BEHAVIOUR OF THE CHLAMYDOSPORES OF *FUSARIUM SOLANI* VAR. *COERULEUM* (SACC.) BOOTH IN SOIL

by

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ABSTRACT

The behaviour of chlamydospores of Fusarium solani var. coeruleum (Sacc.) Booth was examined in soil alone and in soil near potato roots. Some chlamydospores germinated in soil alone but progressively more germinated near potato roots. A similar stimulation of chlamydospore germination was observed near the roots of the non-hosts, barley and broad bean but measurements of the fungal population using a medium selective for F. solani var. coeruleum showed that while the numbers of propagules rose initially they soon declined to levels similar to that of soil alone. In contrast, the population of the fungus continued to increase in soil near young potato roots, stolons and new tubers. Generally, the populations were greatest near the developing tubers and they were higher near stolons than near roots. The results suggest that exudates from the underground parts of the potato specifically favour the growth of F. solani var. coeruleum and that this accounts for the relatively high levels of the fungus on tubers noted by other workers.

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INTRODUCTION

Dry rot of potatoes is caused principally by the fungus, Fusarium solani var. coeruleum (Sacc.) Booth, and is essentially a disease of stored tubers. Much is now known about this disease - the ways in which the fungus enters the tubers, the conditions which affect this process and the methods of control.

Experiments indicate that the fungus is often in soil on the potato tubers at lifting and it is generally believed that it is present as resting spores or chlamydospores. Very little is known, however, about the behaviour of this fungus in soil particularly during the growth of the potato or indeed, during the growth of other crops.

Work with other forms of F. solani suggests that host roots and those of non-hosts might well have substantial effects on the fungal population. The aim of this work was to examine this possibility in relation to the dry-rot pathogen, F. solani var. coeruleum.

REVIEW OF LITERATURE

Causes of dry rot

Various species of Fusarium cause dry rot of potatoes but the most common one is Fusarium solani var. coeruleum (Sacc.) Booth. However, F. avenaceum ((Corda)Fr) Sacc., F. arthrosporioides Sherb., F. tricinctum (Corda) Sacc., F. sporotrichioides Sherb. and F. oxysporum Schlect. are recorded in Europe.

In North America F. sulphureum Schlecht and F. solani var. coeruleum are the most common species but F. trichothecioides Wollenw., F. oxysporum and F. solani (Mart.) Sacc. are also recorded (Boyd, 1972).

In a survey carried out by McKee (1952) in Britain, the parasitic fusaria of potatoes consisted of 33% F. solani var. coeruleum, 6% F. avanaceum and less than 1% F. arthrosporioides and F. trincinctum.

The Fungus

F. solani var. coeruleum

The main causal fungus of dry rot, F. solani var. coeruleum was first described by Libert as Selenosporium coeruleum. It was then placed in the genus Fusarium as F. coeruleum by Saccardo in 1886 and has long been known under this name. In a recent revision Booth (1971) considers it to be a variety of Fusarium solani and this name is used throughout this thesis.

The mycelium of F. solani var. coeruleum is greyish white. On agar media a deep violet-blue pigment develops after a few days. Later a cream coloured mass of macroconidia

form from conidiaphores in the aerial mycelia. Macroconidia are hyaline, slightly fusoid with a rounded, somewhat beaked, apex and a marked apedicellate foot cell. They vary in size but are usually 4-5 celled. Conidial measurements given by Booth are:

4 septate 50-58 x 5 μ
5 septate 60-65 x 5-5.5 μ

Microconidia are formed sparsely in the young cultures. They are oval, 18-12 x 2-4 μ. Globose, thick-walled chlamydospores (8-10 μ diameter) are formed from macroconidia (Booth, 1971) or hyphae. They can be terminal or intercalary and are produced singly, in pairs or in chains. Hypomyces asclepiadis was described as the perfect stage of F. solani var. coeruleum by Zerova (1937) but it has not been confirmed (Booth, 1971).

The Disease

Infection of tubers by F. solani var. coeruleum, causes at first small brown patches to appear. These rapidly increase in size and become sunken. Affected parts of the skin shrink and concentric wrinkles develop around the origin of the infection. Eventually fungal pustules break out through the affected area (Western, 1971). In the later stages, tubers contain cavities with mycelium which often appears bluish-white. Tubers lose much water and eventually become mummified (McKay, 1955). If the tubers have been exposed to light, the pustules on the surface turn a pinkish colour.

Factors affecting the disease

Dry rot is a disease of stored potatoes. The fungus, F. solani var. coeruleum occurs in soil but it has never been observed to cause disease on tubers before lifting (McKay, 1955; Boyd, 1972). It is generally considered, however, that soil on the tuber surface is the main source of inoculum. The spores of the fungus can also be found

on sacks, boxes or even in the air of stores but these are probably relatively unimportant (Lansade, 1949; Pethybridge & Bowers, 1908; Small, 1945). The fungus is mainly a wound parasite (Western, 1971: Boyd, 1972) and infects after damage to the tuber skin during harvesting, grading, transporting or in cutting the tubers before planting. The riddling process (Foister et al, 1952) and grading (Western, 1971) are probably the most important in this respect.

The optimum temperature for infection and rotting was reported by Moore (1945) to be 15°c and the maximum 25°c but Booth (1971) states that the disease develops most rapidly under conditions of high humidity and at temperatures around 20°c. F. solani var. coeruleum can infect tubers even at a relative humidity as low as 50% (Moore, 1945). Low temperatures in potato clamps result in less dry rot than high storage temperatures, but an initial period of high temperature during curing heals the wounds and the chance of infection decreases.

The susceptibility of tubers to dry rot depends both on the variety and the length of the period in store. In Great Britain early varieties have been found, generally, more susceptible than late varieties. Among the varieties (more than forty) tested Arran Pilot and Doon Star were found to be very susceptible, King Edward and Majestic moderately susceptible and Arran Banner and Arran Victory were found to be very resistant.

Within a given variety, the tuber tissue tends to be most resistant to dry rot when the tubers are first lifted (Boyd, 1952b, 1967; Lansade, 1949; Moore, 1924; Pethybridge & Lafferty, 1917). Susceptibility then increases throughout the storage period, reaching a peak in spring (Boyd, 1952b; McKee, 1954; Western, 1971). Other factors which influence susceptibility to dry rot in the store are the weather during the growth of the potato in the field (Mooi, 1950), the length of the growing period

(Boyd, 1967) and the amount of nitrogen applied (Schippers, 1962: Boyd, 1967). There may even be differences in the susceptibility of different parts of the tuber. Thus Boyd (1952a) reported that large tubers were more susceptible than small ones, and the heel end was more susceptible than the rose end.

Control

Good control of the disease can be achieved by dipping tubers in organo-mercury compounds for a few minutes after harvest. Washing the tubers also removes much of the inoculum but may cause bacterial contamination. Dusting, after lifting and before storage with T.C.N.B. (Techazene), which is volatile, is effective for clamped and bulk stored potatoes (Western, 1971). Dithio-carbonates, (zinc + maneb) have also been shown to give effective control (Leach, 1971).

Planting disinfected seed is an important feature of control not only because it prevents contamination of soil but also because there is good evidence that the progeny of infected tubers themselves become contaminated with the fungus and the chances of infection of these tubers are therefore much increased. It is most probable that the disease perpetuates by planting diseased tubers.

Survival of the fungus in soil

The fungus remains viable in soil both in the field and on the tuber surface for a long time. Small (1944) reported that it survived for at least 9 years in soil and McKee & Boyd (1952) showed that the infectivity of field soils could be maintained in the laboratory for at least 5 years. In one instance, Boyd (1970) found the fungus was still viable in soil 10 years after potatoes had been grown and he reported that the level of infect-ivity of the soil hardly decreased after 8 years of storage. However, the period of viability in soil varied

with the conditions of moisture and temperature when stored in the laboratory (Boyd, 1970). Both Boyd (1952) and Schippers (1962) have demonstrated a relationship between the amount of F. solani var. coeruleum in soil and dry rot infection of the stored tubers. However, generally, soil collected from the potatoe riddles showed a higher degree of infestation than the soil in which the potatoes had been grown (Schippers, 1962).

Behaviour of other fusaria in soil

It is generally assumed that F. solani var. coeruleum exists in soil as chlamydospores but little work has been done on its behaviour in soil. However, there have been many investigations with other fusaria of this type in soil, notably F. solani f.sp. phaseoli, and other form species of F. solani. These generally survive as chlamydospores in soil. For example, the existence of F. solani f. phaseoli in soil as chlamydospores was shown by direct observation and also by culture methods (Papavizas, 1967; Cook & Snyder, 1962). Smears of untreated soils on slides readily showed Fusarium chlamydospores and their ability to germinate was established (Nash, Christou & Snyder, 1961).

Most experiments have been concerned with the longevity of chlamydospores and with the factors that influence their subsequent germination. Duration of survival depends on the form of the pathogen concerned, soil type, local environment, cropping history of the site and the cultivation treatments given (Garrett, 1970) The nature of the chlamydospore well may determine the differences between populations of different species and formae speciales with regard to survival.

The factors which most influence germination are the presence of organic matter and exudates of host and non-host roots. It has been established, for example, that chlamydospores of Fusarium require an external source

of carbon and nitrogen for germination and infection (Cook & Schroth, 1965; Schroth, Tousson & Snyder, 1963; Tousson, Nash & Snyder, 1960). These are required probably to overcome the natural fungistasis and there have been many experiments aimed at finding out which C and N compounds are preferred (Cook & Flentje, 1967; Cook & Schroth, 1965; Cook & Snyder, 1965; Schroth & Snyder, 1961; Schroth & Toussoun 7 Snyder, 1963; Smith & Snyder, 1972; Toussoun, Nash & Snyder, 1961). In most instances, the necessary materials come from exudates from plant roots. Generally the response to these exudates is non-specific. The chlamydospores of F. solani f. phaseoli, for example, germinate in response to root exudates of tomato, lettuce and corn (Schroth & Hendrix, 1962) and also near pea and wheat roots (Jackson, 1957). In many of these instances some limited mycelial growth takes place and then further chlamydospores are formed from this mycelium. The result is that the population of the pathogen is maintained and may even be increased. Planting of non-hosts does therefore not always ensure a decrease in the population of the pathogen.

Not all the root system of the host (or non-host) is equally effective. Chlamydospores of F. solani f. phaseoli germinated most consistently when in close proximity to germinating bean seeds and to root tips of primary, lateral and adventitious bean roots. Mature roots had little effect on chlamydospore germination and growth of the fungus in soil (Schroth & Snyder, 1961). This may be related to both the nature and amounts of exudates. Both Pearson and Parkinson (1961) and Schroth & Snyder (1961) showed that the root tips were the most important zone of exudation. Equally, the rubbing-off of moribund cells from the surface of more mature roots might encourage the build-up of micro-organisms which inhibit chlamydospore formation. Indeed, the germination of chlamydospores and

growth of these fungi may well depend upon the balance between certain fungistatic factors and the supply of organic mutrients provided by plant exudates (Schroth, Tousson & Snyder, 1963).

A. PRODUCTION OF CHLAMYDOSPORES

MATERIALS AND GENERAL METHODS

Culture of Fusarium solani var. coeruleum

A freeze-dried culture of F. solani var. coeruleum (CMI 163 397) was obtained from the Commonwealth Mycological Institute.

The freeze-dried spores were suspended in 1 ml sterile distilled water, streaked out on plates of potato-dextrose agar (Oxoid Ltd.), and incubated at 20°c. After colonies of the fungus had formed, these were subcultured to fresh plates and tubes of PDA.

The pathogenicity of the fungus was tested. Several tubers of the variety "Majestic" were thoroughly washed and left to dry on absorbent paper. Then they were immersed in 10% Chloros (1% available Chlorine) for 1 hour, rinsed in a bucket of water, and allowed to drain again. A small plug of tissue was taken out of each tuber with a sterile cork-borer (No.4), a piece of culture of the fungus was inserted and the plug was replaced. The wound was sealed with a molten mixture of 1:1 paraffin wax and Vasaline. Then the inoculated tubers were placed in plastic bins and incubated in a constant temperature room at 25°c.

Approximately 25 days after inoculation, a rot had formed in the inoculated tubers. The fungus was reisolated, and compared under the microscope with the original isolate. It was confirmed that the new isolate was identical to the original one.

Storage and maintenance of cultures

Stock cultures were maintained on slopes of potato sucrose agar (see P.3) in McCartney bottles under sterile liquid paraffin oil. The fungus was allowed to grow at 20°c until colonies were well formed, then these were

covered with sterile liquid paraffin to a depth of 1cm. above the top of the slopes. Stock cultures were kept at 15°c.

The fungus was also maintained in sterile dry soil. Sieved field soil was placed in McCartney bottles so that these were one third full. These were autoclaved for 30 minutes twice, with a 3-day interval between each treatment. Then a 2 ml. spore suspension in sterile distilled water with the concentration of $1x10^6$ per ml. was poured over the sterile soil in each bottle, and these were incubated at 20°c. for about 10 days. Then they were kept at 15°c.

About every 3-4 months, the cultures used in the experiments were renewed from the original cultures which had been kept in sterile soil.

Culture media

Several culture media were tested for growing F. solani var. coeruleum including potato dextrose agar (PDA), potato sucrose agar (PSA), V-8 juice agar, Nash and Snyder's Peptone - PCNB medium (Nash & Snyder 1962) and Papaviza's modified Peptone - PCNB agar (Papavizas, 1967). The composition of these media is given in Table 1. After inoculation, cultures were incubated at 20°c. On the fourth day and then at 3-day intervals up to day 13, the diameter of the colonies were measured. For production of conidia, potato sucrose agar adjusted to Ph 6.5 was used.

Table 1: Composition of media used to grow F. solani var. coeruleum

	Medium	Composition
1)	Potato dextrose agar	39g. Oxoid PDA / 1000 ml. distilled water.
2)	Potato - sucrose agar	500 ml. potato extract, 20g. sucrose, 20g. agar, 500 ml. distilled water (adjusted to Ph 6.5). Method of preparation as described by Booth (1971).
3)	Potato sucrose liquid	Same as PSA but with the agar omitted.
4)	V-8 Juice agar	100 ml. V-8 Juice (Campbells Soups Ltd.), 15g. agar, 900 ml. distilled water.
5)	Peptone -PCNB agar (Nash & Snyder 1962)	15g. Difco peptone, 20g. agar 1g. potassium dihydrogen phosphate (KH_2PO_4), 0.5g. magnesium sulphate ($MgSO_4.7H_2O$) 1g. pentachloronitrobenzene (PCNB 75% wettable powder), 300 ppm streptomycin (when cooled).
6)	Modified Peptone-PCNB agar (Papavizas, 1967)	15g. Difco peptone, 20g. agar, 1g. potassium dihydrogen phosphate (KH_2PO_4), 0.5g. magnesium sulphate $MgSO_4.7H_2O$), 0.5g. PCNB (Terraclor, a commercial product, is 75% active), 0.5 g. oxgall, 100mg. streptomycine sulphate, 50mg. chlortetracycline Hcl. (The last two components should be added to the cooled sugar.)

Testing of optimum temperature

Plates of PSA were inoculated with F. solani var. coeruleum. Five cultures were incubated at each of the following temperatures: 15°, 20° and 25°c. Four days after inoculation and then at 3-day intervals up to day 13 the diameter of the colonies were measured.

Techniques for Producing Chlamydospores

1 - Preliminary experiments

In distilled water

Ten disks cut from the edge of an 18-day old colony on PSA with a sterile cork-borer (No.6) were placed either in a petri dish or in a medical flat containing 25 ml. sterile distilled water. Ten such petri dishes and twenty-eight medical flats were prepared. Chlamydospore formation was examined at intervals.

Portions of mycelia growing from the disks were removed aseptically and examined under the microscope. At later stages in the incubation, the contents of one container were homogenized in a Sorvall Omnimixer and portions of this homogenate were examined under the microscope.

In salt solutions

The fungus was grown for 18 days on PSA in petri dishes. Conidia were washed from the cultures with sterile distilled water. The resulting suspension was passed through five layers of sterile muslin to remove mycelial fragments and collected in a sterile flask. Portions of the conidial suspension were then added to flasks containing a liquid medium consisting of 1g. Monobasic potassium phosphate (KH_2PO_4) and 0.5 g. Magnesium sulphate ($MgSO_4.7H_2O$) in 1 litre of demineralized sterile water as described by Qureshi and Page (1970). In ten flasks, the two salt solution was amended with glucose at two rates, either 0.125mg. or 2mg. per litre. In another ten flasks magnesium carbonate was added, again at the

two rates used for glucose.

Portions of the conidial suspension were also added to flasks containing 0.3 M Na_2SO_4 (Hsu & Lockwood, 1973). All flasks were incubated in darkness at 23°c. Chlamydospore formation was checked at intervals as described above.

In liquid Potato sucrose medium

F. solani var. coeruleum was grown on liquid Potato sucrose in medical flats at 25°c, until mycelial mats were well formed. Then the liquid was drained off. The mycelial mats were washed twice with sterile distilled water and then were refloated on sterile distilled water. The medical flats were again incubated at 25°c. The formation of chlamydospores was examined at intervals, as above.

In acid-washed sand

Sieved, fine sand (0.5kg.) was left in 3% Hcl. for one week. It was then washed fifteen times with distilled water and sterilized in an oven at 200°c for 3 hours. One hundred gram portions of this sterile sand were placed in 150 ml. Erlenmeyer flasks and 5 ml. of liquid potato sucrose medium was poured into each. After mixing the sand and liquid medium thoroughly, each flask was inoculated with one disk taken from an agar culture of the fungus. The flasks were incubated at 20°c for 10 days. The formation of chlamydospores was examined at intervals.

In Soil

Soil, collected from Silwood Park, was air-dried and passed through a No. 10 mesh sieve (1680 µ.). Conidia were washed from 18-day old cultures on PSA and a suspension prepared as described above. Portions of the air-dried soil were then infested with F. solani var.

coeruleum by spraying them with the conidial suspension. Twelve millilitres of suspension were used for each 88g. soil and the concentration of conidia in the suspension was adjusted so that, with thorough mixing, each gram of soil would contain 1 x 10^6 conidia. The infested soil was then sieved three times through a No. 6 mesh (2800 μ). Twenty grams of infested soil were placed in each of ten 100ml. flasks. Five of these were incubated at 25°c and five at 10°c.

A similar number of flasks were set up using sterile soil which had been treated with a conidia suspension as described. The soil was sterilized by autoclaving for 30 minutes at 125°c.

Chlamydospore formation was examined in both types of soil in two different ways:

a. Direct microscopy of soil with lacto-fuchsin

A small amount of soil was mixed with four or five drops of lacto-fuchsin in a staining block. The big particles were allowed to settle down from the suspension for 1-2 minutes. A loopfull of suspension in lacto-fuchsin was examined under a coverslip on a slide, under the low power of the microscope.

b. Direct microscopy of smears of soil suspensions

A small amount of soil was mixed with two to three drops of distilled water and the mixture was spread over the surfaces of the slides. The slides were then dried, the big particles were removed with a needle or forceps and the smear was stained with 0.1% acid-fuchsin in lactic acid. The preparation was examined under the microscope (Cook & Snyder, 1965).

2 - Producing chlamydospores in soil extract

Production of conidia

Medical flats of potato sucrose agar were used to produce conidia. The best results were obtained with PSA flats which were allowed to harden for four or five days in a dark and a cool place before inoculation. After inoculation with a conidial suspension, the flats were incubated initially at 20°c in the dark for 3-4 days. They were then placed in a constant temperature room at 25°c under a bank of two daylight fluorescent tubes (daylight 65/80 w) mounted 30 cm. above them to give a 12h. photoperiod.

Fairly good sporulation was obtained within 16 days under light.

Preparation of conidial suspension

Conidia were washed off with 50 ml. sterile distilled water for each medical flat by hand, shaking for 1-2 minutes. The suspension was filtered through five layers of sterile muslin.

The conidial suspension was centrifuged at 3000 r.p.m. for 3 minutes. The supernatant was decanted and after washing twice, the spores were resuspended in sterile distilled water. The concentration of conidia in the suspension was assessed from a haemocytometer count and the numbers were subsequently adjusted to 15×10^5 conidia/ml.

