

Article

# EarlySeason Morphological and Physiological Responses of Resistant and Susceptible Cotton Genotypes to Reniform Nematode and Soil Nitrogen

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**Abstract:** Soil fertility and reniform nematode (RN) directly affect earlyseason growth and physiology of cotton. The growth responses to soil fertility and RN may, however, vary across germplasm. A greenhouse study was conducted to gain information on the role that host plant resistance plays in influencing RN populations, and cotton growth and physiological response to a range of soil nitrogen (N) levels in the presence and absence of RN. RN-resistant cotton lines (08SS110-NE06.OP and 08SS100) along with susceptible cultivars (Deltapine 16 and PHY 490 W3FE) were subjected to four levels of N from planting until biomass harvesting, 60 days after planting(DAP), under the presence orabsence of RN. The linear and quadratic functions ( $r^2 = 0.72$  to 0.99) bestdescribed measured responses of cotton genotypes to soil N. However, the responses were not different among genotypes, except for plant height at 30 DAP. This study revealed significant increases in several morphological parameters with increasing rates of N. RN population in the pots grown with resistant lines was lower whencompared to susceptible cultivars at biomassharvest. Physiological responses indicated that 08SS110-NE06.OP was more resilient to RN stress than other genotypes. The information from this study could be useful in managing the early season growth of cotton.

Keywords: cotton; resistance; susceptible; nitrogen; reniform nematode; U.S. Mid-South

# 1. Introduction

Negative fiber yield impacts due to *Rotylenchulusreniformis* (RN) in cotton in the U.S. Mid-South have drawn the attention of scientists aiming to mitigate the problem through modifications in management strategies and host plant resistance. According to a recent National Cotton Council Disease Database report, the percent loss in cotton production from reniform nematode ranged between 1.14% and 2.37% during 2000–2019 in the USA (http://www.cotton.org/tech/pest/nematode/index.cfm), while the average loss in cotton production due to reniform nematode in the states of Mississippi,



Louisiana, and Alabama exceeded 8% during 2000–2019. The commercial nematicide materials such as 1,3-dichloropropene (1,3-D) (Dow AgroSciences LLC, Indianapolis, IN, USA), fluopyram 500 SC (Bayer CropScience LP, Research Triangle Park, NC, USA), abamectin (Syngenta, Greensboro, NC, USA), and AgLogic 15G (AgLogic Chemical, LLC, Gig Harbor, WA, USA) are effective in suppressing reniform nematode populations, which significantly contributes to increased lint yield in treated cotton [1-3]. However, the high cost of nematicides, environmental problems, hazards to human health, and lower efficiency of non-fumigant nematicides being highly dependent upon the soil properties are the major limitations on continuous use of nematicides [4]. Some scientists have evaluated variations in fertilizer management as possible tools to minimize the plant-parasitic nematodes' negative impacts without the additional cost of nematicide application [5–7]. Some studies have reported that fertilization can interfere with the lifecycle of plant-parasitic nematodes, thus influencing their reproduction and pathogenicity on crops [5,8,9]. Past studies have recognized that increasing nitrogen applications can mitigate crop losses by nematodes. For instance, Rodriguez-Kabana and King [10] observed that adding urea above 0.4 g/kg soil with blackstrapmolasses in soil reduced the damage caused by Meloidogynearenaria in Cucurbita pepo. Ronan and Queneherve [9] observed chemotactic responses to ionic compounds for different nematode species and found that ammonium salts and ammonium nitrate were strongly repellent to reniform nematodes. Vestergard [11] identified variable responses of endoparasitic and ectoparasitic nematodes to N-fertilization and showed that N-fertilization encourages the activity of ectoparasites but discourages the activity of endoparasites. Limited reports are available oneconomic analysis for efficacy of the nitrogen on reniform nematode in cottonindicatingnet returns above the direct cost of the nitrogen fertilizerusing the assumption of current input prices and the product price. For instance, the economic analysis by McLeann et al. [12] indicated that value of additional yield increase in reniform nematode-infested cotton fields from the application of anhydrous ammoniais USD 7.45/acre considering the market price of USD 0.50/lb minus the input cost. Thus, an additional nitrogen application has been considered economically worthwhile to reduce reinform nematode damage in cotton. Conversely, several other studies have reported either a positive or no influence of soil nutrition on nematode populations [13,14].

Nitrogen (N) has a marked influence on the early vegetative growth of cotton. Morphological and physiological measurements in cotton are powerful tools to assess cotton response to N-fertilization [15–17]. On one hand, excess nitrogen application can cause succulent seedlings, delayed squaring, and increased insect attacks in the early season [18]. On the other hand, nitrogen deficiency can cause uneven stand and poor seedling growth, leading to increased competition from weeds and increased susceptibility of seedlings to diseases [19,20]. Physiologically, nitrogen deficiency can decrease leaf photosynthesis rates through chlorophyll depletion and reductions in hydraulic conductance by reducing leaf expansion, leading to lower stomatal and mesophyll conductance to gas exchange [21]. These physiological changes interact with each other to alter whole-plant morphology. The changes in early season growth and development of cotton under nitrogen deficiency may include chlorotic leaves, reduced leaf expansion, low plant vigor, early maturity, reduced branching, higher root to shoot ratio, increased cell wall thickening, and accumulation of starch and other carbohydrates, which ultimately limits seed cotton yield [19,21,22]. Therefore, optimum N fertilization is necessary to achieve a uniform and even stand establishment, as well as regulate growth and development in cotton.

Reniform nematode (RN) typically parasitizes the pericycle of a root, which leads to the formation of syncytium [23]. Reniform nematode feeding on seedling roots of cotton can cause several morphological and physiological changes such as stunting, reduced shoot to root ratio, fewer secondary roots, delayed maturity, lower chlorophyll content, reduced leaf reflectance, higher leaf water content, and increased light absorption [24–27]. Plant physiological mechanisms such as the phenylpropanoid pathway produce various secondary metabolites such as anthocyanin and flavonoids [28]. These metabolites are involved in the host plant's resistance to various pathogenic soil microorganisms [28]. For instance, Koti et al. [29] observed a strong negative correlation between cotton leaf phenolics and reniform nematode populations during early season growth. Similarly, Thakar and Yadav [30] confirmed that

increased root phenolics in pigeon pea (*Cajanuscajan*) were associated with resistance to the reniform nematode. Additional reports noted variable growth responses among reniform nematode-resistant and -susceptible cultivars to reniform nematode damage in agronomic crops such as pigeon pea [31] and cotton [27]. While the evaluation of RN-resistant lines has mostly been based on nematode development and fecundity [32–34], assessment of morphological and physiological response to reniform nematode offers another approach to verifying resistance.

