Ascospores of *Monosporascus cannonballus*: Germination and Distribution in Cultivated and Desert Soils in Arizona

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ABSTRACT

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Our objectives were to provide an explanation for the observed uniform distribution of vine decline of cantaloupe in commercial fields and determine the role of ascospores of *Monosporascus cannonballus* in the etiology of this disease. Population densities of ascospores in cultivated soils were uniform in distribution, both vertically and horizontally. Additionally, ascospores of the fungus were recovered from all soil samples collected both vertically and horizontally from two native desert sites, and perithecia of the fungus were observed on roots of a native plant, *Lepidium lasiocarpum*, growing in a native habitat. These results indicate that the fungus is an indigenous soilborne fungus, which provides an explanation for the field-wide occurrence of the disease in commercial cantaloupe fields. Ascospores, both native and culturally produced, germinated readily in the rhizosphere and penetrated the roots of susceptible melon seedlings growing in field soil. Up to three germ tubes per ascospore were produced, and germ tubes up to 800 µm in length were observed. Our results confirm the suspected, but previously undocumented, role of ascospores as primary inoculum.

Additional keyword: zone of competency.

A destructive disorder of cantaloupe (*Cucumis melo* L.), characterized by the sudden (commonly within 2 weeks of harvest) and generally uniform collapse of entire fields, has plagued the cantaloupe industry for over 40 years. Common names of the disorder include crown blight, collapse, vine decline, quick decline, and sudden wilt. The disorder is particularly severe in the warmer climatic production regions of the United States (Arizona, California, and Texas).

In the United States, the cause of the disorder was not identified until recently. In 1990, researchers in Texas (4) and Arizona (M. E. Stanghellini, and S. L. Rasmussen, *unpublished*) independently discovered and attributed the cause of the disorder to the root-infecting fungus, *Monosporascus cannonballus* Pollack & Uecker (7). The fungus also has been associated with a similar disorder in Spain, Israel, Tunisia, Taiwan, and Japan (3).

Little is known about the biology of this unusual root-infecting ascomycete. Perithecia (Fig. 1A and C) containing single-spored asci (Fig. 1D) are produced in the cortex of colonized roots. Ascospores of the fungus are spherical, 35 to 50 µm in diameter, and have a glossy black appearance (Fig. 1C). No asexual spore stage has been observed on naturally infected roots or in laboratory cultures of the fungus. The latter suggests that ascospores probably function as the primary survival structure, as well as the primary inoculum, of the fungus in field soil. However, germination of ascospores, collected from naturally infected roots, soil, or pure cultures of the fungus, has not, or only rarely, been observed despite numerous and varied attempts in the laboratory (M. E. Stanghellini, D. H. Kim, and S. L. Rasmussen, *unpublished*; 2,4,7,11). No information exists regarding ascospore germination in soil or its ability to function as inoculum for root infection.

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The objectives of our investigation were to provide an explanation for the observed field-wide occurrence of vine decline of cantaloupe in commercial fields and to determine the role of ascospores of *M. cannonballus* in the etiology of this disease. A preliminary report has been published (10).

MATERIALS AND METHODS

Estimating ascospore population densities of *M. cannonballus* in naturally infested soils. A quantitative extraction technique was used to estimate ascospore population densities in naturally infested soils (9). Recovered ascospores were identified as *M. cannonballus* based on their size, shape, color, texture, and sheen. Additionally, the outer shell of these ascospores was removed physically by a microgrinding procedure, and the internal structure of ascospores recovered from naturally infested soils was compared with ascospores obtained from pure cultures of the fungus (Fig. 1B). The internal morphology of the ascospores from both sources was identical. Ascospores were encased in a darkly pigmented outer shell. A near-hyaline internal spore that had four to six distinct wall layers was observed (Fig. 1F). The microgrinding procedure consisted of gently grinding ascospores between the ground-glass ends of two microscope slides.

Soil sampling methods. *Commercial fields.* Unless otherwise specified, all field studies, initiated in 1991, were conducted in commercial cantaloupe fields located in the Harquahala and Aguila valleys of Arizona. In total, 58 commercial fields (approximately 1,600 ha) were assessed: 33 fields had a known history of the disease, and the remaining 25 fields had been cropped to cantaloupe for the first time following monoculture cotton and had no history of the disease. Fields ranged in size from 16 to 32 ha.

