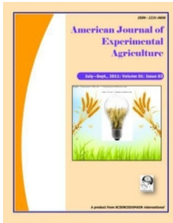

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Impact of Nitrogen on Cotton Growth, Lint Yield, Fiber Quality and Verticillium Wilt

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Authors' contributions

This work was carried out in collaboration between both authors. Author CRA initiated the experiments, collected the data, performed the statistical analysis, managed the literature reviewand wrote the first drafts of the manuscript. Author JEW designed the study, wrote the protocol, assisted with statistical analysis and contributed to the final draft. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: To examine the response of cotton varieties with varying reactions to *Verticillium dahliae* to nitrogen.
Study Design: The experimental design was a randomized complete block in a split-plot arrangement with four replications. Nitrogen rate served as whole plots and cotton variety as sub-plot.
Locations: Trials were conducted at the Texas Tech University New Deal and Quaker Farms in Lubbock, County (2011 and 2012), and the Texas A&M AgriLife Research Halfway Farm in Hale County (1 trial in 2011 and 2 trials in 2012).
Methodology: Plots measured 4.1 m wide (four rows on a 1.2-m spacing) by 10.6 m long. Composite soil samples were collected from four depths to determine nitrogen concentrations and fungal populations. Nitrogen treatments were applied prior to bloom. Plant growth, disease incidence, lint yield and fiber quality were used to compare treatments.
Results: Overall, the application of nitrogen had little affect on cotton growth. Appreciable levels of disease were only observed in one trial. The application of nitrogen slightly increased disease incidence; however, a two-fold increase in disease was observed in susceptible versus partially resistant varieties. Lint yields were only increased with the

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application of nitrogen in 2011. Subtle differences in fiber quality were observed between nitrogen rates, while varietal effects were observed for most all parameters. The fungus was recovered from the entire soil profile with fewer propagates found in the upper layer. Residual nitrate nitrogen increased over the two years and concentrations were highest in the uppermost layer.
Conclusion: Although confounded by adverse environmental conditions, these studies suggest that interactions between *V. dahliae* and nitrogen may exist. Furthermore, variety selection and the vertical distribution of the fungus may affect disease development. Additional studies are needed under more conducive conditions to better understand the interactions between these factors.

Keywords: Gossypium hirsutum; Verticillium wilt; microsclerotia.

1. INTRODUCTION

Upland cotton (*Gossypium hirsutum* L.) is an economically important field crop, with approximately 14% of global production (3.7 million metric tons) occurring in the United States [1]. According to Matocha et al. [2], most production occurs in Texas with the highest concentration being in the High Plains region, which is comprised of the 27 counties surrounding Lubbock. Indigenous to this region, the soilborne fungus *Verticillium dahliae* Kleb., causal agent of Verticillium wilt, has been associated with substantial losses [3].

The fungus has ahost range of more than 400 species [4] and is capable of surviving for many years in the absence of a host, as specialized overwintering structures called microsclerotia [5]. Disease intensity and severity have been correlated to the density of microsclerotia in the soil at planting [6,7,8]. Microsclerotia germinate in response to root exudates [3]. The fungus colonizes the surface of cotton roots behind the root tip, penetrates the root cortex and eventually entersthe xylem vessels [3]. Initial infections of *V. dahliae* occur relatively early in the season; however, symptoms of the disease become more apparent following boll formation and maturation. Foliar symptoms of Verticillium wilt consist of interveinal chlorosis and necrosis, followed by premature defoliation of leaves in the lower canopy [3]. Examination of stems from infected plants will reveal a tan to black discoloration of the xylem vessels. As nutrients within the infected tissues are depleted, new microsclerotia form after several weeks, depending on the presence of moisture [3]. Chawla et al. [9] demonstrated that variety selection could impact densities of microsclerotia. Furthermore, the field application of improperly composted gin trash has been cited as a potential means of moving the pathogen [10] and the removal of crop residue may limit the formation of new microsclerotia [11].

Development of Verticillium wilt is favored by cool wet conditions; whereas, high temperatures and drought conditions do not promote growth and development of the fungus [4,12]. Grimes and Yamada [13] indicated that irrigation amount, frequency and timing, as well as drainage affect disease development. Excessive furrow irrigation lowers soil temperatures, thus promoting infection by the fungus [14]. Extremely low levels of disease are observed in non-irrigated cotton [15] and furrow irrigation is believed to promote Verticillium wilt more so than pivot irrigation or sub-surface drip irrigation (personal observation). Recent studies have shown that higher irrigation rates increased disease incidence and lowered yields [16]. Other cultural practices, such as planting on raised beds [17], the use of higher seeding rates [18] and the utilization of partially resistant varieties

[19,20] in conjunction with rotation schemes including non-hosts [21] have proven effective at managing the disease. Fumigation is the primary chemical management option to manage Verticillium wilt; however, affordable use rates for field crops are not efficacious [22].

Applications of nitrogen are frequently made to cotton to maximize yields production. Lemon et al. [23] found that 28 kg ha^{-1} of available nitrogen is required for every 113.5 kg of lint considering that all other factors are equal. Low to moderate amounts of nitrogen have been shown to substantially increase yields in fields infested with *V. dahlia* with no obvious effect on disease development (personal observation). The avoidance of excessive nitrogen reduces rank vegetative growth, which is believed to exacerbate Verticillium wilt incidence [3]. Increased nitrogen applied in irrigation water through fertigation was associated with a higher incidence of wilt [16]; however, this observation may have been confounded as increased nitrogen fertilizer was applied in conjunction with higher irrigation amounts. Previous studies have shown that split applications of nitrogen can reduce disease incidence and increase yields [17]; however, the overall influence of nitrogen on disease development is poorly understood. The objective of this study was to examine the influence of nitrogen on disease incidence, lint yield and fiber quality of susceptible and partially resistant cotton varieties. A second objective was to determine the vertical distribution of the fungus within the soil profile.

