Factors Influencing Survival of Phialospores of *Chalara elegans* in Organic Soil

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ABSTRACT

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The influence of soil moisture, temperature, crop plant, flooding, and addition of plant residues and $CaCO_3$ on survival of phialospores of *Chalara elegans* in organic soil was studied. The extent of survival of phialospores (number of propagules recovered from artificially infested soil) was monitored over a 19-wk duration using a semiselective medium (TB-2RBA). Survival was significantly greater ($P \leq 0.01$) in soil maintained at a constant soil moisture level (matric potential of about −50 J/kg) than when moisture level gradually declined to about −900 J/kg; propagules could be recovered from both soil moisture treatments for up to 19 wk. The presence of carrot plants did not significantly influence propagule survival when compared with fallow soil, and propagules of *C. elegans* could be detected after 19 wk of soil incubation at 20 C. The presence of onion seedlings, however, decreased recovery of the pathogen to an undetectable level after 15 wk. When soil was flooded and maintained at 4 C, survival was comparable to that in nonflooded, fallow soil. However, with increasing temperature (15, 20, or 25 C), recovery of phialospores from flooded soil was significantly reduced ($P \leq 0.01$) and no propagules were detected after 12 wk. In nonflooded soil at constant soil matric potential of about −50 J/kg, survival was reduced significantly only at 30 C and not at any of the lower temperatures tested. Addition of plant tissues of alfalfa, carrot, rye, and onion all significantly reduced the extent of survival of phialospores compared with the fallow control, with onion tissues having the most pronounced effect. The addition of $CaCO_3$ had no effect. The results from this study indicate that phialospores of *C. elegans* can survive in organic soil for periods greater than 19 wk, especially under moist and cool conditions. Survival of phialospores was reduced significantly by the presence of onion seedlings, flooding the soil at 25 C, and addition of plant residues. These findings could have application for reducing inoculum of *C. elegans* under field conditions.

Additional keywords: black root rot, endoconidia, inoculum density, *Thielaviopsis basicola*

A number of fresh market vegetables, such as carrots, lettuce, celery, onions, and various cole crops, are grown on organic soils in the Fraser Valley of British Columbia, Canada. Commercial fields range in size from 1 to 10 ha and soils are characterized by organic matter contents from 50 to 80% and soil pH values of 4.8–6.5. During the winter months (November–April), many fields, particularly those with a high water table, are saturated and may stay flooded discontinuously over a 2- to 3-mo period. The following spring, the fields are frequently limed (with 1–2 t/ha of $CaCO_3$) to raise the soil pH.

One of the most important diseases affecting carrots (*Daucus carota* L.) grown in organic soils in the Fraser Valley is black root rot, caused by *Chalara elegans* Nag Raj & Kendrick (syn. *Thielaviopsis basicola* (Berk. & Broome) Ferraris) (22). The pathogen develops on the roots postharvest following contact of soilborne inoculum with wound sites incurred during the washing and grading process (23). A number of field soils are known to harbor the pathogen (21,22), although factors that influence survival of inoculum in these organic soils are not well understood. On infected host tissues (2,13,22) and on agar media (19,22), *C. elegans* forms two types of spores: the hyaline, thin-walled phialospores (endoconidia), which generally develop first and are produced in large numbers, followed by the darkly pigmented and segmented chlamydospores (aleuriospores).

Many of the factors that influence survival of the chlamydospores in natural soil have been described (1,4,9,24,25,31), because these spores are reported to represent the major perennating structure (12,31). However, the fate of phialospores, which are produced in large numbers on infected root tissues (13,22), is not well understood. The length of time phialospores can persist in soil is reported to range from 2–3 wk (2,11,31) to 7–15 mo (9,16,18,26). Survival of phialospores may be influenced by soil moisture content (18,26), soil texture (26), temperature (11,18), and addition of plant tissue amendments (1,16,17).