To estimate the interfield variations in population densities of *M. cannonballus*, a composite soil sample was collected from each of the fields. Seven soil cores, each 2.5 cm in diameter and 15 cm long, were collected along each path of a diamond sampling pattern (1,6,8) and pooled into one composite soil sample per field.

Additionally, two fields that had a known history of the disease were selected for estimates of the intrafield variations in the population density of the fungus. Three rows across the length (approximately 200 m) of each commercial melon field, which was recently planted, were selected arbitrarily for this study. The rows were divided into 6-m-long plots. Three soil cores, each 2.5 × 15 cm, were collected from each plot, pooled, and bagged separately. In total, 96 composite soil samples were collected from each field. The ascospore population density in each sample was determined. All soil cores were collected from the tops of beds.

In addition to the studies on the horizontal distribution of the fungus, the vertical distribution also was determined. One arbitrar-

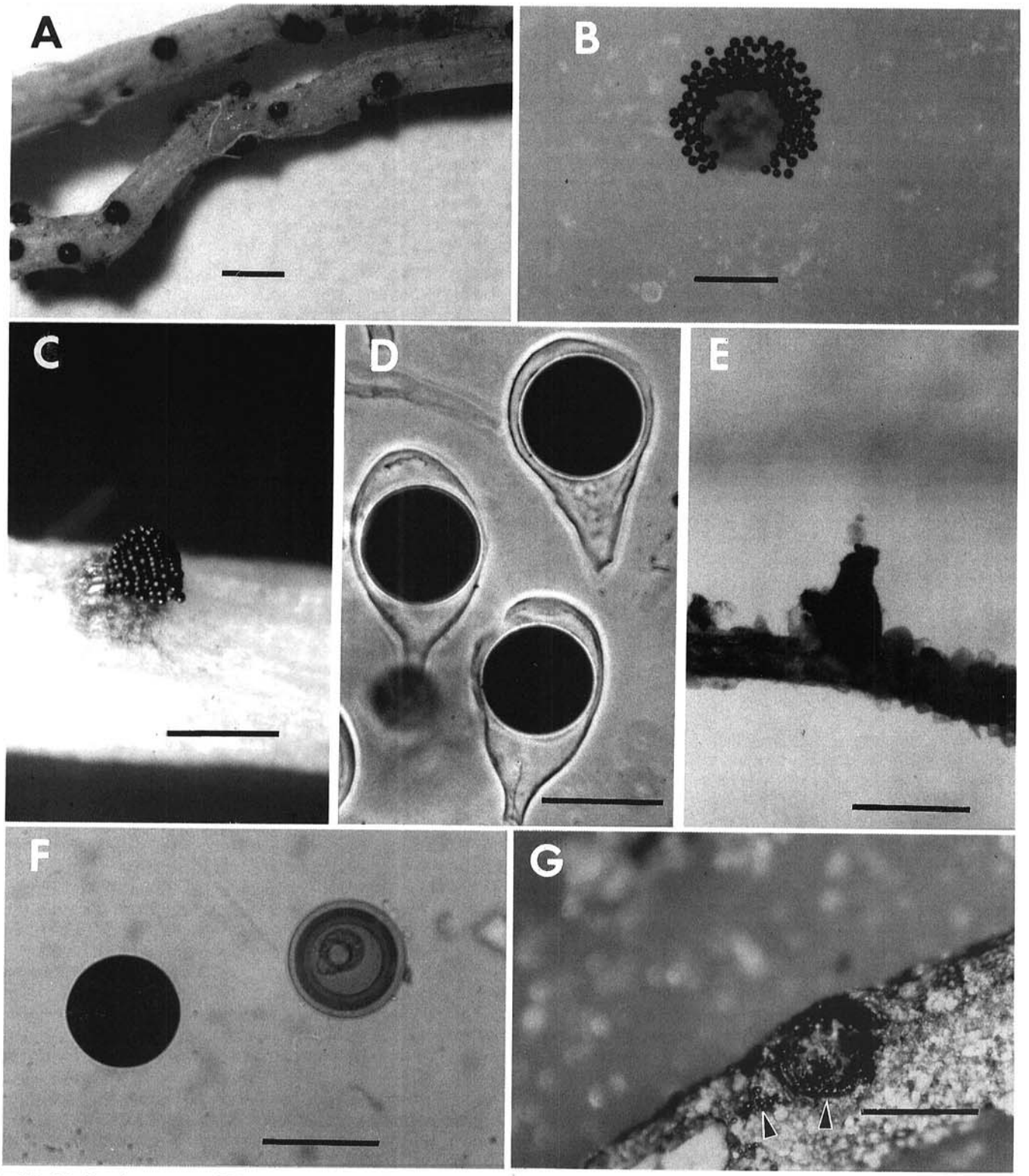

Fig. 1. Perithecia and ascospores of *Monosporascus cannonballus*. **A,** Perithecia in the cortex of a colonized feeder root of a cantaloupe plant (bar = 1 mm); **B,** perithecium and discharged ascospores in a V8-agar culture of the fungus (bar = 0.4 mm); **C,** ascospores released from a perithecium in a colonized root (bar = 1 mm); **D,** asci containing single ascospores (bar = 45 µm); **E,** perithecium on a *Lepidium lasiocarpum* root (bar = 1 mm); **F,** internal structure of an ascospore (right) after removal of the outer pigmented shell (left) (bar = 40 µm); and **G,** ascospores (arrows) in the bottom of a perithecium formed in the cortex of a *L. lasiocarpum* root (bar = 1 mm).

ily chosen site within each of four commercial cantaloupe fields (two fields were drip-irrigated, one field was drip-irrigated and mulched with black plastic, and one field was furrow-irrigated) were assessed for the vertical distribution of ascospore populations as follows: a 30 × 30-cm hole was dug from the tops of beds, and soil samples (200 g each) were collected from the 0 to 5, 6 to 10, 11 to 15, 16 to 20, and 21 to 25 cm soil depths of the exposed profile. Soil samples were processed as described above.

