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Growth and Survival of *Verticillium chlamydosporium* Goddard, a Parasite of Nematodes, in Soil

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Selective media for the isolation of the nematophagous fungus, Verticillium chlamydosporium, *are described. These enabled densities > 500 colony forming units (CFU) g^{-1} soil to be reliably estimated. However, there was little relationship between estimates of the* Verticillium *biomass in a sterilized soil and the numbers of CFU which developed on the selective media. The growth and survival of the fungus in field soils were studied and estimates of the numbers of CFU in soils in which cyst-nematode multiplication was suppressed were greater than in those in which the nematode multiplied. Isolates of the fungus differed in their ability to proliferate in soil, but some increased rapidly from applications of chlamydospores or a mixture of hyphae and conidia in alginate granules containing wheat bran. The energy source (wheat bran) was essential for the establishment of the fungus from granular applications. Numbers of CFU greatly exceeded those of chlamydospores, but there was considerable variation in the relationship in different soils. Some isolates of* V. chlamydosporium *proliferated in soil and survived in considerable numbers for at least 3 months. Hence, pre-cropping applications of the fungus should survive long enough to kill nematode eggs and females that develop on roots of spring-sown crops.*

Keywords: *Nematophagous fungi*, Verticillium chlamydosporium, *biological control, selective media, rootknot nematodes, cyst nematodes*

INTRODUCTION

Verticillium chlamydosporium Goddard is a widespread fungus that parasitizes females and eggs of cyst and rootknot nematodes and is one of the most important parasites responsible for the natural control of both cereal and beet cyst nematodes (Kerry *et al.*, 1982a; Thomas, 1982). The fungus has potential as a biological control agent for *Meloidogyne* spp. when thoroughly incorporated throughout soil (Godoy *et al.*, 1983; de Leij & Kerry, 1991, 1992). Although colonization of the rhizosphere was considered essential for its successful control of *M. arenaria* (Neal) Chitwood, little is known of the proliferation and spread of the fungus in soil.

The study of nematophagous fungi for biological control of nematodes has been severely hampered by a lack of suitable methods to reisolate and quantify the fungi after they have been

added to soil (Stirling, 1988). Crump and Kerry (1981) described a method for the extraction of chlamydospores of *V. chlamydosporium* from soil; spores were more numerous in soils where *Heterodera avenae* Woll. and *H. schachtii* Schmidt failed to multiply than where nematode populations increased. However, chlamydospores are not the only propagules found in soil and their numbers are not a reliable indicator of the activity of the fungus. Although semi-selective media have been described for the isolation of the nematophagous fungi *Paecilomyces lilacinus* Samson (Mitchell *et al.*, 1987; Cabanillas *et al.*, 1989) and *V. chlamydosporium* (Gaspard *et al.*, 1990; de Leij & Kerry, 1991), there is a lack of data on factors that affect the densities of these fungi in soil. Data which are based on the numbers of colony forming units (CFU) from selective media can be misleading (Johnson & Curl, 1972) and do not reflect the fungal biomass in soil or its physiological state.

In this paper, we describe experiments to examine the survival and establishment of different isolates of *V. chlamydosporium* added to soil using selective media to monitor relative changes in fungal densities. Isolates of *V. chlamydosporium* differ markedly in their rate of growth, ability to produce chlamydospores and virulence (Kerry *et al.*, 1986; Irving & Kerry, 1986), and are also likely to differ in their ability to grow and survive in soil. ATP measurements have been shown to provide reliable estimates of total soil microbial biomass (Jenkinson & Ladd, 1981) and were used to monitor changes in the biomass of *V. chlamydosporium* after introduction into sterile soil; these estimates have been compared with relative changes in the numbers of CFU. Proliferation of the fungus from alginate granule formulations was also measured, and the relationship between the numbers of chlamydospores and the total numbers of propagules in several nematode-infested soils examined.

