Longevity of Teliospores of *Ustilago scitaminea* in Soil

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ABSTRACT

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Longevity of teliospores of *Ustilago scitaminea,* the causal agent of sugarcane smut, was studied in soils collected from fields with or without a sugarcane cropping history. Few viable spores (1%) were detected after 4 wk in contact with saturated soil, and none were detected after 6 wk. When teliospores were mixed in nonsterile soils adjusted to three moisture levels, the number of viable spores decreased rapidly after 1–4 wk. Viable spore numbers consistently decreased most rapidly in the wettest soil, but spore longevity was similar for all three moisture levels in each soil. In three experiments, longevity of spores was limited to 7–9 wk in soils containing moisture. The number of viable spores also decreased rapidly with time in sterile soils with different moisture contents, and longevity was limited to 4–7 wk. Variation was observed for different spore collections mixed in air-dried soils maintained at ambient relative humidity. When spores mixed in three air-dried soils were kept under desiccation, the percentage of viable spores did not begin to decrease until after 18 wk. Spores from six locations lost viability after being maintained free of soil for 23 wk at ambient relative humidity. In comparison, germination percentages after 23 wk for the same spore collections maintained under desiccation ranged from 17 to 55%. The results indicate that teliospores of *U. scitaminea* are not long-lived in soil when moisture is present. This represents a limiting factor for the increase of sugarcane smut under subtropical climate conditions in Louisiana, because soilborne inoculum will not persist through the winter and be present when sugarcane is tillering during the spring.

Smut, caused by *Ustilago scitaminea* Syd. & P. Syd., has been an important disease of sugarcane (interspecific hybrids of *Saccharum* L.) in most regions in which this crop is grown (12). The first appearance of the disease in a given region typically results in the development of a severe epidemic in susceptible cultivars and the removal of those cultivars from production.

The infection court for *U. scitaminea* is the sugarcane bud, and germinating buds are most susceptible to infection (2,3). Soilborne teliospores can infect germinating buds of planted seed cane and tillering plants (1,2,10,12). One factor that can affect the development of smut epidemics is the concentration of teliospores in the soil. The number of spores in the soil is determined by proximity to an inoculum source (9) and by conditions affecting spore germination and the duration of viability or longevity. Teliospore germination is inhibited by fungistasis (18,19), and spore longevity is affected by environmental conditions (1,10,13).

The increase of sugarcane smut is affected by subtropical climate conditions in Louisiana. Interactions with the host and environmental factors limit rates of disease increase from season to season during the 3-yr crop cycle (7,8). The production of smut sori ceases when temperatures decrease during the fall (9), and the growth of sugarcane is interrupted for several months during winter (14). Winter freezes adversely affect the overwintering of smut-infected plants and the number of secondary infections that are expressed during the subsequent season (7,8). It is uncertain whether inoculum produced during one season in Louisiana can persist and cause infections in the next season. To provide information on this aspect of the disease cycle, experiments were conducted to study the longevity of teliospores of *U. scitaminea* in Louisiana soils.

MATERIALS AND METHODS

Teliospore and soil collections. Sori of *U. scitaminea* were collected from naturally infected sugarcane plants in the field (Table 1). Sori were allowed to dry for 1 wk, then rubbed on the surface of a sieve with 1-mm openings to free teliospores from the sorus surface. Collected teliospores were placed in a desiccator at room temperature. Field soil was collected from five locations (Table 2). Soils were air dried, passed through a sieve with 1-mm openings, and stored at 4 C.

Determination of teliospore longevity on saturated soil. Cellulose nitrate membranes 50 mm in diameter with 3.0-μm pores were wetted and placed on the surface of saturated soil. Teliospores from collection 1 (Table 1) were dusted onto the membranes, and changes in the percentage of germination over time were determined. In preliminary experiments, the percentage of germination on membranes was similar to that on the soil surface. After 1 day, and then at 4-day intervals, a membrane was removed from the soil, inverted, and the spores pressed onto a water agar plate. The percentage of teliospores already germinated was determined immediately. The agar plate was then incubated 8 hr at 32 C, and teliospore germination was determined again. To determine the percentage of germination, the total number of spores and the number of germinated spores were counted for four replicates with a minimum of 75 spores. Germination was considered to have occurred when a germ tube equal in length to the spore diameter was observed. The experiment was repeated.