Preparation of soil extract

The soil extract was prepared by mixing 1 litre of distilled water with 1 kg. of sandy loam soil taken from Silwoodbottom, Silwood Park. This was dispensed into 250 ml. flasks and these were shaken on a Griffin flask shaker for 30 minutes. The flasks were then stood on a

bench for 30 minutes to allow the large soil particles to settle. The supernatant was filtered through glass-wool. Then it was centrifuged at 10,000 r.p.m. for 10 minutes. The supernatant from this operation was sterilized by passing it through a sterile 0.22 /w millipore filter. To check the efficiency of this sterilization, a few loopfulls of extract were streaked out on the surface of PSA plates.

Chlamydospore production was also tested in unfiltered soil extract.

Assessment of chlamydospores formed

Two millilitre portions of the conidial suspension were added to 30 ml. filtered soil extract in each of twenty-five 150 ml. flasks. These were incubated at 23°c.

Three days after inoculation and then at two day intervals until the 15th day, chlamydospore formation was examined. On each occasion one flask was sampled. Some sterile glass beads were put into the flask and this was placed for 1-2 minutes on a Fison whirlimixer. By this means, any fungal growth on the bottom of the flask was brought into suspension.

The suspension was centrifuged at 3.000 r.p.m. for 3 minutes and then the sediment was resuspended in 2 ml. sterile distilled water. This suspension was homogenized in a Sorvall Omnimixer using a micro-attachment at speed No. 7 for 5 minutes to separate out the chlamydospores.

The chlamydospore suspension was centrifuged again and resuspended in a known amount of sterile distilled water. The number of chlamydospores formed in the soil extract was subsequently calculated from haemocytometer counts of five samples of this suspension.

Testing the viability of the chlamydospores produced

Two methods were used:

a. Washed chlamydospores were suspended in sterile 0.5% glucose to give about 100,000/ ml. Three drops of this chlamydospore suspension were dispensed using a sterile Pasteur pipette onto a sterile microscope slide and this was placed in a humid chamber so as not to dry. After 24h. incubation at 20°c germinated and ungerminated chlamydospores were counted. The readings were taken in three microscope fields on each of three drops on five slides (i.e. forty-five microscope fields.)

b. Suspensions containing 100,000 chlamydospores/ml. in distilled water were streaked out on the modified Peptone-PCNB agar of Papavizas (1967). The plates were incubated at 20°c for 24h and then germination was assessed on at least 100 chlamydospores in each plate.

RESULTS

Culture media

Potato sucrose agar was found to be the best medium for growth (Figure 1 and Appendix Table 1) and as conidia produced abundantly on this medium, it was used generally in most experiements.

Optimum temperature

On potato sucrose agar, the fungus grew best at 20°c (Figure 2 and Appendix Table 2).

Production of Chlamydospores

1 - Preliminary experiments

Chlamydospores formed in all media tested but it took about 1 month or more to obtain abundant chlamydospores. The least number of chlamydospores were formed in the solution of monobasic potassium phosphate plus magnesium sulphate. Adding small amounts of either glucose or magnesium carbonate did not improve chlamydospore formation. In liquid potato-sucrose, mycelial growth was more abundant than chlamydospore formation.

More chlamydospores were produced in sterile soil than in non-sterile soil. In both soils, conidia germinated and produced mycelia and as the soil dried gradually chlamydospores formed. In non-sterile soil some cells of the conidium partly lysed before forming chlamydospores. At the end of one and a half months the fungus existed solely as chlamydospores in both soils.

2 - In soil extract

The production of chlamydospores using non-sterile soil extract was not satisfactory mainly because many bacteria and protozoa developed and the conidial inoculum

Figure 1 **Growth rate of the fungus on different media**

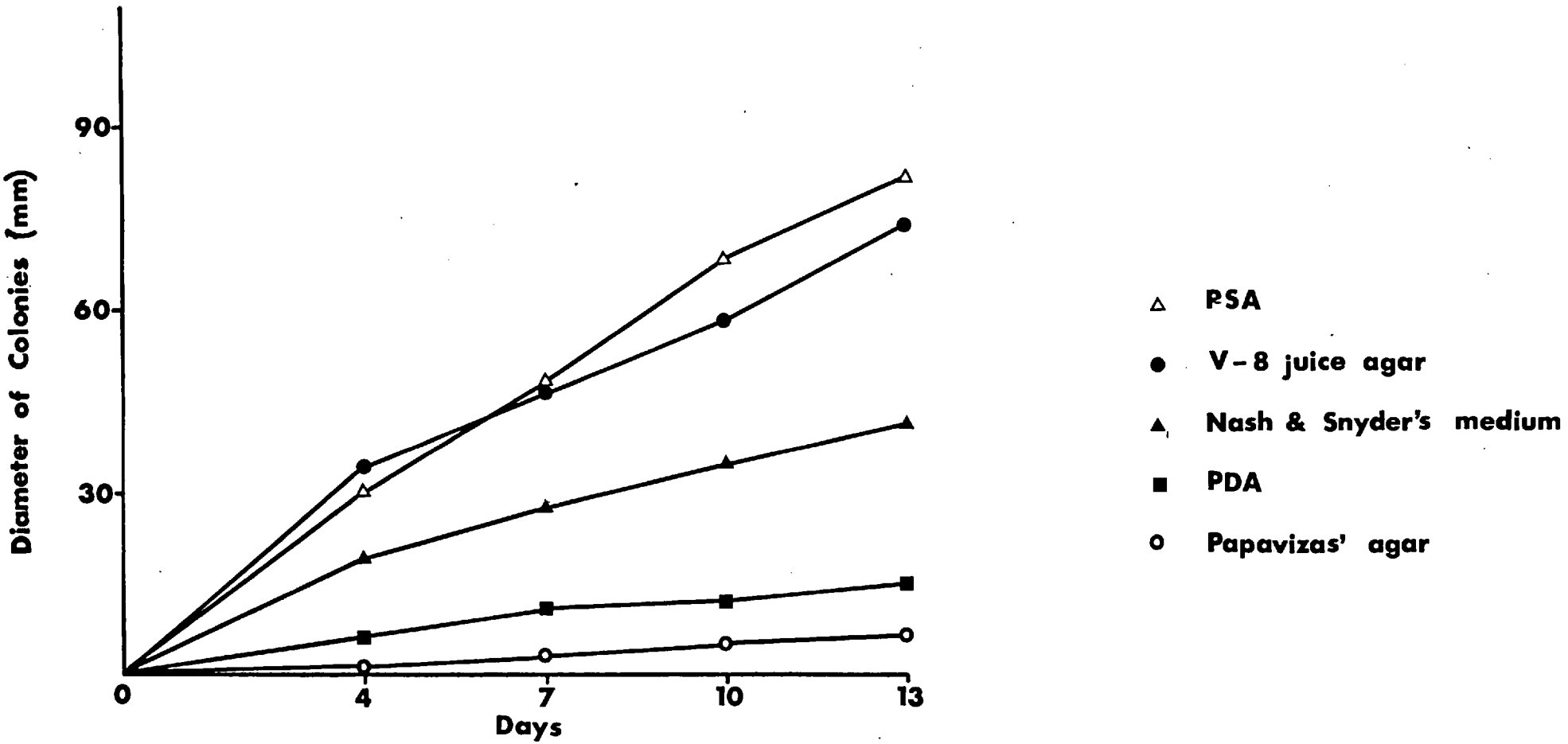
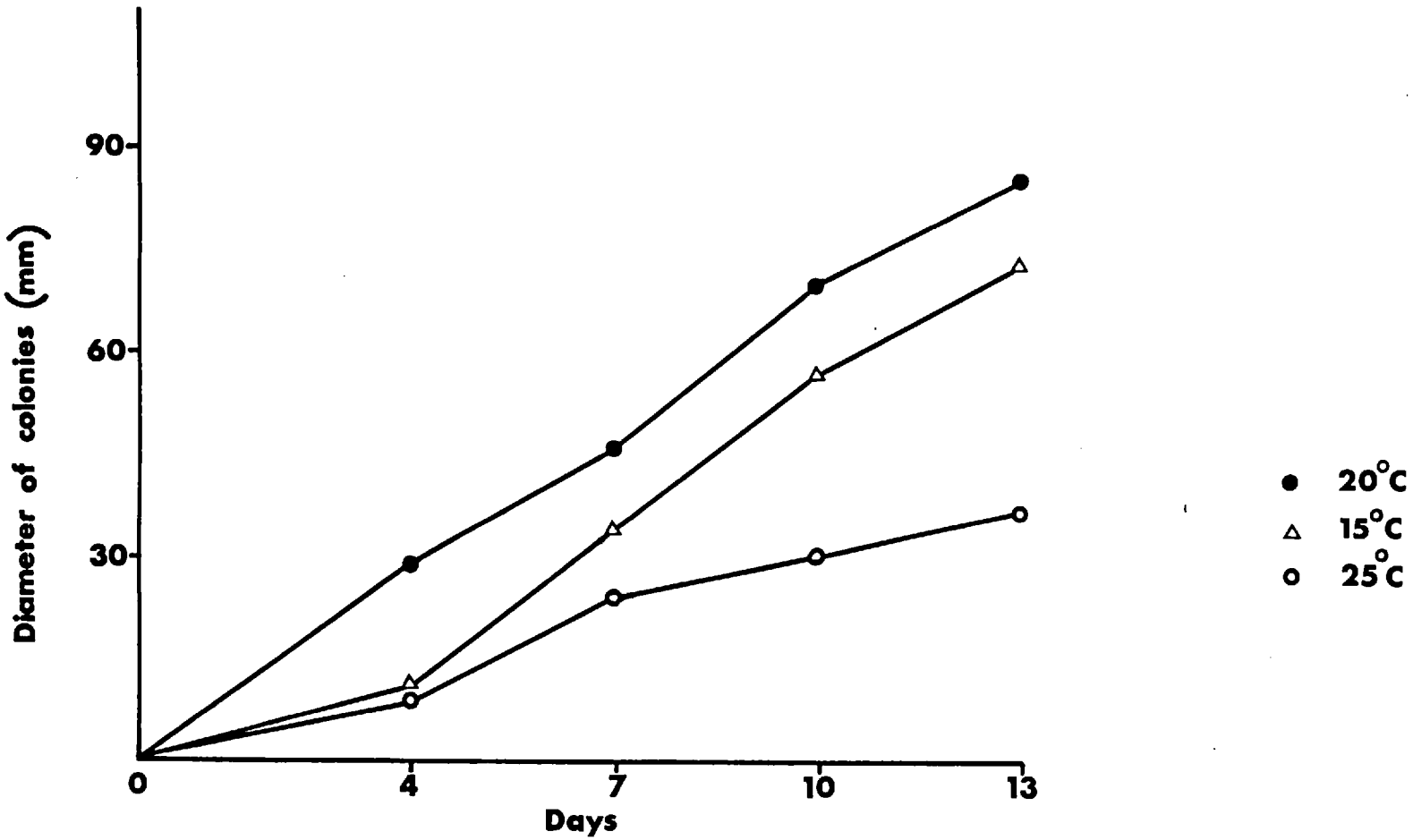


Figure 2 **Growth rate of Fungus at different temperatures**



was lysed. Chlamydospores were produced most readily and were most abundant in sterile soil extract. Conidia added to sterile soil extract mostly did not germinate and generally formed single chlamydospores which was most convenient for experimental work (Figure 3). Some chlamydospores formed 4 days after adding conidia to the sterile soil extract. Then after 7 days, the formation of chlamydospores increased rapidly, and reached at the peak after 15 days (Figure 4 and Appendix Table 3).

The viability of chlamydospores formed 15 days after adding conidia to sterile soil extract was 82.7% in 0.5% sterile glucose and 87.9% on modified Peptone - PCNB agar (Appendix Table 4 & 5).

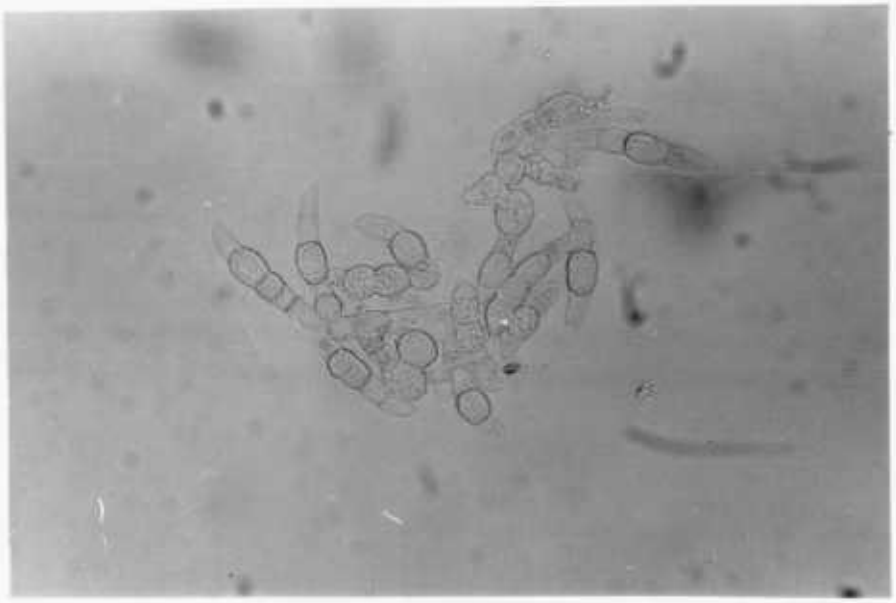
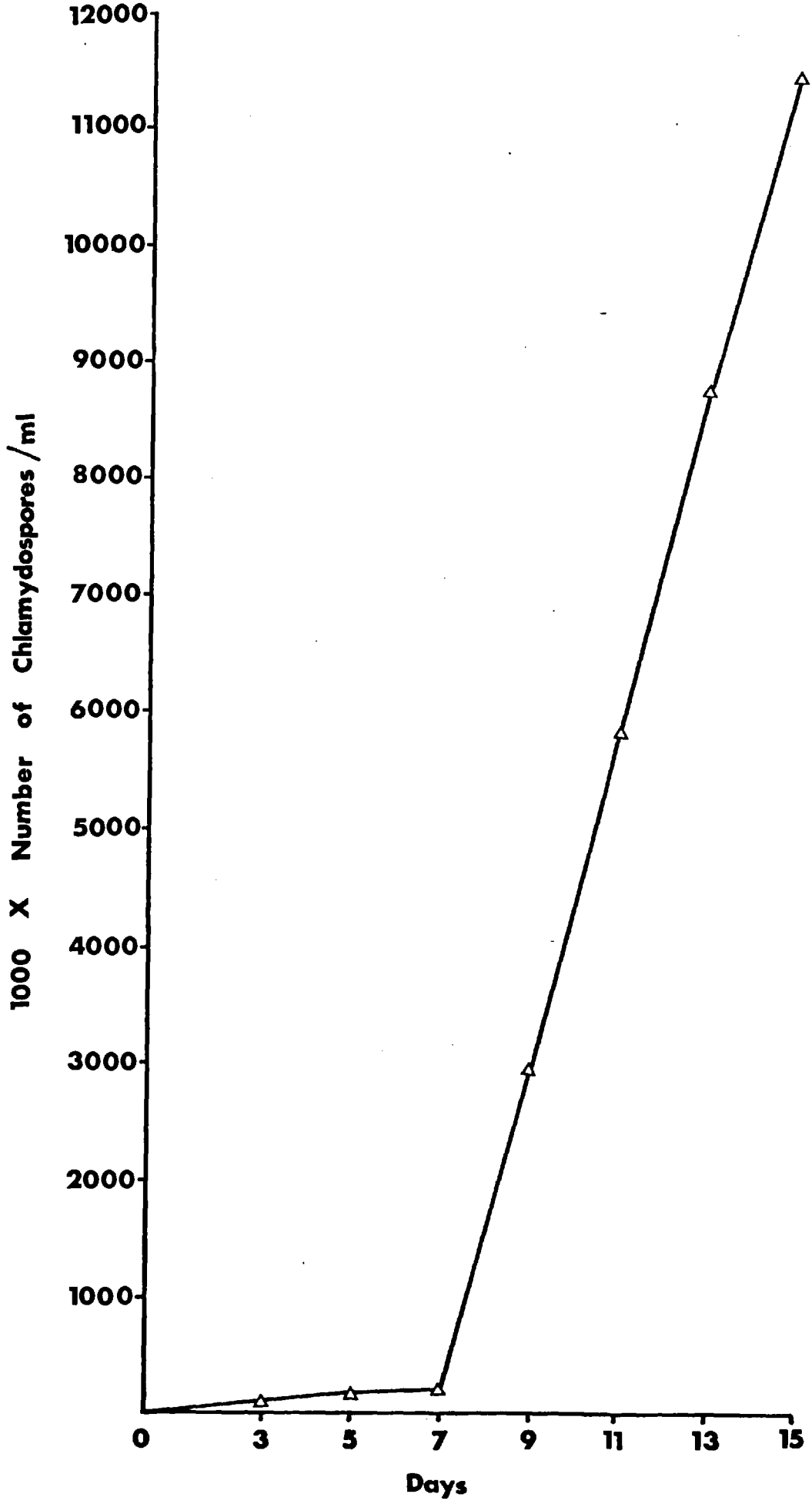


Figure 3 - Chlamydospores formed in soil extract.

Figure 4 Production of chlamydospores of Fusarium Solani var. coeruleum in soil extract



B. LONGEVITY OF THE CHLAMYDOSPORES

Experiment 1: Longevity in dry conditions at different temperatures

In this experiment, the effects of temperature on the longevity of chlamydospores kept in dry conditions in the laboratory were tested.

Materials and Methods

Chlamydospores were produced in soil extract as described in Section A. They were used 20 days after adding the soil extract to the conidial suspension. Dried films of these chlamydospores were prepared on glass coverslips, as follows. Coverslips, 22 mm. diameter, were chemically cleaned by immersing them in a potassium dichromate - sulphuric acid solution for one hour. These were then thoroughly rinsed in distilled water twelve times, so that no toxic material was left on the coverslips and were allowed to dry on clean blotting paper (American Phytopathological Society 1943).

These cleaned coverslips were fixed to glass slides, three per slide, by putting three smears of Vaseline at equal distance from each other on the slide, placing one coverslip over each smear and heating gently in a flame for a few seconds to melt the Vaseline so that it spread out under the coverslip. The slide was then cooled.

A chlamydospore suspension was thoroughly mixed in a sterile 250 ml. flask with sterile glass beads (3 mm diameter) for 1-2 minutes on a whirlimixer. A sample of this suspension was then drawn into an Agla micrometer syringe and a 0.01 ml. drop dispensed onto each coverslip. Each drop was spread out with a sterile loop and allowed to dry in a dessicator. Previous tests indicated that by using a suspension containing 200,000 chlamydospores per ml. and an 0.01 ml.

drop dispensed onto each coverslip. Each drop was spread out with a sterile loop and allowed to dry in a dessicator. Previous tests indicated that by using a suspension containing 200,000 chlamydospores per ml. and an 0.01 ml. drop, films of chlamydospores could be obtained with, on average, 30-70 spores per microscope field (x 150).

After various treatments the viability of chlamydospores deposited on coverslips was determined from their ability to germinate either in 0.5% sterile glucose or in Papavizas' medium. The glucose or the agar medium, cooled to 40-45^{o}c, was pipetted onto each coverslip on the slide with a sterile Pasteur pipette. The slide was incubated at 20^{o}c in a Petri-dish for 24h. It was supported in the dish on a glass angle over water so that the medium did not dry out. Germination was assessed from counts of at least 100 chlamydospores per slide. This was obtained by counting the germinated and non-germinated chlamydospores in three microscope fields per coverslip. A chlamydospore was considered to have germinated if the length of the germ-tube exceeded the width of the spore. If the chlamydospores at each end were counted, as it took more time for those which were inside the chain to germinate. Generally, after adding the drop of 0.5% glucose solution or Papavizas agar to the dry chlamydospores, even those that had been in chains separated readily from each other, as the septa probably broke down during the time in which they had been kept dry.

RESULTS

a. Effect of drying on viability of chlamydospores

A suspension of chlamydospores was prepared as described above. The viability of these chlamydospores was tested in a 0.5% sterile glucose solution as described in Section A. The only difference was that the concentration of the chlamydospore suspension was adjusted to 200,000 spores / ml. After 24h incubation at 20°c, germination was 79.2% (Appendix Table 6).