Phialospores of *C. elegans* are a convenient source of inoculum for use in pathogenicity studies and in cultivar evaluations (10,13,22,23,30), since they are produced in abundance and are easily recovered from agar media. In addition, the phialospores germinate rapidly and can initiate infections within 48–72 hr (13,22). To further elucidate the potential role that phialospores could have in survival of *C. elegans*, especially in organic soils, the influence of environmental factors and of crop residues was evaluated in this study. The objectives were to quantify the effects of moisture level, temperature, crop plants, and crop residues on survival of phialospores of *C. elegans* in organic soil. Preliminary results have been published (20).

MATERIALS AND METHODS

Inoculum production. An isolate of *C. elegans* obtained from diseased carrot roots was used throughout this study. The fungus was grown on V8 agar (canned V8 juice, 150 ml; distilled water, 850 ml; Bacto water agar, 15 g; ampicillin, 100 mg/L) for 14–21 days at 20 C. Phialospores (endoconidia) were obtained by flooding the culture with 10 ml of sterile distilled water and diluting the resulting spore suspension to a concentration of 1×10^4 to 1×10^5 per milliliter. The inoculum was added to an organic field soil (organic matter content of 75%, pH 5.2, soil moisture holding capacity at saturation of 60%) in which no inoculum of *C. elegans* was detected by a carrot root disk assay (22) and a semiselective medium (3). A moisture retention curve was developed for the soil using a ceramic pressure plate apparatus. The matric component of the soil water potential (matric potential) was expressed as joules per kilogram (J/kg) where 1 J/kg = 1 kPa. A soil analyses for nutrient concentrations was conducted by a commercial laboratory (Norwest Labs, Langley, BC). Soil was air-dried to a moisture content of 44% prior to use. Then, 20 ml of the phialospore suspension was added to 200 cm^3 of soil, and the moisture content achieved was determined by drying a subsample at 80 C. The infested soil was placed in 6.5 × 6.5 × 10 cm polystyrene containers with tight-fitting lids or the containers were sealed within polyethylene bags. The initial inoculum density was determined on TB-2RBA medium (3) within 24 hr after addition of the phialospores.

Experimental design and statistical analysis. For each of the experiments described below, treatments were arranged in a randomized complete block design with three replications. Survival of *C. elegans* over time was determined at 2-wk intervals by taking a 1-cm^3 volume

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of soil, diluting it to 10^{-2} or 10^{-3}, and spreading it onto two replicate dishes of TB-2RBA medium (3). All experiments were conducted at least twice. The data from the replications and repetitions of each treatment were used to calculate the mean, standard deviation, and standard error at each sampling time using the SAS statistical package. The treatments were compared by a two-way analysis of variance (ANOVA) with repeated measures on one factor (weeks), followed by a contrast analysis to see which treatments were different, using the SAS program. The 1% level was used to assess a statistical significance. The data were plotted as log propagules per cubic centimeter vs. time.

Influence of soil moisture level. A parallel series of experiments was conducted to determine the extent of survival of phialospores at different soil moisture levels. In one series, the soil moisture content was maintained within the range of 50–55% (between −30 and −100 J/kg matric potential) throughout the experiment; in the second series, the moisture content was allowed to decline gradually over the course of the experiment. At 2-wk intervals, the soil moisture content in both experiments was determined by drying subsamples at 80 C. The required amount of water was added to compensate for the moisture loss in the constant moisture experiment only. The inoculum density of *C. elegans* in each series of experiments was determined at 2-wk intervals, which coincided with the time at which soil moisture was determined. The containers were incubated in a growth chamber set at constant 25 C and a 12-hr photoperiod throughout the 19 wk of the experiment.