Determination of longevity of teliospores in soils with different moisture

Table 1. Location, sugarcane cultivar, and year collected for sources of teliospores of *Ustilago scitaminea*

Teliospore collection	Sugarcane cultivar	Louisiana Parish	Year of collection
1	multiple	Iberville	1987
2	multiple	Iberville	1988
3	multiple	Terrebonne	1988
4	multiple	Iberville	1989
5	CP 65-357	Iberia	1989
6	CP 79-318	Iberia	1989
7	CP 65-357	Iberia	1989
8	CP 79-318	Iberia	1989
9	multiple	Iberville	1990

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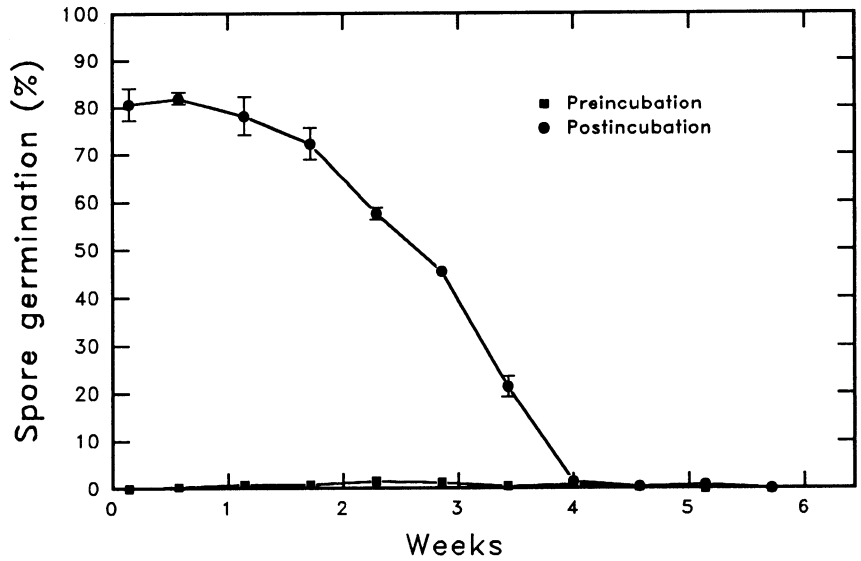

Fig. 1. Percent germination of teliospores of *Ustilago scitaminea* on nitrocellulose membranes on the surface of saturated field soil, determined at 4-day intervals. Percent teliospore germination was determined immediately after transfer to water agar (preincubation) and after incubation for 8 hr at 32 C (postincubation). Data points are means from four replicates, and bars represent mean standard errors.