2. MATERIALS AND METHODS

2.1 Field Trials

Separate trials were established in 2011 and repeated in 2012 at the Texas Tech University New Deal Research Farm and the Texas Tech Quaker Research Farm. Both of these locations were equipped with sub-surface drip irrigation. Drip tape was spaced 2.03 m centers (every other furrow) and was approximately 25.4 to 30.5 cm deep. Soil at the New Deal and Quaker locations was an Acuff clay loam and Amarillo and Acuff clay loam, respectively. Likewise, a trial was initiated at the Texas A&M AgriLife Research Halfway Station (Hale County) in 2011 and repeated in 2012 under center pivot irrigation. A second trial was initiated at the Halfway location in 2012. Soils at Halfway were a Pullman clay loam. All trials were planted between 1-May and 15- May.

Plots measured 4.1 m wide (four rows on a 1.2-m spacing) by 10.6 m long. The experimental design was a six × four factorial split-plot design with four replications. Nitrogen treatments (0, 56, 112, 168, 224 and 280 kg ha^{-1}), comprised of UAN-32, served as whole plots and were applied prior to bloom approximately 30 to 45 days after planting using a wheel-driven side-dress applicator. The varieties Deltapine (DP) 0912B2RF, DP 104B2RF, FiberMax (FM) 9160B2F, NexGen (NG) 3348B2RF (2011 only) and DP 1032B2RF (2012 only) were chosen based on their performance in regional trials[19,20]. All management practices other than nitrogen application and variety selection were in conjunction with local Extension recommendations.

2.2 Data Collection and Analysis

Plant stand was estimated approximately 21 days after planting by counting the number of emerged plants in each row of the plot. Plant height and total nodes were assessed prior to natural senescence. Disease incidence was assessed at the same times by counting the number of plants within the two middle rows that were exhibiting foliar symptoms of

Verticillium wilt. Additionally, five plants were arbitrarily selected from the two outer rows and destructively selected to score vascular discoloration, where 0 = healthy tissue and 1= discoloration.

Plots were harvested between 25-October and 9-November with a John Deere 484 cotton stripper, equipped with a modified cage and load cells to measure plot weights. Bur cotton yields were collected from each plot and sub-samples (~1500 g) were ginned to determine lint and seed turnout. Samples of lint (~200 g) were submitted to the Texas Tech University, Fiber and Biopolymer Institute for HVI analysis. Data were subjected to analysis of variance using Proc ANOVA (Statistical Analysis System, version 9.3, Cary, NC) and means were separated using Fisher's Protected LSD ($P \leq 0.05$).

2.3 Nutrient Analysis and Estimating *V. dahliae* Microsclerotia

Soil samples were taken to a depth of 61 cm. A soil core was randomly collected from the top of the bed in each sub-plot and divided into four layers (0-16, 16-31, 31-46 and 46-61 cm). Composite samples were placed in paper sacks, labeled and transferred to the laboratory, where they were divided into two parts. The first portion was ground using a rolling pin, sent to a commercial laboratory and assayed for nitrate nitrogen as described by Dahnke [24]. The second portion was air-dried at ambient temperature for one week. Dilution plating was conducted using the semi-selective media of NP-10, along with 0.025 N NaOH to enumerate *V. dahliae* microsclerotia in cm^{-3} soil [25,26,27]. Samples were allowed to incubate for 2 weeks in the dark. Soil was gently washed from the surface of the medium and microsclerotia were counted using a stereomicroscope.

3. RESULTS AND DISCUSSION

Rainfall and temperature throughout the duration of this study were well above historical averages (Table 1). Rainfall during the 2011 growing season was 79.6% below normal while the accumulation of heat units [28] were 33.3% above average. While growing conditions moderated slightly the following year, rainfall remained at a deficit (40% below average) and temperatures above average (15.5%). Due to the extreme conditions experienced and lack of uniform varieties, it was decided that years would be analyzed independently. Due to the lack of a trial by treatment interaction data from drip and pivot irrigation trials were combined when possible.

Table 1. Monthly heat unit accumulation and rainfall during the 2011 and 2012 growing seasons compared to the 30-yr average for the High Plains of Texas*

Month	**Heat unit units(DD-60's)**			**Rainfall (mm)**		
	2011	**2012**	**30-yr avg**	**2011**	**2012**	**30-yr avg**
May	363	417	293	7	34	59
June	776	615	515	0	41	76
July	808	683	619	1	7	54
August	800	661	560	9	74	60
September	380	393	343	32	52	65
October	98	25	89	34	7	43
Total	3225 (+33.3%)	2749 (+15.5%)	2419	82 (-79.6%)	214 (-40.1%)	357

**data are from the National Weather Service 2011 and 2012 Lubbock summaries [29,30]. Values in parentheses represent deviations from the 30-yr average*

3.1 Stand Establishment and Plant Growth

Overall, plant stands were unaffected by the application of nitrogen (Table 2). Germination averages ranged from 65% to 78%, with subtle differences being observed for the drip irrigated trials in 2012. This is likely not a direct treatment effect, as stand counts were conducted well before the application of nitrogen. Differences in stand among varieties were observed in both drip and pivot irrigated trials in 2011 and 2012. Stands were consistently higher for DP 104B2RF in both years. In 2011, stands were lowest for NG 3348B2RF, which may have been attributed to a high number of cracked or chipped seed (personal observation). Stands for DP 1032B2RF were lowest in 2012; however, this may have been due to inherently low vigor of the seed lot used in these and other studies [unpublished data]. When considering plant height, higher nitrogen rates typically resulted in taller plants in the drip irrigated trials in both years; whereas, nitrogen had no effect on plant height in the pivot irrigation trials (Table 2). Differences among varieties were observed in all trials with FM 9160B2F averaging 60.0 cm. Total nodes were similar among nitrogen treatments, but varied by variety with FM 9160B2F averaging 2.1 more nodes than all other varieties.