As noted above, several current studies are now looking for sustainable approaches such as altering fertilizer rate in conjunction with effective control practices (crop rotation, nematicides, resistance, etc.) to manage nematode damage on crop production [12,14,34–36]. Importantly, novel, RN-resistant cotton lines (08SS110-NE06.OP and 08SS100) have recently been developed, and critical agronomic characteristics were compared with those of RN-sensitive cultivars (concurrent experiment, unpublished data) in the field under high-RN population conditions in the Mississippi Delta. RN-resistant lines exhibited comparable yields but higher RN suppression than sensitive cultivars under the conditions mentioned above. However, a controlled experiment assessing the early season growth response of these cultivars to nematode presence or nitrogen fertility has not been conducted previously. To better understand the interactions between RN and soil fertility on the growth and physiology of resistant and susceptible cultivars, we conducted the study presented herein. The objective of this study was to gain information on the role that host plant resistance plays in influencing reniform nematode populations, and cotton growth and physiological response to a range of soil N levels in the presence of cotton genotypes under the presence of RN.

#### 2. Materials and Methods

This study was conducted in the year 2018 at the Delta Research and Extension Center, Mississippi State University, Stoneville, Mississippi, USA (33°42' N, 90°92' W). This study was conducted for 60 days in a greenhouse maintained at a temperature of  $30 \pm 5$  °C; 60% average relative humidity; and  $\approx 1500 \ \mu mol$  photons m<sup>-2</sup> s<sup>-1</sup> photosynthetically active radiation (PAR) recorded each day using Li-Cor, LI-1400 datalogger (Li-Cor, Lincoln, NE, USA). Sensors were a Li-Cor LI-190 quantum sensor (Li-Cor, Lincoln, NE, USA), a Li-Cor 1000-16 temperature probe (Li-Cor, Lincoln, NE, USA), and a Vaisala HMP50 relative humidity and temperature sensor (Campbell Scientific, Logan, UT, USA). The study consisted of three-factor treatments and 4replications per treatment arranged in acompletely randomized design. Factor A had 4 levels of cotton genotype (2 resistant upland cotton lines (Gossypium barbadense accession GB 713 introgressions 08SS110-NE06.OP and 08SS100), and 2 cotton cultivars, i.e., Deltapine 16 (Delta and Pine Land Company, Scott, MS, USA) and PHY 490 W3FE (Dow AgroSciences LLC, Indianapolis, IN, USA)). Factor B consisted of 4levels of nitrogen (100% of recommended rates, 150% of recommended, 50% of recommended, and base level (0% N)). Factor C consisted of 2levels of reniform nematodes, i.e., presence and absence of nematodes. Four seeds of cotton genotype were planted in plastic pots (3 kg of soil) filled with a steam-treated (70 °C for 8 h) growth media composed of 1 part Bosket very fine sandy loam soil and 2 parts sand. Before planting, soil was sent to a soil testing laboratory (Southern Soils Lab, Yazoo City, MS, USA) for analysis to determine recommended rates of nitrogen. The soil testing report showed that the soil mixture was deficient in N with base-level averaging 1 mg kg<sup>-1</sup> (very low), and the recommended N rate was 134 kg ha<sup>-1</sup> (i.e., 60 mg kg<sup>-1</sup> soil). The recommended rates for N in the soil testing reports were provided in kg ha<sup>-1</sup>, which were converted into mg kg<sup>-1</sup> for our experiment. Commercial urea (46% N) was used as our source for N fertilization. At the time of planting, each replication was fertilized with an appropriate concentration of commercial urea to generate 4different levels of N. To establish 100, 50, and 150% levels of recommended N urea, we applied urea at the rate of 130, 65, and 195 mg kg<sup>-1</sup> soil. Soil test results also showed a deficiency for phosphorus, potassium, magnesium, and sulfur in the soil medium. Triple superphosphate was used at the rate of 43.5 mg kg<sup>-1</sup> to apply recommended rates of phosphorus (20 mg kg<sup>-1</sup>). Muriate of potash was used at the rate of 150 mg kg<sup>-1</sup> to apply

recommended rates of potassium (90 mg kg<sup>-1</sup>). Deficiencies for magnesium and sulfur were amended by applying the recommended amount (66.7 mg kg<sup>-1</sup>) of magnesium sulfate (15% mg and 20% S). All of the fertilizers needed per pot were first weighed separately and then ground into powder form, mixed, and dissolved in 100 mL of deionized water, and finally applied as a solution over the soil surface at the time of planting. Pots were irrigated using a drip irrigation system twice daily for 1 minute at a rate of 1.9 L h<sup>-1</sup> (0.03 L total). Soil moisture was monitored on the basis of water potential by inserting moisture sensors (METER Teros 21 sensors; Meter Group Inc., Pullman, WA, USA) at a depth of 10 cm in 4 representative pots from the 100% N treatment. Once uniform emergence was obtained, plants were thinned to 1seedling per pot. Plants were inoculated with 5000 reniform nematodes (mixed vermiform life stages) in 1 mL of water at the time of emergence for treatment combinations that include nematodes. The nematodes were pipetted into a 5 cm deep hole in the soil near the plant stem. An isolate of reniform nematode collected at Stoneville, MS, and maintained in greenhouse culture on tomato (*Solanumlycopersicon* 'Rutgers') was used.

#### 2.1. Measurements

#### 2.1.1. Physiological Measurements

Physiological measurements were conducted on the uppermost fully expanded leaf at the first true leaf stage, and 30 and 60 days after planting (DAP). The maximum quantum yield of photosystem II (Fv/Fm) was measured using a portable, pulse amplitude-modulated fluorometer (Model OS5p+, Opti-Sciences, and Hudson, NH, USA) on leaves after they had been dark-adapted for a minimum of 8 h. Initially, ground state fluorescence intensity (Fo) was determined in situ during 0400–0600 h using a modulation light intensity of 1 µmol m<sup>-2</sup> s<sup>-1</sup>.Maximal fluorescence intensity (Fm) was then determined using an excitation light intensity of 15,000 µmol m<sup>-2</sup>s<sup>-1</sup> for 0.8 s. Fv/Fm was finally calculated, according to Maxwell and Johnson [36] (Fv/Fm = (Fm–Fo)/Fm).

Gas exchange and light-adapted fluorescence parameters were obtained using a LI-6800 portable photosynthesis system (Li-Cor, Lincoln, NE, USA) on the uppermost fully expanded leaf. These measurements were determined at 10:00–14:00 h with chamber settings at air temperature equal to the greenhouse daytime temperature (30 °C), light levels of 1500  $\mu$ molm<sup>-2</sup> s<sup>-1</sup>, a flow rate of 600  $\mu$ mol s<sup>-1</sup>, the relative humidity of 60%, and sample CO<sub>2</sub> concentration of 400 ppm. Recorded parameters included transpiration rate (E), leaf temperature (Tleaf), the efficiency of photosystem II ( $\Phi$ PSII), and net photosynthesis (A).