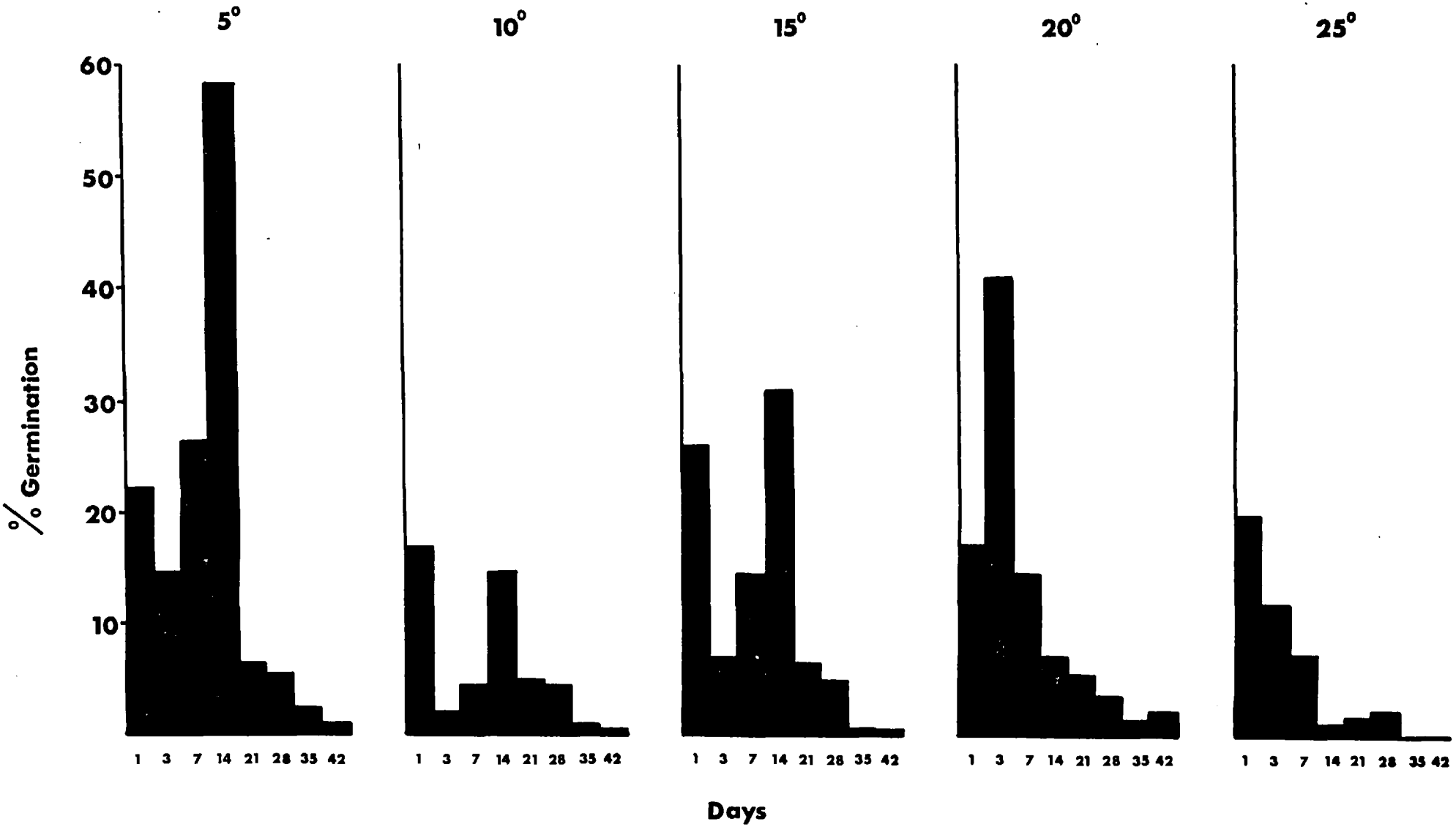
To determine the effect of drying on the chlamydospores, twelve slides (each with coverslips) were inoculated with the chlamydospore suspension and as soon as the films had dried they were covered either with 0.5% sterile glucose (nine slides) or with cooled Papavizas agar (three slides). Each slide was incubated separately in humid chambers at 20°c. After 24h, the number of germinated and ungerminated chlamydospores was counted. To aid counting a drop of 0.1% acid fuchsin in lactic acid was placed on the surface of the agar. In both the glucose and agar treatments 42.7% of the chlamydospores germinated (Appendix Tables 7 and 8).

b. Effect of storing dried chlamydospores at different temperatures

In all, 120 slides were prepared each with three dried films of chlamydospores. Twenty-four slides were then kept at each of the following temperatures: 5°, 10°, 15°, 20° and 25°c. Each slide was placed on a glass angle in a dry petri dish. Germination of chlamydospores in 0.5% glucose was then assessed on a sample of three slides per treatment after 1, 3 and 7 days' storage and subsequently at weekly intervals up to 42 days.

The results are given in Figure 5 and Appendix Table 9. No chlamydospores appeared to be viable after 42 days at 25°c and very few survived after this time at the other temperatures, the figures varying from 0.3% at 10° to 2%

Figure 5 Longevity of Chlamydospores in dry conditions at different temperatures



at 20°c. In most treatments the decline in viability was most marked from the twenty-first day of incubation, but at 25°c there was a steady decline throughout. At 5°c, 10°, 15° and 20° the numbers of chlamydospores up to day 14 varied considerably but generally first reached a peak and then declined. The pattern suggested that storing chlamydospores dry at temperatures of 20°c or below, somehow initially increased their ability to germinate.

Experiment 2: Longevity in soil

In this experiment the aim was to examine the viability of chlamydospores on glass slides in contact with soil when stored at two different temperatures.

Materials and Methods

Soil was collected on 18 March, 1974 from a field at Silwood Park in which potatoes had been grown in the previous year, and left to dry. This soil was sieved through a No. 6 mesh (2800 μ) and then distilled water was sprayed evenly on it so that a moist soil was obtained, suitable for plant growth. This was then used to fill forty 500 ml. beakers.

A suspension of 20 day old chlamydospores was prepared from sterile soil extract cultures and this was adjusted to 200,000 chlamydospores/ml. as in Experiment 1. The viability of the spores in this suspension was tested using both 0.5% glucose and Papavizas agar.

This suspension was then used to prepare dried films of chlamydospores on microscope slides chemically cleaned in the same way as described for coverslips in the previous experiment. One 0.01 ml. drop was placed on each slide using an "Agla" micrometer syringe and this was spread with a sterile loop over an area 2 cm. in diameter. Care was taken to spread the suspension evenly to give a thin layer of chlamydospores. The slides were allowed to dry in the laboratory and were labelled. Each label was fixed on the same side as the dried film and at one end of the slide to a standard depth. The viability of chlamydospores just after drying was tested by taking sample slides and covering the dried films with either 0.5% glucose or with Papavizas agar and incubating for 24h at 20°c as previously described.

The remainder of the labelled slides were buried to a depth of 5 cm. in the soil contained in the beakers as

shown in Figure 6. There were five slides in each beaker. Each beaker was covered with a piece of aluminium foil to prevent evaporation of water. Twenty beakers were incubated at 10°c and twenty at 15°c.

At intervals of 1, 3, 7 and 11 days and then once weekly, two beakers were removed from each temperature-treatment. The soil behind each labelled slide was removed and the slide carefully taken out. The five slides in one beaker were used to assess the number of chlamydospores which had germinated in soil. The chlamydospore films on these slides were stained in 0.1% acid fuchsin in lactic acid to aid counting. Generally not less than 100 chlamydospores were examined on each slide. Three slides from the second beaker were used to assess the viability of chlamydospores. The chlamydospore films on these slides were covered with Papavizas agar and the slides were then placed on glass angles in a plastic box (9 x 11 x 6 cm) lined with moist blotting paper. After incubation for 24h at 20°c, a drop of 0.1% acid fuchsin was placed on each agar-covered film and the number of germinated chlamydospores was counted.

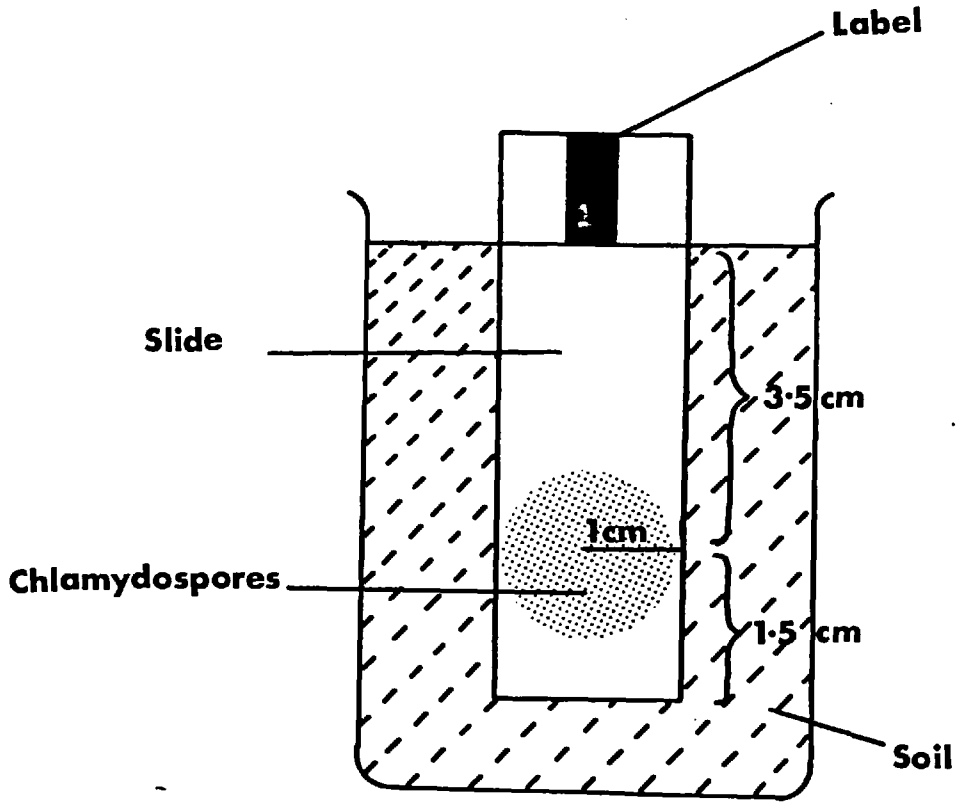


Figure 6 Slide with Chlamydospores buried in soil in a beaker

Results

Before drying, 89.9% of the chlamydospores germinated in 0.5% glucose and 92.4% germinated on Papavizas agar. (Appendix Table 10 and 11). Immediately after drying on glass slides, these figures fell to 39.4% and 44.1% respectively (Appendix Table 12 and 13).

The behaviour of chlamydospores on the slides buried in soil is summarized in Table 2 and detailed results are given in Appendix Table 14 and 15. Over the 28 days of the experiment there was some germination of the chlamydospores in soil. The figures varied but there did seem to be some general increase in germination over this period and more chlamydospores germinated at 15°c than at 10°c. The total number of chlamydospores which appeared viable after 7 days' incubation, when tests with Papavizas agar were started, was greater than expected. At 10°c 83.4% germinated and at 15°c 79.9% germinated, considerably more than the 44.1% germination immediately after drying. The reason for this is not known. Possibly some chlamydospores which germinated during the first week in soil produced mycelia from which further chlamydospores were formed. Certainly towards the end of the experiment many new chlamydospores were formed, especially at the tips of germ-tubes or at the end of mycelia. Generally, the viability of chlamydospores which remained ungerminated in the soil (Column C of Table 2) declined over the experimental period. This decline was somewhat more remarked after 28 days at 15°c than at 10°c.

Table 2: Behaviour of chlamydospores on glass slides in contact with soil at 10°c or 15°c.

Length of incubation (days)	A. % germination in soil		B. % germination in Papavizas agar after 24h at 20°c		C. Viability of 'dormant' spores in soil (B - A)	
	10°	15°	10°	15°	10°	15°
1	2.2	5.3	-	-	-	-
3	3.3	23.0	-	-	-	-
7	15.6	12.3	83.4	79.9	67.8	67.6
11	11.3	7.7	78.0	72.6	66.7	64.9
15	6.1	18.2	76.2	73.9	70.1	55.7
21	13.7	16.8	68.5	70.3	44.8	53.5
28	17.0	30.6	62.0	54.7	45.0	24.1

C. BEHAVIOUR OF CHLAMYDOSPORES NEAR HOST AND NON-HOST PLANTS

Experiment 1: Behaviour of chlamydospores in soil and near potato roots

The aim of this experiment was to find out if there was any effect of potato roots on germination of chlamydospores in soil.

Materials and Methods

Dried films of chlamydospores were prepared on chemically-clean slides as described in Section B. Twenty day old chlamydospores produced in soil extract were used. These were washed by centrifuging in sterile distilled water and the concentration was adjested to 200,000 spores/ml. A drop (0.02 ml) of this suspension was spread over a 10 cm^2 area at the bottom of each slide. In all, three hundred such slides were prepared and each was marked with an adhesive label on the side on which the chlamydospore film had dried.

The viability of chlamydospores prior to drying the film on the slides, was tested as described in Section B and was found to be 90.8% and 92.5% in 0.5% glucose and in Papavizas agar respectively (Appendix Table 16 and 17).

A similar test was carried out just after drying the film on the slides. The viability was then 45.7% and 48.3% in the two media respectively (Appendix Table 18 and 19).

Soil was obtained from Silwood Park on 1 May, 1974, left to dry and then sieved through a No. 6 mesh (2800 /w). Just before potting, it was moistened by spraying with distilled water to a moisture content (c.12%), suitable for plant growth. Thirty 28 cm diameter plastic pots were filled with this soil. They were divided into two groups of fifteen pots and each pot was numbered. Within each group, pairs of numbered pots were chosen

by drawing lots, and the sampling order was thus determined in advance. One group of pots was sown with potatoes and the other used as control.

Seed potato tubers (Solanum tuberosum) of the variety "Majestic" had been selected in early spring from a potato store in Silwood Park. They were washed and after drying, were surface-sterilized by immersing them in 10% chloros (1% available chlorine) for one hour to protect them from bacterial rot. They were then rinsed in distilled water and dried. These potatoes were kept in large clean paper bags in the dark in the laboratory until small sprouts developed.

The experiment was set up on 23 May,1974. Surface sterilized cane sticks were passed through the centre of each tuber to mark the site of the tubers when they were planted to a depth of 5 cm. in a pot. Then ten microscope slides on which chlamydospore films had dried, were buried to a depth of 7 cm. in a circle around the tuber in each pot. These were arranged on a 5cm. radius from the cane stick with the labelled surfaces facing inwards. Slides with chlamydospores were placed in the other fifteen pots containing soil only (control) in a similar way, that is in a circle of 5 cm. radius. The pots with and without potatoes were stood in five trays of water and kept out of doors to provide natural conditions. Where necessary water was added to the trays.

Microscope slides with dried films were examined at 3, 7, 14, 21, 28 and 35 days after burying them in the soil (Appendix Table 20). On each occasion two pots were removed from each treatment (two control and two potato-sown pots).

Germination of chlamydospores was examined by staining five slides from one pot in 0.1% acid-fuchsin in lactic acid. The soil behind each slide was first removed and the slide was then withdrawn carefully so as not to rub

off the spores. The soil particles on the back of the slide were cleaned off with muslin, the slide was tapped to get rid of large soil particles, then one or two drops of stain were placed on the chlamydospore film. This was covered with a long (22 x 44 mm) No.1 coverslip. Germinated and non-germinated chlamydospores were counted under the low power of the microscope (x 150). Chlamydospores were considered to have germinated when the length of the germ-tube exceeded the width of the spore. About fifty spores were counted per slide.

The viability of spores which had not already germinated on slides ('dormant spores') was tested by covering five slides from the second pot which was sampled, with Papavizas agar as described in Section B, expt. 2.

Results

Potatoes sprouted on 3 July. One week after burying the slides in soil, most of the potato root tips reached the slides and soon after, a mass of root tips came in contact with the chlamydospores.

Detailed results of the germination of chlamydospores in soil and near potato roots are given in Appendix Table 21 and these are summarized in Table 3. Some chlamydospores (c.27%) were found to have germinated after 3 days in soil but not many more spores appeared to germinate after that because the percentage germination in soil after 28 days was still only c.38%. In contrast the numbers of germinated chlamydospores near potato roots increased from 35% to 60% during the same period and were significantly more than in soil alone on days 14, 21 and 28. This suggested that the potato roots induced chlamydospores to germinate. Germination of chlamydospores on slides kept near potato roots for 14 days is shown in Figure 7.

Just before day 35 there were heavy rais and the soil in the remaining pots were covered with 4-5 cm. of water. When slides were examined on day 35 it was found that many germ-tubes and spores had lysed. Germination in the control soil apparently dropped from 38.4% to 26.1% and in soil near potato roots from 60.3% to 33.4. (Appendix Table 22). It was also difficult to count the spores because of clay particles on the slides. For these reasons the results from day 35 have been excluded from Table 3.

It was also found that the tests of viability of the non-germinated ('dormant') spores on slides were completely unreliable throughout the experiment. Germ-tubes from spores already germinated in soil masked the non-germinated spores and made further assessments impractible.

Table 3: Germination of chlamydospores of F. solani var. coeruleum in soil and near potato roots

Site	Mean % germination + after 3	7	14	21	28 days
Soil only	26.6	26.3	31.6	30.8	38.4
Near Potato roots	35.0	39.3	53.2	55.1	60.3
Probability (P) that means differ ‡	n.s.	n.s.	0.05	0.05	0.01

\+ based on five samples from each site.

‡ based on t-tests using angular transforms of the data.

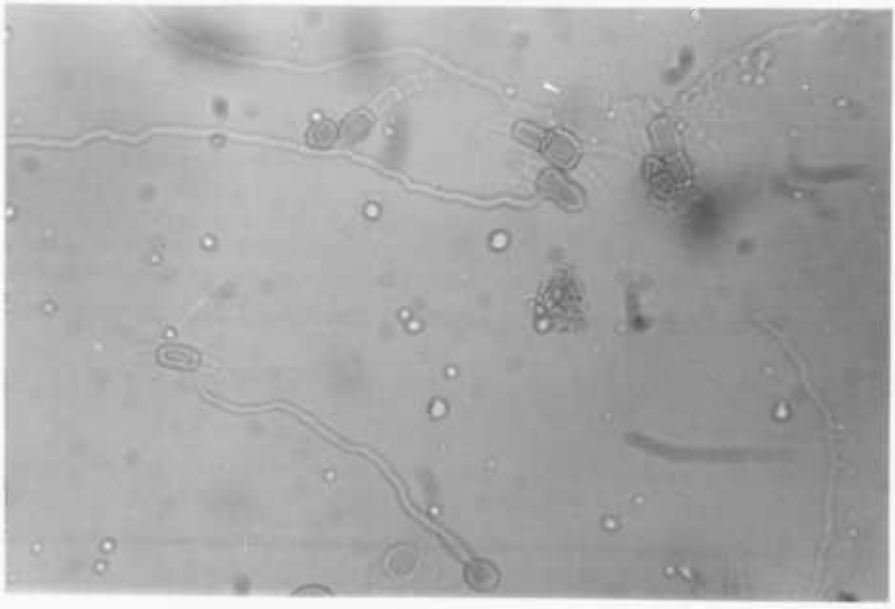


Figure 7: Germinated chlamydospores on slide near potato roots (after 14 days).

Experiments 2 a and b.

Behaviour of chlamydospores near potato, barley and broad bean plants

These experiments were designed to determine the effect of host (potato) and non-host plants (barley and broad bean) on the population of F. solani var. coeruleum in soil. Some studies were also made of the population in soil after potatoes had been harvested and of the inoculum of the fungus on harvested tubers.

(a) Glasshouse Experiment

Materials and Methods

Preparation of chlamydospore suspension.

The method was basically similar to that already described. Medical flats (186) of potato-sucrose agar (hardened for 4-5 days) were inoculated with the fungus. They were incubated at 20°c in the dark for 3-4 days and were then placed under two daylight fluorescent tubes with a 12h photoperiod for 16 days. A conidial suspension was prepared as described in Section A, except that conidia were washed only once by centrifuging. Similarly, the soil extract was prepared initially as described in Section A, but then after centrifuging at 10,000 r.p.m. for 10 min. was sterilized at 125°c for 15 min. in an autoclave because filtration through a millipore filter was impracticable with the large quantity (10.5 l) involved.

The conidial suspension was adjusted to 5×10^6 spores/ml and was added to sterile soil extract at the rate of 1 ml suspension / 10 ml extract in 250 ml and 500 ml flasks, which were incubated at 23°c for 20 days. The resulting cultures were homogenized for 5 min. in a Sorvall omnimixer to produce a suspension of single chlamydospores.

