Quantification of *Monosporascus cannonballus* Ascospores in Three Commercial Muskmelon Fields in South Texas

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ABSTRACT

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Monosporascus cannonballus ascospore populations in the soils of three south Texas muskmelon fields were quantified at planting and at harvest in 1992. Sampling was conducted along eight-row segments (transects) within a 100 × 50 m area in each field according to a stratified random design. In a preliminary study (June 1991), ascospore counts were higher in-beds than between-beds and at a moderate depth of 10–20 cm in field A (annually cropped to muskmelon in fixed, plastic-mulched, drip-irrigated beds). Ascospore numbers were significantly lower in adjacent field B (rotated land, bare soil, and furrow irrigation). In 1992, ascospore counts in A were again significantly higher than in B and also higher than in C, a field similar to B. However, root disease severity at harvest was similar in A and B, and few significant positive correlations between ascospore concentration and root disease severity were found in any field. The presence of other root-damaging pathogens or of infective vegetative mycelium of *M. cannonballus* may explain this lack of correlation, as could inoculum densities exceeding levels necessary for maximum root disease expression. Variance to mean ratios indicated random partitioning of plants with similar root disease severity among transects in each of the three fields.

Additional keywords: cantaloupe, *Cucumis melo,* vine decline

Monosporascus cannonballus Pollack & Uecker is a root rot pathogen associated with vine decline disease of muskmelon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) in warmer areas of the world (12,23,26,27). *M. cannonballus* was first described by Pollack and Uecker (18) in 1974 after they examined an isolate from Arizona where the fungus was associated with a "decay of secondary roots" of cantaloupe (26). *M. cannonballus* subsequently was shown to cause a severe root rot/vine decline disease of muskmelon in the lower Rio Grande Valley (LRGV) of south Texas (12).

Monosporascus root rot/vine decline has reduced muskmelon yields and quality in numerous LRGV fields since 1986. Losses have occurred under traditional cultural practices (bare soil, furrow irrigation, and crop rotation) and in fields under more intensive management (plastic mulch, drip irrigation, and annual cropping). According to some commercial growers, a few muskmelon cultivars perform adequately in fields with a history of root rot/vine decline disease. Their observations are supported by greenhouse experiments which indicate differences between cultivars in response to inoculation with *M. cannonballus* (13). However, the possibility of resistant or tolerant varieties has yet to be demonstrated in replicated field trials. Other than fumigation with chloropicrin, metham-sodium, or methyl bromide, no effective means of disease control have been reported (15,16,21).

Management of Monosporascus root rot/vine decline would be facilitated by developing a method for detecting and quantifying inoculum in the soil. No selective medium has been developed for this organism. However, *Monosporascus* spp. produce large numbers of distinctive, thick-walled ascospores which are amenable to physical extraction and quantification. The ascospores are black, smooth-walled, spherical bodies measuring 30–50 μm in diameter (18), with a specific gravity of approximately 1.25 g/cm^3 (11). A sucrose flotation centrifugation technique traditionally used for soil nematode extraction (7) was applied to the recovery of *Monosporascus* ascospores from the soil by Stanghellini and Rasmussen (25). Their technique was used in this study.

In addition to ascospores, vegetative mycelium of *M. cannonballus* may also function as inoculum. The infectivity of mycelium from young cultures free of ascospores has been demonstrated in greenhouse tests (12). However, little is known about the longevity or infectivity of vegetative mycelium under field conditions. In addition, ascospores of *M. cannonballus* do not germinate or germinate very rarely under laboratory conditions (11,18). Thus, while ascospores can be extracted from the soil and counted, a means for determining their viability and infective potential has not been developed. Therefore, the relative importance of ascospores and vegetative mycelium as components of inoculum potential is unknown. However, since ascospores are the product of previous vegetative and reproductive activity, their numbers may correlate with total inoculum potential and indicate the capacity for future disease. In this study, ascospore numbers in commercial muskmelon fields in the LRGV were quantified and related to cropping system, depth, and disease severity.