The physiological parameters such as leaf pigment content such as anthocyanin index (Anth), epidermal flavanolcontent (FLV), and chlorophyll index (Chl) were determined using a Dualex Scientific+ Chlorophyll and Polyphenol-Meter (Force-A, Centre Universitaire Paris-Sud, France).

#### 2.1.2. Morphological Measurements

At the seedling stage, parameters including the time to 50% emergence and time to first true leaf appearance (FTL) were recorded. Moreover, seedling emergence rate (SER) was calculated as the reciprocal of time to 50% emergence, as described by Reddy et al. [37].

At 30 days after planting(DAP), non-destructive morphological parameters including plant height from soil to main stem apex (PH), mainstem node number (MSN), and leaf thickness (TH) using Outside Micrometer (The Paul N. Gardner Company, Inc., Pompano Beach, FL, USA) were measured.

At the time of final biomass harvesting (60 DAP), measured above-ground growth parameters included plant height from soil to mainstem apex (PH), mainstem node number (MSN), leaf thickness (TH), leaf area per plant (LA) using a table-top leaf area meter (Li-3100, Li-COR Inc., Lincoln, NE, USA), and the number of fruiting structures (FN). Additionally, leaves, stem, and fruiting structures were then harvested and placed in a forced-air dryer oven at 80 °C for 48 h to obtain dry weights (LDW, SDW, and FDW, respectively).

After shoot harvesting, the roots were gently taken from the pots, untangled, washed, measured for taproot length (TRL), and then placed in a forced-air dryer oven at 80 °C for 48 h to obtain root dry weights (RDW). The leaf dry weight, stem dry weight, root dry weight, and fruit dry weight were summed to calculate total dry weight (TDW).

## 2.1.3. Reniform Nematode Population Measurements

Soil sample of 200 g per pot was collected and processed using standard elutriation and sucrosecentrifugation protocols [38–40] for reniform nematode analysis. Vermiform reniform nematodes were counted at a magnification of  $40 \times$  using an inverted microscope. Total reniform nematode population per 200 g soil samples wasdetermined and then converted to reniform nematode population per kilogram soil (RC) for statistical analysis.

## 2.2. Data Analysis

The experimental design was a completely randomized design with 3 factors (4 levels of nitrogen  $\times$  4 levels of genotype  $\times$  2 levels of nematode) and 4replications per treatment. The experiment was repeated, and data from both runs were combined for analysis. Nitrogen, genotype, and reniform nematode treatments were treated as fixed effects, and an experimental run was considered a random effect. Data collected were analyzed using a mixed-effects ANOVA procedure in JMP Pro 12.0 (SAS Institute, Cary, NC, USA). The means were separated using Fisher's protected least significant difference ( $\alpha$  = 0.05). The responses of growth and physiological parameters concerning soil nitrogen were analyzed using linear (Equation (1)) and quadratic (Equation (2)) functions in Sigma-Plot 13 (Systat Software, Inc., San Jose, CA, USA).

$$Y = a + bx \tag{1}$$

$$Y = a + bx + cx^2 \tag{2}$$

where a, b, and c are equation constants and x is the % recommended rate of soil nitrogen.

#### 3. Results

In subsequent sections of the results, we address growth and physiological responses for each sampling period. For brevity, if no significant effects of any treatment or interaction term were observed for a given parameter, the parameter was not presented below. In situations where interaction between treatments was significant, we addressed the interaction rather than the main effects. Thus, the following sections were organized into genotype, nitrogen, and reniform nematode main effects (only when significant and when no interaction waspresent) and significant interactions between treatments (Tables 1 and 2).

Course of Verience	SER	FTL	TH	PH	MSN	TH	PH	MSN	LA	FN	RC	TRL	FDW	RDW	SDW	LDW	TDW
Source of variance				-30 Days	s						60	Days—					
Genotype	**	***	**	NS	**	NS	***	***	*	*	***	*	NS	*	**	*	NS
N	NS	NS	NS	***	***	NS	***	***	***	***	NS	***	NS	***	***	***	NS
Genotype × N	NS	NS	NS	*†	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
RN	NS	NS	NS	NS	NS	*	**	NS	NS	NS	***	**	NS	NS	***	*	NS
Genotype × RN	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	***	NS	NS	NS	NS	NS	NS
$N \times RN$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Genotype $\times$ N $\times$ RN	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

**Table 1.** Analysis of variance across four cotton genotypes, four nitrogen levels, and two reniform nematode levels and their interactions on reniform nematode population and plant morphological parameters measured at 30 and 60 days after planting.

+ The significance levels \*\*\*, \*\*, \*, and NS represent  $p \le 0.001$ ,  $p \le 0.01$ ,  $p \le 0.05$ , and p > 0.05, respectively. Genotypes (G), nitrogen (N), reniform nematode (RN), seedling emergence rate (SER), time to first true leaf (FTL), leaf thickness (TH), plant height (PH), main stem node (MSN), leaf area per plant (LA), number of fruiting structures (FN), reniform nematode population count (RC), taproot length (TRL), weight of fruiting structures (FDW), root dry weight (RDW), stem dry weight (SDW), leaf dry weight (LDW), and total dry weight (TDW).

**Table 2.** Analysis of variance across four cotton genotypes, four nitrogen levels, and two reniform nematode levels and their interactions on physiological parameters at 30 and 60 days after planting.

	Fv/Fm (FLT)	Fv/Fm	Α	Ε	Tleaf	ΦPSI	I Anth	FLV	Chl	Anth	FLV	Chl	Fv/Fm	Α	Ε	Tleaf	ΦPSII
Source of variance						Days—				_			60 Da	ys			
Genotype	NS†	NS	NS	**†	**	NS	NS	***	NS	NS	NS	**	NS	**	**	NS	**
N	**	***†	***	***	**	***	**	***	***	***	NS	***	NS	NS	***	***	*
Genotype $\times$ N	NS	*†	NS	NS	NS	NS	NS	NS	NS	**	NS	**	NS	NS	NS	NS	NS
RN	NS	NS	NS	*	NS	NS	NS	NS	NS	***	NS	***	**	*	NS	*	*
Genotype × RN	NS	NS	NS	NS	NS	*	NS	NS	NS	***	NS	***	**	**	*	NS	**
$N \times RN$	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Genotype $\times$ N $\times$ RN	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

† The significance levels \*\*\*, \*\*, \*, and NSrepresent  $p \le 0.001$ ,  $p \le 0.01$ ,  $p \le 0.05$ , and p > 0.05, respectively. Genotypes (G), four nitrogen (N) levels, reniform nematode (RN), the maximum quantum yield of photosystem II of the first true leaf (Fv/Fm (FTL)), the maximum quantum yield of photosystem II (Fv/Fm), seedling emergence rate (SER), net photosynthesis (A), transpiration rate (E), leaf temperature (Tleaf), chlorophyll content (Chl), the efficiency of photosystem II ( $\Phi$ PSII), anthocyanin index (Anth), and epidermal flavanolcontent (FLV).

