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SURVIVAL OF *APHANOMYCES COCHLIOIDES* OOSPORES FOLLOWING PRECONDITIONING AT DIFFERENT HUMIDITIES

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Aphanomyces cochlioides (= *A. cochlioides*) is a soilborne pathogen that causes damping-off and root rot of sugarbeet throughout the Red River Valley and southern Minnesota. *A. cochlioides* produces two types of spores: zoospores and oospores. Zoospores are motile and swim through water in soil to infect sugarbeet roots; they are motile and infective for 12 hours and then die (3). Oospores, on the other hand, are produced inside infected plant material and are believed to allow the pathogen to survive in the soil for long periods of time. *A. cochlioides* survives in soil in the absence of a sugarbeet crop for as long as 20 years, however, little is known about how the pathogen persists in soil. Previous work has shown that about 90% of oospores in sugarbeet hypocotyls die within 8 weeks after they are buried in soil (4). In these studies, hypocotyls were kept in water before they were buried in soil. In the field, *Aphanomyces*-infected hypocotyls (and the oospores they contain) may desiccate (dry out, dehydrate) before plant tissue collapses on the soil surface or is incorporated into soil. Some species of fungi survive by desiccation but it is unknown if oospores of *A. cochlioides* survive by this mechanism. Understanding the factors that influence oospore survival in soil is important for improving disease management strategies.

OBJECTIVES

The purpose of this research was to determine 1) if survival of *A. cochlioides* is affected by preconditioning oospores at various relative humidities before they are buried in soil, depth of burial in soil, and sugarbeet variety and 2) if preconditioning oospores at different humidities affects pathogenicity on sugarbeet.

MATERIALS AND METHODS

Oospore survival. Two varieties of sugarbeet, ACH 205 (partially resistant to *A. cochlioides*) and ACH 261 (susceptible) were grown in a controlled environment chamber for 2 weeks in a commercial greenhouse soil mix. Seedlings were removed from soil, washed, and one hypocotyl (portion of root between seed and cotyledonary leaves) segment (0.75-inch length) was excised per plant. Two hypocotyls were placed in a Petri dish containing sterile distilled water and inoculated with *A. cochlioides.* Plates were incubated in the dark at $68 \pm 5\ ^{0}F$ for 5 weeks and examined under a stereoscope to confirm presence of oospores within hypocotyls.

Oospores in hypocotyls of both varieties then were preconditioned at <1, 50, and 100% relative humidity for 1 week. The <1 and 50% humidity treatments were established in bell jars with sulfuric acid solutions; 150mL of 80% H_2SO_4 were placed in the bottom of the bell jar to establish a relative humidity of <1% in the air space above; and 50ml of 43.4% H_2SO_4 were placed in the bottom of the bell jar to establish a relative humidity of 50% in the air space above (1). Hypocotyls were placed on filter paper in glass Petri dishes; two glass stirring rods were placed between the Petri dishes so they could be stacked; and then four dishes were placed in each bell jar (114 hypocotyls/bell jar). The bell jars were sealed with petroleum jelly and stored in an incubator at 77 °F. After 1 week, sugarbeet hypocotyls were removed and the filter paper was cut so each piece held one hypocotyl. A single hypocotyl was placed in a nylon mesh bag (1 x 1 inch) of a nylon monofilament mesh fabric (10µ pore size to prevent loss of oospores, which have a 20µ diameter). Bags were closed with monofilament fishing line and buried in the field later in the day. For the 100% relative humidity treatment, hypocotyls were removed from water, put in nylon mesh bags (as previously described), and placed in a pan of water to prevent drying. Five hypocotyls per preconditioning treatment of each sugarbeet variety were selected at random and processed to determine number of viable oospores.

The trial was established on June 3, 2003 in a field at the Northwest Research and Outreach Center, Crookston that had been planted to sugarbeet on May 23. Each treatment (two varieties with oospores preconditioned at three different humidities) were buried at 1- and 6-inch depths (at least 1 ft apart) between two, 20-ft rows of sugarbeet plants. Blocks of these treatments were arranged in a randomized design so all oospore treatments could be sampled 13 times (four replicates per sampling time). Each bag was tied with fishing line to a 10-inch wooden stake, which laid on the soil surface and identified the treatment. A Watchdog 400 Data Logger (Spectrum Technologies, Plainfield, IL 60544) with two external watermark soil moisture sensors and two external temperature sensors were buried at 1- and 6-inch depths in each replicate.