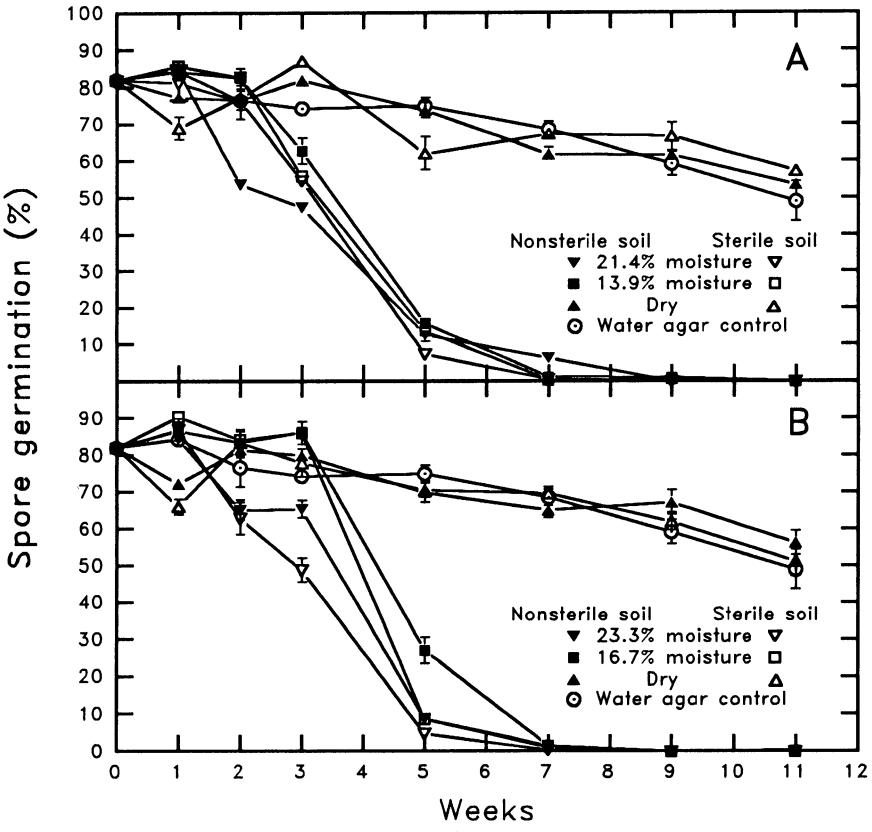

Fig. 2. Longevity of teliospores of *Ustilago scitaminea* from spore collection 1 in nonsterile and sterile field soils 1 **(A)** and 2 **(B)** containing different amounts of moisture. Data points are percent germination means, and bars represent mean standard errors.

contents. Experiments were conducted with teliospore collections 1, 2, 3, and 9 (Table 1) and with five soils (Table 2). A portion of each soil was sterilized by autoclaving twice. Teliospores were mixed into air-dried nonsterile and sterile soils at a rate of 320 mg of spores per 8 g of soil. Soils were then adjusted to matric potentials of −100, −1,000, or −5,000 MPa with a pressure plate extractor and transferred to 6-cm-diameter petri dishes. Because the experiments were conducted over extended periods, the gravimetric moisture content for each soil at different matric potentials was determined. Deionized water was then misted onto and mixed into the different soils each time teliospore germination counts were made, to maintain appropriate moisture levels. Teliospore/soil mixtures were kept in an incubator at 24 C. At weekly intervals, 0.1 g of soil was removed from each treatment and added to 10 ml of deionized water. The samples were vortex-mixed for 2 min, and 0.4 ml of solution was spread over a water agar plate. The plates were incubated for 8 hr at 32 C, and the percentage of spore germination was determined for four replicates of each treatment. Germination rates of teliospores mixed in soil were compared to the rates of teliospores from the same collections plated directly onto water agar at each sampling time. Data were analyzed by repeated-measures analysis of variance and by tests for orthogonal polynomial contrasts of the time variable (17).

Longevity of teliospores from collection 9 (Table 1) was monitored in air-dried soils 1–3 (Table 2) maintained under desiccation and at ambient relative humidity. Teliospores were mixed into the soils, and the percentage of spore germination was determined as described previously. In addition, teliospores from collections 4–9 (Table 2) were maintained in petri dishes free of soil under the same conditions, and the percentages of germination of spores under desiccation and at ambient relative humidity were compared at the conclusion of the experiment. Relative humidity, determined with a sling psychrometer, ranged from 28 to 70% during the experiments; however, relative humidity was not determined inside the petri dishes containing the spores. Results were analyzed by repeated-measures analysis of variance and by tests for orthogonal polynomial contrasts of the time variable (17).

Table 2. Characteristics of soils used in experiments to determine the longevity of teliospores of *Ustilago scitaminea*

Soil collection	Previous crop	Texture class	Particle fractions (%)			Nitrogen content (g/kg)	Organic matter (%)	pH
			Sand	Silt	Clay			
1	sugarcane	silt loam	30.4	54.4	15.2	0.76	0.94	5.8
2	sugarcane	silt loam	19.7	58.9	21.4	0.91	0.99	6.3
3	pasture	silt loam	17.4	65.1	17.4	1.42	1.89	6.9
4	soybean	silt loam	15.3	62.1	22.6	1.19	1.55	6.8
5	sugarcane	clay	7.4	24.9	67.7	1.12	2.07	5.5

RESULTS

Teliospore longevity on saturated soils. The number of germinated spores detected immediately at each sampling ranged from 0 to 1.9% and averaged 0.6% (Fig. 1). The percentage of germination of these spores after incubation on agar exceeded 80% initially, then decreased with increasing time in contact with saturated soil. No viable spores could be detected after 4 wk (Fig. 1). Teliospore longevity in the repeat experiment was similar.

Teliospore longevity in soils with different moisture levels. The repeated-measures analysis of variance and the tests for orthogonal polynomial contrasts indicated that all variables, high-order contrasts, and interactions were significant in each of the three experiments. Repeated measures effects and interactions were evaluated with Greenhouse-Geisser adjusted F values (17). These results indicate that the pattern of change in the number of viable spores (decrease in percent germination) over time was different for each combination of factors; therefore, the data for each treatment were plotted for comparison in Figures 2–4.