Preparation of infested soil

The soil used in this experiment was a sandy soil obtained from agricultural land in Silwood Park on which potatoes had been grown previously. It was taken in the first week of March 1975, and passed through a 1 cm x 1 cm mesh, and then left to dry for one and a half months with several mixings. Then it was passed through a mesh of 0.5 cm x 0.5 cm pores and just before potting it was seived with a No. 6 mesh (2800 μ).

The physical structure of the soil was as shown below (Sayles, 1973):

	%
Organic matter	10.2
Sand	93.1
Silt	2.2
Clay	4.7

The mineral content, per 100g. soil, was found by Sayles (1973) to be:

Fe^{+++}	Mg^{++}	Ca^{++}	Na^{+}	K^{+}	PO_4^{--}
910mg	45.9mg	65mg	21.5mg	81.5mg	16mg

About 288 kg. of sieved and air-dried field soil was treated with 34.6 l of chlamydospore suspension in order to obtain an infested soil with about 12% moisture content containing 3.6×10^4 spores / g. fresh soil. The procedure was as follows. The soil and the chlamydospore suspension were divided into four equal parts to make the infesting process easier. A thin layer of soil was laid on a concrete floor and was sprayed with chlamydospore suspension using a hand-held "ASL Spraymist" sprayer. Then another thin layer of soil was laid on the sprayed soil and more suspension sprayed on to it. This

procedure was continued until the one-quarter lot of soil was infested with its appropriate amount of chlamydospores. The infested soil was then thoroughly mixed. Each quarter of the soil was treated in this way then the four portions of infested soil were bulked and mixed thoroughly. The pile of soil was covered with a nylon sheet and left overnight. The next day the soil was mixed again and passed three times through a riddle of 0.5 x 0.5 cm mesh. Then approximately 1 kg. samples were taken at random from five different places in the pile. These were bulked, mixed thoroughly and divided into five sub-samples. One sub-sample was taken at random and three 100g. portions taken from it. One was used to determine the moisture content and the other two to estimate the population of F. solani var. coeruleum. Portions of the other samples were used to determine the soil pH (see below).

Determination of soil moisture content

The soil sample (100g) was sieved through a No. 10 mesh (1680/w) and transferred to a 250 ml glass beaker. This was placed in an oven at 105°c for 24h, then cooled in a dessicator and weighed. This procedure was repeated until a constant weight was achieved. Percentage soil moisture content was calculated from the formula:

$$\% \text{ Soil Moisture Content} = \frac{\text{Weight of original sample} - \text{Weight of dried sample}}{\text{Weight of orignal sample}} \times 100$$

It was sometimes necessary during the experiment to determine moisture content on smaller (20-50g) samples of soil, especially when collecting this near stolons or young tubers.

Measurement of soil pH

Five 20g portions of the sieved soil were each mixed with 20ml of distilled water in a 50 ml beaker. Each suspension was stirred for 30 min. and then the soil particles were allowed to settle out. The pH was then measured on a Beckman pH meter using a glass electrode.

Plant material

The following was used:

(i) Potato. Healthy tubers of the variety "Majestic" about 4-5 cm diameter were selected from the potato store at Silwood Park in February, 1975. They were thoroughly washed, surface-sterilized and sprouted as in Experiment 1.

(ii) Barley. Undressed seed of the variety "Midas" was obtained from Miln Marsters of Chester. This had an 89% germination after 3 days on wet blotting paper at 25°c.

(iii) Broad Bean. Seed of the variety "Meteor" was obtained from Sutton's of Reading. When a sample was incubated at 25° for 12 days in a seed germination tray, 88% germinated.

Design and management of the experiment

Thirty-two 28 cm diameter plastic pots were filled with infested soil, 9 kg in each. They were divided into four groups of eight pots, the sampling order of the pots in each group was determined by drawing lots and the pots were labelled appropriately. One sprouted potato tuber was planted to depth of 5 cm in each of the pots of one group. In a second group, thirty-nine barley seeds were sown, 4 cm apart from each other and 1 cm deep, in each pot. In a third group, seven unsoaked broad bean seeds were sown, 10 cm apart and 2 cm deep in each. The remaining eight pots with infested soil were left as controls. The pots were then placed in trays

of water in an unheated glasshouse in the completely random manner shown in Figure 8. The experiment was set up on 25 April, 1975.

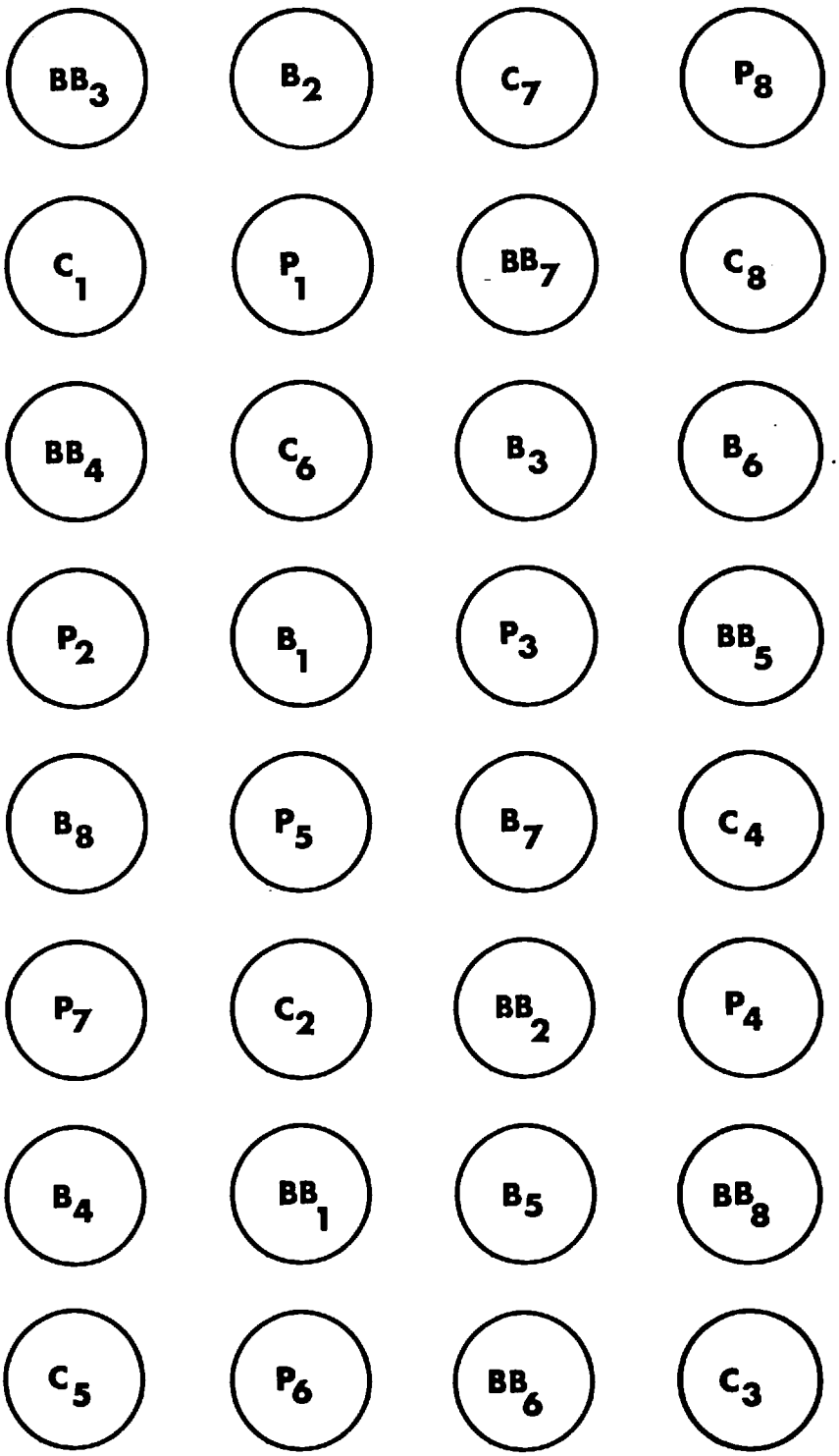
The pots were weeded twice, 15 and 29 days after sowing. Plants were sprayed twice (25 July and 4 August) with 'Biospray' (Pan Britannica Industries Ltd.) to control aphids and white flies.

One pot was sampled from each treatment on the dates shown in Table 4.

Before taking soil samples, the above ground parts of the plants were removed and the plastic pots were cut into four pieces and separated from the soils. Then the bulk of the soil was put up-side down on a 2 lt. beaker. After removing the wet clay soil region (close to the water in the tray) portions of soil near roots were separated with a small spatula and collected. Where necessary, the bulk of soil was divided into two or four portions then stolons were followed and soil from around them, and the soil which was in immediate contact with young tubers was collected both by scraping the tubers and the cavities when the tubers were removed. If the seeds and old tubers decayed and completely rotted, no sample was taken.

Each soil sample taken was separately passed through a No. 10 mesh (1680 μw) and mixed thoroughly. Then 20g portions were taken from each sample for soil dilution plates (see below) and other sub-samples (100g) were taken to determine soil moisture content. Each time after sampling, spatula, sieves and hands were washed thoroughly. As soon as the soil samples were taken, dilution series were prepared and plated out. If more than one sample was taken (e.g. from pots with potatoes), the other samples were stored temporarily in a refrigerator at 4^{o}c to avoid drying.

Figure 8 Experimental layout and sampling order (Glasshouse Experiment 2a)



Key: B = Barley
BB = Broad Bean
C = Control
P = Potato

Table 4: Sampling intervals and dates of soil near potato, barley and broad bean

Intervals (Week)	Sampling Dates (1975)
Immediately after infesting soil	25th April
3 week	15th May
6 "	5th June
9 "	26th June
12 "	17th July
15 "	7th August
18 "	28th August
21 "	18th September
After Potato Harvest:	
24	9th October

Estimation of the population of F. solani var. coeruleum.

Estimates of the population of F. solani var. coeruleum were made immediately after infesting the soil and then throughout the experiment on the dates shown in Table 4. A serial dilution of the soil sample was first prepared in sterile distilled water in medical flats containing glass beads (3mm daim.) to facilitate disintegration of soil aggregates. After several tests of different dilution serious, it was established that the best dilution for these pot soils was 10^{-3} and so this was then used throughout the experiment. This dilution gave twenty-five to thirty colonies per petri-dish.

The medium chosen was a selective one developed by Boyd to detect the propagules of this fungus in soil. This contained, per litre:

20 g	Sucrose
2 g	Potassium nitrate (KNO_3)
70 ppm	Dodine acetate
20 g	Agar
1 g	Potassium dihydrogen phosphate (KH_2PO_4)
0.5 g	Magnesium sulphate ($Mg\ SO_4.7H_2O$)
1 g	Pentachloronitrobenzene (PCNB 75% wettable powder)
300 ppm	Streptomycin (added on cooling)

On this medium colonies of F. solani var. coeruleum give a deep-blue colour which distinguishes them from other fusaria. Very low populations of the fungus in soil can be dedected by this medium and the results agree well with those obtained by the biological method previously developed by Boyd (1973). Figure 9 shows blue coloured colonies of the fungus formed on Boyd's agar.

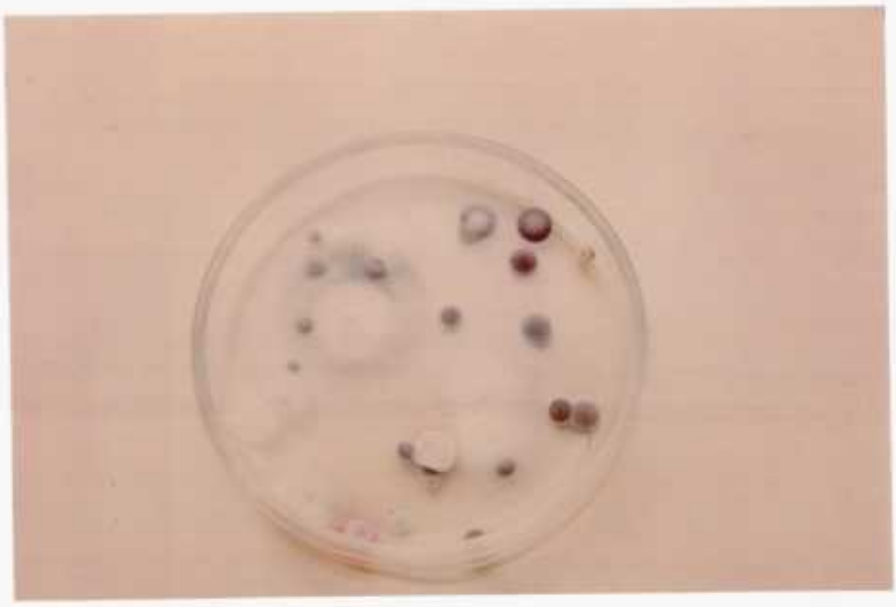


Figure 9: F. solani var coeruleum colonies isolated from soil (on Boyd's agar).

The best results were obtained by using 'hardened plates' as the soil suspension was then readily absorbed and there was less tendency for bacteria and yeasts to spread and inhibit the fusaria (Booth, 1971; Nash & Snyder, 1962). About 18 ml of medium was used per petri-dish (so that the agar would not dry during the long incubation). Plates were then kept in a cool, dry, dark place for 12-13 days before use.

In preparing the soil plates, a 1 ml portion of a freshly-agitated 10^{-3} dilution was pipetted onto the surface of the agar and was then spread out with a flame-sterilized loop. Fifteen such plates were prepared for each sample. Inoculated plates were kept at 20°c for 18 days in an incubator containing a plastic box with water to create a high humidity so that the cultures would not dry out. The deep blue colour associated with colonies of F. solani var. coeruleum started to appear after 14 days and colonies were counted after 18 days' incubation. The numbers obtained were converted to numbers of propagules per gram oven-dry soil.

Microscopic examination of chlamydospores on roots and in soil adjacent to roots.

Six weeks after sowing seeds in the pots (5 June), plant roots and soil adjacent to host and non-host plant roots were examined microscopically.

(a) Examining of chlamydospores in soil adjacent to plant roots

For direct assay of germinated and non-germinated chlamydospores near potato, barley and broad bean, the roots were removed from soil with care and the adhering soil was washed with a wash-bottle on to hardened Boyd agar. The plate was swirled a few seconds until the suspension was spread uniformly and then left to allow the soil particles and fungal propagules to settle.

The excess water was removed with a blotting paper and the plate was left in order to allow the suspension to dry. As the agar surface had already hardened for 12-13 days in the dark, the suspension was easily absorbed by the agar. Then three drops of lacto-fuchsin were added on the agar surface and two No. 1 coverglasses (22 x 40 mm) were placed on them. The coverglasses were pressed gently onto the agar surface with a pencil eraser. Then they were examined immediately under the microscope (Papavizas, 1967). Three soil samples adjacent to roots were examined in this way for each treatment.

(b) Examining of chlamydospores on roots

The roots of potato, barley and broad bean were removed from the pot soil with care and the adhering soil particles were washed into sterile petri-dishes. Then the wet roots were dried between sheets of sterile blotting paper for a few seconds. Small pieces of epidermis from roots were peeled off with the aid of a sterile scalpel and they were placed onto a slide. They were stained with lacto-fuchsin (0.1% acid-fuchsin in lactic acid) to aid counting and examined under microscope. As much tissue as possible was examined for germinated and non-germinated chlamydospores. Three preparations of roots were examined for each plant.

Experimental

Soil pH and moisture capacity

The soil pH was found to be 5.06 ± 0.03 and the moisture capacity 36.6%.

Crop development

Potatoes sprouted on 28 April and began to produce stolons on 15 May. By 26 June (9 weeks after sowing) they had produced young tubers, 1 cm diameter and on that day they started flowering. By 15 weeks after planting the new tubers were about 3-6 cm diameter. By the 26 August all the plants had dried completely. The last potatoes were harvested on 18 September. By then the potato roots had already died, the stolons had started to turn brown and in some instances young tubers had lost their connections with the plant as some of the stolens had rotted.

The barley emerged on 2 May, reached maturity on 17 July and was ready for harvesting on 25 July.

The broad beans emerged on 9 May. The bean pods matured on 30 July and the leaves were completely dried by 15 August.

Populations of F. solani var. coeruleum

(a) During growth of the plants

The results of the microscopic examination of chlamydospores in soil near roots and on the roots themselves are given in Tables 5 and 6. These indicated no significant differences in the germination of the chlamydospores in the different situations. The soil isolations using Boyd's agar, however, revealed marked changes in the population of F. solani var. coeruleum. The numbers of propagules in the control pots and in soil near potato, barley and broad bean plants, from sowing (25 April) until potato harvest (18 September) are given in full in Appendix Table 23 and are summarized in

Table 5: Microscopic examination of chlamydospores in soil adjacent to roots.

	Replicate	No. Counted	No. Germ-inated	% Germ-ination	Angular Trans-form
B. Bean	1	10	2	20.00	26.56
	2	12	2	16.66	24.04
	3	10	1	10.00	18.44
			Mean	15.55	23.01
Barley	1	10	1	10.00	18.44
	2	8	3	37.50	37.76
	3	9	1	11.11	19.46
			Mean	19.53	25.22
Potato	1	13	4	30.76	33.65
	2	13	3	23.07	28.66
	3	11	6	54.54	47.58
			Mean	36.12	36.63

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatments	2	320.47	160.23	2.33 N.S.
Error	6	411.10	68.51	
Total	8	731.57		

Table 6: Microscopic examination of chlamydospores on roots.

	Replicates	No. Counted	No. Germ-inated	% Germ-ination	Angular Trans-form
B. Bean	1	6	-	0.0	0.0
	2	13	1	7.69	16.00
	3	12	2	16.66	24.04
			Mean	8.11	13.34
Barley	1	15	1	6.66	14.89
	2	9	-	0.00	0.00
	3	11	1	9.09	17.46
			Mean	5.25	10.78
Potato	1	19	3	15.78	23.34
	2	15	2	13.33	21.39
	3	11	5	45.45	42.36
			Mean	24.85	29.03

Analysis of Variance

	D.F.	S.S.	M.S.	F.	
Treatments	2	585.48	292.74	2.35	N.S.
Error	6	745.66	124.27		
Total	8	1331.14			

Figure 10.