Samples were removed nine times during 2003 (June 10, 17, 24, 30; July 10, 15, 22; August 19; September 20). Four sets of treatments remain for sampling in 2004. After removal from soil, bags were gently washed with tap water to remove dirt and cut open. Hypocotyl tissue was removed with a fine-point tweezers, placed on a glass slide, and examined microscopically to rate the amount of tissue on a 0-5 scale: 0 = no tissue present, 1 = 1-20% of original tissue present (or only vascular tissue remaining), 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, and 5 = 81-100% of original tissue intact (4).

To extract oospores after rating, the tissue was transferred to a microcentrifuge tube containing 1 mL of sterile deionized water (4). The contents of each tube were poured into a 2 mL Wheaton tissue grinder and macerated by depressing a plunger 15 times. The solution was returned to the original microcentrifuge tube and centrifuged for 10 minutes at 10,000 rpm. A 750 µl aliquot of supernatant (not containing oospores) was decanted and the remaining 250 µl (containing oospores and macerated hypocotyl tissue) was vortex-mixed for several seconds. Subsamples were placed in a Speirs-Levy eosinophil counting chamber and oospores were microscopically examined and counted for viability at 400 X magnification. Viable (living) oospores have densely organized and uniformly granular (DOUG) cytoplasm and non-viable oospores (dead) have loosely organized and non-uniformly granular (LONG) cytoplasm (2). A double-sided piece of scotch tape removed oospores remaining on the surface of the nylon bags. The tape was placed on a glass slide and microscopically examined for oospore viability.

Pathogenicity Trial. Oospores were produced in hypocotyls of ACH 205; preconditioned at < 1, 50, and 100% relative humidity; and extracted, as previously described. For each preconditioning treatment, oospores were mixed with an *Aphanomyces*-free field soil at the rates of 9, 17, or 35 living oospores/cm^3 soil. The control consisted of non-inoculated field soil. For each soil mix, 550 cm^3 was dispensed into a round plastic pot (4.5-inch diameter), and sown with 25 seeds of ACH 261 treated with 0.625 + 2.7 g a.i. metalaxyl + thiram, respectively (five replicates/treatment). Pots were placed in a growth chamber at 69 + 2 °F and a 14 hour day length. When plants emerged 5 days after planting, the temperature was raised to 77 + 2 °F and pots were watered daily to favor disease. Stand counts were made twice a week for 3 weeks; 10 dead seedlings were removed from each pot and assayed to identify the pathogen. At 4 weeks after planting, dying seedlings were rated for disease and a root rot index was calculated (0 to 100 scale; 0 = healthy and 100 = all seedlings dead).

Statistical Analysis. For each date oospores were sampled in the field, data were analyzed by a one-way analysis of variance (ANOVA). For pathogenicity trials, root rot index values were transformed using arc sin and then analyzed by a three-way ANOVA.

RESULTS

Oospore survival. Prior to burial in soil, the average number of oospores per hypocotyl preconditioned at <1, 50, or 100% humidity (averaged across varieties) was 6,610, 4,810, and 5,990, respectively; oospores in each preconditioning treatment averaged 84% viability (data not shown). The average number of oospores per hypocotyl of ACH 205 and ACH 261 (averaged across preconditioning treatments) was 5,110 and 6,500, respectively; oospores in both varieties averaged 85% viability (data not shown).

There were no significant differences in oospore survival among treatments (preconditioning humidity, variety, or depth of burial), or interactions among treatments for most sampling dates from June 7 through September 20, 2003. Therefore, data are combined and averaged across all treatments (Fig. 1). Throughout the 109 days of sampling, total number of oospores per hypocotyl were higher than number of living oospores. This is because the total number includes living and dead oospores. The number of total and living oospores decreased rapidly and linearly until 37 days after burial in soil (Fig. 1). Then, the population of total and living oospores started to stabilize. By 109 days after burial, only 13% of oospores were still alive.

