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SPORE SURVIVAL, GERMINATION AND SPORELING GROWTH OF *PENICILLIUM EXPANSUM* IN SOIL*

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Abstract

Conidia of *Penicillium expansum* did not germinate in natural soil but remained viable (68%) for upto at least one year. In autoclaved soil, however, conidia germinated to develop and form conidia microcyclically.

Introduction

Soil invading fungi lack the ability to develop into an active form since they are unable to colonize dead organic substrates (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). Of the soilborne fungi *P. expansum* has been isolated from a wide range of soils throughout the world (Domasch *et al.*, 1980). However, isolation from soil does not demonstrate that it can grow and complete its life cycle in this environment. It is possible that isolation from soil arose from conidia which had developed on aerial plant parts and then arrived in the soil. *P. expansum* may thus be soil invader rather than a soil inhabitant.

The present work was carried out to determine whether conidia of a pathogenic isolate of *P. expansum*, isolated from apple fruit, could survive and germinate in natural soil, and to compare its behaviour with a parasitic and a saprophytic species of *Penicillium.*

Materials and Methods

The method of Old (1967) was followed for the survival and germination of *P. expansum* conidia in soil. Soil was collected from the Botany Experimental Garden, Glasgow, Scotland and dried at room temperature before sieving through 2mm mesh sieve. Half of the bulk was sterilized by autoclaving. Conidia of *P. expansum* (an isolate from apple and obtained from Commonwealth Mycological Institute, Kew, Surrey, England (CM1 39761) were mixed with autoclaved sterilized and unsterilized soils using a small drum mixer designed in our workshop and spray gun (Zardari, 1984) to give approximately 15 x 10^3 conidia g^{-1} oven dry soil. Approximately 60g of inoculated soil was

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Table 1. Behaviour of *Penicillium expansum*. *P. digitatum* and *P. brevicompactum* conidia in soils.

Culture	Soil	Time period				
		16 hr	45 hr	3 days	7 days	12 days
P. expansum	Sterilized	82.9*	94.9*	More conidia produced than after 45 h Germtubes and hyphae have started to lyse	Conidia present Greater lysis of germtubes and hyphae	Many young conidia present Hyphae completely disintegrated
		36-108**	547.0**			
	Unsterilized	0*	0*	0*	0*	0*
P. digitatum	Sterilized	68.9*	72.8*	More conidia produced than after 45 h Germtubes and hyphae have started to lyse	Conidia present Greater lysis of germtubes	Many young conidia present Germ tubes lysed but outline of their walls still noticeable
		9-54**	29-216**			
	Unsterilized	0*	0*	0*	0*	0*
P. brevicompactum	Sterilized	43.3*	64.6*	More conidia produced than after 45 h Few spores still ungerminated Germtubes and hyphae have started to lyse	Conidia present Greater lysis of germtubes and hyphae	Many young conidia present Hyphae completely lysed but outline of their walls still noticeable
		3.6-45**	18-234**			
	Unsterilized	0*	0*	0*	0*	0*

*percentage conidial germination, **Germtube length (μm)

significantly higher compared with unsterilized soil ($P < 0.001$). The highest number of colonies in sterilized soil was recorded about 8 weeks after inoculation which declined with time ($P < 0.01$). In contrast, there was no growth in the unsterilized soil where a number of viable propagules had declined. However, the number of viable propagules remained 68% of the inoculum level after 48 weeks. As *P. expansum* was never recovered from uninfested soils, all isolations from the infested soils must have originated from the inoculum of *P. expansum.*

The results of comparative study of different species of *Penicillium* (Table 1) show that only the sterilized soil supported the germination of all the species. Germination perplaced in 9cm plastic Petri dish 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. The plates were buried individually in 17.8cm plastic pots containing either sterilized or unsterilized garden soil. The pots were incubated in dark at 25°C and watered once a week. After various periods of incubation, 5 plates of each soil containing conidia and 3 plates of each soil without conidia were retrieved and a number of viable propagules present were determined by plating the samples onto the slightly modified malt extract medium (Zardari, 1984). The plates were incubated at 23°C for 7 to 12 days before colonies were scored.

