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PLANT PATHOLOGY & NEMATOLOGY

Effect of Cropping Systems on Densities of *Verticillium dahliae*

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ABSTRACT

The density of *Verticillium dahliae* (causal agent of Verticillium wilt of cotton) microsclerotia was monitored over a 7-yr period in fields located in the Southern High Plains of Texas. Included in the project were eight sites that were essentially in continuous cotton (short to no rotation from cotton); six sites that were planted with hosts for *V. dahliae* approximately 50 to 71% of the time (intermediate rotation from cotton); and 12 sites that were primarily planted to grain crops (< 2 cotton crops) (long rotation from cotton). There was a decline in the density of microsclerotia at a rate of 1.4 microsclerotia (MS)/cm^3 soil per year in fields that were in a long rotation from cotton, but no consistency in microsclerotia dynamics in the intermediate and short rotations from cotton over time. There was an average of 6.7, 6.9, and 26.1 MS/cm^3 soil, for the long, intermediate, and short/no rotation from cotton, respectively, in the tested sites. Irrigation rate was associated positively with microsclerotia density for both continuous cotton and in a cotton/cotton/sorghum rotation. Microsclerotia density was correlated (R^2 = 0.23) with the incidence of wilt when combined across 26 sites. In sites that have a high density of microsclerotia, it might be necessary to manage irrigation to create a less conducive environment for wilt development, rather than using crop rotation to reduce microsclerotia density.

Verticillium wilt is an important disease of cotton (*Gossypium hirsutum* L.) worldwide (Bell, 1992). *Verticillium dahliae* Kleb. is the predominant species causing this disease in many regions. Microsclerotia produced by *V. dahliae* serve as the initial inoculum (Isaac, 1953). The fungus colonizes roots externally and then internally (Huisman, 1988), eventually reaching the vascular system, where conidia spread upward in the xylem (Presley et al., 1966). The density of microsclerotia has been associated with infection frequency, symptom severity, and yield loss (Ashworth et al., 1972, 1979; Paplomatas et al., 1992).

Management of this disease requires an integrated approach (El-Zik, 1985), because no single tool has been successful, especially for lower-value crops where soil fumigation at efficacious rates has been cost prohibitive (Woodward et al., 2011). An important tactic of managing monocyclic diseases is to reduce the density of infective propagules. However, this is difficult for microsclerotia of *V. dahliae*. Microsclerotia survive for long periods of time (Schreiber and Green, 1962; Wilhelm, 1955) and have the ability to germinate repeatedly when stimulated with water and nutrients (Farley et al., 1971). Rotation with non-hosts to reduce microsclerotia density has been relatively unsuccessful with no real decline in density with short rotations (Butterfield et al., 1978), or a slow decline with longer rotations with non-hosts (Ben-Yephet and Szmulewich, 1985; Huisman and Ashworth, 1976). Although a fast decline in microscelotia occurred (94% in 2 yr) with the use of the non-host broccoli (*Brassica oleracea* v. *botrytis* L.) when the broccoli residue was plowed back into the soil (Xiao et al., 1998). This example however, represents another mechanism besides using a non-host, because the broccoli residue amendment itself reduced the fungal colonization rate on roots and might have had a toxic effect on microsclerotia (Shetty et al., 2000).

Verticillium wilt is a substantial problem in irrigated cotton in the Southern High Plains of Texas (Wheeler and Woodward, personal observations). Losses can be severe even when using management tactics such as partially resistant cultivars (Wheeler et al., 2012) and higher seeding rates (Wheeler et al., 2010). Crop rotation with non-hosts or with fallow land is one of the few other options available to producers who have land infested with *V. dahliae*. The purpose of this project was to characterize the dynamics of *V. dahliae* microsclerotia with long, intermediate, and short-to-no crop rotation patterns with non-hosts, and subsequent wilt incidence on cotton when cultivars with partial resistance to Verticillium wilt were utilized.