## 3.1. Seedling Emergence and First True Leaf

#### 3.1.1. Genotype Effects

There was significant variability (p < 0.01) among genotypes for seedling emergence rate (SER) and time to reach first true leaf (FTL), such that Phytogen 490 W3FE had a significantly lower SER and took longer for FTL than other genotypes (Table 3). However, no effect of nitrogen (N) or reniform nematode (RN) treatments was observed for SER or FTL.

Table 3. Genotype effect on seedling emergence rate (SER) and first true leaf (FTL) of cotton.

Genotype	SER	FTL
	days <sup>-1</sup>	days <sup>-1</sup>
08SS110-NE06.OP	0.013 <sup>a†</sup>	6.37 <sup>b</sup>
08SS100	0.013 <sup>a</sup>	6.25 <sup>b</sup>
Deltapine 16	0.012 <sup>a</sup>	6.13 <sup>b</sup>
PHY 490 W3FE	0.011 <sup>b</sup>	6.89 <sup>a</sup>

<sup>+</sup> Values in a column sharing a letter are not significantly different (p > 0.05) for the genotype treatment effect. Data are means (n = 64 plantsin total for fourreplications and two runs).

#### 3.1.2. Nitrogen Effects

The maximum quantum yield of photosystem II (F*v*/F*m*) was significantly higher at 100% N (0.826) than 0% N (0.814) and 150% N (0.819) (Figure 1). F*v*/F*m* showed a quadratic ( $r^2 = 0.99$ ) response concerning soil N. F*v*/F*m* increased at the rate of 2.59% recommended N rate<sup>-1</sup> for soil nitrogen (Figure 1). There were no significant interaction effects observed for F*v*/F*m* of the first true leaf.



**Figure 1.** Nitrogen treatment effect on the maximum quantum yield of photosystem II of the first true leaf; Fv/Fm (FTL) of cotton. Data are means ± standard error (n = 64 plants total for four four eplications and two runs).

#### 3.2. ThirtyDays Measurements

No effect of RN treatment was observed on morphological parameters at 30 DAP (Tables 1 and 2).

Leaf thickness (TH) of 08SS100 was significantly greater than Deltapine 16 and 08SS110-NE06.OP (Table 4). Mainstem node number (MSN) and flavanol index of 08SS110-NE06.OP was significantly higher than other genotypes across all treatments (Table 4). The leaf temperatures were significantly lower in Phytogen 490 W3FE by 1.3% among other genotypes. The transpiration rates of Phytogen 490 W3FE (0.0083 mol m<sup>-2</sup> s<sup>-1</sup>) were significantly higher than 08SS100 and Deltapine 16, which were not different from each other (Table 4).

**Table 4.** Genotype effect on leaf thickness (TH), main stem node (MSN), transpiration rate (E), leaf temperature (Tleaf), and epidermal flavonol content (FLV) of cotton measured at 30 days after planting.

Genotype	TH	MSN	Ε	Tleaf	FLV
	μm	No. plant <sup>-1</sup>	mol m <sup>-2</sup> s <sup>-1</sup>	°C	Flavonol Index
08SS110-NE06.OP 08SS100 Deltapine 16 PHY 490 W3FE	8.25 <sup>b†</sup> 8.89 <sup>a</sup> 8.44 <sup>b</sup> 8.60 <sup>ab</sup>	5.67 <sup>a</sup> 5.34 <sup>b</sup> 5.22 <sup>b</sup> 5.17 <sup>b</sup>	0.0078 <sup>ab</sup> 0.0073 <sup>b</sup> 0.0073 <sup>b</sup> 0.0083 <sup>a</sup>	29.6 <sup>a</sup> 29.7 <sup>a</sup> 29.8 <sup>a</sup> 29.4 <sup>b</sup>	1.109 <sup>a</sup> 1.013 <sup>b</sup> 0.978 <sup>b</sup> 0.876 <sup>c</sup>

<sup>+</sup> Values in a column sharing a letter are not significantly different (p > 0.05) for the genotype treatment effect. Data are means (n = 64 plantsin total for four plications and two runs).

#### 3.2.2. Nitrogen Effects

Mainstem node numbers showed a quadratic ( $r^2 = 0.97$ ) increase at the rate of 0.04 nodes per percentagerecommended N with increasing soil N levels (Figure 2). The MSN ranged from a maximum of 6.34 nodes per plant in150% N treatment to a minimum of 3.56 nodes per plant in 0% N treatment (Figure 2).



**Figure 2.** Nitrogen treatment effect on main stem node number (MSN) of cotton at 30 days after planting. Data are means  $\pm$  Standard error (n = 64 plants total for four four plications and two runs).

#### 3.2.3. Interaction Effects

Genotype × nitrogen treatmenteffects were observed for plant height in 30-day-old plants (Table 1), such that no differences were observed among genotypes at 100 and 150% N, while 08SS110 had 14% greater PH than Phytogen 490 W3FE at 50% N (Figure 3). Deltapine 16, Phytogen 490 W3FE, and 08SS100 were significantly shorter by 4.1 cm on average than 08SS110-NE06.OP at the lowest N level

(Figure 3). The response of plant height in all the four genotypes to soil nitrogen was best explained by quadratic function ( $r^2 = 0.78$  to 0.99; Figure 3). The rate of increase in PH for 08SS110-NE06.OP was nearly half of the rates for Deltapine 16 and Phytogen 490 W3FE (Table 5). The resistant genotypes showed no difference in  $\Phi$ PSII across RN environments and had  $\Phi$ PSII equivalent to that obtained for susceptible genotypes under no RN pressure, i.e., 0.26 on average (Table 6).



**Figure 3.** Interaction effect of nitrogen and genotype on plant height (PH) of cotton at 30 days after planting. Data are means  $\pm$  Standard error (n = 16 plants in total for four plications and two runs).

**Table 5.** Quadratic equation constants and coefficient of determination for plant height of four cotton genotypes to nitrogen treatment at 30 days after planting.