MATERIALS AND METHODS

1991 Study. In June 1991, soil samples were taken from two adjacent fields (fields A and B) in a commercial farm in the LRGV. Field A was intensively managed, and had been cropped to muskmelon during each of the previous 3 yr, while field B was traditionally managed, with muskmelon grown during alternate years in a crop rotation system. At the time of sampling, the muskmelon harvest was completed in field A, and field B was planted recently to corn. Cropping histories, cultural practices, and other characteristics of these two fields and a third field sampled during a later portion of the study (field C), are presented in Table 1.

In each field, a representative area approximately 50 m (24 beds) in width and 100 m in length was subdivided into eight 25 × 25 m sectors (Fig. 1). Sampling was done along a 10-m row segment (transect) randomly located in each sector, according to a stratified random sampling design (6). Samples were taken

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Table 1. Cultural practices and other characteristics of three commercial muskmelon fields in south Texas[y]

Characteristic or practice	Field A	Field B	Field C
Soil			
Texture (% sand, silt, clay)	6, 49, 45	14, 49, 37	50, 24, 26
pH	7.7	7.8	7.7
Conductivity (Sm^{-1})	0.19	0.11	0.14
Organic matter (%)	0.9	0.8	0.4
Tillage			
1989–1990	Rotovate, paratill, bedshape	Plow, disk, row sweep	Plow, disc, row sweep
1991	Plow, disk, bed formation	Plow, disk, bed formation	Plow, disc, bed formation
Crop history			
1989	Muskmelon	Cotton	Onion, fallow
1990	Muskmelon	Muskmelon, onion	Muskmelon, onion
1991	Muskmelon	Onion, corn	Onion, bell pepper
1992	Muskmelon cv. Cruiser	Muskmelon cv. Cruiser	Muskmelon cv. Honeydew Green Flesh
Irrigation			
1989–1991	Buried drip	In furrow	In furrow
1992	Buried drip	Buried drip	Buried drip
Mulch			
1989–1991	Black plastic	None (bare soil)	None (bare soil)
1992	Black plastic	Black plastic	Black plastic
Crop protection chemicals[z]	Fungicides, herbicides, nematicides	Fungicides, herbicides, nematicides	Fungicides, herbicides, nematicides

[y] Fields A and B are adjacent fields 13 km east of Rio Grande City, Texas; field C is 4 km west of Rio Grande City.
[z] 1992 Fungicides = foliar applications of metalaxyl/chlorothalonil and triadimefon as needed; herbicides = bensulide (2.2 L a.i./ha preplant, 25–30 cm band), trifluralin (0.5 L a.i./ha between rows); and nematicides = 1,3-dichloropropene (31–50 L a.i./ha preplant), oxamyl (0.6–1.2 L a.i./ha through drip-line).

with a 7-cm-diameter soil auger at 2-m intervals along a transect, with each sample composited from two soil cores (Fig 1). In field A, samples were taken adjacent to the row of plants in the up-raised beds (in-bed) and from depressed areas between beds (between-bed) at 0–10, 10–20, and 20–30 cm depths for a total of 30 samples per transect. In field B, five 0–30-cm in-row samples were taken in each transect; samples were not taken from furrows between rows or separated into depth increments because of the extensive cultivation associated with traditional field management and its disruptive effect on inoculum patterns (17). The selection of 30 cm as the maximum sample depth was based on a study of *Macrophomina phaseolina* (Tassi) Goidanich, a soilborne fungus which causes a similar vine decline disease of muskmelon. In cultivated soils of the LRGV, most of the inoculum of this pathogen was found in the upper 30 cm of the soil profile (3). All samples were placed in plastic bags, stored at 5 C, and processed within 40 days.