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MATERIALS AND METHODS

Fields in various parts of the Southern High Plains of Texas were selected to monitor the population density of *V. dahliae*. Fields naturally infested with *V. dahliae* and associated with three producers in three different areas of the Southern High Plains of Texas were selected for this study. In addition, a research field located in Hale Co., TX was utilized that had areas maintained both in continuous cotton and with a cotton/cotton/sorghum rotation. This research field began showing symptoms of Verticillium wilt in 2007 (the two different cropping systems began in 2001). The producer fields were sampled each year from 2007 to 2013 for microsclerotia density, and the research field was sampled from 2008 to 2014.

Producer 1 was located in Lamb Co., TX and developed a severe Verticillium wilt problem on a number of his fields by 2007. He stopped growing cotton on any of those fields until 2010, and then only grew cotton on a small area within his fields, and on few to no fields each year after that. Corn (*Zea mays* L.) or sorghum (*Sorghum bicolor* (L.) Moench), which are non-hosts of *V. dahliae* (Benson and Ashworth, 1976), were the main crops produced along with a small amount of sunflower (*Helianthus annuus* L.), which is a host of *V. dahliae* (Sackston and Martens, 1959). A total of 12 fields were monitored for microsclerotia density and four fields were monitored for Verticillium wilt incidence when cotton was grown by producer 1. These fields represent the long-rotation group of fields (Table 1). Field F310 had part of the field in cotton during 2010 to 2012; Bearden field had part of the field in cotton during 2011; Dads field had part of the field in cotton during 2011 to 2013; and S-Davis field had part of the field in cotton during 2012. All of the fields were irrigated with center pivot systems.

Producer 2 was located near Gaines Co., TX (Table 1) and produces both cotton and peanut (*Arachis hypogaea* L.), which is also a host of *V. dahliae* (Woodward et al., 2011). Microsclerotia density was monitored in three fields from 2007 to 2013. In the S-Doc field, peanut or cotton was grown from 2007 to 2011, and then the field was left fallow in 2012 and 2013. Wilt incidence was monitored in this field. In the N-Doc field, cotton was grown in 2008, 2010, and 2012, and left fallow in 2007, 2009, 2011, and 2013. Wilt incidence was monitored in 2010 and 2012. In the Ratliff field, half of the field was left fallow each year and half was planted to cotton. All three fields were irrigated with center pivot systems. These fields represent an intermediate level of rotation.

Producer 3 was located in Lynn Co., TX (Table 1) and grew continuous cotton unless that crop was lost to inclement weather. Microsclerotia density was monitored in five fields from 2007 to 2013. The Barn field was in continuous cotton throughout the study and was irrigated with a center pivot system. Wilt incidence was monitored on this field. The remaining four fields were each hailed out one year, so cotton was grown for six of the seven years. The Swan-pivot field was irrigated initially with a center pivot system and then changed to subsurface drip irrigation in 2013. This field was monitored for Verticillium wilt incidence. The Swan-drip field was in subsurface drip irrigation for the duration of the study and was monitored for Verticillium wilt incidence. The Lumsdun and Cievers fields changed to drip irrigation around 2010 and were not monitored for Verticillium wilt incidence. These five fields represent a short to non-rotation group.

A research field located in Hale Co., TX was in a cotton/cotton/sorghum rotation (three wedges representing half of the circle = 24.3 ha) and continuous cotton (one wedge representing 1/6 of the circle = 8.1 ha) since 2001. Verticillium wilt first appeared in the field in 2007, and a soil sampling program was initiated in January of each year from 2008 to 2014. A portion of this work from 2008 to 2010 was published previously (Wheeler et al., 2012). The research field also had three irrigation rates superimposed over the two cropping systems. The base (B) irrigation rate represented the rate that would be applied to replace 80% (2008 and 2009) or 60% (2010-2013) of the evaporative transpiration needs of the crop, when pumping capacity was sufficient to meet those needs. The other irrigation treatments were 50% above (B+50%) or 50% below (B-50%) this rate (Wheeler et al., 2012). This field represented three intermediate rotation sites (Rot-High, Rot-Med, Rot-Low in Table 1) (one for each irrigation rate) and three non-rotated sites (CC-High, CC-Med, CC-Low in Table 1).