Native desert soil. Preliminary studies showed that ascospores of *M. cannonballus* were recovered readily from native desert (Sonoran Desert) soils. Two native desert sites (designated A and B) that were approximately 20 km apart and at least 4 km from cultivated fields in the Harquahala Valley, were chosen arbitrarily for evaluation of the horizontal and vertical distribution of the fungus. The vertical distribution was determined, as described above, at each site.

The horizontal distribution at native desert sites A and B was determined as follows: a cork borer, 1.5 cm in diameter and 10 cm in length, was employed to collect soil samples. A soil core was collected from each of the corners and the center of each of three nested-square (100 × 100, 30 × 30, and 10 × 10 cm) sampling patterns (Fig. 2). Each soil core (approximately 15 g) was bagged and assayed separately.

In addition to soil samples, the root systems of indigenous plants were excavated carefully from each site, transported to the laboratory, and microscopically examined for the presence of perithecia.

Ascospore germination. As previously mentioned, the role of ascospores of *M. cannonballus* as inoculum is unknown. However, during the course of our investigations, we observed microscopically the presence of germinated ascospores on roots of washed cantaloupe seedlings that had been collected from a commercial field 11 days after planting. The population density of the fungus in the latter field was estimated at 2.0 ascospores per g of soil. The observed ascospores were attached firmly to the roots of the seedlings by germ tubes whose lengths ranged from 50 to 500 µm (Fig. 3B). Microscopic examination also revealed that the germ tubes had penetrated the roots. Cultures of the fungus were obtained from excised root segments bearing single-ascospore germlings as well as from single-ascospore germlings that were removed physically from the roots. These observations led to the following studies.

