

Behavior of Chlamydospores and Endoconidia of *Thielaviopsis basicola* in Nonsterilized Soil

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ABSTRACT

Chlamydospores and endoconidia of *Thielaviopsis basicola* obtained as separate pure preparations from clones isolated from cotton and tobacco and grown on potato-dextrose agar were added to nonsterilized cotton soils. The chlamydospore germination percentage was dependent on the soil moisture regime. Thirty-five to 58% germinated in moist soil in the presence of 10% carrot juice or raw carrot slices. Only 11-28% germinated with the same nutrients after being kept 11 weeks in dry soil. Similar results were obtained on water agar plates. The low chlamydospore germination in dry soil could be

increased by wetting the soil several days prior to testing. When the soil was allowed to dry for several days, the germination again was low. The amount of germination was changed in this manner several times during a 1-month period. Some chlamydospores kept wet in soil for several weeks germinated spontaneously with a thin, branched germ tube. Endoconidia did not respond in this manner but germinated 6 hr after nutrients were applied and a few produced new conidia. Some endoconidia added to soil were found to survive at least 7 months.

Thielaviopsis basicola (Berk. & Br.) Ferraris is a soil-borne fungus that attacks the roots and hypocotyls of many plants. This pathogen has two main spore forms, namely, hyaline endoconidia and large, dark, thick-walled chlamydospores. Both spore forms are produced in considerable abundance in nature in or on infected tissue. Yet relatively little is known about their behavior or their role in nature. Patrick et al. (2) recently demonstrated the details and mechanisms of chlamydospore germination. Certain physiological requirements for chlamydospore and endoconidium germination under sterile conditions on agar substrates have also been reported (1). While chlamydospores would appear to be the major soil-borne propagules responsible for the long-period survival of this fungus in soil (4), the exact role of the hyaline, thin-walled conidia in survival and pathogenicity in nature is still a matter of some uncertainty (2, 3, 4). Very little is known about the environmental and nutritional requirements for chlamydospore and endoconidium germination and growth of this fungus in natural soil. This paper gives the results of laboratory studies on the survival and germination of chlamydospores and endoconidia in nonsterilized soil.

MATERIALS AND METHODS.—The isolates used were obtained from diseased cotton (C) and tobacco (T), and were grown on potato-dextrose agar (PDA) slants from which the chlamydospores and endoconidia were obtained. Pure endoconidia were obtained from 1-2-week-old cultures before chlamydospores had formed. Chlamydospore chains were separated from endoconidia and mycelial fragments by first washing 3-4-week-old agar slants in running water. The surface of the slants was then removed and homogenized for 1-2 min in a Virtis "45" homogenizer. The resulting suspension was freed of agar by repeated washings and slow-speed centrifugation. The chlamydospore chains could then be separated from the remaining hyphal fragments and endoconidia by filtration through a coarse filter paper or by means of differential sedimentation. A much better method, however, was to pour

the resuspended fungus pellet into large empty petri dishes. The suspension was allowed to dry overnight. The hyphal fragments and endoconidia adhered to the glass surface; the chlamydospore chains, which projected upward, were easily removed with a fine brush. By this means, chlamydospore preparations of high purity could readily be obtained. The chlamydospore chains were broken up by placing them on Millipore filter discs over wet nonsterilized soil as previously described (2), or by placing them directly in approximately 100 g of wet soil. In either case, chlamydospore chains were broken up in 1 week.

Germination was tested at room temperature (ca. 23 C) by wetting samples of soils fortified with chlamydospores in well dishes with a 10% solution of canned proprietary carrot juice or by spreading the soil samples over slices of raw carrot (5). The behavior of the propagules was determined by direct microscopic observation of soil smears. The preparations were either unstained or stained with a dilute solution of cotton blue in water. The soils used were from cotton fields in Tulare County, Calif. Chlamydospores, in most experiments, were left in soil for at least 1 month before germination studies were made.

RESULTS.—*Chlamydospore germination.*—Preliminary experiments with the C isolate showed that the germination process was similar to that previously described for the T isolate (2). The percentage germination of the C isolate was generally lower than that of the T isolate, however. In addition, the percentage germination of both isolates fluctuated widely depending on the soil moisture content. Such observations led to a study of the effect of drying and wetting the soil on chlamydospore germination.

Chlamydospores kept in moist soil appeared similar to those found in culture. Their contents were uniformly granular in appearance (Fig. 1). When the soil was air dried for prolonged periods, the chlamydospore contents were transformed into a few large globules and the chlamydospores appeared disorganized (Fig. 2). Most such chlamydospores failed to germinate in the