3.2 Disease Incidence

Foliar symptoms were not observed in either of the drip irrigation fields in 2011 and incidence under pivot irrigation was <1.0%, similarly levels of vascular discoloration were extremely low (data not shown). The moderation of growing conditions in 2012 proved to be more conducive for disease development. While relatively low, differences between nitrogen rates were observed with more disease occurring where higher rates of nitrogen were applied. Higher levels of disease were found in the susceptible varieties DP 0912B2RF and DP 1032B2RF compared to the partially resistant varieties DP 104B2RF and FM 91602B2F.

In 2012, vascular discoloration in the drip-irrigated trials did not differ for any of the nitrogen rates (Table 3). Subtle differences among varieties were found, but overall the level of disease averaged 0.6%. Appreciable levels of disease were observed in both of the pivot irrigation trials. Although variable, the application of nitrogen tended to result in higher levels of Verticillium wilt compared to the non-treated control. Similar to trends observed in the drip irrigation trials, higher levels of disease were observed in the susceptible varieties under pivot irrigation than in the partially resistant varieties averaging 12.3% and 6.4%, respectively. Examination of lower stems revealed a high percentage, 35%, of plants exhibiting vascular discoloration. Values ranged from 28.8% to 38.3% for FM 9160B2F and DP 1032B2RF.

3.3 Lint Yield

Lint yields differed among the two drip irrigation locations in 2011. Yields were greatest at New Deal compared to Quaker (data not shown). Overall, the application of nitrogen had a positive effect on yield compared to the non-treated control. Lint yields were highest for the susceptible DP 0912B2RF at 1259 kg ha^{-1}, and similar for the other three varieties averaging 1086 kg ha^{-1}. Differences in yield were observed between locations and varieties but not nitrogen rates (Table 4). In 2012, yields were greater at New Deal compared to Quaker (data not shown), with DP 0912B2RF and DP 1032B2RF having higher yields than the partially resistant varieties.

Table 2. Effect of nitrogen on plant stand, height and nodes of cotton varieties grown under drip or pivot irrigation in 2011 and 2012*

Factor, level	Stand counts (plants m^{-1})				Plant height (cm)				Nodes (# $plant^{-1}$)			
Nitrogen rate (kg ha^{-1})	Drip		Pivot		Drip		Pivot		Drip		Pivot	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0	10.3 a	9.6 ab	8.3 a	10.3 a	50.4 c	67.8 ab	58.0 a	58.8 a	18.6 a	16.6 a	15.1 a	17.1 a
56	10.4 a	8.9 b	8.4 a	10.3 a	52.7 b	66.5 ab	64.0 a	59.4 a	18.9 a	16.4 a	15.5 a	17.0 a
112	10.1 a	10.0 a	9.3 a	10.1 a	53.9 a	63.8 c	57.0 a	62.3 a	19.1 a	15.8 b	15.9 a	17.0 a
168	10.1 a	9.7 ab	8.3 a	10.2 a	53.9 a	66.6 ab	60.3 a	63.3 a	19.0 a	16.7 a	16.5 a	17.3 a
224	10.2 a	9.2 ab	8.6 a	10.2 a	53.9 a	65.4 bc	61.7 a	63.1 a	19.3 a	16.5 a	16.2 a	17.6 a
280	10.2 a	9.9 ab	8.2 a	10.3 a	53.5 a	68.1 a	61.8 a	62.2 a	18.6 a	16.7 a	16.1 a	17.1 a
Variety												
DP 0912B2RF	9.8 c	9.1 c	9.3 a	9.8 c	54.5 b	69.6 ab	63.1 a	67.4 a	17.9 b	16.4 b	15.3 b	17.0 b
DP 104B2RF	11.0 a	10.7 a	8.4 b	11.3 a	50.1 d	60.5 c	60.4 a	54.7 b	18.3 b	15.9 c	15.3 b	17.0 b
DP 1032B2RF	n/t	8.3 d	n/t	9.2 d	n/t	70.1 a	n/t	66.0 a	n/t	16.4 b	n/t	17.1 b
NG 3348B2RF	9.9 c	n/t	7.7 c	n/t	51.7 c	n/t	57.9 a	n/t	18.4 b	n/t	11.0 c	n/t
FM 9160B2F	10.3 b	10.1 b	8.7 b	10.9 b	56.0 a	65.2 bc	60.4 a	58.0 b	21.1 a	17.2 a	17.9 a	17.7 a

**Means for a factor with a column followed by the same letter are not different, according to Fisher Protected LSD (P=0.05). Values are the mean of combined trials (there were a total of 2 drip irrigated trials in 2011 and 2012, and 1 pivot irrigated trial in2011 and 2 in 2012).n/t = not tested, as DP1032B2RF replaced NG 3348B2RF in 2012*

Table 3. Effect of nitrogen on Verticillium wilt incidence and vascular discoloration on cotton varieties grown under drip or pivot irrigation in 2012*