Soil (1 kg) was collected from the field, transported to the laboratory, sieved, and allowed to air-dry. Approximately one-half of the volume of soil was infested artificially with ascospores that were obtained from a 1-month-old V8 agar (100 ml of V8 juice, 1 g of $CaCO_3$, 20 g of agar, and 900 ml of sterile distilled water) culture of *M. cannonballus*. The resulting ascospore population density was estimated at 300 ascospores per g of soil. Twenty-gram subsamples of soil (naturally infested soil and naturally infested soil amended with ascospores) were dispensed into tubes, 8 cm in length and 1.5 cm in diameter, and irrigated with sterile distilled water until drainage occurred (Fig. 3A). A 2-day-old cantaloupe (cv. PMR 45) seedling (pregerminated on water agar) was transplanted into each tube. There were 28 tubes per treatment. Planted tubes were transferred to a growth chamber (12 h of light, 12 h of dark) and incubated at 24°C. At 3, 6, 9, and 12 days after transplanting, seven seedlings from each treatment were removed from the tubes, and the root system of each seedling was gently washed in water and placed in petri dishes containing approximately 15 ml of sterile distilled water. The root system of each seedling was examined microscopically at 10 to 40× for the presence of ascospore germlings of *M. cannonballus* attached to roots. The number of ascospore germlings attached to the root system of each seedling, as well as the length and number of germ tubes, was recorded. Excised root segments bearing ascospore germlings and germlings physically removed from roots were plated onto water agar and incubated at 24°C. The experiment was repeated once.

RESULTS

Horizontal and vertical distribution of *M. cannonballus* in commercial melon fields. Ascospores of *M. cannonballus* were recovered from all 58 commercial melon fields surveyed (Table 1). Populations in fields with a known history of the disease ranged from 0.75 to 4.5 (mean = 2.49 ± 1.05) ascospores per g of soil, whereas fields with no history of the disease ranged from 0.5 to 5.1 (mean = 1.70 ± 1.04) ascospores per g of soil. Mean ascospore population densities were significantly higher (analysis of variance [ANOVA], $P = 0.006$) in fields with a disease history compared to fields with no disease history.