Soil sampling for *V. dahliae* microsclerotia was conducted during the winter (January and February). For those sites that would also be monitored for wilt incidence, the field was divided into 300 to 400 squares, and then either 20 or 30 squares at random were selected and the longitude and latitude for the center of those squares was obtained. A square with 30 m on each side was marked around each center point and 20 soil cores were taken in a regularly spaced pattern in the square to a depth of 30 cm. Approximately 75 cm^3 soil was removed from each soil core and all cores were mixed together and represented a composite sample. The same locations were sampled each year. For those fields that were not monitored for wilt incidence, the field was divided into four to eight equal sized areas and a composite sample consisting of 20 cores was sampled in each area. At the research field, soil samples (composite samples with 20 cores per sample) were taken on a four-row strip that went through an entire plot, with typically eight samples taken per irrigation treatment for the continuous cotton wedge, and 24 samples taken per irrigation treatment for the three wedges involved in the cotton/cotton/sorghum rotation.

Details of the soil assays for *V. dahliae* are provided in Wheeler et al., 2012. However, in brief, soil was dried for 1 wk, and then two dilution assays were run per sample, each using 20 cm^3 soil in 80 ml of water. Both dilutions were stirred vigorously and plated out with 1 ml/plate (on to semi-selective media) on each of five plates for each assay, or 10 plates total (Wheeler and Rowe, 1995). Plates were incubated at room temperature in the dark for 14 d, washed free of soil, and *V. dahliae* microsclerotia were counted using a dissecting microscope.

Table 1. Attributes of fields sampled for *Verticillium dahliae* microsclerotia over a 7-yr period in the Southern High Plains of Texas.

County	Field	Latitude	Longitude	Soil Texture	Rotation Group[z]	Mean *V. dahliae* /cm^3 soil[y]
Lamb	F310	34.2386	-102.1284	Clay loam	long	8.3
Lamb	Bearden	34.1968	-102.1586	Sandy clay loam	long	3.2
Lamb	Bovey	34.2279	-102.1069	Loam	long	5.2
Lamb	Dads	34.2389	-102.1372	Clay loam	long	5.6
Lamb	N-Davis	34.1842	-102.2114	Sandy clay loam	long	10.3
Lamb	S-Davis	34.1771	-102.2109	Sandy clay loam	long	9.9
Lamb	Fin-circle	34.2040	-102.1190	Loam	long	0.9
Lamb	Fin-drip	34.2040	-102.1125	Loam	long	2.9
Lamb	Holland	34.2108	-102.2094	Loam	long	10.0
Lamb	Home-Place	34.2178	-102.2097	Loam	long	5.9
Lamb	RG	34.2029	-102.1585	Loam	long	18.1
Lamb	W-Brown	34.2127	-102.2519	Fine sandy loam	long	1.7
Gaines	N-Doc	32.8256	-103.0056	Loamy sand	intermediate	12.3
Gaines	S-Doc	32.8182	-103.0100	Sandy loam	intermediate	15.8
Gaines	Ratliff	32.7504	-102.9556	Loamy sand	intermediate	8.9
Hale	Rot-High	34.1506	-101.9454	Clay loam	intermediate	5.0
Hale	Rot-Med	34.1506	-101.9454	Clay loam	intermediate	1.9
Hale	Rot-Low	34.1506	-101.9454	Clay loam	intermediate	0.9
Hale	CC-High	34.1519	-101.9510	Clay loam	none	67.4
Hale	CC-Med	34.1519	-101.9510	Clay loam	none	27.2
Hale	CC-Low	34.1519	-101.9510	Clay loam	none	11.5
Lynn	Barn	33.3287	-101.7537	Sandy clay loam	none	10.8
Lynn	Swan-drip	33.3842	-101.7798	Loam	short	38.7
Lynn	Swanpivot	33.3767	-101.7876	Loam	short	29.0
Lynn	Cievers	33.3345	-101.7194	Sandy clay loam	short	9.1
Lynn	Lumsdun	33.3338	-101.7744	Sandy clay loam	short	15.4

