to be much greater than Co. sativus or G. graminis. However, G. graminis is very similar to P. expansum in that it can survive in soil in substrates colonized before addition to the soil.

Thus P. expansum can survive in soil in litter which was colonized before arrival in the soil, but until more types of litter have been tested it is not possible to conclude that P. expansum cannot also colonize litter within the soil. It was certainly unable to colonize apple root or leaf litter well.

GENERAL CONCLUSIONS

It is clear from this work that P. expansum spores do not germinate in the fertile sandy loam soil which was used in these investigations. However, they do not die rapidly because about 68% still remained viable after 48 weeks. Thus some spores can remain dormant but viable for one year or more in this soil.

The dormancy of the spores could be broken by adding nutrients, and so shortage of nutrients may be one factor inhibiting spore germination. However, the growth was limited since the germtubes produced rapidly developed to produce microcyclic conidia. Spores incorporated into autoclaved soil germinated, but also only developed to produce microcyclic conidia. Growth in the latter case could have been supported by the nutrients released from micro-organisms killed by the heat treatment, but the limited growth and other observations indicate that inhibitory factors are also involved.

Thus treating the natural soil at 60°C had no effect on spore dormancy, but the treatment did kill over 90% of all micro-organisms isolatable on malt extract agar or on the modified Hutchinson's agar. Treating the soil at 80°C or above broke spore dormancy almost completely. If the release of nutrients from the killed micro-organisms were the sole explanation for spore germination in the autoclaved soil, then germination would be expected to occur in soils treated at 60°C. The effect of the higher temperature treatment must be on some other factor, possibly the inactivation of inhibitors themselves and/or the micro-organisms producing them.

Spore germination was inhibited on agar discs placed on cellophane on the soil surface but not on discs separated from the soil by glass slides. Thus there is evidence for the presence of diffusible non-volatile inhibitors in these soils. The germination of spores on agar discs placed on autoclaved soils shows that micro-organisms may be one of the producers of the

non-volatile inhibitors.

Penicillium expansum was able to germinate and grow in the rhizospheres of various plants. The most rapid growth occurred during the early stages of plant growth after which there was a sharp reduction. The induction of germination in the rhizosphere was probably due to the increased availability of the nutrients from the root exudates. Fungal development was probably microcyclic, and the sharp reduction in colony forming propagules indicates that propagules (spores) formed during growth in the rhizosphere lose their viability more rapidly than the spores which were used as the inoculum in the first place. The spores used for the inoculum were produced in culture on a rich medium and were much larger than the microcyclic conidia formed in autoclaved soils, or in natural soils supplemented with nutrients. Thus microcyclic conidia may contain limited amounts of nutrients and have a low potential for prolonged survival.

Penicillium expansum was found to readily colonize the root surfaces of tomato from a high inoculum but to a lesser extent from a low inoculum. Maize and broad bean were also colonized to a limited extent from a low inoculum. It was never found to invade the internal root tissues of tomato even from a high inoculum.

However, its presence in the rhizospheres of tomato did affect plant growth to a limited extent. It caused a slight but consistent reduction in stem height and in total leaf area, but did not affect total dry matter production or the total number of leaves produced. This reduction in growth may be due to phytotoxic substances produced by P. expansum in the rhizospheres. One phytotoxin known to be produced by P. expansum is patulin.

The ability of P. expansum to colonize root and leaf litter of apple within soil in the face of competition by other soil micro-organisms was very low. However, it is not possible to conclude from this one example that P. expansum cannot colonize and grow within litter from other plant species in the soil. P. expansum grew and survived in apple root litter within soil if the litter was colonized before addition to the soil.

This suggests that P. expansum may be able to grow and survive in litter in the soil, if the litter is colonized before arrival on the soil and before colonization by other soil micro-organisms.

Thus these studies indicate that P. expansum can survive in soil in the form of dormant spores, and that it has limited competitive saprophytic ability in that it can grow in soil under certain conditions in the rhizospheres of plants and in organic matter, possibly colonized before incorporation into the soil. These experiments thus can account for the frequent isolation of P. expansum from soil.

APPENDICES

APPENDIX TABLES 1A to 1C

Comparison of methods for mixing spores into the soil.

Appendix Table 1A. Recovery of P. expansum after mixing spores into soil by hand.

	Replicates					Recovery of spores g^{-1} soil			
Sample	1	2	3	4	5	Mean	Sample Mean	* %	Overall Mean ± S.E.
1	10	7	13	8	8	9.2	3680	85.581	
2	8	5	5	9	10	7.4	2960	68.837	3120 ± 288.45
3	8	5	8	7	6	6.8	2720	63.256	

* Number of colonies expressed as a percentage of the number of spores added to soil (4300 spores g^{-1} soil).

Appendix Table 1B. Recovery of P. expansum after mixing spores into soil by shaking manually in plastic container.

Samples	Replicates 1	2	3	4	5	Spores g^{-1} soil Mean	Sample Mean	* %	Overall Mean ± S.E.
1	6	8	4	8	4	6.0	2400	55.814	
2	5	15	11	10	7	9.6	3840	89.302	3040 ± 423.33
3	8	7	6	9	6	7.2	2880	66.976	

* Number of colonies expressed as a percentage of the number of spores added to soil (4300 spores g^{-1} soil).

Appendix Table 1C. Recovery of P. expansum after mixing of spores into soil by concrete mixer.

Samples	Replicates 1	2	3	4	5	Mean	Recovery of spores g^{-1} (oven dry) soil: Sample Mean	* %	Overall Mean ± S.E.
1	20	15	25	25	15	20.0	1025.64	85.47	
2	17	22	21	17	29	21.2	1087.18	90.59	
3	26	12	16	18	19	18.2	933.33	77.78	
4	13	17	24	21	19	18.8	964.10	80.34	
5	17	14	15	15	18	15.8	810.25	67.52	
6	23	15	14	16	16	16.8	861.54	71.79	975.49 ± 38.049
7	20	22	20	14	16	18.4	943.58	78.63	
8	18	20	21	18	17	18.8	964.10	80.34	
9	23	16	31	24	22	23.2	1189.74	99.14	

* Number of colonies expressed as a percentage of the number of spores added to soil (1200 spores g^{-1} soil).

APPENDIX TABLES 2A to 2C

Analysis of organic matter, particle size and chemical of soil.

Appendix Table 2A. Organic Matter.

Methods	Samples	1	2	3	4	5	6	7	8	Mean ± S.E.
Organic matter, by loss on Ignition	(%)	9.73	11.64	13.9	13.08	10.88	11.07	10.97	10.82	11.51 ± 0.48
Organic matter by Walkley Black method	(%)	9.26	7.55	10.06	7.84	-	-	-	-	8.68 ± 0.59

Appendix Table 2B. Particle size fractionation by mechanical analysis.

Name of Fraction	Samples	1	2	3	Mean ± S.E.
Coarse + Medium sand %		35.0	31.0	34.6	33.5 ± 1.27
Fine sand %		25.8	24.8	25.2	25.3 ± 0.29
Clay %		13.3	12.4	12.1	12.6 ± 0.36
Silt %		24.9	21.5	22.0	22.8 ± 1.06

Appendix Table 2C. Chemical analysis of soil.

	Samples	1	2	3	4	5	Mean ± S.E.
Cation exchange capacity (me/100 g oven dry soil)	K	21.203	25.827	27.505	29.754	26.623	26.182 ± 1.407
Exchangeable Bases (me/100 g oven dry soil)	K	1.089	1.045	1.036	1.049	1.027	1.049 ± 0.0106
	Mg	2.523	1.747	1.697	1.862	2.180	2.002 ± 0.155
	Na	0.616	0.381	1.109	0.783	0.787	0.735 ± 0.119
	Ca	18.093	18.660	17.403	19.309	17.133	18.119 ± 0.399
Available (mg/Kg oven dry soil)	K	214.982	292.514	285.473	222.034	214.982	245.997 ± 17.636
	P	229.076	229.076	202.643	216.747	223.789	220.266 ± 4.953

Appendix Table 3. Survival and growth of P. expansum in soil.

Soil	Samples	Number of viable propagules $x10^4$ g^{-1} oven dry soil								
		0	1 Week	2 Weeks	4 Weeks	8 Weeks	16 Weeks	24 Weeks	36 Weeks	48 Weeks
Unsterilized	1	1.238	1.307	1.132	0.946	0.855	0.629	0.619	0.641	0.388
	2	1.495	1.324	1.106	0.971	0.960	0.661	0.661	0.437	0.431
	3	1.066	1.376	1.100	1.039	1.030	0.662	0.691	0.521	0.492
	4	1.381	1.433	1.223	1.206	1.044	0.789	0.725	0.544	0.356
	5	1.470	1.791	1.353	1.314	1.129	0.897	0.731	0.464	-
Mean ± S.E.		1.33 ± 0.079	1.446 ± 0.089	1.183 ± 0.048	1.095 ± 0.071	1.004 ± 0.046	0.728 ± 0.050	0.685 ± 0.021	0.521 ± 0.035	0.417 ± 0.029
Sterilized	1		152.43	75.78	212.95	1027.02	1182.60	10.04	26.60	3.93
	2		283.83	83.47	275.15	1451.42	1482.66	16.45	4.61	23.63
	3		631.35	62.36	347.05	1805.40	893.50	47.52	15.96	15.38
	4		834.13	277.77	988.10	3353.42	1221.05	434.13	5.55	-
	5		1351.79	1435.89	994.28	8544.00	10.18	510.27	-	-
Mean ± S.E.			650.71 ± 213.13	387.05 ± 265.19	563.51 ± 175.89	3236.25 ± 1383.83	957.99 ± 254.71	203.68 ± 110.47	13.18 ± 5.16	14.31 ± 5.71

APPENDIX TABLES 4A AND 4B

Percentage germination and germtube lengths of *P. expansum* in different concentrations of glucose after 24 hrs incubation at 23°C.

