Survival of Endoconidia and Chlamydospores of Thielaviopsis basicola as Affected by Soil Environmental Factors

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ABSTRACT

A high percentage of chlamydospores of *Thielaviopsis basicola* survived for at least 4 months in various autoclaved soils and for 2 months in nonautoclaved soils at 45-50% of the moisture-holding capacity (MHC). Soil moisture content was the most important single factor affecting germinability of endoconidia and chlamydospores. Germinability of chlamydospores declined rapidly at 45-50% of the MHC. Decline in germinability was intermediate at 30%; and no decline was observed at 15% and in air-dry soil maintained constantly at 20 C. When the temperature was varied, in air-dry soil germinability was 90% at 10 and 18 C after 3 months, 42% at 26 C, and 30% at 34 C. In moist soil (45-50% MHC), the effect of temperature on germinability was masked by that of soil moisture. In the moist soil at 26 C, germinability dropped to less than 10% after 1 month and to less than 5% after 2 months. Reduction in chlamydospore germinability was not pronounced in wet soil at 10 C. CO_2 did not greatly affect chlamydospore germinability in air-dried soil, but had some effect in moist soil. Viability and numbers of endoconidia were greatly reduced by high soil moisture content (45-50% MHC) and high temperature (26 and 34 C). Phytopathology 61:108-113.

Additional key words: Survival, root-infecting fungi, soil moisture.

Thielaviopsis basicola (Berk. & Br.) Ferr. survives in soil for long periods of time as large, dark, thick-walled chlamydospores (10, 11). Endoconidia, the small, hyaline propagules of this fungus, are considered shortlived (10) and less important than chlamydospores in the epidemiology of black root rot (4). Recently, however, some endoconidia were found unchanged and viable in soil after 7 months (2) or even after 10 months (8).

Very little information is available on soil environmental factors affecting survival of endoconidia and chlamydospores. Linderman & Toussoun (2) showed a definite dependence on abundant soil moisture for germination of chlamydospores but not of endoconidia. In their experiments, chlamydospores kept for 11 weeks in dry soil germinated only 11-28% in soil to which 10% carrot juice was added; however, these investigators obtained 40-60% germination when they wetted the soil several days before testing. Martinson (3) also reported that endoconidia and chlamydospores survived best in soil at 9 and poorest at 24 C.

We performed this investigation to learn more about the effects of moisture, temp, and CO_2 on survivability of endoconidia and chlamydospores in natural soils.

MATERIALS AND METHODS.—The characteristics of six soils used in this work are given in Table 1. Prior to use the soils were air-dried, passed through a 5-mm sieve, and mixed thoroughly. The moisture-holding capacity (MHC) of each soil was determined by the use of Hilgard cups and was expressed as the per cent of MHC.

We performed all experiments with isolates Tb 3 and Tb 5. For the chlamydospore experiments we used isolate Tb 3. For the experiments involving endoconidia, we used isolate Tb 5 because its endoconidia, and not those of Tb 3, are relatively sensitive to soil fungistasis (1). Endoconidia and chlamydospores were obtained as previously described (9) from cultures grown on Czapek-Dox agar containing 0.25% yeast extract.

Chlamydospores were added to autoclaved (15 psi and 121 C for 1 hr) and nonautoclaved soils as aq suspensions, and the soils were air-dried and kept at 10 C. When needed, chlamydospore-infested soil was brought to the desired moisture and used for the experiments. In preliminary experiments, we found that chlamydospores survived for long periods of time in air-dried soil at 10 C. An aq suspension of endoconidia was added to 2 kg of nonautoclaved soil so that the soil moisture was increased to about 45-50% of MHC. One kg was immediately air-dried. The other portion was used moist. The dry and wet endoconidia- and chlamydospore-infested soils were subdivided into 200-g portions and placed in 400-ml beakers. The beakers were weighed, covered with household plastic wrap, and punctured 5 times with a transfer needle to facilitate air exchange. The beakers were weighed once a week and, when necessary, additional water was added to bring the moisture content to the desired level. Immediately after each addition of water, the soil was mixed thoroughly and the beakers were covered. With this procedure, less than 1% of moisture was lost each week.

To determine the effect of CO_2 on germinability, 5-liter desiccators were used. The desiccators contained 200 ml of saturated KH_2PO_4 to maintain a relative humidity of 96%. KH_2PO_4 does not absorb CO_2. Atmospheric air and mixtures of CO_2 and air at 5, 20, and 50% CO_2 were used. The desiccators were filled with the gases by a method described by Umbreit et al. (12). For periodic sampling, soils were removed from the beakers, and the desiccators were again filled with the desired gases.