Comparative study of the behaviour of *P. expansum, Penicillium digitatum* Sacc. (CMI 91956) and *Penicillium brevicompactum* Dierkx (CMI 1746) conidia in soil was carried out as per Matturi & Stenton (1964).

Results

Figure 1 presents the survival and germination of *P. expansum* conidia in soil. Analysis of variance shows that the number of colonies of *P. expansum* from sterilized soil was centage and germ tube length in sterilized soil increased continuously upto 45 h beyond which measurement was impossible. Soon after germination some germ tubes apparently stopped growing, producing at their apex 1 to 2 phialides in *P. expansum* and 1 to 5 phialides in *P. digitatum* followed by conidia formation (Fig. 2 A & D). Some germ tubes grew longer and developed one or two lateral conidiophores with phialides and conidia formation (Fig. 2 B & C). In *P. brevicompactum* phialides and conidia never developed at the tip of germ tubes, rather on lateral conidiophores. *P. brevicompactum* occasionally developed lateral conidiophores forming up to 3 metulae with several phialides. Autolysis of the protoplasm of germtubes and hyphae started at 3rd day incubation and continued till 12th day.

Discussion

Failure of *P. expansum* conidia to germinate in unsterilized soil may be attributed to the absence of exogenous nutrients as has been suggested by Cochrane (1960). Microbial

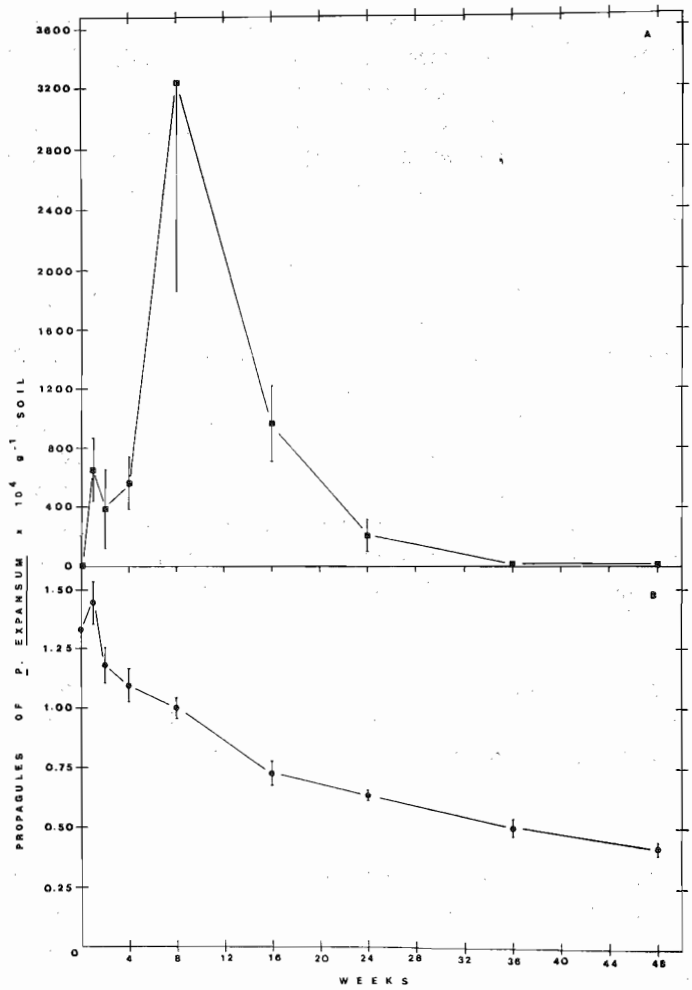

Fig. 1. Survival and growth of *Penicillium expansum* in sterilized (A) and unsterilized (B) soil.

activities may proceed in the presence of low levels of these nutrients since they compete with the spores for the limited nutrients (Ko & Lockwood, 1967). Lingappa & Lockwood (1964) found increased microbial activity when the spores of certain fungi were incorporated into soil. This was shown to be due to substances released from spores. Thus, the competition may be both for exogenous nutrtients and for nutrients that diffused out of the spores (Lockwood, 1977).