MATERIALS AND METHODS

Cultures and Inoculum

The five isolates (2, 8, 13, 40 and 104) used in these studies were collected from infected cyst-nematode eggs using standard techniques (Kerry & Crump, 1977) and stored on silica gel at 4°C (Smith & Onions, 1983) for 3 years. Silica gel crystals were aseptically transferred to 1.7% corn meal agar (CMA, Oxoid) and incubated at 18°C in the dark for 10 days before a plug (5 mm diameter) taken from the edge of an actively growing colony was added to 150 ml 3.34% Czapek Dox broth (CDB, Oxoid) for inoculum production. Each fungus was grown in shaken culture using CDB supplemented with a salts mixture (Kerry *et al.*, 1986) except that the salts were omitted when inoculum was to be encapsulated in alginate granules; salts caused gelation of the alginate–fungus mixture before exposure to the gelant and prevented the formation of granules. Chlamydospores are not readily produced in CDB culture and, when required, were washed from the surface of CMA; up to 500×10^3 chlamydospores were collected from a 6-week-old culture (diameter 9 cm).

The selection of isolates was based on a standard laboratory test (Irving & Kerry, 1986); isolates 8, 13 and 40 were more able to infect nematode eggs than isolates 2 and 104. Also, isolates 13, 40 and 104, but not 2 or 8 were capable of extensive colonization of the rhizosphere of barley plants in tests based on those described by Kerry *et al.* (1984). Hence, isolate 2 lacked virulence and the ability to colonize the rhizosphere, whereas isolates 13 and 40 were both effective parasites and effective colonizers.

Selective Media

In preliminary tests, alcohol agar (Nadakavukaren & Horner, 1959), soil extract agar (Menzies & Griebel, 1967) and Christen's medium (Christen, 1982), all developed for the estimation of *Verticillium* spp. in soil, were not effective for the isolation of *V. chlamydosporium*. The medium (A) used initially contained 17 g CMA, 17.5 g NaCl, 75 mg Rose Bengal, 75 mg carbendazim and 50 mg each of the antibiotics chloramphenicol, aureomycin and streptomycin sulphate per litre; the antibiotics and fungicide were added to the cooled agar after autoclaving. This medium

proved useful for reisolating the fungus after large numbers of propagules had been added to soil, but was not sufficiently selective to be used at dilutions greater than 1:500. Hence, it could not be used reliably to isolate the fungus in many naturally infested soils where $< 10^4$ propagules g^{-1} soil were usually found (Kerry *et al.*, 1982b), as fewer than 20 colonies would be observed on each Petri dish. A second medium (B), described by de Leij and Kerry (1991), was used for this purpose; medium B contained similar materials to medium A except that half the concentration of carbendazim was used and 37.5 mg of thiabendazole and 3 ml of Triton X-100 were added. The fungicides and antibiotics helped to suppress the growth of other fungi and bacteria respectively, and NaCl and Triton X-100 reduced the rate of colony growth so that colonies remained discrete even after 2 weeks at 20°C, by which time they had sporulated and could be identified. Rose Bengal acts as an antimicrobial agent and also slows colony growth, but Petri dishes should be kept in the dark as exposure to sunlight causes considerable inhibition of fungal development (Pady *et al.*, 1960). In tests with isolate 13, medium B decreased the growth of *V. chlamydosporium* by $< 20\%$ whereas medium A decreased growth by up to 50% compared to that on CMA.

A dilution-plate technique was used with the selective medium to estimate changes in the relative abundance of *V. chlamydosporium*. Soil dilutions were prepared from 2-g samples taken at random from thoroughly mixed soils in the experiments described below. Each sample was added to 18 ml of 0.05% water agar (1:10 dilution) and shaken vigorously for 15 s before a 2-ml subsample was transferred to 18 ml of dilute agar as part of a dilution series; this was repeated to give a series of 1:10, 1:100, 1:1000 dilutions. On each sampling, three 0.2-ml aliquots of suspension from each of the last two dilutions were aseptically spread on the surface of the selective media in 9-cm Petri dishes. The numbers of CFU that developed on each Petri dish (six per sample) were counted after incubation at room temperature in the dark for 2 weeks.