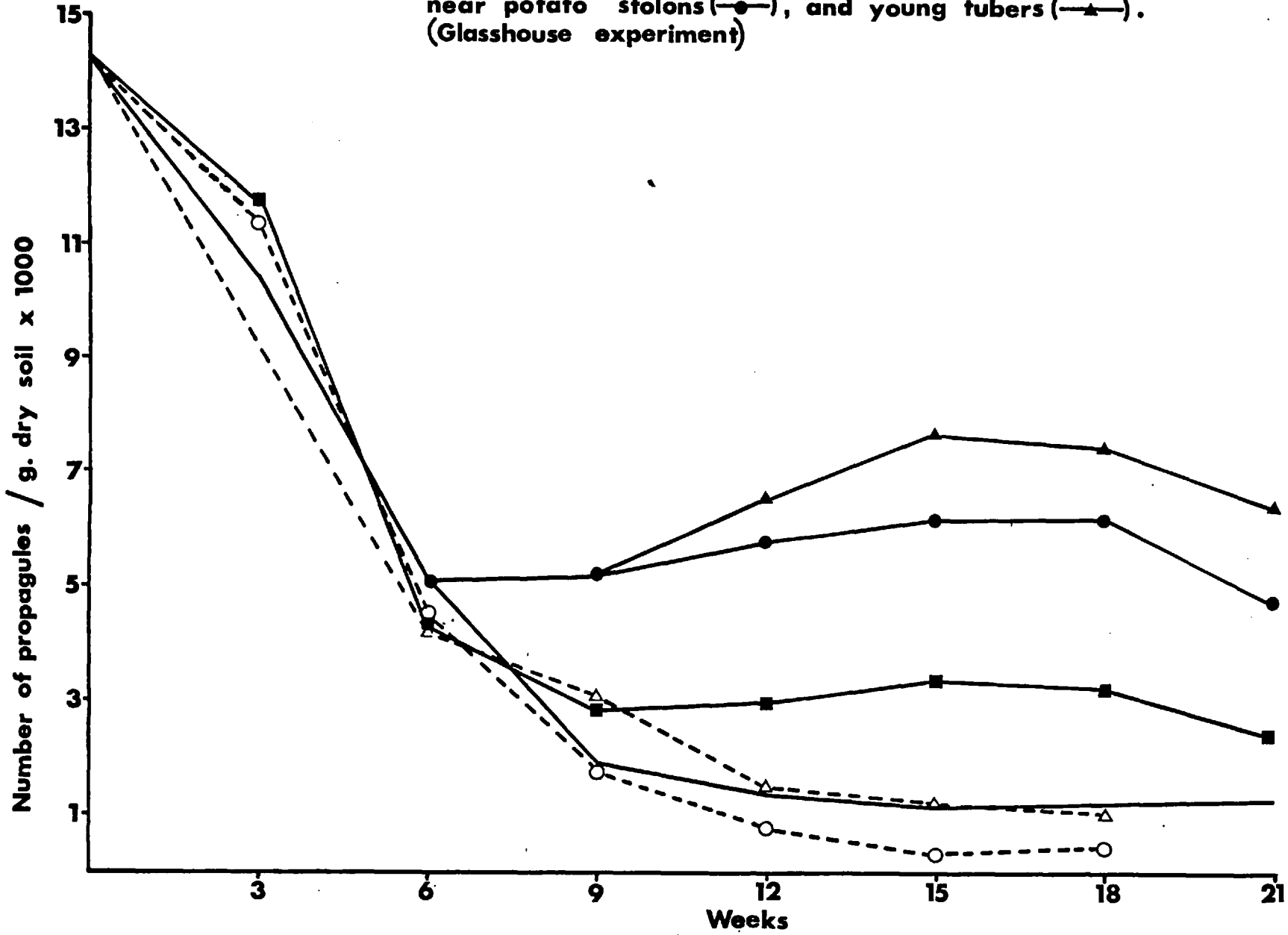
Immediately after infesting the soil the number of propagules was c. 14,500 per gram dry soil (Appendix Table 24). In all treatments, the numbers of propagules declined rapidly during the first 6 weeks, possibly because as in Experiment 1 some chlamydospores germinated and the germ-tubes were then lysed. The numbers of propagules in the control and also near the roots of the non-hosts, barley and broad bean continued to decline until the twelfth week. Then they remained steady at a low level of about 500-1000 propagules per gram dry soil. Old tubers and broad bean seeds also did not appear to affect the population of the fungus at all throughout the experiment. In contrast, from the ninth week onwards the population of the fungus in soil near potato roots, stolons and young tubers increased consistently. At week 9 there were significantly more propagules near stolons and young tubers than near potato roots, near those of the non-hosts or in the control. By week 12 the numbers of propagules near potato roots, stolons and developing tubers were all substantially greater than in the control or near roots of non-hosts. In this and later samplings there were also marked differences in the numbers of propagules associated with the underground parts of the potato. There were always most propagules near the newly-formed tubers. There were somewhat fewer near stolons and least near the roots.

After week 18 the numbers of propagules near these potato structures declined slightly. This was associated with the death of the shoots.

During isolations, it was noted that in soils with barley, broad bean and potato and also (to some extent) in the control soil, the number of other Fusarium species isolated increased towards the end of the experiment, whereas at the beginning most colonies isolated were F. solani var. coeruleum. Possibly drying the soil

Figure 10

Population of Fusarium solani var. coeruleum in control soil (——), in soil near roots of barley (--△--), broad bean (--○--), potato (—■—), near potato stolons (—●—), and young tubers (—▲—). (Glasshouse experiment)



before setting-up the experiment favoured the fungus (F. solani var. coeruleum) but later more competitive fusaria, like F. solani, F. roseum, F. oxysporum increased in soil.

(b) After harvest (potatoes only)

After the potato shoots had completely dried, tubers were left in the ground until 18 September (week 21) to allow them to produce a thick cork tissue. Then they were harvested and soils collected near young tubers, stolons and potato roots were mixed with all the remaining soil in the pot. The mixture was sieved three times through a No. 6 mesh (2800 μ). The control soil was similarly mixed and sieved. The numbers of propagules in both soils was assessed just after harvest (18 September). Both soils were then put back in their pots and these were stood in a tray of water as they were during the growing period. A further count of the propagules in both soils was made 3 weeks later. The results are given in Table 7. Even after mixing, the 'potato soil' contained significantly more propagules of the fungus than the control soil both on 18 September and 9 October (as determined by t-tests). However, for each soil there was no significant difference in the numbers detected on these dates (Table 8). That is, the population of the fungus did not change significantly in either soil within 3 weeks of harvest.

(c) On the tuber surface

The numbers of propagules on the tuber surface were determined after harvest in the following way. With a sterile No. 12 cork borer, disks were cut in the skin of tubers, about 1 mm deep. These disks were removed with a sterile scalpel and the disks from the surface only or including 'eyes' were collected separately in two sterile mortars. The numbers and areas of the disks are given in Appendix Table 25. A small amount of sterile

Table 7: Number of propagules per g. dry soil of Fusarium solani var. coeruleum after harvest.

Replicate	Week 21 (18 Sep)		Week 24 (3 Oct)	
	Control Soil	'Potato Soil'	Control Soil	'Potato Soil'
1	2328	6825	2333	2436
2	4656	2275	3500	3654
3	2328	4550	0	4872
4	0	3412	1166	1218
5	1164	2275	0	2436
6	1164	2275	1166	4872
7	0	3412	0	2436
8	0	2275	2333	0
9	3492	5688	3500	1218
10	0	2275	1166	3654
11	2328	2275	2333	1218
12	2328	5688	0	2436
13	0	1137	2333	3654
14	0	4550	0	2436
15	0	2275	1166	4872
Total	19788	51187	20996	41412
Mean	1319.2	3412.4	1399.7	2760.8
t - value	3.60**		2.69*	
D.F.	28		28	

Significance at $P \leqslant 0.05$ and 0.01 denoted by * and ** respectively.

Table 8: (a) Difference between numbers of propagules in 'potato soils' after harvest.

Mixed Potato soil	(21 week)	3412.4
" " "	(24 week)	2760.8
t - value	0.4 N.S.	
D.F.	28	

(b) Difference between the numbers of propagules in control soils after harvest.

Control	(21 week)	1319.0
Control	(24 week)	1399.7
t - value	0.15 N.S.	
D.F.	28	

distilled water was poured on the disks in each mortar and these were crushed and well macerated separately. The suspensions were transferred to two sterile 250 ml. flasks with 25 sterile spherical glass beads in each, and the contents were shaken on a Griffin flask-shaker for ten minutes. After removing, the flasks were shaken vigorously by hand for a few seconds and then immediately the suspensions were passed separately through sterile muslin lining sterile funnels. Any spores remaining in the muslin were removed by washing with further sterile distilled water and the suspensions were made up to 100 ml each. Further dilutions were prepared from these suspensions and these were used to inoculate plates of Boyd's agar which were then incubated as previously described. The best results were obtained with a 3×10^{-3} dilution. Two samples of tubers were used: one of four tubers immediately after lifting and another of three tubers which were stored at 15^{o}c in paper bags for 3 weeks. The full results of these isolations are given in Appendix Table 26 and the mean results are given in Table 9. Storing the tubers for 3 weeks did not appear to affect the numbers of F. solani var. coeruleum on the tuber surface. On the second sample (3 weeks after harvest) there appeared to be more propagules associated with the 'eyes' but as the tubers were not washed, this might only reflect differences in the numbers of small soil particles trapped around eyes and on the tuber surface.

Table 9: Numbers of propagules per cm^2 of tuber surface

	Isolation date	
	Immediately after harvest	3 weeks later
Surface only	5.2	3.9
Surface including 'eyes'	7.6	7.4
t - value	1.49 (N.S.)	2.93**
D.F.	18	18

** Significant at $P < 0.01$

(b) Field Experiment

The experiment was set up with the aim of finding out the effect of host and non-host plants on the population of F. solani var. coeruleum under natural conditions in a soil with a much lower inoculum of the fungus.

Materials and Methods

The trial was sited in Silwood bottom, Silwood Park, facing N.E. on a light , sandy, well-drained soil. Physical structure of the soil is given below (Sayles, 1973):

Gravel	< 2 mm
25.4%	74.6%

Organic Mat. %	Inorganic Mat. %
10.2	89.8

Sand%	Silt%	Clay%
93.1	2.2	4.7

Mineral contents were similar to those mentioned in Glasshouse Experiment 2 a.

The experiment was laid down on 30 April (1975), in an area 4.9 x 10.1 m in which potatoes had been grown in the previous season, and was a completely randomized design with four treatments and eight sampling times. The treatments provided, soils near potato, barley and broad bean roots and soil in an unplanted (control) plot.

Just before sowing, the soil was superficially cultivated by using a rake, then divided into thirty-two 1 m^2 plots separated by 30 cm width gourdrows. Plots were divided into four groups and within each group the sampling order was determined in advance at

random by drawing lots. Each group was labelled with different colour labels attached to canes. The experimental lay out is shown in Figure 11.

Soil samples were taken before sowing to assess the initial level of F. solani var. coeruleum in the soil and also to measure the pH and moisture content. For the fungal counts about 500 g. of soil was taken at random from each of ten different plots. These samples were sieved through a No. 10 mesh (1680 μ) and then bulked. Two 100 g. sub-samples were then taken from which two soil dilutions were made. One ml portions of the dilutions were spread on plates of Boyd's medium which had been hardened for 15-16 days. These tests showed that a 10^{-2} dilution was the best for this soil and throughout the experiment this dilution was used.

Before sowing an area 28 cm in diameter was marked in each 1 m^2 plot and the soil was deeply cultivated with a spade. Then the same number of potato, barley or broad bean seeds were sown in the same way as described in Glasshouse Experiment 2. As the soil was very dry, all plots were watered with 3 litres of water each after sowing. The experimental area was protected with wire netting against rabbits. After crop emergence all plots were hoed and all weeds removed on 25 May, 14 June and 30 June. All necessary cultural practices were completed during stolon formation and on 30 June soil around the potato plants was hoed up to encourage tuber production. As there was a very long dry period the experimental plots were each watered twice with 3 litres of water at the end of May and in mid-June.

Throughout the experiment soils near host (potato) and non-host plants (barley and broad bean) were sampled at 3 week intervals from sowing (30 April) until potato harvest (4 September) in the pre-determined order, on the dates shown in Table 10. On each occasion one

Figure 11 Layout of field trial and sampling order (Silwood Park, 1975)

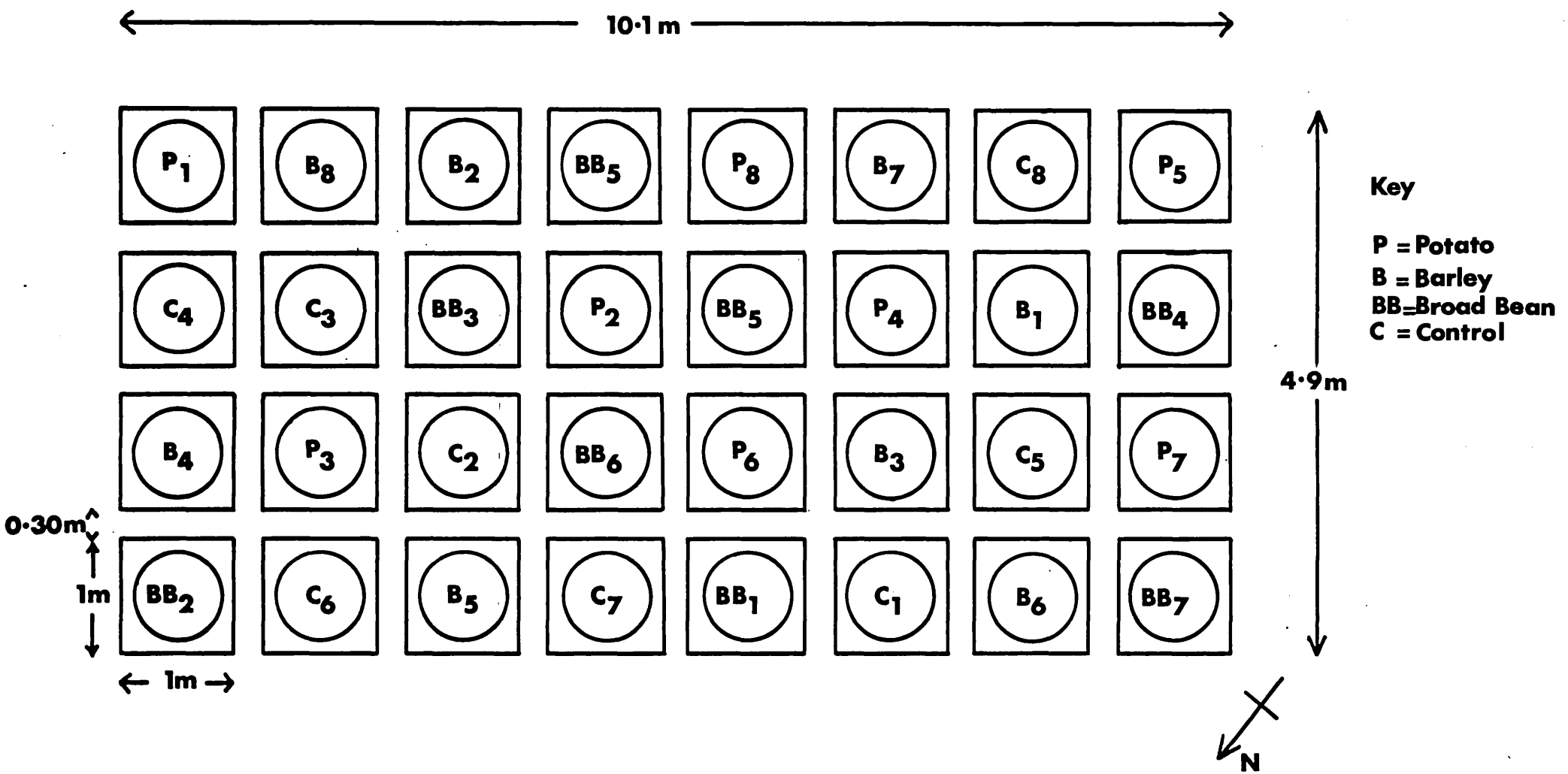


Table 10: Sampling dates (Field Experiment, 1975)

Intervals (week)	Date
Just after sowing	1 May
3	22 May
6	12 June
9	3 July
12	24 July
15	14 August
18	4 September

plot was sampled from each treatment (four plots in all). Soil near roots was taken out with a spade and soil samples were collected near roots of potato, barley and broad beans and also near stolons, young and old tubers and around old bean seeds as described in experiment 2 a. These samples were sieved through a No. 10 mesh (1680 μ) separately. Each sample was mixed well and then 20 g. sub-samples were taken and dilutions of 10^{-2} were prepared. The methods were otherwise similar to those described for the Glasshouse Experiment.

Experimental

Soil pH and moisture capacity.

The soil pH was found to be 5.5 (± 0.06) and the moisture capacity 31.92%.

Crop development

Potatoes produced stolons by the sixth week (12 June) and by the ninth week they had produced young tubers. The plants rather stunted because of the dry periods throughout the season but they produced a good yield.

Broad beans started to produce pods on 10 July and completely dried on 28 August.

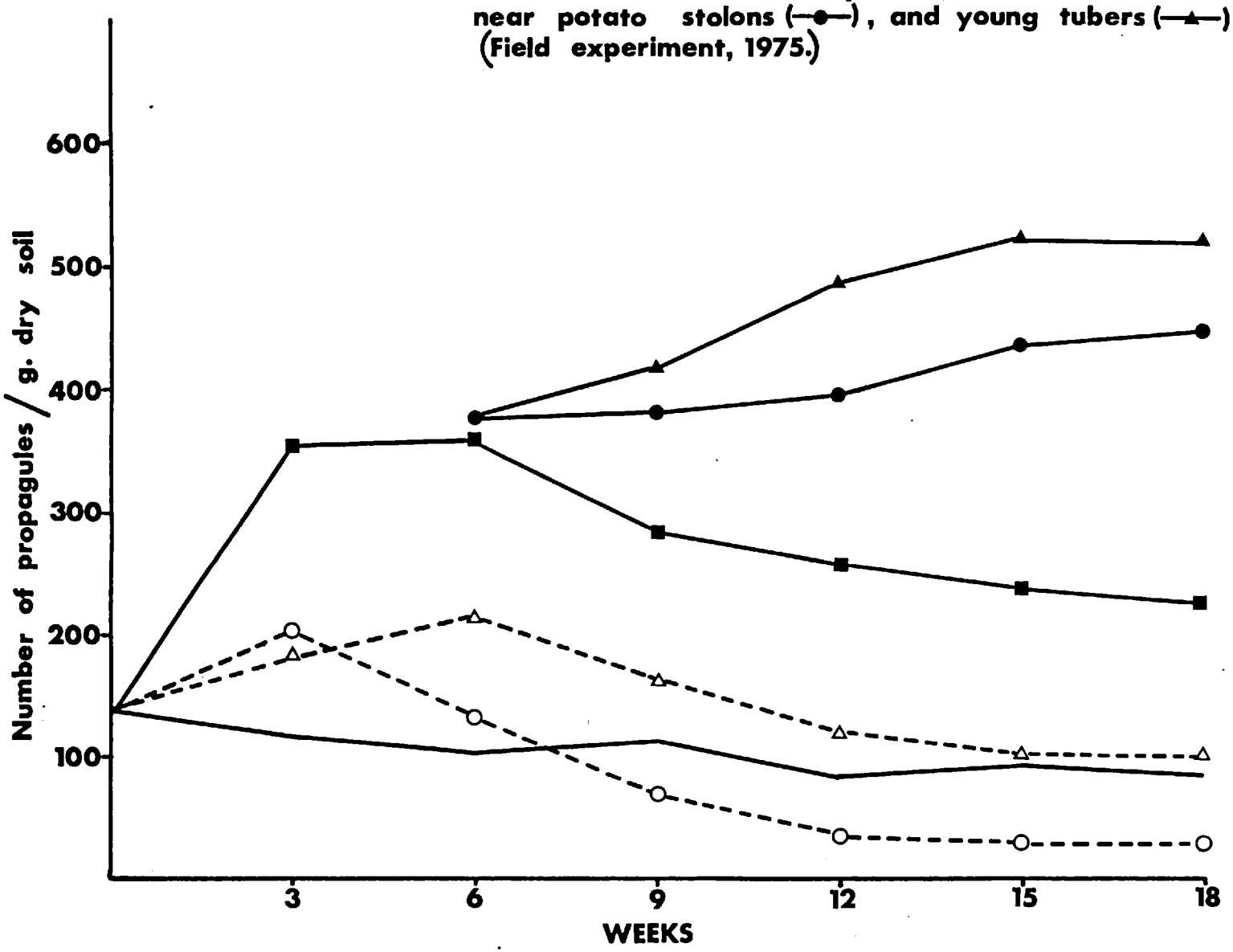
The barley reached maturity by August by which time the broad beans had started to yellow. By the beginning of September the plants were all dried.

Behaviour of chlamydospores near potato, barley and broad bean

The numbers of propagules isolated from the various plots throughout the growing season are given in Appendix Tables 27 and 28. The population of the fungus was very low throughout the site at the beginning of the experiment (Appendix Table 27) and in the unplanted (control) plots it remained low throughout the experiment (Figure 12). In soil near barley and broad bean roots there were slight increases in numbers of propagules 3-6 weeks after planting but then the numbers decreased again and by week 15, were not different to those of the control. In plots with potatoes the number of propagules of the fungus rapidly increased within the first 3 weeks and stayed stable for another 3 weeks. These increases were associated with the presence of potato roots. When stolons and young tubers began to form, after week 6, there were marked increases in the numbers of *F. solani* var. *coeruleum* in soil near them

Figure 12

Population of Fusarium solani var. coeruleum in control soil (——), in soil near roots of barley (--△--), broad bean (--○--), potato (—■—), near potato stolons (—●—), and young tubers (—▲—). (Field experiment, 1975.)



and these continued to week 18. During this period the numbers associated with roots declined. The overall result was that there were significant differences between the populations around stolons and new tubers and those around potato roots. Similarly there were significantly more propagules in soil taken around potato roots than in soil around either barley or broad bean roots or in soil from the unplanted control.

These results were similar to those found in Glasshouse Experiment 2 a.

The old potato (mother) tuber had some effect on the population up to week 6, whereas the broad bean seeds had no effect at all.

DISCUSSION

The isolate of F. solani var. coeruleum used in these experiments grew best and produced most conidia on potato-sucrose agar of the five media tested (Figure 1; Appendix Table 1). On this medium at 20°c it formed a colony c. 3.0 cm diameter in 4 days and this agrees with the results of Booth (1971) for this fungus. The optimum temperature for growth was 20°c, also similar to that found by Booth. (Figure and Appendix Table 2).