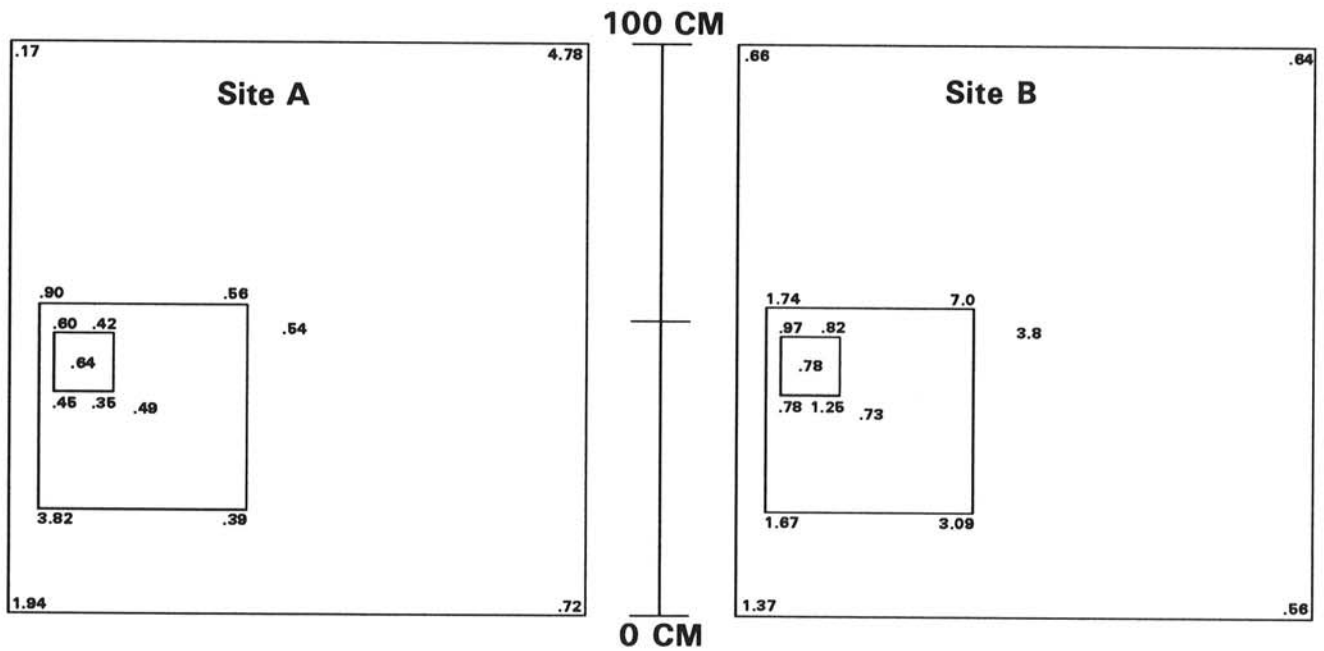

Fig. 2. Horizontal distribution of ascospores of *Monosporascus cannonballus* at two locations (A and B) in a native desert habitat. A soil core, 1.5 cm in diameter and 10 cm in length, was collected from each of the corners and the center of each of three nested-square sampling patterns. Numbers represent ascospores per g of soil at each sampling site.