Comparison of Estimates of Colony Forming Units and Biomass

An organic calcareous loam soil (Sutton Veny) in the Icknield soil series (pH 7.9) which had been screened through a 1-mm aperture sieve was weighed in 30-g aliquots into 28 small glass vials and autoclaved twice for 50 min with a 2-day interval between autoclavings. Each vial was aseptically inoculated with 2 ml of a conidia suspension of isolate 40 containing 60×10^6 conidia ml^{-1}. The vials were kept in an airtight container with a vial containing 1 M NaOH to absorb CO_2 and a layer of water to maintain humidity. The vials were kept at 18°C and four were removed on each sampling occasion after 4, 8, 13, 19, 25 and 32 days to estimate the density of the fungus.

Soil ATP was extracted using the method of Jenkinson and Oades (1979), and measured by the firefly luciferin-luciferase enzyme system (Tate & Jenkinson, 1982). On each sampling occasion, including immediately after the addition of the inoculum, the ATP contents were measured in triplicate portions of moist soil (5 g oven-dry) taken from each of the four vials. At the same time, two 2-g samples were also aseptically removed from each vial, immediately passed through a dilution series (1:100–1:10 000) and 0.2-ml subsamples transferred to Petri dishes containing CMA or medium B as described; the numbers of CFU were counted after 2 weeks at room temperature. After 8 and 32 days the chlamydospores in soil were extracted and the numbers estimated as described (Crump & Kerry, 1981).

Addition of *V. chlamydosporium* to Three Soils

Two calcareous loams (Tidworth and Devizes) in the Upton soil series (both pH 8.1) and an organic (Sutton Veny) soil were screened through a 1-mm aperture sieve and the moisture content of each adjusted to 50% water holding capacity (WHC). The mixture of hyphae and conidia (fermentation biomass) from 4-week-old liquid cultures of isolates 2 or 13 was comminuted for 15 s in a blender and 5-ml aliquots thoroughly mixed with three 50-g aliquots of each soil in small plastic pots (18 pots in total). Chlamydospores of both isolates were washed from 4-week-old cultures on CMA on to a 53-μm aperture sieve and the chlamydospores, along

with some hyphal fragments, were collected on a 10-μm aperture sieve beneath. A further 18 pots were inoculated with 5 ml of the chlamydospore suspension from each isolate (9 pots per isolate) as before. Each inoculum contained a mixture of spores and hyphal fragments of varying size. Hence, it was not possible to standardize the amount of viable inoculum added and the initial inoculum was estimated from 2-g soil samples taken with a sterilized spatula immediately after the fungus had been mixed in soil. Thereafter, samples were taken after 24 h and at weekly intervals for 8 weeks. Three samples of uninoculated soil from each source were also taken as controls on each occasion. Pots were covered with aluminium foil to reduce water loss and maintained at 50% WHC and 20°C. On each occasion the number of CFU g^{-1} soil was estimated as described using selective medium A.

Establishment of Three Isolates of *V. chlamydosporium* Added to Soil in Alginate Granules

Isolates 8, 13 and 104 were grown for 3 weeks in shaken liquid culture at room temperature and comminuted in a blender as before; each isolate produced about $30–40 \times 10^6$ spores and hyphal fragments ml^{-1}. The fungal suspensions were encapsulated in 2% (w/v) sodium alginate using methods described by Fravel *et al.* (1985). The alginate was mixed either with 100 g kaolin l^{-1} or with 100 g wheat bran l^{-1}; the latter had been milled through a 0.4-mm aperture sieve. The mixture of fungus, alginate and kaolin or bran was gelled using 0.1 M calcium gluconate. Granules for control treatments were produced in a similar procedure, but the fungus was not incorporated. The granules that formed were dried overnight in a laminar flow hood; 250 ml of the mixture produced 15–20 g of dried granules, which were stored for 24 h at 5°C before use. Prior to addition to soil, 20 granules were disintegrated in 10 ml of a mixture of 8.7×10^{-2} M KH_2PO_4 and 3.0×10^{-2} M Na_2HPO_4 (pH 7.7) and the number of CFU assayed by dilution plating (Fravel *et al.*, 1985).

