Investigation of biotic and abiotic factors contributing to yield loss caused by Cotton leafroll dwarf virus

by

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Abstract

Cotton leafroll dwarf virus (CLRDV) is an aphid transmitted virus that has been reported in cotton producing states across the southeastern United States, initially causing significant yield losses. Yield losses in recent years have not been widespread. Factors contributing to yield losses caused by CLRDV-infection remain unclear and symptomology is not well defined. This research aims to better understand the interactions between certain abiotic and biotic factors, CLRDV, and yield loss. Three studies were conducted to investigate the influence of plant age, nutrient deficiencies, and elevated temperatures on yield loss. Timing of infection only impacted yield in one of three years, but environmental conditions were different each year. Nutrient deficiencies and CLRDV infection caused a significant yield reduction in one year of a two-year study. Symptoms were variable, and the only symptom consistently associated with infected plants was stunted plant height. In the third study, cotton grown under high heat conditions that were infected with CLRDV showed a significant reduction in yield in both years of the study. Taken together, these results show yield loss only occurs under certain environmental conditions and may be exacerbated by plant age at infection and nutrient deficiencies.

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Table of Contents

Abstract 2

Acknowledgments 3

Table of Contents 4

List of Tables 6

List of Figures 7

List of Abbreviations 8

Chapter :1 Literature Review 9

Cotton 9

Cotton Viruses 10

Cotton leafroll dwarf virus 11

Cotton Aphid 12

Age-related Plant Resistance to Pathogens 13

Nutrition 14

Conclusion 16

References 18

Chapter 2: Investigating the Interaction of Plant Age and Timing of Cotton leafroll dwarf virus Infection on Yield Loss 28

Introduction 28

Methods and Materials 30

Results 33

Discussion 35

References 38

Chapter 3: Cotton Leafroll Dwarf Virus Infection and the Effects on Yield Loss and Symptoms 56
Introduction 56
Materials and Methods 57
Data Collection 60
Results 61
Discussion 63
References 66
Chapter 4: Effects of temperature and Cotton leafroll dwarf virus infection in cotton 97
Introduction 97
Methods and Materials 98
Results 101
Discussion 102
References 105

List of Tables

Table 1 Final Virus Incidence in Cage Study Trial 43

Table 2 Means comparisons of average lint quality measurements in Cage Study Trial 44

Table 3 Means comparisons of average plant mapping measurements in Cage Study Trial 46

Table 4 Means comparisons of average plant mapping measurements by year at Cullars 71

Table 5 Means comparisons of average plant mapping measurements by treatment in 2021 at Cullars 72

Table 6 Means comparisons of average plant mapping measurements by treatment in 2022 at Cullars 75

Table 7 Means comparisons of average lint yield by year at Cullars 78

Table 8 Means comparisons of average seed count by year at Cullars 79

Table 9 Means comparisons of average lint quality by year at Cullars 80

Table 10 Means comparisons of average lint quality by treatment in 2021 at Cullars 81

Table 11 Means comparisons of average lint quality by treatment in 2022 at Cullars 83

Table 12 Means comparisons of average plant mapping measurements in Screenhouse Trial ..... …109

List of Figures

Figure 1 Means comparison of average lint yield in 2019, 2020, and 2021 49

Figure 2 Means comparison of average temperature and soil moisture content..... 50

Figure 3 Means comparison of average temperature of Inside versus outside of Cages 51

Figure 4 Means comparison of average PAR of inside versus outside of ages 52

Figure 5 Means comparison of average relative humidity of inside versus outside of cages …53

Figure 6 Means comparison of average soil moisture of inside versus outside of cages 54

Figure 7 Means comparison of average lint yield of control and border rows 55

Figure 8 Examples of symptom severity at Cullars 86

Figure 9 Symptom Severity Rating: Stunting..... 87

Figure 10 Symptom Severity Rating: Red Stem..... 88

Figure 11 Symptom Severity Rating: Red Petiole..... 89

Figure 12 Symptom Severity Rating: Bronzing..... 90

Figure 13 Symptom Severity Rating: Red Leaf Vein..... 91

Figure 14 Symptom Severity Rating: Tenting..... 92

Figure 15 Symptom Severity Rating: Rugosity..... 93

Figure 16 Symptom Severity Rating: Cupping..... 94

Figure 17 Means comparison of average lint yield by treatment at Cullars 95

Figure 18 Means comparison of average seed count by treatment at Cullars..... 96

Figure 19 Means Comparison of average lint yield in Screenhouse Trial..... 110

Figure 20 Means comparison of average seed count in Screenhouse Trial 111

Figure 21 Means comparison of average root weight..... 112

Figure 22 Means comparison of temperature and dewpoint..... 113

List of Abbreviations

CLRDV	Cotton leafroll dwarf virus
°C	Degrees Celsius
L	Liter
cm	Centimeter
m	Meter
ha	Hectare

Chapter 1

Literature Review

Cotton (*Gossypium spp.*) is an important textile fiber that makes up roughly 25% of the world's fiber use (Cotton and Wool 2022). The seed can be used as a food source for livestock (Luttrell et al. 1994), and cottonseed oil can be consumed by people (Chandra Sekhar 2011). The United States is the top cotton exporter, responsible for one-third of the world's raw cotton trade and $21 billion in products and services (Cotton and Wool 2022). Globally, the harvested area of cotton equaled 32 million hectares (Tarazi and Vaslin 2022). Within the United States, Texas is generally the top producer, followed by Georgia and Mississippi (Raper et al. 2020). Alabama alone produced 833,000 bales in 2022 (USDA-NASS). Upland cotton (*Gossypium hirsutum* L.) makes up the majority of the cotton grown in the U.S., although some areas in California, Arizona, Texas, and New Mexico produce Pima cotton (*Gossypium barbadense* L.) (Raper et al. 2019).

Certain environmental factors can influence lint yield. The optimal temperature for cotton growth and development is reported to be between 23.5 and 32 °C (Burke et al. 1988). Elevated temperature has been shown to cause a reduction in yield, boll set, boll size, and seed count (Constable and Bange 2015; Gao et al. 2021; Pettigrew 2008b). Drought stress can also cause a reduction in yield, particularly if stress occurs during the flowering or boll development stage (Gao et al. 2021; Hu et al. 2018; Loka and Oosterhuis 2012).

Besides yield, lint quality is another important aspect of cotton production. The quality of lint generally depends on the variety of cotton planted, environmental factors, and agronomic practices. The value of the lint is influenced by lint quality (Brown and Sandlin 2022; Chohan et

al. 2020) . To measure lint quality, HVI (high volume instrument) or AFIS (Advanced Fiber Information System) can be used. First developed in the 1960's, HVI is used to measure fiber length, micronaire, uniformity, and strength (Negm et al. 2015). Later in the 1980's, AFIS was developed and could measure fiber length, short fiber content, maturity ratio, and fineness (Negm et al. 2015). Larger samples can be measured using HVI, while AFIS can be used for single fiber samples (Calhoun and Bargeron 2997; Negm et al. 2015). While some metrics of lint quality are largely controlled by genetics, the environment can affect lint quality as well (Brown and Sandlin 2022). Fiber length was found to decrease with drought stress, whereas micronaire increased (Hu et al. 2018; Oosterhuis 2000).

Cotton Viruses

In addition to environmental factors, lint yield and quality can be adversely affected by virus infection. The main vectors of cotton viruses are aphids (Hemiptera: Aphididae) and whiteflies (Hemiptera: Aleyrodidae) (Tarazi and Vaslin 2022). One economically significant virus is cotton leafcurl virus (CLuV) (Family: Geminiviridae; Genus: *Begomovirus*). Cotton leafcurl virus was devastating to Pakistan in the early 90's, causing a reported five-billion-dollar loss between 1992 and 1997 (Briddon and Markham 2000). Current management strategies involve the use of resistant cultivars, cultural and chemical practices such as clearing out alternate host plants and chemical control of the whitefly vector (Singh et al. 1999).

The only two viruses reported to infect cotton in the United States are cotton leaf crumple virus (CLCrV) (Family: *Geminiviridae*; Genus: *Begomovirus*), and cotton leafroll dwarf virus (Family: *Solemoviridae*; Genus*: Polerovirus*). Cotton leaf crumple caused significant yield losses in the 1950s and 1960s, but is currently managed through the use of resistant cultivars and the banning of stub cotton (Chohan et al. 2020; Idris and Brown 2004). More recently, cotton

leafroll dwarf virus (CLRDV) was reported in the United States (Avelar et al. 2019). As it is a relatively new virus to the United States, factors influencing yield loss in CLRDV infected plants require further study.

Cotton leafroll dwarf virus

Cotton leafroll dwarf virus is a phloem-limited polerovirus (Family: Solemoviridae) that has been reported globally in South America, North America, and Asia (Avelar et al. 2019; Mukherjee et al. 2012.; Ray et al. 2016.; Silva et al. 2008). In South America, plants showing symptoms of shortened internodes, stunting, leaf rolling, intense green foliage, and yellowing veins, were tested using PCR methods. The virus was identified as a polerovirus and characterized as CLRDV (Corrêa et al. 2005). In susceptible cotton, major yield losses due to CLRDV infection were reported as high as 80% (Silva et al. 2008). However, a resistant cultivar was developed and used to manage disease (Agrofoglio et al. 2017). In 2006, a new strain was discovered and characterized as atypical cotton blue disease. Symptoms of atypical cotton blue disease includes the typical symptoms of CLRDV infection, as well as reddening and withering of the leaves (Silva et al. 2008).

At the end of the cotton growing season in 2017 in the United States, cotton fields in Alabama exhibited virus-like symptoms, and RNA sequencing revealed this was due to CLRDV infection (Avelar et al. 2019). The genetic sequence from isolates in the United States were distinct from the strains found in South America. The symptoms were variable and included leaf curling or rolling, reddening of the stems and petioles, rugosity, shortened internodes and a reduced boll set (Avelar et al. 2019). When CLRDV was first reported in the United States, significant yield losses occurred, estimated at $19 million, but more recently, losses have been

more sporadic, and asymptomatic infections occur (Brown et al. 2020; Lawrence et al. 2019, 2020, 2021, 2022).

The cotton aphid (*Aphis gossypii* Glover) is the only vector reported to transmit CLRDV to cotton. Two other aphid species, *Myzus persicae* Sulzer and *A. craccivora* Koch, have been reported as vectors of CLRDV in India but did not transmit to cotton in the United States (Cauquil J and Vaissayre M 1971; Michelotto and Busoli 2007; Mukherjee et al. 2016; Heilsnis et al. 2023). The cotton aphid transmits CLRDV in a non-propagative, persistent and circulative manner (Heilsnis et al. 2022; Michelotto and Busoli 2007, 2003). Apterous aphids can acquire CLRDV in 30 minutes, inoculate the virus in 45 minutes, and retain CLRDV for 15 days. Alates can acquire CLRDV in 24 hours, inoculate in 24 hours, and retain the virus for 23 days (Heilsnis et al. 2023).

Cotton Aphid

Aphids comprise of a group of roughly 4700 species. Out of that total, 227 species have been found to transmit viruses to plants (Fereres and Moreno 2009). Like other hemipterans, aphids use piercing-sucking mouthparts to feed on sap in vascular tissue. In order to feed, aphids insert their stylet into the plant and pass between cells to reach the target tissue, phloem or xylem. If an aphid has acquired a virus, saliva and virus particles are released as the aphid feeds (Mitchell 2004).

The cotton aphid is reported to transmit over 50 viruses to plants (Im et al. 2022). The aphid can damage the plant indirectly through transmission of viruses, and directly through feeding. Infestations in younger plants can cause cupping and crinkling of the leaves, as well as a reduction in yield (Chen et al. 2018). Honeydew is a sugary substance produced by the aphid,

and infestations occurring after bolls are open can cause lint to become contaminated with honeydew. It makes the cotton sticky which can increase the trash in cotton, decrease efficiency at textile mills, and decrease profits (Slosser et al. 2002). Honeydew on the lint also causes sooty mold, which can stain the fiber, lowering its value (Godfrey et al. 2000). The general lifecycle of the aphid is based on the environment. In warmer areas, the aphid goes through an anholocyclic life cycle, in which the aphid reproduces asexually by thelytokous parthenogenesis year-long because live host plants are available to support populations. In cooler climates aphids will go through a heteroecious or autoecious holocyclic life cycle in which the aphid reproduces sexually and produces eggs that overwinter (Ebert and Cartwright 1997). The optimum temperature for development and reproduction is between 25°C and 30°C. Temperatures above this optimum results in increased mortality, decreased fecundity, and prolonged development (Kersting et al. 1999; Nimbalkar et al. 2010). Aphids are also subject to predation by natural enemies, as well as an entomopathogenic fungus, *Neozygites fresenii* (Nowakowski) Batko. This fungus occurs around mid-July and is capable of greatly reducing aphid populations (Abney et al. 2008).

Cotton aphids exhibit phenotypic plasticity and can produce different sized and colored morphs based on resource availability and environmental conditions. Alates are produced when host conditions decline due to overcrowding or poor host quality so the aphids can disperse to find a new host plant (Ebert and Cartwright 1997). A morph termed “yellow dwarf” can occur too. These aphids are about ¼ to 1/3 of the normal sized aphids and remain pale yellow and small their entire lifespan. Conditions such as high temperatures, overcrowding, and poor host quality can cause the production of yellow dwarfs. It is suggested this occurs because this morph

develops slower and has a lower rate of population increase, therefore reducing damage and nutrient drain on the host plant. (Watt and Hales 1996).

Age-related Plant Resistance to Pathogens

Age-related resistance, or mature plant resistance, is the phenomenon of plants developing resistance to pathogens as they age and develop. This type of resistance often corresponds to different stages of plant development, such as flowering, or vegetative stages, while some types correspond with leaf size, plant age, or synthesis of secondary metabolites or defense compounds (Develey-Rivière and Galiana 2007; Hu and Yang 2019; Panter and Jones 2002; Rankenberg et al. 2021).

Age-related resistance has been documented against other viruses in the Solemoviridae family. One study looking at a resistant line of wheat found that resistance to cereal yellow dwarf virus was not effective if plants were inoculated at the first-leaf stage (Wiangjun and Anderson 2004). The *Bdv1* gene is associated with age-related resistance to leaf rust and yellow rust diseases, as well as barley yellow dwarf virus (Aradottir and Crespo-Herrera 2021). Symptoms of potato leafroll virus developed faster in younger potatoes compared to more mature ones. There was an increase in virus incidence in the younger plants as well (Knutson and Bishop 1964.). Additionally, cotton plants have been reported to demonstrate age-related resistance against two cotton-infecting viruses, CLuV and CLCrV (Brown 1987; El nur and Abu salih 2009).

Nutrition

An important aspect of cotton production is nutrient management. Different macronutrients and micronutrients are essential for cotton to grow and develop, and deficiencies

can cause a variety of symptoms in cotton (Xiao and Yin 2020). One major nutrient required by cotton is nitrogen. As the plant begins to grow, the importance and demand of nitrogen increases (Rinehardt et al. 2006). Nitrogen is a major component of both structural and nonstructural organs, and influences leaf expansion, fruit production and fruit retention (Khan et al. 2017). Deficiency of nitrogen leads to a reduction in chlorophyll and photosynthesis. Since nitrogen is able to relocate in the plant, the symptoms of nitrogen deficiency start at the bottom of the plant in older leaves, and move up the plant to younger leaves (Xiao and Yin 2020). Other symptoms might include a reduction in plant height, shortened petioles, reddening of the leaves, and a decrease in boll production (Xiao and Yin 2020 2020; Rinehardt et al. 2006). Too much nitrogen may lead to excessive vegetative growth, boll rot, delayed maturity and decrease the effectiveness of pesticides (Rinehardt et al. 2006). Excess nitrogen has been shown to increase aphid size, fecundity, and growth rate (Cisneros and Godfrey 2001; Hosseini et al. 2010).

Another essential nutrient for plant metabolism, growth, and development is potassium. Potassium is involved with several key processes such as stomatal opening, photosynthesis, and transport of photosynthates (Pettigrew 2008a). It has been reported to be involved in resistance to abiotic and biotic stress (Oosterhuis et al. 2013). Late-season potassium deficiency has been reported across the cotton belt in more recent years. This deficiency has been attributed to early-season, high yielding cotton varieties. The high-yield varieties have a greater demand for potassium, especially during boll development (Adeli and Varco 2006), as cotton takes up most of the potassium during bloom and boll-filling periods (Pettigrew 2008a). Potassium deficiency can cause a variety of issues in cotton such as a reduction in plant height, boll mass and lint yield. Other symptoms include interveinal chlorosis, necrosis, and bronzing. Excess potassium

can lead to increased boll rot, increased plant height, and delayed maturity (Oosterhuis et al. 2013).

Phosphorus is taken up in the fourth largest amount, behind nitrogen, potassium, and calcium, and is important for root growth and fruit setting, respiration, cell division, and photosynthesis (Arif et al. 2018). A lack of phosphorus lowers photosynthesis, metabolism, and yield (Wang et al. 2018). A phosphorus deficiency can cause stunted growth, reduction in leaf area and number, purpling of the leaves, and may cause chlorosis or necrosis if the deficiency is severe enough (Wang et al. 2018; Xiao and Yin 2020).

A reduction in boll maturation and yellowing of the younger leaves can be a result of a sulfur deficiency (Kamprath et al. 1957; Xiao and Yin 2020). A distinctive symptom is stunted chlorotic growth. The stems are shorter and thinner, and leaf area is reduced, which leads to reduced fruiting. Seedlings can die if the deficiency is severe (Jordan and Ensminger 1959).

A lack a micronutrients such as boron, manganese, iron, copper, zinc, and molybdenum can cause various symptoms such as chlorosis, leaf malformation, or leaf curling. Deficiencies can decrease the number of bolls and yield (Yaseen et al. 2013; Xiao and Yin 2020).

Deficiencies in nutrients can result in increased susceptibility to viruses. Several studies have shown that as potassium levels decreased, susceptibility to CLCuV infection increased. Plants in low potassium treatments had higher incidence and greater symptom severity (Pervez et al. 2007; Ullah Zafar and Athar 2013; Panhwar et al. 2022). Proper management of nutrients in cotton is needed to maximize yield and minimize disease incidence and severity.

Conclusions

It’s important to understand how different abiotic and biotic factors interact with a virus and influence yield loss in crops. With no resistant cotton varieties in the United States, and

management of aphids being an ineffective method to control of CLRDV infection (Mahas et al. 2022), understanding what factors contribute to yield losses caused by CLRDV infection may provide information that will help develop strategies to minimize yield loss in the future. The focus of this thesis aims to better understand how some of these abiotic and biotic factors might interact with CLRDV infection. The second chapter of this thesis investigates the effect of plant age at time of infection on yield loss. The third Chapter examines how various nutrient deficiencies influence yield loss and symptomatology in infected plants. The fourth chapter of this thesis quantifies yield loss between infected and non-infected plants grown in high heat conditions on a per plant basis to more accurately measure the effects of CLRDV infection on yield.

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Chapter 2

Investigating the Interaction of Plant Age and Timing of Cotton leafroll dwarf virus Infection on Yield Loss

Introduction

Cotton leafroll dwarf virus (CLRDV) was first reported in the United States in 2017 when virus-like symptoms were observed in cotton, *Gossypium hirsutum* L., fields in Alabama (Avelar et al. 2019). CLRDV is a phloem limited polerovirus from the family *Solemoviridae* (Parkash et al. 2021). The cotton aphid, *Aphis gossypii* Glover, transmits CLRDV to cotton in a persistent and circulative manner (Heilsnis et al. 2022; Michelotto and Busoli 2007, 2003). Worldwide, CLRDV has been confirmed using RT-PCR in South America, Asia, and North America (Avelar et al. 2019; Mukherjee et al. 2012; Ray et al. 2016; Silva et al. 2008). Within the United States, CLRDV has also been reported in North Carolina, South Carolina, Georgia, Florida, Mississippi, Louisiana, Texas, Arkansas, Oklahoma, and Kansas (Aboughanem-Sabanadzovic et al. 2019; Alabi et al. 2020; Ali and Mokhtari 2020; Ali et al. 2020; Faske et al. 2020; Iriarte et al. 2020; Price et al. 2020; Tabassum et al. 2019; Thiessen et al. 2020; Wang et al. 2020). Symptoms of CLRDV infection are variable, and include stunting, leaf curling or rolling, leaf rugosity, reduced boll set, and shortened internodes (Avelar et al. 2019). In 2017, yield losses of 50,000 bales of cotton valued at $19 million dollars was reported in Alabama (Avelar et al. 2019). Since then, both symptomatic and asymptomatic infections have been detected (Brown et al. 2020; Mahas et al. 2022), but widespread losses have not been reported (Roberts, Conner, Jacobson, personal observation; Lawrence et al. 2019, 2020, 2021, 2022) even

though incidence of CLRDV based on molecular testing can be high in some areas of Alabama and Georgia (Mahas et al. 2022; Sedhain et al. 2021). The variation in apparent yield losses suggest that factors other than infection contribute to yield loss outcomes.