Influence of crop plant. Artificially infested soil with an initial inoculum density of 4.8×10^5 cfu/cm^3 and moisture content of 55% (about −30 J/kg) was used. Either the soil was left fallow or 1-mo-old carrot seedlings (cv. Danvers) or green (bunching) onion seedlings (*Allium cepa* L.) were transplanted into the soil, with two plants per container. The containers were sealed within polyethylene bags and incubated at 25 C in the growth chamber. At 2-wk intervals, the moisture content of the soil was determined as before and adjusted as required. Occasionally, it was necessary to replace carrot or onion seedlings that were lost to damping-off caused by *Pythium* spp.

Influence of flooding, crop plant, and $CaCO_3$. Samples (200 cm^3) of artificially infested soil with an initial inoculum density of 4.1×10^5 cfu/cm^3 were placed in polystyrene containers and the moisture content was adjusted to 55% (about −30 J/kg) or the soil was saturated with water to achieve a 1-cm layer of water on the soil surface. In the unflooded experiment, either the soil was planted to green (bunching) onion seedlings as before or 1% (w/v) of $CaCO_3$ was thoroughly mixed into the soil. The moisture content was monitored at 2-wk intervals and water was added as needed to maintain the moisture content at 50–55%. The containers with flooded soil were left fallow and incubated at either constant 4 or 25 C in a growth chamber. For comparison, a fallow treatment in which the moisture content was allowed to decline gradually over the course of the experiment was included.

Influence of plant tissue amendments. Leaf segments (0.5 cm^2) of alfalfa, fall rye, or onion and root segments of carrot were air-dried to about 10–15% moisture content and added to infested soil at the rate of 0.5% (w/v), except for rye, which was added at 0.25%. The soil moisture content was maintained at 50–55% and the temperature at 25 C throughout the experiment. The inoculum density was estimated at 2-wk intervals.

Influence of temperature. Soil that was infested with *C. elegans* was adjusted to 55% moisture content or was flooded. Containers with soil from each treatment were placed at 4, 15, 20, or 30 C. The inoculum density was estimated at 2-wk intervals.

RESULTS

Inoculum production and soil analysis. Phialospores of *C. elegans* were produced in large numbers on V8 agar and were uniformly rectangular and hyaline. The morphology and size of the phialospores and chlamydospores are compared in Figure 1. After the phialospores were added to soil, colony-forming units were readily detected on TB-2RBA medium and could be quantified (3). The analysis for nutrient concentrations of the soil used throughout this study indicated the levels, in micrograms per milliliter, to be: ammonium-N, 44; nitrate-N, 117; phosphate, 160; potassium, 419; sulfate, 35; calcium, 9,078; magnesium, 797; and aluminum, 1.6.

Influence of soil moisture level. The moisture release curve for the organic soil used in this study is shown in Figure 2. At saturation, the soil moisture content was 60%; at 55% moisture content, the matric potential was approximately −30 to −50 J/kg (Fig. 2). Recovery of phialospores of *C. elegans* decreased gradually over time at a moisture level of 50–55% (Fig. 3A) and was reduced significantly ($P \leq 0.01$) after 19 wk. With declining moisture content, the survival of phialospores was significantly lower, although propagules were also recovered after 19 wk (Fig. 3A). The moisture content at the end of the experiment was 41% (about −900 J/kg).

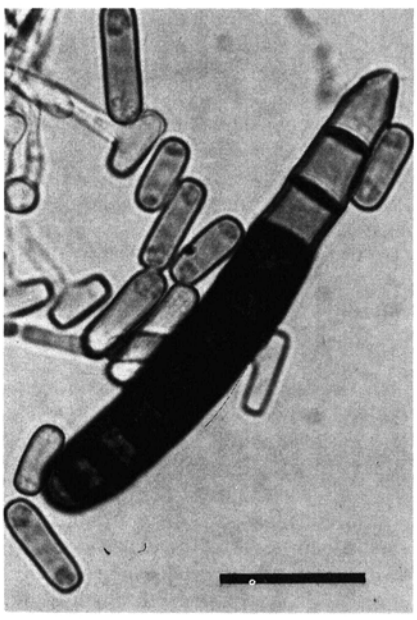

Fig. 1. Phialospores of *Chalara elegans* from V8 agar adjacent to a chlamydospore. Scale bar = 20 μm.