Appendix Table 4A. Percentage germination of P. expansum in glucose.

	UN-WASHED SPORES					WASHED SPORES			
% Glucose	Replicates 1	2	3	4	Mean ± S.E.	Replicates 1	2	3	Mean ± S.E.
0	1.0*	0.4	21.19	1.82	6.103 ± 5.038	5.84	4.18	-	5.01 ± 0.83
0.125 g	28.68	31.34	40.68	53.36	38.515 ± 5.577	33.42	13.75	17.72	21.63 ± 6.006
0.25 g	54.19	59.29	58.24	55.77	56.873 ± 1.159	71.1	60.54	58.86	63.5 ± 3.831
0.5 g	67.19	77.31	-	-	72.25 ± 5.06	76.07	58.2	84.71	72.993 ± 7.806
1.0 g	75.78	72.27	85.61	-	77.887 ± 3.993	87.32	91.84	70.04	83.067 ± 6.643
2.0 g	70.33	70.0	94.06	70.83	76.305 ± 5.921	93.42	95.75	-	94.585 ± 1.165

* After 24 hours

Appendix Table 4B. Mean length (μm) of germtubes of P. expansum after 24 hours in glucose.

		UN-WASHED SPORES					WASHED SPORES			
% Glucose		Replicates 1	2	3	4	Mean±.S.E.	Replicates 1	2	3	Mean±S.E.
0	Mean	5.9[a]	-	-	-		6.5[c]	-	-	
	S.E.	0.4					1.3			
0.125 g	Mean	16.06[a]	16.76[a]	20.16[a]	13.6[a]	16.645±1.353	11.8[a]	10.2[b]	9.92[c]	10.64±0.586
	S.E.	1.33	1.71	1.93	0.99		1.14	1.2	2.21	
0.25 g	Mean	31.03[a]	24.35[a]	17.22[a]	26.04[a]	24.66±2.857	17.33[a]	18.1[a]	15.06[a]	16.83±0.912
	S.E.	2.07	1.87	1.27	2.43		1.02	1.61	1.29	
0.5 g	Mean	18.72[a]	17.96[a]	-	-	18.34±0.38	13.36[a]	16.1[a]	13.4[a]	14.287±0.906
	S.E.	2.17	1.59				1.38	1.31	1.23	
1.0 g	Mean	17.45[a]	20.34[a]	19.65	-	19.147±0.871	20.58[a]	15.81[a]	14.73[b]	17.04±1.797
	S.E.	1.35	1.83	1.27			1.43	1.38	2.0	
2.0 g	Mean	17.65[a]	18.06[b]	26.49[a]	17.51[b]	19.927±2.191	17.28[a]	15.49[a]		16.385±0.895
	S.E.	1.33	1.56	2.2	1.83		1.3	1.31		

a. Values are means of measurements of 40 to 54 germtubes.

b. Values are means of measurements of 25 to 30 germtubes.

c. Values are means of measurements of 10 germtubes.

APPENDIX TABLES 5A AND 5B

Percentage germination and germtube lengths of *P. expansum* in different concentrations of sodium nitrate after 24 hrs incubation at 23°C.

Appendix Table 5A. Percentage germination of P. expansum in sodium nitrate.

	UN-WASHED SPORES						WASHED SPORES					
	Replicates					Mean	Replicates					Mean
% $NaNO_3$	1	2	3	4	5	± S.E.	1	2	3	4	5	± S.E.
0	9.04	9.88	33.20	-	-	17.373 ± 7.917	13.39	19.14	8.74	4.71	-	11.495 ± 3.105
19 mg	30.60	35.81	47.39	68.40	7.97	38.034 ± 9.934	23.48	14.21	14.06	6.74	12.93	14.284 ± 2.677
37 mg	16.66	22.22	33.10	17.57	32.16	24.342 ± 3.516	6.98	19.42	21.33	19.62	-	16.838 ± 3.314
75 mg	16.66	20.18	23.12	23.05	-	20.753 ± 1.526	19.74	6.09	16.35	5.08	-	11.815 ± 3.668
150 mg	15.92	32.11	40.48	26.37	34.84	29.944 ± 4.178	14.21	7.44	12.44	15.12	-	12.303 ± 1.714
300 mg	8.43	41.25	4.35	0	0.56	10.918 ± 7.733	0	0	0	1.16	17.29	3.69 ± 3.407
500 mg	19.85	8.13	1.16	19.23	20.06	13.686 ± 3.855	4.41	0	0	1.15	0	1.112 ± 0.854

Appendix Table 5B. Mean length (μm) of germtubes of P. expansum after 24 hrs in sodium nitrate.

		UN-WASHED SPORES					WASHED SPORES				
% $NaNO_3$		Replicates 1	2	3	4	Mean ± S.E.	Replicates 1	2	3	4	Mean ± S.E.
0	Mean	10.31^{c}	6.15^{b}	9.85^{a}	-	8.77±1.316	10.22^{a}	8.75^{b}	-	-	9.485±0.735
	S.E.	1.96	0.66	1.0			1.09	0.8			
19 mg	Mean	15.56^{b}	26.89^{b}	18.04^{b}	30.34^{a}	22.708±3.519	8.0^{a}	10.68^{a}	10.48^{b}	10.68^{b}	9.96±0.655
	S.E.	1.67	4.06	2.54	2.58		0.72	1.47	1.49	1.05	
37 mg	Mean	17.83^{a}	14.82^{a}	19.44^{a}	15.45^{a}	16.885±1.07	5.76^{c}	10.08^{b}	11.26^{b}	13.48^{a}	10.145±1.623
	S.E.	1.09	1.32	1.67	1.41		0.86	1.67	1.41	1.18	
75 mg	Mean	12.15^{b}	15.08^{a}	18.69^{a}	14.89^{a}	15.203±1.341	10.08^{c}	7.06^{c}	6.62^{c}	4.6^{c}	7.09±1.131
	S.E.	1.97	1.49	1.84	1.55		2.65	1.04	0.96	1.73	
150 mg	Mean	6.72	11.69^{a}	14.6^{a}	10.71	10.93±1.628	10.13^{a}	14.4^{b}	12.47^{a}	6.88^{b}	10.97±1.618
	S.E.	1.42	1.02	1.27	1.31		0.89	2.3	1.12	0.75	
300 mg	Mean	10.92^{a}	-	-	-	-	14.08^{b}	-	-	-	-
	S.E.	0.83					0.99				
500 mg	Mean	7.92^{a}	7.03^{c}	8.86^{a}	6.75^{a}	7.64±0.477	5.04^{c}	-	-	-	-
	S.E.	0.85	1.05	0.73	0.53		1.47				

a Values are means of measurements of 40 to 50 germtubes.

b Values are means of measurements of 25 to 30 germtubes.

c Values are means of measurements of 5 to 15 germtubes.

APPENDIX TABLES 6A to 6D.

Effect of malt-extract broth supplementation on the germination and growth of *P. expansum* in soil.

Appendix Table 6A. Percentage germination of P. expansum in unsterilized soil amended by the addition of malt-extract broth.

Strength of malt-extract broth in soil	Experiment No. 1				Experiment No. 2			
	Replicates			Mean ± S.E.	Replicates			Mean ± S.E.
	1	2	3		1	2	3	
No addition	0*	0	0	0	0	0	0	0
1/8x	7.39	4.56	3.11	5.02 ± 1.256	3.54	3.45	3.62	3.536 ± 0.049
1/2x	24.44	34.35	23.55	27.447 ± 3.461	13.06	16.50	12.89	14.15 ± 1.176
Normal	53.69	23.16	38.33	38.393 ± 8.814	45.17	37.74	29.79	37.566 ± 4.44
2x	70.90	76.11	70.32	72.443 ± 1.841	66.21	74.17	39.96	60.11 ± 10.335
3x	98.82	84.92	85.21	89.65 ± 4.586	76.52	65.64	78.65	73.603 ± 4.029
4x	96.52	92.11	89.63	92.753 ± 2.015	86.15	85.95	88.22	86.773 ± 0.726

* After 24 hours

Appendix Table 6B. Mean length (μm) of germtubes produced after 24 hrs in unsterilized soil amended by the addition of malt-extract broth.

Strength of malt-extract broth in soil		EXPERIMENT No. 1 Replicates 1	2	3	Mean ± S.E.	EXPERIMENT No. 2 Replicates 1	2	3	Mean ± S.E.
No addition	Mean	0	0	0	0	0	0	0	0
	S.E.								
1/8x	Mean	9.74	5.21*	7.7*	7.55 ± 1.309	4.8*	3.24*	4.73*	4.257 ± 0.508
	S.E.	0.85	0.79	0.99		1.3	1.36	0.89	
1/2x	Mean	16.96	24.58	14.21	18.583 ± 3.102	12.83	13.66	15.51	14.0 ± 0.079
	S.E.	1.65	2.09	1.02		1.47	2.15	1.18	
Normal	Mean	27.81	14.4	18.89	20.366 ± 3.941	19.78	19.42	19.34	19.513 ± 0.135
	S.E.	3.60	1.26	1.93		1.39	2.14	1.67	
2x	Mean	38.13	45.05	62.57	48.583 ± 7.273	33.22	40.56	35.18	36.32 ± 2.194
	S.E.	2.85	8.23	4.26		3.74	3.6	2.67	
3x	Mean	110.49	96.31	45.96	84.253 ± 19.579	53.82	57.17	40.75	50.58 ± 5.009
	S.E.	8.26	5.34	3.48		3.52	3.91	3.2	
4x	Mean	182.85	132.42	108.92	141.396 ± 21.809	85.23	60.04	85.87	77.046 ± 8.506
	S.E.	9.22	7.42	6.33		7.51	5.95	4.63	

Values are means of measurements of 30 to 50 germtubes.
* Values are means of measurements of 9 to 4 germtubes.