Factor, level	Disease incidence (%)		Vascular discoloration (%)	
Nitrogen rate (kg ha^{-1})	**Drip**	**Pivot**	**Drip**	**Pivot**
0	1.1 b	7.2 c	0.6 a	30.6 a
56	1.0 b	11.2 ab	0.5 a	36.9 a
112	0.8 b	6.5 c	0.5 a	34.4 a
168	1.1 b	12.0 a	0.4 a	31.9 a
224	1.8 a	10.4 b	0.5 a	39.4 a
280	1.4 ab	8.7 ab	0.8 a	35.0 a
Variety				
DP 0912B2RF	1.6 a	12.7 a	0.5 b	35.4 a
DP 104B2RF	0.8 b	7.3 b	0.4 b	36.3 a
DP 1032B2RF	1.9 a	11.8 a	0.6 ab	38.3 a
FM 9160B2F	0.5 b	5.5 b	0.8 a	28.8 a

**Means for a factor with a column followed by the same letter are not different, according to Fisher Protected LSD (P=0.05)*

Lint yield across the six nitrogen treatments were not different under pivot irrigation in 2011; however, yields were generally higher in plots receiving nitrogen compared to the non-treated control (Table 4). There were differences when considering varieties with DP 0912B2RF out yielding other varieties by 124 kg ha^{-1}. In 2012, yields were similar for the two locations averaging 1276kg ha^{-1}, ranging from 1232 to 1364 kg ha^{-1} (Table 4). Yields did differ among varieties with FM 9160B2F, DP 0912B2RF and DP 1032B2RF out performing DP 104B2RFby approximately 250 kg ha^{-1}.

Table 4. Effect of nitrogen on plant stand, height and nodes of cotton varieties grown under drip or pivot irrigation in 2011 and 2012*

Factor, level	Lint yield (kg ha^{-1})			
Nitrogen rate (kg ha^{-1})	**Drip**		**Pivot**	
	2011	**2012**	**2011**	**2012**
0	994 b	1797 a	830 a	1232 a
56	1129 a	1741 a	872 a	1242 a
112	1156 a	1750 a	844 a	1255 a
168	1136 a	1718 a	879 a	1271 a
224	1175 a	1727 a	892 a	1364 a
280	1187 a	1826 a	895 a	1294 a
Variety				
DP 0912B2RF	1259 a	1827 a	961 a	1340 a
DP 104B2RF	1101 b	1660 b	833 b	1092 b
DP 1032B2RF	n/t	1828 a	n/t	1326 a
NG 3348B2RF	1071 b	n/t	830 b	n/t
FM 9160B2F	1087 b	1725 b	850 b	1348 a

**Means for a factor with a column followed by the same letter are not different, according to Fisher Protected LSD (P=0.05). Values are the mean of combined trials (there were a total of 2 drip irrigated trials in 2011 and 2012, and 1 pivot irrigated trial in 2011 and 2 in 2012). n/t = not tested, as DP1032B2RF replaced NG 3348B2RF in 2012*

Recent reports indicate that the response of cotton varieties to Verticillium wilt under normal growing conditions is relatively consistent among years [19,20]. The varieties included in this study represented both determinate and indeterminate growth habits, as well as susceptible and partially resistant disease reactions. Although included as a susceptible variety, yields of DP 0912B2RF consistently ranked high in each of the trials, supporting previous studies indicating the high yield potential of this variety in the absence of disease [31,32]. Likewise, Wheeler et al. [16] found that susceptible varieties grown under low disease pressure out-yielded more resistant varieties.

3.4 Fiber Quality

With the exception of strength, fiber quality parameters in the 2011 drip irrigated trials were generally unaffected by the application of nitrogen (Table 5). Micronaire and length values averaged 4.6 units and 1.08 in, respectively. Higher nitrogen rates resulted in increased strength where values were highest for nitrogen rates of 224 and 280 kg ha^{-1} and lowest for the non-treated control. Differences were experienced across varieties for all fiber parameters (Table 4). Micronaire values were lowest for DP 104B2RF and highest for DP 0912B2RF ranging from 4.2 to 5.1, respectively. Length was greatest for FM 9160B2F at 1.12 in and lowest for 1.04 for DP 0912B2RF. Strength ranged from 28.9 to 30.3 g tex^{-1} for DP 0912B2RF and DP 104B2RF, respectively.

In 2012, micronaire ranged from 4.10 to 4.29 for the 224 and 112 kg ha^{-1} rates, respectively. Values were lowest for DP 104B2RF and FM 9160B2F at 3.91 and 3.97 units, respectively, and highest for DP 0912B2RF (4.5 units) and averaged 4.2 units intermediate for DP 1032B2RF (4.29 units). Length and strength values were higher at New Deal than Quaker (data not shown); however, differences in nitrogen treatment were not observed. When combined across locations, staple length was greatest for FM 9160B2F and lowest for DP 0912B2RF, whereas, strength was greatest for DP 104B2RFand lowest for DP 1032B2RF.

The application of nitrogen did not affect micronaire, length or strength in the 2011 pivot irrigation trial (Table 4). While micronaire and strength values were similar among nitrogen rates in 2012, staple length was slightly higher (0.01 in) for plots treated with nitrogen compared to the non-treated control. Similar to what was observed in the drip irrigation trials, fiber quality parameters differed among varieties (Table 4). Higher micronaire values were associated with DP 0912B2RF, and lower values were associated with DP 104B2RF. Conversely, staple length was lowest for DP 0912B2RF and greatest for FM 9160B2F. Strength ranged from 29.3 to 31.0g $tex^{-1}$and 27.6 g to 29.5 g tex^{-1} in 2011 and 2012, respectively and was highest for DP 104B2RF in both years.