The calcareous loam soil from Devizes was sieved, adjusted to 50% WHC and twenty-four 50-g aliquots were weighed into small plastic pots as before. The aliquots were mixed with 0.5 g of granules (*c.*60 granules) of either formulation containing one of the fungal isolates; each treatment combination was replicated three times. Pots were loosely covered with aluminium foil to reduce water loss and maintained at 50% WHC and 15°C. The soils were sampled at random with a spatula immediately after inoculation, after 3 days and thereafter at weekly intervals for 12 weeks. On each occasion the granules were sieved from the sample and excluded from the population estimate, otherwise the methods of sampling and determining the numbers of CFU which developed on medium A were as described.

Numbers of CFU and Chlamydospores in Nematode-infested Soils

Eleven soils infested with cereal or beet cyst nematodes and known to differ in their ability to suppress reproduction of the nematodes on susceptible hosts (B. R. Kerry, unpublished; Crump, 1987) were collected and screened through a 4-mm aperture sieve. Approximately 1 kg of each soil sample (Table 1) was stored at 4°C for 4 weeks before extraction. Three 2-g subsamples were taken at random from the well-mixed sample from each site and the numbers of CFU that developed on medium B were assessed on dilution plates as described. At the same time, the chlamydospores were extracted from three 25-g subsamples by the sieving and flotation method described by Crump and Kerry (1981). The soil moisture content was measured in two additional samples.

RESULTS

Comparison of Estimates of CFU and Biomass

In sterile conditions, the numbers of CFU of isolate 40 detected on the non-selective CMA medium increased steadily for 19 days to a maximum 2×10^6 g^{-1} soil (Figure 1). The selective medium (B) decreased the recovery of the fungus by approximately 10% compared to that on CMA, but changes in density were similar on both media. No ATP was detected in the sterilized

TABLE 1. Density (CFU g^{-1}) of *V. chlamydosporium* in cyst nematode-infested soils which differ in their levels of suppression of nematode multiplication

Location	No. soil samples examined	Cyst nemtode infestation	Suppressive (+) or conducive (–) soil[b]	Mean CFU g^{-1}[a]	SE
Tidworth	2	*H. avenae*	+	1792	360
Sutton Veny	2	*H. avenae*	+	7525	760
Devizes	2	*H. avenae*	+	2000	625
Crux Easton	2	*H. avenae*	+	2875	744
Mansel Lacey A	2	*H. avenae*	–	178	111
Mansel Lacey B	3	*H. avenae*	–	119	46
Burlington Cross	1	*H. avenae*	–	334	217
Little Downham	3	*H. schachtii*	+	5133	259
Broom's Barn	3	*H. schachtii*	+	4306	456
Woburn	3	*H. schachtii*	±	900	44
Great Barton	3	*H. schachtii*	±	1050	292

[a]Means of six replicates per sample assayed by dilution plating on medium B.
[b]Suppressive soils are those where the nematode fails to multiply on susceptible crops; conducive soils support nematode multiplication. The soils have been characterized by B.R. Kerry (unpublished) and Crump (1987).

soil prior to inoculation and estimates thereafter are assumed to relate to the increase in biomass of *V. chlamydosporium*. Changes in ATP content were not closely related to changes in CFU counts; in fact, levels declined when CFU counts were still increasing. ATP content reached a maximum at about day 2 (Figure 1), but the number of CFU, on either corn meal agar or selective medium B, did not reach an asymptote until about day 18. By this time the ATP content of the fungus was falling quite sharply. The decline in ATP content in soil was associated with a substantial increase in chlamydospore production from 12×10^3 g^{-1} soil after 8 days to 97×10^3 g^{-1} soil at the end of the experiment.