Appendix Table 6C. Percentage of germtubes of P. expansum developing phialides and conidia in unsterilized soil amended by the addition of malt-extract broth.

Strength of malt-extract broth in soil	EXPERIMENT NO. 1				EXPERIMENT NO. 2			
	Replicates				Replicates			
	1	2	3	Mean ± S.E.	1	2	3	Mean ± S.E.
1/8x	-	-	-	-	-	-	-	-
1/2x	2.89*	3.42	0.48	2.263 ± 0.904	0	0	0	0
Normal	1.72	4.23	5.26	3.737 ± 1.051	1.38	0	1.51	0.963 ± 0.483
2x	3.12	0	7.11	3.41 ± 2.05	0.68	5.97	0.49	2.38 ± 1.796
3x	14.19	4.21	5.55	7.983 ± 3.127	3.25	9.77	1.79	4.937 ± 2.453
4x	0	0.22	0.51	0.243 ± 0.148	0.89	0	0.57	0.487 ± 0.260

* After 24 hours

Appendix Table 6D. Percentage of germtubes of P. expansum showing autolysis in unsterilized soil amended by the addition of malt-extract broth.

Strength of malt-extract broth in soil	EXPERIMENT NO. 1				EXPERIMENT NO. 2			
	Replicates				Replicates			
	1	2	3	Mean ± S.E.	1	2	3	Mean ± S.E.
1/8x	-	-	-	-	-	-	-	-
1/2x	0*	1.36	1.9	1.086 ± 0.565	15.91	11.76	27.86	18.51 ± 4.826
Normal	60.08	30.98	32.33	41.130 ± 9.483	5.52	21.0	21.97	16.163 ± 5.329
2x	21.03	45.98	22.02	29.677 ± 8.157	4.14	16.42	4.97	8.51 ± 3.962
3x	82.71	42.05	42.36	55.707 ± 13.502	11.55	10.15	5.71	9.137 ± 1.76
4x	53.17	41.09	18.88	37.713 ± 10.041	4.46	11.54	0	5.333 ± 3.36

* After 24 hours

APPENDIX TABLES 7A AND 7B

Effect of diffusible inhibitors in soil on germination and germtube growth of *P. expansum*.

Appendix Table 7A. Effect of diffusible inhibitors in the soil on P. expansum spore germination.

Treatment		Percentage spore germination after 12 hr.			
		Replicates			
	Agar discs	1	2	3	Mean ± S.E.
Unsterilized soil	Preactivated* (24hr)	9.22	25.97	8.67	14.62 ± 5.677
	Continuing** activation	9.76	9.03	5.88	8.223 ± 1.191
Sterilized soil (control)	Preactivated* (24hr)	99.76	97.50	98.29	98.517 ± 0.662
	Continuing** activation	98.52	99.51	99.29	99.106 ± 0.300
1% Agar (control)		98.29	95.35	95.26	96.30 ± 0.995

* Agar discs were removed after 24hr in contact with the soil and placed on sterilized glass slides in petridishes before inoculation with spores.
** Agar discs were in contact with soil before and after inoculation with spores.

Appendix Table 7B. Effect of diffusible inhibitors in the soil on germtube growth of P. expansum.

Treatment	Agar discs		Germtube length (µm) after 12 hr. Replicates 1	2	3	Mean ± S.E.
Unsterilized soil	Preactivated* (24hr)	Mean	11.15	7.26	9.75	9.387 ± 1.138
		S.E.	1.07	0.6	0.91	
	Continuing** activation	Mean	13.19	9.68	4.32[a]	9.063 ± 2.579
		S.E.	1.08	0.96	0.83	
Sterilized soil (control)	Preactivated* (24hr)	Mean	47.32	40.23	30.59	39.38 ± 4.848
		S.E.	2.36	2.07	1.54	
	Continuing** activation	Mean	72.99	57.97	55.84	62.267 ± 5.397
		S.E.	3.67	2.79	2.67	
1% Agar (control)		Mean	30.83	28.51	30.13	29.823 ± 0.687
		S.E.	1.44	1.32	2.08	

Values are means of measurements of 50 germtubes.
a Mean of 10 measurements only.
* Agar discs were removed after 24hr in contact with the soil and placed on sterilized glass slides in petri dishes before inoculation with spores.
** Agar discs were in contact with soil before and after inoculation with spores.

APPENDIX TABLES 8A AND 8B

The volatility of the diffusible inhibitors of spore germination and germtube growth of *P. expansum*.

Appendix Table 8A. Volatility of the diffusible inhibitors of spore germination.

Treatment		Percentage of spore germination after 12 hr.			
		Replicates			
	Agar discs	1	2	3	Mean ± S.E.
Unsterilized soil	Preactivated* (24hr)	97.85	98.31	99.01	98.39 ± 0.337
	Continuing** activation	99.75	99.27	99.08	99.367 ± 0.199
Sterilized soil (control)	Preactivated* (24hr)	99.50	97.57	98.84	98.637 ± 0.566
	Continuing** activation	97.96	97.14	98.58	97.893 ± 0.417
Without soil (control)		98.29	98.28	98.38	98.316 ± 0.032

* Agar discs were removed after 24hr in contact with the soil and placed on sterilized glass slides in petridishes before inoculation with spores.
** Agar discs were in contact with soil before and after inoculation with spores.

Appendix Table 8B. Volatility of the diffusible inhibitors of germtube growth of P. expansum.

Treatment			Germtube length (μm) after 12 hr.			
			Replicates			
	Agar discs		1	2	3	Mean ± S.E.
Unsterilized soil	Preactivated* (24hr)	Mean	56.30	43.95	35.10	45.117 ± 6.148
		S.E.	3.07	2.97	1.8	
	Continuing** activation	Mean	65.40	56.88	56.94	59.74 ± 2.83
		S.E.	3.52	3.19	3.11	
Sterilized soil (control)	Preactivated* (24hr)	Mean	55.99	51.03	49.26	52.093 ± 2.014
		S.E.	2.84	3.18	2.397	
	Continuing* activation	Mean	61.89	70.33	69.29	67.17 ± 2.657
		S.E.	2.80	2.98	3.66	
Without soil (control)		Mean	56.02	53.87	49.58	53.157 ± 1.893
		S.E.	2.75	2.71	2.53	

Values are means of measurements of 50 germtubes.

* Agar discs were removed after 24hr in contact with the soil and placed on sterilized glass slides in petridishes before inoculation with spores.

** Agar discs were in contact with soil before and after inoculation with spores.

APPENDIX TABLES 9A AND 9B

Effect of diluting unsterilized soil with sterilized soil on germination and germtube growth of *P. expansum*.

Appendix Table 9A. Percentage germination of Penicillium expansum in unsterilized/sterilized soil mixtures.

Percentage sterilized soil in the soil mixture	Replicates 1	2	3	4	5	Mean ± S.E.
100	54.87*	78.49	55.28	68.65	72.91	66.04 ± 4.741
75	51.82	46.51	52.52	56.69	51.24	51.756 ± 1.624
50	23.81	22.22	13.14	20.0	25.53	20.94 ± 2.152
25	10.37	5.07	9.80	6.84	-	8.02 ± 1.251
0	4.51	4.85	3.7	7.07	-	5.033 ± 0.721

* After 24 hours

Appendix Table 9B. Mean length (μm) of germtubes after 24hrs in unsterilized/sterilized soil mixtures.

Percentage sterilized soil in the soil mixture		Replicates 1	2	3	4	5	Mean ± S.E.
100	Mean	33.27	66.29	43.25	70.91	84.79	59.702 ± 9.402
	S.E.	5.96	8.91	4.88	10.15	13.69	
75	Mean	13.82	10.71	14.05	13.76	12.27	12.922 ± 0.636
	S.E.	1.89	0.86	1.69	2.27	1.44	
50	Mean	12.27	10.59	7.43	8.18	13.19	10.332 ± 1.119
	S.E.	1.38	0.69	0.63	0.98	1.84	
25	Mean	8.81	10.66	6.91	10.54	-	9.23 ± 0.881
	S.E.	0.99	1.28	0.82	1.90		
0	Mean	14.26	9.12*	4.89*	5.76*	-	8.507 ± 2.123
	S.E.	3.87	2.17	1.73	2.09		

Values are means of measurements of 25 germtubes.

* Mean of ten measurements only.

APPENDIX TABLES 10A AND 10B

The effect of different heat treatments of soil on germination and germtube growth of *P. expansum*.

Appendix Table 10A. Percentage germination of P. expansum spores in soil which has been treated at various temperatures.

	EXPERIMENT No. 1				EXPERIMENT No. 2					
	Replicates				Replicates					
	1	2	3	Mean ± S.E.	1	2	3	4	5	Mean ± S.E.
Control	0*	0	0	0	0.19*	0.66	0	0	0	0.17±0.128
60°C	3.03	0	0	1.01±1.01	0.29	0.35	0.25	0.26	0	0.23±0.06
70°C	17.24	11.68	21.23	16.716±2.769	3.17	4.56	6.15	7.79	9.94	6.322±1.189
80°C	36.36	62.13	54.47	50.986±7.641	26.86	28.56	33.96	33.61	58.17	36.232±5.657
Autoclaved Soil	32.56	18.4	-	25.48±7.081	39.9	50.29	-	-	-	45.095±5.195

* After 24 hours

Appendix Table 10B. Mean length (μm) of germtubes of P. expansum produced after 24 hours in soil which has been treated at various temperatures.