3.5 Residual Nitrogen

A broad range of residual nitrate levels were observed among locations (Fig. 1). Opposite trends were observed when comparing nitrogen rates from 2011 to 2012 in drip fields versus the pivot irrigation study. Treatments were not different either of the two years, at the Quaker location; however, there was a substantial increase in residual nitrogen at the end of the study. Extremely low nitrogen levels were found at the New Deal site in 2010, prior to the start of this study (data not shown). Results from samples collected at the end of 2011 revealed higher residual nitrogen levels in plots treated during the season compared to the non-treated control (Fig. 1). As was the case at the Quaker location, higher levels of residual nitrogen were observed at the conclusion of the study; however, the increase may have mostly resulted from a broadcast application of nitrogen that was applied between the 2011 and 2012 growing season. No differences in residual nitrogen were observed among nitrogen treatments following either of the two growing seasons at the Halfway pivot irrigation trial (Fig. 1). Final amounts of nitrogen in 2012 were 29.9% lower than final amounts found in 2011. Such nitrogen utilization, in addition to improved rainfall, attributed to the 408 kg ha^{-1} increase in lint yield observed in 2012 over 2011.

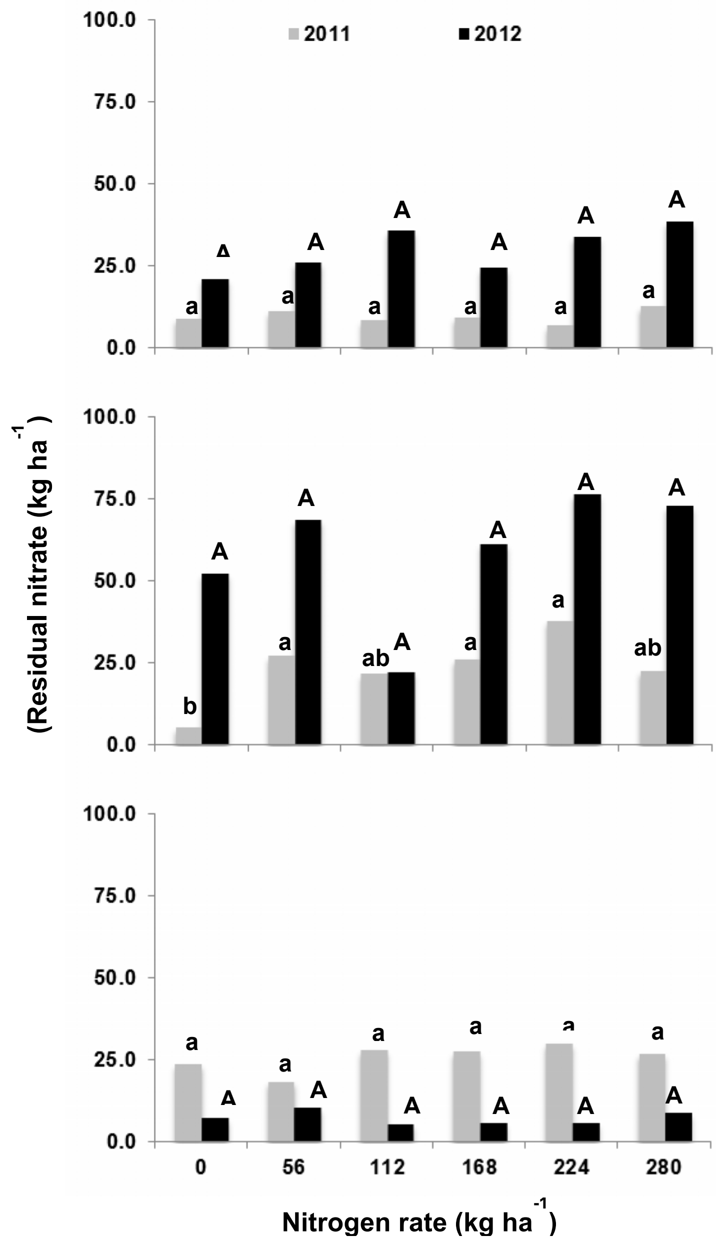

Fig. 1. Residual nitrate nitrogen levels for three field sites Quaker (Top), New Deal (Middle) and Halfway (Bottom) in 2011 and 2012

Table 5. Effect of nitrogen on plant stand, height and nodes of cotton varieties grown under drip or pivot irrigation in 2011 and 2012*

Factor, level	Micronaire(units)				Length (in)				Strength(g tex^{-1})			
Nitrogen rate	Drip		Pivot		Drip		Pivot		Drip		Pivot	
(kg ha^{-1})	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
0	4.6 a	4.2 b	3.6 a	4.2 a	1.08 a	1.10 a	1.05 a	1.06 c	29.3 c	30.3 a	30.0 a	28.1 a
56	4.5 a	4.2bc	3.6 a	4.1 a	1.08 a	1.10 a	1.06 a	1.07 ab	29.4 b	30.0 a	30.1 a	28.5 a
112	4.6 a	4.3 a	3.6 a	4.2 a	1.08 a	1.09 a	1.04 a	1.06 bc	29.2 b	29.9 a	29.6 a	28.3 a
168	4.6 a	4.1bc	3.6 a	4.2 a	1.07 a	1.10 a	1.06 a	1.08 a	29.5 b	30.1 a	30.3 a	28.6 a
224	4.6 a	4.1 c	3.6 a	4.1 a	1.08 a	1.09 a	1.05 a	1.07 abc	29.7 a	29.8 a	30.3 a	28.6 a
280	4.6 a	4.2ab	3.7 a	4.1 a	1.08 a	1.10 a	1.05 a	1.08 a	29.7 a	30.1 a	30.2 a	28.5 a
Variety												
DP 0912B2RF	5.05 a	4.53 a	4.3 a	4.5 a	1.04 d	1.07 d	1.02 c	1.04 d	28.9 b	29.4 d	29.3 c	27.6 c
DP 104B2RF	4.24 d	3.91 c	3.3 d	3.9 c	1.09 b	1.09 c	1.05 b	1.07 c	30.3 a	30.7 a	31.0 a	29.5 a
DP 1032B2RF	n/t	4.29 b	n/t	4.2 b	n/t	1.11 b	n/t	1.08 b	n/t	29.8 c	n/t	28.2 b
NG 3348B2RF	4.41 c	n/t	3.4 c	n/t	1.06 c	n/t	1.05 b	n/t	29.0 b	n/t	29.9 b	n/t
FM 9160B2F	4.50 b	3.97 c	3.6 b	4.1 c	1.12 a	1.12 a	1.08 a	1.09 a	29.9 a	30.3 b	30.2 b	28.5 b