Germinability and numbers of the two spore forms of *T. basicola* were assayed by the propagule assay method (7). Briefly, 5-g soil samples were suspended in 95 ml

TABLE 1. Characteristics of six soils used in viability studies with *Thielaviopsis basicola*

Soil	pH Value	Moisture-holding capacity	Organic matter	Total nitrogen
		%	%	%
Lakeland sand (LS)	5.0	23	0.6	0.015
Codorus loam (CL)	6.7	53	1.0	0.074
Collington loam (Coll L)	5.5	44	1.2	0.101
Chester sandy loam (CSL)	7.5	30	1.6	0.100
Galestown-Evesboro sandy loam (GESL)	5.0	41	2.9	0.120
Muck soil[a] (Indiana) (MS)	5.5	125	42.7	0.720

[a] The Indiana muck soil was provided by R. J. Green, Jr.

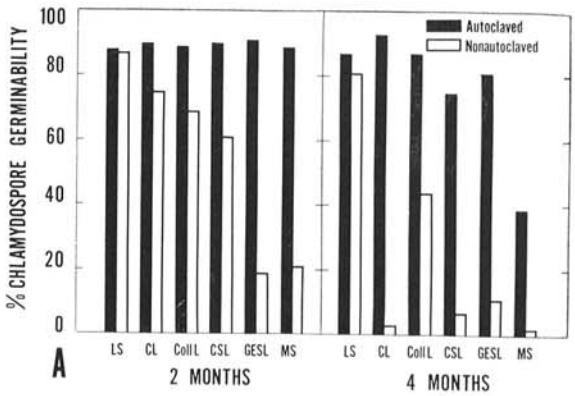
of 0.5% carboxymethylcellulose, and 1-ml aliquots were incubated on the VDYA-PCNB medium (6) at 25 C for 12-18 hr. The ability of 400 propagules/soil treatment per collection date to germinate on the agar was determined by microscopic observations. Populations of endoconidia and chlamydospores were assayed with the dilution-plate method on the VDYA-PCNB medium (6).

RESULTS.—*Effect of soil type on chlamydospore survival.*—Six chlamydospore-infested natural and autoclaved soils were brought to 45-50% of their MHC and incubated at 24 C. Samples for chlamydospore germinability were removed after 1, 2, 3, and 4 months.

In all six autoclaved soils, chlamydospore germinability was high after 2 months (Fig. 1-A). After 4 months of incubation, chlamydospore germinability remained high in five of the six soils. Germinability in muck soil, however, declined to 38%. During microscopic examination, we observed unusually high numbers of bacteria and streptomycetes that became established in this high organic content soil. All soils had been autoclaved 4 weeks before chlamydospore addition, and became contaminated during experimental handling.

In nonautoclaved soils, chlamydospore germinability declined with time and the rate and amount of decline varied from soil to soil. With some exceptions, germinability appeared to decline more in soils of high organic content than in those of low content. Even after 4 months of incubation at 45-50% MHC and 24 C, we observed no decline in Lakeland sand containing 0.6% organic matter, but considerable decline in muck soil containing 42.7% organic matter.

Chlamydospore survival in nonautoclaved soil.—Portions of Chester sandy loam containing chlamydospores were moistened to 4, 15, 30, 45, 60, and 75% of the MHC and incubated at 20 C. Chlamydospore survival differed considerably with variation in the moisture content of the soil (Fig. 1-B). Germinability declined rapidly at 45, 60, and 75% of MHC, and after 90 days in soil, germinability at these moistures dropped to less than 5%. Germinability declined less rapidly at 30% of soil MHC, with 36% of the chlamydospores germinating after 3 months. Germinability was high (90-93%) when soils were incubated for 90 days at 4 and 15% of MHC.