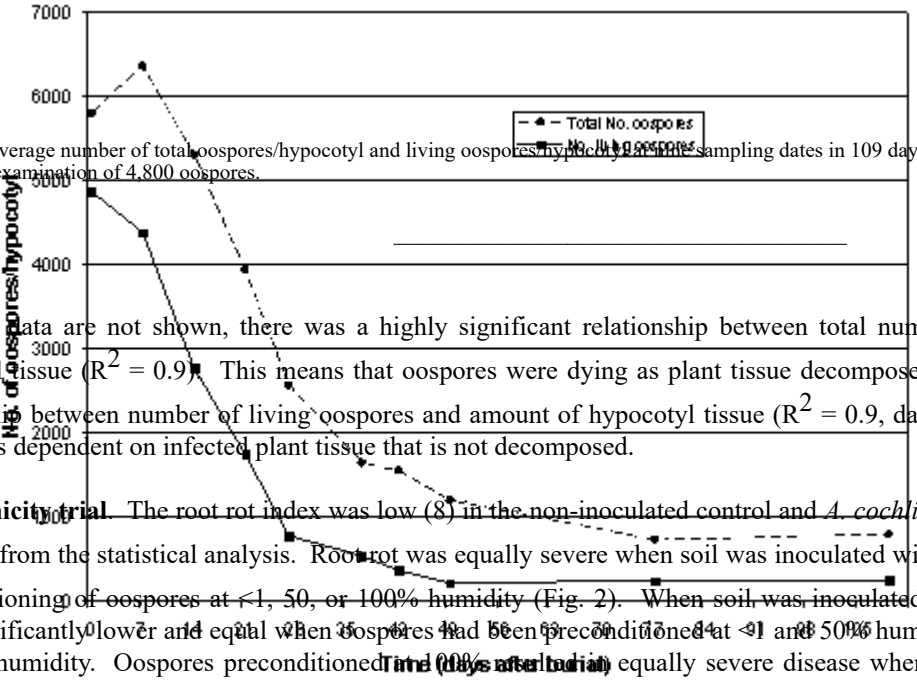


Fig. 1. Average number of total oospores/hypocotyl and living oospores/hypocotyl at nine sampling dates in 109 days. Each data point is based on microscopic examination of 4,800 oospores.

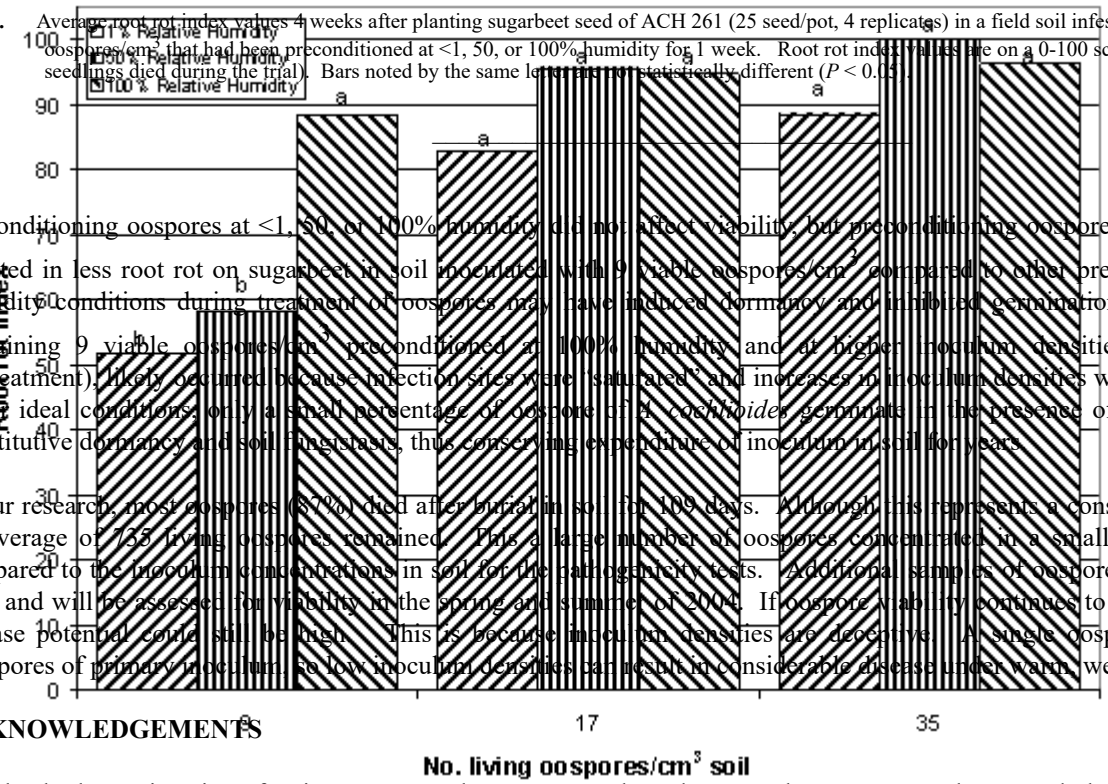
Although data are not shown, there was a highly significant relationship between total number of oospores and amount of intact hypocotyl tissue ($R^2 = 0.9$). This means that oospores were dying as plant tissue decomposed. There also was a highly significant relationship between number of living oospores and amount of hypocotyl tissue ($R^2 = 0.9$, data not shown), which indicates oospore survival is dependent on infected plant tissue that is not decomposed.