[z] **A long rotation had < 2 host crops to *V. dahliae* in seven years; an intermediate rotation had host crops to *V. dahliae* 51-71% of the time; a none rotation indicated that all crops were susceptible to *V. dahliae*; a short rotation indicated that one crop in seven years was not a host of *V. dahliae*.**

[y] **Averaged over seven years.**

Verticillium wilt incidence ratings were taken on one row of cotton, 10.7 m in length centered at the points that were soil sampled. In the research farm, three, 10.7-m length row areas were evaluated for Verticillium wilt within each plot. The total number of plants in the 10.7-m row length was counted, and then all plants with Verticillium wilt symptoms on leaves or defoliation were counted to give an incidence value (number of plants with symptoms/total number of plants). Ratings were taken in late August.

Regression analysis (Proc Reg, SAS version 9.3, SAS Institute, Cary, NC) was conducted on the three rotation groups (none and low rotation away from cotton were combined; moderate rotation away from cotton; high rotation away from cotton), and within individual fields to determine the effect of time (with a 1-7-yr scale) on microsclerotia density. Mean microsclerotia density/field (or with a square root transformation) for each year was used as the y-variable and time as the x-variable. Time was fitted with both linear and quadratic functions. Models were acceptable if the F-value was significant at $p < 0.10$, and the individual slope parameter was significant at $p < 0.10$ (for quadratic functions). If multiple models could be fitted, then the model with the highest R^2 value was selected. If no model could be fitted to the data, then the mean MS/cm^3 soil over time was examined visually for a general evaluation of increase or decrease in time. Means were calculated for each rotation group over the entire time span to discuss general trends. The relationship between average Verticillium wilt incidence per field and average MS/cm^3 soil per field were correlated using regression analysis across all the years and sites.

RESULTS

The average microsclerotia density for the long, medium, and short/non-rotation was 6.7, 6.9, and 26.1 MS/cm^3 soil, respectively. Microsclerotia density from the long rotation could be fitted with a linear model: $(MS/cm^3) = 12.22 - 1.39(Time)$, $p = 0.004$, $R^2 = 0.10$, where Time is years, with 2007 = 1 and 2013 = 7. This model predicts the average rate of decline in microsclerotia was 1.4 MS/cm^3 soil/year. Microsclerotia density could not be fitted with a linear or quadratic model for the intermediate and short/non-rotation sites as a group.

Eight individual fields of the 26 monitored could be fit with a model predicting microsclerotia density. At the research farm, microsclerotia density for the continuous cotton oscillated widely as irrigation rate increased (Fig. 1). None of the individual irrigation rates could be fitted with a model predicting MS density. At the short/non-rotation fields in Lynn Co., MS density increased over time in the Cievers field, $(MS)^{1/2} = 1.56 + 0.33(Time)$, $p = 0.09$, $R^2 = 0.48$ (Fig. 2A). At the Swan-pivot field, MS density deceased over time, $MS = 60.12 - 7.78(Time)$, $p = 0.096$, $R^2 = 0.46$ (Fig. 2B). At the Barn field, MS density was fitted with a quadratic model, $MS = 34.23 - 12.31(Time) + 1.29(Time)^2$, $p < 0.001$, $R^2 = 0.97$ (Fig. 2B). At the Lumsdun field, MS density was fitted with a quadratic model $MS = 54.19 - 20.73(Time) + 2.21(Time)^2$, $p = 0.052$, $R^2 = 0.77$ (Fig. 2B). At the Swan-drip field, no model could be fitted to MS density (Fig. 2B). To summarize the results for the short/non-rotation fields, there was one field where MS increased consistently (Cievers), one field where MS decreased consistently (Swan-pivot), four situations where MS oscillated greatly (three were at the research farm, plus Swan-drip), and two sites where MS did not change much (and were fitted with quadratic models) (Barn and Lumsdun fields). There was a complete lack of consistency of MS density over time from site to site for short/non-rotated fields.