Plant age at time of virus infection is one factor that has been shown to influence disease severity of plant viruses (Hu and Yang 2019). Age-related resistance, which may also be called mature seedling resistance, adult seedling resistance, developmental resistance, or ontogenic resistance, refers to plants becoming less susceptible to disease as the plant ages (Hu and Yang 2019). Age-related resistance often occurs as the plant goes through different developmental stages (Develey-Rivière and Galiana 2007) such as when a plant transitions from the embryonic stage to the juvenile vegetative stage, or from the juvenile vegetative stage to adult vegetative stage (Hu and Yang 2019). Age-related resistance has also been reported to develop during transition to the flowering stage or at the start of senescence (Develey-Rivière and Galiana, 2007). Age-related resistance has been observed for other insect-transmitted cotton viruses, other cotton pathogens, and other crops infected with insect-transmitted viruses. Two whitefly-transmitted viruses, cotton leaf curl virus (CLuV) (Family: Geminiviridae; Genus: *Begomovirus)* and cotton leaf crumple virus (CLCrV) (Family: Geminiviridae; Genus: *Begomovirus*), have been shown to cause greater yield loss in cotton as plant age decreases at the time of infection (El Nur and Abu salih 2009; Brown 1987), and cause an increase in symptom severity when younger plants are infected with CLCrV (Brown 1987). While not a virus, another study found that five day old cotton developed symptoms faster than 12 day old cotton when inoculated with the fungus *Rhizoctonia solani* (Hunter et al. 1978).

This study was conducted to examine whether age-related resistance influences yield losses caused by CLRDV infections in cotton. The objective of this experiment was to inoculate

cotton at different growth stages to compare the effect of plant age at the time of CLRDV infection on yield. We hypothesized that the younger the plants are when they are infected with CLRDV, the greater the yield loss.

Materials and Methods

A three-year small plot field experiment was conducted from 2019 to 2021 at the E.V. Smith Agricultural Experiment Station in Shorter, Alabama. Infection timing was controlled in small plots by manually infesting plants with viruliferous aphids maintained on CLRDV-infected cotton in the greenhouse. Aphids used for the experiment were from an *A. gossypii* colony maintained in a greenhouse and originally collected from a cotton field in Tallassee, AL, in 2019. Aphids were reared on one to two true-leaf 'DP1646' (DeltaPine®, Dekalb Genetics Corporation, Dekalb, IL) seedlings. Each week two adults were transferred to new seedlings and allowed to reproduce for one week before repeating the process. This also enabled a large number of wingless adult morphs being available for experiments and minimized production of winged aphid morphs in the colony. Wingless adult morphs were preferred because winged adult morphs are less prone to settle and colonize on plants due to their dispersal behaviors (Johnson 1958). Infected plants were collected from a field in Tallassee, AL, in 2018, and infected plants were maintained in the greenhouse with annual aphid transmission as described in Heilsnis et al. (2022). Infected plants were tested each spring for CLRDV through RT-PCR (Mahas et al., 2022) to confirm infection status before experiments began. Viruliferous aphids used in the field experiments were generated by infesting CLRDV-infected source plants with aphids for three to seven days prior to infesting experimental plots; allowing for a minimum acquisition access period of 72 hours to maximize transmission rates (Michelotto and Busoli 2007, 2003).

Research plots consisted of two-rows x 6.10m of ‘DP1646’ cotton without an insecticide seed treatment. Plots were planted on 5/30/2019, 5/26/2020, and 5/18/2021. The day after planting, plots were covered with insect-proof cages to exclude insects and confine aphids released into plots. The cages were constructed by stretching mesh 50 anti-insect screen (Green-Tek, Baldwin, GA) over a PVC pipe (2.50 cm diameter) frame (622.30cm L x 142.24cm W x 129.54cm D). A Hobo RX3000 data logger with sensors that measured PAR (photosynthetically active radiation), relative humidity, temperature, and soil moisture content (Onset Computer Corporation, Bourne, MA) were used to monitor environmental conditions inside and outside of the cages. EC5 Soil Moisture Smart Sensors were installed at a 15.24 cm and 30.38 cm depths (Onset Computer Corporation, Bourne, MA). Two data loggers were placed between the two rows of cotton inside two cages. A third data logger with sensors was placed between two border rows of cotton outside of the cages; four border rows of cotton were planted along two sides of the research plots, parallel to the caged plots/block design and planted the same date, with the same variety.

To examine the influence of age-related resistance on yield, six treatments were arranged in a randomized complete block design with four replications: no aphids released (control), release of nonviruliferous aphids (aphid control), viruliferous aphids released one week after emergence at the cotyledon stage (week 1 release), viruliferous aphids released two weeks after emergence at the one to two true-leaf stage (week 2 release), viruliferous aphids released three weeks after emergence at the three to four true-leaf stage (week 3 release), and viruliferous aphids released four weeks after emergence at the five to six true leaf stage (week 4 release). Weekly releases were conducted over four weeks in 2020 and 2021; in 2019 there were only three release dates, beginning at the one to two true leaf growth stage (week 2 release - week 4

release). Due to space constraints and colony rearing limits, we could not replicate non-viruliferous aphid releases on each of the four weekly release dates. Therefore, one of the four plots in the aphid control treatment were infested each week from emergence to the five to six true-leaf growth stage.

CLRDV was transmitted to plots by placing aphid infested leaves from CLRDV infected source plants onto every healthy cotton plant in cages to minimize handling effects on aphid mortality, and to facilitate colonization and transmission of virus throughout each plot. One week after aphids were released (seven-day inoculation access period), plants were sprayed with 1.02 L/ha flupyradifurone (Sivanto® Prime, Bayer CropScience LP, St. Louis, MO) to kill aphids. Plants in each caged plot were inspected weekly until cages were removed. First, we checked plots that had not been infested to ensure that unintended infestations did not occur in the cages, and plots sprayed the week before to ensure aphids were killed. Then we monitored aphids in cages where we infested to make sure that aphids colonized every plant.

Cages were removed approximately eight weeks after planting when cotton growth reached the top of the cage on 7/23/2019, 7/21/2020, and 7/22/2021. Cage removal corresponded to approximately two weeks after the last aphids were released into cages, and after natural infestations of aphids in the area had crashed due to natural epizootics of entomopathogenic fungi. Season-long management was conducted according to standard local recommendations (Integrated Pest Management Guides - Alabama Cooperative Extension System 2021).

Final CLRDV incidence was determined by individually testing ten randomly selected plants from each plot on 9/13/2019, 9/14/2020, and 9/22/2021 with RT-PCR (Mahas et al., 2022). Lint was harvested from all plants in both rows of each plot on 10/24/2019,10/27/2022, and 11/17/2021-11/19/2021. A JD 9920 cotton picker (John Deere, Moline, IL) was used to

harvest the lint in 2019, and a Case IH 2555 cotton picker (Case Corporation, Racine, WI) was used in 2020. Lint was hand harvested in 2021 due to weather and equipment issues. Seed cotton yield for each plot was weighed after harvest, and plot samples were sent to the University of Georgia Microgin (Tifton, GA) for processing (Li et al. 2011). Fiber samples were sent to the USDA Classing Office in Macon, GA for the 2019 season and the USDA Classing Office in Memphis, TN for the 2020 and 2021 growing seasons to obtain HVI measures of fiber length, strength, uniformity, and micronaire (United States Department of Agriculture-Agriculture Marketing Service 2001).

Data were analyzed using the GLIMMIX procedure in SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA). Lint yield and quality were compared among treatments in separate analyses with lint yield, fiber length, micronaire, uniformity, or strength as the dependent variable, and treatment as the independent variable. Block was run as a random effect when preliminary analyses showed no significant differences between blocks. Weather data were analyzed separately to compare the average temperature, soil moisture, PAR, relative humidity, and dew point (dependent variables) among years (independent variable) for caged plots. Means comparisons were performed among treatments using Tukey's method at P=0.05 level.

Results

CLRDV Incidence

In the field experiments CLRDV was detected in all plots (Table 1). A higher proportion of plants tested positive in the plots where viruliferous aphids were released (56-98%) compared to the control (2-58%) and aphid only control plots (10-78%), except in 2021 when there were no statistical differences among the aphid only and week 1-4 release treatments. Overall, these results suggest CLRDV was successfully transmitted to caged plants during the prescribed time

treatment, and additional virus spread occurred late-season after cages were removed because unintended infestations were not observed while plots were caged. The magnitude of natural mid- to late-season spread was evident in the Aphid Control and Control plots, and was variable among years.

Lint Yield and Quality

There was a significant effect of treatment on yield in one year of this study (Fig. 1). In 2019, week 2 release plots had significantly lower lint yield (1402.65 kg ha-1) than the control treatment (2035.42 kg ha-1). While not statistically significant, there was a numerical trend across the three release dates, with the week 2 treatment having the lowest yield and week 4 treatment having the highest yield (1818.73 kg ha-1) of the three weekly inoculation treatments (Fig. 1A; $F_{4,13.04}$=1.50, *P*=0.2598). In 2020 and 2021, there were no significant differences in yield among any treatments (Fig. 1B; $F_{5,17}$=0.09, *P*=0.9929 and 1C; $F_{5,15}$=0.41, *P*=0.8351).

Length, micronaire, uniformity, and strength per plot were compared among treatments in separate analyses. In 2019 and 2021 there were no significant differences among treatments observed for any lint quality metric (Table 2). In 2020, fiber length of the control treatment was significantly greater than all other treatments, and micronaire was significantly greater in the week1-4 release treatments compared to the aphid control treatment (Table 2).

Cage-Related and Environmental Effects

The environment inside of the cages differed from the environment outside of the cages. The caged environment was significantly warmer (Fig 3), and had reduced PAR (Fig 4) and reduced relative humidity (Fig 5) compared to the environment outside of the cages. However, soil moisture measured at the 15.24 cm depth showed no significant differences between the caged environment and the environment outside of the cages (Fig 6). Compared to the border

rows, cotton generally grew taller in the cages until they were removed, but by harvest there were no visual differences between cotton in caged plots and cotton in the four border rows (Schlarbaum and Jacobson, personal observation). There were no significant differences in yield between the border rows and control in 2019 and 2021, but there was a significant reduction in yield in the border rows compared to control in 2020 (Fig 7). Although the cages did alter the environment, they did not appear to exert any effect that decreased expected yield in these plots.

Abiotic variables among years were also examined to determine variation among the three years of this trial. The environmental conditions among these three growing seasons were significantly different. The average temperature in the caged plots from May to July was significantly higher in 2019 (28.42 °C) than in 2020 (26.5 °C) and 2021 (25.97 °C) (Fig. 2A; $F_{2,281}$=22.03, *P*=0.0001). Each year also had significantly different soil moisture content; 2019 had the lowest soil moisture content (0.09 m^3/m^3) and 2021 had the highest soil moisture content (0.21 m^3/m^3) (Fig. 2B; $F_{2,281}$=103.21, *P*=0.0001). There were no significant differences in PAR ($F_{2,136}$=1.93, *P*=0.1489) and relative humidity ($F_{2,143}$=0.38, *P*=0.6824) across the three years of the trial

Discussion

This is the first study that examines whether age-related resistance can help protect cotton plants from yield loss caused by CLRDV. In this study, there were significant differences in lint yield by treatment in 2019, but not in 2020 or 2021 (Fig. 1). Although other cotton viruses have been shown to reduce lint quality (Monga and Sain 2021), variables associated with quality were only affected in one year. The biological significance of the observed differences is unclear, and quality was not different among treatments in 2019 when yield differences occurred (Table 2). Therefore, these are likely due to other environmental variables not measured in this study. High

CLRDV incidence was achieved by infesting plots with viruliferous aphids, and infested plots generally had statistically similar levels of incidence. Assuming the levels of incidence accurately reflected the true incidence among the plants in each plot, variation in CLRDV incidence should not have significantly influenced the results.

Yield was the primary indicator of disease we used in this study because there is a lack of defined symptomatology for CLRDV. To better understand any yield loss that may occur, plant mapping was also performed on plants tested for CLRDV in each plot after cutout in all three years, but there were few differences among treatments in plant height, total number of nodes, retention of first and second position bolls, and the sum of retention for first and second position bolls (Table 3). The only significant differences by treatment occurred in the 2019 week 2 treatment (one to two true-leaf growth stage) when yield was significantly lower than the control. The plants in the week 1 treatment were also significantly shorter, and retention of first position bolls was higher. This suggests that the reduction in yield observed in 2019 was due to a reduction in lint produced per boll, not a reduction in the number of first and second position bolls per plant. At the time plant mapping occurred there were no apparent symptoms or visual differences among plots (Schlarbaum and Jacobson, personal observation).

The variation in yield losses and symptomatology reported for CLRDV in the U.S. (Avelar et al. 2019; Brown et al. 2020; Mahas et al. 2022) suggest that environmental effects may strongly influence the expression of disease. If specific CLRDV x environment interactions are required for yield loss outcomes, those conditions might not have been replicated among years in this trial because both temperature and precipitation were significantly different among all three years (Fig. 2). The conditions in 2019 were significantly warmer and drier compared to the other two years, and the 2019 plots experienced visible heat and drought stress while the trial

was ongoing. Other studies have found that temperature and/or drought stress can cause a reduction in lint yield and reduce the number of bolls produced (Constable and Bange 2015; Gao et al. 2021; Hu et al. 2018). The environment is an important part of the disease triangle and may be another source of variation in lint yield between the years. The influence of specific environmental variables should be examined in future studies on CLRDV.

CLRDV detection in control plots also indicated that mid- to late-season virus spread occurred in addition to the inoculations performed with the caged plants. How multiple transmission events alters disease caused by CLRDV is unknown, but if this were a factor in the study it should have exerted a larger effect the two years no yield loss was observed because more late season virus spread occurred (Table 1, Fig. 1). Currently there is no way to prevent virus spread without mechanically blocking the plants from aphids because frequent insecticide application does not eliminate aphid infestations or reduce virus spread (Mahas et al. 2022). Due to logistical constraints aphids were not specifically monitored after cages were removed, but no infestations were observed during regular scouting activities.

CLRDV is a recently discovered cotton virus in the United States, and little is known about what factors may influence yield loss in infected plants. Plant age at the time of infection is one factor that may influence yield loss in CLRDV infected plants, but was only observed when plants were inoculated within two weeks after emergence in one year of the three-year study. This indicates multiple interactions between the pathogen, plant, vector, and environment are likely contributing to yield loss outcomes. Drought and heat stress were also observed the year yield loss occurred and should be investigated in future studies along with other biotic or abiotic factors that could promote plant stress and impact yield.

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	Final Virus Incidence		
	2019	**2020**	**2021**
Treatment			
Aphid Control	0.10 (0.05) c	0.07 (0.04) c	0.78 (0.07) bc
Control	0.02 (0.02) c	0.24 (0.07) d	0.58 (0.08) c
Week 1	N/A*	0.76 (0.07) b	0.81 (0.06) ab
Week 2	0.56 (.08) a	0.84 (0.06) ab	0.86 (0.06) ab
Week 3	0.98 (0.02) b	0.96 (0.03) a	0.95 (0.03) a
Week 4	0.73 (0.07) ab	0.91 (0.04) ab	0.86 (0.06) ab
Significance of Main Effects			
Treatment	$F_{4,190}$=12.31, P<0.0001	$F_{5,231}$=14.47, P<0.0001	$F_{5,231}$=3.40, P<0.0055
Block	$F_{3,190}$=4.12, P=0.0073	$F_{3,231}$=2.47, P=0.0624	$F_{3,231}$=1.40, P=0.2427

*Plots were not infested before the one to two true-leaf stage in 2019.

Table 1: Final cotton leafroll dwarf virus incidence reported as the average proportion (±standard error) of plants infected per plot. Virus testing using RT-PCR was performed on ten random plants per plot. Means comparisons were performed using Tukey's method at P=0.05.

Treatment	Length (mm)	Micronaire	Uniformity (%)	Strength (grams per textile)
2019				
Aphid Control	1.21 (0.01)	4.56 (0.17)	82.46 (0.69)	31.73 (0.45)
Control	1.22 (0.01)	4.62 (0.14)	83.24 (0.58)	31.98 (0.39)
Week 2	1.21 (0.01)	4.52 (0.14)	83.14 (0.58)	31.70 (0.39)
Week 3	1.21 (0.01)	4.65 (0.14)	83.04 (0.58)	31.80 (0.39)
Week 4	1.22 (0.01)	4.60 (0.14)	82.64 (0.58)	32.20 (0.39)
Significance of Main Effects				
Treatment	$F_{4,14}$=0.42, *P*=0.7918	$F_{4,13.07}$=0.45, *P*=0.7726	$F_{4,13.06}$=0.97, *P*=0.4549	$F_{4,14}$=0.28, *P*=0.8885
2020				
Aphid Control	1.23 (0.01) b	4.03 (0.05) a	82.14 (1.67)	30.93 (0.23)
Control	1.29 (0.01) a	4.08 (0.05) ab	82.72 (1.67)	30.65 (0.23)
Week 1	1.25 (0.01) b	4.23 (0.06) bc	82.67 (1.82)	30.42 (0.27)
Week 2	1.25 (0.01) b	4.25 (0.05) c	82.54 (1.67)	30.38 (0.23)
Week 3	1.24 (0.01) b	4.18 (0.05) bc	82.02 (1.67)	30.70 (0.23)
Week 4	1.25 (0.01) b	4.20 (0.05) bc	82.19 (1.67)	30.95 (0.23)
Significance of Main Effects				
Treatment	$F_{5,17}$=6.20, *P*=0.0019	$F_{5,17}$=2.99, *P*=0.0410	$F_{5,16.01}$ =0.13, *P*=0.9822	$F_{5,14}$=1.02, *P*=0.4419
Block				$F_{5,14}$=7.84, *P*=0.0026
2021				
Aphid Control	1.22 (0.01)	3.53 (0.10)	83.03 (0.26)	31.35 (0.43)
Control	1.24 (0.01)	3.83 (0.10)	83.45 (0.26)	31.83 (0.43)

Week 1	1.23 (0.01)	3.83 (0.10)	83.40 (0.26)	32.18 (0.43)
Week 2	1.23 (0.01)	3.58 (0.10)	83.23 (0.26)	32.20 (0.43)
Week 3	1.24 (0.01)	3.93 (0.10)	83.93 (0.26)	31.65 (0.43)
Week 4	1.23 (0.01)	3.80 (0.10)	83.30 (0.26)	32.00 (0.43)
Significance of Main Effects				
Treatment	$F_{5,18}$=1.11, P=0.3895	$F_{5,18}$=2.67, P=0.0566	$F_{5,18}$=1.49, P=0.2506	$F_{5,18}$ =0.58, P=0.7136

Table 2: The average (±standard error) of lint quality measurements per plot was compared among treatments using Tukey's method at P=0.05.

Treatment‡	Plant Height (inches)	Total Number of Nodes	1st Position Bolls	2nd Position Bolls	Sum of 1st and 2nd position bolls
2019					
Aphid Control	45.34 (1.01) a	19.37 (0.41)	7.17 (0.51) ab	5.04 (0.56)	11.84 (0.98)
No Aphids	47.77 (2.14) a	19.78 (0.87)	6.56 (1.08) ab	5.34 (1.19)	11.71 (2.08)
Week 2	41.15 (0.67) b	19.01 (0.27)	8.44 (0.34) a	4.83 (0.37)	13.18 (0.65)
Week 3	43.67 (2.14) ab	20.31 (0.87)	6.52 (1.08) b	3.81 (1.19)	10.24 (2.08)
Week 4	44.61 (0.73) a	19.09 (0.30)	6.99 (0.37) ab	4.18 (0.42)	11.00 (0.71)
	Significance of Main Effects				
Treatment	$F_{4,185}$= 5.54, *P*=0.0003	$F_{4,185}$= 0.72, *P*=0.5796	$F_{4,185}$= 1.85, *P*=0.0243	$F_{4,174}$= 0.69, *P*=0.5967	$F_{4,185}$= 1.52, *P*=0.1993
Block	$F_{3,185}$= 18.58, *P*=0.0001	$F_{3,185}$= 9.62, *P*=0.0001	$F_{3,185}$= 0.75, *P*=0.5258	$F_{3,174}$= 3.50, *P*=0.0168	$F_{3,185}$= 2.28, *P*=0.0806
2020					
Aphid Control	57.01 (1.47)	23.19 (0.58)	10.73 (0.67)	8.08 (0.81)	18.79 (1.38)
No Aphids	59.27 (0.72)	21.77 (0.29)	10.09 (0.33)	7.27 (0.40)	17.16 (0.68)
Week 1	59.03 (0.89)	21.41 (0.35)	9.60 (0.41)	6.21 (0.51)	15.42 (0.84)
Week 2		22.39 (0.40)	10.27 (0.46)	7.56 (0.56)	17.82 (0.95)

	60.70 (1.01)				
Week 3	62.09 (1.80)	22.08 (0.71)	9.05 (0.83)	6.54 (0.99)	15.41 (1.69)
Week 4	59.46 (1.26)	21.61 (0.50)	9.40 (0.58)	6.86 (0.70)	16.20 (1.19)
Significance of Main Effects					
Treatment	$F_{5,233}$= 1.35, *P*=0.2435	$F_{5,233}$= 1.79, *P*=0.1165	$F_{5,233}$= 0.96, *P*=0.4428	$F_{5,227}$= 1.16, *P*=0.3307	$F_{5,233}$= 1.42, *P*=0.2188
Block	$F_{3,233}$= 6.44, *P*=0.0003	$F_{3,233}$= 2.34, *P*=0.0745	$F_{3,233}$= 2.62, *P*=0.0514	$F_{3,227}$= 0.89, *P*=0.4450	$F_{3,233}$= 2.12, *P*=0.0981
2021					
Aphid Control	44.24 (1.28)	24.74 (0.53)	7.82 (0.48)	4.63 (0.50)	11.28 (0.89)
No Aphids	40.43 (1.10)	23.54 (0.45)	7.09 (0.41)	3.86 (0.49)	9.61 (0.77)
Week 1	43.89 (1.40)	23.40 (0.58)	7.91 (0.56)	4.12 (0.54)	10.50 (0.98)
Week 2	41.42 (1.50)	23.19 (0.62)	6.36 (0.56)	2.72 (0.52)	8.81 (1.05)
Week 3	44.96 (2.43)	24.32 (1.00)	8.03 (0.91)	3.20 (0.85)	10.94 (1.70)
Week 4	42.35 (1.51)	24.16 (0.62)	7.47 (0.56)	4.45 (0.58)	11.62 (1.06)
Significance of Main Effects					
Treatment	$F_{5,218}$= 1.60, *P*=0.1622	$F_{5,212}$= 1.10, *P*=0.3615	$F_{5,216}$= 1.23, *P*=0.2968	$F_{5,173}$= 1.82, *P*=0.116	$F_{5,218}$= 1.15, *P*=0.3345

Block	$F_{3,218}$= 8.62, *P*=0.0001	$F_{3,212}$= 5.71, *P*=0.0009	$F_{3,216}$= 4.24, *P*=0.0061	$F_{3,173}$= 2.86, *P*=0.0385	$F_{3,218}$= 4.80, *P*=0.0029

Table 3. The average (±standard error) of plant mapping measurements from 10 random plants per plot performed after cutout on 9/13/2019, 9/18/2020, and 9/22/2021. Data were analyzed using GLIMMIX procedure in SAS (version 9.4). Plant mapping data were compared among treatments in separate analyses for plant height, total number of nodes, retention of first position bolls, retention of second position bolls, or the sum of first and second boll retention as the dependent variable, and treatment as the independent variable. Block was run as a random effect if preliminary analyses showed no significant differences between blocks. Means comparisons were performed among treatments using Tukey's method at *P*=0.05.