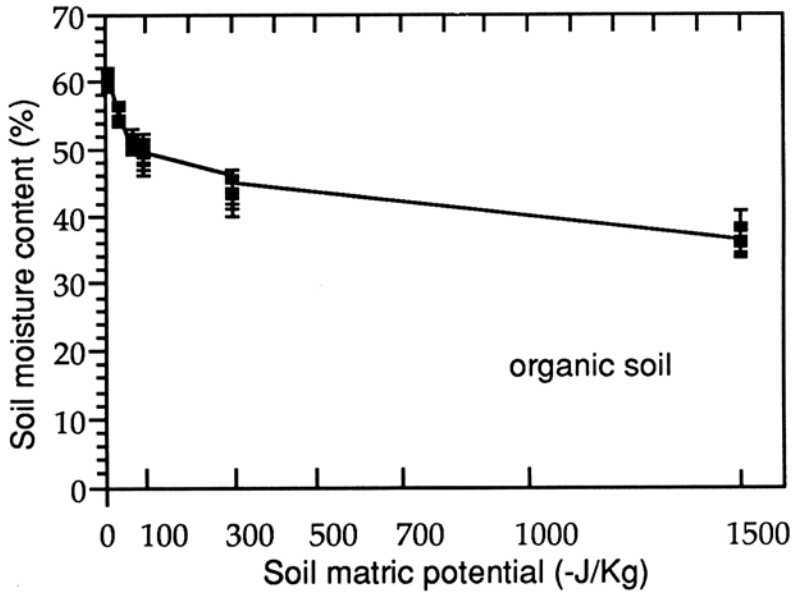

Fig. 2. Moisture retention curve for the organic soil used in this study.

Influence of crop plant. The presence of carrot plants in infested soil did not significantly affect the extent of survival of phialospores of *C. elegans* when compared with fallow soil, at either constant or declining soil moisture (Fig. 3A and B). However, in the presence of onion seedlings, recovery was reduced significantly after 17 wk and no colonies were observed on TB-2RBA medium at 19 wk (Fig. 3B).

Influence of flooding, crop plant, and $CaCO_3$. When infested soil was flooded and incubated at 4 C, there was no decline in propagule numbers from the initial level even after 19 wk (Fig. 3C). At 25 C, however, soil flooding reduced survival dramatically, and no propagules were recovered after 15 wk. The addition of $CaCO_3$ had no effect when compared with fallow soil, whereas the presence of onion seedlings reduced recovery to undetectable levels after 19 wk. On TB-2RBA medium, large numbers of *Penicillium* colonies, and some of *Trichoderma* and *Gliocladium*, were observed from soil samples in which onion seedlings had been grown (*data not shown*).

Influence of plant tissue amendments. The addition of any tissues tested (carrot, alfalfa, rye, onion) reduced the propagule numbers significantly compared with the fallow control after 18 wk (Fig. 4A), with onion tissues showing the most pronounced effect.

Influence of temperature. In nonflooded soil incubated at 4 C, there was no significant decline in propagule numbers from the initial level even after 18 wk (Fig. 4B). At 15 and 20 C, there was a slight decline in recovery, while the most significant reduction was observed at 30 C (Fig. 4B). When the soil was flooded and incubated at 15 or 20 C, propagule numbers were dramatically reduced after 8 wk and no colonies were recovered after 10 wk (Fig. 4C).