In the laboratory, each sample was passed through a 4-mm screen. A 20-g subsample was mixed with 200 ml of tap water, agitated on a magnetic stirrer for 8–10 min, and washed through nested 75 and 38 μm metallurgical sieves. Material retained on the 38-μm sieve was backwashed into a 50-ml tube and centrifuged at 2,000 *g* for 4 min. The supernatant was discarded by decanting, and the pellet was resuspended in 30–40 ml of 50% sucrose and centrifuged for 2 min at 2,000 *g*. Ascospores and other materials floating or suspended in the sucrose were decanted onto a small (8-cm-diameter, 38-μm) sieve and washed into a clean centrifuge tube. A second sucrose extraction was performed on the residual soil pellet to salvage spores not recovered during the first extraction. The resulting suspension from the second extraction was added to the first, and the combined suspension was stored at 5 C until counts were made. Characteristic ascospores (12,18) were counted in a rectangular plastic dish at 25–30× on a dissecting microscope using lateral and basal light sources.

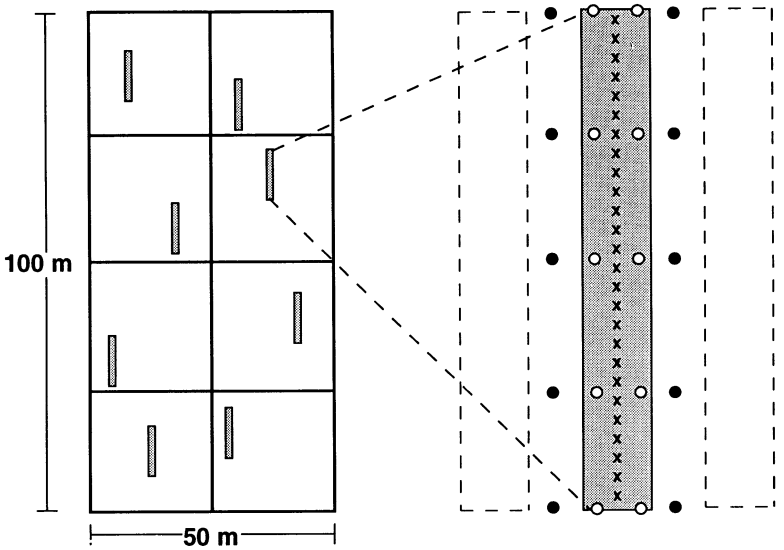

Fig. 1. Representative subfield sample area (left) and sampling transect (expansion on right). ● = between-bed soil core, ○ = in-bed soil core, and x = location of plants within a transect.

1992 Study. Based on the results of work done in 1991, several experimental procedures were modified for the main portion of this study. First, samples were air-dried in paper bags at room temperature prior to screening and processing. Extraction efficiency was increased by careful vacuum aspiration of the aqueous supernatant after the first centrifugation and by allowing the soil suspensions to settle for 1–2 min following agitation and prior to each centrifugation step. Second, samples were taken exclusively from within the rows at the 10–20 cm depth, because the initial study indicated higher populations of ascospores at this depth. Finally, the sampling distance was shortened to 1 m to increase the number of comparisons between ascospore concentration and disease severity along individual transects and within fields.

In 1992, samples were collected and ascospores counted from fields A, B, and C in January, when the muskmelon seedlings in each field were in the first to second true leaf stage. At the May harvest, all plants within the transects were excavated; the tops were discarded and the root systems were washed and evaluated. Primary and major secondary

Table 2. In-bed, between-bed, and vertical distribution of *Monosporascus cannonballus* ascospores in fields A and B (June 1991 sample period)

Field[w]	Sample: Location[x]	Sample: Depth (cm)	Ascospores/g dry soil (transects 1–8)[y]: 1	2	3	4	5	6	7	8	Avg[z]
A	In-bed	0–10	4.0	2.6	3.9	3.0	3.0	2.7	3.6	4.4	3.4 a
A	In-bed	10–20	3.9	3.8	4.0	4.0	2.9	3.6	7.0	4.1	4.2 a
A	In-bed	20–30	3.3	3.7	3.4	3.4	4.2	2.4	4.9	3.6	3.6 a
A	Between-bed	0–10	3.0	2.6	3.1	3.4	2.3	2.1	3.4	3.0	2.9 b
A	Between-bed	10–20	4.6	3.4	6.3	3.5	3.3	5.4	4.1	3.8	4.3 a
A	Between-bed	20–30	2.4	2.8	2.5	3.5	2.7	2.4	4.2	4.5	3.1 ab
A	In-bed avg.	0–30	3.7	3.4	3.8	3.5	3.4	2.9	5.2	4.1	3.7 a
A	Between-bed avg.	0–30	3.3	2.9	4.0	3.5	2.8	3.3	3.9	3.7	3.4 a
A	Overall avg.	0–30	3.5	3.1	3.9	3.4	3.1	3.1	4.6	3.9	3.6 a
B	In-row avg.	0–30	2.6	2.3	2.0	2.8	2.9	2.3	3.2	2.9	2.6 b