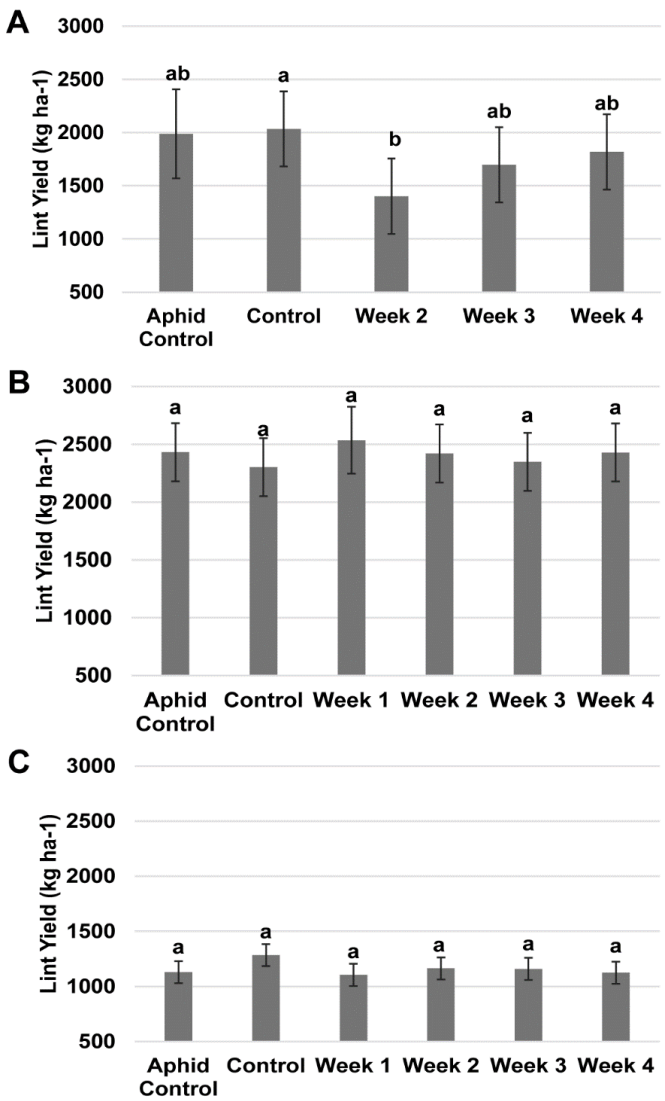

Figure 1: The average (±standard error) of lint yield (kg ha-1) in (A) 2019, (B) 2020, and (C) 2021. Means comparisons among treatments were performed using Tukey's method at *P*=0.05

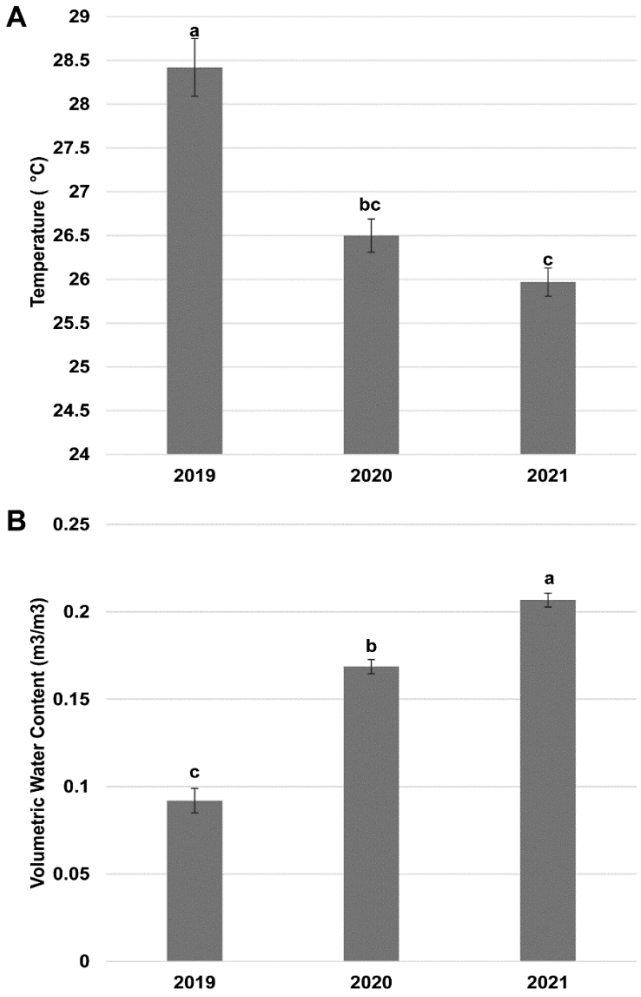

Figure 2: (A) The average (±standard error) of (A) temperature and (B) soil moisture measured at a 15.24 cm depth inside of caged plots between May and July. Data were collected from 6/19/2019-7/22/2019 in 2019, from 6/02/2020-7/20/2020 in 2020, and from 5/20/2021-7/21/2021 in 2021. Means comparisons were performed using Tukey's method at *P*=0.05.

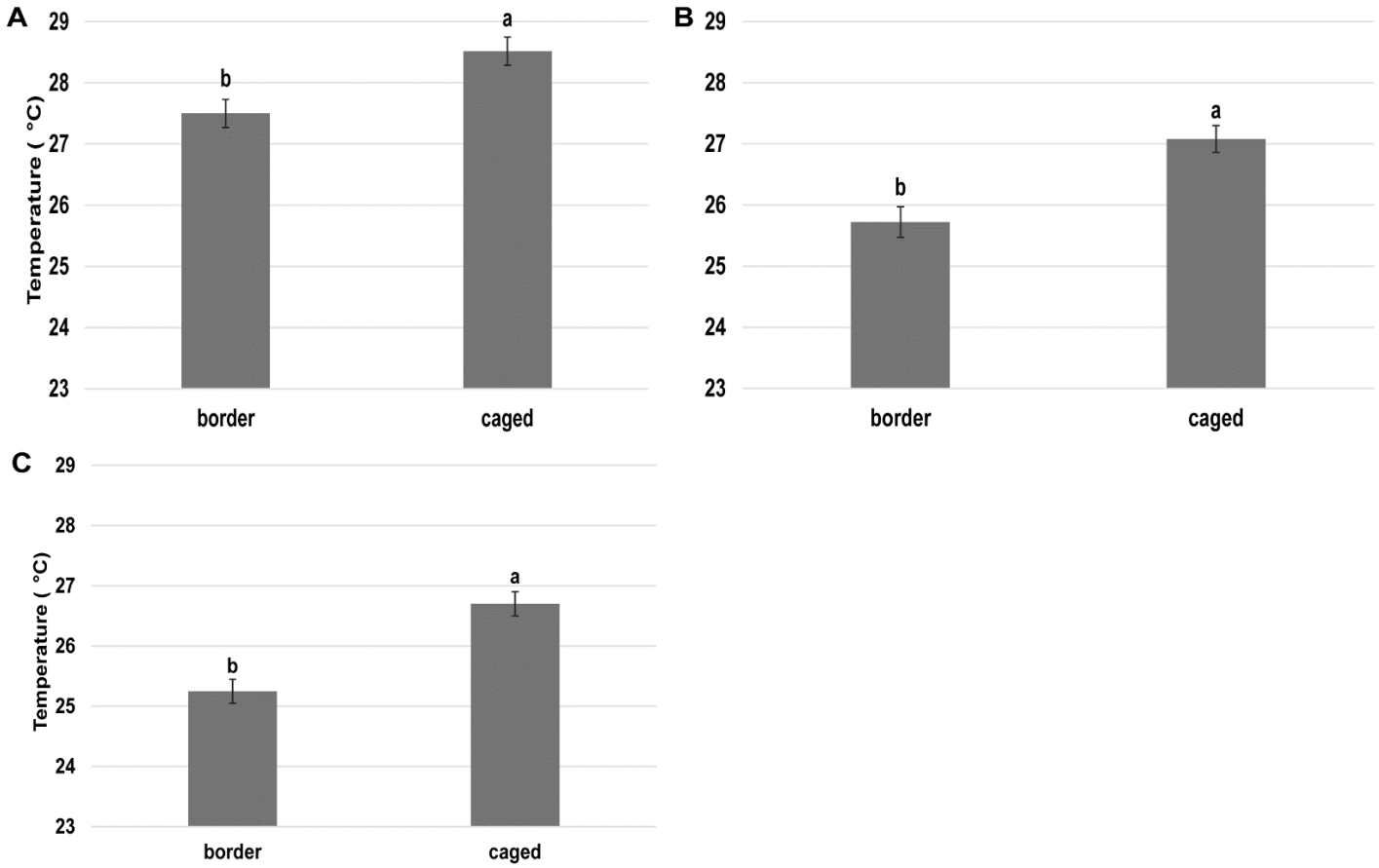

Figure 3: The average (±standard error) of temperature inside cages and in non-caged border rows between the months of May and July in (A) 2019; $F_{1,66}$=9.99, *P*=0.0024 (B) 2020; $F_{1,84}$=16.48, *P*=0.0001, and (C) 2021; $F_{1,124}$=27.38, *P*=0.0001. Data were collected from 6/19/2019-7/22/2019 in 2019, from 6/02/2020-7/20/2020 in 2020, and from 5/20/2021-7/21/2021 in 2021. Means comparisons were performed using Tukey's method at *P*=0.05.

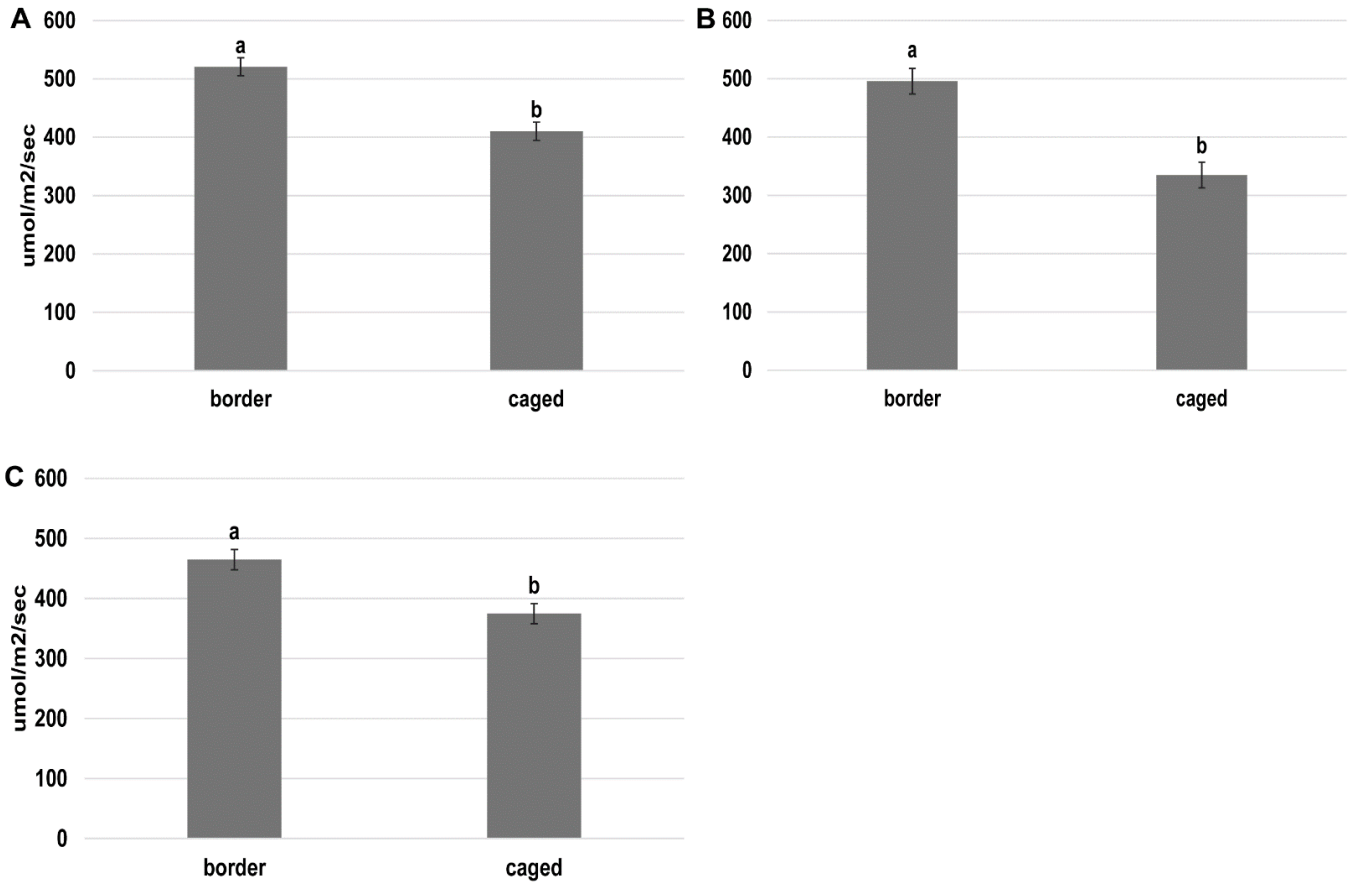

Figure 4: The average (±standard error) of PAR inside cages and in non-caged border rows between the months of May and July in (A) 2019; $F_{1,66}$=25.10, *P*=0.0001, (B) 2020; $F_{1,86}$=26.67, *P*=0.0001, and (C) 2021; $F_{1,120}$=14.49, *P*=0.0002. Data were collected from 6/19/2019-7/22/2019 in 2019, from 6/02/2020-7/20/2020 in 2020, and from 5/20/2021-7/21/2021 in 2021. Means comparisons were performed using Tukey's method at *P*=0.05.

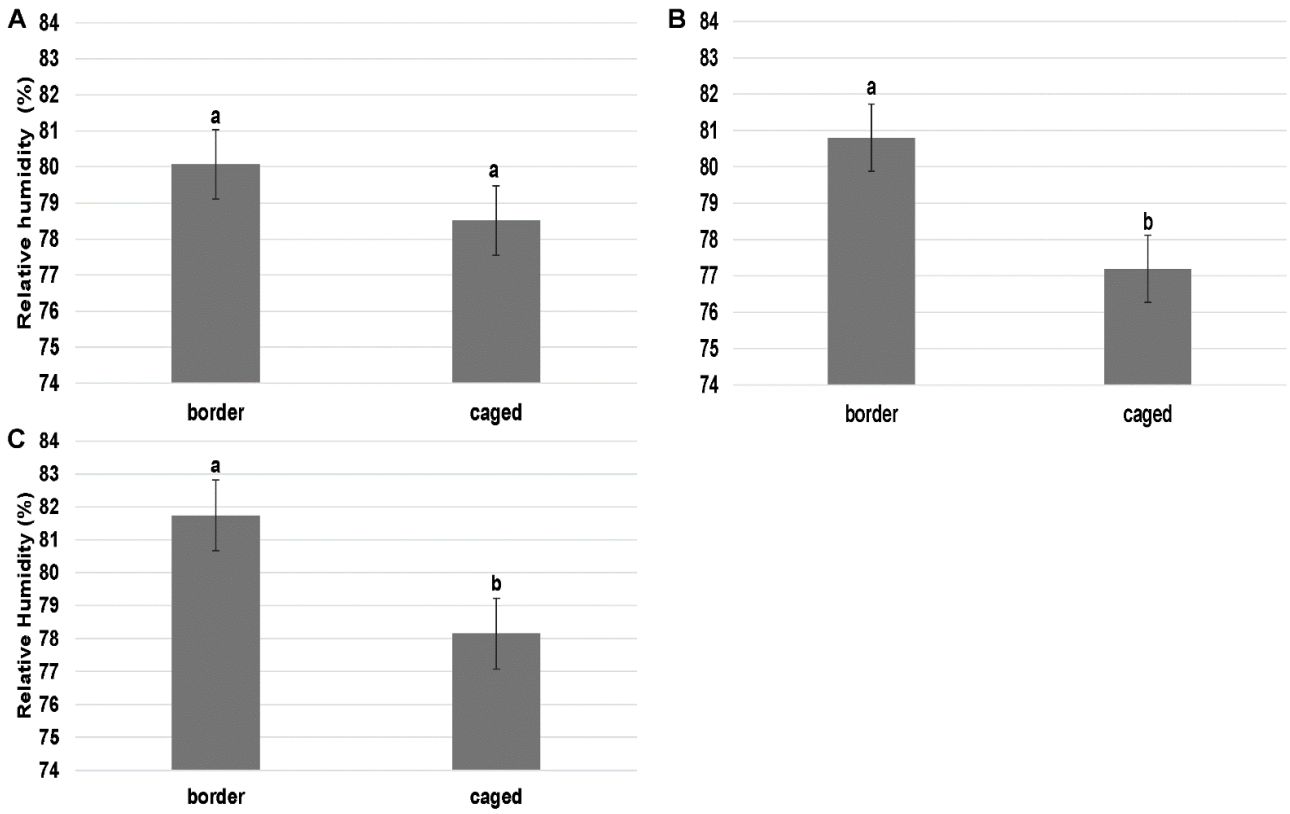

Figure 5: The average (±standard error) of relative humidity inside cages and in non-caged border rows between the months of May and July in (A) 2019; $F_{1,66}$=1.32, P=0.2547, (B) 2020; $F_{1,66}$=9.99, P=0.0024, and (C) 2021; $F_{1,124}$=5.47, P=0.0210. Data were collected from 6/19/2019-7/22/2019 in 2019, from 6/02/2020-7/20/2020 in 2020, and from 5/20/2021-7/21/2021 in 2021. Means comparisons were performed using Tukey's method at P=0.05 level.