Abundant chlamydospores were formed in all media tested, except the solution containing monobasic potassium phosphate and magnesium sulphate amended with glucose or magnesium carbonate. However, the time taken to produce these chlamydospores was rather longer than that reported by other workers. For example, Qureshi & Page (1970) stated that abundant chlamydospores of F. oxysporum formed within 3-4 days in a two-salt solution but F. solani var. coeruleum did not produce many chlamydospores in this time. Even when chlamydospores were formed many of them apparently were not mature because on drying chlamydospores on slides the germination fell from about 79% to 43%. When dried films were stored at different temperatures most of the chlamydospores lost their viability within 42 days and this decline was especially rapid at the higher temperatures. At temperatures below 20°c there was initially an improvement in germination following the first few days of storage. The reasons for this are not clear but it could have been due to a further maturation of some spores induced by the lower temperatures.

When slides with dried chlamydospores were placed in soil some spores germinated. This is in contrast to the lack of germination found by Schroth & Hendrix (1962) when chlamydospores of F. solani f. phaseoli were added to non-rhizosphere soil. It could be that in this soil

there were sufficient organic materials left from the previous potato crop to provide sufficient carbon and nitrogen compounds to overcome the soil fungistasis. Alternatively, this germination could be similar to the so-called 'spontaneous germination' observed when some resting spores are first added to soil, for example, those of Plasmodiophora brassicae and Theilaviopsis basciola (Garrett, 1970). On slides placed in soil only the amount of germination did not increase much over one month. In contrast, germination of chlamydospores on similar slides placed near potato roots continued to rise, a clear indication that the chlamydospores of F. solani var. coeruleum are stimulated to germinate near potato roots. It is particularly interesting that in this experiment the final waterlogging (on day 35) of the soil resulted in lysis of many germ tubes and spores. Obviously under these excessively wet conditions which probably result in lack of oxygen the fungus is unable to produce new chlamydospores. This has been noted with other soil fusaria (Newcombe, 1960).

Further evidence for a stimulation of germination by potato roots was obtained from the experiment in which potato, barley and broad beans were grown in pots of soil with a high population of chlamydospores. This experiment indicated, however, that chlamydospores germinated equally well near the roots of the non-host barley and broad bean. In this respect the fungus behaved similarly to F. solani f. phaseoli, the chlamydospores of which were found by Schroth & Hendrix (1962) to germinate near the roots of sixteen non-susceptible plants. These authors found that later the germ-tubes lysed. It would seem likely that a similar sequence of events occurred with F. solani var. coeruleum because counts of propagules near roots of barley and broad bean using Boyd's agar showed an initial rise in numbers followed by a decline to levels similar to those in soil alone. In contrast the fungal population increased near potato roots,

stolons and young tubers (Experiment 2a and 2b). The extent of these increases varied. The population near young tubers was always higher than that near stolons and this in turn was higher than that near young roots. The reasons for this are not known but possibly the exudates from these plant parts differ (Frenzel, 1960; Rovira, 1965; Schroth & Snyder, 1961). This could be related to differences in the quality and quantity of the amino-acids and sugars exuded (Garrett, 1970). Equally, the greater number of propagules near young tubers could be due to the large surface area which these present to the surrounding soil. This line of research needs further investigation. It is especially interesting because whereas the association of F. solani f. phaseoli with the bean host leads to a pathogenic relationship in the growing plant, with F. solani var. coeruleum it does not. There thus appears to be a host specific effect distinct from a parasitic or pathogenic relationship. The results suggest that F. solani var. coeruleum continues to grow near or on the underground parts of the potato following chlamydospore germination and that this growth is particularly favoured by exudates from young tissue. Thus there appeared to be a levelling-off of the number of propagules near roots as these matured. Support for the present results comes from the work of Schippers (1962) and of Boyd (1971), Both investigators found a higher level of F. solani var. coeruleum in soils from potato riddles than in the field soil in which the potatoes had been grown. This itself suggests that the tubers influence the population of the fungus.

In the pot experiments described, the number of propagules after harvesting the potatoes was still much higher than in the control soil, even after the soil had been mixed. Unfortunately there was not sufficient time to study further the population changes in these soils. This would be worth investigating especially in relation

to the field situation. Presumably in the field the population of F. solani var. coeruleum will also be high in the areas previously occupied by potatoes. These levels will inevitably be decreased by the mixing of the soil through cultivation but it is not known how well these levels are then maintained or indeed how they are affected by further crops.

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A P P E N D I X

APPENDIX

Table 1: Growth rate of the fungus on different media (cm)

Intervals Day	Replica.	MEDIA PSA	Nash & Sn.	V-8 Juice	PDA	Papa-vizas
4	1	3.2	2.1	3.4	0.6	0.1
	2	3.0	1.8	3.6	0.6	0.2
	3	2.9	2.0	3.4	0.8	0.1
	Mean	3.0	1.9	3.4	0.6	0.1
7	1	4.8	2.8	4.7	1.1	0.3
	2	5.0	2.8	4.8	1.0	0.3
	3	4.7	2.7	4.7	1.2	0.4
	Mean	4.8	2.7	4.7	1.1	0.3
10	1	7.0	3.5	6.0	1.3	0.5
	2	6.9	3.4	5.8	1.1	0.5
	3	6.6	3.3	5.8	1.4	0.6
	Mean	6.8	3.4	5.8	1.2	0.5
13	1	8.4	4.3	7.5	1.5	0.6
	2	8.3	4.0	7.3	1.4	0.7
	3	8.0	4.1	7.4	1.7	0.7
	Mean	8.2	4.1	7.4	1.5	0.6

APPENDIX

Table 2: Growth rate of the fungus at different temperatures. (cm)

Intervals (Day)	Replica	Temperature 15°c	20°c	25°c
4	1	1.1	3.0	0.8
	2	1.2	2.8	1.0
	3	1.2	2.9	1.0
	Mean	1.1	2.9	0.9
7	1	3.6	4.8	2.4
	2	3.4	4.5	2.5
	3	3.3	4.6	2.3
	Mean	3.4	4.6	2.4
10	1	5.7	7.0	3.1
	2	5.8	7.2	3.1
	3	5.7	6.9	3.0
	Mean	5.7	7.0	3.0
13	1	7.3	8.5	3.7
	2	7.5	8.6	3.6
	3	7.3	8.6	3.6
	Mean	7.3	8.5	3.6

APPENDIX

Table 3: Production of Chlamydospores of Fusarium solani var. coeruleum in soil extract with time.

Time (day)	Sample	Chlamydospore/ml.
3	1	48.000
	2	58.000
	3	48.000
	4	40.000
	5	64.000
	Mean	51.600
5	1	152.000
	2	128.000
	3	160.000
	4	144.000
	5	168.000
	Mean	150.400
7	1	152.000
	2	160.000
	3	136.000
	4	136.000
	5	176.000
	Mean	152.400
9	1	3.176.000
	2	2.968 000
	3	2.848.000
	4	2.976.000
	5	2.992.000
	Mean	2.992.000

APPENDIX

Table 3: (continued)

Time (day)	Sample		Chlamydospore/ml
11	1		5.872.000
	2		6.440.000
	3		6.312.000
	4		5.624.000
	5		5.112.000
		Mean	5.872.000
13	1		8.808.000
	2		7.976.000
	3		9.520.000
	4		9.336.000
	5		8.400.000
		Mean	8.808.000
15	1		11.496.000
	2		11.920.000
	3		11.576.000
	4		11.864.000
	5		10.624.000
		Mean	11.496.000

APPENDIX

Table 4: Viability of the Chlamydospores produced in soil extract. (Response to 0.5% Glucose).

Replicates	Chl. No. Counted	No. Germ.	% Germ.
1	266	216	81.20
2	307	244	79.48
3	257	215	83.66
4	292	244	83.56
5	257	220	85.60
		Mean	82. 7

Table 5: Viability of the Chlamydospores produced in soil extract. (Response to Papavizas' (1967) Peptone-PCNB Modified Agar).

Replicates	Chl. No. Counted	No. Germ.	% Germ.
1	117	99	84.62
2	116	108	93.10
3	113	100	88.49
4	140	122	87.14
5	123	106	86.17
		Mean	87. 9

APPENDIX

Table 6: Viability of the Chlamydospores prior to drying on coverslips. (Response to 0.5% Glucose).

Replicates	No.Counted	No.Germinated	% Germination
1	380	309	81.31
2	272	228	83.82
3	324	252	77.77
4	432	363	84.02
5	347	299	86.17
6	395	274	69.36
7	463	352	76.02
8	507	389	76.72
9	473	369	78.01
		Mean	(79.24)

APPENDIX

Table 7: Viability of Chlamydospores just after drying films of suspension on slides. (Response to 0.5% Glucose).

Replicate	No.Counted	No.Germinated	% Germination
1	98	33	33.67
2	111	53	47.75
3	85	58	68.24
4	64	21	32.81
5	90	28	31.11
6	96	26	27.08
7	93	48	51.61
8	113	46	40.71
9	95	49	51.58
		Mean	42.73

Table 8: Viability of Chlamydospores just after drying films of suspension on slides. (Response to Papavizas' agar).

Replicate	No.Counted	No.Germinated	% Germination
1	98	50	51.02
2	70	27	38.57
3	114	44	38.60
		Mean	42.73

APPENDIX

Table 9: Longevity of Chlamydospores in dry conditions at different temperatures. (Response to 0.5% Glucose).

Day	Temperature °C	Replicates	No. Counted	No. Germ.	% Germ.	Angular Transformation Figure
1	5°c	1	132	28	21.21	27.42
		2	179	51	28.49	32.20
		3	165	27	16.36	23.81
				Mean	22.02	27.81
	10°c	1	199	33	16.58	23.97
		2	204	35	17.15	24.43
		3	141	26	18.43	25.40
				Mean	17.38	24.60
	15°c	1	147	39	19.72	26.35
		2	202	44	21.78	27.76
		3	99	37	37.37	37.64
				Mean	26.29	30.58
	20°c	1	186	28	15.05	22.79
		2	125	23	18.40	24.50
		3	147	24	16.32	23.81
				Mean	16.59	24.00
	25°c	1	138	23	16.66	24.04
		2	185	40	21.62	27.69
		3	228	45	19.73	26.35
				Mean	19.33	26.03

APPENDIX

Table 9: (Continued)

Day: 1

Analysis of Variance

	Degrees of Freedom (D.F.)	Sums of Squares (S.S.)	Means of Squares (M.S.)	Variance Ratio (F.)
Tempera-tures	4	82.25	21.31	1.74 N.S.
Error	10	122.45	12.24	
Total	14	207.70		

S.E. = $\bar{+}$ 2.02

APPENDIX

Table 9: (Continued)

Days	Temperature °C	Replicates	No. Counted	No. Germ.	% Germ.	Angular Transformation Figure
3	5°c	1	146	15	10.27	18.63
		2	169	26	15.38	23.03
		3	138	26	18.84	27.70
				Mean	14.83	22.45
	10°c	1	171	3	1.75	7.49
		2	108	3	2.77	9.46
		3	163	2	1.22	6.29
				Mean	1.91	7.75
	15°c	1	152	20	13.15	21.22
		2	229	9	3.93	11.39
		3	114	4	3.50	10.78
				Mean	6.86	14.46
	20°c	1	264	145	54.92	47.81
		2	142	68	47.88	43.74
		3	141	28	19.85	26.42
				Mean	40.88	39.32
	25°c	1	156	33	21.15	27.35
		2	130	4	3.07	9.98
		3	101	10	9.90	18.34
				Mean	11.37	18.56

APPENDIX

Table 9: (Continued)

Day: 3

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Temperatures	4	1682.99	420.74	8.27**
Error	10	508.23	50.82	
Total	14	2191.22		

S.E. = $\bar{+}$ 4.1 ** Significant at 1% level

To test differences between temperatures (Duncan's test).

$$D_n = R_n \cdot S\bar{x} \qquad S\bar{x} = \sqrt{\frac{S^2}{n}}$$

$$S\bar{x} = 4.11$$

10°	15°c	25°c	5°c	20°c
7.75	14.45	18.56	22.45	39.42

Means not underscored by the same line are significantly different ($P \leqslant 0.05$): Duncan's new multiple range test.

APPENDIX

Table 9: (Continued)

Days	Temperature °C	Replicates	No. Counted	No. Germ.	% Germ.	Angular Transformation Figure
7	5°c	1	188	18	9.57	17.95
		2	179	81	42.25	40.51
		3	179	47	26.25	30.79
				Mean	26.02	29.75
	10°c	1	255	19	7.45	15.79
		2	226	14	6.19	14.30
		3	184	2	1.08	5.74
				Mean	4.90	11.94
	15°c	1	161	11	6.83	15.12
		2	179	50	27.93	31.88
		3	180	16	8.88	17.36
				Mean	14.54	21.45
	20°c	1	92	11	11.95	20.18
		2	189	17	8.99	17.36
		3	117	26	22.22	28.11
				Mean	14.38	21.88
	25°c	1	189	14	7.40	15.79
		2	125	13	10.40	18.81
		3	133	4	3.00	9.98
				Mean	6.93	14.86

APPENDIX

Table 9: (Continued)

Day: 7

Analysis of Variance

S.V.	D.F.	S.S.	M.S.	F.
Temperatures	4	576.14	144.03	2.47 N.S.
Error	10	582.91	58.29	
Total	14	1159.05		

S.E. = $\pm$ 4.40

APPENDIX

Table 9: (Continued)

Days	Temperature °C	Replicates	No. Counted	No. Germ.	% Germ.	Angular Transformation Figure
14	5°c	1	181	108	59.66	50.53
		2	314	145	46.17	42.76
		3	202	136	67.32	55.12
				Mean	57.71	49.47
	10°c	1	217	28	12.90	21.05
		2	141	5	3.54	10.78
		3	423	120	28.36	32.14
				Mean	14.93	21.32
	15°c	1	244	100	40.98	39.76
		2	191	37	19.37	26.06
		3	224	71	31.69	37.20
				Mean	30.68	33.34
	20°c	1	112	8	7.14	15.45
		2	107	8	7.47	15.79
		3	104	7	6.73	15.00
				Mean	7.11	15.41
	25°c	1	242	1	0.41	3.67
		2	238	2	0.84	5.26
		3	214	4	1.86	7.71
				Mean	1.03	5.55

APPENDIX

Table 9: (Continued)

Day: 14

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Temperatures	4	3456.56	864.14	21.08**
Error	10	409.85	40.98	
Total	14	3866.41		

** Significant at 1% level

S.E. = $\pm$ 3.69

Duncan's Test

5.55	15.41	21.32	33.34	49.47
25°c	20°c	10°c	15°c	5°c

Means not underscored by the same line are significantly different ($P \leqslant 0.05$)

APPENDIX

Table 9: (Continued)

Days	Temperature °C	Replicates	No. Counted	No. Germ.	% Germ.	Angular Transformation Figure
21	5°c	1	106	4	3.77	11.09
		2	193	15	7.77	16.11
		3	173	9	5.30	13.18
				Mean	5.58	13.46
	10°c	1	126	7	5.55	13.56
		2	201	10	4.97	12.79
		3	204	10	4.90	12.79
				Mean	5.14	13.04
	15°c	1	208	21	10.09	19.28
		2	162	9	5.55	13.56
		3	148	5	3.37	10.47
				Mean	6.60	14.43
	20°c	1	68	3	4.41	12.11
		2	77	7	9.09	17.46
		3	72	2	2.77	9.46
				Mean	5.42	13.01
	25°c	1	148	1	0.67	4.69
		2	176	5	2.84	9.63
		3	126	1	0.79	5.10
				Mean	1.43	6.47

APPENDIX

Table 9: (Continued)

Day: 21

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Temperatures	4	122.07	30.51	3.01 N.S.
Error	10	101.31	10.13	
Total	14	223.38		

S.E. = $\bar{+}$ 1.83

APPENDIX

Table 9: (Continued)

Days	Temp-erature	Repli-cates	No. Counted	No. Germ.	% Germ.	Angular Trans-formation Figure
28	5°c	1	133	12	9.02	17.46
		2	258	14	5.42	13.44
		3	151	3	1.98	7.92
				Mean	5.47	12.94
	10°c	1	190	12	6.31	14.54
		2	217	11	5.06	12.92
		3	157	4	2.54	9.10
—				Mean	4.63	12.18
	15°c	1	216	10	4.62	12.39
		2	206	12	5.82	13.94
		3	174	7	4.02	11.54
				Mean	4.82	12.62
	20°c	1	191	6	3.14	10.14
		2	151	4	2.64	9.28
		3	151	7	4.63	12.39
				Mean	3.47	10.60
	25°c	1	258	9	3.48	10.63
		2	127	2	1.57	7.04
		3	193	2	1.03	5.74
				Mean	2.02	7.80

APPENDIX

Table 9: (Continued)

Day: 28

Analysis of Variance

	D.F.	S.S.	M.S.	F.	
Temperatures	4	53.74	13.43	1.62	N.S.
Error	10	82.42	8.24		
Total	14	136.16			

S.E. = $\bar{+}$ 1.65

APPENDIX

Table 9: (Continued)

Day	Temp-erature	Repli-cates	No. Counted	No. Germ.	% Germ.	Angular Trans-formation Figure
35	5°c	1	124	2	1.61	7.27
		2	182	7	3.84	11.24
		3	157	4	2.54	9.10
				Mean	2.66	9.20
	10°c	1	144	2	1.38	6.55
		2	120	-	0	0
		3	98	1	1.02	5.74
				Mean	0.80	4.09
	15°c	1	169	1	0.59	4.40
		2	148	0	0.0	0
		3	144	0	0.0	0
				Mean	0.19	1.46
	20°c	1	66	0	0.0	0
		2	87	3	3.44	10.63
		3	60	0	0.0	0
				Mean	1.14	3.54
	25°c	1	169	2	1.18	6.02
		2	76	0	0.0	0
		3	149	0	0.0	0
				Mean	0.39	2.00

APPENDIX

Table 9: (Continued)

Day: 35

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Temperatures	4	112.99	28.24	1.93 N.S.
Error	10	145.78	14.57	
Total	14	258.77		

S.E. = $\bar{+}$ 2.2

APPENDIX

Table 9: (Continued)

Days	Temp-erature °C	Repli-cates	No. Counted	No. Germ.	% Germ.	Angular Trans-formation Figure
42	5°c	1	170	2	1.17	6.02
		2	107	1	0.93	5.53
		3	82	0	0.0	0
				Mean	0.70	3.85
	10°c	1	112	1	0.89	5.41
		2	100	0	0.0	0
		3	92	0	0.0	0
				Mean	0.29	1.80
	15°c	1	117	1	0.85	5.29
		2	138	1	0.72	4.87
		3	94	1	1.06	5.74
				Mean	0.87	5.3
	20°c	1	156	3	1.92	7.92
		2	105	1	0.95	5.59
		3	92	3	3.26	10.31
				Mean	2.04	7.94
	25°c	1	95	0	0	0
		2	129	0	0	0
		3	102	0	0	0
				Mean	0.00	0.00

APPENDIX

Table 9: (Continued)

Day: 42

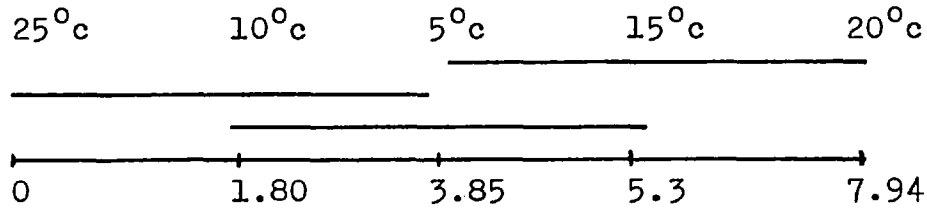
Analysis of Variance

	D.F.	S.S.	M.S.	F.
Temperatures	4	113.44	28.36	5.31 *
Error	10	53.37	5.33	
Total	14	166.81		

* Significant at 5% level.

S.E. = $\pm$ 1.33

Duncan's Test:



Means not underscored by the same line are significantly different ($P \leq 0.05$).

APPENDIX

Table 10: Viability of the chlamydospores prior to drying films of chlamydospores on slides (Response to 0.5% Glucose).