Physical and chemical sterilization of soil can remove general fungistasis. Ko & Lockwood (1967) observed that sterilizing natural soils by autoclaving increased the con-

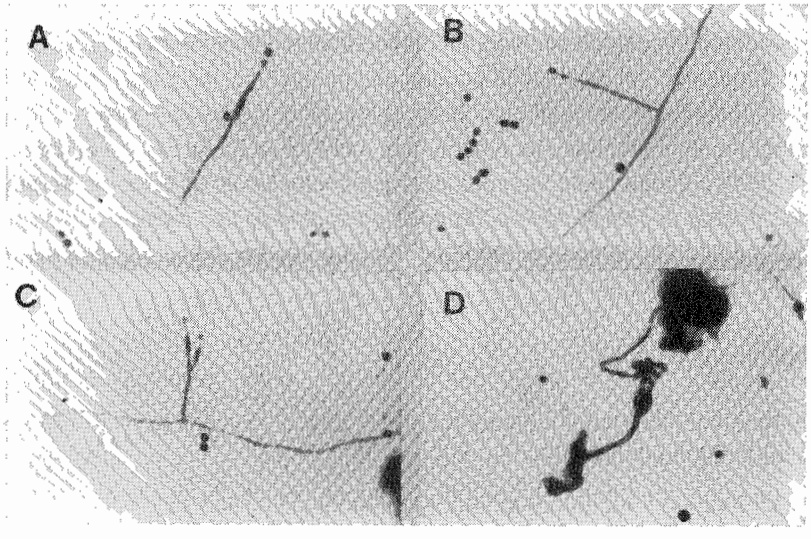

Fig. 2. Photomicrographs of germinated conidia producing microcyclic conidiation in sterilized soil. (x 80). A. Germinated conidium of *Penicillium expansum* with microcyclic conidiation at the tip of a germtube. B&C. Germinated conidia of *P. expansum* with microcyclic conidiation on lateral conidiophore. D. Germinated conidia of *P. digitatum* with microcyclic conidiation at the tip of germtube.

centrations of soluble carbohydrates and amino acids. If nutrients had been released in our experiments they would have been responsible for the growth of all the three investigated species of *Penicillium*.

An alternative explanation for the failure of conidial germination in soil calls for the release of diffusable microbial inhibitory substances (Lingappa & Lockwood, 1964). Soil emanation agar method has been used to differentiate between inhibitors diffused in solution and those capable of diffusing as volatile gases (Hora & Baker, 1970; Romine & Baker, 1972). Thus, evidence has been provided for the presence of volatile germination inhibitors in different types of soil. It seems that the failure of *P. expansum* conidia to germinate in natural soil may be due to the shortage of nutrients or due to the presence of inhibitors.

Sporeling growth of *Penicillium* species in sterilized soil after conidial germination has been observed. However, the amount of growth was very limited, leading to microcyclic conidiation only. Anderson & 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 & Bashi (1969) proposed that sporulation may be induced by factors which inhibit vegetative growth. In *Geotrichum candidum* induction of sporulation was due either to a diffusible fungal metabolite or to depletion of a factor

essential for the maintenance of vegetative growth (Park & Robinson, 1969). Morton (1961) examined the conditions required for conidiation by several *Penicillium* species in submerged cultures and concluded that the most suitable condition was the absence or exhaustion of available nitrogen even in the presence of assimilable carbon source. However, carbohydrate starvation can also induce conidiation in certain *Penicillium* species (Jicinska, 1968). Temperature variation may also be the cause of microcyclic conidiation in the soil (Cortat & Turian, 1974). The results reported in this paper would support that microcyclic conidia formation in sterilized soil may be due to any one or a combination of the factors discussed above.

Acknowledgement

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