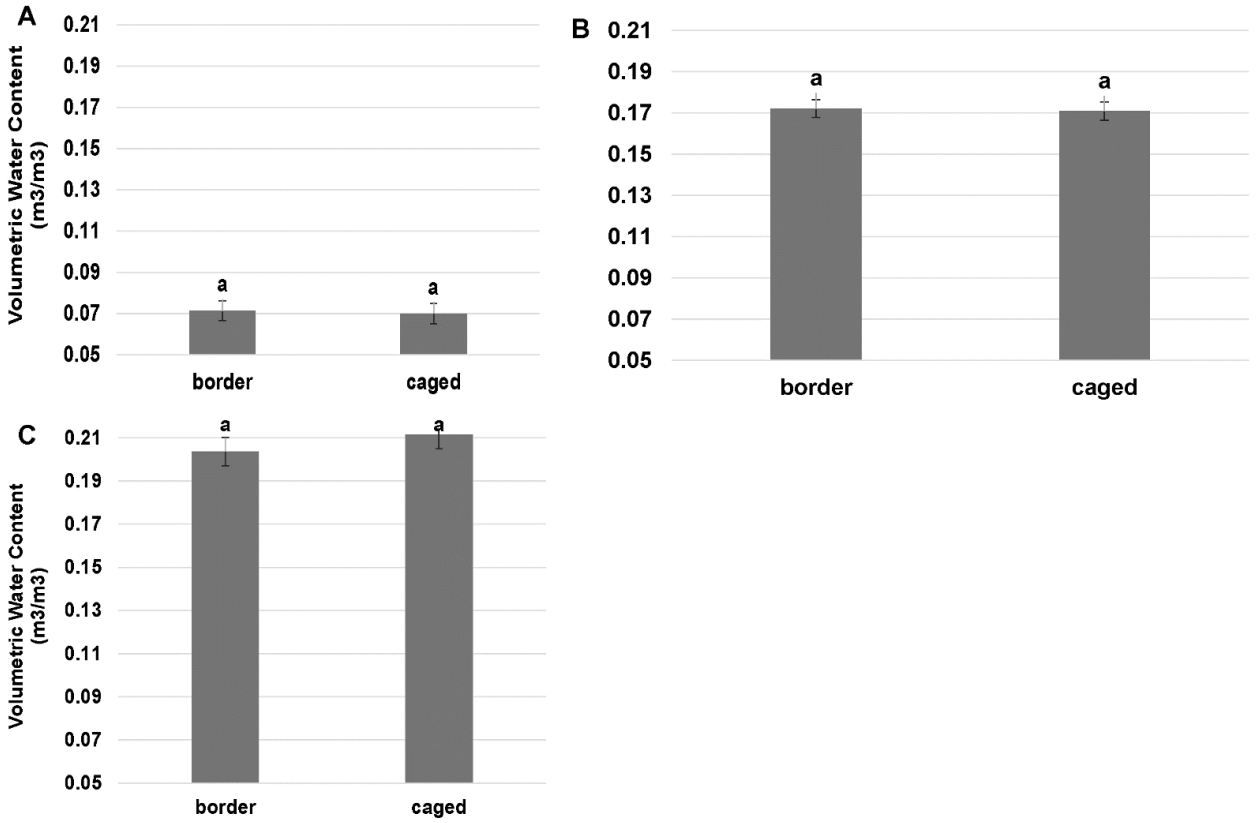

Figure 6: The average (±standard error) of soil moisture at a 15.24 cm depth in cages and in non-caged border rows between the months of May and July in (A) 2019; $F_{1,66}$=0.04, *P*=0.8339, (B) 2020; $F_{1,96}$=0.04, *P*=0.8392, and (C) 2021; $F_{1,124}$=0.73, *P*=0.3936. Data were collected from 6/19/2019-7/22/2019 in 2019, from 6/02/2020-7/20/2020 in 2020, and from 5/20/2021-7/21/2021 in 2021. Means comparisons were performed using Tukey's method at *P*=0.05.

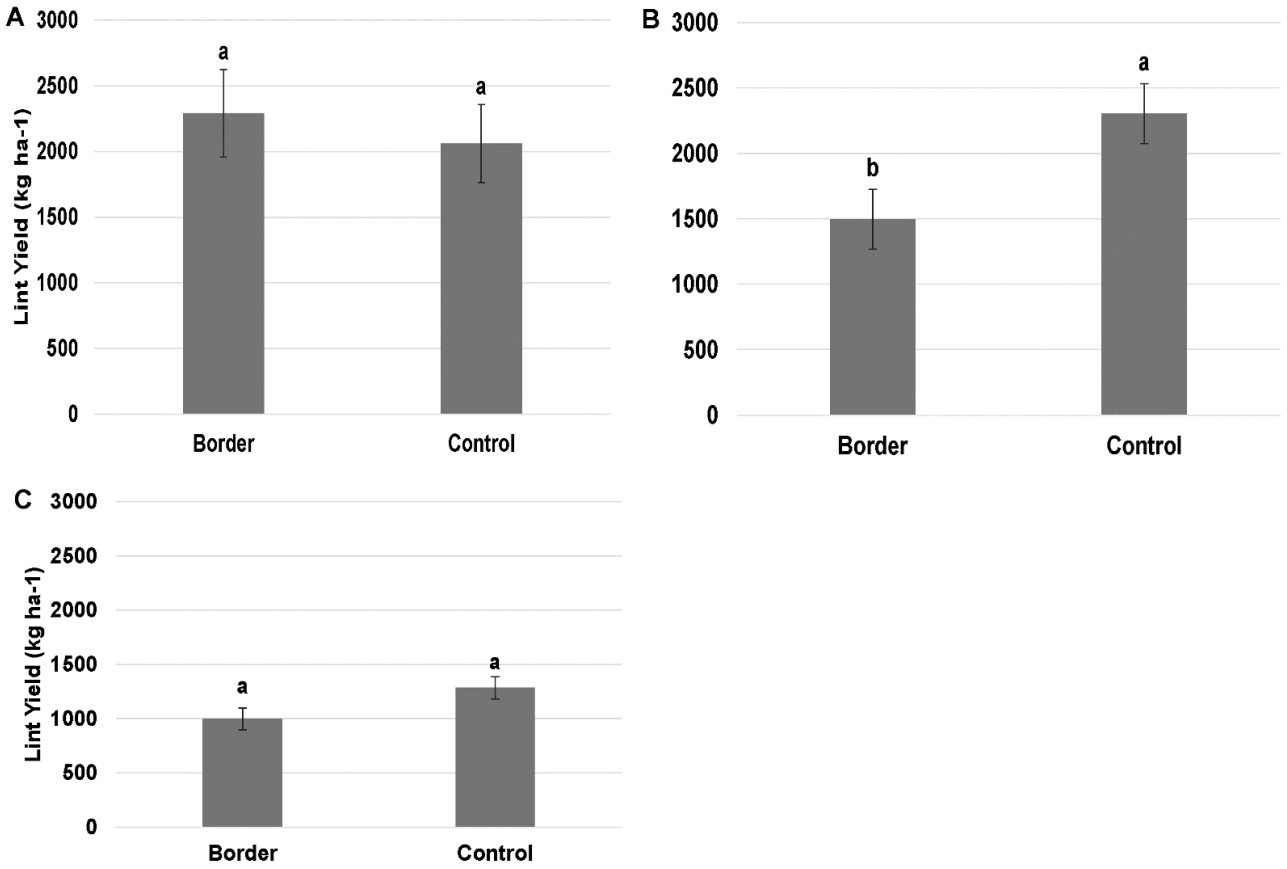

Figure 7: The average (±standard error) of lint yield (kg ha-1) compared between the Control and the border rows in (A) 2019; $F_{5,16.04}$=2.35, *P*=0.0879, (B) 2020; $F_{7,23}$=2.02, *P*=0.0968, and (C) 2021; $F_{6,18}$=0.69, *P*=0.6569. A length of the inner two rows of the four border rows that were equal to the length of caged plots were harvested from each experimental block. Means comparisons were performed using Tukey's method at *P*=0.05 to examine differences between yield in caged and non-caged environments.

Chapter 3

Introduction

Cotton leafroll dwarf virus (CLRDV) is a polerovirus (family: Solemoviridae) transmitted by the cotton aphid, *Aphis gossypii* Glover, that has been reported to infect cotton, *Gossypium hirsutum* L. CLRDV has been found from Virginia to west Texas in the United States Cotton Belt (Aboughanem-Sabanadzovic et al. 2019; Alabi et al. 2020; Ali and Mokhtari 2020; Ali et al. 2020; Faske et al. 2020; Iriarte et al. 2020; Price et al. 2020; Tabassum et al. 2019; Thiessen et al. 2020; Wang et al. 2020). Yield losses as a result of CLRDV infection have been reported (Avelar et al. 2019; Parkash et al. 2021), but are not commonly observed (Brown et al. 2020; Lawrence et al. 2019, 2020, 2021, 2022; Mahas et al. 2022). The lack of reported losses due to CLRDV and inconsistent yield effects observed in research studies suggest that environmental conditions are an important component of the disease triangle driving yield loss outcomes. Symptomatology of CLRDV is not defined because a wide range of symptoms are associated with virus detection in cotton, and asymptomatic infections can occur (Brown et al. 2020; Edula et al. 2023). Definitive confirmation of CLRDV requires the use of RT-PCR (Brown et al. 2020). Symptoms associated with CLRDV-infected plants include stunted growth, cupping or curling of the leaves, rugosity, leaf bronzing, tenting, reduced boll set, and reddening of the leaves, stems, or petioles (Brown et al. 2020; Parkash et al. 2021). Many of these symptoms overlap with symptoms associated with nutrient deficiencies in cotton (Xiao and Yin 2020), but CLRDV-nutrient interactions have not been studied.

There are several key nutrients that cotton requires to grow, and deficiencies in any one of these nutrients can lead to the development of various symptoms in the plant and a reduction

in yield (Xiao and Yin 2020). Nitrogen deficiencies cause pale green or yellowing of the lower leaves, as well as a reduction in plant height, shortened petioles, and reddening of the leaves and stems in more severe cases (Xiao and Yin 2020; Khawar Jabran and Singh Chauhan 2020). Potassium deficiencies cause a reduction in plant height boll mass and lint yield, and may be accompanied by foliar symptoms including interveinal chlorosis, necrosis, and bronzing (Oosterhuis et al. 2013). Symptoms of phosphorus deficiency are chlorosis or necrosis of leaves, purpling of the leaves, as well as stunted plant growth and a reduction in leaf number and size (Wang et al. 2018; Xiao and Yin 2020). A sulfur deficiency can cause a reduction in the number of bolls, yellowing of the younger leaves, chlorosis, and stunted plant growth (Jordan and Ensminger 1959). A lack of micronutrients such as zinc, boron, or iron can cause various symptoms such as chlorosis, leaf malformation, or leaf curling (Xiao and Yin 2020). Nutrient deficiencies are also known to increase a plant's susceptibility to disease and increase disease severity. Other studies have found that potassium deficiency increased disease severity and incidence when plants were infected with cotton leaf curl virus (CLCUV) (Panhwar et al. 2022; Pervez et al. 2007; Ullah Zafar and Athar 2013). There is currently no information on the impact of nutrient stress on disease caused by CLRDV.

The objectives of this research were to compare yield and symptoms between CLRDV-infected and virus-free cotton plants grown under specific nutrient deficiencies. Data from this study can be used to help identify which symptoms can be associated with CLRDV-infection. A comparison of yield on a per plant basis will allow us to determine if yield losses due to nutrient deficiencies are more severe when plants are infected with CLRDV.

Materials and Methods

‘DP1646’ (DeltaPine®, Dekalb Genetics Corporation, Dekalb, IL) seed without an insecticide seed treatment was planted at the Cullars Rotation (Mitchell et al. 2012) in Auburn, AL on May 17, 2021, and 2022. The Cullars Rotation is a historic crop rotation that began as a soil fertility experiment in 1911. It includes 14 soil nutrient treatments and a three-year rotation of 1)cotton, followed by crimson clover, 2) corn, followed by winter wheat, and 3) soybean double cropped after wheat is harvested, which allows for observations on the effects of nutrient deficiencies on the crops grown there (Mitchell et al. 2012a). Nutrient treatments are as follows: 1) winter legume- no nitrogen, 2) no winter legume- no nitrogen, 3) no amendments, 4) nitrogen added, no winter legume, 5) no phosphorous, 6) no micronutrients, 7) high potassium (4/3) rate, 8) rock phosphate, 9) no potassium, 10) low potassium (2/3) rate, 11) no lime, 12) no sulfur, 13) complete fertilizer with micronutrients, and 14) low potassium (1/3 rate). Rate adjustments were based off the recommended rate according to Auburn Soil, Forage and Water Laboratory (Mitchell et al. 2012b). All treatments were used in these experiments except for those with no amendments and no lime, as cotton will not grow there. Plots for each treatment at the Cullars rotation are eight rows wide and 30.18 m long with a row spacing of 0.76 m. Approximately 15.24 m of the middle two rows were used for these experiments to exclude edge effects and plots began approximately 2 m from the plot ends.

Plots were maintained to try and increase the likelihood that both CLRDV-infected and non-infected plants would be present to compare yield and symptoms between them. In the U.S. CLRDV is a low titer virus and low transmission rates are observed under controlled conditions (Heilsnis et al. 2022, 2023). Despite this, incidence due to natural virus spread can range from 60-100% in some areas (Mahas et al. 2022; Pandey et al. 2022; Sedhain et al. 2021). To ensure there were virus-free plants in this study, a 6.10 m L x 1.42 m W x 1.14 m H cage was placed

over part of each plot; cages were constructed with mesh 50 anti-insect screen (manufactured by Green-tek) stretched over a 2.50 cm diameter PVC pipe frame to exclude natural infestations of aphids. Cages were removed mid-season when plant growth reached the top, and this was after the time *A. gossypii* populations naturally crash due to annual fungal epizootics (Abney et al. 2008). Cages were removed July 16, 2021, and July 13, 2022.

To generate CLRDV-infected plants, a two row by 6.10 m area was measured to serve as the non-caged area and a buffer of 0.91 m of row was left between the cages and the start of the uncaged area in each plot. To increase the likelihood of CLRDV infection above what was expected from natural infestations, plants were infested with viruliferous *A. gossypii* during the seedling stage. Cotton aphids used in this experiment were from an *A. gossypii* colony maintained in the greenhouse that originated from multiple individuals that were collected from a cotton field in Tallassee, AL in 2019. The colony was maintained by transferring two adults each week to one to two true-leaf 'DP1646' (DeltaPine®, Dekalb Genetics Corporation, Dekalb, IL) cotton seedlings. CLRDV infected plants were originally collected from a field in Tallassee, AL in 2018. Plants were maintained in a greenhouse according to methods of Heilsnis et al. (2022). Confirmed CLRDV-infected source plants were infested with aphids for a minimum of a 72 hour acquisition access period (Michelotto and Busoli 2007, Heilsnis et al. 2022).

To infest plants in the plots, aphid infested leaves from the CLRDV infected plants were removed from the source plant and placed onto marked non-caged plants in the field. Plants outside of the cage were infested with viruliferous aphids three times in 2021: May 31, June 8, and June 22, and three times in 2022: June 3, June 6, and June 10. The presence or absence of aphids was recorded weekly to ensure colonization of the infested plants, and cages were monitored to ensure they were successful in keeping out insects. Prior to removal of the cages,

plants were sprayed with 1.02 L/ha flupyradifurone (Sivanto® Prime, Bayer CropScience LP, St. Louis, MO) July 1 and July 3, 2021 and June 22, 2022.

Data Collection

Due to the limited area of the historic rotation, and the expectation that nutrient deficiencies would be uniform after 110 years of maintenance, a completely randomized design was used for experiments. Forty plants were randomly selected and marked for data collection in each nutrient plot; 20 from each caged and each non-caged area of every plot. Data collection occurred on each plant so that main effects could be compared between CLRDV infected and non-infected plants. CLRDV detection was performed at the end of the season to account for natural spread by local aphid populations that can occur throughout the growing season (Mahas et al. 2023). Final virus testing was performed using RT-PCR September 10, 2021, and September 21, 2022. Plants testing positive for the virus will hereafter be referred to as CLRDV+ plants, and plants testing negative will be referred to as CLRDV- plants.

Symptom rating began July 6 in 2021 and 2022, approximately two months after planting. Symptoms monitored included cupping of leaves, reddening of stem, leaf veins and petioles, bronzing of the leaf, leaf tenting, cupping, rugosity, and stunted plant growth (Fig 8). Symptoms were rated on a severity scale of one to five, with one being least severe, and five being most severe. The entire plant was looked at for stem and petiole symptoms, and the top 1/3rd of plant was observed for foliar symptoms. Ratings were performed every other week for a total of five times each year.

All marked cotton was tested for CLRDV after cut-out using PCR-based methods on September 10, 2021, and September 21, 2022, roughly 4 months after planting (Mahas et al., 2022). Plant mapping was conducted on September 24, 2021, and September 28-30, 2022;

fruiting branch position, number of first and second position bolls, and plant height were recorded. Lint was hand harvested from each marked plant and the number of harvestable bolls was recorded on October 20, 2021, September 30, 2022, and October 7, 2022. Lint was collected from open bolls in September 2022 due to concerns about the loss of lint that may occur during an incoming tropical storm. Final lint harvest occurred in October 2022 when the remaining bolls had opened. The seed cotton weight per plant was recorded, then cotton was ginned using a 10-saw tabletop cotton gin (Dennis Manufacturing Co., Inc.). Lint was weighed, and seeds were counted using a Uline Economy Counting scale (Uline, Pleasant Prairie, WI). Lint quality analyses were conducted using AFIS (Advanced Fiber Information System).

Data were analyzed using the GLIMMIX procedure in SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA). Means comparisons were performed separately for plants in each nutrient treatment and in each year across all treatments to compare CLRDV+ and CLRDV- plants. Plants were grouped as CLRDV+/- regardless of whether they were from caged or non-caged areas as yield is not expected to significantly differ between the two areas, as observed in Chapter 2 (Fig 7) of this thesis. Each symptom, lint yield, seed count, and lint quality were analyzed in separate analyses as dependent variables to compare the main effects of treatment, infection, and the interaction term, using Tukey's method at the P =0.05 level.

Results

Symptom rating analyses from September 1, 2021 and September 14, 2022, showed similar results for both years (Fig 9-16). Only data from the last are presented because symptoms were at the highest severity during the last rating compared to the earlier ratings performed. In both years, the CLRDV+ plants were more severely stunted in most nutrient treatments compared to CLRDV- plants (Fig 9) and reddening of the stems and petioles were generally

rated as more severe in the CLRDV- plants (Fig 10 and 11). Bronzing and red leaf veins were observed in most treatments, but both symptoms were variable among treatments and years (Fig 12 and 13). Tenting was not observed in the 'no sulfur,' 'no potassium,' and 'rock phosphate,' treatments in 2021. Treatments 'no micronutrient' and 'winter legume, no nitrogen' showed significantly more severe tenting in CLRDV- plants. Tenting was not observed in 2022 (Fig 14). Rugosity was rarely observed in 2021, with only 11 out of 467 plants being rated for rugosity. This symptom occurred more commonly in 2022, but there were only two significant differences among treatments with no consistent trends across treatments or by year (Fig 15). Cupping was rarely observed in 2021 with only 29 out of 467 plants being rated for this symptom. Cupping was more commonly seen in 2022, but there were no trends across treatments or years (Fig 16).

In 2021, there was an overall reduction in measured plant height, retention of first position bolls, and the sum of first and second position bolls in the CLRDV+ plants. The total number of nodes were significantly higher in CLRDV+ plants (Table 4). In 2022, there was again an overall reduction in plant height, but no significant differences in any of the other plant mapping variables measured (Table 4).

Because differences between treatments due to nutrient deficiencies are expected, additional analyses were conducted to specifically compare CLRDV+ and CLRDV- plants in each treatment for 2021 (Table 5) and 2022 (Table 6) data. A statistically significant height reduction in CLRDV+ plants was observed in some treatments, but there were few significant differences with other plant mapping variables in both years.

The analysis that compared lint yield between CLRDV+ and CLRDV- plants across all nutrient treatments showed a statistically significant 31% reduction in yield in CLRDV+ plants in 2021 (Table 7). There were also significant differences among treatments, but the interaction

term was not significant. In a separate analysis that compared CLRDV+ and CLRDV- plants separately for each nutrient treatment, statistically significant differences were only observed in the 'no micronutrient' and 'high potassium (4/3 rate)' treatments, and while not significant, there was a general trend of infected plants yielding less than CLRDV- plants in most other treatments (Fig. 17A). In 2022, there were no significant differences in yield between CLRDV+ and CLRDV- plants in analyses that included all treatments (Table 7), or in analyses conducted separately for each nutrient treatment (Fig 17B).

Similar to lint yield, there was a 23% reduction in the number of seeds per plant in the CLRDV+ plants compared to the CLRDV- plants in 2021 (Table 8). There was also a nonsignificant trend of CLRDV- plants having a reduced number of seeds in most treatments (Fig 18A). There were no significant differences in 2022 (Table 8, Fig 18B).

Mean fiber length, fineness, and maturity ratio were measured and compared between CLRDV+ and CLRDV- plants in both years. The maturity ratio was significantly reduced in CLRDV+ plants, but there were no significant differences in fiber length or fineness in both years (Table 9). There were very few significant differences when plants were analyzed by treatment in both years (Table 10 and Table 11).

Discussion

Despite few significant differences in yield in each of the nutrient treatments (Figure 17), there was an overall significant reduction in yield in CLRDV+ plants in 2021 (Table 7). A possible explanation for the lack of significant differences in some of the treatments may be due to a low sample size of CLRDV+ plants in most treatments. Despite having similar sample sizes both years, the same trends in yield loss were not observed in 2022.

There were few significant differences in lint quality. Genetics of the variety greatly influences lint quality, but the environment can have some affect too. Temperature, moisture, and nutrient stress can reduce fiber length. Temperature stress can also increase micronaire, which is a measure of fineness and maturity (Brown and Sandlin 2022). There was an overall increase in fineness and maturity ratio in 2022 compared to 2021; fiber length, however, was very similar in both years across treatments (Tables 10 and 11). Overall, there were few differences in lint quality and no consistencies between years, with the exception of maturity ratio which showed a significant decrease in CLRDV+ plants both years (Table 9).