[w] Fields A and B are characterized in Table 1.
[x] In-bed = samples from within the beds; between-bed = samples from furrows between the beds; overall avg. = mean of all in-bed and between-bed samples in a given transect; in-row avg. = mean of all 0–30 cm composite samples taken from within rows of corn, a rotation crop grown in field B in 1991.
[y] Each value in transects 1–8 and at depths 0–10, 10–20, and 20–30 cm is an average of five samples.
[z] Four groups (indicated by spacing) were analyzed separately. In-bed and between-bed results for the three depths (groups 1 and 2) were analyzed by repeated measure analyses of variance (ANOVAs) with contrast statements. In-bed and between-bed averages (group 3) were analyzed by a two-way ANOVA followed by Tukey's test for means comparison. Overall averages in field A and in-row averages in field B (group 4) were analyzed by a one-way ANOVA. Numbers within a group followed by the same letter are not significantly different at the $P = 0.05$ level.

Table 3. Root disease severity indices and average ascospore concentrations of *Monosporascus cannonballus* along transect lines in three south Texas muskmelon fields

Field	Transect	DSI[w] (0–4)	Ascospores[x] (correlation coefficients): Jan. 1992 (*r*1)[y]	June 1992 (*r*2)[y]	(*r*3)[y]
A	1	2.0	14.4 (−0.33)	12.1 (0.55)	(−0.25)
A	2	2.3	14.8 (−0.02)	10.3 (0.05)	(−0.62)
A	3	1.5	10.0 (−0.52)	10.9 (0.33)	(−0.26)
A	4	1.5	8.6 (0.30)	9.9 (−0.26)	(0.12)
A	5	1.7	10.6 (0.02)	11.1 (0.34)	(0.25)
A	6	1.8	10.4 (0.01)	13.0 (−0.11)	(0.90*)
A	7	2.1	8.8 (0.29)	10.5 (0.66*)	(0.31)
A	8	2.0	8.9 (−0.25)	11.2 (−0.28)	(0.12)
B	1	1.5	4.4 (0.16)	3.6 (−0.11)	(−0.13)
B	2	2.1	3.8 (0.32)	3.3 (0.56)	(0.30)
B	3	1.7	4.0 (−0.32)	3.5 (−0.47)	(−0.22)
B	4	2.0	4.2 (0.66*)	3.1 (0.13)	(0.28)
B	5	2.4	4.2 (−0.17)	3.9 (0.16)	(0.70*)
B	6	2.9	3.9 (0.42)	3.8 (0.14)	(−0.07)
B	7	1.9	3.8 (−0.53)	3.7 (0.27)	(−0.44)
B	8	2.1	3.6 (−0.27)	3.6 (0.02)	(0.01)
C	1	1.4	5.7 (−0.71*)	7.8 (−0.14)	(0.00)
C	2	0.8	4.7 (−0.23)	7.0 (−0.72*)	(0.36)
C	3	1.6	5.0 (0.47)	6.3 (−0.09)	(0.48)
C	4	1.4	6.2 (0.08)	7.4 (0.22)	(0.55)
C	5	2.2	4.7 (−0.06)	6.4 (−0.14)	(−0.24)
C	6	1.5	3.8 (−0.65)	5.3 (0.10)	(−0.22)
C	7	1.7	5.1 (0.21)	5.8 (0.21)	(0.26)
C	8	1.7	4.4 (−0.53)	5.4 (−0.64*)	(0.67*)
A	all	1.9	10.8 a[z] (0.50)	11.1 a[z] (0.07)	(0.20)
B	all	2.1	4.0 b (−0.29)	3.6 c (0.35)	(0.06)
C	all	1.5	5.0 b (−0.05)	6.4 b (−0.42)	(0.80*)