DISCUSSION

All of the previous studies on factors influencing survival of phialospores of *C. elegans* have been conducted using mineral soils, with organic matter contents from 0.5 to 3% (1,2,9,16–18,26). Differences in characteristics between these soils and the organic soil used throughout this study would account, in part, for some of the differences between our results and those of previous investigators. Schippers (26) reported that the percentage of viable phialospores was higher after 1 yr in a clay loam soil than in a sandy soil and that lysis of the spores was more rapid in moist than in drier soil. When nutrients or other substances that stimulated germination of the phialospores were added to sandy loam soil, lysis of the germ tubes occurred and survival was reduced (1,16,17,26). In our study, phialospore survival was higher when the soil was constantly moist (about −50 J/kg) than when it was allowed to dry out (to about −900 J/kg). Phialospores have been reported to be fairly sensitive to drying (12). In laboratory studies, germination of the phialospores was highest at 0 to −250 J/kg osmotic potential and at pH 4.5–6.0 (19). Following germination, germ tubes either developed into hyphae or produced secondary phialospores (19). Because of the presence of high organic matter in the soil in our study, stimulation of phialospore germination in moist soil may have been followed by saprophytic growth of the fungus, which could have maintained the high propagule counts. Johnson (7) observed that high organic matter con-

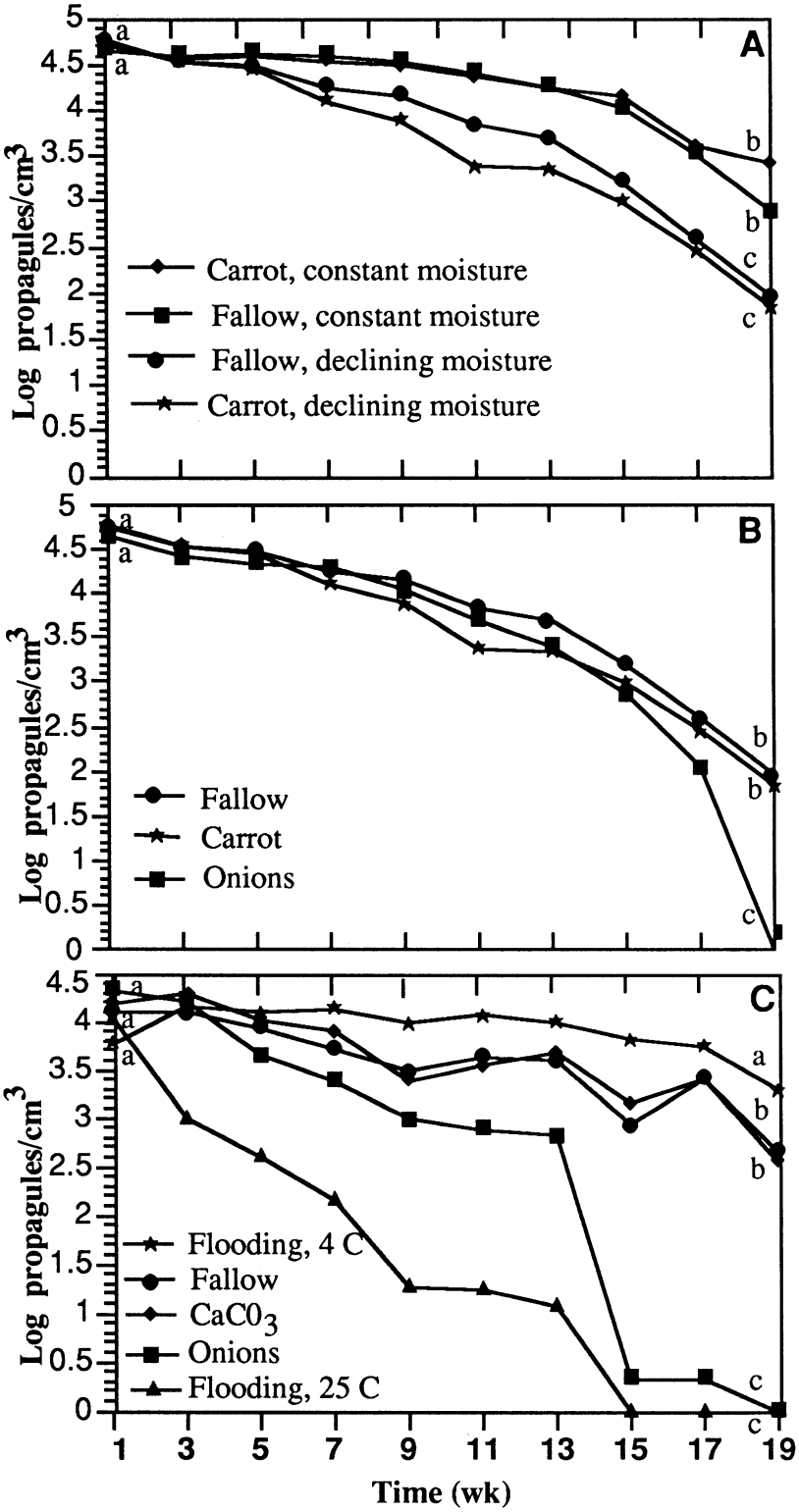

Fig. 3. Survival of phialospores of *Chalara elegans* in organic soil: **(A)** Influence of soil moisture level, **(B)** influence of crop plant, and **(C)** influence of flooding and onion plants. Data represent the average of two experiments, each with three replications. Treatments with different letters are significantly different ($P \leq 0.01$); these are indicated only at weeks 1 and 19 to illustrate maximum differences.

tent increased soil infestation by and persistence of *C. elegans* in soil. Peat-based potting media also were found to contain the pathogen, and infested peat debris extended survival in the greenhouse over winter (6).