Cotton leafroll dwarf virus does not have well defined symptomatology. Symptom data was collected to try and identify which symptoms were associated with CLRDV infection versus nutrient deficiencies. The only symptom that seemed to be consistent with CLRDV-infected plants was a reduction in plant height. There was both an observable difference in plant height as shown by the symptom rating results, as well as a measurable difference in plant height, shown by the plant mapping results. Reddening of the stems and petioles was significantly worse in some of the CLRDV- plants, and the majority of the treatments that had significant differences were treatments with no nitrogen added, suggesting that this symptom might be as a result of a nutrient deficiency rather than CLRDV-infection (Xiao and Yin 2020). The variability in symptoms is consistent with other studies reporting a range of symptoms associated with CLRDV infection (Avelar et al. 2019; Brown et al. 2020; Edula et al. 2023; Parkash et al. 2021). It has been found that symptoms of CLRDV may even vary based on location or time of infection (Brown et al. 2020; Edula et al. 2023). These results suggest that PCR methods still remain the most reliable diagnostic tool for determining CLRDV incidence.

In conclusion, stunted plant height seems to be the only symptom consistent with CLRDV-infection, and CLRDV continues to lack well defined symptomology. Yield loss was not consistent across years, and it is not clear why yield loss was only observed in 2021. Low sample sizes in both years or differences in the environment may have contributed to low statistical power to detect differences. Further research is needed on factors that might influence symptoms and yield loss.

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Infection **2021**	**Sample size**	**Plant Height**	**Total Nodes**	**1st position bolls**	**2nd position bolls**	**Sum of 1st and 2nd position bolls**
CLRDV - CLRDV +	337 115	40.91 (0.38) a 33.53 (0.73) b	17.35 (0.16) b 18.60 (0.26) a	5.09 (0.15) a 4.47 (0.24) b	2.64 (0.16) a 2.00 (0.27) a	5.25 (0.21) a 4.82 (0.37) b
Significance of Main Effects						
Infection		$F_{1,354}$ = 65.30, *P*= 0.0001	$F_{1,348}$ = 0.60, *P*= 0.4373	$F_{1,371}$ = 5.14, *P*= 0.0239	$F_{1,137}$ = 3.59, *P*= 0.0616	$F_{1,429}$ = 6.33, *P*= 0.0123
Treatment		$F_{9,354}$ = 14.53, *P*= 0.0001	$F_{9,348}$ = 8.17, *P*= 0.0001	$F_{10,371}$ = 3.89 *P*= 0.0001	$F_{10,137}$ = 0.81, *P*= 0.6172	$F_{11,429}$ = 6.81, *P*= 0.0001
Infection* Treatment		$F_{9,354}$ = 1.68, *P*= 0.0930	$F_{9,348}$ = 1.12, *P*= 0.3462	$F_{10,340}$ = 1.36, *P*= 0.1952	$F_{10,137}$ = 0.23, *P*= 0.9930	$F_{11,429}$ = 0.89, *P*= 0.5549
2022						
CLRDV -	342	32.37 (0.30) a	17.50 (0.14) a	4.32 (0.13) a	1.98 (0.10) a	6.29 (0.20) a
CLRDV +	110	30.65 (0.59) b	17.09 (0.27) a	4.69 (0.25) a	1.73 (0.20) a	6.42 (0.39) a
Significance of Main Effects						
Infection		$F_{1,428}$ = 6.77, *P*= 0.0096	$F_{1,399}$ = 1.80, *P*= 0.1801	$F_{1,398}$ = 1.76, *P*= 0.1851	$F_{1,399}$ = 1.21, *P*= 0.2720	$F_{1,399}$ = 0.09, *P*= 0.7611
Treatment		$F_{11,428}$ = 34.61, *P*= 0.0001	$F_{10,399}$ = 14.33, *P*= 0.0001	$F_{10,398}$ = 4.31, *P*= 0.0001	$F_{10,399}$ = 6.44, *P*= 0.0001	$F_{10,399}$ = 4.22, *P*= 0.0001
Infection* Treatment		$F_{11,428}$ = 0.91, *P*= 0.5279	$F_{10,399}$ = 1.14, *P*= 0.3286	$F_{10,398}$ = 1.47, *P*= 0.1479	$F_{10,399}$ = 0.50, *P*= 0.8913	$F_{10,399}$ = 1.05, *P*= 0.4002

Table 4: The average (±standard error) of plant height, total number of nodes, retention of first position or second boll position, and the total sum of bolls of both positions were compared between CLRDV+ and CLRDV- plants, treatments and their interaction term. Separate analyses were conducted for 2021 and 2022. Means comparisons were performed using Tukey's method at *P*=0.05.

	Infection	Sample Size	Plant Height	Total Nodes	1st position bolls	2nd position bolls	Sum of 1st and 2nd position bolls
Treatment‡							
Winter Legume, No nitrogen	CLRDV -	31	38.66 (1.18) a	16.26 (0.37) a	5.52 (0.40) a	2.20 (0.51) a	6.23 (0.56) a
	CLRDV +	5	32.06 (3.27) a	16.00 (1.03) a	3.00 (1.11) b	1.00 (1.62) a	3.25 (1.56) a
No Winter legume, No nitrogen	CLRDV -	29	35.41 (1.51) a	16.68 (0.59) a	5.11 (0.47) a	3.00 (0.77) a	5.76 (4.21) a
	CLRDV +	6	25.58 (3.31) b	16.20 (1.41) a	5.60 (1.10) a	2.00 (1.54) a	5.33 (1.92) a
Nitrogen added, no winter legume	CLRDV -	33	40.81 (1.02) a	18.13 (0.33) a	5.10 (0.50) a	2.59 (0.34) a	6.12 (0.72) a
	CLRDV +	7	36.93 (2.22) a	18.29 (0.69) a	4.71 (1.05) a	2.00 (0.82) a	5.57 (1.57) a
No Phosphorus	CLRDV -	33	27.64 (0.80) a	18.64 (0.43) a	4.00 (0.31) a	1.40 (0.26) a	3.97 (0.36) a
	CLRDV +	3	24.50 (2.67) a	18.00 (1.42) a	6.00 (1.22) a	1.50 (0.44) a	5.00 (1.21) a
No micronutrients	CLRDV -	22	46.24 (0.84) a	18.36 (0.36) a	6.45 (0.46) a	2.61 (0.48) a	8.00 (0.67) a
	CLRDV +	17	35.13 (0.96) b	17.65 (0.41) a	4.29 (0.52) b	1.00 (1.23) a	4.41 (0.76) b
High potassium (4/3 rate)	CLRDV -	24	40.08 (0.87) a	16.46 (0.50) a	6.04 (0.37) a	3.00 (0.57) a	6.92 (0.65) a
	CLRDV +	16	35.47 (1.06) b	17.81 (0.61) a	4.00 (0.44) b	2.00 (0.61) a	5.00 (0.80) a
Rock Phosphate	CLRDV -	26	43.1 (0.98) a	19.84 (0.51) a	4.14 (0.59) a	3.42 (0.43) a	5.57 (0.85) a
	CLRDV +	10	40.33 (1.59) a	21.70 (0.80) a	4.10 (0.88) a	2.50 (0.75) a	5.60 (1.42) a
No Potassium	CLRDV -	33	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
	CLRDV +	5	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
Low Potassium (2/3 rate)	CLRDV -	22	44.42 (1.55) a	18.57 (0.46) a	6.14 (0.57) a	3.00 (0.65) a	7.23 (0.92) a

	CLRDV +	15	37.55 (1.83) a	18.43 (0.56) a	5.62 (0.73) a	2.40 (0.92) a	5.67 (1.11) a
No Sulfur	CLRDV -	30	37.27 (1.75) a	17.30 (0.30) a	5.93 (0.40) a	2.36 (0.58) a	6.80 (6.22) a
	CLRDV +	9	30.28 (3.20) a	18.22 (0.55) a	5.22 (0.73) a	1.50 (0.96) a	5..89 (1.14) a
Complete fertilization with micronutrients	CLRDV -	22	47.88 (1.07) a	18.23 (0.36) a	5.86 (0.55) a	2.18 (0.65) a	6.95 (0.87) a
	CLRDV +	16	37.44 (1.26) b	18.44 (0.42) a	4.50 (0.64) a	2.43 (0.82) a	5.56 (1.04) a
Low potassium (1/3 rate)	CLRDV -	32	**N/A**	**N/A**	2.43 (0.26) a	1.50 (0.25) a	2.13 (0.31) a
	CLRDV +	6	**N/A**	**N/A**	2.00 (0.62) a	1.00 (0.35) a	2.00 (0.72) a
Significance of Main Effects							
Winter Legume, No nitrogen			$F_{1,33} = 3.60$, $P = 0.0664$	$F_{1,33} = 0.06$, $P = 0.8150$	$F_{1,33} = 4.58$, $P = 0.0399$	$F_{1,9} = 0.50$, $P = 0.4977$	$F_{1,33} = 3.21$, $P = 0.0822$
No Winter legume, No nitrogen			$F_{1,33} = 7.29$, $P = 0.0108$	$F_{1,31} = 0.10$, $P = 0.7562$	$F_{1,31} = 0.17$, $P = 0.6829$	$F_{1,8} = 0.34$, $P = 0.5776$	$F_{1,33} = 0.05$, $P = 0.8188$
Nitrogen added, no winter legume			$F_{1,38} = 2.53$, $P = 0.1199$	$F_{1,36} = 0.04$, $P = 0.8393$	$F_{1,36} = 0.11$, $P = 0.7442$	$F_{1,18} = 0.44$, $P = 0.5157$	$F_{1,38} = 0.10$, $P = 0.7517$
No Phosphorus			$F_{1,34} = 1.27$, $P = 0.2670$	$F_{1,34} = 0.18$, $P = 0.6706$	$F_{1,31} = 2.53$, $P = 0.1217$	$F_{1,5} = 0.04$, $P = 0.8457$	$F_{1,34} = 0.67$, $P = 0.4200$
No micronutrients			$F_{1,37} = 75.74$, $P = 0.0001$	$F_{1,37} = 1.70$, $P = 0.1999$	$F_{1,37} = 9.57$, $P = 0.0037$	$F_{1,13} = 1.50$, $P = 0.2417$	$F_{1,37} = 12.48$, $P = 0.0011$
High potassium (4/3 rate)			$F_{1,38} = 11.37$, $P = 0.0017$	$F_{1,38} = 2.95$, $P = 0.0938$	$F_{1,37} = 12.47$, $P = 0.0011$	$F_{1,15} = 1.44$, $P = 0.2481$	$F_{1,38} = 3.47$, $P = 0.0701$
Rock Phosphate			$F_{1,34} = 2.36$, $P = 0.1338$	$F_{1,33} = 3.82$, $P = 0.0592$	$F_{1,30} = 0.0$, $P = 0.9730$	$F_{1,23} = 1.14$, $P = 0.2970$	$F_{1,36} = 0.00$, $P = 0.9863$

No Potassium	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
Low Potassium (2/3 rate)	$F_{1,34} = 8.19$, $P = 0.0072$	$F_{1,33} = 0.04$, $P = 0.8444$	$F_{1,32} = 0.33$, $P = 0.5723$	$F_{1,13} = 0.28$, $P = 0.6040$	$F_{1,35} = 1.17$, $P = 0.2866$
No Sulfur	$F_{1,37} = 3.68$, $P = 0.0628$	$F_{1,37} = 2.14$, $P = 0.1520$	$F_{1,37} = 0.72$, $P = 0.4010$	$F_{1,13} = 0.60$, $P = 0.4531$	$F_{1,37} = 0.49$, $P = 0.4862$
Complete fertilization with micronutrients	$F_{1,36} = 39.84$, $P = 0.0001$	$F_{1,36} = 0.14$, $P = 0.7085$	$F_{1,36} = 2.62$, $P = 0.1142$	$F_{1,16} = 0.06$, $P = 0.8171$	$F_{1,36} = 1.04$, $P = 0.3152$
Low potassium (1/3 rate)	$F_{1,36} = 0.30$, $P = 0.5864$	$F_{1,33} = 0.95$, $P = 0.3361$	$F_{1,31} = 0.41$, $P = 0.5265$	$F_{1,4} = 1.33$, $P = 0.3125$	$F_{1,36} = 0.16$, $P = 0.6913$

Table 5: The average (±standard error) of plant height (in inches), total number of nodes, count of bolls at 1st position, count of bolls at 2nd position, sum of 1st and 2nd position bolls were compared between CLRDV+ and CLRDV- plants separately for each nutrient treatment in 2021. Means comparisons were performed using Tukey's method at P =0.05 level. N/A indicates no bolls were present at a particular boll position, or that plant height and total number of node data were not collected because stem samples were used for CLRDV testing because all leaves and petioles had fallen off of the plant.

	Infection	Sample Size	Plant Height inches	Total Nodes	1st position bolls	2nd position bolls	Sum of 1st and 2nd position bolls
Treatment‡							
Winter Legume, No nitrogen	CLRDV -	29	33.14 (0.74) a	15.48 (0.41) a	4.69 (0.39) a	1.45 (0.27) a	6.14 (0.57) a
	CLRDV +	11	32.02 (1.21) a	15.73 (0.66) a	6.00 (0.63) a	1.64 (0.43) a	7.64 (0.93) a
No Winter legume, No nitrogen	CLRDV -	34	33.29 (0.80) a	14.68 (0.25) a	6.50 (0.34) a	1.41 (0.28) a	7.91 (0.53) a
	CLRDV +	4	27.13 (2.33) b	12.50 (0.74) b	4.75 (1.00) a	0.50 (0.82) a	5.25 (1.55) a
Nitrogen added, no winter legume	CLRDV -	28	37.34 (0.79) a	18.82 (0.33) a	4.57 (0.45) a	3.61 (0.42) a	8.18 (0.68) a
	CLRDV +	10	33.90 (1.31) b	18.50 (0.55) a	5.00 (0.75) a	2.60 (0.70) a	7.60 (1.14) a
No Phosphorus	CLRDV -	37	31.37 (1.22) a	18.05 (0.43) a	4.65 (0.37) a	1.24 (0.26) a	5.89 (0.55) a
	CLRDV +	3	34.17 (4.27) a	19.67 (1.51) a	3.00 (1.29) a	0.67 (0.92) a	3.67 (1.93) a
No micronutrients	CLRDV -	30	36.56 (0.66) a	18.77 (0.37) a	5.40 (0.47) a	2.57 (0.38) a	7.97 (0.76) a
	CLRDV +	9	33.28 (1.20) b	18.67 (0.67) a	6.11 (0.86) a	2.78 (0.69) a	8.89 (1.38) a
High potassium (4/3 rate)	CLRDV -	33	33.15 (0.75) a	16.55 (0.42) a	3.67 (0.36) a	3.82 (0.40) a	7.48 (0.61) a
	CLRDV +	7	32.71 (1.64) a	15.71 (0.92) a	4.14 (0.79) a	3.14 (0.88) a	7.29 (1.33) a
Rock Phosphate	CLRDV -	24	32.48 (0.95) a	20.26 (0.48) a	3.00 (0.47) b	1.83 (0.31) a	4.83 (0.69) a
	CLRDV +	15	31.27 (1.14) a	19.63 (0.58) a	4.88 (0.56) a	1.81 (0.38) a	6.69 (0.83) a
No Potassium	CLRDV -	19	14.29 (1.02) a	**N/A**	**N/A**	**N/A**	**N/A**
	CLRDV +	12	12.75 (1.29) a	**N/A**	**N/A**	**N/A**	**N/A**
Low Potassium (2/3 rate)	CLRDV -	27	35.17 (1.15) a	15.78 (0.34) a	5.11 (0.46) a	2.00 (0.40) a	7.11 (0.74) a
	CLRDV +	11	30.59 (1.80) b	16.09 (0.53) a	5.00 (0.72) a	2.36 (0.62) a	7.36 (1.16) a

No Sulfur	CLRDV -	34	35.57 (1.12) a	20.26 (0.57) a	2.52 (0.44) a	1.71 (0.29) a	4.15 (0.63) a
	CLRDV +	5	34.50 (2.93) a	17.40 (1.50) a	4.40 (1.13) a	2.00 (0.76) a	6.40 (1.64) a
Complete fertilization with micronutrients	CLRDV -	27	36.79 (1.47) a	16.93 (0.45) a	4.96 (0.42) a	1.19 (0.26) a	6.15 (0.63) a
	CLRDV +	10	35.33 (2.42) a	16.60 (0.73) a	4.70 (0.68) a	0.60 (0.42) a	5.30 (1.04) a
Low potassium (1/3 rate)	CLRDV -	20	29.25 (1.45) a	16.95 (0.79) a	2.40 (0.36) b	0.95 (0.29) a	3.35 (0.58) a
	CLRDV +	13	30.17 (1.80) a	17.54 (0.98) a	3.62 (0.45) a	0.92 (0.36) a	4.54 (0.72) a
Significance of Main Effects							
Winter Legume, No nitrogen			$F_{1,38}$ =0.62, *P*= 0.4369	$F_{1,38}$ = 0.10, *P*= 0.7537	$F_{1,38}$ = 3.13, *P*= 0.0849	$F_{1,38}$ = 0.14, *P*= 0.7124	$F_{1,38}$ = 1.90, *P*= 0.1761
No Winter legume, No nitrogen			$F_{1,36}$ =6.25, *P*= 0.0171	$F_{1,36}$ = 7.78, *P*= 0.0084	$F_{1,36}$ = 2.72, *P*= 0.1080	$F_{1,36}$ = 1.10, *P*= 0.3009	$F_{1,36}$ = 2.63, *P*= 0.1138
Nitrogen added, no winter legume			$F_{1,36}$ =5.04, *P*= 0.0310	$F_{1,36}$ = 0.25, *P*= 0.6217	$F_{1,36}$ = 0.24, *P*= 0.6271	$F_{1,36}$ = 1.52, *P*= 0.2257	$F_{1,36}$ = 0.19, *P*= 0.6666
No Phosphorus			$F_{1,38}$ =0.40, *P*= 0.5326	$F_{1,38}$ = 1.06, *P*= 0.3096	$F_{1,38}$ = 1.52, *P*= 0.2250	$F_{1,38}$ = 0.36, *P*= 0.5523	$F_{1,38}$ = 1.23, *P*= 0.2753
No micronutrients			$F_{1,37}$ = 5.71, *P*= 0.0001	$F_{1,37}$ = 0.02, *P*= 0.8965	$F_{1,37}$ = 0.53, *P*= 0.4709	$F_{1,37}$ = 0.07, *P*= 0.7902	$F_{1,37}$ = 0.34, *P*= 0.5613
High potassium (4/3 rate)			$F_{1,38}$ = 0.06, *P*= 0.8099	$F_{1,38}$ = 0.67, *P*= 0.4175	$F_{1,38}$ = 0.30, *P*= 0.5852	$F_{1,38}$ = 0.49, *P*= 0.4877	*$F_{1,38}$ = 0.02*, *P*=0.8929
Rock Phosphate			$F_{1,37}$ = 0.66, *P*= 0.4211	$F_{1,37}$ = 0.71, *P*= 0.4041	$F_{1,37}$ = 6.61, *P*= 0.0143	$F_{1,37}$ = 0.00, *P*= 0.9780	$F_{1,37}$ = 2.96, *P*= 0.0937
No Potassium			$F_{1,29}$ = 0.88,	**N/A**	**N/A**	**N/A**	**N/A**

	P= 0.3570				
Low Potassium (2/3 rate)	$F_{1,36}$ = 4.59, P= 0.0390	$F_{1,36}$ = 0.25, P= 0.6220	$F_{1,36}$ = 0.02, P= 0.8971	$F_{1,36}$ = 0.24, P= 0.6266	$F_{1,36}$ = 0.03, P= 0.8560
No Sulfur	$F_{1,37}$ = 0.12, P= 0.7357	$F_{1,37}$ = 3.20, P= 0.0819	$F_{1,36}$ = 2.40, P= 0.1301	$F_{1,37}$ = 0.13, P= 0.7202	$F_{1,37}$ = 1.65, P= 0.2066
Complete fertilization with micronutrients	$F_{1,35}$ = 0.27, P= 0.6089	$F_{1,35}$ = 0.14, P= 0.7065	$F_{1,35}$ = 0.11, P= 0.7440	$F_{1,35}$ = 1.40, P= 0.2447	$F_{1,35}$ = 0.48, P= 0.4912
Low potassium (1/3 rate)	$F_{1,31}$ = 0.16, P= 0.6921	$F_{1,31}$ = 0.22, P= 0.6422	$F_{1,31}$ = 4.41, P= 0.0440	$F_{1,31}$ = 0.00, P= 0.9538	$F_{1,31}$ = 1.63, P= 0.2113

Table 6: The average (±standard error) of plant height (in inches), total number of nodes, count of bolls at 1st position, count of bolls at 2nd position, sum of 1st and 2nd position bolls were compared between CLRDV+ and CLRDV- plants separately for each nutrient treatment in 2022. Means comparisons were performed using Tukey's method at P =0.05 level. N/A indicates no bolls were present at a particular boll position.