		EXPERIMENT No. 1				EXPERIMENT No. 2					
		Replicates				Replicates					
		1	2	3	Mean ± S.E.	1	2	3	4	5	Mean ± S.E.
Control	Mean	0	0	0	0	0*	0*	0	0	0	0
	S.E.										
60°C	Mean	0*	0	0	-	0*	0*	0*	0*	0*	-
	S.E.										
70°C	Mean	20.7[a]	10.99[a]	24.27[a]	18.653±3.968	3.64[a]	6.22[a]	9.52[a]	5.29[a]	4.32[a]	5.798±1.028
	S.E.	2.31	1.59	2.64		0.36	0.99	1.81	0.62	1.09	
80°C	Mean	34.29[a]	33.6[a]	38.66[a]	35.517±1.584	17.93[a]	12.27[a]	25.73[a]	29.43[a]	17.25[a]	20.522±3.098
	S.E.	3.71	3.39	3.97		1.97	1.19	2.33	1.76	1.09	
Autoclaved Soil	Mean	46.44[a]	29.8[a]		38.12±8.321	30.89[a]	30.29[a]				30.59±0.300
	S.E.	5.11	4.04			2.29	2.81				

* Too few to measure

a Values are means of measurements on at least 40 germtubes

APPENDIX TABLES 11 to 13

Colonization of the rhizosphere and non-rhizosphere soils of maize, broad bean and tomato, grown in *P. expansum* infested sterilized soils.

Appendix Table 11A. *Penicillium expansum* in rhizosphere and non-rhizosphere of maize grown in *P. expansum* infested sterilized soil .

		Number of viable propagules $x10^5$ g^{-1} oven dry soil				
Soil	Replicates	30 Days	60 Days	90 Days	127 Days	145 Days
Rhizosphere	1	146.59	70.97	7.59	41.51	18.97
	2	231.63	76.04	6.42	22.84	67.84
	3	189.11	132.34	10.47	72.39	18.33
MEAN ± S.E.		189.11 ± 24.55	93.11 ± 19.66	8.16 ± 1.2	45.58 ± 14.45	35.04 ± 16.4
Non-rhizosphere	1	18.63	16.72	2.19	16.96	2.70
	2	8.97	6.02	1.72	17.16	3.86
	3	12.25	10.24	2.08	10.20	5.61
MEAN ± S.E.		13.28 ± 2.83	10.99 ± 3.11	1.99 ± 0.14	14.77 ± 2.28	4.06 ± 0.84

Initial number of *P. expansum* spores 500 g^{-1} soil

Appendix Table 11B. *Penicillium* spp other than *P. expansum* in rhizosphere and non-rhizosphere of maize grown in *P. expansum* infested sterilized soil.

Soil	Replicates	Number of viable propagules $x10^3$ g^{-1} oven dry soil				
		30 Days	60 Days	90 Days	127 Days	145 Days
Rhizosphere	1	39.8	41.03	43.0	15.09	18.28
	2	43.24	16.16	7.38	21.05	13.5
	3	36.36	17.39	6.63	0	0
Mean ± S.E.		39.8 ± 1.986	24.86 ± 8.093	19.003 ± 12.00	12.046 ± 6.264	10.593 ± 5.473
Non-rhizosphere	1	0	0	0.85	2.13	4.57
	2	0	0	0	11.59	6.76
	3	0	0	0.54	23.18	3.38
Mean ± S.E.		0	0	0.463 ± 0.248	12.3 ± 6.087	4.903 ± 0.989

Appendix Table 12A. *Penicillium expansum* in rhizosphere and non-rhizosphere of broad bean grown in *P. expansum* infested sterilized soil.

Number of viable propagules $x10^3$ g^{-1} oven dry soil				
Soil	Replicates	16 Days	37 Days	69 Days
Rhizosphere	1	204.0	100.34	80.0
	2	86.91	75.35	5.27
	3	5.85	62.86	40.0
Mean ± S.E.		98.92 ± 57.517	79.517 ± 11.019	41.756 ± 21.591
Non-rhizosphere	1	25.11	0.41	4.95
	2	1.01	8.92	2.53
	3	5.58	0.61	3.16
Mean ± S.E.		10.567 ± 7.391	3.313 ± 2.804	3.547 ± 0.725

Initial number of *P. expansum* spores 500 g^{-1} soil.

Appendix Table 12B. *Penicillium* spp other than *P. expansum* in rhizosphere and non-rhizosphere of broad bean grown in *P. expansum* infested sterilized soil.

		Number of viable propagules $x10^3$ g^{-1} oven dry soil		
Soil	Replicates	16 Days	37 Days	69 Days
Rhizosphere	1	26.0	272.54	3.2
	2	3.74	2.58	8.2
	3	296.00	7.62	433.33
Mean ± S.E.		108.58 ± 93.933	94.246 ± 89.161	148.243 ± 142.555
Non-rhizosphere	1	0.25	3.88	0
	2	0	3.08	1.01
	3	0.24	0.1	7.89
Mean ± S.E.		0.163 ± 0.082	2.353 ± 1.150	2.966 ± 2.479

Appendix Table 13A. Penicillium expansum in rhizosphere and non-rhizosphere of tomato grown in P. expansum infested sterilized soil.

		Number of viable propagules $x10^3$ g^{-1} oven dry soil				
Soil	Replicates	16 Days	35 Days	65 Days	105 Days	125 Days
	1	2626.66	436.36	24.78	75.50	28.00
Rhizosphere	2	8.66	29.45	52.92	153.60	16.80
	3	1.2	365.71	56.00	70.15	25.60
Mean ± S.E.		878.84 ± 873.938	277.173 ± 125.533	44.566 ± 9.933	99.75 ± 26.97	23.466 ± 3.404
	1	1357.89	100.26	20.69	47.33	5.59
Non-rhizosphere	2	28.11	3.48	3.84	19.59	4.00
	3	6.31	39.49	15.78	24.63	7.08
Mean ± S.E.		464.103 ± 446.951	47.743 ± 28.242	13.436 ± 5.003	30.516 ± 8.532	5.556 ± 0.889

Initial number of P. expansum spores 500 g^{-1} soil

Appendix Table 13B. *Penicillium* spp other than *P. expansum* in rhizosphere and non-rhizosphere of tomato grown in *P. expansum* infested sterilized soil.

Soil	Replicates	Number of viable propagules $x10^3$ g^{-1} oven dry soil				
		16 Days	35 Days	65 Days	105 Days	125 Days
Rhizosphere	1	82.666	6.909	4.304	2.000	4.571
	2	3.333	1.818	7.385	9.600	2.400
	3	12.000	18.857	6.634	6.769	12.800
Mean ± S.E.		32.666 ± 25.125	9.194 ± 5.050	6.107 ± 0.927	6.123 ± 2.217	6.590 ± 3.167
Non-rhizosphere	1	12.631	0.213	0.853	0.333	0.657
	2	0.216	0.103	0	0.820	1.600
	3	0.421	0.911	0.540	0.526	3.589
Mean ± S.E.		4.422 ± 4.104	0.409 ± 0.253	0.464 ± 0.249	0.559 ± 0.141	1.948 ± 0.864

APPENDIX TABLES 14-17

Colonization of the rhizosphere and non-rhizosphere soils of maize, broad bean, tomato and apple in *P. expansum* infested unsterilized soil.

Appendix Table 14A. Penicillium expansum in rhizosphere and non-rhizosphere of maize grown in P. expansum infested unsterilized soil.

Soil	Replicates	Number of viable propagules $x10^3$ g^{-1} oven dry soil			
		30 Days	60 Days	90 Days	127 Days
Rhizosphere	1	0	5.454	2.285	0.615
	2	1.306	1.469	0.888	0.716
	3	1.353	30.250	0.375	1.531
Mean ± S.E.		0.886 ± 0.443	12.391 ± 9.003	1.183 ± 0.571	0.954 ± 0.29
Non-rhizosphere	1	0.238	0.123	0.266	0.492
	2	0.238	0	0.129	0.250
	3	0.464	0.387	0.387	0.492
Mean ± S.E.		0.313 ± 0.075	0.170 ± 0.114	0.261 ± 0.074	0.411 ± 0.081

Initial number of P. expansum spores 500 g^{-1} soil.

Appendix Table 14B. *Penicillium* spp other than *P. expansum* in rhizosphere and non-rhizosphere of maize grown in *P. expansum* infested unsterilized soil.

Soil	Replicates	Number of viable propagules $x10^3$ g^{-1} oven dry soil			
		30 Days	60 Days	90 Days	127 Days
Rhizosphere	1	10.666	9.939	6.628	11.938
	2	12.898	20.897	3.288	10.626
	3	9.969	5.875	8.750	9.021
Mean ± S.E.		11.178 ± 0.883	12.237 ± 4.486	6.222 ± 1.589	10.528 ± 0.843
Non-rhizosphere	1	10.507	4.676	5.200	8.246
	2	7.880	8.676	5.935	9.875
	3	7.072	5.806	5.935	7.015
Mean ± S.E.		8.486 ± 1.037	6.386 ± 1.191	5.690 ± 0.245	8.379 ± 0.828

Appendix Table 15A. Penicillium expansum in rhizosphere and non-rhizosphere broad bean grown in P. expansum infested unsterilized soil.