**Means for a factor with a column followed by the same letter are not different, according to Fisher Protected LSD (P=0.05). Values are the mean of combined trials (there were a total of 2 drip irrigated trials in 2011 and 2012, and 1 pivot irrigated trial in 2011 and 2 in 2012). n/t = not tested, as DP1032B2RF replaced NG 3348B2RF in 2012*

When comparing nitrogen levels within the soil profile, differences were observed at all three locations (Fig. 2). A gradual increase in the amount of residual nitrogen was observed for all four levels throughout the duration of the study. Overall, higher levels of nitrogen were observed from 0-30.5 cm than from lower levels. This accumulation likely resulted from a lack of rainfall to leach nitrates into lower levels of the profile, coupled with the fact that plant roots were grew towards the drip tape, which was found in the lower portions of the profile. Similarly, higher levels of nitrogen were found in the uppermost soil layers; however, there was a sharp decrease in the amount of nitrogen at the end of the study, which may have resulted from the application of overhead irrigation.

Nitrogen is the nutrient most frequently applied to cotton to maximize production. Deficiencies can negatively affect cotton growth, development and yield; whereas, excessive nitrogen can delay maturity, lower fiber quality and increase pest problems. El-Zik [17] suggested that Verticillium wilt severity is positively correlated with nitrogen levels. Furthermore, the amount, form and time of application may influence the development of diseases. In the High Plains of Texas, nitrogen can be applied in a single application prior to planting; however, splitting applications during this time and during squaring typically result in greater use efficiency. Little information exists regarding the influence of nitrate nitrogen on Verticillium wilt development. Wheeler et al. [16] found that the application of irrigation with higher levels of nitrogen in combination with a susceptible variety resulted in greater losses than combinations of partially resistant varieties and irrigation/nitrogen rates. In this study, variability was observed in residual nitrate levels and drought conditions undoubtedly affected nitrogen use efficiency.

3.6 Recovery of *V. dahliae*

Baseline *V. dahliae* populations estimated in 2010 average 1.2, 1.4 and 0.8 microsclerotia cc soil^{-1}for Quaker, New Deal and Halfway, respectively (data not shown). Subtle differences in microsclerotial densities for the different nitrogen treatments were observed at all locations; however, these differences were not correlated with increasing nitrogen rates (Fig. 3) and may have resulted from aggregation of the pathogen within the field. When combined across nitrogen treatments, *V. dahlia* densities increased slightly at the Quaker and New Deal sites, but more than doubled at the Halfway locations, respectively between 2011 and 2012. Recovery of the pathogen from the different soil depths varied at each location for the three years (Fig. 4). At the Quaker location, populations within the soil profile were similar, except at the end of 2011 when differences between the lower and upper most layers were observed. Although not different, the fewest microsclerotia were recovered from the upper layer at the New Deal location all three years. Differences were observed between depthsamong years at the Halfway location. The highest level of microsclerotia were recovered from the 45.7-61.0 cm depth, whereas, fewer propagules were recovered from the top layer. This contradicts results from Ben-Yephet and Szmulewich, [33], where the majority of microsclerotia were recovered from the top 10 cm of soil and densities decreased to almost undetected at 40 cm.