Infection Status	Sample size	Lint Yield (g/plant)
2021		
CLRDV -	340	9.68 (0.42) a
CLRDV +	115	6.85 (0.83) b
Significance of Main Effects:	Infection	$F_{1,431} = 9.14$, $P = 0.0026$
	Treatment	$F_{11,431} = 5.62$, $P = 0.0001$
	Infection* Treatment	$F_{11,431} = 0.86$, $P = 0.5829$
2022		
CLRDV -	342	10.89 (0.58) a
CLRDV +	111	11.74 (1.11) a
Significance of Main Effects:	Infection	$F_{1,429} = 0.46$, $P = 0.5000$
	Treatment	$F_{11,429} = 5.97$, $P = 0.0001$
	Infection* Treatment	$F_{11,429} = 0.68$, $P = 0.7611$

Table 7: The average (±standard error) of lint yield (g/plant) were compared between CLRDV+ and CLRDV- plants, treatments and their interaction term. Separate analyses were conducted for 2021 and 2022. Means comparisons were performed using Tukey's method at P=0.05.

Infection	**Sample size**	**Seed Count** (per plant)
2021		
CLRDV -	340	149.13 (6.62) a
CLRDV +	115	115.44 (12.93) b
Significance of Main Effects	Infection	$F_{1,429}$ = 5.38, *P*= 0.0209
	Treatment	$F_{11,429}$ = 4.75, *P*= 0.0001
	Infection*Treatment	$F_{11,429}$ = 0.74, *P*= 0.6997
2022		
CLRDV -	342	154.06 (8.12) a
CLRDV +	111	170.38 (15.71) a
Significance of Main Effects	Infection	$F_{1,429}$ = 0.85, *P*=0.3567
	Treatment	$F_{11,429}$ = 6.55, *P*=0.0001
	Infection* Treatment	$F_{11,429}$ = 0.77, *P*=0.6656

Table 8: The average (±standard error) of seed count (number of seeds per plant) were compared between CLRDV+ and CLRDV- plants, treatments and their interaction term. Separate analyses were conducted for 2021 and 2022. Means comparisons were performed using Tukey's method at *P*=0.05.

Infection	**Sample size**	**Fiber length**	**Fineness**	**Maturity Ratio**
2021		inches	mTex	
CLRDV -	289	1.01 (0.01) a	160.62 (0.74) a	0.84 (0.002) a
CLRDV +	97	1.00 (0.01) a	159.90 (1.26) a	0.83 (0.004) b
Significance of Main Effects	Infection	$F_{1,364}$= 0.81, *P*= 0.3675	$F_{1,364}$ = 0.22, *P*= 0.5459	$F_{1,364}$ = 3.77, *P*= 0.0529
	Treatment	$F_{10,364}$= 9.10, *P*= 0.0001	$F_{10,364}$= 6.29, *P*= 0.0001	$F_{10,364}$= 13.63, *P*= 0.0001
	Infection* Treatment	$F_{10,364}$= 1.29, *P*= 0.2369	$F_{10,364}$= 0.89, *P*= 0.5459	$F_{10,364}$= 1.81, *P*= 0.0571
2022				
CLRDV -	298	1.04 (0.01) a	170.76 (0.72) a	0.87 (0.002) a
CLRDV +	95	1.03 (0.01) a	167.81 (1.40) a	0.86 (0.004) b
Significance of Main Effects	Infection	$F_{1,371}$= 0.36, *P*= 0.5463	$F_{1,371}$ = 3.53, *P*= 0.0611	$F_{1,371}$ = 5.96, *P*= 0.0151
	Treatment	$F_{10,371}$= 9.04, *P*= 0.0001	$F_{10,371}$= 11.29, *P*= 0.0001	$F_{10,371}$= 11.94, *P*= 0.0001
	Infection*Treatment	$F_{10,371}$= 1.75, *P*= 0.0683	$F_{10,371}$= 1.39, *P*= 0.1825	$F_{10,371}$= 2.07, *P*= 0.0261

Table 9: The average (±standard error) of fiber length, fineness, and maturity ratio were compared between CLRDV+ and CLRDV- plants, treatments and their interaction term. Separate analyses were conducted for 2021 and 2022. Means comparisons were performed using Tukey's method at *P*=0.05.

	Infection	Sample Size	Fiber Length inches	Fineness mTex	Maturity Ratio
Treatment[‡]					
Winter Legume, No nitrogen	CLRDV -	32	1.02 (0.01) a	166.17 (1.69) a	0.86 (0.004) a
	CLRDV +	5	1.00 (0.03) a	163.80 (4.27) a	0.86 (0.01) a
No Winter legume, No nitrogen	CLRDV -	30	1.00 (0.01) a	158.97 (2.52) a	0.84 (0.01) a
	CLRDV +	4	0.98 (0.03) a	154.60 (6.08) a	0.84 (0.02) a
Nitrogen added, no winter legume	CLRDV -	30	1.02 (0.02) a	165.40 (2.41) a	0.84 (0.01) a
	CLRDV +	7	1.02 (0.03) a	159.43 (5.00) a	0.82 (0.01) a
No Phosphorus	CLRDV -	29	0.91 (0.01) a	152.45 (1.75) a	0.81 (0.003) a
	CLRDV +	2	0.87 (0.03) a	153.50 (6.65) a	0.80 (0.01) a
No micronutrients	CLRDV -	22	1.08 (0.01) a	162.32 (2.27) a	0.85 (0.01) a
	CLRDV +	17	1.06 (0.01) a	165.94 (2.59) a	0.85 (0.01) a
High potassium (4/3 rate)	CLRDV -	23	0.99 (0.017) a	169.09 (2.12) a	0.86 (0.01) a
	CLRDV +	15	1.01 (0.02) a	163.80 (2.62) a	0.83 (0.01) a
Rock Phosphate	CLRDV -	22	1.01 (0.02) a	157.50 (2.48) a	0.82 (0.01) a
	CLRDV +	8	1.09 (0.04) a	159.25 (4.11) a	0.82 (0.01) a
No Potassium	CLRDV -	---	---	---	---
	CLRDV +	---	---	---	---
Low Potassium (2/3 rate)	CLRDV -	21	1.06 (0.02) a	159.76 (2.38) a	0.83 (0.01) a
	CLRDV +	10	1.03 (0.02) a	162.10 (3.45) a	0.83 (0.01) a
No Sulfur	CLRDV -	29	1.01 (0.01) a	153.79 (2.09) a	0.82 (0.01) a
	CLRDV +	9	1.03 (0.02) a	155.67 (3.75) a	0.82 (0.01) a
Complete fertilization with micronutrients	CLRDV -	22	1.07 (0.01) a	167.55 (2.30) a	0.87 (0.01) a
	CLRDV +	16	1.03 (0.01) b	172.81 (2.70) a	0.88 (0.01) a
Low potassium (1/3 rate)	CLRDV -	29	0.93 (0.02) a	153.83 (2.43) a	0.82 (0.01) a
	CLRDV +	4	0.90 (0.05) a	148.00 (6.53) a	0.78 (0.02) b
Significance of Main Effects					

Winter Legume, No nitrogen	$F_{1,35} = 0.50$ $P = 0.4827$	$F_{1,35} = 0.27$ $P = 0.6099$	$F_{1,35} = 0.22$, $P = 0.6398$
No Winter legume, No nitrogen	$F_{1,32} = 0.65$, $P = 0.4263$	$F_{1,32} = 0.44$, $P = 0.5118$	$F_{1,32} = 0.23$, $P = 0.6377$
Nitrogen added, no winter legume	$F_{1,35} = 0.02$, $P = 0.8993$	$F_{1,35} = 1.16$, $P = 0.2895$	$F_{1,35} = 1.78$, $P = 0.1905$
No Phosphorus	$F_{1,29} = 1.60$, $P = 0.2162$	$F_{1,29} = 0.02$, $P = 0.8795$	$F_{1,29} = 0.50$, $P = 0.4847$
No micronutrients	$F_{1,37} = 1.68$, $P = 0.2032$	$F_{1,37} = 1.11$, $P = 0.2994$	$F_{1,37} = 0.46$, $P = 0.5009$
High potassium (4/3 rate)	$F_{1,37} = 0.37$, $P = 0.5446$	$F_{1,36} = 2.46$, $P = 0.1256$	$F_{1,36} = 12.36$, $P = 0.0012$
Rock Phosphate	$F_{1,28} = 3.02$, $P = 0.0934$	$F_{1,28} = 0.13$, $P = 0.7184$	$F_{1,28} = 0.06$, $P = 0.8046$
No Potassium	---	---	---
Low Potassium (2/3 rate)	$F_{1,29} = 0.74$, $P = 0.3980$	$F_{1,29} = 0.31$, $P = 0.5808$	$F_{1,29} = 0.22$, $P = 0.6421$
No Sulfur	$F_{1,36} = 0.47$, $P = 0.4970$	$F_{1,36} = 0.19$, $P = 0.6652$	$F_{1,36} = 0.03$, $P = 0.8723$
Complete fertilization with micronutrients	$F_{1,36} = 5.32$, $P = 0.0269$	$F_{1,36} = 2.21$, $P = 0.1458$	$F_{1,36} = 0.88$, $P = 0.3541$

Low potassium (1/3 rate)	$F_{1,31} = 0.28$, $P = 0.6012$	$F_{1,31} = 0.70$, $P = 0.4095$	$F_{1,31} = 5.24$, $P = 0.0290$

Table 10: The average (±standard error) of fiber length, fineness, and maturity ratio were compared between CLRDV+ and CLRDV- plants separately for each nutrient treatment in 2021. Means comparisons were performed using Tukey's method at P =0.05 level. N/A indicates that an insufficient amount of lint was produced for measurement in that treatment.

	Infection	**Sample Size**	**Fiber Length** inches	**Fineness** mTex	**Maturity Ratio**
Treatment‡					
Winter Legume, No nitrogen	CLRDV -	28	1.02 (0.01) a	173.25 (1.40) a	0.88 (0.005) a
	CLRDV +	11	1.03 (0.02) a	173.36 (2.24) a	0.8755 (0.01) a
No Winter legume, No nitrogen	CLRDV -	34	1.01 (0.01) b	168.76 (1.37) a	0.87 (0.004) a
	CLRDV +	4	1.07 (0.02) a	173.00 (3.99) a	0.88 (0.01) a
Nitrogen added, no winter legume	CLRDV -	27	1.11 (0.02) a	177.96 (1.90) a	0.89 (0.005) a
	CLRDV +	10	1.03 (0.03) b	177.40 (3.12) a	0.87 (0.01) a
No Phosphorus	CLRDV -	37	0.99 (0.01) a	168.81 (1.92) a	0.85 (0.01) a
	CLRDV +	3	1.03 (0.05) a	165.67 (6.75) a	0.84 (0.02) a
No micronutrients	CLRDV -	30	1.02 (0.02) a	166.63 (2.41) a	0.85 (0.01) a
	CLRDV +	7	1.08 (0.04) a	170.00 (4.99) a	0.87 (0.01) a
High potassium (4/3 rate)	CLRDV -	32	1.12 (0.02) a	180.84 (2.09) a	0.90 (0.01) a
	CLRDV +	7	1.12 (0.04) a	178.29 (4.46) a	0.89 (0.01) a
Rock Phosphate	CLRDV -	18	1.12 (0.02) a	172.39 (2.53) a	0.86 (0.01) a
	CLRDV +	16	1.06 (0.03) a	166.13 (2.69) a	0.85 (0.01) a
No Potassium	CLRDV -	0	N/A	N/A	N/A

	CLRDV +	0			
Low Potassium (2/3 rate)	CLRDV -	25	1.04 (0.02) a	174.20 (2.85) a	0.88 (0.01) a
	CLRDV +	11	0.98 (0.03) a	160.00 (4.30) b	0.83 (0.01) b
No Sulfur	CLRDV -	24	1.02 (0.02) a	168.25 (3.33) a	0.86 (0.01) a
	CLRDV +	5	1.00 (0.04) a	155.80 (7.29) a	0.82 (0.02) a
Complete fertilization with micronutrients	CLRDV -	27	1.01 (0.02) a	176.63 (2.08) a	0.90 (0.01) a
	CLRDV +	10	0.98 (0.03) a	175.70 (3.42) a	0.89 (0.01) a
Low potassium (1/3 rate)	CLRDV -	16	0.93 (0.02) a	150.63 (4.58) a	0.82 (0.01) a
	CLRDV +	11	0.94 (0.02) a	150.55 (5.52) a	0.82 (0.01) a
Significance of Main Effects					
Winter Legume, No nitrogen			$F_{1,37} = 0.73$, $P = 0.3977$	$F_{1,37} = 0.00$, $P = 0.9659$	$F_{1,37} = 0.00$, $P = 0.9916$
No Winter legume, No nitrogen			$F_{1,36} = 7.00$, $P = 0.0120$	$F_{1,36} = 1.01$, $P = 0.3224$	$F_{1,36} = 0.78$, $P = 0.3830$
Nitrogen added, no winter legume			$F_{1,35} = 6.49$, $P = 0.0154$	$F_{1,35} = 0.02$, $P = 8785$	$F_{1,35} = 2.64$, $P = 0.1129$
No Phosphorus			$F_{1,38} = 0.47$, $P = 0.4956$	$F_{1,38} = 0.20$, $P = 0.6566$	$F_{1,38} = 0.23$, $P = 0.6360$
No micronutrients			$F_{1,35} = 1.79$, $P = 0.1896$	$F_{1,35} = 0.37$, $P = 0.5478$	$F_{1,35} = 1.69$, $P = 0.2018$
High potassium (4/3 rate)			$F_{1,37} = 0.00$, $P = 0.9927$	$F_{1,37} = 0.27$, $P = 0.6065$	$F_{1,37} = 0.43$, $P = 0.5171$
Rock Phosphate			$F_{1,32} = 2.88$, $P = 0.0993$	$F_{1,32} = 2.88$, $P = 0.0995$	$F_{1,32} = 3.02$, $P = 0.0917$

No Potassium	N/A	N/A	N/A
Low Potassium (2/3 rate)	$F_{1,34} = 2.15$, $P = 0.1516$	$F_{1,34} = 7.58$, $P = 0.0094$	$F_{1,34} = 10.88$, $P = 0.0023$
No Sulfur	$F_{1,27} = 0.19$, $P = 0.6677$	$F_{1,27} = 2.41$, $P = 0.1321$	$F_{1,27} = 3.58$, $P = 0.0692$
Complete fertilization with micronutrients	$F_{1,35} = 1.17$, $P = 0.2878$	$F_{1,35} = 0.05$, $P = 0.8178$	$F_{1,35} = 0.52$, $P = 0.4767$
Low potassium (1/3 rate)	$F_{1,25} = 0.03$, $P = 0.8659$	$F_{1,25} = 0.00$, $P = 0.9912$	$F_{1,25} = 0.01$, $P = 0.9372$

Table 11: The average (±standard error) of fiber length, fineness, and maturity ratio were compared between CLRDV+ and CLRDV- plants separately for each nutrient treatment in 2022. Means comparisons were performed using Tukey's method at P =0.05 level. N/A indicates that an insufficient amount of lint was produced for measurement in that treatment.

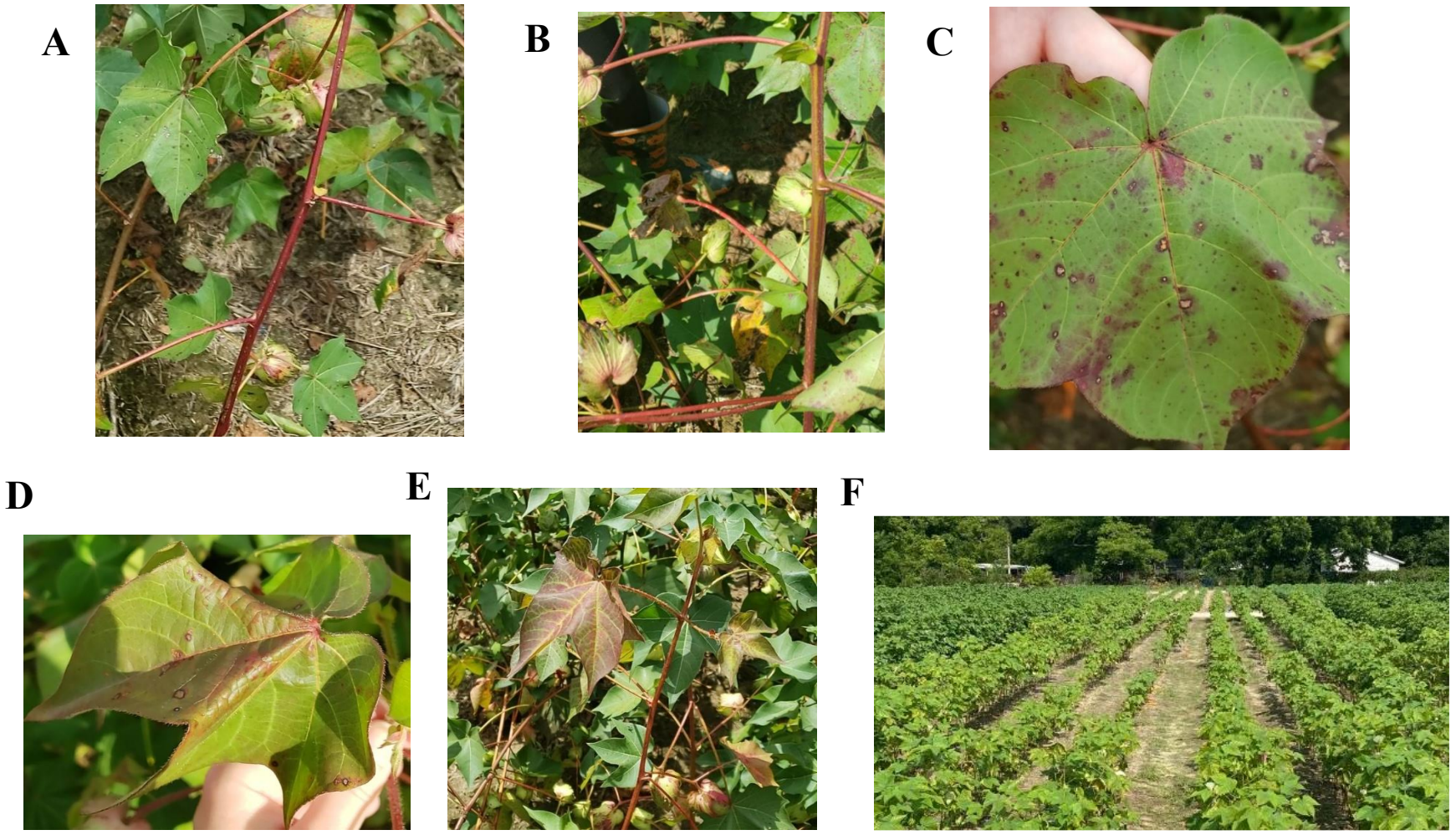

Figure 8: Examples of symptoms at highest severity rating observed, starting at the top left: A) Red stem (Symptom rating=5), B) Red petiole (Symptom rating= 5), C) Red leaf vein (Symptom rating =3), D) Tenting (Symptom rating= 3), E) Bronzing (Symptom rating= 4), F) Stunting (Symptom rating= 5)

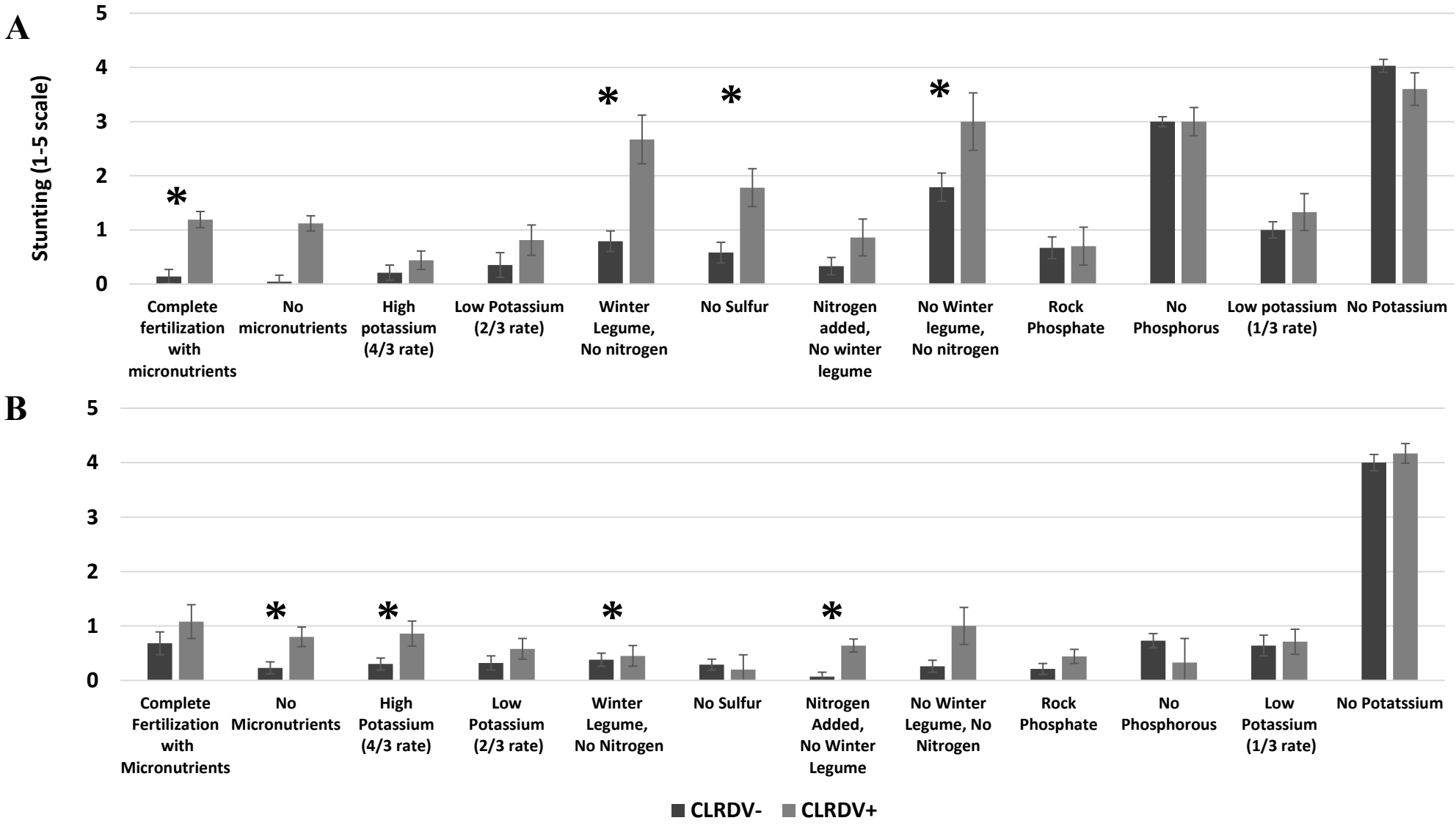

Figure 9: The average (±standard error) of stunting severity (symptom severity scale 1-5) in (A) 2021 and (B) 2022. Means comparisons between plant testing positive (CLRDV+) and negative (CLRDV-) for CLRDV were performed separately for each nutrient treatment using Tukey's method at *P*=0.05. Asterisks indicate numbers are significantly different, and absence indicates no difference.

