Survival and Colonization Potential of *Fusarium moniliforme* var. *subglutinans* in Soil

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ABSTRACT

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Conidia of *Fusarium moniliforme* var. *subglutinans* were short-lived in soil in the absence of host tissues; they survived 6–13 wk depending on soil moisture and incubation temperature. However, conidia colonized sterilized pineapple stem and leaf segments that were previously mixed in soil of 10% moisture content, which enabled the fungus to survive at least 12 mo. In general, free conidia survived better in air-dried soil than in soil adjusted to 10, 25, or 35% moisture content and survived better at soil temperatures of 4 and 18 C than at 25 and 30 C. *F. moniliforme* var. *subglutinans* also colonized sterilized dead stem segments of corn, bean, and soybean, which indicates that the fungus exists mainly by colonizing plant tissues and less frequently as conidia in a field.

Additional key words: pineapple fruit rot, basal rot.

Fusarium moniliforme var. *subglutinans* Wr. & Reink., attacks various economically important plant species (2) including corn (*Zea mays* L.) (12), sugarcane (*Saccharum officinarum* L.) (14), slash pine (*Pinus elliottii* Englam var. *elliottii*), and loblolly pine (*Pinus taeda* L.) (8). In Brazil, *F. moniliforme* var. *subglutinans* attacks pineapple (*Ananas comosus* (L.) Merr.) and induces fruit rot and basal rot of the asexual propagative parts (11,16). The disease is widespread and limits the production of pineapple in Brazil. Despite repeated fungicide applications (1,7), 30–80% losses often occur (1,16).

Factors affecting survival of *F. moniliforme* var. *subglutinans* have not been studied. The fungus does not produce chlamydospores and it is not clear how it survives between pineapple crops. Several investigations showed the importance of soil moisture or temperature on the survival of *Fusarium* spp. in soil (5,6,15), but similar studies with *F. moniliforme* var. *subglutinans* have not been made. The objectives of this study were to determine the influence of soil moisture and temperature on the survival and colonization of *F. moniliforme* var. *subglutinans* in soil under controlled conditions.

MATERIALS AND METHODS

Isolate UnB 302 (ATCC 38067) of *F. moniliforme* var. *subglutinans,* originally isolated from naturally infected pineapple fruit was used throughout the study. Conidia for soil infestations were obtained as previously described (1). The soil was a red latosol with a pH of 5.2. It was sifted through a 2-mm mesh sieve and air-dried for 48 hr at room temperature (24 ± 2 C), which resulted in approximately −910 bars matric potential. Soil moisture potential was determined (Fig. 1) by the filter paper method of Fawcett and Collis-George (9).

Survival in soil independent of host tissue. Forty milliliters of conidia suspension (5×10^6 conidia per milliliter) was thoroughly mixed with 400 g of soil in plastic pots (13 × 11 × 11 cm). Soil moisture was adjusted to 10, 25, and 35% by weight (resulting in approximately −86, −3, and −0.4 bars matric potential, respectively), or the soil was air-dried again to its original water content (−910 bars matric potential). Soils were incubated at 4, 18, 25, or 30 C in incubators (Freas 815 Low Temperature incubator, Precision Scientific Co., Chicago, IL 60647), and moisture contents were maintained by adding water twice weekly. Soils prepared similarly but not infested with conidia were controls. Two replicates per treatment were used, and the experiment was repeated twice.

Soil was sampled for assay of *F. moniliforme* var. *subglutinans* immediately after conidia were added to soil and 7 days thereafter until the fungus could no longer be recovered. Three 5-g soil samples were removed from each pot and separately diluted to 10^{-4} with sterile water at each time interval. One milliliter of the diluted soil suspension was then pipetted into a sterile petri plate and approximately 10 ml of cooled (45 C) selective medium (13) was added. Plates were prepared in quadruplicate per soil sample assayed. The number of colonies of *F. moniliforme* var. *subglutinans* developed on the selective medium (13) were counted after 1 wk of incubation at room temperature (24 ± 2 C). Throughout the investigation, colonies from representative numbers of plates were transferred to potato dextrose agar and compared with an original culture of *F. moniliforme* var. *subglutinans.* All data were analyzed statistically and means were grouped by Duncan's multiple range test ($P = 0.05$).