[w] Root disease severity indices based on a scale of 0 (healthy) to 4 (severely lesioned or necrotic).
[x] Ascospore concentrations expressed as number/g dry soil; each value is the mean of 10 samples.
[y] Correlation coefficients *r*1 and *r*2 reflect relationship of DSI and ascospore concentration for January 1992 and June 1992 sample periods, respectively. *r*3 is the correlation coefficient between ascospore concentrations during the two sample periods. * indicates significance at $P = 0.05$.
[z] Means in a column followed by the same letter are not significantly different at the $P = 0.05$ level according to Duncan's multiple range tests.

roots were rated separately for root disease severity on a scale of 0 (healthy) to 4 (severely lesioned or necrotic) and averaged for each plant, resulting in a total of nine rating categories. Root systems were also inspected for perithecia indicative of colonization by *M. cannonballus*. Ten plants were removed from a single transect in each field for isolations, and the remaining root systems were reburied where they were dug up. In June, a replicate set of soil samples was taken from the same transects in each field, and ascospores were counted. Additional samples were collected along a diamond-shaped path in each sample area and composited for soil analysis. Electrical conductivity and pH were determined from soil water extracts obtained by vacuum suction of saturated soil pastes (22). Soil texture and organic matter determinations were made by the soil testing laboratory of the Texas Agricultural Experiment Station.

Ascospore count data were subjected to analyses of variance (ANOVA) using the GLM procedure of SAS (SAS Institute, Cary, NC). A two-way ANOVA was performed to compare in-bed and between-bed ascospore concentrations in field A, and a one-way ANOVA by transect averages compared overall concentrations in fields A and B. Repeated measures ANOVAs with contrast statements were used to compare spore numbers at different sample depths in field A and changes in ascospore numbers over time. Simple linear regression was used to determine potential correlations between root disease severity at harvest and ascospore numbers found at planting and at harvest. Disease-severity data were analyzed by Field Runner, a computer program for the analysis of disease incidence, severity, and spatial patterns (5).

RESULTS

1991 Study. The 1991 study demonstrated significantly higher ascospore numbers in intensively managed field A than in traditionally managed field B (Table 2). This relationship held true for both in-bed and between-bed comparisons of field A to field B, suggesting an association between intensive crop management practices and the higher inoculum levels in field A.

In field A, in-bed and between-bed ascospore numbers did not differ significantly when averaged across the three sampling depths (Table 2). However,

ascospore numbers within the beds (3.4 per gram of soil) were significantly higher than between the beds (2.9 per gram of soil) at the 0–10-cm level ($P = 0.03$). The highest average concentrations (4.2 and 4.3) occurred at 10–20 cm for in-bed and between-bed samples, respectively. In most transects, ascospore concentrations were lower at 0–10 and 20–30 cm than at 10–20 cm, but overall differences between depths were significant only for between-bed samples.

1992 Study. The average numbers of ascospores in fields A, B, and C at planting (January 1992) were 10.8, 4.0, and 5.0 per gram of soil, respectively (Table 3). In June, when the original transects were resampled, ascospore concentrations averaged 11.1 in field A, 3.6 in field B, and 6.4 in field C. Significant changes in ascospore abundance occurred in fields B and C between January and June, while ascospore numbers remained relatively stable in field A. During this interval, ascospore counts decreased by 0.4 per gram of soil in field B, but increased by 1.4 in field C. Although the magnitude of change was small for field B, it was significant ($P = 0.014$), as was the change in field C ($P = 0.001$).