Genotype	a	b§	С	r <sup>2</sup>
	cm plant <sup>−1</sup>	Recommended Rate, %		
08SS110	20.46 (20.20-20.72)	0.11 (0.10-0.12)	-0.0005 (-0.0006-0.0005)	0.99
08SS100	17.13 (-23.23-61.48)	0.16 (-1.27-1.58)	-0.0007 (-0.0098-0.0084)	0.78
Deltapine 16	16.44 (15.06–17.83)	0.20 (0.16-0.25)	-0.0009 (-0.0012-0.0006)	0.99
PHY 490 W3FE	13.15 (11.68–14.62)	0.22 (0.17–0.26)	-0.0008 (-0.0011-0.0005)	0.99

08SS110-NE06.OP (08SS110). § Rate constant. Values in parentheses indicate 95% confidence intervals. Data are means (n = 16 plants total for four plications and two runs).

**Table 6.** Interaction effect of genotype (G) and reniform nematode (RN) treatments on the efficiency of photosystem II ( $\varphi$ PSII) of cotton genotypes at 30 days after planting.

$\mathbf{G} \times \mathbf{RN}$	φPSII
PHY 490 W3FE, NO	0.2675 <sup>a†</sup>
08SS110-NE06.OP, RN	0.2673 <sup>a</sup>
08SS100, NO	0.2604 <sup>ab</sup>
08SS100, RN	0.2576 <sup>ab</sup>
Deltapine16, NO	0.2495 <sup>abc</sup>
08SS110-NE06.OP, NO	0.2480 <sup>abc</sup>
Deltapine16, RN	0.2436 <sup>bc</sup>
PHY 490 W3FE, RN	0.2334 <sup>c</sup>

<sup>+</sup> Values in a column sharing a letter are not significantly different (p > 0.05) for the interaction effect.Data are means (n = 16 plants in total for four plications and two runs). NO, absence of reniform nematode; RN, presence of reniform nematode.

A significant nitrogen × RN treatment interaction effect was also observed for  $\Phi$ PSII. The highest N level produced the highest  $\Phi$ PSII, and the lowest produced the lowest  $\Phi$ PSII, but the nematode effect was not significant at these rates. However, at 100%, RN presence produced lower efficiencies, and at 50% RN presence produced higher values (Figure 4). A positive and linear increase in  $\Phi$ PSII with increasing N was observed both in the presence and absence of RN ( $r^2 = 0.93$  to 0.96; Figure 4). Moreover, the rate of increase in  $\Phi$ PSII under the presence of RN was not different from RN absence (Table 7).



**Figure 4.** Nitrogen treatment affects the efficiency of photosystem II of cotton under the presence and absence of the reniform nematode. Measurements were taken at 30 days after planting. Data are means  $\pm$  SE (n = 32 plants in total for four plications and two runs).

**Table 7.** Quadratic equation constants, coefficients of determination, and levels of significance for the efficiency of photosystem II of cotton for nitrogen treatment under the presence and absence of reniform nematode (RN) measured at 30 days after planting.

RN	a	b§	$r^2$	<i>p</i> -Value
		Recommended Rate, %		
Absence	0.21 (0.15-0.26)	0.0007 (0.0001-0.0012)	0.93	0.03
Presence	0.19 (0.16-0.24)	0.0007 (0.0003-0.0012)	0.96	0.02

Reniform nematode (RN); § Rate constant. Values in parentheses indicate 95% confidence intervals. Data are means (n = 32 plants in total for four replications and two runs).

#### 3.3. 60Days Measurements

#### 3.3.1. Genotype Effects

Significant effects of genotype were observed for PH, MSN, and LA at the time of final biomass harvest such that the resistant genotype 08SS110-NE06.OP (37.9 cm) had significantly greater height followed by 08SS100 (34.2 cm), Deltapine 16 (31.9 cm), and PHY 490 W3FE (31.5 cm) (Table 8). The mainstem node number was significantly higher in 08SS110-NE06.OP by 13% on average in comparison with theother three genotypes. Resistant genotypes had an averaged leaf area of 355.5 cm<sup>2</sup>, which was greater than the mean leaf area (321.7 cm<sup>2</sup>) for susceptible genotypes. At the time of biomass harvesting, the taproot length (TRL) of Phytogen 490 W3FE was 15.2% smaller than other genotypes. Genotype 08SS110-NE06.OP had significantly greater stem (3.54 g) and root (2.01 g) dry weights than

the other genotypes that had an average stem and root dry weights of 2.85 and 1.73 g, respectively (Table 8). The leaf dry weight of 08SS110-NE06.OP (3.48 g) was significantly greater than susceptible genotypes (2.93 g), but not different from 08SS100 (3.20 g).

 Table 8. Genotype effect on morphological and physiological parameters of cotton at 60 days after planting.

Genotype	PH	MSN	LA	TRL	RDW	SDW	LDW
	cm	No. plant <sup>-1</sup>	cm <sup>2</sup>	cm		g	
08SS110-NE06.OP	37.9 <sup>a†</sup>	8.25 <sup>a</sup>	362 <sup>a</sup>	27.1 <sup>a</sup>	2.01 <sup>a</sup>	3.54 <sup>a</sup>	3.48 <sup>a</sup>
08SS100	34.2 <sup>b</sup>	7.27 <sup>b</sup>	349 <sup>a</sup>	26.4 <sup>a</sup>	1.73 <sup>b</sup>	3.04 <sup>b</sup>	3.20 <sup>ab</sup>
Deltapine 16	31.9 <sup>bc</sup>	7.08 <sup>b</sup>	309 <sup>b</sup>	28.2 <sup>a</sup>	1.76 <sup>b</sup>	2.80 <sup>b</sup>	2.95 <sup>b</sup>
PHY 490 W3FE	31.5 <sup>c</sup>	7.11 <sup>b</sup>	334 <sup>ab</sup>	23.1 <sup>b</sup>	1.71 <sup>b</sup>	2.70 <sup>b</sup>	2.91 <sup>b</sup>

<sup>+</sup> Values in a column sharing a letter are not significantly different (p > 0.05) for the genotype treatment effect. Data are means (n = 64 plants in total for four plications and two runs). Plant height (PH), main stem node number (MSN), leaf area (LA), taproot length (TRL), root dry weight (RDW), stem dry weight (SDW), and leaf dry weight (LDW).