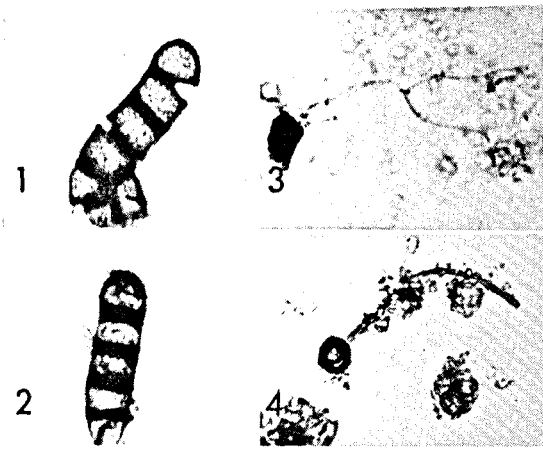


Fig. 1-4. Chlamydospores of *Thielaviopsis basicola* in nonsterilized soil. 1) Chlamydospores kept in moist soil. 2) Chlamydospores kept in air-dried soil for several months. 3) Chlamydospore germinating spontaneously (without addition of any nutrients) after being kept in moist soil for several weeks. Note the thin, branched germ tube (stained with cotton blue). 4) Chlamydospore germinating 24 hr after the addition of 10% carrot juice. Note the robust nature of the germ tube (stained).

presence of 10% carrot juice or when the soil was placed on raw carrot slices for 1-4 days. Germination was also very low on water agar and was only slightly better on water agar containing 300 ppm streptomycin. This low incidence of germination could be sharply increased, however, by wetting the soil with distilled water. Twenty-four to 48 hr after this treatment, most of the chlamydospores had regained their granular appearance. Germination followed a lag phase of 1-3 days depending on the isolate. Three to 5 days after the soil had been wet, germination reached a peak and then declined (Fig. 5).

In a typical experiment (Fig. 6), chlamydospores of the C and T clones were first added to soil that was air dried after the chlamydospore chains had been

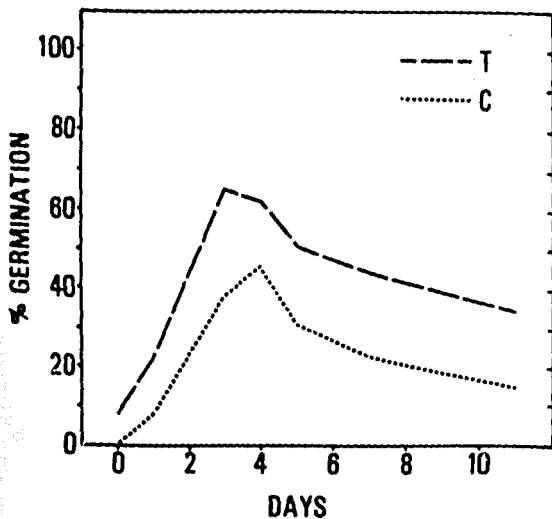


Fig. 5. Chlamydospore germination of *Thielaviopsis basicola* in nonsterilized soil at daily intervals after the soil was wet with water. Germination of two clones isolated from tobacco (T) and cotton (C), respectively, was tested with 10% carrot juice.

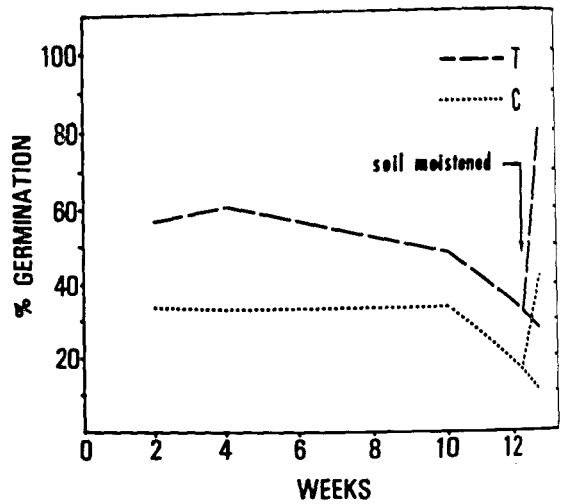


Fig. 6. Effect of prolonged periods of drying of nonsterilized soil on chlamydospore germination of *Thielaviopsis basicola* using tobacco (T) and cotton (C) clones of the fungus tested on raw carrot discs. Germination was increased rapidly to above the original level by wetting the soil for 5 days prior to testing.

broken up. The germination percentages on carrot discs were 35 and 58, respectively, based on the observation of 100 chlamydospores/clone. Eleven weeks later, during which time the soil was allowed to air dry further, germination was down to 10 and 28%, respectively. When half of this soil was then removed and moistened to above saturation for 5 days, germination was 42 and 80%, respectively. These percentages are higher than those obtained at the start of the experiment. Figure 7 shows a similar picture. Here the chlamydospores of both clones were broken up after 1 week in wet nonsterilized soil. Then the soils were allowed to dry for nearly 4 months at room temperature. At this time the soils were moistened to above saturation for 4 days and germination was tested with 10% carrot juice. Then the samples were air dried were 4 days and germination was tested again. This alternation of the moisture