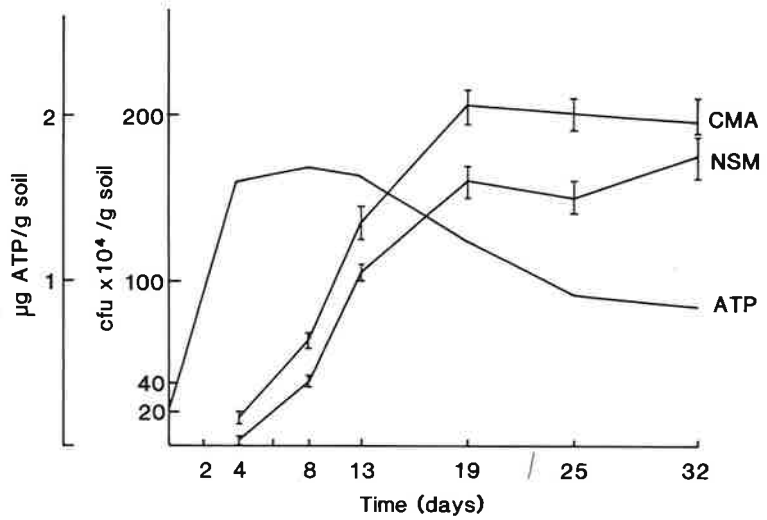

FIGURE 1. Mean number of CFU of *V. chlamydosporium* isolate 40 growing on corn meal agar (CMA) and the new selective medium B (NSM) and changes in the ATP content after introduction of the fungus to a sterile soil. (Means for ATP estimates from four replicates per sampling occasion; CFU counts from duplicate samples/replicates assayed by dilution plating.)

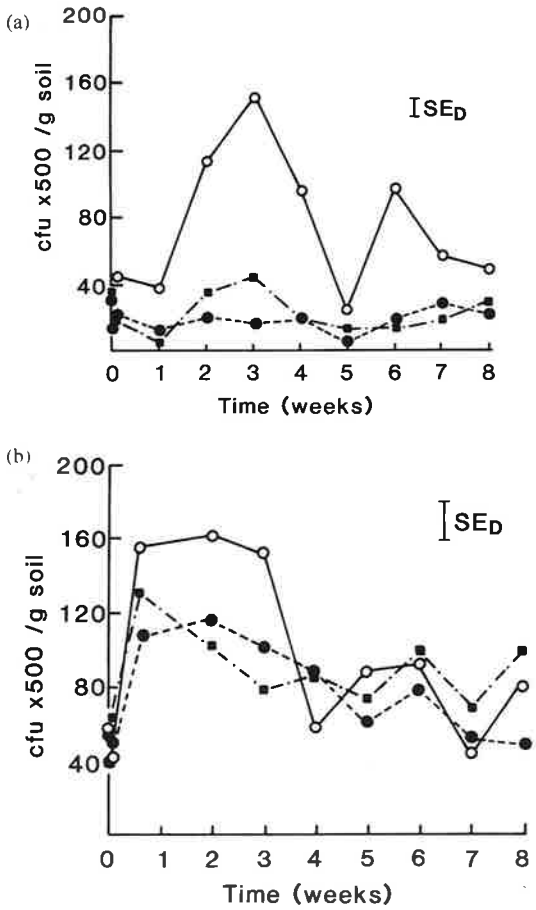

FIGURE 2. Changes in the numbers of CFU of *V. chlamydosporium* isolate 2(A) and 13(B) inoculated as an aqueous suspension of chlamydospores into three soils. Sutton Veny: ○ (organic); Tidworth: ● (calcareous loam); Devizes: ■ (calcareous loam). (Means of three replicates assayed by dilution plating on medium A.)

Addition of *V. chlamydosporium* to Three Soils

In general, the mean numbers of CFU that proliferated from chlamydospores of both isolates were greater ($P < 0.001$) in the organic soil than in the other two, and this effect was more marked for isolate 2 (Figure 2(a)) than 13 (Figure 2(b)). Similar trends were observed in soils inoculated with the mixture of conidia and hyphae or chlamydospores and so only data from the latter are presented. Isolate 2 did not establish in the mineral soils and growth in the organic soil was slower than that of isolate 13, which reached a similar maximum density 2 weeks earlier. At the beginning of the experiment the soils contained the following numbers of CFU g^{-1}: Tidworth, 1833; Devizes, 333; Sutton Veny, 6083.