#### 3.3.2. Nitrogen Effects

Plant height, MSN, and LA increased in a quadratic manner ( $r^2 = 0.99$ ) with increasing levels of N (Figure 5A–C). The rate of increases in PH, MSN, and LA with respect to soil N were 0.29 cm per percentagerecommended N, 0.04 counts per percentagerecommended N, and 4.20 cm<sup>2</sup> per percentagerecommended N, respectively. Plant height was significantly greater under nitrogen levels of 150 and 100% N than 50 and 0% N (Figure 5A). The MSN increased from 5.47 nodes  $plant^{-1}$  at 0% N to 8.67 nodes plant<sup>-1</sup> at 150% N with increasing levels of N (Figure 5B). Leaf area increased from 115  $\rm cm^2$  to 507 cm<sup>2</sup> with increasing N levels from 0 to 150% (Figure 5C). Dry weights for various plant components also increased with increasing N levels from 0 to 150% and ranged between 0.65 and 2.49 g for root, 0.82 and 4.72 g for the stem, and 0.96 and 4.75 g for leaf (Figure 6A–C). Root, stem, and leaf dry weights increased at the rate of 0.03, 0.04, and 0.04 g per percentagerecommended N (Figure 6A–C;  $r^2 = 0.98-0.99$ ). The quadratic response of taproot length (TRL) was observed concerning soil N (Figure 7;  $r^2 = 0.99$ ). Taproot length was reduced from 30.8 to 19.2 cm with declines in N from 100 to 0%. The leaf temperature (Tleaf) increased at a linear rate ( $r^2 = 0.99$ ; Figure 8A) with increasing N levels such thatTleaf ranged from 29.9 °C at 0% N level to 30.5 °C at 150% N (Figure 8A).The efficiency of Photosystem II ( $\Phi$ PSII) was also significantly affected by N treatments at the time of biomass harvest (Figure 8B).  $\Phi$ PSII wassignificantly lower at 50% N than other levels of N. Transpiration rates declined linearly ( $r^2 = 0.90$ ) at the rate of  $1.06e^{-5}$  mol m<sup>-2</sup> s<sup>-2</sup> per percentagerecommended N with respect to soil N (Figure 8B).



**Figure 5.** Nitrogen treatment effect on plant height (**A**), main stem node number (**B**), and leaf area (**C**) of cotton at 60 days after planting. Data are means  $\pm$  Standard error (n = 64 plants in total for fourreplications and two runs).



**Figure 6.** Nitrogen treatment effect on root (**A**), stem (**B**), and leaf (**C**) dry weights of cotton at 60 days after planting. Data are means  $\pm$  Standard error (n = 64 plants in total for four plications and two runs).



**Figure 7.** Nitrogen treatment effect on taproot length of cotton at 60 days after planting. Data are means  $\pm$  Standard error (*n* = 64 plantsin total for fourreplications and two runs).



**Figure 8.** Nitrogen treatment effect on leaf temperature (**A**) and transpiration (**B**) of cotton at 60 days after planting. Data are means  $\pm$  Standard error (n = 64 plants in total for four plications and two runs).

Reniform nematode treatment significantly decreased SDW and LDW by 26% and 8.5%, respectively (Table 9), but no effect of RN was observed for RDW at 60 DAP. Further, PH was significantly higher under no nematode pressure (35.1 cm) than the nematode presence (32.6 cm). Reniform nematode presence caused thinner leaves (leaf thickness, TH) at 60 DAPcompared to RN absence. TRL was reduced by 14% under the presence of RN compared to RN absence (Table 9). Reniform nematode also decreased Tleaf compared to no RN (Table 9).

**Table 9.** Reniform nematode effect on morphological and physiological parameters of cotton at 60 days after planting.

RN	Tleaf	TH	РН	TRL	SDW	LDW
	°C	Mm	Cm	cm		g—–
Absence Presence	30.2 <sup>a†</sup> 30.1 <sup>b</sup>	10.20 <sup>a</sup> 9.67 <sup>b</sup>	35.1 <sup>a</sup> 32.6 <sup>b</sup>	28.1 <sup>a</sup> 24.2 <sup>b</sup>	3.26 <sup>a†</sup> 2.67 <sup>b</sup>	3.27 <sup>a</sup> 2.99 <sup>b</sup>

<sup>+</sup> Values in a column sharing a letter are not significantly different (p > 0.05) for the reniform nematode treatment effect. Data are means (n = 32 plantsin total for fourreplications and two runs). Leaf temperature (Tleaf), leaf thickness (TH), plant height (PH), taproot length (TRL), stem dry weight (SDW), and leaf dry weight (LDW).

#### 3.3.4. Interaction Effects

An interaction effect was not observed amongany treatments for any of the morphological parameters, including PH, MSN, and LA, at the time of biomass harvesting (Table 1).

However, significant G × N interactions were observed for anthocyanin and chlorophyll indices at 60 DAP. 08SS110-NE06.OP showed a lower Anth when N was applied above the recommended rate compared to N applied below the recommended rate, while PHY 490 W3FE showed no difference in Anth between 50 and 150% N (Figure 9A). The rate of increase in Anth with respect to soil N was not different among genotypes (Table 10). PHY 490 W3FE showed higher Chl at base level among N levels, with no change between 50 and 150% N levels, while 08SS110-NE06.OP showed stepwise and quadratic increase in Chl with increasing levels of N from 50 to 150% (Figure 9B). Similar to Anth, genotypes were not significantly different for the rate of increase in Chl with increasing N and averaged 0.06 chlorophyll index per percentagerecommended N (Table 11).

A genotype by reniform nematode treatment (G  $\times$  RN) effect was observed for Fv/Fm determined on the uppermost fully expanded leaf at 60 DAP (Table 2). Resistant genotypes showed no differences in Fv/Fm under the presence and absence of RN and averaged 0.78, which was equivalent to mean Fv/Fm obtained for susceptible genotype under the absence of RN (Table 12). However, Fv/Fm for susceptible genotypes was lowered (p < 0.05) in the presence of RN, averaging 0.77, when compared to no nematode pressure (Table 12). The Anth content of susceptible genotypes and resistant genotype 08SS100 significantly increased in the presence of reniform nematode compared to no RN pressure. In contrast, no differences in Anthwere observed under the presence or absence of RN for resistant genotype 08SS110-NE06.OP. However, mean Anth values for 08SS110-NE06.OP was lower than for susceptible genotypes in the presence of RN (Table 12). The Chl content significantly declined in susceptible genotypes and resistant genotype 08SS100 under the presence of RN compared to no RN. In contrast, no differences in Chlwere observed across two levels of RN treatment for resistant genotype 08SS110-NE06.OP. Interestingly, Chl in 08SS110-NE06.OP was comparable to susceptible genotypes and resistant genotype 08SS100 in the presence of RN (Table 12). Reniform nematode presence significantly increased E in 08SS100 by 18% compared to RN absence, while no change was observed in other genotypes (Table 12). Resistant genotypes showed no change in  $\Phi$ PSII across RN environments, while RN presence significantly decreased  $\Phi$ PSII in susceptible genotypes. Phytogen 490 W3FE and Deltapine 16 in RN absence had  $\Phi$ PSII comparable to 08SS110-NE06.OP ( $\approx$ 0.15) followed by 08SS100 (0.13), which was not different from the lowest  $\Phi$ PSII (0.12) obtained for susceptible genotypes under the presence of RN (Table 12).