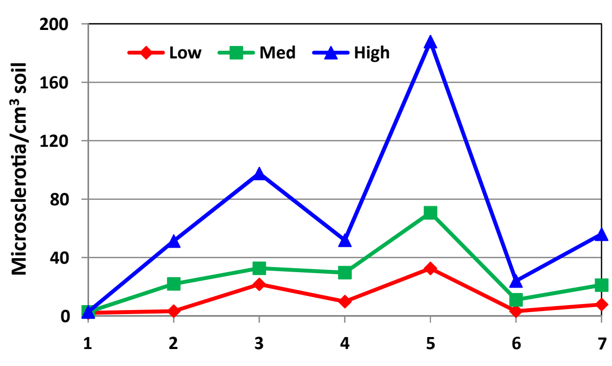

Figure 1. Average microsclerotia density of *Verticillium dahliae* measured from 2008 to 2014 in continuous cotton. There were three irrigation rates at this site: Medium (Med), 50% higher than the medium rate (High) and 50% lower than the medium rate (Low).

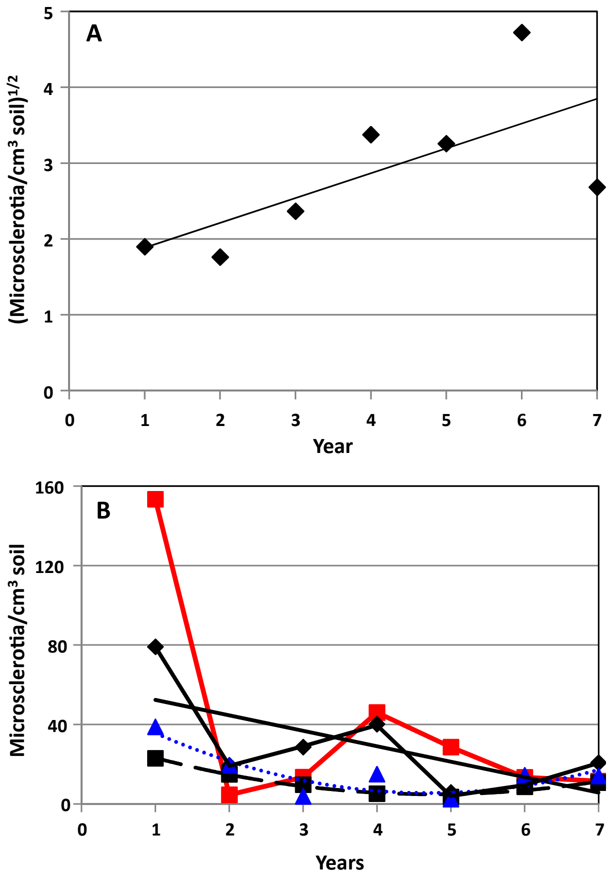

Figure 2. Dynamics of *Verticillium dahliae* microsclerotia (MS) over a 7-yr period in commercial production fields that were in cotton at least six of the seven years. A) Cievers field, $(MS)^{1/2} = 1.6 + 0.3(Time)$, $R^2 = 0.48$. B) Swanpivot (—◆—) MS = 60.1 – 7.8(Time), $R^2 = 0.46$; Barn (■ —) MS = 34.2 – 12.3(Time) + $1.3(Time)^2$, $R^2=0.97$; Lumsdun (··▲··) MS = 54.2 – 20.7(Time) + $2.2(Time)^2$, $R^2 = 0.77$. Swan-drip (—■—).