Intensively managed field A contained significantly higher populations of ascospores than traditionally managed fields B and C based on the results obtained in 1992. However, higher ascospore numbers in field A (10.8 per gram of soil) relative to field B (4.0 per gram of soil) at planting did not result in greater disease severity at harvest (Table 3). In fact, the average root disease severity indices (DSI) at harvest were slightly higher in field B (2.1) than in field A (1.9). The average DSI for field C (1.5) was lower than for the other two fields, possibly because of the cultivation of a honeydew melon (Honeydew Green Flesh) in this field rather than the muskmelon cultivar Cruiser grown in the other two fields. The incidence of perithecia and the frequency of isolation of *M. cannonballus* were highest in field B, which also had the highest DSI, and lowest in field C, which had the lowest DSI (Tables 3 and 4). Correlation coefficients (r) relating ascospore numbers to disease severity were calculated for individual transects and for each of the three fields. While positive for the majority of transects, the r values varied widely and were rarely significant at the $P = 0.05$ level.

Analyses by the computer program Field Runner were based on disease-severity data from 30 root systems per transect or 240 root systems per field. Although an unusually high number of severely diseased root systems was found in field B, the frequency distributions for disease severity indicated that a majority of plants in each field was lightly to moderately diseased (Table 5). Variance to mean ratios for the severity classes ranged from 0.6 to 2.3 in field A, 0.3 to 2.1 in field B, and 0.6 to 5.8 in field C.

Table 4. Number of plants in transects 1–8 colonized by *Monosporascus cannonballus*, as indicated by isolation frequency and field observations of perithecia on diseased roots

Field	Plants with perithecia/no. of plants excavated									Isolation freq (%)[z]
	1	2	3	4	5	6	7	8	Totals (%)[y]	
A	1/31	1/36*	1/33	0/36	1/34	2/34	1/35	4/32	11/271 (4.1)	10
B	0/37	1/35	0/35	1/38	3/38	5/32*	0/33	2/33	12/281 (4.3)	50
C	0/33	0/30	0/34	1/30	1/31*	2/32	0/34	1/31	5/255 (2.0)	10

[y] Perithecia of *M. cannonballus* observed on the roots of excavated plants.
[z] Isolations were made from 10 randomly selected plants in transects indicated by asterisks. Frequencies based on number of plants (out of 10) from which *M. cannonballus* was isolated.

Table 5. Frequency distributions and variance to mean ratios for disease severity evaluations of muskmelon root systems in three south Texas fields

DSI class[x]	Frequency (%)[y]			Variance/mean ratio[z]		
	Field A	Field B	Field C	Field A	Field B	Field C
0	0.0	0.0	0.4	...	...	...
0.5	1.3	2.9	10.8	1.5	2.1	5.8
1.0	13.3	20.8	24.6	1.8	1.9	1.6
1.5	34.6	27.1	32.1	0.9	0.3	1.5
2.0	29.6	14.1	16.7	0.6	1.3	1.5
2.5	12.5	10.0	7.5	2.3	1.1	2.0
3.0	3.3	2.9	4.2	0.9	0.8	2.4
3.5	1.7	3.8	2.1	2.2	0.9	0.9
4.0	3.8	18.3	1.7	0.9	1.4	0.6
Means	...	...	...	1.4	1.2	2.0

[x] Disease severity indices (DSI) for primary and secondary roots were determined separately on a scale of 0 (healthy) to 4 (extensively lesioned or necrotic) and averaged for each plant, producing the fractional classes.
[y] Plants with root systems that segregated into each disease severity class.
[z] Calculated by the computer program Field Runner (5). Values close to unity indicate random partitioning of plants in the specified disease category among the transects, while values exceeding unity indicate a tendency for clustering of plants in the specified disease category in one or a few transects.

DISCUSSION

The main purpose of this study was to quantify the numbers of ascospores of the root rot/vine decline fungus *M. cannonballus* in the soils of three commercial muskmelon fields in south Texas and to relate these numbers to cropping systems and root rot severity. Two fields were examined in 1991. The first (field A) was mulched with black plastic film and cropped to muskmelon each year since 1989, and had not been deep cultivated since the installation of buried drip irrigation lines in 1988. For these reasons, it was sampled intensively to determine if natural stratification of ascospores had developed within the beds, between the beds, and at different depths. In-bed ascospore numbers were higher, but not significantly higher, than between-bed numbers (Table 2). Much of the effect was due to differences at the 0–10-cm level and may be related to lower root densities, extreme moisture fluctuations, and soil compaction found in shallow soils between the beds. When individual depths were compared, more ascospores were found between 10 and 20 cm than between 0 and 10 or 20 and 30 cm. This was true for both in-bed and between-bed samples, and may be associated with root density. Higher numbers of roots at moderate depths may provide abundant substrate for the production of perithecia and ascospores. Based on these results, samples for the 1992 study were taken within the beds at the 10–20-cm level.