In the first experiment, teliospores from collection 1 (Table 1) were mixed into soils 1 and 2 (Table 2). In nonsterile soils containing moisture, teliospore viability decreased sharply after 2–3 wk (Fig. 2). Few viable spores were detected after 7 wk, and none at 9 wk. Germination of teliospores mixed in air-dried soils showed a gradual decrease with time, and the percentages of germination ranged from 51 to 57% at 11 wk (Fig. 2). The teliospores of the control treatment were maintained free of soil but not under desiccation, and these spores showed a decrease in the percentage of germination over time similar to spores in dry soil (Fig. 2). Changes over time in the numbers of viable teliospores in sterile soils were similar to those in nonsterile soils (Fig. 2).

In the second experiment, teliospores from collection 2 (Table 1) were mixed with nonsterile and sterile soil from collections 1–3 (Table 2). The numbers of viable teliospores in nonsterile soils collected from sugarcane fields (Fig. 3A and B) decreased rapidly when moisture was present, and few viable spores were detected after 4 wk. Germination of teliospores in a pasture soil ranged from 40 to 59% after 4 wk (Fig. 3C). However, as in experiment 1, spore longevity was limited to 7 wk in all soils when moisture was present. The number of viable spores decreased most rapidly in all three sterile soils with the highest moisture content (Fig. 3), and the decrease in the number of viable spores was more rapid in the sterile pasture soil at all three moisture levels than in the corresponding nonsterile soil (Fig. 3C). Few viable spores were detected in any sterile soil at 4 wk, and none were detected after 5 wk. Teliospores in air-dried soils showed similar decreases in the percentage of germination in nonsterile and sterile soils (Fig. 3). At the conclusion of the experiment, teliospore viability levels in dry soils ranged from 6 to 13%. Teliospores in the control treatment were kept under desiccation and showed only a 15% decrease in germination percentage after 7 wk.

In the third experiment, teliospores from collection 3 (Table 1) were mixed with nonsterile and sterile soils 3–5 (Table 2). Decreases in viable spore numbers with time showed variation among treatments and soils; however, teliospore longevity was similar in all three soils. Viable spores were not detected after 8 wk in any of the four replicates per treatment (Fig. 4). Teliospores in dry soils showed decreases in numbers over time similar to spores in soils containing moisture (Fig. 4). Extensive scanning of plates, without counting, detected rare viable spores in all three soils after 13 wk at the conclusion of the experiment.

Comparisons of teliospore longevity under desiccation and at ambient relative humidity. Because all of the variables and many of the higher-order contrasts and interactions were significant, the data for each treatment were plotted and compared in Figure 5. No viable spores were detected after 23 wk in three air-dried soils maintained at ambient relative humidity, whereas the percentage of viable spores in soils maintained under desiccation did not begin to decrease until that time (Fig. 5). Decreases in the number of viable teliospores were similar in different soils within the desiccation