In both moist and dry organic soil, a high proportion of the phialospores survived for at least 19 wk in our study, substantiating some of the earlier reports on the persistence of these spores in various types of soil (9,16,18,26). Although it has been suggested that phialospores may be converted into secondary chlamydospores through cell wall thickening (26,29), which would permit their prolonged survival, the conversion phenomenon has been observed only under laboratory conditions and may not occur in nature (9,11,16). Through direct microscopic observations of soil smears, it was shown that viable phialospores may survive in an ungerminated and morphologically unaltered form (26) or they may germinate (9,11,17,26).

The host range of *C. elegans* is extensive and includes both cultivated plants and weed species (5). The fungus may also be associated with the roots of various plant species in the absence of any visible symptoms (32). Root infections on susceptible hosts invariably result in an increase in inoculum levels in the rhizosphere (2,6,13,24,25). When carrot was compared with fallow soil for effect on survival of phialospores in this study, there was no difference in propagule numbers after 19 wk. Although carrot roots are highly susceptible to infection by *C. elegans* (22), most infections occur postharvest and not on the growing root (23), which would explain why there was no change in propagule numbers when carrots were planted into infested soil. The extended survival in fallow organic soil indicates that presence of a host plant is not necessarily required.

The addition of dried alfalfa plant tissues to sandy loam soil was shown to stimulate germination and reduce survival of phialospores of *C. elegans* within 1 wk following addition (1,16,17). Lysis of germ tubes through enhanced activities of antagonistic microorganisms was one of the reasons for the decline in phialospore survival (17). Similar effects have been obtained using residues of rye (24) and hairy vetch (8). Enhanced microbial activity, especially of bacteria, is known to reduce the extent of survival of chlamydospores of *C. elegans* (4,17, 18,24,25). In our study, a gradual reduction in recovery of propagules of *C. elegans* was observed over time when a number of unrelated plant tissues were added to organic soil. Thus, a general increase in microbial activity may have contributed to nonspecific competition with *C. elegans*, which is generally regarded to be a poor competitor in soil (2,16). However, in the presence of either onion residues or plants, survival of *C. elegans* was reduced to a much greater extent.