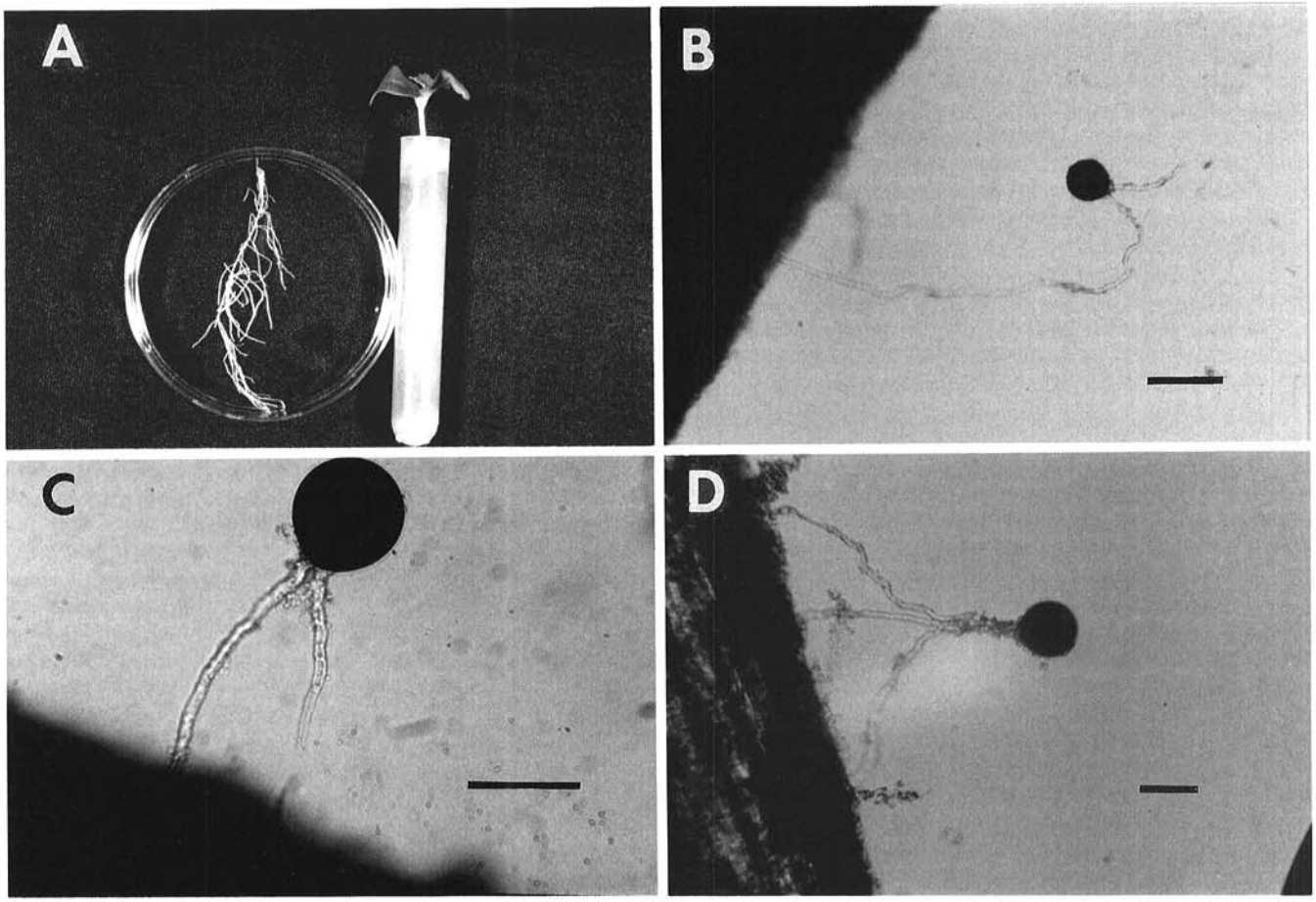

Fig. 3. Germinated ascospores of *Monosporascus cannonballus* attached to roots of cantaloupe seedlings. **A,** Seedlings were grown in naturally infested soil or soil amended with culturally produced ascospores. Roots, after various periods of incubation, were washed and placed in a petri dish containing sterile distilled water and were examined for the presence of ascospore germlings attached to roots; **B,** ascospore germling observed on the root of a cantaloupe seedling obtained from a commercial field (bar = 100 μm); and **C and D,** ascospore germlings, with two to three germ tubes, attached to seedling roots (bar = 45 μm).

TABLE 1. Population densities of ascospores of *Monosporascus cannonballus* in commercial cantaloupe fields[a]

Fields with a disease history		Fields with no disease history	
No. of fields	Ascospores/ g of soil	No. of fields	Ascospores/ g of soil
2	0.1–1	6	0.1–1
10	1.1–2	12	1.1–2
13	2.1–3	5	2.1–3
6	3.1–4	1	3.1–4
1	4.1–5	0	4.1–5
1	5.1–6	1	5.1–6

[a] One composite soil sample, consisting of 28 soil cores, was collected from each field. Ascospore population densities were estimated in soil samples by a soil extraction technique (9).

TABLE 2. Vertical distribution of ascospores of *Monosporascus cannonballus* in four commercial cantaloupe fields and two native desert sites[a]

Soil depth (cm)	Commercial field				Native desert site	
	1	2	3	4	A	B
1–5	1.75	1.60	2.40	1.40	1.75	1.55
6–10	1.25	1.70	1.90	1.15	0.80	1.30
11–15	1.30	1.95	2.00	1.35	1.45	0.85
16–20	1.60	2.05	2.00	1.65	1.15	1.00
21–25	1.65	2.35	1.55	1.90	1.70	1.40

[a] Fields 1 and 4 were drip-irrigated; field 2 was mulched with black plastic and drip-irrigated; and field 3 was furrow-irrigated. Two native desert sites, designated A and B, that were approximately 20 km apart and at least 4 km from cultivated fields in the Harquahala Valley in Arizona were chosen arbitrarily for the study. Values are the number of ascospores per g of soil.

The horizontal distribution of ascospores in an intensively surveyed field is presented in Figure 4. Ascospores were recovered from all 96 composite soil samples. The mean population was 1.86 ± 0.58 (range = 1 to 4.3) ascospores per g of soil. According to a chi-square analysis, a normal (uniform) distribution best fit the data, and the variance-to-mean ratio was 0.18. A second commercial field was surveyed intensively, as described above, and the results were similar and, therefore, are not presented.

The vertical distribution of ascospores in commercial fields is presented in Table 2. The fungus was recovered from all soil depths in all fields surveyed. Similar population densities, irrespective of irrigation method, were detected in all four fields. There were no significant differences (ANOVA, $P \geq 0.05$) between ascospore populations at the various soil depths.

Distribution of ascospores of *M. cannonballus* in native desert soils. Ascospores of the fungus were recovered from all soil depths at each of the two sampling sites (Table 2). The horizontal distribution of ascospores is presented in Figure 2. The mean ascospore population at site A was 1.118 ± 1.36 (variance-to-mean ratio = 1.6) and at site B was 1.72 ± 1.73 (variance-to-mean ratio = 1.7). Additionally, perithecia of the fungus that contained ascospores were found on the roots of a native desert annual, *Lepidium lasiocarpum* Nutt. ex Torr. & A. Gray. (Fig. 1E and G).