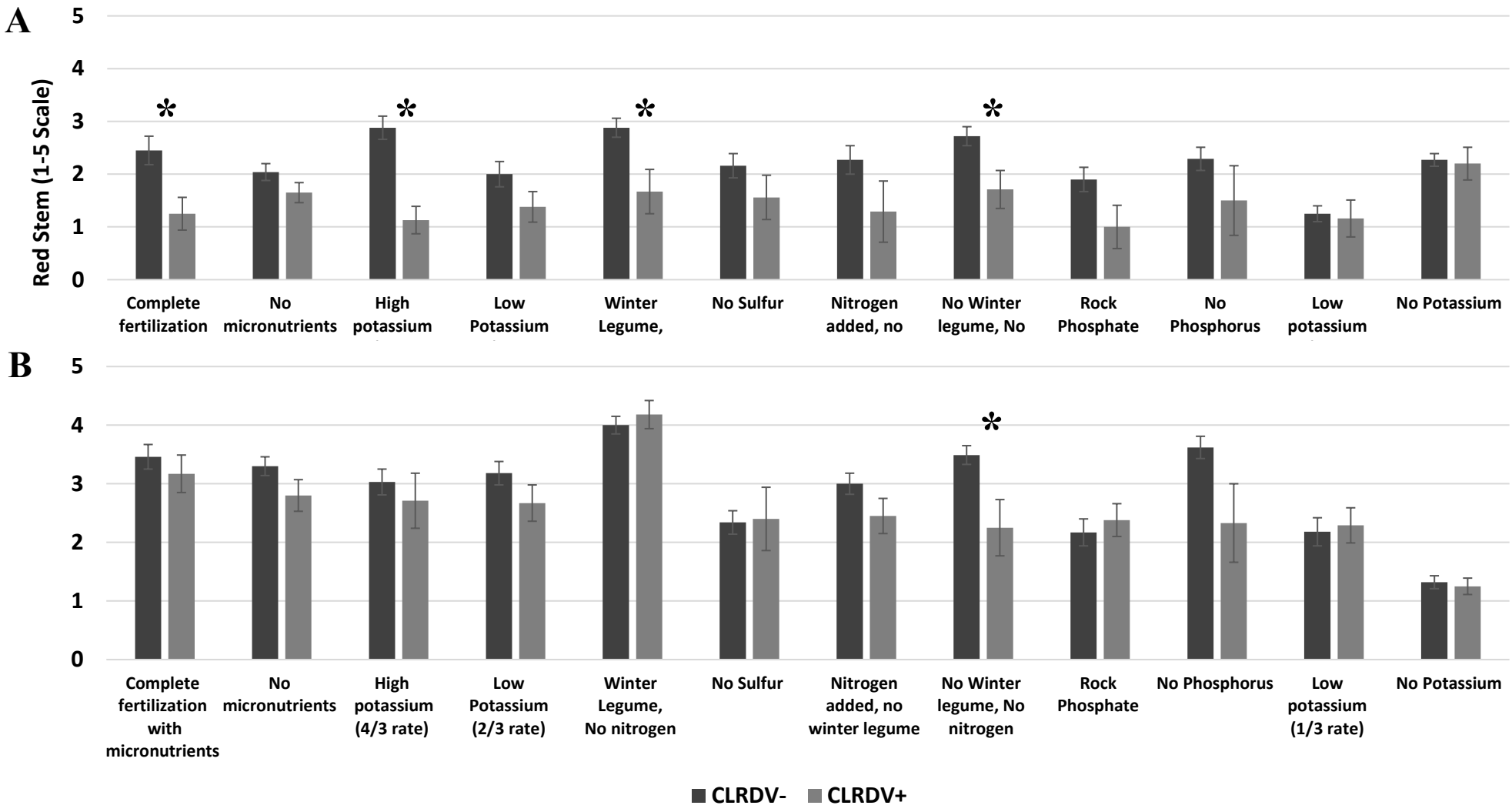

Figure 10: The average (±standard error) of red stem severity (symptom severity rating, scale of 1-5) in (A) 2021 and (B) 2022. Means comparisons between plant testing positive (CLRDV+) and negative (CLRDV-) for CLRDV were performed separately for each nutrient treatment using Tukey's method at P=0.05. Asterisks indicate numbers are significantly different, and absence indicates no difference.

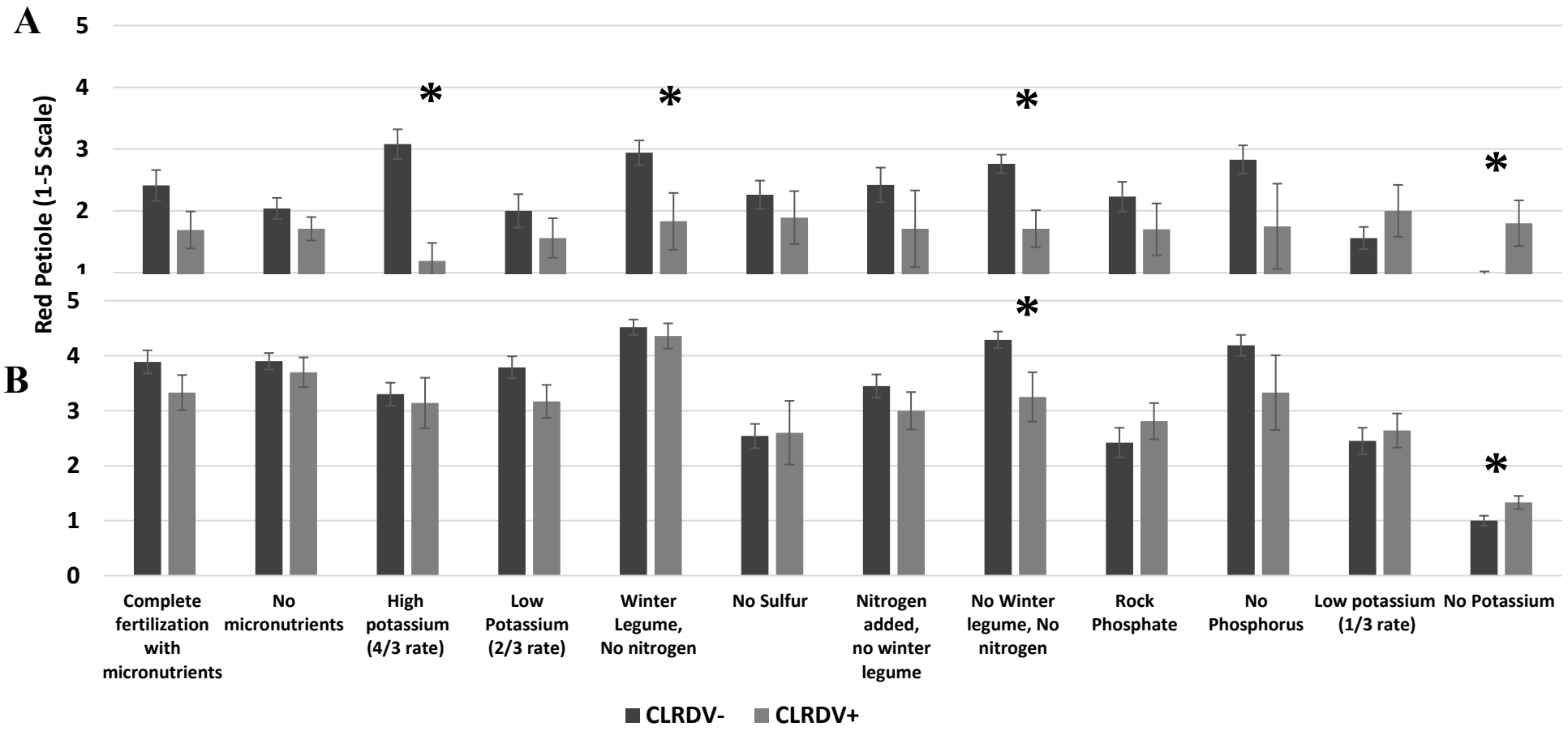

Figure 11: The average (±standard error) of red petiole severity (symptom severity scale 1-5) in (A) 2021 and (B) 2022. Means comparisons between plant testing positive (CLRDV+) and negative (CLRDV-) for CLRDV were performed separately for each nutrient treatment using Tukey's method at *P*=0.05. Asterisks indicate numbers are significantly different, and absence indicates no difference.

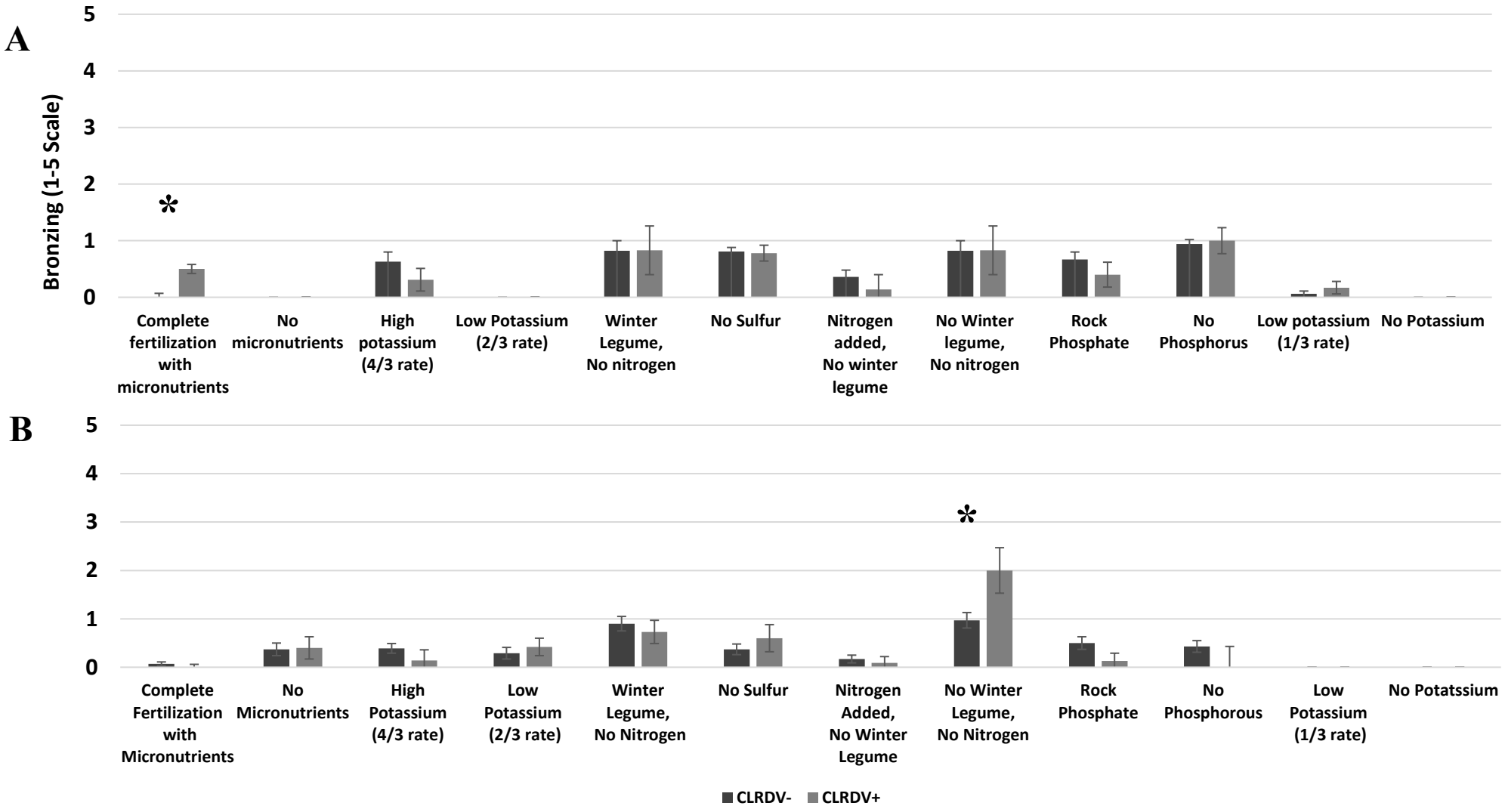

Figure 12: The average (±standard error) of leaf bronzing severity (symptom severity rating, scale of 1-5) in (A) 2021 and (B) 2022. Means comparisons between plant testing positive (CLRDV+) and negative (CLRDV-) for CLRDV were performed separately for each nutrient treatment using Tukey's method at P=0.05. Asterisks indicate numbers are significantly different, and absence indicates no difference. Treatments without bars indicate no observations of the symptom.

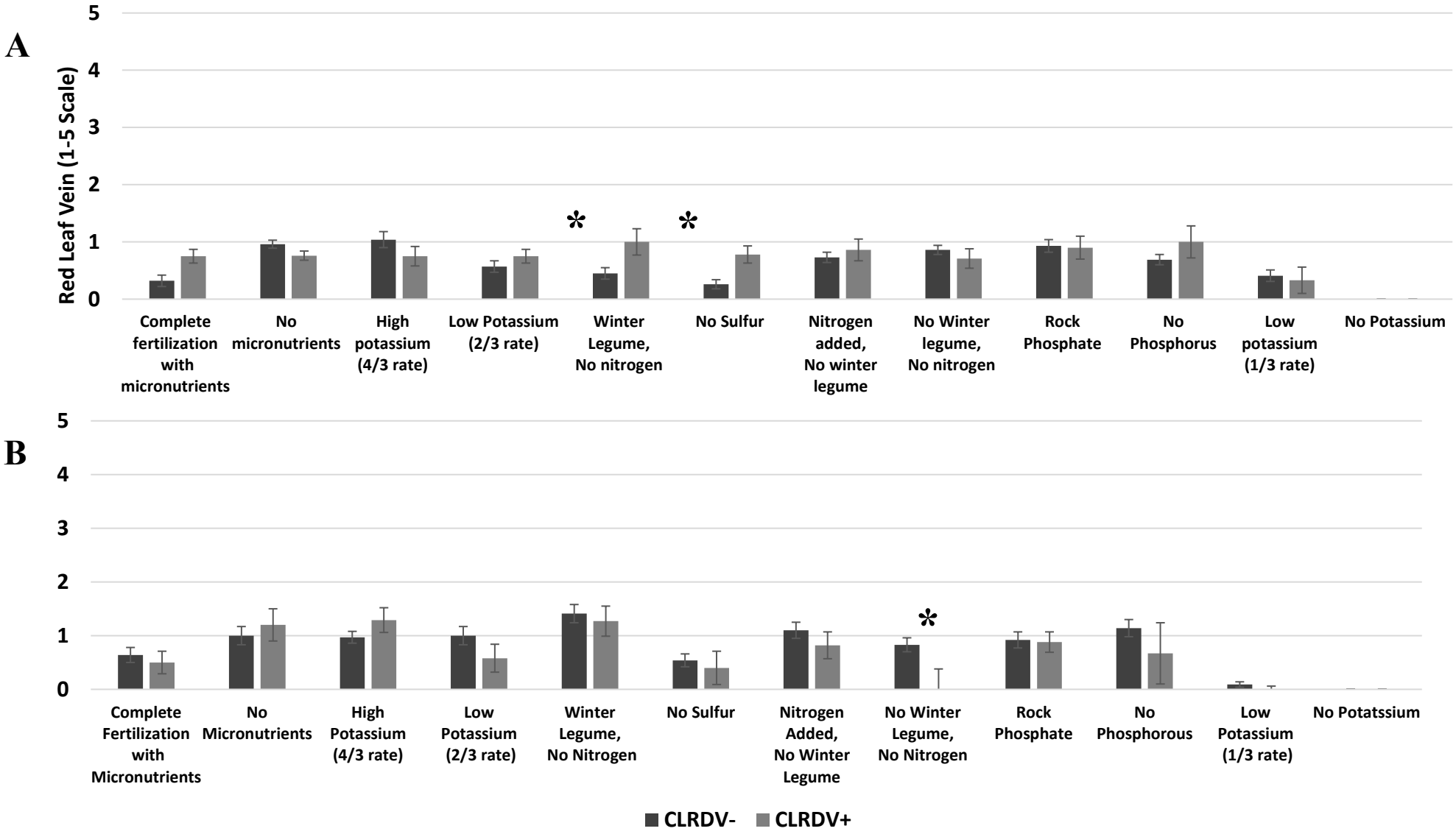

Figure 13: The average (±standard error) of red leaf vein severity (symptom severity rating, scale of 1-5) in (A) 2021 and (B) 2022. Means comparisons between plant testing positive (CLRDV+) and negative (CLRDV-) for CLRDV were performed separately for each nutrient treatment using Tukey's method at P=0.05. Asterisks indicate numbers are significantly different, and absence indicates no difference. Treatments without bars indicate no observations of the symptom.

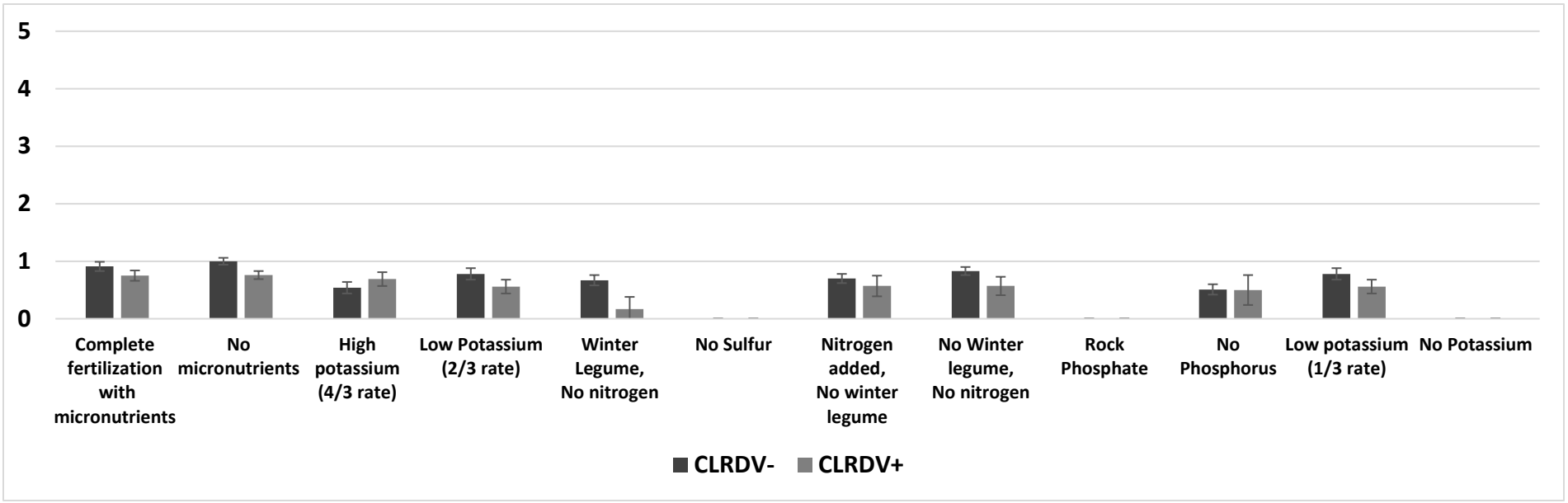

Figure 14: The average (±standard error) leaf tenting severity (symptom severity rating, scale of 1-5) in 2021. Means comparisons between plant testing positive (CLRDV+) and negative (CLRDV-) for CLRDV were performed separately for each nutrient treatment using Tukey's method at *P*=0.05. Asterisks indicate numbers are significantly different, and absence indicates no difference. Treatments without bars indicate no observations of the symptom.