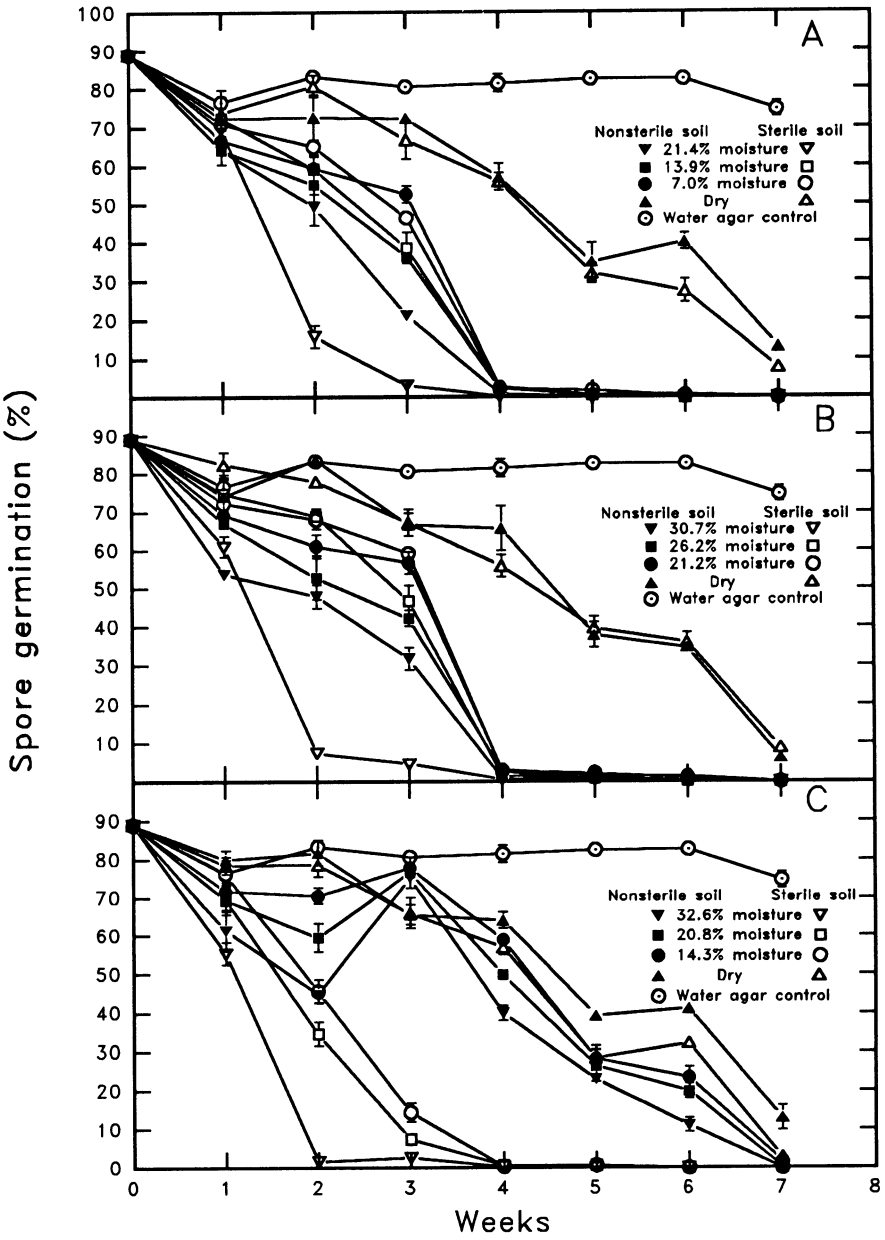

Fig. 3. Longevity of teliospores of *Ustilago scitaminea* from spore collection 2 in nonsterile and sterile field soils 1 **(A)**, 2 **(B)**, and 3 **(C)** containing different amounts of moisture. Data points are percent germination means, and bars represent mean standard errors.

and ambient-relative-humidity treatments.

The percentages of germination for teliospores from six collections maintained free of soil under desiccation and at ambient relative humidity were determined at the beginning of the experiment and after 23 wk. At ambient relative humidity, the germination rate from all collections had decreased to less than 1% after 23 wk. For spores maintained under desiccation, the percent losses after 23 wk compared with the germination rates at the beginning of the experiment were 55, 58, 44, 40, 28, and 17% for teliospore collections 4 through 9 (Table 1), respectively.

DISCUSSION

Longevity of teliospores of *U. scitaminea* was similar for different spore collections, in soils with different characteristics and moisture levels, and in soils with and without a sugarcane cropping history. Teliospore longevity was limited to 7–9 wk in nonsterile soils with moisture levels ranging from 7 to 33%, and spores persisted only 4 wk in contact with saturated soil.

The longevity of teliospores in these experiments was generally similar to the results of previous studies. In India, the longevity of teliospores buried in the field was limited to 3 wk under wet conditions, whereas viable spores could be detected after 31 wk under dry conditions (14). In Florida, teliospores mixed in an organic soil lost viability after 4 wk in saturated soil but persisted 14 wk in soil containing 61% moisture (1). When sugarcane was planted in soil at various times after the addition of teliospores, viable spore concentrations sufficient for infection were present after 8–16 days under wet conditions and after 32–64 days under dry conditions (10).