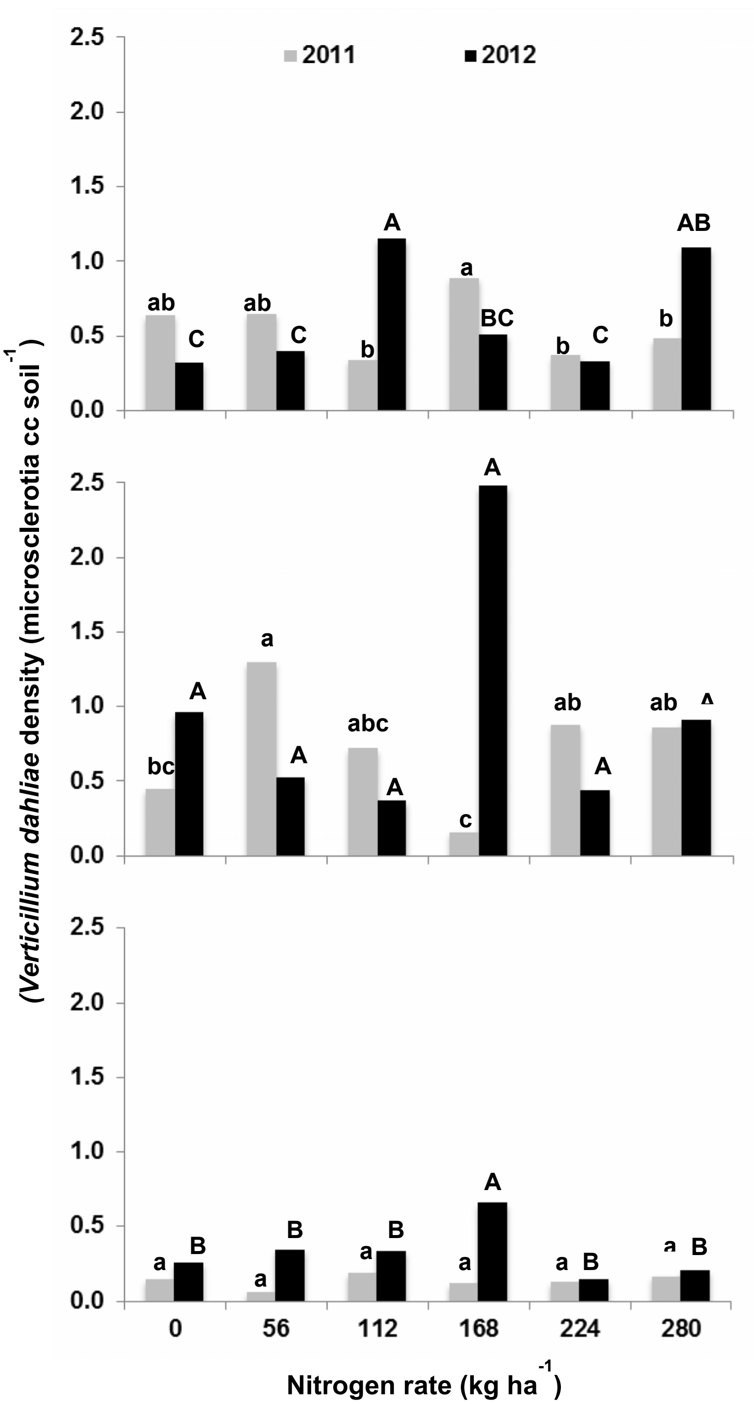

Fig. 2. Densities of Verticillium dahliae microsclerotia from plots treated with nitrogen at Quaker (Top), New Deal (Middle) and Halfway (Bottom) in 2011 and 2012

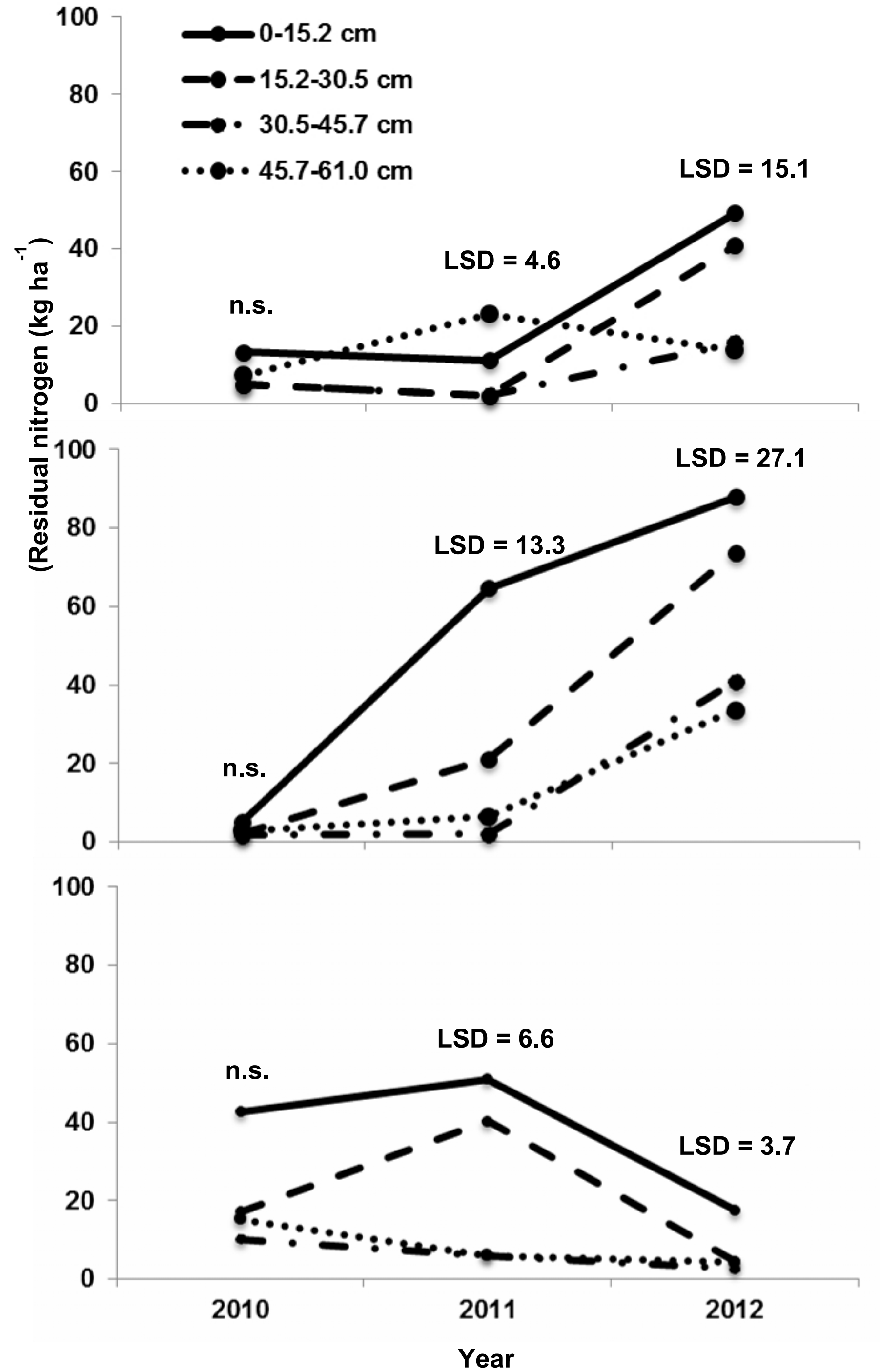

Fig. 3. Residual nitrogen levels from four strata at Quaker (Top), New Deal (Middle) and Halfway (Bottom). Data are pooled across six nitrogen rates (n=24)