Pathogenicity trial. The root rot index was low (8) in the non-inoculated control and *A. cochlioides* was not detected, so this data was excluded from the statistical analysis. Root rot was equally severe when soil was inoculated with 17 or 35 oospores/cm^3, regardless of preconditioning of oospores at <1, 50, or 100% humidity (Fig. 2). When soil was inoculated with 9 oospores/cm^3, root rot indices were significantly lower and equal when oospores had been preconditioned at <1 and 50% humidity compared to those preconditioned at 100% humidity. Oospores preconditioned at 100% humidity caused equally severe disease when soil was inoculated with 9, 17, or 35 oospores/cm^3 (Fig. 2).

DISCUSSION

This research shows that desiccation is a not survival mechanism for oospores of *A. cochlioides.* In fact, loss of viable oospores was the same, regardless of preconditioning them at different humidities, depth of burial, or variety of sugarbeet. The rapid rate in loss of viable oospores within 5 weeks after burial is similar to population losses typically observed when fungal spores are added to soil. In the present study it was assumed that hypocotyl tissue, as well as the thick wall of oospores, would protect oospores from dying for an extended period of time. Hypocotyls, however, rapidly decomposed in soil and there was a significant relationship between tissue decomposition and loss of oospore viability. Since the pore size of the mesh bags was too small to allow oospores to be lost, it is reasonable to conclude that oospores died as plant tissue decayed. This outcome indicates that oospore survival is dependent on intact plant tissue, although it has been suggested that oospores remain free in the soil matrix as debris decomposes (3). Older plant tissues, which decompose slower in soil, may ensure oospore viability for a longer period of time. On the other hand, perhaps most oospores die very quickly, while a small proportion of the population survives for longer periods of time.

Fig. 2. Average root rot index values 4 weeks after planting sugarbeet seed of ACH 261 (25 seed/pot, 4 replicates) in a field soil infested with 9, 17, or 35 oospores/cm³ that had been preconditioned at <1, 50, or 100% humidity for 1 week. Root rot index values are on a 0-100 scale (0 = no disease, 100 = all seedlings died during the trial). Bars noted by the same letter are not statistically different ($P < 0.05$).



Preconditioning oospores at <1, 50, or 100% humidity did not affect viability, but preconditioning oospores at <1 and 50% humidity resulted in less root rot on sugarbeet in soil inoculated with 9 viable oospores/cm³ compared to other pretreatments. Low relative humidity conditions during treatment of oospores may have induced dormancy and inhibited germination. Severe root rot in soil containing 9 viable oospores/cm³ preconditioned at 100% humidity and at higher inoculum densities (regardless of oospore pretreatment) likely occurred because infection sites were 'saturated' and increases in inoculum densities were inconsequential. Even under ideal conditions, only a small percentage of oospore of *A. cochlioides* germinate in the presence of sugarbeet (3) because of constitutive dormancy and soil fungistasis, thus conserving expenditure of inoculum in soil for years.

In our research, most oospores (87%) died after burial in soil for 109 days. Although this represents a considerable loss in inoculum, an average of 733 living oospores remained. This a large number of oospores concentrated in a small volume, especially when compared to the inoculum concentrations in soil for the pathogenicity tests. Additional samples of oospores are overwintering in the field and will be assessed for viability in the spring and summer of 2004. If oospore viability continues to decline to lower numbers, disease potential could still be high. This is because inoculum densities are deceptive. A single oospore produces 100 to 300 zoospores of primary inoculum, so low inoculum densities can result in considerable disease under warm, wet conditions.

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