Germination of teliospores of *U. scitaminea* in soil is inhibited by fungistasis (18,19). In addition, teliospores require liquid water for germination (19). Means for immediate spore germination rates decreased from 7 ± 2% (standard error) and 63 ± 26% in nonsterile and sterile saturated soil, respectively, to 1 ± 0.6% and 14 ± 6%, respectively, in soils adjusted to −100 MPa (J. W. Hoy, *unpublished*). A continuous low germination rate for spores followed by germ tube lysis could account for the consistently rapid decrease in numbers of viable spores detected in nonsterile and sterile soils with the highest moisture (−100 MPa) content. Water availability in soils with lower moisture contents should not have been sufficient to allow a high frequency of spore germination. As a result, much of the observed decrease in the number of viable spores must be attributed to a loss of the ability to germinate. The similar longevity of teliospores in sterile and nonsterile soils containing intermediate levels of moisture provides additional evidence that short-term spore survival in moist soil is an inherent characteristic of *U. scitaminea*.

Smut sori often are colonized by other microorganisms, including parasitic fungi and bacteria with antibiotic activity (5). However, colonization of teliospores by other fungi or high numbers of bacteria was not observed in spore platings from sterile or nonsterile soil treatments. The appearance of spores did not change during the course of the experiments. Spores with low germination percentages in soil were plated on potato-dextrose agar in one experiment to determine if germination was still possible in the presence of nutrients or if colonization by microorganisms would become evident, and neither situation was detected (J. W. Hoy, *unpublished*).

Viability and longevity of teliospores of different smut fungi vary among spore collections (5). Differences in longevity were observed among *U. scitaminea* collections when spores were mixed in air-dried soils or kept under desiccation, but different spore collections all showed similar longevity in soils when moisture was present.

The longevity of teliospores in storage is variable among the smut fungi (5). However, there is little information on teliospore longevity in soil under natural conditions. Teliospores of some smut fungi, such as *Sphacelotheca reiliana* (Kühn) Clinton (6), *Tilletia indica* Mitra (11,15), and *U. maydis* (DC.) Corda (4), regularly overwinter and provide soilborne inoculum the following season. Longevity of teliospores of *U. scitaminea* may be prolonged under dry conditions or by desiccation, but teliospores of this smut fungus cannot persist for long