Soil	Replicates	Number of viable propagules x10^3 g^{-1} oven dry soil 16 Days	37 Days	69 Days
	1	0.551	0	0.228
Rhizosphere	2	1.159	1.306	0.145
	3	0.423	1.353	0
Mean ± S.E.		0.711 ± 0.227	0.886 ± 0.443	0.124 ± 0.067
	1	0.117	0.238	0
Non-rhizosphere	2	0.231	0.238	0.105
	3	0.242	0.463	0.105
Mean ± S.E.		0.197 ± 0.04	0.313 ± 0.075	0.070 ± 0.035

Initial number of P. expansum spores 500 g^{-1} soil.

Appendix Table 15B. *Penicillium* spp other than *P. expansum* in rhizosphere and non-rhizosphere of broad bean grown in *P. expansum* infested unsterilized soil.

Soil	Replicates	Number of viable propagules $x10^3$ g^{-1} oven dry soil 16 Days	37 Days	69 Days
	1	15.448	15.578	14.628
Rhizosphere	2	16.231	17.548	8.436
	3	2.962	10.833	11.675
Mean		11.547	14.653	11.579
±		±	±	±
S.E.		4.298	1.993	1.788
	1	6.823	6.144	8.333
Non-rhizosphere	2	6.608	4.108	7.368
	3	8.848	5.297	4.105
Mean		7.426	5.183	6.602
±		±	±	±
S.E.		0.713	0.591	1.279

Appendix Table 16A. *Penicillium expansum* in rhozosphere and non-rhizosphere of tomato grown in *P. expansum* infested unsterilized soil.

Soil	Replicates	Number of viable propagules $x10^3$ g^{-1} oven dry soil				
		16 Days	35 Days	65 Days	105 Days	125 Days
Rhizosphere	1	0.342	0.470	0.571	0.444	0.266
	2	0.800	4.000	0.800	0.225	0.800
	3	0	1.333	0.177	0.114	0.400
Mean ± S.E.		0.381 ± 0.232	1.934 ± 1.062	0.516 ± 0.182	0.261 ± 0.097	0.489 ± 0.160
Non-rhizosphere	1	0.432	0.235	0	0.117	0.238
	2	0.103	0.347	0.369	0.123	0.438
	3	0.106	0.235	0.250	0.250	0.228
Mean ± S.E.		0.214 ± 0.109	0.272 ± 0.037	0.206 ± 0.109	0.163 ± 0.043	0.301 ± 0.068

Initial number of *P. expansum* spores 500 g^{-1} soil.

Appendix Table 16B. *Penicillium* spp other than *P. expansum* in rhizosphere and non-rhizosphere of tomato grown in *P. expansum* infested unsterilized soil.

		Number of viable propagules $x10^3$ g^{-1} oven dry soil				
Soil	Replicates	16 Days	35 Days	65 Days	105 Days	125 Days
	1	8.457	10.353	16.000	10.666	16.000
Rhizosphere	2	10.800	10.666	12.000	11.428	36.000
	3	7.800	11.333	11.200	8.000	5.333
Mean ± S.E.		9.019 ± 0.910	10.784 ± 0.289	13.067 ± 1.485	10.031 ± 1.039	19.111 ± 8.989
	1	10.810	7.764	9.159	6.618	7.403
Non-rhizosphere	2	6.545	10.319	7.261	5.661	12.274
	3	8.746	7.88	8.500	12.875	6.857
Mean ± S.E.		8.700 ± 1.231	8.654 ± 0.833	8.307 ± 0.556	8.385 ± 2.262	8.845 ± 1.722

Appendix Table 17A. Penicillium expansum in rhizosphere and non-rhizosphere of apple grown in P. expansum infested unsterilized soil.

		Number of viable propagules $x10^3$ g^{-1} oven dry soil			
Soil	Replicates	32 Days	67 Days	97 Days	139 Days
	1	11.275	14.451	0.680	13.104
Rhizosphere	2	24.918	2.039	0.387	17.872
	3	18.691	23.111	10.210	1.481
Mean ± S.E.		18.295 ± 3.943	13.2 ± 6.115	3.759 ± 3.226	10.819 ± 4.868
	1	6.400	8.597	0.500	6.470
Non-rhizosphere	2	6.880	0	0.250	8.00
	3	7.520	7.428	5.333	0.235
Mean ± S.E.		6.933 ± 0.324	5.342 ± 2.692	2.027 ± 1.654	4.901 ± 2.374

Initial number of P. expansum spores 10,000 g^{-1} soil.

Appendix Table 17B. *Penicillium* spp other than *P. expansum* in rhizosphere and non-rhizosphere of apple grown in *P. expansum* infested unsterilized soil.

		Number of viable propagules $x10^3$ g^{-1} oven dry soil			
Soil	Replicates	32 Days	67 Days	97 Days	139 Days
Rhizosphere	1	19.865	15.483	9.872	13.103
	2	36.721	8.627	5.935	11.063
	3	109.158	21.037	6.105	10.222
Mean ± S.E.		55.248 ± 27.391	15.049 ± 3.589	7.304 ± 1.285	11.463 ± 0.855
Non-rhizosphere	1	2.560	2.388	0.750	3.294
	2	1.440	1.575	0.375	4.387
	3	3.680	1.942	1.333	1.176
Mean ± S.E.		2.560 ± 0.647	1.968 ± 0.235	0.819 ± 0.279	2.952 ± 0.942

APPENDIX TABLES 18A to 18C

Colonization of the rhizosphere of tomato grown in *P. expansum* infested sterilized soil.

Appendix Table 18A. *Penicillium expansum* in rhizosphere of tomato grown in sterile soil.

		Number of viable propagules $x10^3$ g^{-1} oven dry soil						
	Replicates	0 Weeks	2 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks
Rhizosphere	1	0.123	10.59	13.09	31.82	1.85	12.72	10.69
	2	0.154	10.66	30.47	32.99	9.7	19.6	15.91
	3	0.156	16.00	32.0	36.25	15.79	30.2	16.36
	4	0.174	16.42	89.7	54.9	16.8	33.0	24.91
	5	0.182	54.3	97.82	77.42	39.88	69.7	31.58
	Mean ± S.E.	0.205 0.232	21.594 ± 8.272	52.616 ± 17.17	46.676 ± 8.745	16.804 ± 6.355	33.044 ± 9.863	19.89 ± 3.706
Control soil*	1		2.62	8.77	5.97	1.48	3.36	1.63
	2		3.05	10.15	10.92	4.73	4.8	3.35
	3		7.14	10.95	11.17	5.78	5.08	4.55
	4		12.28	22.03	12.44	6.03	6.98	8.62
	5		14.77	22.63	14.73	9.23	22.3	9.85
	Mean ± S.E.	0.175 ± 0.0135	7.972 ± 2.432	14.906 ± 3.052	11.046 ± 1.437	5.45 ± 1.235	8.504 ± 3.496	5.6 ± 1.567

* Pots of control soils were without plants.

Appendix Table 18B. *Penicillium* spp other than *P. expansum* in rhizosphere of tomato grown in *P. expansum* infested sterilized soil.

		Number of viable propagules $x10^3$ g^{-1} oven dry soil						
Soil	Replicates	0 Weeks	2 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks
Rhizosphere	1	0	35.24	33.94	1.03	14.81	321.21	181.81
	2	0.03	6.54	50.57	2.68	5.26	14.68	131.81
	3	0.02	44.92	7.27	1.52	10.19	235.80	155.26
	4	0.07	13.65	9.52	2.5	10.28	137.86	16.97
	5	0.02	60.0	5.33	1.10	10.13	129.49	24.48
Mean ± S.E.		0.03 ± 0.4	32.07 ± 9.86	21.32 ± 8.96	1.76 ± 0.35	10.13 ± 1.51	167.8 ± 51.94	102.06 ± 34.16
Control soil*	1		0.37	0.25	0.51	1.72	2.32	0.25
	2		0	0.12	0.38	0.49	7.49	0.37
	3		0.13	0.48	0.25	0.12	2.28	3.50
	4		0.49	0.26	12.22	0.37	0.26	55.38
	5		0.37	0.50	0.38	0.36	23.60	16.77
Mean ± S.E.		0.08 ± 0.05	0.27 ± 0.09	0.32 ± 0.07	2.75 ± 2.36	0.61 ± 0.28	7.19 ± 4.27	15.25 ± 10.48

* Pots of control soils were without plants.

Appendix Table 18C. Fungi other than *Penicillium* in rhizosphere of tomato grown in *P. expansum* infested sterilized soil.

		Number of viable propagules $x10^3$ g^{-1} oven dry soil						
Soil	Replicates	0 Weeks	2 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks
Rhizosphere	1	0.041	4.11	9.69	5.16	36.11	233.33	1078.78
	2	0.03	1.70	4.14	31.96	11.28	262.24	918.18
	3	0.01	1.23	218.18	213.64	21.25	108.67	1176.31
	4	0.084	1.28	22.86	23.75	16.57	151.46	344.24
	5	0.041	2.32	0	33.73	21.30	228.77	18.62
Mean ± S.E.		0.01 ± 0.34	2.13 ± 0.53	50.97 ± 41.98	61.65 ± 38.33	21.30 ± 4.14	196.89 ± 28.69	707.22 ± 224.56
Control soil*	1		1.12	0.12	0.25	0.25	4.00	8.61
	2		0.12	0	0.25	0.12	1.90	4.55
	3		0.37	0.47	0.38	0.12	2.79	1.63
	4		0	0.52	1.01	0.25	0.53	9.85
	5		0.62	0.87	0.38	0.36	44.59	3.35
Mean ± S.E.		0.08 ± 0.04	0.45 ± 0.2	0.39 ± 0.15	0.45 ± 0.14	0.22 ± 0.04	10.76 ± 8.48	5.60 ± 1.56

* Pots of control soils were without plants.