Replicates	No. Chl. Counted	No. Germinated	% Germination
1	240	221	92.08
2	172	148	86.04
3	245	225	91.84
4	134	120	89.55
5	158	142	89.87
		Mean	89.87

Table 11: Viability of the chlamydospores prior to drying films of chlamydospores on slides. (Response to Papavizas agar)

Replicates	No. Chl. Counted	No. Germinated	% Germination
1	104	98	94.23
2	122	117	95.90
3	115	90	85.71
4	116	110	94.82
5	140	128	91.42
		Mean	92.41

APPENDIX

Table 12: Viability of the Chlamydospores just after drying films of Chlamydospores on slides. (Response to 0.5% Glucose).

Replicate	No. Counted	No. Germinated	% Germination
1	100	39	39.00
2	102	30	29.14
3	112	55	49.10
4	161	79	49.06
5	112	37	33.03
		Mean	39.90

Table 13: Viability of the Chlamydospores just after drying films of Chlamydospores on slides. (Response to Papavizas Agar).

Replicate	No. Counted	No. Germinated	% Germination
1	114	63	55.26
2	92	31	33.70
3	125	96	76.80
4	109	23	21.10
5	161	54	33.54
		Mean	44.08

APPENDIX

Table 14: Germination of the Chlamydospores in soil at 10° and 15°c

Day: 1

Temperature °C	Replicate	No. Counted	No. Germ.	% Germ.	Angular Transform
10°c	1	125	5	4.00	11.54
	2	98	1	1.02	6.29
	3	205	2	0.97	5.65
	4	131	4	3.05	9.98
	5	111	2	1.80	7.71
			Mean	2.17	8.23
15°c	1	180	16	8.88	17.26
	2	120	3	2.50	9.10
	3	131	6	4.58	12.25
	4	137	8	5.84	13.94
	5	146	7	4.79	12.52
			Mean	5.32	13.01

APPENDIX

Table 14: (Continued)

Day: 3

Temperature °C	Replicate	No. Counted	No. Germ.	% Germ.	Angular Transform
10°c	1	112	6	5.35	13.31
	2	161	6	3.72	11.09
	3	159	1	0.62	4.52
	4	147	5	3.40	10.63
	5	116	4	3.45	10.63
			Mean	3.31	10.03
15°c	1	202	39	19.31	26.06
	2	265	69	26.03	30.66
	3	298	49	16.44	23.89
	4	108	34	31.48	34.08
	5	155	34	21.94	27.90
			Mean	23.04	28.51

APPENDIX

Table 14: (Continued)

Day: 7

Temperature °C	Replicate	No. Counted	No. Germ.	% Germ.	Angular Transform
10°c	1	281	55	19.57	26.21
	2	143	22	15.38	23.03
	3	144	11	7.63	16.00
	4	172	35	20.34	26.78
	5	138	21	15.21	22.95
			Mean	15.63	22.99
15°c	1	113	18	15.92	23.50
	2	165	8	4.84	12.66
	3	116	14	12.06	20.27
	4	156	34	21.79	27.76
	5	300	21	7.00	15.34
			Mean	12.32	19.90

APPENDIX

Table 14: (Continued)

Day: 11

Temperature °C	Replicate	No. Counted	No. Germ.	% Germ.	Angular Transform
10°c	1	200	22	11.0	19.37
	2	324	56	17.2	24.50
	3	245	13	5.3	13.31
	4	246	28	11.3	19.64
	5	211	25	11.85	20.09
			Mean	11.33	19.38
15°c	1	118	5	4.2	11.83
	2	144	11	7.6	16.00
	3	126	13	10.3	18.72
	4	269	20	7.4	15.79
	5	158	14	8.8	17.26
			Mean	7.66	15.92

APPENDIX

Table 14: (Continued)

Day: 15

Temperature °C	Replicate	No. Counted	No. Germ.	% Germ.	Angular Transform
10°c	1	368	28	7.60	16.00
	2	456	21	4.60	12.39
	3	189	11	5.82	13.94
	4	246	16	6.50	14.77
	5	275	17	6.18	14.30
			Mean	6.14	14.28
15°c	1	339	78	23.01	28.66
	2	173	24	13.87	21.81
	3	293	55	18.77	25.62
	4	196	28	14.29	22.14
	5	176	37	21.02	27.28
			Mean	18.19	25.10

APPENDIX

Table 14: (Continued)

Day: 21

Temperature °C	Replicate	No. Counted	No. Germ.	% Germ.	Angular Transform
10°c	1	141	2	1.41	6.80
	2	73	10	13.69	21.64
	3	145	9	6.20	14.42
	4	112	28	25.00	30.00
	5	68	15	22.05	27.97
			Mean	13.67	20.16
15°c	1	100	21	21.00	27.28
	2	137	25	18.25	21.25
	3	232	29	12.50	20.70
	4	165	33	20.00	26.56
	5	210	26	12.38	20.53
			Mean	16.83	23.26

APPENDIX

Table 14: (Continued)

Day: 28

Temperature °C	Replicate	No. Counted	No. Germ.	% Germ.	Angular Transform
10°c	1	103	30	29.13	32.65
	2	157	12	7.64	16.00
	3	113	15	13.27	21.30
	4	104	22	21.15	27.35
	5	130	18	13.85	21.81
			Mean	17.00	23.82
15°c	1	104	6	5.77	13.81
	2	100	36	36.00	36.87
	3	101	29	28.71	32.39
	4	94	30	31.91	34.39
	5	107	54	50.47	45.23
			Mean	30.57	32.53

APPENDIX

Table 15: Viability of Chlamydospores incubated in soil. (Response to Papavizas Agar).

Time (day)	Temperature	Replicate	No Count.	No. Germ.	% Germ.	Angular transform
7	10°c	1	262	168	64.12	53.19
		2	76	75	98.68	83.20
		3	79	69	87.34	69.12
				Mean	83.38	68.50
	15°c	1	278	209	75.17	60.07
		2	299	200	66.88	54.82
		3	165	161	97.57	80.90
				Mean	79.87	65.26

APPENDIX

Table 15: (Continued)

Time (day)	Temper-ature	Repli-cate	No. Count.	No. Germ.	% Germ.	Angular trans-form
11	10°c	1	118	92	77.97	61.96
		2	156	107	68.59	55.86
		3	158	138	87.34	69.12
				Mean	77.94	62.31
	15°c	1	206	141	68.44	55.80
		2	173	121	69.94	56.73
		3	200	159	79.50	63.08
				Mean	72.62	58.53

APPENDIX

Table 15: (Continued)

Time (Day)	Temper-ature	Repli-cate	No. Counted	No. Germ.	% Germ.	Angular Trans-form
15	10°c	1	184	162	88.04	69.73
		2	105	93	88.57	70.18
		3	167	87	52.10	46.20
				Mean	76.24	62.03
	15°c	1	163	123	75.46	60.27
		2	257	153	59.53	50.48
		3	186	161	86.56	68.44
				Mean	73.85	59.73
21	10°c	1	118	83	70.34	56.98
		2	268	173	64.55	53.43
		3	122	86	70.49	57.04
				Mean	68.46	55.81
	15°c	1	149	94	63.08	52.53
		2	160	116	72.50	58.37
		3	224	169	75.44	60.27
				Mean	70.34	57.05

APPENDIX

Table 15: (Continued)

Time (Day)	Temper-ature	Repli-cate	No. Counted	No. Germ.	% Germ.	Angular Trans-form
28	10°c	1	123	58	47.15	43.34
		2	131	74	56.48	48.68
		3	135	111	82.22	65.05
				Mean	61.95	52.35
	15°c	1	127	65	51.18	45.63
		2	122	75	61.47	51.59
		3	216	111	51.39	45.75
				Mean	54.68	47.65

APPENDIX

Table 16: Viability of the Chlamydospores prior to drying the films of Chlamydospore suspension on slides. (Response to 0.5% Glucose).

Replicate	No. Counted	No. Germinated	% Germination
1	230	218	94.78
2	282	244	86.52
3	148	134	90.54
4	236	215	91.10
5	137	125	91.24
		Mean	90.83

Table 17: Viability of the Chlamydospores prior to drying the films of Chlamydospore suspension on slides. (Response to Papavizas Agar).

Replicate	No. Counted	No. Germinated	% Germination
1	168	156	93.85
2	212	195	91.98
3	170	162	95.29
4	212	202	95.28
5	115	99	86.08
		Mean	92.49

APPENDIX

Table 18: Viability of the Chlamydospores just after drying the films of Chlamydospore suspension on slides. (Response to 0.5% Glucose).

Replicate	No. Counted	No. Germinated	% Germination
1	111	58	52.25
2	123	48	39.02
3	143	60	41.95
4	117	55	47.00
5	127	61	48.08
		Mean	45.66

Table 19: Viability of the Chlamydospores just after drying the films of Chlamydospore suspension on slides. (Response to Papavizas agar).

Replicate	No. Counted	No. Germinated	% Germination
1	102	50	49.01
2	138	79	57.24
3	109	44	40.36
4	128	75	58.59
5	132	48	36.36
		Mean	48.31

APPENDIX

Table 20: Sampling dates of buried slides with dried films of chlamydospores.

Intervals (Day)	Sampling Dates (1974)
3	9 June
7	13 June
14	20 June
21	27 June
28	4 July
35	11 July

APPENDIX

Table 21: Behaviour of the Chlamydospores in soil and near potato roots.

Days	Treatments	Replicates	No. Counted	No. Germ.	No. Germ.	Angular Transform
3	In soil	1	22	7	31.81	32.33
		2	26	4	15.38	23.03
		3	18	7	38.88	38.53
		4	38	6	15.78	23.34
		5	29	9	31.03	33.83
				Mean	26.57	30.61
	Near potato roots	1	34	14	41.17	39.87
		2	31	7	22.58	28.32
		3	31	11	35.48	36.51
		4	70	36	51.42	45.80
		5	58	14	24.13	29.40
				Mean	34.95	35.98

t = 1.184

N.S.

APPENDIX

Table 21: (Continued)

Days	Treatments	Replicates	No. Counted	No. Germ.	% Germ.	Angular Transform
7	In soil	1	89	13	14.60	22.46
		2	92	39	42.39	40.57
		3	111	16	14.41	22.30
		4	29	12	41.37	39.99
		5	70	13	18.57	25.48
				Mean	26.26	30.16
	Near Potato roots	1	26	8	30.76	33.65
		2	70	40	57.14	49.08
		3	23	7	30.43	33.46
		4	39	22	56.41	48.68
		5	46	10	21.73	27.76
				Mean	39.29	38.52

t=1.385
N.S.

APPENDIX

Table 21: (Continued)

Days	Treatments	Replicates	No. Counted	No. Germ.	% Germ.	Angular Transform
14	In soil	1	44	15	34.09	35.67
		2	42	7	16.66	24.04
		3	60	25	41.66	40.16
		4	18	8	44.44	41.78
		5	19	4	21.05	27.08
				Mean	31.58	33.74
	Near Potato roots	1	68	28	41.17	39.87
		2	50	31	62.00	51.94
		3	41	26	63.41	52.77
		4	56	25	44.64	41.90
		5	20	11	55.00	47.87
				Mean	53.24	46.87

$t = 2.99$

$P \leq 0.05$

APPENDIX

Table 21: (Continued)

Days	Treatments	Replicates	No. Counted	No. Germ.	% Germ.	Angular Transform
21	In soil	1	33	9	27.27	31.44
		2	38	13	34.21	35.79
		3	29	9	31.03	33.83
		4	39	11	28.20	32.08
		5	36	12	33.33	35.24
				Mean	30.80	33.67
	Near Potato roots	1	41	27	65.85	54.21
		2	24	10	41.66	40.16
		3	99	40	40.40	39.47
		4	74	45	60.81	51.24
		5	30	20	66.66	54.70
				Mean	55.07	47.95

d = 4.0998 with D.f 4.239 $P \leq 0.05$ (Variances unequal)

APPENDIX

Table 21: (Continued)

Days	Treatments	Replicates	No. Counted	No. Germ.	% Germ.	Angular Transform
28	In soil	1	26	9	34.61	36.03
		2	21	6	28.57	32.27
		3	30	11	36.66	37.23
		4	15	8	53.33	46.89
		5	41	16	39.02	38.65
				Mean	38.43	38.21
	Near Potato roots	1	69	42	60.86	51.24
		2	39	22	56.41	48.68
		3	34	25	73.52	59.02
		4	22	13	59.09	50.18
		5	54	28	51.85	46.03
				Mean	60.34	51.03

$t = 3.94$

$P \leq 0.01$

APPENDIX

Table 22: Behaviour of the Chlamydospores in soil and near potato roots.

Days	Treatments	Replicates	No. Counted	No. Germ.	% Germinat.
35	In soil	1	5	1	20.00
		2	9	3	33.33
		3	17	6	35.29
		4	15	4	26.66
		5	13	2	15.38
				Mean	26.13
	Near Potato roots	1	31	14	45.16
		2	14	6	42.86
		3	45	6	13.33
		4	32	6	18.75
		5	32	15	46.88
				Mean	33.39

APPENDIX

Table 23: Changes in the number of propagules of Fusarium solani var. coeruleum (per g. dry soil) during the growth of potato, barley and broad bean.
(Glasshouse experiment, 1975)

(a) Summary

Soil from pots planted with	Weeks after planting 0	3	6	9	12	15	18	21
No Crop (Control)		10426	5068	1882	1310	1098	1240	1319
Barley - near roots		9138	4179	3043	1464	1146	991	-
Broad Bean - near roots		11359	4449	1732	789	296	434	-
Potato - near roots		11793	4102	2838	2996	3352	3163	2375
- near stolons		-	5043	5111	5790	6129	6196	4633
- near new tubers		-	5189	5201	6511	7631	7364	6413
S.E. Diff. ±		835.2	592.3	541.3	453.0	395.7	413.4	429.6

APPENDIX

Table 23: (Continued)

(b) Detailed results for each sampling date

3rd week

	Control	Barley Root	Broad Bean Root	Broad Bean Seed	Potato Root	Old Tuber
1	13546	12239	11194	16199	15970	8668
2	7389	11015	11194	8722	12285	6191
3	12315	9791	14925	7476	8599	8668
4	8620	7343	13681	9968	13513	4953
5	12315	4895	12437	9968	11056	4953
6	8620	4895	11194	9968	11056	9907
7	12315	13463	1194	8722	13513	7430
8	7389	6119	8706	6320	11056	7430
9	7389	14687	11194	7476	9829	4953
10	13546	6119	8706	6320	14742	6191
11	11083	8567	11194	8722	13513	7430
12	9852	9791	13681	9968	11056	7430
13	11083	11015	12437	7476	9828	6191
14	12315	9791	8706	7476	9828	6191
15	8620	7343	9950	11214	8599	8668
Total	156397	137073	170393	135995	176899	108970
Mean prop/gr. dry soil	10426.4	9138.2	11359.5	9066.3	11793.2	7264.6
Soil moisture content	% 18.8	% 18.3	% 19.6	% 19.75	% 18.6	% 19.25

APPENDIX

Table 23: (Continued)

3rd Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatments	5	212873828	42574765	8.13 **
Error	84	439490254	5232026	
Total	89	652364082		

** Significant at level 1%

To test differences between treatments (Tukey's method)

$W = q_{\alpha}(p, n_2)\ S\bar{x}$

$W_{0.05} = 4.13 \times 590.59$

$W_{0.05} = 2439.13$

Potato Roots	Broad Bean Roots	Control	Barley	Broad Bean Seed	Old Tuber
11793.2	11359.5	10426.4	9138.2	9066.3	7264.6

APPENDIX

Table 23: (Continued)

6th Week

Control	Barley Root	Broad Bean Root	Old Bean Seed	Potato Root	Stolon	Young Tuber	Old Tuber
5847	4250	6355	3177	3296	6578	4385	2229
3508	2125	2118	4237	4395	3289	6578	3344
3508	4250	5296	3177	3296	4385	5482	3344
8187	5313	2118	6355	5494	3289	5482	2229
3508	5313	3177	2118	7692	3289	4385	4459
5847	2125	3177	2118	5494	6578	3289	6688
7017	3188	5296	3177	3296	3289	3289	0
5847	4250	3177	3177	1098	8771	5482	2229
2339	4250	4237	5296	5494	3289	5482	0
4678	4250	7415	1059	2197	3289	6578	3344
3508	4250	3177	2118	2197	6578	6578	3344
4678	3188	4237	4237	4395	6578	6578	3344
7017	5313	4237	4237	7692	5482	5482	4459
5847	5313	4237	3177	3296	6578	4385	2229
4678	5313	8474	1059	2197	4385	4385	5574
Total							
76014	62691	66728	48719	61529	75647	77840	46816
Mean							
5067.6	4179.4	4448.5	3247.9	4101.9	5043.1	5189.3	3121.(
Soil Moisture Content %							
14.5	5.9	5.6	5.6	9.0	8.8	8.8	10.3

APPENDIX

Table 23: (Continued)

6th Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatments	7	67 574 033.0	9 653 433.2	3.66**
Error	112	294 711 950.7	2 631 356.7	
Total	119	362 285 983.8		

** Significant at 1% level

To test differences between treatments (Tukey's Method)

$W = q_{\alpha}(p, n_2)\ S\bar{x}$ $\qquad S\bar{x} = \sqrt{S^2/n}$

q_{α} for 5% = 4.35 $\qquad S\bar{x} = 418.8$

$W_{0.05} = 1821.7$

Young Tuber	Control	Stolon	Broad Bean	Barley	Potato Root	Old Bean Seed	Old Tuber
5189.3	5067.6	5043.1	4448.5	4179.4	4101.9	3247.9	3121.0

Means not underscored by the same line are significantly different ($P \leq 0.05$)

APPENDIX

Table 23: (Continued)

9th Week

Replicate	Control	Barley Root	Broad Bean (Root)	Old Bean Seed	Potato Root	Potato Stolon	Young Tuber	Old Tuber
1	4705	3504	0	0	3546	7777	6593	4667
2	2352	2336	2475	0	0	3333	6593	0
3	2352	1168	0	0	2364	5555	5494	1166
4	2352	5481	1237	1237	7092	7777	4395	2333
5	4705	0	0	1237	3564	5555	4395	1166
6	1176	5481	1237	1237	1182	4444	7692	2333
7	1176	5481	1237	1237	1182	4444	7692	2333
8	0	3504	2475	1237	2364	5555	3296	2333
9	0	1168	1237	1237	1182	5555	3296	1166
10	1176	3504	3712	0	3546	3333	4395	3500
11	1176	5481	4950	2475	3546	4444	5494	0
12	2352	2336	4950	0	3546	3333	6593	3500
13	0	2336	0	1237	3546	4444	5494	2333
14	4705	3504	1237	1237	2364	5555	4395	2333
15	0	1168	2475	0	3546	5555	4395	1166
Total	28227	45643	25985	11134	42570	76659	78014	29162
Mean	1881.8	3042.8	1732.2	742.2	2838.0	5110.6	5200.9	1944.1
% Soil Moisture cont.	15.0	14.4	19.2	19.2	15.4	10.0	9.0	14.3

APPENDIX

Table 23: (Continued)

9th Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatments	7	271 687 191.7	38 812 455.9	17.66 **
Error	112	246 147 564.6	2 197 746.1	
Total	119	517 834 756.3		

Tukey's W procedure

$W = q_{\alpha}(p, n_2) \quad S\bar{x}$

$W_{0.05} = 4.35 \times 382.7$

$W_{0.05} = 1665.0$

Young Tuber	Stolon	Barley	Potato Root	Old Tuber	Con-trol	B.Bean Root	Old Bean Seed
5200.9	5110.6	3042.8	2838.0	1944.1	1881.8	1732.3	742.2

APPENDIX

Table 23 (Continued)

12th Week

	Control	Barley Root	Broad Bean Root	Potato Root	Potato Stolon	Potato Young Tuber	Potato Old Tuber
1	3468	0	1183	5617	5714	6976	0
2	1156	3468	0	2247	4571	6976	0
3	1156	4624	0	3370	6857	5813	1176
4	2312	3468	1183	5617	5714	4651	1176
5	0	0	0	3370	4571	5813	0
6	2312	1156	0	2247	5714	5813	2352
7	0	3468	0	3370	6857	6976	0
8	0	0	0	3370	5714	6976	0
9	1156	0	0	0	5714	8139	0
10	1156	0	0	3370	5714	6976	1176
11	0	2312	4733	1123	8000	6976	0
12	2312	1156	1183	0	4571	5813	2352
13	2312	0	1183	4494	6857	6976	0
14	2312	1156	1183	3370	4571	5813	1176
15	0	1156	1183	3370	5714	6976	0
Total	19652	21964	11831	44935	86853	97663	9408
Mean prop/g dry soil	1310.1	1464.2	788.7	2995.6	5790.2	6510.8	627.2
% Soil Moisture Cont.	13.5	13.5	15.5	11.0	12.5	14.0	15.0