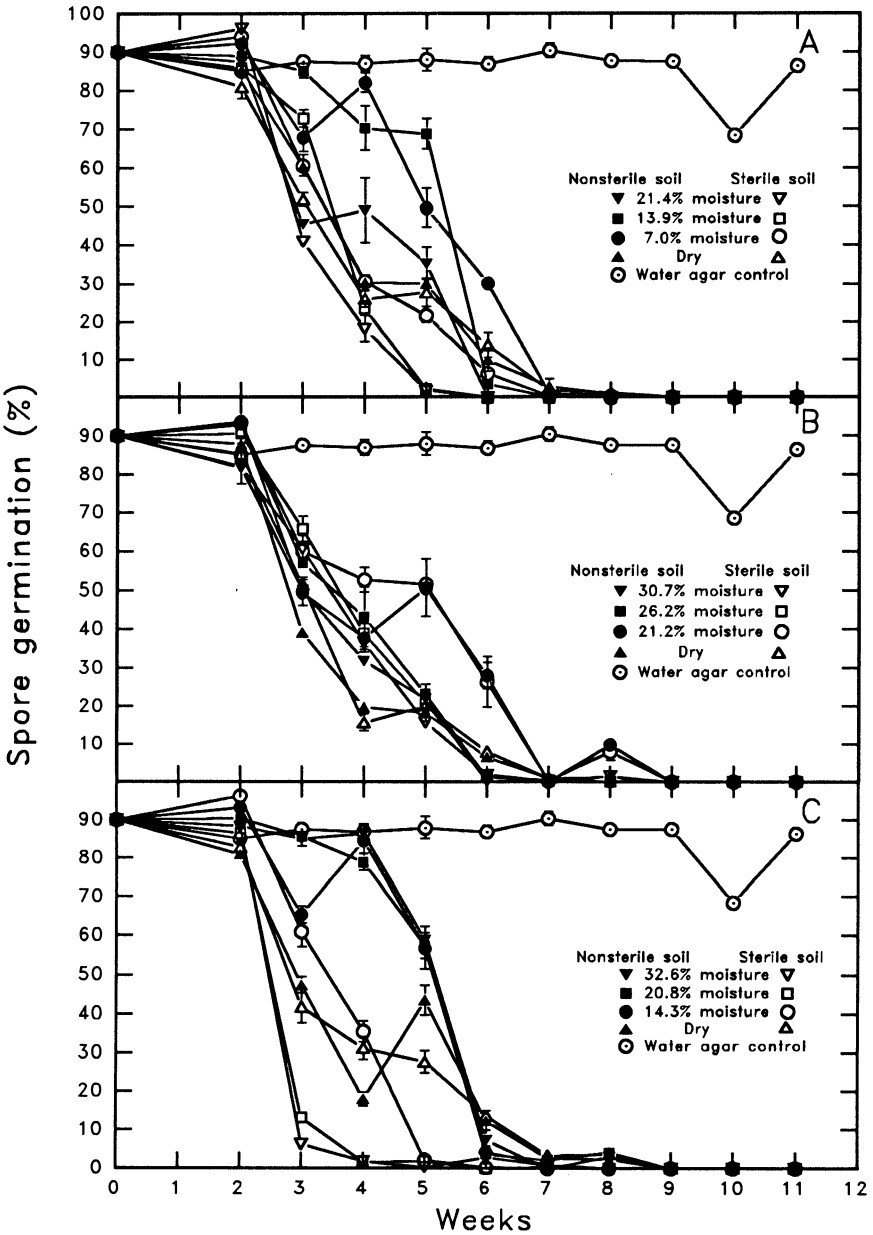

Fig. 4. Longevity of teliospores of *Ustilago scitaminea* from spore collection 3 in nonsterile and sterile field soils 3 **(A)**, 4 **(B)**, and 5 **(C)** containing different amounts of moisture. Data points are percent germination means, and bars represent mean standard errors.

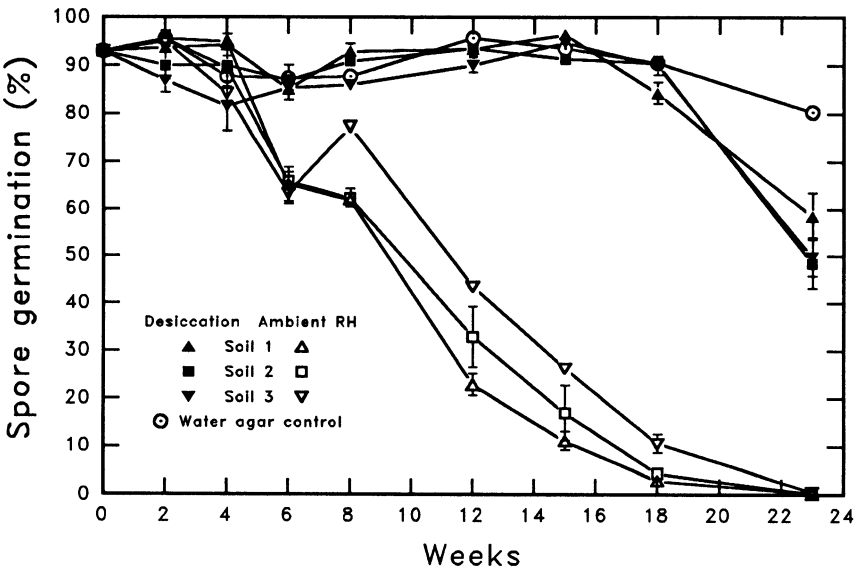

Fig. 5. Longevity of teliospores of *Ustilago scitaminea* mixed in three air-dried field soils, then maintained under desiccation or at ambient relative humidity. Data points are percent germination means, and bars represent mean standard errors.

periods in moist soils. This suggests that overwintering of smut teliospores is unlikely in Louisiana or in other subtropical regions in which sugarcane is grown.

Smut sori are produced from May through October in Louisiana (9). The failure of teliospores produced during the season to overwinter would have an adverse effect on disease increase the next season. The pathogen can overwinter in infected plants, but infected plants often do not survive winter freezes (7,8). The results of the studies reported here suggest that few viable teliospores are present in the soil in spring, and that the sugarcane tillering phase occurs with little risk of infection until May. Observations of tillering in sugarcane have found that the average maximum shoot populations developed by the end of April are 32% in plant cane and 72% in ratoon (16). All of these factors interact to effectively lower potential rates of disease increase in moderately susceptible sugarcane cultivars in Louisiana (7,8,9). If the initial incidence of smut infection is kept low by continuously introducing and maintaining sources of smut-free seed cane, development of severe epidemics during the 3-yr crop cycle is unlikely.

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