In soil planted to onions, a higher frequency of fungi, especially species of *Penicillium* and occasionally *Trichoderma* and *Gliocladium*, was recovered (*unpublished*). The presence of onion seedlings or tissues may have enhanced populations of *Penicillium* species selectively, which in turn reduced survival of *C. elegans*. The addition of spores of *Penicillium* spp. recovered from these soils to soils infested with *C. elegans* was observed to drastically reduce survival of *C. elegans* within 2 wk (*unpublished*). Species of *Penicillium* have been shown to have pronounced antagonism to *C. elegans* when tested in dual cultures (27). Further research into the role of onion plants in reducing

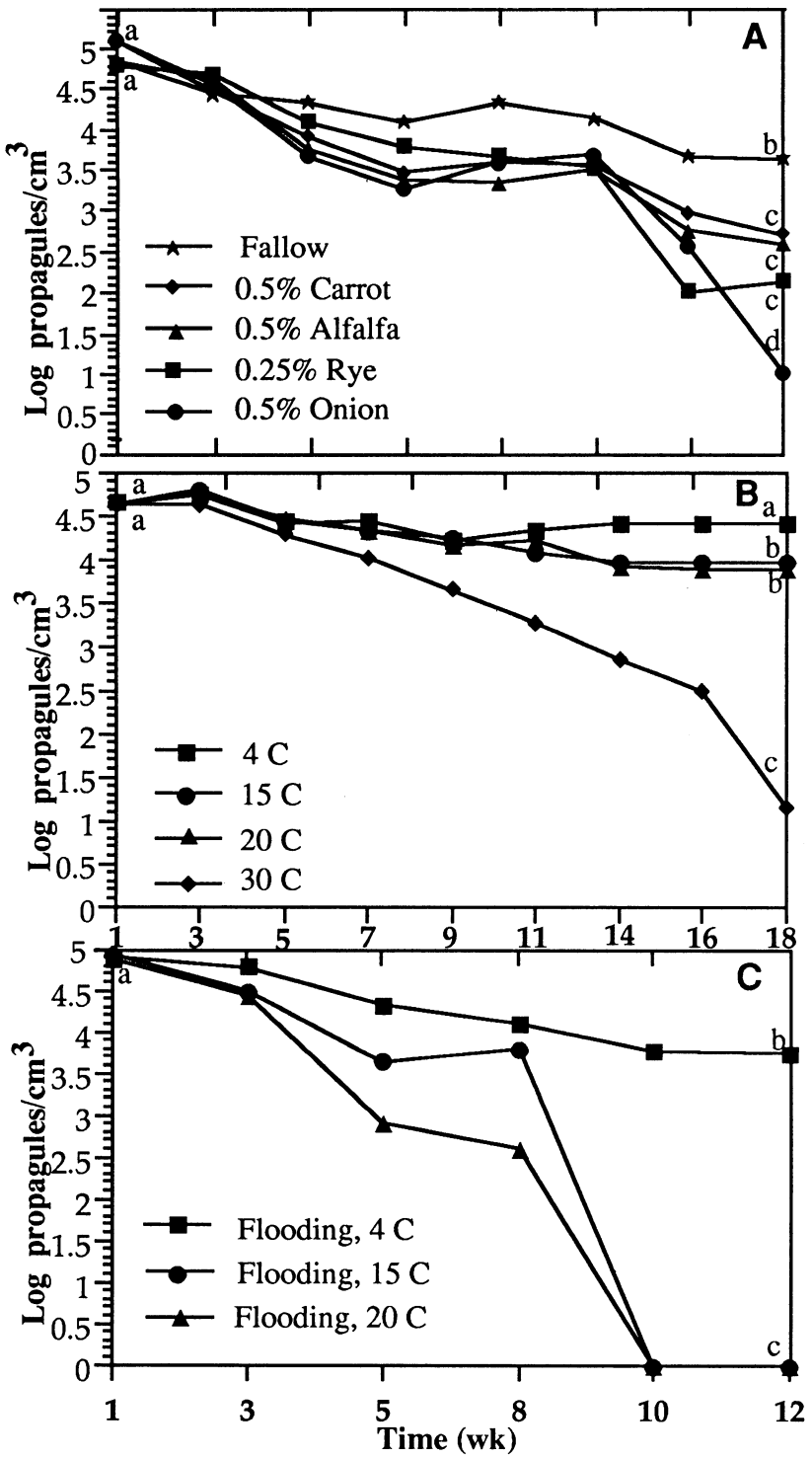

Fig. 4. Survival of phialospores of *Chalara elegans* in organic soil: **(A)** Influence of plant tissue amendments, **(B)** influence of temperature, and **(C)** influence of flooding at different temperatures. Data represent the average of two experiments, each with three replications. Treatments with different letters are significantly different ($P \leq 0.01$); these are indicated only at weeks 1 and 18 for A and B and weeks 1 and 12 for C to illustrate maximum differences.

inoculum levels of *C. elegans* is warranted, especially if onions are to be included as part of a cropping sequence in vegetable production. The role of *Penicillium* spp. in reducing inoculum density of *C. elegans* also requires further study.

The organic soil used in this study had very high levels of nitrogen and calcium. Survival of phialospores of *C. elegans* was reported to be prolonged by the addition of various forms of nitrogen to soil (16). Similarly, calcium enhanced growth of the fungus in culture (28), and high soil calcium levels were correlated with increased infection and disease development on susceptible tobacco cultivars (15). Thus, soil nutrient levels in the organic soil may also have contributed to the extended survival of phialospores observed in our study. Soils that were suppressive to black root rot caused by *C. elegans* in western North Carolina were characterized by low base saturation, low calcium levels, aluminum levels greater than 1 meq/100 g of soil, and pH <5.0 (14). Since the organic soil used in this study had a high base saturation level, a high level of calcium, and a very low level of aluminum, it should not be expected to suppress disease development in the field or to influence pathogen survival.