APPENDIX TABLE 19

Colonization of internal root tissues and/or the root surfaces of tomato grown in *P. expansum* infested sterilized soil.

Appendix Table 19. Colonization of tomato roots in P. expansum infested sterilized soil.

Weeks	P. expansum Replicates 1	2	3	Mean ± S.E.	Other Penicillium species Replicates 1	2	3	Mean ± S.E.	Other fungi Replicates 1	2	3	Mean ± S.E.
2	0*	0	5	1.7 ± 1.7	0	5	5	3.3 ± 1.7	0	2.5	0	0.8 ± 0.8
4	4	0	2	2 ± 0.6	4	0	0	1.3 ± 1.3	0	10	0	3.3 ± 3.3
8	0	4	-	2 ± 2	2	0	-	1.0 ± 1.0	8	10	-	9 ± 1.0
13	2	2	0	1.3 ± 0.66	4	6	0	3.3 ± 1.76	12	6	2	6.7 ± 2.9

* % number colonized of 50 2mm root segments plated onto the selective medium.

APPENDIX TABLES 20A to 20C

Colonization of the rhizosphere of tomato grown in *P. expansum* infested unsterilized soil.

Appendix Table 20A. *Penicillium expansum* in rhizospheres of tomato grown in unsterile soil.

Soil	Replicates	Number of viable propagules x10^4 g^{-1} oven dry soil						
		0 Weeks	2 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks
Rhizosphere	1	22.1	40.53	40.94	36.66	36.55	21.09	32.1
	2	21.16	37.14	38.56	36.32	23.91	20.46	22.09
	3	19.6	35.2	37.86	32.17	20.0	18.8	21.76
	4	19.0	35.11	34.0	30.0	19.44	12.8	20.62
	5	18.9	28.8	33.0	27.89	16.07	9.01	20.53
	Mean	16.7	35.356	36.872	32.608	23.194	16.432	23.42
	± S.E.	14.78	± 1.911	± 1.477	± 1.724	± 3.563	± 2.364	± 2.192
Control*	1		28.51	27.1	20.13	20.66	16.63	20.56
	2		21.12	24.0	19.09	18.15	14.84	18.65
	3		19.22	23.03	18.88	18.4	14.29	16.53
	4		18.77	21.5	16.98	16.5	13.58	14.35
	5		17.57	20.95	15.87	13.55	13.33	14.54
	Mean	18.891	21.038	23.314	18.19	17.452	14.534	16.926
	± S.E.	± 0.974	± 1.953	± 1.09	± 0.771	± 1.18	± 0.588	± 1.197

* Pots of control soils were without plants.

Appendix Table 20B. *Penicillium* spp other than *P. expansum* in rhizosphere of tomato grown in *P. expansum* infested unsterilized soil.

		Number of viable propagules $x10^3$ g^{-1} oven dry soil						
Soil	Replicates	0 Weeks	2 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks
Rhizosphere	1	64.0	177.78	154.67	84.21	125.0	131.34	253.13
	2	48.0	128.0	172.31	171.43	164.28	116.48	147.37
	3	56.0	165.33	140.00	142.86	172.41	186.04	117.65
	4	43.54	154.29	181.17	116.67	121.74	69.33	53.49
	5	52.66	117.33	148.33	156.52	111.11	122.73	122.67
Mean ± S.E.		36.46 56.0	148.55 ± 11.33	159.29 ± 7.62	134.34 ± 15.4	138.91 ± 12.3	125.18 ± 18.63	138.86 ± 32.5
Control*	1		81.29	141.93	95.48	74.67	43.25	74.04
	2		83.87	58.41	49.84	66.03	58.91	60.25
	3		70.82	68.19	54.74	54.60	58.94	48.76
	4		60.94	83.25	45.90	53.33	56.38	69.82
	5		68.57	87.84	58.06	49.03	46.97	38.36
Mean ± S.E.		50.95 ± 3.44	73.1 ± 4.22	87.92 ± 14.49	60.80 ± 8.91	59.53 ± 4.71	52.89 ± 3.26	58.25 ± 6.61

* Pots of control soils were without plants.

Appendix Table 20C. Fungi other than Penicillium in rhizosphere of tomato grown in P. expansum infested unsterilized soil.

Soil	Replicates	Number of viable propagules x10^3 g^{-1} oven dry soil						
		0 Weeks	2 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks
Rhizosphere	1	10.0	13.33	40.0	31.58	200.0	77.61	68.75
	2	4.0	20.0	10.26	100.0	157.14	164.84	136.84
	3	7.0	32.0	12.0	85.71	137.93	83.72	68.53
	4	11.14	17.14	28.24	75.0	82.61	154.67	100.0
	5	5.06	18.67	16.67	52.17	116.67	59.09	56.0
Mean ± S.E.		4.05 ± 5.0	20.23 ± 3.15	21.43 ± 5.6	68.89 ± 12.17	138.87 ± 19.66	107.99 ± 21.58	86.02 ± 14.64
Control*	1		7.74	12.9	10.32	10.67	7.63	7.93
	2		11.6	3.81	5.25	13.97	6.55	10.25
	3		1.31	6.56	12.63	7.62	5.12	6.59
	4		8.75	5.20	7.87	4.0	6.56	7.76
	5		10.16	9.41	9.03	1.29	10.16	6.16
Mean ± S.E.		6.61 ± 1.09	7.91 ± 1.77	7.58 ± 1.62	9.02 ± 1.23	7.51 ± 2.27	7.2 ± 0.84	7.83 ± 0.67

* Pots of control soils were without plants.

APPENDIX TABLE 21

Colonization of the internal root tissues and/or the root surfaces of tomato grown in *P. expansum* infested unsterilized soil.

Appendix Table 21. Colonization of tomato roots in P. expansum infested unsterilized soil.

	P. expansum				Other Penicillium spp.				Other fungi			
	Replicates				Replicates				Replicates			
Weeks	1	2	3	Mean ± S.E.	1	2	3	Mean ± S.E.	1	2	3	Mean ± S.E.
2	12.0*	16.0	40.0	22.66±8.74	6.0	4.0	18.0	9.33±4.37	8.0	0	8.0	5.3±2.66
4	24.0	18.0	20.66	20.66±1.76	20.0	6.0	2.5	9.5±5.35	9.0	0	0	3.0±3.0
7	8.0	4.0	4.0	5.33±1.33	2.0	8.0	4.0	4.66±1.76	2.0	2.0	2.0	2.0±0
11	16.0	15.0	16.0	15.66±0.33	6.0	2.0	2.0	3.33±1.33	4.0	8.0	6.0	6.0±1.15
15	54.0	48.0	24.0	42.0±9.16	26.0	36.0	14.0	25.33±6.36	4.0	10.0	6.0	6.66±1.76
19	36.0	24.0	32.0	30.67±3.53	28.0	32.0	40.0	33.33±3.53	16.0	18.0	8.0	14.0±3.05

* % number colonized of 50 2mm root segments plated onto the selective medium.

APPENDIX TABLES 22A to 22F

Data on growth of tomato plants after transplanting in *P. expansum* infested and uninfested sterilized soils.

Appendix Table 22A. 1st Harvest (2 weeks)

Soil	Replicates	Stem Height (cm)	Dry weight (g)			Total Leaf area (dm^2)	No.of greem leaves	No. of senescent leaves
			Stem	Root	Leaves			
Infested	1	11.34	0.0408	0.0188	0.2517	0.7908	7.7	0
	2	10.81	0.0356	0.0148	0.1293	0.7362	7.6	0
	3	10.7	0.0312	0.0143	0.1088	0.5388	7.1	0
	4	11.07	0.0405	0.0181	0.1488	0.7916	6.6	0
	Mean ± S.E.	10.98 ± 0.143	0.0370 ± 0.0023	0.0165 ± 0.0011	0.1597 ± 0.0318	0.7143 ± 0.0599	7.25 ± 0.253	0
Control Uninfested	1	10.41	0.0397	0.0134	0.2019	0.7914	7.2	0
	2	11.89	0.0488	0.0192	0.15722	0.9226	7.6	0
	3	11.87	0.0490	0.0146	0.1688	1.0238	7.8	0
	Mean ± S.E.	11.39 ± 0.490	0.0458 ± 0.0031	0.0157 ± 0.0018	0.1759 ± 0.0134	0.9126 ± 0.0673	7.53 ± 0.176	0

All figures are means of 9 plants.

Appendix Table 22B. 2nd Harvest (4 weeks)

Soil	Replicates	Stem Height (cm)	Dry weight (g)			Total Leaf area (dm^2)	No. of green leaves	No. of senescent leaves
			Stem	Root	Leaves			
Infested	1	23.65	0.4853	0.2605	1.0	3.2898	10.5	0
	2	26.93	0.5645	0.245	0.5415	3.4345	9.3	0
	3	25.0	0.5813	0.2513	1.3695	3.3453	9.7	0
	4	20.2	0.4143	0.2503	1.2580	3.1473	9.5	0
	Mean ± S.E.	23.945 ± 1.418	0.5114 ± 0.0385	0.2518 ± 0.0032	1.0629 ± 0.1836	3.3042 ± 0.0602	9.75 ± 0.263	0
Control Uninfested	1	22.08	0.3638	0.1843	1.2198	3.5553	9.8	0
	2	25.38	0.4945	0.187	1.3828	4.0330	9.3	0
	3	23.23	0.4410	0.1886	1.5167	3.7537	9.0	0
	Mean ± S.E.	23.563 ± 0.967	0.4331 ± 0.0379	0.1866 ± 0.0013	1.3731 ± 0.0858	3.7807 ± 0.1386	9.37 ± 0.233	0

All figures are means of 4 plants.