Changes in the numbers of CFU of isolate 13 showed similar trends in the three soils and so data comparing the effects of inoculum type are averages of the numbers in all soils (Figure 3); data for isolate 2 are not presented because it failed to establish in mineral soils. Mean CFU densities that developed from the chlamydospore inoculum of isolate 13 increased rapidly in the first week from 2.5 to 6.6×10^4 g^{-1} soil and even after 8 weeks were greater ($P < 0.001$) than at the beginning of the experiment (Figure 3). The proliferation of the fungus introduced as

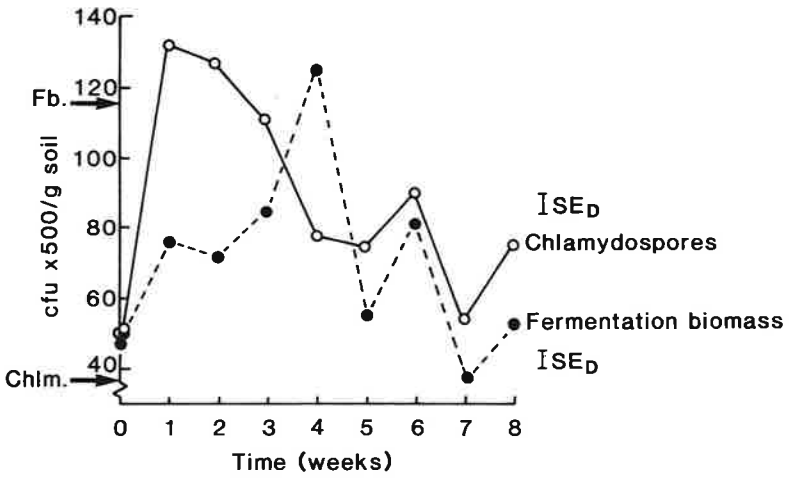

FIGURE 3. Changes in the numbers of CFU of *V. chlamydosporium* isolate 13 introduced into soil as fermentation biomass (hyphae and conidia) or as an aqueous suspension of chlamydospores. (Counts are averages for the three soils, nine replicates per inoculum type on each sampling occasion; arrows indicate estimated inoculum density prior to addition to soil.)

'fermentation biomass' (hyphae and conidia) was slower than when chlamydospores were used as inoculum. However, numbers of CFU increased ($P < 0.001$) after 1 week, and by 4 weeks they had reached similar numbers to those following inoculation with chlamydospores (Figure 3). After 8 weeks the numbers of CFU in soil had decreased and were similar to those at the beginning of the experiment.

Establishment of Three Isolates Added to Soil in Alginate Granules

The mean numbers of CFU of the three isolates of the fungus added to soil in alginate granules containing kaolin failed to increase, whereas granules containing wheat bran supported more growth ($P < 0.001$) on all sampling occasions (Figure 4). Growth from bran-based granules was markedly dependent on the isolate used (Figure 5); isolate 13 proliferated more ($P < 0.001$) than did 104 and isolate 8 failed to grow. Approximately 1.4×10^2 CFU g^{-1} of *V. chlamydosporium* were detected in the soil before inoculation; the dried granules added approximately 5×10^3 CFU g^{-1} soil of each isolate. After only 1 week the numbers of CFU of isolate 13 had increased to 4.5×10^4 g^{-1} soil and after 12 weeks 2.1×10^4 g^{-1} soil remained. The considerable fluctuations

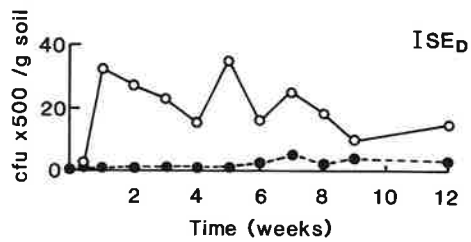

FIGURE 4. Changes in the mean numbers of CFU of *V. chlamydosporium* added to soil in alginate granules containing 10% wheat bran (○) or kaolin (●). (Counts are averages for the three isolates; nine replicates per inoculum type on each sampling occasion.)