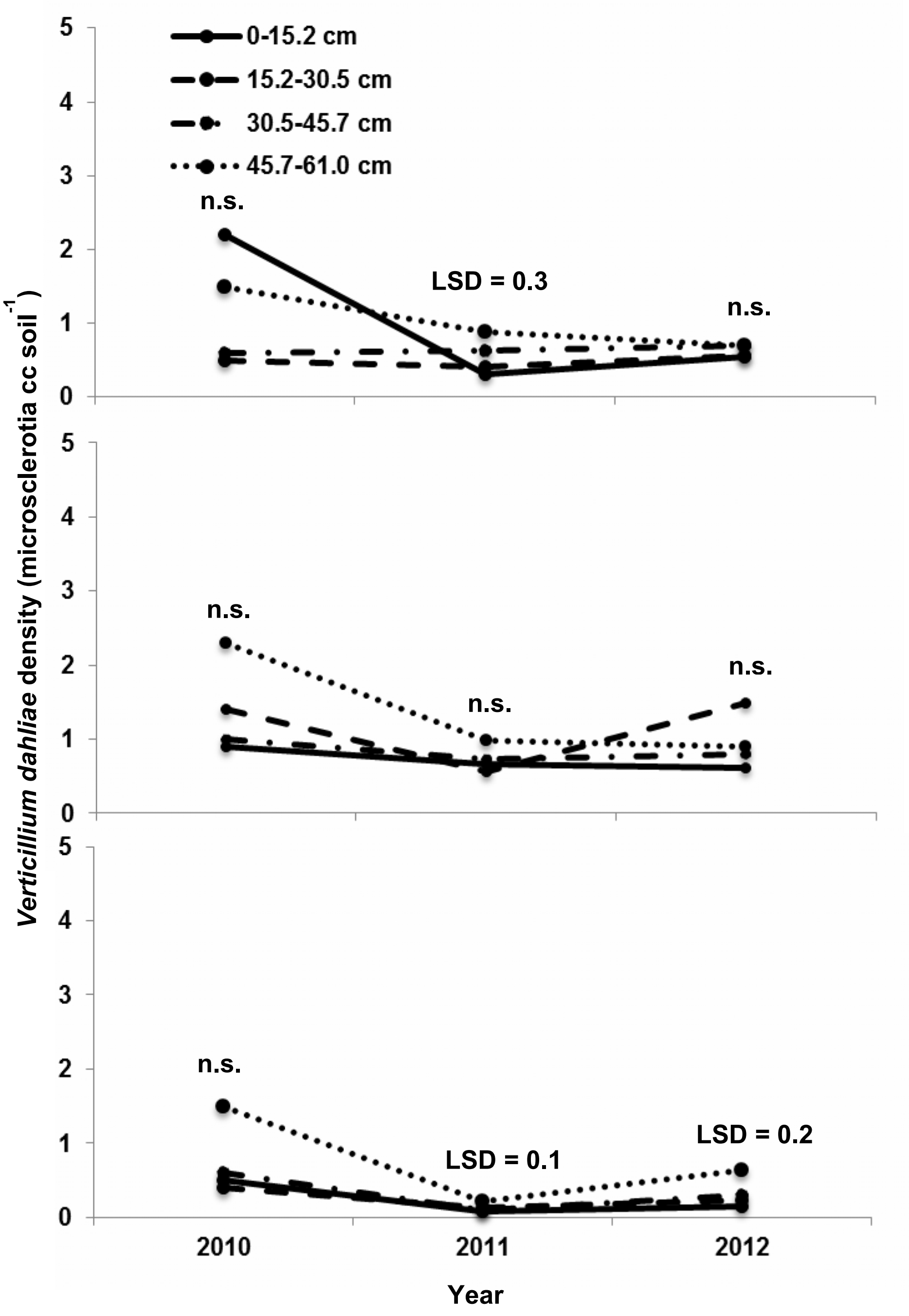

Fig. 4.Vertical distribution of *Verticillium dahliae* microsclerotia at Quaker (Top), New Deal (Middle) and Halfway (Bottom). Data are pooled across six nitrogen rates (n=24)

4. SUMMARY AND CONCLUSION

Verticillium wilt is an increasingly important disease of cotton throughout the High Plains of Texas. Currently, variety selection is the primary means of managing the disease; however, other cultural practices can also be implemented to minimize losses to the disease. This study was initiated to determine the affect of nitrogen fertility on disease development. While adverse conditions were experienced throughout the duration of this study, subtle differences were observed. Disease incidence was greater for susceptible varieties when compared to partially resistant varieties. A slight increase in disease was observed with higher nitrogen rates; however, yield and fiber quality were generally unaffected by the application of nitrogen. Varietal effects were more pronounced than nitrogen effects for most of the parameters evaluated, thus additional studies, under more conducive conditions, are needed to better understand the role nitrogen plays in Verticillium wilt development in cotton. These studies provide the first description of the vertical distribution of *V. dahliae* under different irrigation strategies in cotton on the High Plains of Texas. More work is needed to determine how vertical distribution of the pathogen affects disease development in different soil types.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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