Survival in soil in the presence of host tissue. Two-hundred milliliters of conidia suspension (5×10^6 conidia per milliliter) was thoroughly mixed with 2 kg of air-dried soil in plastic trays (37 × 27 × 12 cm). Stem and leaf segments (10 × 5 mm) of 7-mo-old pineapple, sterilized by overnight exposure to propylene oxide, were mixed into infested soil. Soil was incubated at 25 C and the moisture content was maintained at 10% by adding water periodically. At various intervals for 1 yr, 50 stem and leaf segments were removed from soil, washed in running tap water for 15 min and plated on the selective medium (13). The number of segments colonized by *F. moniliforme* var. *subglutinans* was recorded.

Colonization of plant tissues. To determine whether *F. moniliforme* var. *subglutinans* can colonize plant tissues other than pineapple, 30 ml of conidia suspension at 10^6, 10^4, and 10^2/ml were separately mixed with 300 g of air-dried soil in plastic pots (13 × 11 × 11 cm). One-hundred dead stem segments (10 × 5 mm) of pineapple, corn, bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* (L.) Merr.), obtained after harvest and sterilized as previously described, were buried separately in infested soil. Soil was adjusted to 10% moisture and held at 25 C. After 1 mo of incubation, all stem segments from each treatment were removed from soil and assayed separately for *F. moniliforme* var. *subglutinans.*

RESULTS

Survival in soil independent of host tissue. Conidia of *F. moniliforme* var. *subglutinans* survived in soil relatively briefly in

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the absence of host tissue; survival was influenced by soil moisture and incubation temperature (Fig. 2). At 25 C, *F. moniliforme* var. *subglutinans* was recovered for 10 wk from air-dried soil but only for 6 wk from soil adjusted to 35% moisture at the same temperature (Fig. 2C). Similarly, the mean number of colonies developing in dilution plates from soil adjusted to 10% moisture declined to undetectable levels after 8 wk at 30 C (Fig. 2D), but colonies were still recoverable from soil of the same moisture level until the 11th wk at 4 C (Fig. 2A). One week after infestation the mean number of *F. moniliforme* var. *subglutinans* colonies developing in the dilution plates sharply declined at all soil moistures and temperatures. Within this period, however, the sharpest deline in the number of colonies developing in the dilution plates was detected in air-dried soil at 30 C and in soil adjusted to 10% moisture and held at 4 and 30 C. The number of colonies obtained on the selective medium from air-dried soil at 30 C and from soil adjusted to 10% moisture held at 4 and 30 C, was reduced 90, 92, and 91% from the initial concentration, respectively (Fig. 2A and D). The frequency of isolation of *F. moniliforme* var. *subglutinans* fluctuated after 1 wk of incubation at 4 C with 10% soil moisture (Fig. 2A) and after 3 wk at 18 C with air-dried soil (Fig. 2B). At 4 C the number of colonies appearing on the selective medium increased from 4×10^3 to 7.7×10^3/g of soil adjusted to 10% moisture (Fig. 2A) and, at 18 C, from 2.5×10^3 to 5.3×10^3/g of air-dried soil (Fig. 2B). The frequency of isolation of the fungus gradually declined to undetectable levels, regardless of soil moisture or incubation temperature.

The longest time in which *F. moniliforme* var. *subglutinans* was detected in air-dried soil held at 18 C in the absence of host tissue was 13 wk (Fig. 2B). The shortest time was 6 wk in soil adjusted to 35% moisture and held at 25 C (Fig. 2C). The fungus survived significantly longer ($P = 0.05$) in air-dried soil and soil adjusted to 10% moisture incubated at 4 and 18 C than at 25 and 30 C (Fig. 2). There were no significant differences in the survival of *F. moniliforme* var. *subglutinans* among propagules in soil incubated at 4 and 18 C at both soil moistures and at 25 and 30 C in air-dried soil or in soil adjusted to 10% moisture (Fig. 2). No colonies of *F. moniliforme* var. *subglutinans* were detected on dilution plates from soil used as control.

Survival in soil in the presence of host tissue. When conidia were added to soil of 10% moisture content previously mixed with sterilized pineapple stem and leaf segments, the fungus was isolated from these tissues. *F. moniliforme* var. *subglutinans* was isolated from all dead host tissue segments after 1 wk of soil infestation and thereafter for a 1-yr period.

Colonization of plant tissues. The percentage of dead stem segments from which *F. moniliforme* var. *subglutinans* was isolated at 10^5 and 10^3 conidia per gram of soil was about the same for all plant species (Table 1). At 10 conidia per gram of soil, however, the fungus was isolated less frequently from bean, soybean, and corn stem segments than from pineapple stem segments. The percent colonization of stem segments of bean, soybean, and corn did not differ significantly (Table 1).