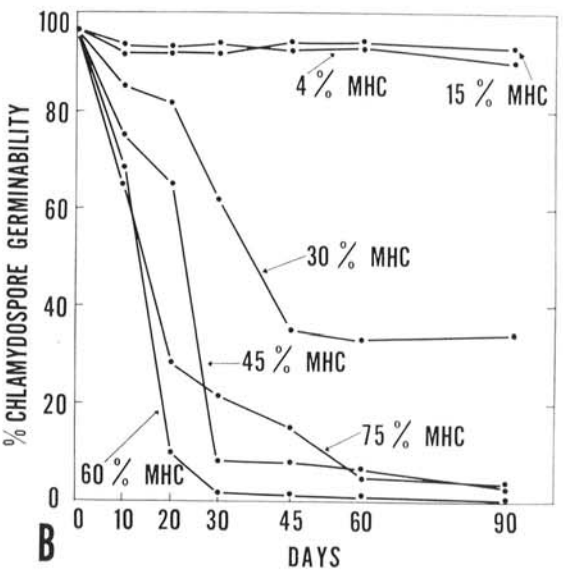


Fig. 1. Germinability of chlamydospores of *Thielaviopsis basicola* at various intervals of time. **A)** Effect of soil type on chlamydospore germinability. **B)** Effect of soil moisture on chlamydospore germinability.

After 90 days of incubation, soil with chlamydospores from the 30% MHC treatment was rewetted to about 50% of MHC to see whether reduction of germinability was due to desiccation. After a 5-day rewetting period, chlamydospore germination was 22%, about the same obtained without rewetting. At the two low moisture contents (4 and 15% MHC), per cent germinability of chlamydospores did not increase by rewetting the soils.

CO_2 did not affect chlamydospore germinability in air-dry soil even at the concn of 50% CO_2 in atmospheric air (Fig. 2-A). In the moist soil, the highest CO_2 concn used retarded the rate of decline of chlamydospore germinability produced by soil moisture.