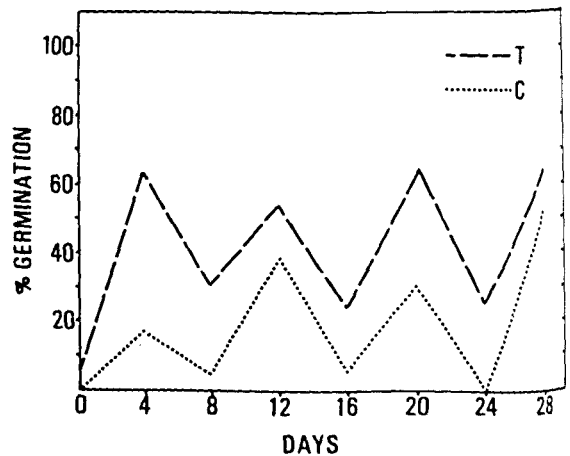


Fig. 7. Effect of fluctuating moisture regimes on chlamydospore germination of *Thielaviopsis basicola* in nonsterilized soil. Germination of the tobacco (T) and cotton (C) clones of the fungus was tested with 10% carrot juice following 4-day periods of keeping the soil wet or dry. Following a wet period, germination was increased; following a dry period, germination was decreased.

regime was continued over a 1-month period. The germination percentages obtained reflect these treatments. Germination was consistently higher following wetting of the soil and lower after drying of soil.

The T clone consistently had higher germination percentages than the C clone, regardless of the nutrient source. This indicates that there are quantitative clonal differences in the sensitivity of chlamydo-spores to soil moisture changes.

Also, where chlamydo-spores were kept in saturated soils continuously for more than 3 weeks, they germinated spontaneously without the addition of nutrients. The germ tubes produced under such conditions were branched and very thin (Fig. 3), unlike the robust sort produced in the presence of nutrients (Fig. 4). The percentage of chlamydo-spores germinating spontaneously, although difficult to determine, appeared not to exceed 10-15%. Such spontaneous germination occurred sooner with the T clone than with the C clone. Chlamydo-spores here generally produced hyphae only.

Survival and germination of endoconidia.—Endoconidia of the C clone produced on PDA were added to four cotton soils. Half of each replicate was allowed to air-dry and the other half was kept moist with distilled water for 7 months. Germination obtained with 10% carrot juice or raw carrot slices occurred after 6 hr of exposure to the nutrients in all treatments, indicating that these spores were not sensitive to differences in soil moisture regimes. Endoconidia germinated only in the presence of nutrients; no spontaneous germination in wet soil was seen. The experiments were terminated after 7 months, at which time viable endoconidia were still present. Microscopic examination at the end of the experiment showed that the original endoconidia were still germinating. Endoconidia germinated and often produced new endoconidia, which could be recognized by their rapid absorption of the cotton blue dye. They were usually smaller and thinner than the original endoconidia produced in culture or on host tissue. "Secondary chlamydo-spores" as described by Stover (3) in cultures were never seen in soil. Occasionally, conidia were seen to produce what appeared to be chlamydo-spore initials in 10% carrot juice after 48 hr.

DISCUSSION.—Chlamydo-spores and endoconidia of *T. basicola* are produced in abundance on diseased tissue, but the fate of these spores when they are liberated into the surrounding soil has remained unknown, largely because of difficulties in finding and studying them in situ. The methods presented in this paper whereby quantities of these spores can be prepared in high purity should facilitate such studies. Although these spores were obtained from PDA cultures, they were placed in soil early enough to allow conditions to approach those existing in nature.

One of the more surprising results was the dependence of chlamydo-spore germination on abundant soil moisture. The apparent requirement for a prior treatment with water has interesting implications with respect to survival of the organism, and in studies on

nutrition, pathogenicity, and populations in the field. The spontaneous germination of chlamydo-spores but not of endoconidia is also of interest, and further investigations are needed to explain this behavior. Meanwhile, this type of chlamydo-spore germination should not be confused with that due to nutrients under study. The increase in the percentage of chlamydo-spore germination following water treatment may indicate that the water in root exudates plays a significant role in disease epidemiology, particularly under arid soil conditions, and may explain the frequently reported increased incidence of disease in wet soils. The decline in the percentage germination which followed the peak at 3-5 days after wetting is still another unexplained observation. The practice of air-drying soils prior to making soil dilutions should also be reviewed.

Another significant result was the longevity of at least a small percentage of culture endoconidia in non-sterilized soil. Their delicate appearance would not indicate such a capacity. Stover (3) previously reported that some endoconidia were resistant to adverse conditions of heat, drying, and long periods of dormancy in culture. Tsao and Bricker (4) have recently reported that no colonies originated from conidia when natural soils were plated out on selective agar media. However, our studies certainly indicate that endoconidia must be considered with respect to survival, at least during the growing season of the host. Although endoconidia are rapidly lysed when placed on Millipore filters over saturated soil (2), some do survive for much longer periods when placed directly in soil, whether maintained wet or dry. Perhaps the conditions on the soil surface are more favorable for lysis, especially when the soil is at or above water-holding capacity. The observation that conidia placed in soil do not convert to any other form would suggest that the "secondary chlamydo-spore" reported by Stover (3) is a cultural phenomenon. In our studies, endoconidia did not germinate in soil wet with water only, but did germinate very rapidly in the presence of carrot juice. This germination may result in the immediate production of numerous new conidia. Occasionally, chlamydo-spore initials may be produced. These facts reinforce the importance of endoconidia in survival and possibly in pathogenicity.

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