DISCUSSION

Conidia of *F. moniliforme* var. *subglutinans* can survive only a short time in soil independent of host tissue. Relatively high soil moistures (35% moisture) and high temperatures (25 and 30 C) decrease survival time. Free conidia of *F. moniliforme* var. *subglutinans* survived twice as long in air-dried soil as in soil of 35% moisture content and survived significantly longer in soil incubated at 18 C than at 30 C. Differences in survival time in dry and wet soil may be explained by more rapid or increased germination at high soil moisture, thus reducing the population of viable conidia; conidia either fail to germinate or germinate poorly under dry conditions, as occurs with other fungi (3,10). The increase in the mean number of viable propagules of *F. moniliforme* var. *subglutinans* in dilution plates from air-dried soil at 18 C (Fig. 2B) is not understood at this time.

Our results indicate that long-term survival of *F. moniliforme* var. *subglutinans* in soil is by colonization of host tissue. The fungus remained viable for at least 12 mo in the presence of pineapple stem and leaf segments. The fact that *F. moniliforme* var. *subglutinans* survived in plant tissues other than those of pineapple suggests that it survives mainly by colonizing plant tissues and less frequently as free conidia in soil. Therefore, the fungus can prolong its survival by colonizing residues of plants that are not hosts.

The results also suggest that *F. moniliforme* var. *subglutinans* has competitive saprophytic ability and may colonize dead plant material, since the fungus was isolated from 67% of pineapple stem segments at a density as low as 10 conidia per gram of soil (Table 1). However, experimental conditions such as incubation temperature, water content of the soil, and exposure of the tissues to propylene oxide, may have contributed to the high percentage of colonization of the tissues. Such a high degree of saprophytic colonization (4) by *F. moniliforme* var. *subglutinans* may not occur in the field.

Although the perfect stage of *F. moniliforme* var. *subglutinans* occurs (2), it has not yet been found in Brazil. Because the fungus is not known to produce chlamydospores (2), we conclude that *F.*

TABLE 1. Percent colonization of pineapple, bean, soybean, and corn stem segments by *Fusarium moniliforme* var. *subglutinans* in soil adjusted to 10% moisture and incubated at 25 C for 1 mo

Conidia per gram of soil	Stem segments and colonization (%)[y]			
	Pineapple	Bean	Soybean	Corn
1×10^5	100 A[z]	98 A	95 A	100 A
1×10^3	100 A	93 A	98 A	94 A
1×10	67 A	39 B	33 B	41 B

[y] Average of 100 stem segments (10 × 5 mm) per treatment. Stem segments were obtained after harvest and sterilized by overnight exposure to propylene oxide.

[z] Mean values in each row followed by the same letter do not differ significantly ($P = 0.05$) from each other by Duncan's multiple range test.

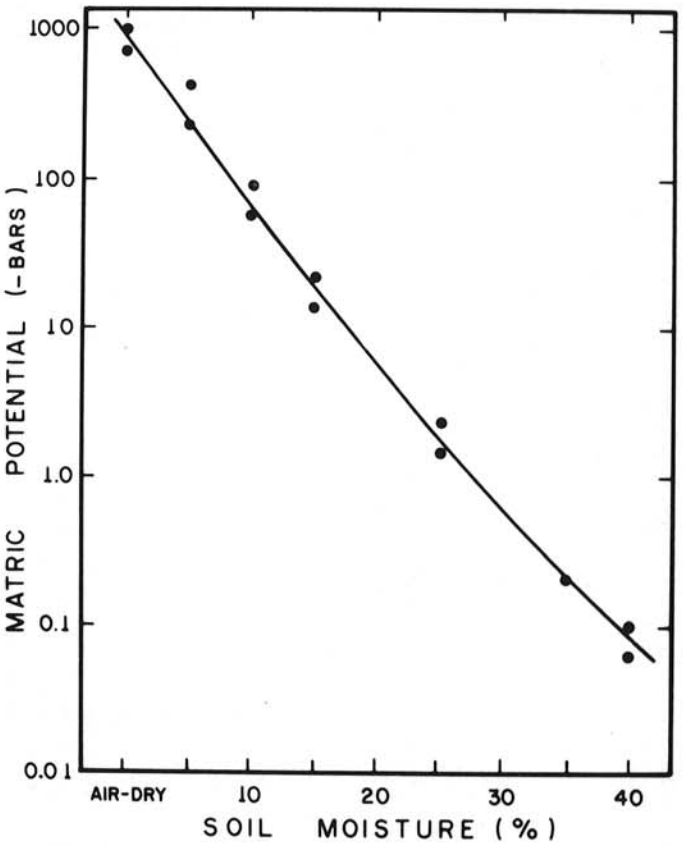

Fig. 1. Matric potential vs. soil moisture curve for soil used in the survival studies of *Fusarium moniliforme* var. *subglutinans*.