Nitrogen, % recommended rate

**Figure 9.** Interaction effect of nitrogen and genotype on anthocyanin (**A**) and chlorophyll (**B**) indices of cotton at 60 days after planting. Data are means  $\pm$  Standard error (n = 16 plantsin total for fourreplications and two runs).

**Table 10.** Quadratic equation constants and coefficient of determination for anthocyanin index (Anth) of four cotton genotypes with respect to nitrogen treatment at 60 days after planting.

Genotype	а	b§	c	$r^2$	<i>p</i> -Value
	Anthocyanin Index	Recommended Rate, %			
08SS110	0.19 (0.03-0.36)	0.0054 (-0.0053-0.0054)	-0.00002 (-0.00003-0.00003)	0.87	0.040
08SS100	0.19 (0.17-0.22)	0.0003 (-0.0005-0.0010)	-0.000002 (-0.000007-0.000002)	0.99	0.005
DP 16	0.18 (0.06-0.31)	0.0005 (-0.0035-0.0044)	-0.000005 (-0.00003-0.00002)	0.92	0.030
PHY 490	0.17 (-0.06-0.39)	0.0008 (-0.0065-0.0081)	-0.000004 (-0.00005-0.00004)	0.72	0.010

08SS110-NE06.OP (08SS110), Deltapine 16 (DP 16), and PHY 490 W3FE (PHY 490). § Rate constant. Values in parentheses indicate 95% confidence intervals.Data are means (n = 16 plants total for four plications and two runs).

Genotype	a b§		с	$r^2$	<i>p</i> -Value
	Chlorophyll Index	Recommended Rate, %			
08SS110-NE06.OP	14.03 (2.36-25.69)	-0.04 (-0.41-0.34)	-0.0005 (-0.0019-0.0029)	0.96	0.040
08SS100	14.42 (2.91-25.93)	-0.05 (-0.42-0.32)	-0.0004 (-0.0020-0.0028)	0.87	0.030
Deltapine 16	13.55 (6.57-20.52)	-0.06 (-0.29-0.16)	-0.0006 (-0.0008-0.0021)	0.92	0.020
PHY 490 W3FE	15.23 (-0.12-30.34)	-0.08 (-0.57-0.40)	-0.0004 (-0.0027-0.0035)	0.72	0.040

**Table 11.** Quadratic equation constants and coefficients of determination for chlorophyll index (Chl) of four cotton genotypes to nitrogen treatment measured at 60 days after planting.

§ Rate constant. Values in parentheses indicate 95% confidence intervals. Data are means of 16 replications (n = 16 plants total for four replications and two runs) measured at 60 days after planting.

**Table 12.** Interaction effect of genotype (G) and reniform nematode (RN) treatment effects on reniform nematode population and physiological parameters of cotton genotypes at 60 days after planting.

G×RN	RC	Anth	Chl	Fv/Fm	Α	Е	φPSII
<b>G</b> / <b>R</b> (	nematodeskg-	1			$\mu mol \ m^{-2} \ s^{-1}$	mol m $^{-2}$ s $^{-1}$	
Deltapine16,RN	5306 <sup>a†</sup>	0.20 <sup>ab</sup>	11.95 <sup>c</sup>	0.77 <sup>c</sup>	9.40 <sup>d</sup>	0.0039 <sup>bc</sup>	0.12 <sup>b</sup>
PHY 490 W3FE,RN	5198 <sup>a</sup>	0.22 <sup>a</sup>	9.81 <sup>d</sup>	0.78 <sup>bc</sup>	9.63 <sup>cd</sup>	0.0045 <sup>ab</sup>	0.13 <sup>b</sup>
08SS100,RN	1309 <sup>b</sup>	0.19 <sup>bc</sup>	13.21 <sup>c</sup>	0.78 <sup>ab</sup>	11.20 <sup>bc</sup>	0.0045 <sup>ab</sup>	0.13 <sup>b</sup>
08SS110-NE06.OP,RN	973 <sup>bc</sup>	0.17 <sup>def</sup>	15.47 <sup>ab</sup>	0.78 <sup>bc</sup>	13.06 <sup>a</sup>	0.0051 <sup>a</sup>	0.16 <sup>a</sup>
08SS110-NE06.OP,NO	188 <sup>c</sup>	0.18 <sup>cd</sup>	15.20 <sup>b</sup>	0.78 <sup>bc</sup>	12.23 <sup>ab</sup>	0.0047 <sup>a</sup>	0.15 <sup>a</sup>
PHY 490 W3FE,NO	171 <sup>c</sup>	0.16 <sup>ef</sup>	15.70 <sup>ab</sup>	0.80 <sup>a</sup>	12.37 <sup>ab</sup>	0.0048 <sup>a</sup>	0.15 <sup>a</sup>
08SS100,NO	131 <sup>c</sup>	0.17 <sup>de</sup>	15.45 <sup>ab</sup>	0.78 <sup>abc</sup>	10.26 <sup>cd</sup>	0.0037 <sup>c</sup>	0.13 <sup>b</sup>
Deltapine16,NO	120 <sup>c</sup>	0.15 <sup>f</sup>	17.18 <sup>a</sup>	0.79 <sup>a</sup>	12.20 <sup>ab</sup>	0.0045 <sup>ab</sup>	0.15 <sup>a</sup>

Genotype (G), reniform nematode (RN), reniform nematode population count (RC), anthocyanin index (Anth), chlorophyll index (Chl), the maximum quantum yield of photosystem II (Fv/Fm), net photosynthesis (A), transpiration rate (E), and efficiency of photosystem II ( $\varphi$ PSII) of cotton measured at 60 days after planting. Data are means (n = 8 plants total for four plications and two runs). <sup>†</sup> Values in a column sharing a letter are not significantly different (v > 0.05) for the interaction effect.

Genotypes (G) and G × RN interaction effects were observed for net photosynthetic rate (A) at 60 days after planting (Table 2). Overall, genotype 08SS110-NE06.OP had significantly higher A (12.64  $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) than the other three genotypes (<11  $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>). The susceptible genotypes exhibited a significant decline in A under RN's presence compared to no RN, while A in resistant genotypes remained unaffected by RN (Table 12). Further, A observed for resistant genotypes under RN was comparable to A for susceptible genotypes under no RN. A significant G × RN effect was observed for reniform nematode populations at 60 DAP. The final RN population was 78% lower in pots planted with resistant genotypes compared to pots planted with susceptible genotypesfor treatments that included nematodes. The maximum and minimum counts for RN population at 60 DAP were obtained in Deltapine 16 (5306 nematodes/kg) and 08SS110-NE06.OP (973 nematodes kg<sup>-1</sup>), respectively, for treatments that included nematodes (Table 12).