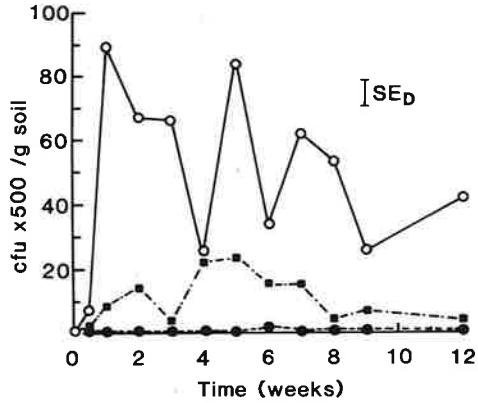

FIGURE 5. Changes in the numbers of CFU of three strains of *V. chlamydosporium* added to soil in alginate/wheat bran granules. Isolate 13, ○; isolate 8, ●; isolate 104, ■. (Means of three replicates assayed by dilution plating on medium A.)

in the numbers of CFU isolated on each occasion may have been due to uneven fungal growth from the granules.

Numbers of CFU and Chlamydospores in Nematode-infested Soils

Numbers of CFU, as determined using medium B, were greater in nematode suppressive than in conducive soils (Table 1). Soils that suppressed the multiplication of the cereal and beet cyst nematodes contained 1.7–7.5 and 4.3–5.1 $\times 10^3$ CFU g^{-1} soil respectively. Soils that supported nematode reproduction contained $< 10^3$ CFU g^{-1} soil. The fungus could be detected at densities below 10^3 CFU g^{-1} soil, but numbers < 500 g^{-1} were based on counts of < 10 colonies per replicate plate and such estimates tended to be variable. The correlation between the numbers of CFU and the numbers of chlamydospores in samples taken from different soils with different cropping histories was significant ($P < 0.01$) but highly variable (Figure 6). In some soils the numbers of chlamydospores were similar to or even exceeded the estimates of total CFU which suggested that a considerable proportion of the natural inoculum did not germinate on the selective medium.

DISCUSSION

The selective media proved useful for estimating changes in the relative abundance of several isolates of *V. chlamydosporium* in soil. Medium B could be used at dilutions of 1:50 and even though several colonies of other fungi developed it was still possible to estimate reliably the numbers of *V. chlamydosporium* in field soils and relatively small populations ($> 10^2$ g^{-1} soil) were detected. More testing is required to determine the numbers of samples required to give acceptable standard errors. Growth of some isolates was very slow and their numbers were inevitably underestimated. However, when known numbers of chlamydospores were added to soil, similar CFU counts were obtained from samples taken immediately afterwards. *Trichoderma* isolates varied in their growth rates on selective media (Papavizas & Lumsden, 1982). Hence, comparisons between isolates should be treated with caution; when selective media are used to isolate *V. chlamydosporium* from field soils different isolates may not be detected with the same efficiency.

ATP does not occur extracellularly in soil and is therefore only associated with living organisms (Jenkinson & Ladd, 1981). Soil ATP content and amounts of total soil microbial biomass are closely and linearly related, so that ATP can be used as an independent measure of

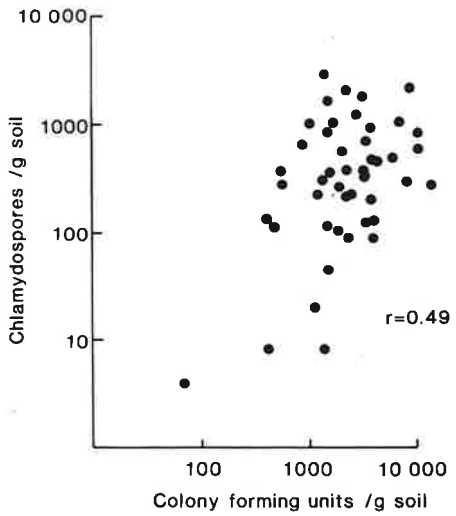

FIGURE 6. Relationship between numbers of chlamydospores and CFU of *V. chlamydosporium* in different soils with different cropping histories. (CFU estimates from triplicate samples assayed by dilution plating on medium B; chlamydospore estimates are means of triplicate samples.)

the amount of total soil microbial biomass (Jenkinson & Ladd, 1981). Increases in the biomass of *V. chlamydosporium* in sterile soil preceded increases in the numbers of CFU. This may suggest that the ATP concentration in *V. chlamydosporium* declines as the fungal population ages and spores predominate; certainly, there was a significant increase in the production of chlamydospores when ATP content was declining, but CFU densities remained high. Hence, the numbers of CFU may not be linearly related to the amount of fungal biomass as the population ages. More work clearly needs to be done to determine the relationship between ATP and fungal population growth.