Appendix Table 22C. 3rd Harvest (8 weeks)

Soil	Replicates	Stem Height (cm)	Dry weight (g)			Total leaf area (dm^2)	No. of green leaves	No. of senescent leaves	No. of flower buds	No. of flowers	Total No. of fruits	Fruit fresh weight (g)
			Stem	Root	Leaf							
Infested	1	93	9.85	1.62	12.782	24.991	19	2	16	9	3*	0.47
	2	82	9.81	2.91	13.693	24.395	19	2	16	11	4	8.54
	3	91.1	10.748	2.02	14.94	27.165	17	0	24	8	0	0
	4	90	9.935	1.84	13.326	23.363	19	2	10	9	3	0.30
	5	88	10.937	2.12	15.239	27.036	18	0	17	11	2	0.37
	Mean ± S.E.	88.82 ± 1.887	10.256 ± 0.242	2.102 ± 0.219	13.996 ± 0.471	25.39 ± 0.746	18.4 ± 0.4	1.2 ± 0.49	16.6 ± 2.227	9.6 ± 0.60	2.4 ± 0.678	1.936 ± 1.653
Control Uninfested	1	96.5	10.028	1.52	12.637	25.748	19	0	19	8	3	0.27
	2	92.0	7.8	1.2	12.139	27.909	17	1	26	10	0	0
	3	91.5	9.87	1.71	14.028	28.02	16	1	18	11	3	0.42
	Mean ± S.E.	93.33 ± 1.59	9.232 ± 0.718	1.476 ± 0.149	12.935 ± 0.565	27.226 ± 0.739	17.333 ± 0.882	0.666 ± 0.333	21 ± 2.516	9.667 ± 0.882	2 ± 1.0	0.23 ± 0.122

* Fruits are not differentiated into unexpanding and expanding fruits.

Appendix Table 22D. 4th Harvest (12 weeks)

Soil	Replicates	Stem Height (cm)	Dry Weight (g)			Total leaf area (dm^2)	No. of green leaves	No. of senescent leaves	No. of flower buds	No. of flowers	Total No. of fruits	Fruit fresh weight (g)
			Stem	Root	Leaf							
Infested	1	137.5	20.9	3.295	19.948	21.199	20	6	0	21	12*	151.12
	2	154	17.348	1.946	16.52	19.456	18	4	0	23	13	193.1
	3	157	13.276	1.904	15.121	14.775	17	7	4	20	9	162.7
	4	127	18.414	3.004	17.018	-	-	-	-	-	-	-
	5	148	20.176	1.99	18.636	-	-	-	-	-	-	-
	Mean ± S.E.	144.7 ± 5.540	18.023 ± 1.342	2.428 ± 0.298	17.449 ± 0.840	18.477 ± 1.918	18.333 ± 0.882	5.667 ± 0.882	1.33 ± 1.33	21.333 ± 0.882	11.333 ± 1.202	168.973 ± 12.518
Control Uninfested	1	133	19.163	2.928	17.221	18.020	18	4	0	12	23	137.4
	2	154	21.717	2.214	19.763	30.511	20	6	0	14	18	87.1
	3	137	19.324	2.601	18.725	22.41	16	5	0	20	13	183.2
	Mean ± S.E.	141.33 ± 6.438	20.068 ± 0.826	2.581 ± 0.206	18.569 ± 0.738	23.647 ± 3.658	18.0 ± 1.155	5 ± 0.577	0	15.333 ± 2.404	18 ± 2.887	135.9 ± 27.753

* Fruits are not differentiated in unexpanding and expanding fruits.

Appendix Table 22E. 5th Harvest (16 weeks)

Soil	Replicates	Stem Height (cm)	Dry Weight (g)			Total leaf area (dm^2)	No. of green leaves	No. of senescent leaves	No. of flower buds	No. of flowers	Total No. of fruits	No. of expanding fruits	Fruit fresh weight (g)
			Stem	Root	Leaf								
Infested	1	168	22.728	2.55	17.037	21.455	21	8	2	2	55	33	639.6
	2	143.5	13.041	3.384	11.181	15.804	21	7	4	0	53	21	636.9
	3	167.5	14.6	3.063	10.964	15.602	22	7	3	1	28	20	570.9
	4	186.0	22.442	2.383	15.165	15.117	21	10	6	5	41	36	476.7
	5	165.5	19.829	2.888	12.366	14.785	22	8	3	3	32	21	634.6
	Mean ± S.E.	166.1 ± 6.755	18.528 ± 2.00	2.854 ± 0.1789	13.343 ± 1.189	16.553 ± 1.239	21.4 ± 0.245	8 ± 0.548	3.6 ± 0.678	2.2 ± 0.860	41.8 ± 5.417	26.2 ± 3.426	591.74 ± 31.493
Control Uninfested	1	170.5	23.99	2.242	14.047	21.712	22	7	5	2	39	32	624.2
	2	159.5	19.537	2.618	18.659	20.604	23	7	4	1	27	16	603.9
	3	178.0	17.643	1.838	15.493	30.152	21	7	5	1	29	22	743.5
	Mean ± S.E.	169.33 ± 5.372	20.39 ± 1.881	2.233 ± 0.225	16.066 ± 1.362	24.156 ± 3.015	22 ± 0.577	7 ± 0	4.667 ± 0.333	1.333 ± 0.333	31.667 ± 3.712	23.333 ± 4.667	657.2 ± 43.547

Appendix Table 22F. 6th Harvest (20 weeks)

Soil	Replicates	Stem Height (cm)	Dry Weight (g)			No. of green leaves	No. of senescent leaves	No. of flower buds	No. of flowers	Total No. of fruits	No. of expanding fruits	Fruit fresh weight (g)
			Stem	Root	Leaves							
Infested	1	197.5	26.31	2.871	15.147	28	10	0	4	29	19	459.4
	2	230.0	25.135	3.275	13.909	27	9	2	13	46	35	288.7
	3	217.0	33.218	2.668	19.889	23	10	0	6	31	24	529.2
	4	206.0	21.4	2.229	16.993	25	7	3	4	37	25	535.0
	5	186.0	23.764	2.759	17.304	27	6	0	8	26	18	446.1
Mean ± S.E.		207.3 ± 7.619	25.965 ± 1.988	2.760 ± 0.169	16.648 ± 1.02	26 ± 0.894	8.4 ± 0.812	1.0 ± 0.63	7.0 ± 1.673	33.8 ± 3.541	24.2 ± 3.023	451.68 ± 44.501
Control Uninfested	1	227.0	27.713	2.79	12.6	26	10	0	7	52	38	331.7
	2	231.5	25.135	2.339	15.276	24	7	2	10	36	25	384.8
	3	243.5	37.39	2.568	17.726	26	7	4	8	67	57	610.3
Mean ± S.E.		234.0 ± 4.924	30.079 ± 3.73	2.566 ± 0.130	15.2006 ± 1.483	25.333 ± 0.667	8 ± 1.00	2.0 ± 1.155	8.333 ± 0.882	51.667 ± 8.951	40.0 ± 9.292	442.267 ± 85.406

APPENDIX TABLES 23A to 23F

Data on growth of tomato plants after transplanting in *P. expansum* infested and uninfested unsterilized soils.

Appendix Table 23A. 1st Harvest (2 weeks)

Soil	Replicates	Stem Height (cm)	Dry weight (g)			Total Leaf area (dm^2)	No. of green leaves	No. of senescent leaves
			Stem	Root	Leaves			
Infested	1	16.16	0.0722	0.0254	0.2078	1.2276	7.6	0
	2	18.16	0.09	0.0308	0.208	1.1394	7.6	0
	3	18.82	0.0894	0.0280	0.1998	1.1506	7.6	0
	4	18.30	0.0914	0.0280	0.21	1.0724	8.2	0
	5	18.18	0.0986	0.0362	0.2396	1.1460	8.2	0
	Mean ± S.E.	17.924 ± 0.457	0.0893 ± 0.0034	0.0297 ± 0.0018	0.2130 ± 0.0069	1.1472 ± 0.0246	7.84 ± 0.147	0
Control Uninfested	1	15.30	0.0758	0.0244	0.2036	1.0902	7.6	0
	2	18.72	0.1032	0.0312	0.232	1.3570	8.2	0
	3	16.46	0.0782	0.0184	0.184	1.0526	7.4	0
	Mean ± S.E.	16.827 ± 1.004	0.0857 ± 0.0088	0.0247 ± 0.0037	0.2065 ± 0.0139	1.1666 ± 0.0958	7.733 ± 0.240	0

All figures are means of 4 plants.

Appendix Table 23B. 2nd Harvest (4 weeks)

Soil	Replicates	Stem Height (cm)	Dry weight (g)			Total leaf area (dm^2)	No. of green leaves	No. of senescent leaves	No. of flower buds
			Stem	Root	Leaves				
Infested	1	45.35	1.081	0.2245	1.5878	5.4373	12.25	0	3.25
	2	53.75	1.1865	0.1965	1.4238	4.7328	11.75	0	3.75
	3	55.95	1.0825	0.1773	1.2403	4.5805	12.25	0	3.25
	4	54.25	1.1103	0.17	1.2688	4.5465	13.25	0	2.75
	5	50.35	1.1788	0.166	1.5768	5.5585	12.5	0	4.25
	Mean ± S.E.	51.93 ± 1.88	1.1278 ± 0.023	0.1869 ± 0.0108	1.4195 ± 0.0734	4.9711 ± 0.2182	12.4 ± 0.245	0	3.45 ± 0.255
Control Uninfested	1	54.85	1.3038	0.2588	1.3363	5.4105	12.75	0	2.0
	2	66.63	1.4228	0.191	1.3573	5.8313	12.0	0	3.75
	3	60.30	1.2855	0.2183	1.3713	6.7668	11.75	0	4.75
	Mean ± S.E.	60.593 ± 3.404	1.3373 ± 0.043	0.2227 ± 0.0197	1.3549 ± 0.0101	6.0029 ± 0.4008	12.1667 ± 0.3005	0	3.5 ± 0.804

All figures are means of 4 plants.