Studies on the effect of soil temperature and moisture on survival of both phialospores and chlamydospores of *C. elegans* have shown that high temperatures (above 24 C) coupled with high soil moisture resulted in a rapid decline of propagules (4,18,25). Enhanced microbial activity under these conditions promoted lysis and decay of the spores (4, 18,25). At lower temperatures (5–10 C), survival of phialospores in moist soil was observed over a 5- to 8-mo period (18). Our results also showed that phialospore survival in moist soil was reduced significantly at 30 C but not at 4, 15, or 20 C. However, when the soil was flooded, survival was reduced at all temperatures above 15 C and there was no effect at 4 C. The prolonged survival of phialospores of *C. elegans* in flooded soil maintained at 4 C implies that the pathogen may be tolerant of anaerobic conditions (low O_2/high CO_2). Papavizas and Lewis (18) reported that chlamydospore survival in moist soil incubated at 26 C was not affected by a CO_2 concentration of 20% when compared with air and that survival was enhanced at 10 C. They postulated that a reduction in general microbial activity at 10 C and high CO_2 may have prolonged chlamydospore survival (18).

The organic soils in the Fraser Valley frequently remain saturated with water for 2–3 mo during the winter. The results from our study indicate that phialospores of *C. elegans* can persist under these conditions for at least 5 mo with little loss of viability. In spite of the fact that phialospores are frequently used as a source of inoculum in pathogenicity studies (10,13,22,23,30), their role in initiation of disease in nature is unknown. Whereas chlamydospores produced in vitro were found to survive to a lesser extent than those formed in nature (25), a similar comparison has not been made for phialospores.

The soil and environmental conditions in the Fraser Valley of British Columbia appear to be conducive to the long-term persistence of *C. elegans*, which can be found in a large number of commercial fields (21). Frequent liming, high soil nitrogen levels, cool and moist weather conditions in the spring, and flooded soil conditions during the winter would all appear to favor survival. The potential of summer flooding, crop rotations that include onions, and biocontrol agents such as *Penicillium* spp. for reducing inoculum density of *C. elegans* warrants further study.

ACKNOWLEDGMENTS

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