We performed an experiment with chlamydospore-

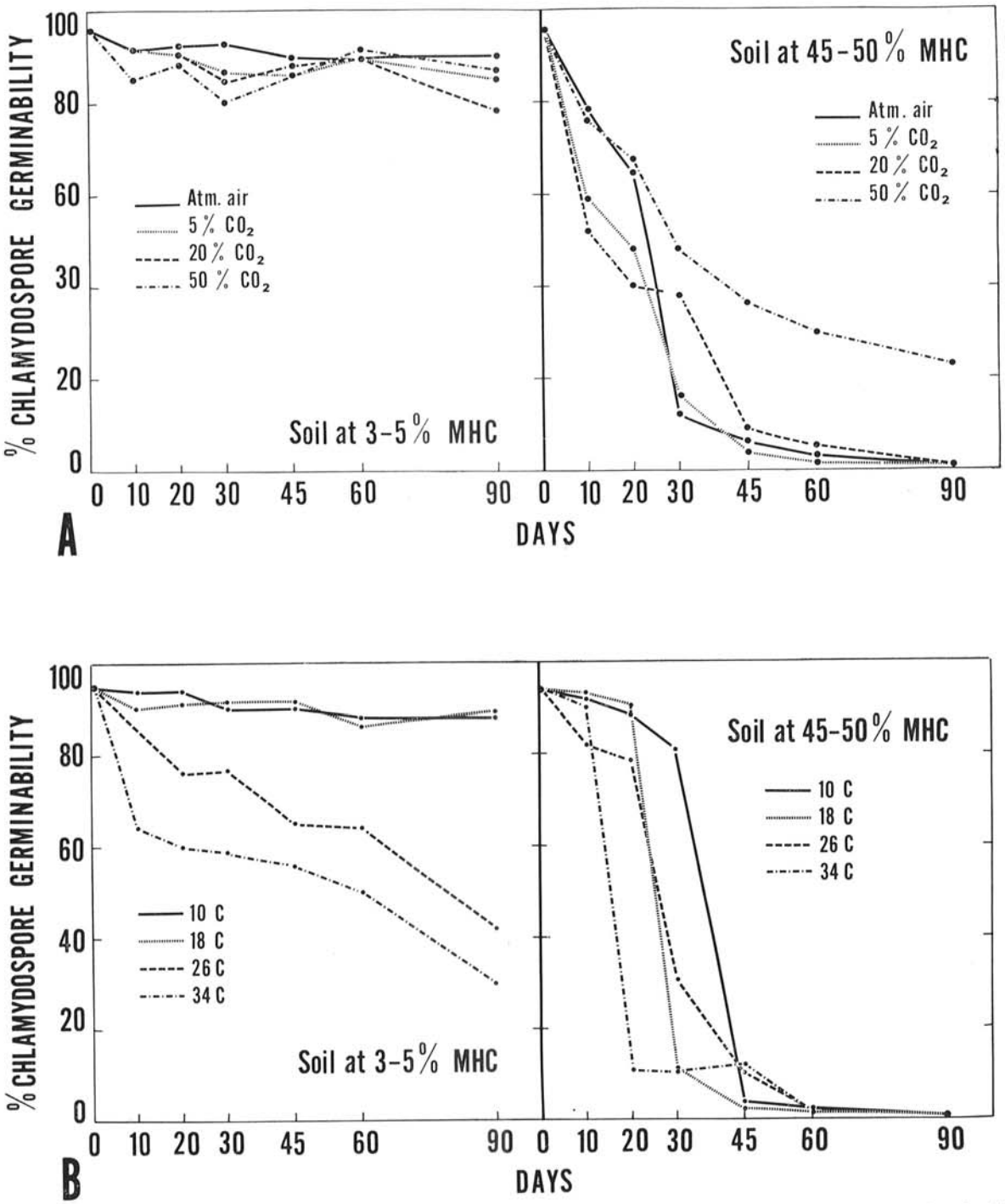


Fig. 2. Germinability of chlamydospores of *Thielaviopsis basicola* at various intervals of time in nonautoclaved soil. **A)** Effect of increasing concentrations of CO_2 on chlamydospore germinability. **B)** Effect of soil temp on chlamydospore germinability.

infested Chester sandy loam to determine the effect of temp on the viability of chlamydospores incubated at two moisture regimes, 3-5% (air-dry) and 45-50% of the soil MHC. In air-dry soil, temp of 10 and 18 C did not reduce germinability (Fig. 2-B). At 26 and 34 C, in air-dry soil germinability declined gradually until about 40% of the chlamydospores germinated after 90 days. In the moist soil, germinability fell off rapidly irrespective of incubation temp.