Appendix Table 23C. 3rd Harvest (8 weeks)

Soil	Replicates	Stem Height (cm)	Dry weight (g)			Total leaf area (dm^2)	No. of green leaves	No. of senescent leaves	No. of flower buds	No. of flowers	Total No. of fruits	No. of expanding fruits	Fruits fresh weight (g)
			Stem	Root	Leaves								
Infested	1	113.0	11.546	1.698	11.036	28.472	19	1	14	12	7	3	13.49
	2	119.5	13.194	2.09	14.094	34.99	20	3	14	11	7	4	7.11
	3	103.0	14.502	2.506	15.467	30.31	20	1	8	15	14	8	52.19
	4	110.5	14.242	2.207	15.572	31.881	20	2	20	20	20	11	43.27
	5	111.5	14.158	1.826	14.739	28.903	21	1	13	9	10	6	16.18
	Mean ± S.E.	111.5 ± 2.641	13.528 ± 0.543	2.065 ± 0.143	14.182 ± 0.831	30.911 ± 1.182	20 ± 0.316	1.6 ± 0.4	13.8 ± 1.908	13.4 ± 1.913	11.6 ± 2.462	6.4 ± 1.435	26.448 ± 8.925
Control Uninfested	1	108.5	13.528	2.249	15.795	30.637	20	1	19	13	12	4	15.1
	2	122.0	14.214	1.716	15.536	35.622	22	0	13	9	10	5	29.8
	3	130.0	14.817	2.289	15.205	37.671	20	2	15	9	11	6	19.15
	Mean ± S.E.	120.167 ± 6.274	14.186 ± 0.372	2.085 ± 0.185	15.512 ± 0.171	34.643 ± 2.088	20.667 ± 0.667	1 ± 0.577	15.667 ± 1.764	10.333 ± 1.333	11.0 ± 0.577	5 ± 0.577	21.35 ± 4.384

Appendix Table 23D. 4th Harvest (12 weeks)

Soil	Replicates	Stem Height (cm)	Dry weight (g)			Total leaf area (dm^2)	No. of green leaves	No. of senescent leaves	No. of flower buds	No. of flowers	Total No. of fruits	No. of expanding fruits	Fruits fresh weight (g)
			Stem	Root	Leaves								
Infested	1	146.0	17.902	2.177	13.82	30.741	21	5	3	4	32	8	420.1
	2	152.5	17.918	1.784	13.658	27.535	18	5	3	2	38	11	355.9
	3	154.0	20.445	2.055	14.682	30.648	19	4	2	4	34	10	351.2
	4	170.5	23.704	2.483	16.599	29.691	20	5	3	6	49	7	381.0
	5	155.5	16.135	1.751	13.216	33.328	21	4	2	3	40	16	339.3
	Mean ± S.E.	155.7 ± 4.039	19.221 ± 1.315	2.05 ± 0.135	14.395 ± 0.600	30.389 ± 0.934	19.8 ± 0.583	4.6 ± 0.245	2.6 ± 0.245	3.8 ± 0.663	38.6 ± 2.96	10.4 ± 1.568	369.5 ± 14.361
Control Uninfested	1	164.0	15.931	2.427	12.913	34.729	20	4	3	6	33	12	295.1
	2	164.5	13.478	1.884	14.268	37.681	21	5	7	1	35	13	409.4
	3	162.0	17.461	2.786	13.386	34.509	20	6	3	3	35	9	420.1
	Mean ± S.E.	163,5 ± 0.764	15.623 ± 1.16	2.366 ± 0.262	13.522 ± 0.397	35.64 ± 1.023	20.333 ± 0.333	5.0 ± 0.577	4.333 ± 1.333	3.333 ± 1.453	34.333 ± 0.667	11.333 ± 1.202	374.867 ± 40.004

Appendix Table 23E. 5th Harvest (16 weeks)

Soil	Replicates	Stem Height (cm)	Dry weight (g)			Total leaf area (dm^2)	No. of green leaves	No. of senescent leaves	No. of flower buds	No. of flowers	Total No. of fruits	No. of expanding fruits	Fruit fresh weight (g)
			Stem	Root	Leaves								
Infested	1	168.0	17.955	2.346	16.969	34.928	24	6	5	0	38	22	702.7
	2	146.0	16.871	3.295	15.992	26.106	21	7	5	0	43	30	818.9
	3	178.5	16.225	2.547	14.918	28.785	22	7	8	0	43	32	725.3
	4	151.5	15.143	2.44	16.612	37.017	22	5	4	0	34	28	778.1
	5	179.0	14.304	1.617	15.549	26.259	23	7	10	0	36	24	614.6
	Mean ± S.E.	164.6 ± 6.818	16.1 ± 0.64	2.449 ± 0.267	16.008 ± 0.366	30.619 ± 2.261	22.4 ± 0.51	6.4 ± 0.4	6.4 ± 1.123	0	38.8 ± 1.828	27.2 ± 1.855	727.92 ± 34.843
Control Uninfested	1	160.0	12.579	1.937	12.828	33.851	23	6	7	0	33	20	565.3
	2	193.4	17.982	2.096	17.536	48.768	28	5	4	0	35	20	645.4
	3	178.5	12.554	2.154	15.280	32.856	23	7	10	4	36	18	676.9
	Mean ± S.E.	177.3 ± 9.66	14.372 ± 1.805	2.062 ± 0.065	15.215 ± 1.36	38.492 ± 5.146	24.667 ± 1.667	6.0 ± 0.578	7.0 ± 1.732	1.333 ± 1.333	34.667 ± 0.882	19.333 ± 0.667	629.2 ± 33.22

Appendix Table 23F. 6th Harvest (20 weeks)

Soil	Replicates	Stem Height (cm)	Dry weight (g)			Total leaf area (dm^2)	No. of green leaves	No. of senescent leaves	No. of flower buds	No. of flowers	Total No. of fruits	No. of expanding fruits	Fruit fresh weight (g)
			Stem	Root	Leaves								
Infested	1	145.8	12.921	2.033	9.173	12.395	16	10	2	0	33	23	737.7
	2	156.5	12.462	1.676	13.396	28.161	23	6	4	0	25	23	697.2
	3	158.5	12.702	1.352	11.183	27.309	21	7	0	0	36	25	745.7
	4	150.0	13.345	1.914	14.535	28.101	23	7	6	0	35	26	836.5
	5	162.2	14.814	2.602	13.825	28.472	24	6	4	0	32	25	876.1
	Mean ± S.E.	154.6 ± 2.959	13.249 ± 0.417	1.915 ± 0.207	12.422 ± 0.987	24.888 ± 3.129	21.4 ± 1.435	7.2 ± 0.735	3.2 ± 1.02	0	32.2 ± 1.934	24.4 ± 0.6	778.64 ± 33.348
Control Uninfested	1	208	14.838	1.334	14.656	42.778	25	5	6	0	31	24	746.0
	2	213.5	15.41	1.880	14.917	44.835	30	6	6	4	26	21	793.4
	3	184.0	11.594	2.184	12.485	31.561	25	8	3	0	41	23	824.7
	Mean ± S.E.	201.83 ± 9.057	13.947 ± 1.188	1.799 ± 0.248	14.019 ± 0.771	39.725 ± 4.125	26.667 ± 1.667	6.333 ± 0.882	5.0 ± 1.0	1.333 ± 1.333	32.667 ± 4.409	22.667 ± 0.882	788.033 ± 22.877

Appendix Table 24. Colonization of apple root litter in *P. expansum* infested soils.

Colonizer	Replicates	STERILISED SOIL						UNSTERILISED SOIL					
		5 days	7 days	10 days	15 days	32 days	42 days	5 days	7 days	10 days	15 days	32 days	42 days
P. expansum	1	50*	78	75	86	97	51.7	0	4	0	0	0	0
	2	30	76	68	64	98	42	0	2	0	4	6	2
	3	90	87.5	88	88	65	80	0	0	0	0	2	0
	Mean ± S.E.	56.67 ± 17.64	80.5 ± 3.54	77.0 ± 5.86	79.33 ± 7.69	86.67 ± 10.83	57.9 ± 11.4	0	2.0 ± 1.15	0	1.33 ± 1.33	2.66 ± 1.76	0.67 ± 0.67
Other *Penicillium* spp	1	2	0	4	2	0	2	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0
	Mean ± S.E.	0.67 ± 0.67	0	1.33 ± 1.33	0.67 ± 0.67	0	0.67 ± 0.67	0	0	0	0	0	0
Other fungal species	1	2	14	27	20	22.5	51.7	92	62	84	94	95	86
	2	2	8	14	18	20	66	68	48	76	96	92	72
	3	2	4	16	16	35	52	40	50	70	56	98	100
	Mean ± S.E.	2 ± 0	8.66 ± 2.91	19.0 ± 4.04	18.0 ± 1.15	25.83 ± 4.64	56.56 ± 4.72	66.66 ± 15.03	53.33 ± 4.37	76.67 ± 4.06	82.0 ± 13.01	94.33 ± 1.2	86.0 ± 8.08

* Number of root lengths expressed as a percentage of 50 (2mm)root lengths plated onto the selective medium.

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