The second field sampled in 1991 (field B) was under traditional management, i.e., furrow irrigation, crop rotation, and deep tillage. Field B was adjacent to A and had produced a spring crop of onions followed by corn (Table 1). Deep tillage (such as that which preceded the non-cucurbit crops in field B) has been shown to decrease aggregation of sclerotia of *Macrophomina phaseolina* (17) and would be expected to disrupt any vertical stratification of *Monosporascus* ascospores which may have developed when the field was cropped to muskmelon in 1990. Therefore, 0–30-cm samples were taken for comparison to combined averages for the three depth increments in field A. As anticipated, ascospore concentrations in field B were significantly lower than either in-bed or between-bed averages in field A (Table 2). In 1975, Johnson et al (8) grew three successive cucurbit crops in plastic-mulched and trickle-irrigated plots in Georgia, and found that the numbers of propagules of three *Fusarium* spp. all increased during individual crop cycles and from the beginning to the end of the study. *M. cannonballus* may respond with similar increases in spore numbers when

muskmelons are cultivated annually in intensively managed fields in south Texas.

During the 1991 study, considerable variability in ascospore numbers occurred between samples within a transect (*data not shown*), although transect averages at a given depth and location were similar (Table 2). Some variability in spore counts was also noted when repeated extractions were made from single, well-mixed soil samples (coefficient of variation [CV] = 29–33%). These observations suggested a need for improvements in the spore extraction procedure and more intensive sampling along the transects. In 1992, the number of sample sites along each transect were increased to 10, and refinements in the extraction procedure lowered the CV to 14–17% and increased recoveries of ascospores by a factor of 1.2–1.5.

In 1992, fields A and B and an additional field C were sampled at planting (January) and after the last harvest (June). At harvest, all plants in each transect were excavated and evaluated for root disease severity to correlate ascospore numbers at individual sample sites with symptom severity at those sites. Few strong positive correlations were found along individual transects or when data were pooled for an entire field (Table 3). The failure to show a strong positive relationship between inoculum density and disease incidence or severity is not unusual in field studies or when natural field inoculum and soils are used. Similar results have been reported for *Macrophomina phaseolina* on muskmelon (2), *Rhizoctonia solani* Kühn on radish (9), and *Peronosclerospora sorghi* (W. Weston & Uppal) C.G. Shaw (= *Sclerospora sorghi* W. Weston & Uppal) on sorghum (19).

In the latter study, Pratt and Janke (19) found that the numbers of oospores of *P. sorghi* in the soils of south Texas sorghum fields were not well correlated with downy mildew incidence because "little or no disease developed in some soils with high oospore densities." We encountered a similar situation in 1992 when ascospore concentrations two to three times higher in field A than in field B failed to produce more severe disease symptoms. Pratt and Janke (19) attributed their findings to a lack of knowledge of the inoculum potential of *P. sorghi,* of which inoculum density is only one component. Lockwood (10) attributed results such as these to 1) decreased infectivity of the inoculum or 2) interference with infection or disease development, usually via microbial antagonism. With respect to 1), ascospores of *M. cannonballus* do not germinate in the laboratory (18) or have done so only rarely (11). Therefore, the viability and infectivity of these propagules, like the oospores of *P. sorghi,* are difficult to assess.