A successful biological control agent for a soil-borne pest or pathogen must be able to grow, survive and proliferate in soil (Baker & Cook, 1974). These characteristics are particularly important if the agent is used in arable crops, in which inundative application rates are impractical. Although there are many difficulties in establishing an introduced organism in soil (Garrett, 1956), selected organisms have proved active against some soil-borne pests and diseases (Cook & Baker, 1983). Successful establishment has generally been associated with the addition of an energy source to help the organisms overcome competition from the residual soil microflora. Adding an energy source to alginate granules containing *V. chlamydosporium* markedly improved the establishment of the fungus. If actively growing hyphae already occupy the food base, they do not appear to be subject to fungistasis when added to soil and such intimate contact between mycelium and food enables the fungus to grow relatively unimpeded through soil (Lewis & Papavizas, 1985a). However, the addition to soil of comminuted 'fermentation biomass', which contained hyphal fragments and conidia that had limited food reserves, resulted in the eventual establishment of *V. chlamydosporium* in soil in appreciable numbers. Proliferation tended to be much greater in organic rather than mineral soils. As in previous studies (de Leij & Kerry, 1991), chlamydospores added to soil in aqueous suspensions had sufficient food reserves to enable the fungus to establish rapidly in soil without the addition of other energy sources.

Addition of *V. chlamydosporium* isolate 13 in granules containing bran resulted in a 300-fold increase in the numbers of CFU and, even after 12 weeks, populations were still 150 times greater than at the time of inoculation. These increases were not as great as those using alginate preparations of *Trichoderma hamatum* (Bonorden) Bainier and *Gliocladium virens* (Miller)

Giddens & Foster (Lewis & Papavizas, 1985b), but were similar to those of *Talaromyces flavus* (Papavizas *et al.*, 1987). Conidia of *T. flavus* were more efficient than ascospores or fermentation biomass in establishing the fungus in soil; in our tests no attempt was made to separate the types of propagules produced in liquid culture. The thick-walled chlamydospores, which are only produced in large numbers on solid media, might be expected to survive better in soil and have a longer shelf-life than other propagules; if these spores could be produced more easily, they might prove a more efficient inoculum for addition to soil. The growth pattern of *V. chlamydosporium*, as determined by increases in CFU from alginate granules or chlamydospore inocula, was similar to that observed for the other fungi mentioned above. It is not clear what caused the rapid increase in CFU in the first week; the rate of hyphal extension as measured on nylon meshes buried in soil (Kerry, 1991) seemed too slow to account for such increases. The numbers of CFU resulting from granule applications of single isolates of *V. chlamydosporium* sometimes greatly exceeded those found in cyst nematode suppressive soils, but the rates of application were large. It remains to be demonstrated whether fungal densities that are required to control nematodes can be established in soil from practical rates of application. In spring-sown crops in the UK, young female cyst nematodes appear on root systems about 8 weeks after planting. In our experiments, soils contained about 4×10^4 CFU g^{-1} (a density at which effective nematode control might be expected; see Table 1) 6–8 weeks after inoculation of a 1% application rate.

In this paper, we demonstrated that *V. chlamydosporium* can be substantially increased in soil in the absence of nematodes or their host plants and that the fungus can survive for at least 3 months, which is long enough to allow infection of developing female cyst nematodes on roots. Although nematode control is dependent on sufficient fungal inoculum in soil, isolates of the fungus that survived in soil in large numbers failed to infect eggs of *M. arenaria* unless they were able to colonize the rhizosphere (de Leij & Kerry, 1991). Hence, once established in soil, factors that affect rhizosphere colonization are likely to determine the efficacy of *V. chlamydosporium* as a biological control agent. The selective media described provide a useful way of estimating relative changes in the abundance of *V. chlamydosporium*. However, care is needed in the interpretation of CFU counts on these media as such population estimates may not reflect fungal biomass or the level of activity.

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