Ascospore germination. Germinating ascospores were attached to roots of seedlings grown for 12 days in soils that were either amended or nonamended with culturally produced ascospores (Table 3). The number of germlings of the fungus attached to roots was 40-fold higher, however, on the root system of seedlings grown in ascospore-amended soil. Up to 35 germlings were observed on the root system of a single seedling grown in soil amended with

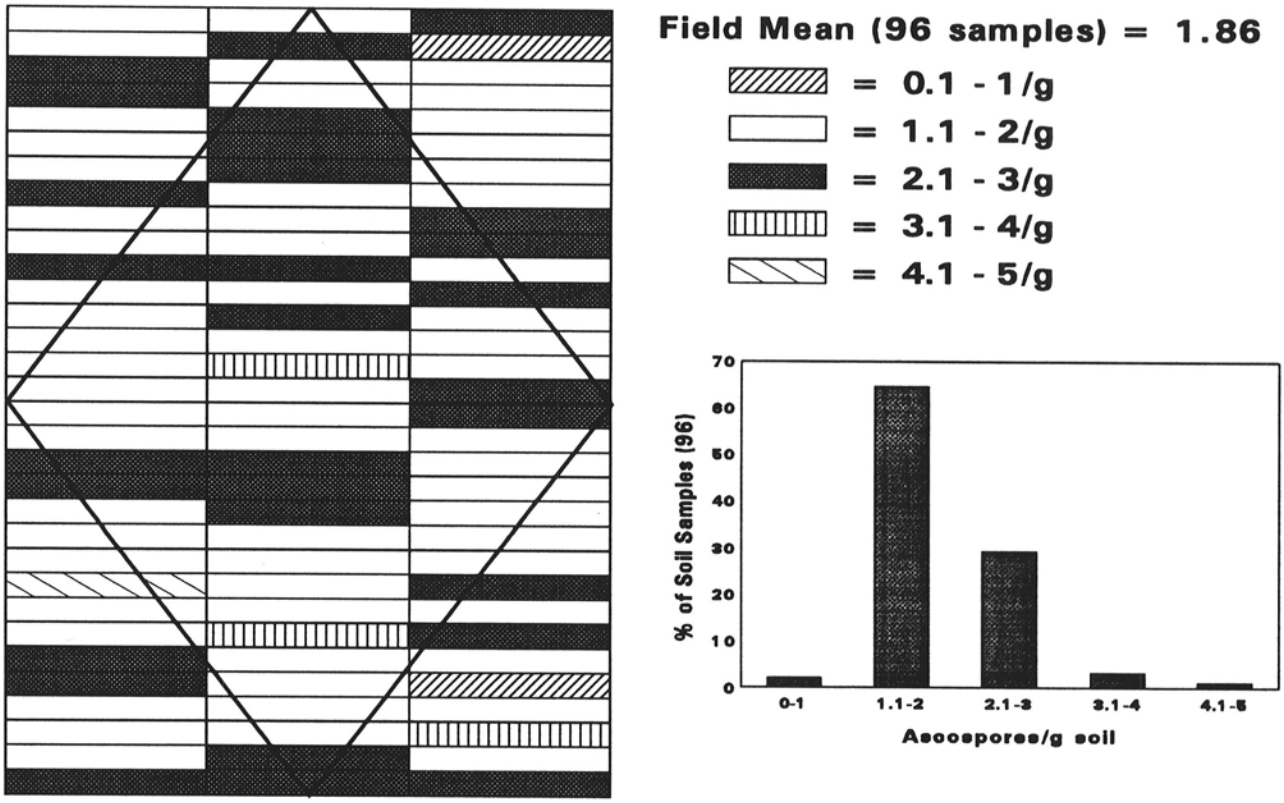

Fig. 4. Horizontal distribution of ascospore populations of *Monosporascus cannonballus* in a commercial cantaloupe field. Ascospore populations in each of 96 composite soil samples, collected at 6-m intervals across the length of each of three arbitrarily selected rows, were determined. Additionally, five soil cores were taken along each path of a diamond sampling pattern and composited into a single soil sample. The ascospore population in the latter composite sample was 1.80 ascospores per g of soil.

culturally produced ascospores. Germ tube lengths of germlings attached to roots ranged from 50 to 800 µm. Up to three germ tubes per ascospore were observed (Fig. 3C and D). Microscopic observation also showed that the germ tubes of attached germlings had penetrated the roots, and the immediate area of host penetration appeared sunken. Cultures of the fungus were obtained from excised portions of these infected roots as well as from germlings physically removed from roots. No ascospore germlings were observed on roots of seedlings sampled at 3, 6, or 9 days after transplanting.