## 4. Discussion

The present study evaluated the growth and physiological responses of twonovel, RN-resistant cotton lines, 08SS110-NE06.OP and 08SS100, along with two susceptible cultivars (Deltapine 16 and PHY 490 W3FE) to a range of soil N levels in the presence and absence of RN. The reniform nematode (RN) and N fertility affected various morphological and physiological parameters measured during the early season (seedling emergence, first true leaf, 30- and 60-day-old plants). Previous studies mostly focused on either degree of nematode infection or some impact on nematode reproduction in evaluating RN-resistant cotton lines/cultivars, and very little information is available on plant growth responses [32,33]. To our knowledge, this is the first study that has described earlyseason growth and physiological response of novel, RN-resistant cotton lines (08SS110-NE06.OP and 08SS100) to soil N and reniform nematode.

The lowered RN populations observed in the pots with resistant cotton lines compared to susceptible cotton genotypes suggest that resistant cotton lines have the potential to inhibit reniform nematode reproduction. This present study agrees with [32,33], whoreported inhibition of RN reproduction by host plant resistance in upland cotton. Further, no effects of nitrogen treatment on RN population indicate that the reduction in RN population observed was solely due to host plant resistance and not a chemotactic response of urea, as has been reported in previous studies [9,11]. However, we are unsure if the reduced RN pressure contributed to greater early-season vigor in resistant genotypes compared to susceptible genotypes because the interaction effects of genotype by reniform nematode were not observed for cotton morphological parameters. This is in contrast with the results of Anver and Alam [31], who reported less of a decline in growth and development characteristics in resistant cultivars when compared to susceptible cultivars in pigeon pea (*Cajanuscajan*). The species or genus-specific responses to RN damage could be one of the reasons for the contrasting results.

The damage from RN on growth and physiological traits of cotton genotypes were not observed at emergence and 30 DAP, except for  $\Phi$ PSII, whichwas, however, not biologically significant. The present study results gree with past greenhouse studies [24,26,29,31] conducted under the pure RN environment. We observed RN damage such as reduced shoot to root ratio and leaf area andfewer secondary roots in susceptible cotton cultivars at 60 DAP. The damage from RN on cotton included delayed maturity [25] and reduced boll size [40], whichwere also reported late in the growing season. Consistent with thepresent study results, our sister study also observed no difference for growth and development traits until squaring( $\approx$ 50 days after planting) among these genotypes when grown under RN-infested field conditions [41]. Previous studies have reported that responses to RN such as stunting and higher root to shoot ratio were more pronounced on cotton seedlings than at later stages of crop growth and development [42–44], but such symptoms were primarily due to synergistic interaction between RN with other soil-borne pathogens such as *Fusariumsolani* and abiotic stresses such aschilling [45].

The growth and physiological responses of cotton genotypes observed in the present study are in congruence with the cotton response to N fertility [19,20,22] reported in the literature. In addition to nitrogen, growth and physiological traits such as SER (seedling emergence rate), PH (plant height), MSN (mainstem nodes), TRL (taproot length), dry weights of plant components, A (plant photosynthesis), RC (reniform nematode population count), and Fv/Fm (maximum quantum yield of photosystem II) were successfully exploited to determine abiotic and biotic stress tolerance in major agronomic crops during earlyseason growth [46–52]. Although genotypes varied for some morpho-physiological traits at given N levels, growth rates of resistant lines were comparable with elite commercial cultivar PHY 490 W3FE, indicating that novel resistant lines could be selected to accommodate fertilization management strategies for cotton in the U.S. Mid-South upon future testing under field conditions. An interaction effect of N × RN was not observed on growth throughout the study, which suggests that increasing N levels might not be an effective strategy to improve the performance of cotton genotypes used in this study under reniform nematode-infested conditions. The result also supports Elbert et al. [14], whoreported no influence of soil nutrition on nematode damage in cotton under field conditions.

RN × G interaction effects were observed for physiological traits but only on 60-day-old plants. The leaf physiological traits of resistant genotype 08SS110-NE06.OP were not affected by RN presence and had similar values to susceptible genotypes under the absence of RN, which indicates that leaf phenolic compounds, as evidenced by stable anthocyanin index, might be associated with mechanisms to reduce RN reproduction in this genotype. Thus, the building up of anthocyanin index in susceptible genotypes under RN presence further suggests that the pigment might play a role in mitigating damage by RN in cotton. The results support [30], whoshowed a negative correlation between cotton leaf or root phenolic compounds with reniform nematode populations. Interestingly, 08SS100 behaved more like susceptible genotypes in terms of anthocyanin index response to RN × G interaction effect, with values increased under the RN presence when compared to no RN, which suggests that the mode of RN suppression by 08SS100 could be different from 08SS110-NE06.OP. Further, resistant genotype 08SS110-NE06.OP maintained A, Chl, Fv/Fm, and  $\Phi$ PSII under the presence or absence of RN, unlike susceptible genotypes. Thus, leaf

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physiological traits of 08SS110-NE06.OP showed more resilience to RN pressure and simultaneously showed comparable growth rate performance to susceptible cultivars. Future studies should evaluate the resistant lines' agronomic responses under management approaches, linking the intensive agricultural system to the integrated crop-nematode system for sustainable future cotton production.

# 5. Conclusions

Genotypes showed variable morphological and physiological responses to RN treatment at first bloom (60 days after planting). Genotype 08SS110-NE06.OP performed better across all RN levels, possibly by maintaining anthocyanin index, Chl, and E compared to other genotypes. Resistant genotypes inhibited RN reproduction when compared to susceptible genotypes. The increased leaf phenolic compounds such as anthocyanin could be one possible mechanism in 08SS110-NE06.OP to suppress RN population, which is unlikely for 08SS100. Overall, the growth and physiology of the genotypes showed quadratic or linear responses concerning soil N. The genotypes were not significantly different for the rate of change in growth and physiological traits concerning soil N, except for plant height at 30 DAP. There was no relationship between N and RN treatments observed on the basis of the responses of growth parameters determined in this study. The information on growth responses from this study could be useful for future research to identify mechanisms to suppress reniform nematode.

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# Abbreviations

RN, reniform nematode; N, nitrogen; DAP, days after planting; FTL, time to first true leaf; SER, seedling emergence rate; PH, plant height; MSN, mainstem nodes; FN, number of fruiting structures; TH, leaf thickness; LA, leaf area per plant; LDW, leaf dry weight; SDW, stem dry weight; FDW, weight of fruiting structures; TRL, taproot length; RDW, root dry weight; and TDW, total dry weight.

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