At the research farm within the cotton/cotton/sorghum cropping system, a linear model could be fitted to the B+50% irrigation rate, $(MS)^{1/2} = 0.41 + 0.68(Time)$, $p = 0.014$, $R^2 = 0.73$ (Fig. 3). There was a strong increase over time in microsclerotia density, compared to other irrigation treatments. A quadratic model was fitted to the Base irrigation rate, $(MS)^{1/2} = 0.17 + 0.77(Time) – 0.079(Time)^2$, $p = 0.06$, $R^2 = 0.76$. No model could be fitted to MS density for the B-50% irrigation rate and little change in MS density occurred over the seven years. Similar to results in the continuous cotton wedge, there was a positive association between irrigation rate and microsclerotia density in the cotton/cotton/sorghum cropping system (Fig. 3).

The commercial production fields with intermediate rotation (Fig. 4) included one field with a reduction in microsclerotia density over time (N-Doc) $(MS)^{1/2} = 5.50 - 0.59(Time)$, $p = 0.053$, $R^2 = 0.56$. A second field had microsclerotia density that exhibited a quadratic relationship (Ratliff) $(MS)^{1/2} = 0.99 + 1.18(Time) – 0.14(Time)^2$, $p = 0.039$, $R^2 = 0.80$. For the third field in this category (S-Doc), microsclerotia density fluctuated widely (Fig. 4). To summarize results in the intermediate rotated fields, one field had a consistent increase in MS, one field had a consistent decrease in MS, and in four fields there wasn't much change in MS over time (either no model fitted or a quadratic model was fitted). However, where no model was fitted, the MS densities were typically lower than in the continuous cotton sites, and the oscillations in MS densities were much smaller. Overall, there was an increase in the incidence of wilt with increasing microsclerotia density (Fig. 5). The model fitted was %Wilt = $4.5 + 2.75(MS/cm^3 soil)^{1/2}$, $p < 0.001$, $R^2 = 0.23$.

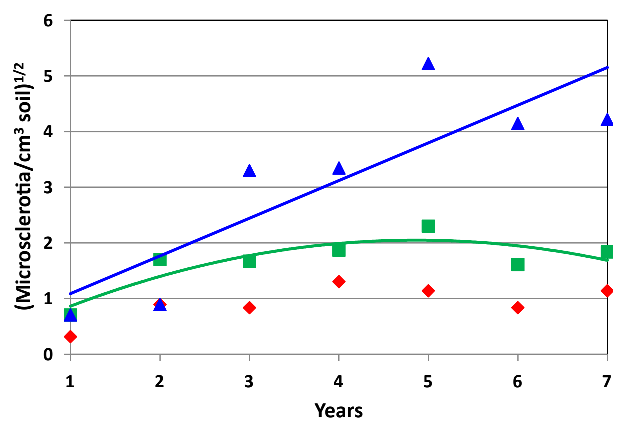

Figure 3. Average microsclerotia density (MS) of *Verticillium dahliae* measured over seven years in a field at a research farm that had a long-term 2-yr cotton and 1-yr grain (primarily sorghum) rotation. There were three irrigation rates superimposed on the rotation, a base (B) rate (■), a rate that was 50% higher than the base rate (▲), and a rate that was 50% lower than the base rate (◆). The B+50% rate (——) was fitted with the model $(MS)^{1/2} = 0.41 + 0.68(Time)$, $R^2 = 0.73$. The Base irrigation rate (——) was fitted with the model $(MS)^{1/2} = 0.17 + 0.77(Time) – 0.079(Time)^2$, $R^2 = 0.76$.