The air-dry, chlamydospore-infested soil exposed to

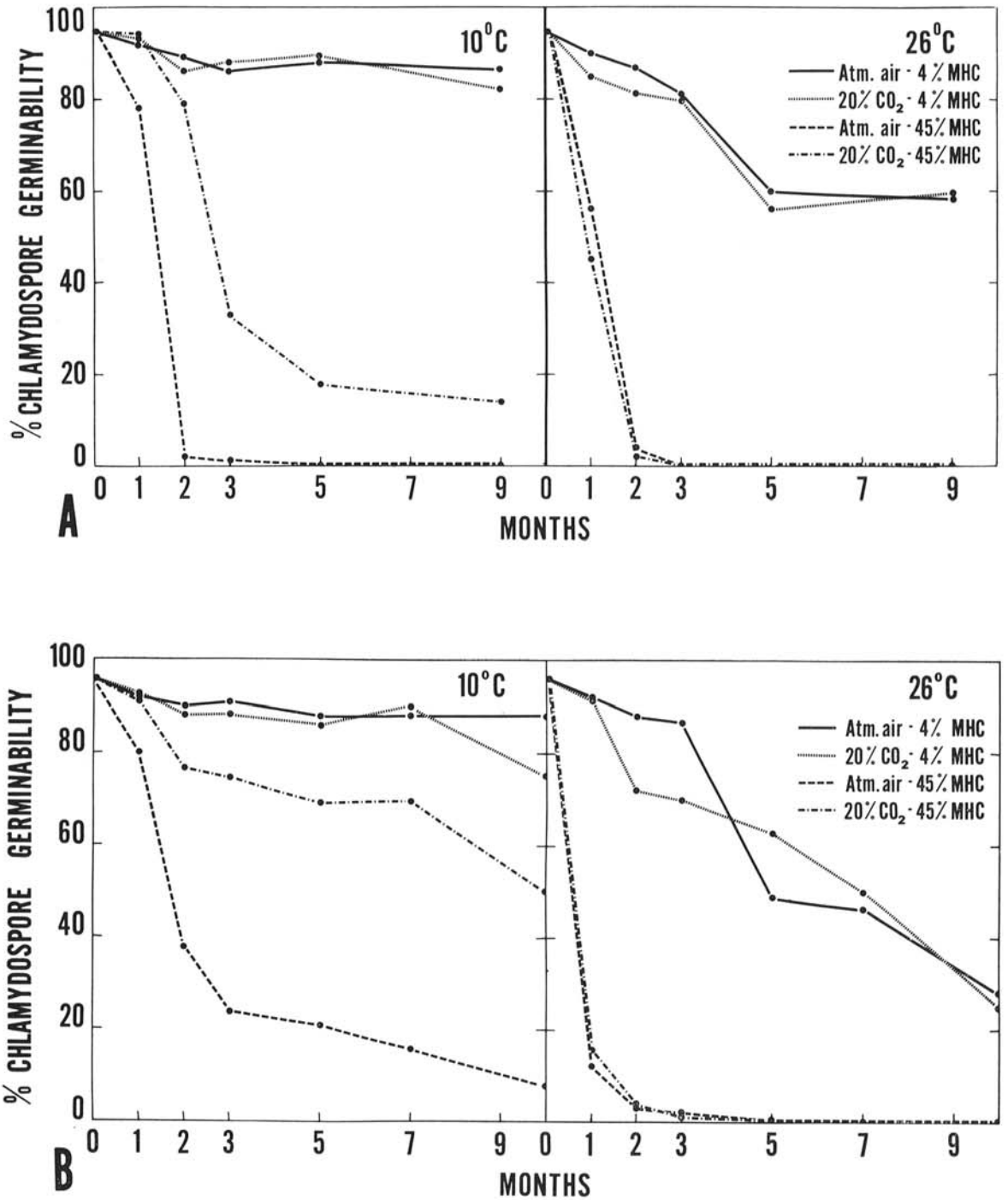


Fig. 3. Germinability of chlamydospores of *Thielaviopsis basicola* at various intervals of time in two chlamydospore-infested soils incubated dry (4% MHC) and moist (45% MHC) at 10 and 26 C and in two CO_2 concentrations. **A)** Nonautoclaved Chester sandy loam. **B)** Nonautoclaved Galestown-Evesboro sandy loam.

26 and 34 C for 90 days was rewetted and kept at 25 C for 5 days. Germinability assays were performed in the rewetted soils, and the data were compared with those from air-dry soil. Per cent germinability was 42 and 30% for 26 and 34 C, respectively, and 69 and 76% after rewetting.

A separate experiment was performed with Chester sandy loam and Galestown-Evesboro sandy loam to determine the effect of interactions of temp, moisture, and CO_2 on chlamydospore germinability. Assays were performed at intervals up to 9 months with the first soil and 10 months with the second (Fig. 3-A, B). In

the air-dry soils at 10 C, germinability was not reduced by air and 20% CO_2. At this low temp, 20% CO_2 delayed the rate of germinability decline, and the amt of delay was more pronounced in Galestown-Evesboro sandy loam (pH 5.0) than in Chester sandy loam (pH 7.5). In both air-dry soils incubated in air or 20% CO_2 at 26 C, germinability declined gradually to about 40-60%. Again, moisture content was the most important factor affecting chlamydospore germinability. For instance, chlamydospores did not survive in either soil at 26 C after 2-3 months of incubation in wet soil incubated in air or 20% CO_2.

In all previous experiments, we performed assays with the dilution-plate method to see whether chlamydospore populations actually declined over the periods of time used. In a typical experiment, for instance, per cent chlamydospore germinability after 90 days at 4, 30, 45, and 75% of MHC was 88, 8, 0, and 0, respectively; and the numbers of propagules/g soil determined by the dilution-plate method were 48.2×10^3, 3.6×10^3, 1.0×10^3, and 0.2×10^3, respectively. Similar data were obtained in the other foregoing experiments. The germinability data, therefore, on the recovered chlamydospores represent an accurate picture of the reduction of viability of chlamydospores as affected by the various environmental factors tested.

Survival of endoconidia in nonautoclaved soil.—The endoconidia-infested portions of Galestown-Evesboro sandy loan were incubated at 5, 10, 18, 26, and 34 C. A combination of high moisture content (45-50% of MHC) and high temp (26 or 34 C) resulted in rapid decline of numbers of endoconidia in soil (Table 2) and in a rapid decline of viability of the nonlysed endoconidia (Fig. 4). In 1 month, no endoconidia could be seen in wet soil portions incubated at 26 and 34 C, and very few remained nonlysed in wet soil at 18 C. Observations at 26 and 34 C are not included in Fig. 4 because only occasional endoconidia could be observed.

TABLE 2. Effect of soil moisture and temperature on numbers of endoconidia of *Thielaviopsis basicola* (Isolate Tb 5) in natural soil as determined by the quantitative propagule assay method

Soil moisture and temp	Endoconidia estimated by the propagule assay method[a]	
	3 Months	5 Months
	10^3/g of soil	
Dry soil (3–5% of MHC)		
C		
5	2,560	1,500
10	2,470	1,580
18	2,390	1,200
26	2,050	1,580
34	2,070	840
Wet soil (45–50% of MHC)		
5	1,480	580
10	120	60
18	2	0
26	0	0
34	0	0

[a] Populations at zero time were $2,850 \times 10^3$/g.