The germinability of culturally produced ascospores was not a unique character of our Arizona isolate. Ascospores from an isolate of the fungus obtained from Texas and California (M. E. Stanghellini, D. H. Kim, and S. L. Rasmussen, *unpublished*) also germinated readily in the rhizosphere of melon roots using the assay system described above (data not presented).

DISCUSSION

The results of our studies show that ascospores of *M. cannonballus* are distributed uniformly, both vertically and horizontally, in commercial melon field soils. These results support and extend a previous study (5) on the distribution of ascospores of the fungus in fields with a known history of disease. However, we also found that similar population densities occurred in commercial fields that had no history of disease as well as in native desert habitats.

The latter results indicate that the fungus is an indigenous soilborne fungus. This conclusion is strengthened further by our observation of perithecia containing ascospores on roots of a native annual plant, *L. lasiocarpum*, that was collected from desert habitats. The indigenous nature of the pathogen provides a logical explanation for the distribution of ascospores of the fungus in cultivated soils, which in turn accounts for the field-wide occurrence of the disease. It does not, however, account for the occurrence of the disease in only some commercial fields. Although populations of recovered ascospores were similar in numbers between fields with a known history of disease and those with no disease history, the percentage of the resident ascospore populations that are germinable may be quite different. To date, quantitative determina-

TABLE 3. Number of germinated ascospores of *Monosporascus cannonballus* attached to roots of cantaloupe seedlings[a]

	Naturally infested soil		Artificially infested soil	
	Seedling number	No. of germlings on root system	Seedling number	No. of germlings on root system
Exp. 1				
	1	0	1	7
	2	1	2	15
	3	0	3	10
	4	0	4	13
	5	0	5	3
	6	1	6	6
	7	0	7	6
Exp. 2				
	1	0	1	15
	2	0	2	5
	3	2	3	35
	4	0	4	29
	5	0	5	7
	6	0	6	21
	7	0	7	5

[a] Pregerminated seeds were planted in naturally infested soil that contained two ascospores per g of soil or naturally infested soil that was amended with culturally produced ascospores (300 ascospores per g of soil). The root systems of seedlings were recovered from soil, washed, and examined microscopically for the presence of ascospore germlings attached to roots. Data are from seedlings that were recovered after 12 days of incubation at 24°C. There were seven seedlings per treatment.

tion of the viability of ascospores recovered from naturally infested soil has not been achieved. Lack of such knowledge currently precludes establishment of threshold populations for prediction of the occurrence and severity of the disease. However, our discovery of the germination of naturally as well as culturally produced ascospores in soil may soon permit achievement of the latter objective.

Ascospores of *M. cannonballus* germinated and functioned as inoculum for root infection both in the field and in the laboratory. The actual percentage of germination of the resident ascospore soil populations, however, is unknown. There is no question, however, that a high percentage did germinate. Up to 35 germinated ascospores of the fungus were observed attached to roots of a cantaloupe seedling growing in soil artificially infested with culturally produced ascospores. Germ tubes ranging in length from 50 to 800 μm were observed. Assuming that germ tube growth occurred perpendicular to the growing root, these findings indicate that the width of the rhizosphere effect, i.e., zone of competency (8), is rather large, which provides an explanation for the apparently high inoculum efficiency of low ascospore populations of *M. cannonballus* in fields with a known history of the disease.

Although germination of ascospores occurred in the rhizosphere of roots of susceptible cantaloupe plants, the exact nature of the stimulating factor(s) responsible for germination is unknown. Extensive studies on identification of the specific nature of the germination stimulant(s) are currently underway.

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