APPENDIX

Table 23: (Continued)

12th Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatments	6	537 234 894.8	89 539 149.1	58.16**
Error	98	150 872 236.1	1 539 512.6	
Total	104	688 107 130.9		

Tukey's W Procedure

$W = q_{\alpha}(p, n_2)\ S\bar{x}$

$W = 4.26 \times 320.36$
0.05

$W = 1364.7$
0.05

Young Tuber	Stolon	Potato Root	Barley	Control	Broad Bean	Old Tuber
6510.8	5790.2	2995.6	1464.2	1310.1	788.7	627.2

APPENDIX

Table 23: (Continued)

15th Week

	Control	Barley Root	Broad Bean Root	Potato Root	Potato Stolon	Potato Young Tuber	Potato old Tuber
1	0	1146	0	4571	8325	8325	1162
2	0	2293	0	3428	4705	8325	1162
3	1176	1146	2222	2285	7058	5882	0
4	1176	1146	0	3428	5882	5882	0
5	0	0	0	3428	4705	7058	0
6	0	2293	0	5714	7058	9411	0
7	2352	2293	1111	2285	5882	7058	2325
8	2352	1146	0	2285	5882	5882	1162
9	1176	1146	0	4571	5882	8325	1162
10	1176	0	0	4571	4705	8325	0
11	1176	0	0	3428	4705	9411	0
12	3529	1146	0	0	5882	9411	1162
13	2352	1146	0	2285	7058	7058	1162
14	2352	1146	0	2285	5882	7058	0
15	0	1146	1111	5714	8325	7058	0
Total		17193	4444	50278	91936	114469	9297
Mean	1097.7	1146.2	296.2	3351.8	6129.0	7631.2	619.8
% Soil Moisture Cont.	15.0	12.8	10.0	12.5	15.0	15.0	14.0

APPENDIX

Table 23: (Continued)

15th Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatments	6	761 638 472.4	126 939 745.4	108.0**
Error	98	115 140 595.1	1 174 906.7	
Total	104	876 779 337.5		

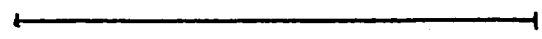
Tukey's Test

$W = q_{\alpha}(p,n_2)\ S\bar{x}$

$W_{0.05} = 4.26 \times 279.86$

$W_{0.05} = 1192.2$

Young Tuber	Stolon	Potato Root	Barley	Control	Old Tuber	Broad Bean
7631.2	6129.0	3351.8	1146.2	1097.7	619.8	296.2



APPENDIX

Table 23: (Continued)

18th Week

	Control	Barley Root	Broad Bean Root	Potato Root	Potato Stolon	Potato Young Tuber
1	2325	1144	0	3389	7058	5813
2	1162	1144	0	2259	5882	5813
3	1162	0	2169	3389	5882	8139
4	2325	0	1084	3389	5882	9302
5	1162	2288	0	3389	7058	5813
6	1162	1144	0	4519	2352	5813
7	1162	1144	0	2259	7058	6976
8	0	2288	0	3389	5882	6976
9	0	2288	0	0	5882	8139
10	1162	0	0	0	5882	8139
11	1162	0	1084	3389	5882	9302
12	2325	1144	0	2259	5882	9302
13	2325	1144	0	2259	5882	9302
14	1162	1144	0	3389	7058	4651
15	0	0	0	4519	5882	9302
Total	18596	14872	6505	47446	92933	110456
Mean	1239.7	991.0	433.6	3163.0	6195.5	7363.7
% Soil Moisture Content	14.0	12.6	7.8	11.5	15.0	14.0

APPENDIX

Table 23: Continued

18th Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatment	5	640 174 254.8	128 034 850.9	99.87**
Error	84	107 687 831.5	1 281 997.9	
Total	89	747 862 086.3		

Tukey's Test

$W = q_{\alpha}(p, n_2)\ S\bar{x}$

$W = 4.13 \times 292.34$
0.05

$W = 1209.1$
0.05

Young Tuber	Stolon	Potato Root	Control	Barley	Broad Bean
7363.7	6195.5	3163.0	1239.7	991.0	433.6

APPENDIX

Table 23: (Continued)

21st Week

Replicate	Control	Potato Root	Potato Stolon	Young Tuber
1	2328	9195	5605	4750
2	4656	4597	8968	7125
3	2328	1149	3363	13064
4	0	4597	7847	9501
5	1164	1149	3363	5938
6	1164	2298	3363	5938
7	0	2298	4484	8313
8	0	0	4484	3562
9	3492	4597	3363	9501
10	0	0	1121	4750
11	2328	1149	4484	7125
12	2328	0	1121	5938
13	0	0	4484	4750
14	0	2298	8968	2375
15	0	2298	4484	3562
Total	19788	35625	69502	96192
Mean	1319.2	2375.0	4633.4	6412.8
% Soil Moisture Content	14.1	13.0	10.8	15.8

APPENDIX

Table 23: (Continued)

21st Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatments	3	1 049 605 277.3	349 868 425.7	63.17**
Error	56	310 132 418.5	5 538 078.9	
Total	59	1 359 737 695.8		

** Significant at level 1%

Tukey's W Procedure

$W = q_{\alpha}(p, n_2)\, S\bar{x}$

$W_{0.05} = 3.75 \times 607.62$

$W_{0.05} = 2278.5$

Young Tuber	Stolon	Potato Root	Control
6412.8	4633.4	2375.0	1319.2

APPENDIX

Table 24: The number of propagules in soil just after infesting soil with chlamydospores.

Replicate	Number of chlamydospores /gr dry soil	
	Sample A	Sample B
1	18244	11402
2	13683	17103
3	11402	15963
4	13683	15963
5	18244	11402
6	11402	11402
7	13683	12542
8	13683	21664
9	20254	19384
10	10262	15963
11	13683	12542
12	14823	13683
13	13683	14823
14	11402	15963
15	13683	17103
Total	212084	226902
Mean	14138.9	15126

General Mean: 14632

Soil Moisture Content: 12.3%

APPENDIX

Table 25: Numbers and areas of disks of tuber skins

	Tuber No.	Surface including 'eyes'		Surface only	
		No. of disks	cm^2 area	No. of disks	cm^2 area
Immediately after harvest	1	6	17.01	13	36.85
	2	8	22.68	10	28.35
	3	5	14.17	14	39.69
	4	9	25.51	15	42.52
	Total	28	79.37	52	147.41
3 weeks later	1	7	19.84	12	34.02
	2	7	19.84	13	36.85
	3	5	14.17	11	31.18
	Total	19	53.85	36	102.05

APPENDIX

Table 26: Numbers of propagules per cm^2 of tuber surface.

Immediately after harvest

Replicate	Surface only		Surface including 'eyes'	
	No.colonies /petri-dish	No. propagules /cm^2	No. colonies /petri-dish	No. propagules /cm^2
1	5	11.30	1	4.19
2	3	6.78	2	8.39
3	2	4.53	2	8.39
4	3	6.78	1	4.19
5	0	0	3	12.59
6	3	6.78	1	4.19
7	0	0	2	8.39
8	4	9.06	1	4.19
9	2	2.26	2	8.39
Total	23	52.02	18	75.50
Mean	2.3	5.20	1.8	7.55

APPENDIX

Table 26: (Continued)

3 weeks later

Replicate	Surface only		Surface including 'eyes'	
	No.colonies /petri-dish	No. prop-agules /cm^2	No.colonies /petri-dish	No. prop-agules /cm^2
1	3	5.87	1	3.71
2	4	7.83	2	7.42
3	2	3.91	2	7.42
4	2	3.91	1	3.71
5	1	1.95	3	11.14
6	3	5.87	2	7.42
7	1	1.95	1	3.71
8	2	3.91	3	11.14
9	0	0	3	11.14
10	2	3.91	2	7.42
Total	20	39.11	21	74.23
Mean	2.0	3.91	2.1	7.42

APPENDIX

Table 27: Numbers of propagules in field soil before sowing.

Replicate	Sample A	Sample B
1	116	233
2	116	116
3	0	116
4	233	0
5	116	233
6	233	0
7	467	116
8	116	0
9	0	466
10	233	0
11	0	116
12	233	116
13	116	0
14	233	233
15	116	350
16	116	0
17	233	233
18	0	0
19	116	116
20	0	116
Total	2793	2560
Mean	139.6	128.0
% Soil Moisture Content	14.5	14.3

APPENDIX

Table 28: Changes in the number of propagules of Fusarium solani var. coeruleum (per g. dry soil) during the growth of potato, barley and broad bean. (Field experiment, 1975).

(a) Summary

Soil from plots planted with:	Weeks after planting 0	3	6	9	12	15	18
No crop (Control)		119	101	111	83	90	84
Barley - near roots		183	216	161	118	99	100
Broad bean - near roots		201	130	70	34	27	28
Potato - near roots		356	357	282	256	237	224
- near stolons		-	378	382	395	439	447
- near new tubers		-	-	418	486	523	517
S.E. Diff. ±		41.4	34.9	32.5	36.9	32.5	39.6

APPENDIX

Table 28: (Continued)

(b) Detailed results for each sampling date.

3rd Week

Replicate	Control	Barley	Broad Bean Root	Broad Bean Seed	Potato Root	Old Tuber
1	223	220	224	224	465	0
2	0	330	112	0	582	348
3	111	0	224	0	232	348
4	0	110	224	112	232	232
5	223	220	224	337	232	0
6	111	330	224	224	349	465
7	223	110	224	224	465	348
8	111	220	112	112	465	348
9	111	0	224	112	465	0
10	111	220	224	224	232	232
11	111	220	337	112	349	348
12	111	330	112	224	582	348
13	223	110	112	112	232	465
14	111	110	224	224	232	0
15	0	220	224	224	232	232
Total	1780	2750	3025	2465	5346	3714
Mean prop/gr dry soil	118.6	183.3	201.6	164.3	356.4	247.6
% Soil Moist.	10.5	9.3	11.0	11.0	14.1	14.0

APPENDIX

Table 28(b): (Continued)

3rd Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatments	5	510 457.4	102 091.4	7.91**
Error	84	1 083 856 5	12 903.0	
Total	89	1 594 313.9		

Significance at $P \leqslant 0.01$ denoted by **

Tukey's W Procedure

$W = q_{\alpha}(p, n_2) . S\bar{x}$

$W_{0.05} = 4.14 \times 29.3$

$W_{0.05} = 121.3$

Potato Root	Old Tuber	Broad Bean	Barley	Broad Bean Seed	Control
356.4	247.6	201.6	183.3	164.3	118.6

Means not underscored by the same line are significantly different ($P \leqslant 0.05$)

APPENDIX

Table 28(b): (Continued)

6th Week

	Control	Barley Root	Broad Bean Roots	Broad Bean Seed	Potato Root	Stolon	Old Tuber
1	217	216	109	109	328	656	328
2	108	325	109	109	438	437	328
3	0	325	328	109	328	218	218
4	0	216	0	218	328	328	218
5	108	108	109	0	219	437	109
6	0	325	218	0	328	437	218
7	217	325	0	109	328	218	109
8	108	0	0	0	438	328	328
9	217	216	0	109	438	328	218
10	108	108	218	109	328	328	0
11	108	216	218	0	438	437	328
12	108	325	218	218	328	547	109
13	108	216	109	218	328	328	328
14	108	216	109	109	328	328	218
15	0	108	218	0	438	328	437
Total	1515	3245	1963	1417	5361	5683	3494
Mean	101.0	216.3	130.8	94.4	357.4	378.8	232.9
% Soil Moisture cont.	8.0	7.7	8.6	8.6	8.8	8.6	8.6

APPENDIX

Table 28(b): (Continued)

6th Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatments	6	1 230 740.8	205 123.4	22.37 **
Error	98	898 369.1	9 167.0	
Total	104	2 129 109.9		

Tukey's W Procedure

$W = q_{\alpha}(p, n_2) \; S\bar{x}$

$W_{0.05} = 4.26 \times 24.7$

$W_{0.05} = 105.2$

Stolon	Potato Root	Old Tuber	Barley	Broad Bean	Control	Broad Bean Seed
378.8	357.4	232.9	216.3	130.8	101.0	94.4

Means not underscored by the same line are significantly different ($P \leqslant 0.05$)

APPENDIX

Table 28(b): (Continued)

9th Week

Replicate	Control	Barley Root	Broad Bean Root	Bean Seed	Potato Root	Stolon	Young Tuber	Old Tuber
1	111	105	211	0	217	319	638	210
2	111	210	105	105	326	319	531	315
3	111	0	105	210	326	319	319	0
4	222	315	0	0	217	425	425	315
5	0	210	0	0	217	531	425	0
6	111	210	0	0	434	531	425	315
7	111	210	0	0	217	319	531	210
8	222	105	0	105	217	425	212	210
9	0	105	105	105	326	319	319	0
10	0	105	105	0	217	319	425	105
11	222	210	105	105	105	217	425	0
12	222	210	105	105	326	319	425	105
13	111	105	105	0	326	425	425	315
14	0	105	0	0	217	425	531	210
15	111	210	105	105	434	319	212	210
Total	1665	2415	1051	840	4234	5739	6268	2520
Mean	111.0	161.0	70.0	56.0	282.2	382.6	417.8	168.0
% Soil Moisture Cont.	10.0	5.0	5.3	5.0	8.0	6.0	6.0	5.0

APPENDIX

Table 28(b): (Continued)

9th Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatment	7	2 030 451.6	290 064.5	36.55 **
Error	112	888 813.2	7 935.8	
Total	119	2 919 264.8		

** Significant at 1% level

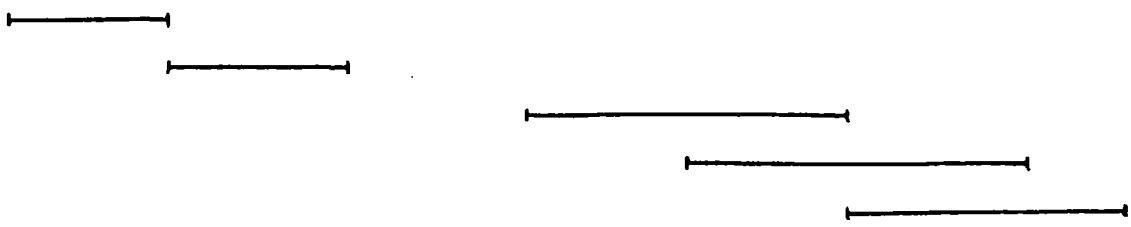
Tukey's W Procedure

$W = q_{\alpha}(p, n_2) \; S\bar{x}$

$W_{0.05} = 4.39 \times 23$

$W_{0.05} = 100.9$

Young Tuber	Stolon	Potato Root	Old Tuber	Barley	Control	Broad Bean	Bean Seed
417.8	382.6	282.2	168.0	161.0	111.0	70.0	56.0



APPENDIX

Table 28(b): (Continued)

12th Week

	Control	Barley	Broad Bean Root	Potato Root	Stolon	Young Tuber	Old Tuber
1	104	0	103	427	634	529	0
2	104	208	0	213	423	529	217
3	0	208	0	320	423	423	108
4	209	104	0	213	211	423	217
5	0	104	103	320	317	423	326
6	209	208	0	213	317	529	217
7	209	104	0	320	317	529	217
8	0	104	103	320	423	317	108
9	0	104	103	0	317	211	217
10	104	104	0	213	317	423	0
11	104	208	0	320	634	634	326
12	0	0	0	320	423	846	217
13	0	104	103	213	529	423	217
14	104	0	0	213	317	529	0
15	104	208	0	213	317	529	108
Total	1251	1768	515	3838	5919	7297	2495
Mean	83.4	117.86	34.3	255.8	394.6	486.4	166.3
% Soil Moist. Cont.	4.5	4.0	3.5	6.5	5.5	5.5	8.0

APPENDIX

Table 28(b): (Continued)

12th Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatments	6	2 538 283.1	423 047.1	41.31 **
Error	98	1 003 475.1	10 239.5	
Total	104	3 541 758.2		

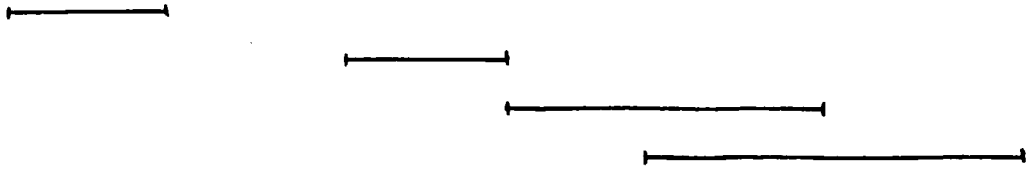
Tukey's Test

$W_{0.05} = q_{\alpha}(p, n_2)\ S\bar{x}$

$W_{0.05} = 4.26 \times 26.12$

$W_{0.05} = 111.2$

Young Tuber	Stolon	Potato Root	Old Tuber	Barley	Control	Broad Bean
486.4	394.6	255.8	166.3	117.8	83.4	34.3



APPENDIX

Table 28(b): (Continued)

15th Week

	Control	Barley Root	Broad Bean Root	Potato Root	Stolon	Young Tuber
1	0	106	0	209	416	313
2	0	213	0	418	416	418
3	104	213	0	313	520	523
4	104	106	104	313	520	627
5	104	106	0	209	416	523
6	104	0	0	313	520	627
7	0	213	0	313	312	418
8	208	106	104	209	520	523
9	104	106	0	313	312	627
10	208	0	0	209	625	523
11	104	106	0	104	312	627
12	0	106	0	209	416	418
13	208	106	0	104	416	732
14	104	0	104	313	312	418
15	0	0	104	0	520	523
Total	1352	1487	416	3549	6553	7840
Mean	90.1	99.1	27.7	236.6	438.8	522.6
% Soil Moist. Cont.	4.0	6.4	4.4	4.4	4.0	4.4

APPENDIX

Table 28(b): (Continued)

15th Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatment	5	3 088 631.3	617 726.2	77.85**
Error	84	666 473.1	7 934.2	
Total	89	3 755 104.4		

Tukey's Test

$W = q_{\alpha}(p, n_2)\ S\bar{x}$

$W_{0.05} = 4.13 \times 22.9$

$W_{0.05} = 95.0$

Young Tuber	Stolon	Potato Root	Barley	Control	Broad Bean
522.6	438.8	236.6	99.1	90.1	27.7

APPENDIX

Table 28(b): (Continued)

18th Week

	Control	Barley	Broad Bean Root	Potato Root	Potato Stolon	Young Tuber
1	105	107	0	210	524	744
2	0	107	0	105	314	638
3	0	0	0	210	314	531
4	105	0	106	105	629	531
5	105	214	0	421	209	425
6	105	0	0	210	419	425
7	0	107	0	315	419	638
8	105	107	106	210	209	531
9	0	107	106	210	629	425
10	105	0	106	315	734	531
11	105	214	0	421	314	425
12	210	107	0	210	524	531
13	105	107	0	105	629	319
14	105	321	0	210	629	638
15	105	0	0	105	209	425
Total	1260	1498	424	3362	6705	7757
Mean	84.0	99.8	28.2	224.1	447.0	517.1
% Soil Moist. Cont.	5.0	6.8	6.0	5.0	4.7	6.0

APPENDIX

Table 28(b): (Continued)

18th Week

Analysis of Variance

	D.F.	S.S.	M.S.	F.
Treatment	5	3 126 699.4	625 339.8	53.16**
Error	84	988 086.1	11 762.9	
Total	89	4 114 785.5		

Tukey's Test

$W = q_{\alpha}\ (p, n_2)\ S\bar{x}$

$W_{0.05} = 4.13 \times 28.0$

$W_{0.05} = 115.8$

Young Tuber	Stolon	Potato Root	Barley	Control	Broad Bean
517.1	447.0	224.1	99.8	84.0	28.2

APPENDIX 2

Determination of Soil Moisture Capacity

About one hundred grams of soil sample was wetted thoroughly so as not to lose its structure, and the excess water was allowed to drain away. The wetted soil was weighed and then placed in an oven at 105°c and was dried for 24h. After cooling in a dessicator, it was reweighed. It was reheated and weighed again until a constant weight was achieved. Then soil moisture capacity was calculated from the formula.

$$\frac{\text{Weight of fresh sample} - \text{weight of dried sample}}{\text{Weight of dried sample}} \times 100$$