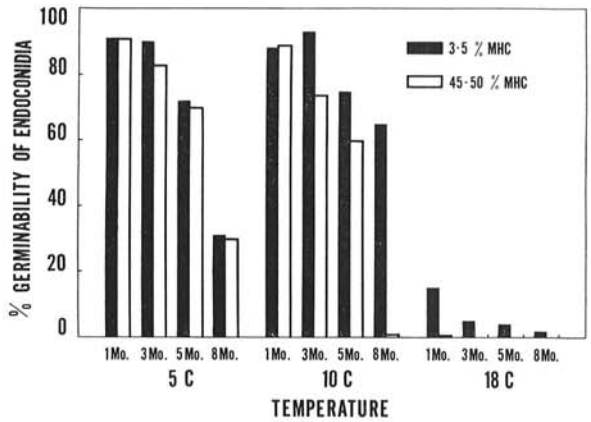


Fig. 4. Germinability of endoconidia of *Thielaviopsis basicola* at various intervals of time in dry and moist nonautoclaved soil maintained at temp of 5, 10, and 18 C.

Although a great reduction in numbers of endoconidia was observed in wet soil at 5 and 10 C, considerable numbers still survived even after 5 and 8 months; and the viability of the nonlysed endoconidia was greater than 50% after 5 months.

In the air-dry soil, lysis of endoconidia was not as pronounced as in the moist soil as is indicated by the numbers of nonlysed endoconidia estimated by visual observations (Table 2). Viability of nonlysed endoconidia, however, was greatly reduced at 18, 26, and 34 C in both dry and moist soil.

DISCUSSION.—Moisture was the most important single factor affecting viability of endoconidia and chlamydospores in soil. The moisture effect at high moisture contents was so pronounced that it overshadowed that of temp. For instance, at 45-50% MHC (Fig. 2-B), chlamydospores lost their germinability rapidly after 20-45 days in soil irrespective of incubation temp. Also, endoconidia lysed rapidly in wet soil at temp of 18 C or higher; and those that were not lysed lost their viability rapidly. The detrimental effect of high soil moisture on propagule viability may have some applications in the field for cultural control of *T. basicola*. If a field is kept moist during periods of high soil temp, an appreciable reduction of the inoculum density of *T. basicola* might result.

It is well known that soil microbial activities are more intense at 26 and 34 C than at 5, 10, and 18 C. In the results obtained by Martinson (3) and by us, temp itself played an important role in the survival of endoconidia (Fig. 4) and chlamydospores (Fig. 2-B, Fig. 3-A, B) in soil. The fact that germinability of both spore types declined more rapidly in microbially active moist soils than in inactive dry soils suggests that microbial activity may be important for survival of these propagules in soil. This assumption is based on the fact that the inoculum added to soils does not grow and resporulate (1, 8). This assumption is also supported by the fact that CO_2 (50% in air) delayed or reduced loss of viability in wet soils, presumably by reducing activities of some microbial groups in soil. Old (5) made similar observations on the importance of mi-

crobial activities on survival of *Cochliobolus sativus*. On the other hand, gradual loss of viability of endoconidia and chlamydospores in dry soils at 26 C or higher, but not at 10 C, may be explained by assuming that protracted desiccation at high temp irreversibly damages the propagule protoplast.

Germinability of chlamydospores incubated in dry soil at 26 or 34 C could be increased to some extent in some experiments by rewetting the soils prior to germinability assays. In other experiments, viability loss under these conditions was irreversible. On the other hand, rewetting of dry, chlamydospore-infested soil that had been incubated at 10 C for 9-10 months was not needed for good germination (Fig. 3-A, B). This observation is in contrast to observations made by others (2) concerning the effect of soil dehydration on chlamydospore germinability. The discrepancy may be due to differences in isolates used or in temp of incubation of chlamydospores in air-dry soil.

From the results in Fig. 4, and from previous observations (2, 8), it appears that survival of endoconidia of *T. basicola* may have been underestimated in the past. High numbers of endoconidia survived at 5 and 10 C in both dry and wet soil for 8 months. This means that endoconidia produced on infected plant roots in the fall may survive, and constitute an effective source of inoculum the following spring in areas where temp fall below 10 C in soil. Although the effect of organic debris and the depth of its burial in soil on survivability of *T. basicola* propagules are not known, it is possible that large amounts of organic matter in the soil may be detrimental to the survival of the resistant chlamydospores. Perhaps propagules of *T. basicola* may survive for longer periods of time under adverse conditions of moisture and temp when they occur free in the soil matrix than when they are embedded in organic debris.

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