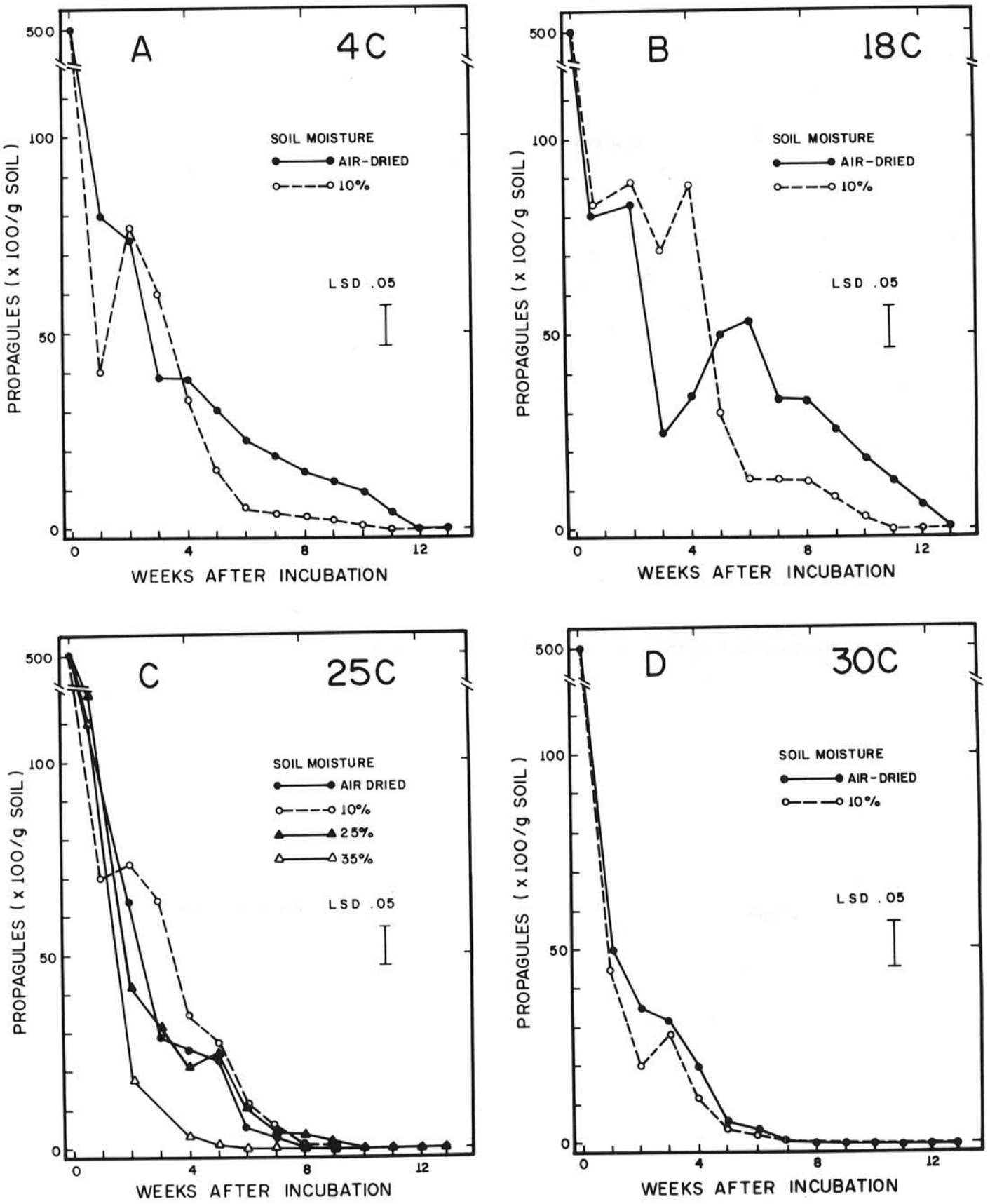

Fig. 2. Effect of various soil moisture levels on the population of *Fusarium moniliforme* var. *subglutinans* in red latosol during 13 wk: **A,** at 4 C; **B,** at 18 C; **C,** at 25 C; and **D,** at 30 C. Each point represents the average number of colonies developing in 48 dilution plates (three subsamples per soil sample per replicate with four plates per subsample, repeated twice).

moniliforme var. *subglutinans* can survive saprophytically from season to season by colonizing plant debris.

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