Determinations of inoculum potential for *M. cannonballus* are complicated further by the ability of vegetative mycelium to act as inoculum. The infectivity of mycelial fragments from young cultures of *M. cannonballus* lacking ascospores has been demonstrated in greenhouse tests (12), but the importance of vegetative inoculum in the field and the relationship of ascospore numbers to mycelial mass is unknown. This may explain the lack of response of disease severity to widely differing levels of ascospores in fields A and B. By themselves, the relatively high numbers of ascospores in field A may not constitute high levels of potentially infective inoculum or indicate high inoculum potential, because of the presence of vegetative mycelium.

One objection to interfield comparisons in the present study is the different management system in field A compared to the other two fields (Table 1). However, at the end of 1991 and before the main portion of this study, fields B and C were converted to plastic mulch/drip irrigation by the growers, and field A was completely reworked to install new drip lines. These changes afforded the unique opportunity to study the relationship of inoculum density to disease severity in fields historically under different management systems, but later standardized.

There are at least two other explanations for the lack of an apparent relationship between ascospore numbers and disease severity in this study. The most intriguing is that inoculum levels in the three fields may have exceeded a minimum threshold needed for full disease expression. Susceptible muskmelon and other cucurbit crops have been grown regularly in all three fields for more than 10 yr, ample time for ascospore numbers to increase to relatively high levels. Another possibility is that disease symptoms rated at harvest were the result of infections by multiple organisms. While *M. cannonballus* produced perithecia on the roots of some plants and was isolated from additional plants during the harvest period (Table 4), *Fusarium* spp., *Macrophomina phaseolina,* and *Stagonospora* spp. were also identified on the isolation plates and, according to previous studies (2,4,12), may contribute to root disease symptoms. Muskmelon root rots/vine declines in the LRGV have been attributed to disease complexes rather than to single pathogens (1). Therefore, the inoculum density of *M. cannonballus* may be only one of several variables needed to predict disease incidence and severity.

In our study, perithecia of *M. cannonballus* were observed on unusually low numbers of plants in all three fields (Table 4). Higher frequencies probably would have been noted if the observations had been made later in the harvest period, or if the roots had been examined at 20–30× under laboratory conditions. However, when isolations were made with root segments from 20 plants in fields A and B, one or more segments from 18 of the plants yielded the mycelial growth characteristic of *M. cannonballus.* Subsequent perithecial production on the plates confirmed these observations for 10 and 50% of the plants in the two respective fields. These lower frequencies may have been due to the growth of other fungi which interfered with perithecial development on the isolation plates.

Although our study was not designed to investigate the horizontal arrangement of inoculum, a consistent pattern can be seen from the data. For any given field and sample period, ascospore concentrations were remarkably similar when transect averages were compared (Tables 2 and 3). This suggests a nonclustered pattern of inoculum across broad areas of the field, although clustering on a smaller scale (e.g., within transects) would not be detected by the sampling methods employed in this study. Disease severity indices (DSI) were more variable than ascospore concentrations from transect to transect (Table 3). However, when DSI data were analyzed by Field Runner (5), variance to mean ratios for the different disease categories generally fell between 0.6 and 2.4, indicating negligible to slight clustering of plants with identical disease severities among transects (Table 5). This corresponds to an apparent lack of aggregation of inoculum and a uniformity of disease incidence (lesions or other symptoms were found on the roots of more than 99% of the plants examined). Other studies have shown distinct aggregations of inoculum or disease for *Macrophomina phaseolina* (14) and *Sclerotium rolfsii* Sacc. (20,24). Additional studies of the spatial patterns of *M. cannonballus* ascospores in the field are needed to confirm our findings.

This report is the first to apply Stanghellini and Rasmussen's (25) extraction technique to an actual field investigation. The technique is used for the detection and quantification of ascospores of *Monosporascus* in the soil; but with appropriate modifications, other applications such as separation of various propagules from soil and irrigation water can be envisioned. The extraction procedure is time-consuming but precise, because replicate extractions from the same soil samples yielded spore counts with relatively low coefficients of variation. While ascospore concentrations at planting could not be correlated with root disease severity in 1992, data of this type may become an important component of a disease-prediction model for root rot/vine decline in the future. Additional information on the relative contributions of cultural and edaphic factors to disease severity and inoculum potential of *M. cannonballus* are also needed.

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