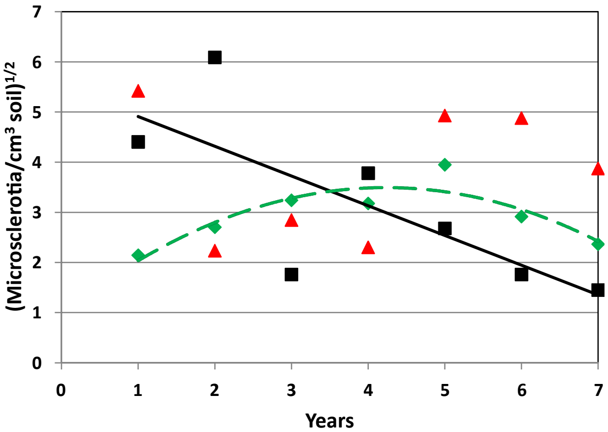

Figure 4. Dynamics of *Verticillium dahliae* microsclerotia (MS) over a 7-yr period in commercial production fields that were in cotton or peanut 57-71% of those years. N-Doc (■) was fitted with a linear model (——) $(MS)^{1/2} = 5.5 - 0.59(Time)$, $R^2 = 0.56$. Ratliff was fitted with a quadratic model (◆ –) $(MS)^{1/2} = 0.99 + 1.18(Time) - 0.14(Time)^2$, $R^2 = 0.80$. S-Doc (▲) could not be fitted with a linear or quadratic model.

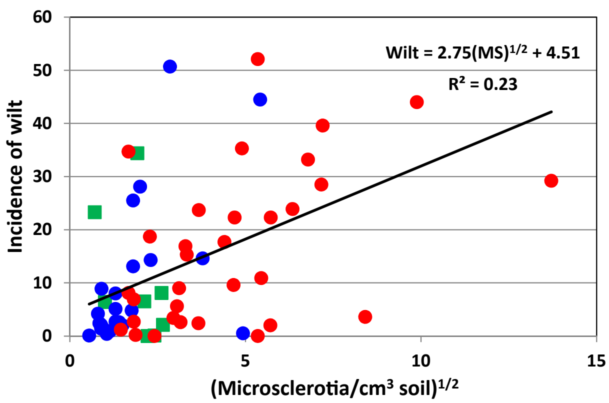

Figure 5. The relationship between microsclerotia of *Verticillium dahliae* (MS)/cm³ soil and incidence of Verticillium wilt the following growing season. ■ = fields with minimal rotation with hosts susceptible to *V. dahliae*; ● = fields with moderate rotation with hosts susceptible to *V. dahliae*; ● = fields in continuous cotton or < 2 non-hosts for *V. dahliae* in 7 yr.

DISCUSSION

Microsclerotia density in the Southern High Plains of Texas declined slowly (average of 1.4 MS/cm³ soil per year) when fields went into a long rotation without susceptible hosts to *V. dahliae*. Similar results were reported in other studies (Butterfield et al., 1978; Huisman and Ashworth, 1976). Fields with intermediate rotations with non-hosts for *V. dahliae* did not show any consistent decline in microsclerotia density, but did have lower densities of microsclerotia with less intense oscillation in annual microsclerotia recovery than did fields with minimal or no rotations from cotton. Huisman and Ashworth (1976) suggested that rotations served to slow the time necessary for initial microsclerotia buildup, but eventually the field can become heavily infested. Our data was also consistent with the premise that crop rotation slows the time necessary for microsclerotia buildup; however to become heavily infested, it may also be necessary for field to have high irrigation or rainfall.

Irrigation rate played a critical role in the buildup of microsclerotia. This was apparent in both the continuous cotton and the cotton/cotton/sorghum cropping system at the research site. Whereas microsclerotia density is a measure for the potential of disease, the actual expression of disease might be dependent on irrigation rate (Wheeler et al., 2012). The amount of in-season water had a large impact on both the increase in microsclerotial density and on disease expression. Though, even without including any factor for irrigation rate or rainfall, there was a correlation between microsclerotia density and incidence of wilt across a wide range of fields and growing conditions. Crop rotation with non-hosts was beneficial in maintaining a lower overall density of microsclerotia than a short/no-rotation system. However, in fields where the microsclerotia density already was sufficient to cause substantial wilt, crop rotation could not consistently lower the density of microsclerotia. It might also be necessary to modify irrigation practices so that conditions are less favorable for disease expression.

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