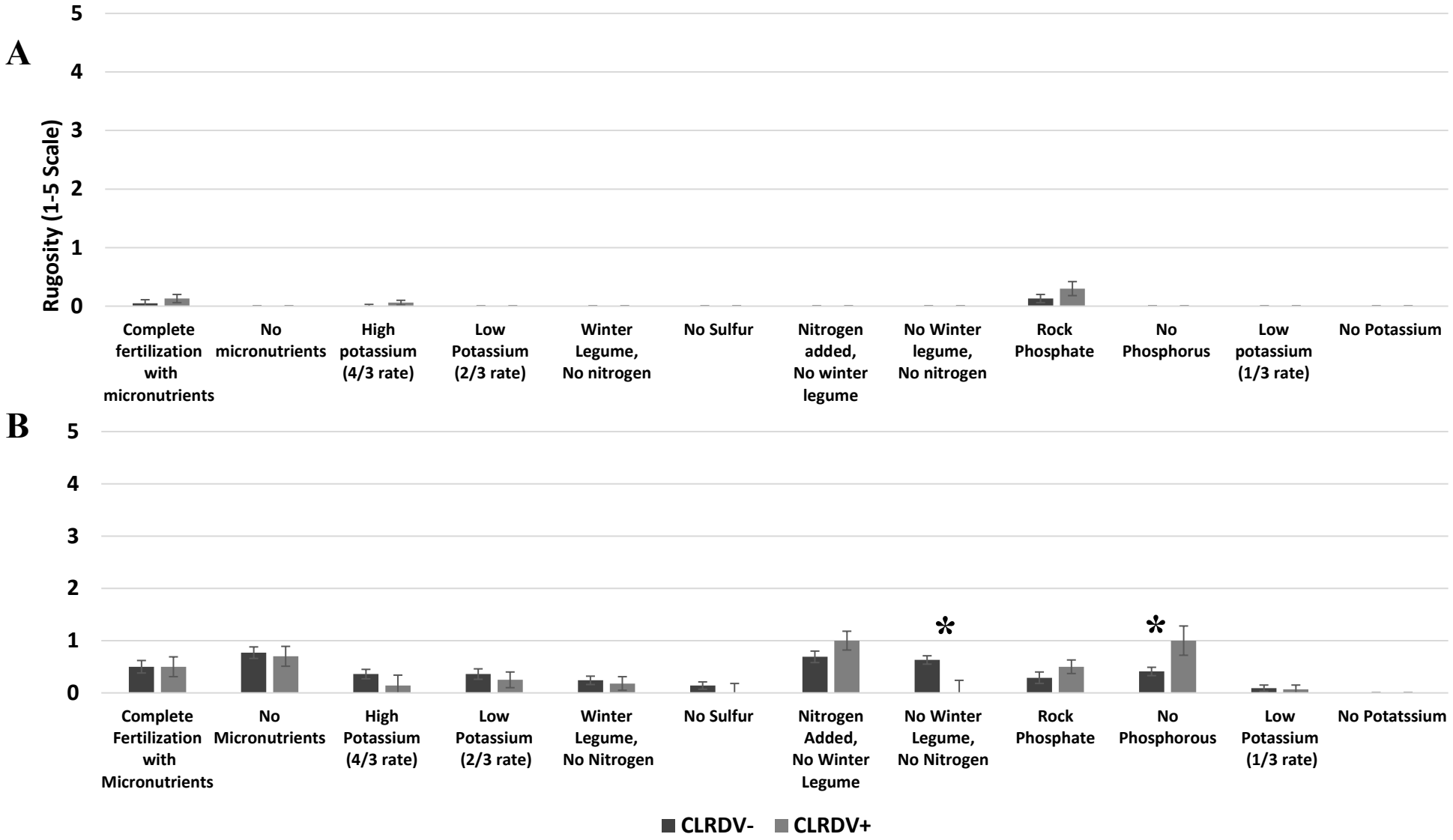

Figure 15: The average (±standard error) of rugosity (symptom severity rating, scale of 1-5) in (A) 2021 and (B) 2022. Means comparisons between plant testing positive (CLRDV+) and negative (CLRDV-) for CLRDV were performed separately for each nutrient treatment using Tukey's method at *P*=0.05. Asterisks indicate numbers are significantly different, and absence indicates no difference. Treatments without bars indicate no observations of the symptom.

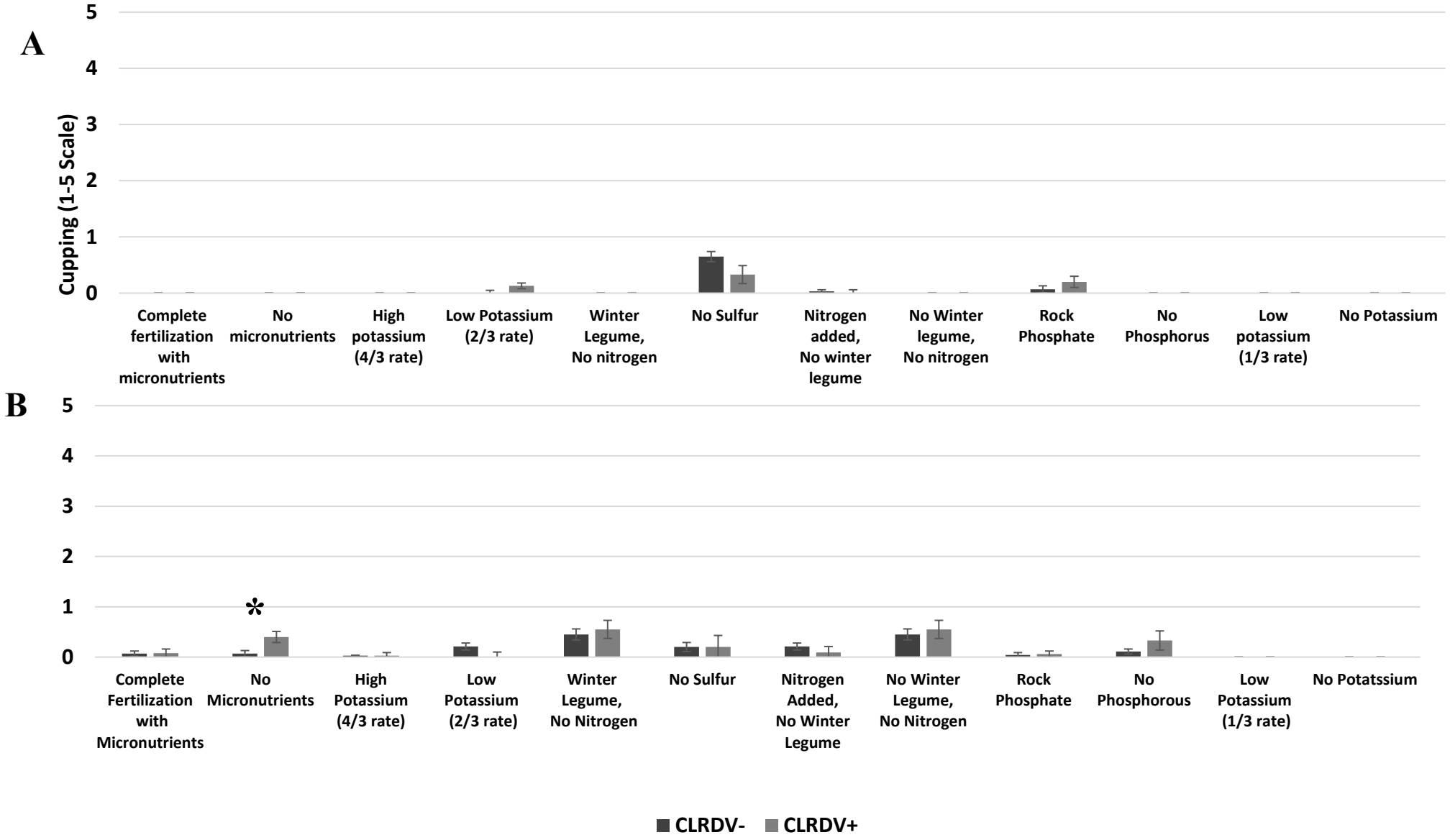

Figure 16: The average (±standard error) of leaf cupping severity (symptom severity rating, scale of 1-5) in (A) 2021 and (B) 2022. Means comparisons between plant testing positive (CLRDV+) and negative (CLRDV-) for CLRDV were performed separately for each nutrient treatment using Tukey's method at P=0.05. Asterisks indicate numbers are significantly different, and absence indicates no difference. Treatments without bars indicate no observations of the symptom.

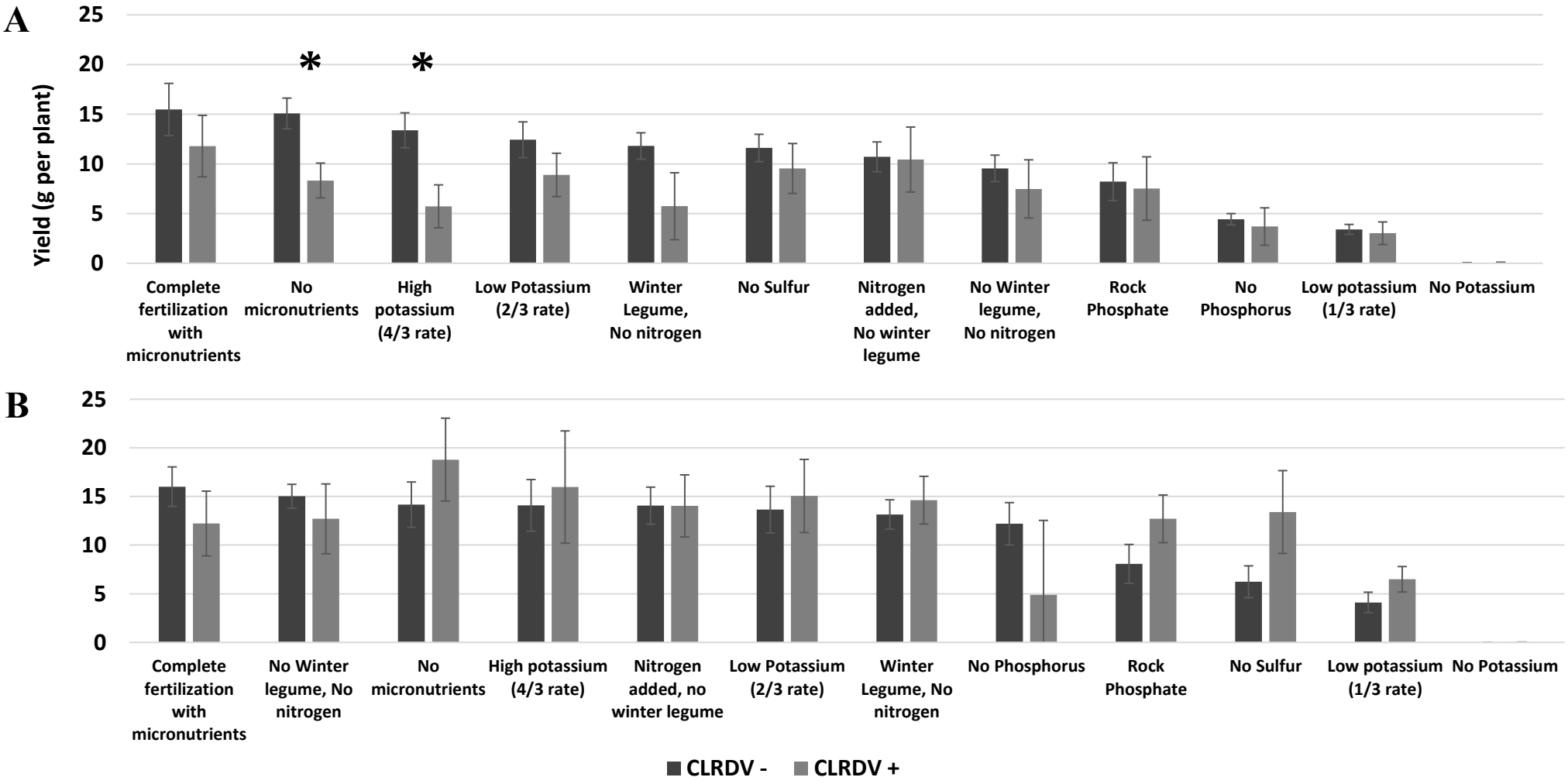

Figure 17: The average (±standard error) of lint yield (g/plant) in (A) 2021 and (B) 2022. Means comparisons between plant testing positive (CLRDV+) and negative (CLRDV-) for CLRDV were performed separately for each nutrient treatment using Tukey's method at *P*=0.05. Asterisks indicate numbers are significantly different, and absence indicates no difference. Treatments without bars indicate no observations of the symptom.

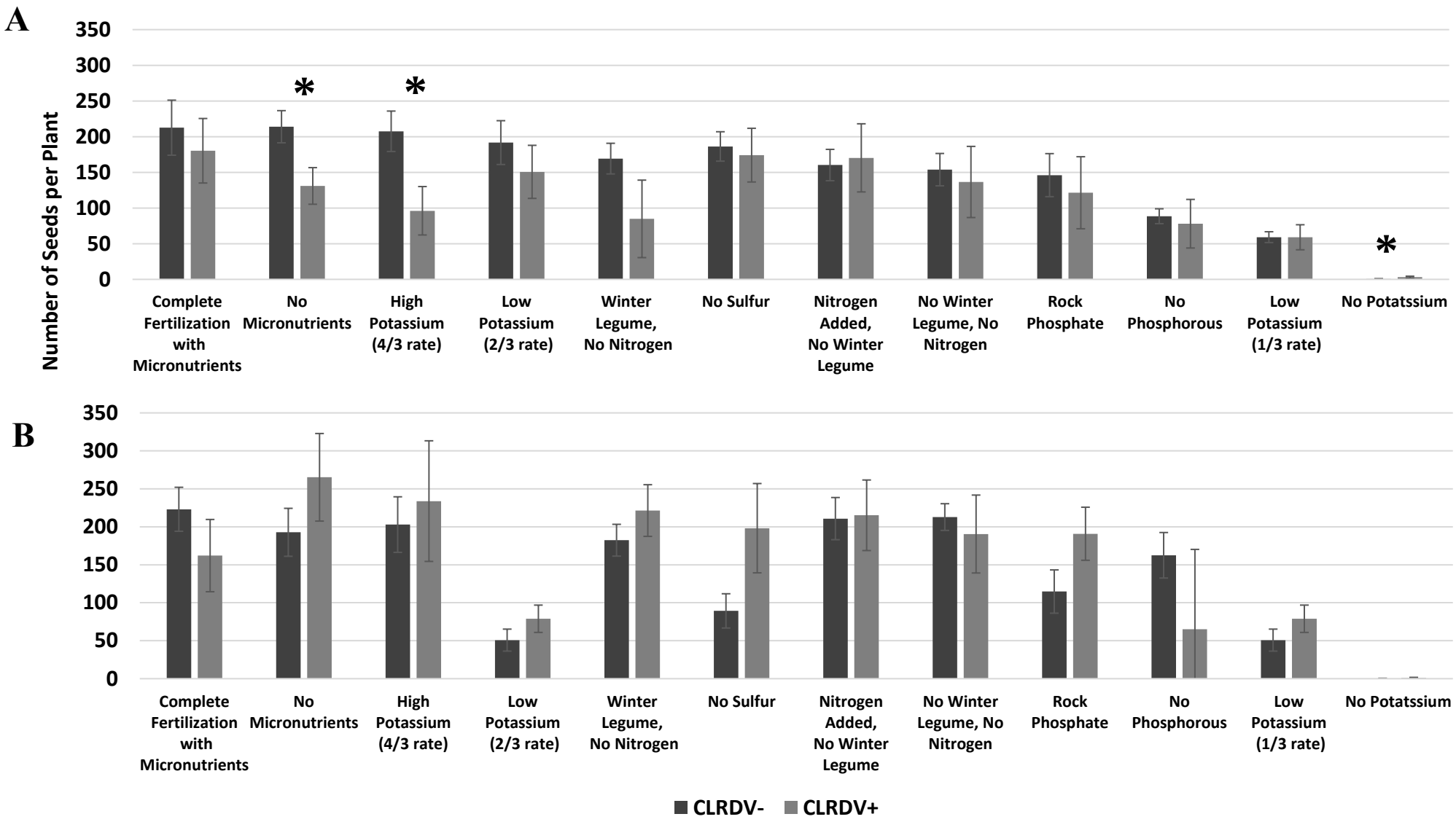

Figure 18: The average (±standard error) of seed count (number of seeds per plant) in (A) 2021 and (B) 2022. Means comparisons between plant testing positive (CLRDV+) and negative (CLRDV-) for CLRDV were performed separately for each nutrient treatment using Tukey's method at *P*=0.05. Asterisks indicate numbers are significantly different, and absence indicates no difference.

Chapter 4

Effects of temperature and Cotton leafroll dwarf virus infection in cotton

Introduction

Cotton leafroll dwarf virus (CLRDV) is an aphid transmitted polerovirus (family: Solemoviridae) capable of causing yield loss in cotton, *Gossypium hirsutum* L. (Avelar et al. 2019). The cotton aphid, *Aphis gossypii* Glover, is the vector of CLRDV and transmits the virus in a persistent, circulative manner (Heilsnis et al. 2023, 2022; Michelotto and Busoli 2007, 2003). The virus has been reported across cotton producing states in the Southeastern and Mid-southern U.S. as far as west Texas (Aboughanem-Sabanadzovic et al. 2019; Alabi et al. 2020; Ali and Mokhtari 2020; Ali et al. 2020; Faske et al. 2020; Iriarte et al. 2020; Price et al. 2020; Tabassum et al. 2019; Thiessen et al. 2020; Wang et al. 2020). CLRDV was first reported in Alabama with yield losses in 2019 (Avelar et al. 2019). Severe yield losses in commercial fields have not been reported since (Lawrence et al. 2019, 2020, 2021, 2022), but have been observed in research studies (Parkash et al. 2021) and research conducted for this thesis (Chapter 2 and 3). Yield loss caused by CLRDV in field settings is difficult to assess because the symptomatology of CLRDV remains undefined, asymptomatic infections occur, and costly RT-PCR is required to confirm infection status and field-level incidence (Brown et al. 2020). Results from previous research and the lack of apparent yield loss in fields with high incidence of CLRDV suggest the environment plays a role in disease development and yield loss. Further research is needed to better understand how interactions between environmental stressors and CLRDV infection in cotton impact yield.

Chapter 2 of this thesis found a significant reduction in yield in infected plants in one year of a three-year study when plants were visibly heat and drought stressed. It has been reported that heat stress can increase disease severity in other pathosystems (Prasad et al. 2022). This study aimed to quantify and compare yields, plant growth and root development in CLRDV infected plants versus healthy plants grown in elevated temperatures. Data was collected on a per plant basis so that effects between infected and healthy plants could be more accurately compared.

Materials and Methods

Cotton variety 'Deltapine 1646' (Deltapine®, Dekalb Genetics Corporation, Dekalb, IL) without an insecticide seed treatment was planted into 606 cell packs in peatlite (PRO-MIX 'BX', Quakertown, PA) on May 21, 2021 and 2022. Cotton was grown in an insect free environmental chamber (Percival Scientific Inc., Perry, IA) at 25 °C, 12:12 light:dark cycle and 50% RH until seedlings reached the first true-leaf growth stage. Seedlings were then placed in a screenhouse for the remainder of the experiment or used for aphid colony maintenance (see below). In the screenhouse, cotton was transplanted into tree pots with a 25.40cm diameter x 45.72cm filled with approximately 40 lbs. of 70:30 sand:peatlite mixture on July 7, 2021, and June 6, 2022. Pots were spaced 10.16cm apart in rows, with rows spaced 0.85m apart. Plants were fertilized using half-strength Hoagland's solution every two weeks for a total of three applications after cotton began squaring in both years (Hoagland and Arnon 1950). Plants were scouted weekly for unintended insects; no unintended infestations occurred throughout the course of this study.

The screenhouse was a 9.45m W x 15.24m L x 7.62m H rectangular structure with a semicircular arch, covered with plastic from the top of the screenhouse to 3.8’ from the bottom to exclude rain. The area inside was divided in half, and each half was enclosed using mesh 50 anti-insect screen (Green-Tek, Baldwin, GA) to exclude insects. The area was divided to prevent cross-contamination between healthy plants and plants infected with CLRDV. A HOBO RX3000 weather station (Onset, Bourne MA) was placed in the screenhouse to record temperature, PAR, dewpoint, and soil moisture content.

The aphid colony used for the experiment originated from multiple aphid individuals collected from a cotton field in Tallassee, AL in 2019. The colony was maintained in the greenhouse and reared on one to two true-leaf ‘DP1646’ (DeltaPine®, Dekalb Genetics Corporation, Dekalb, IL) seedlings. Every week two adult aphids were transferred to a one-true leaf cotton seedling (36 seedlings total) and allowed to reproduce for a week before repeating the process. CLRDV infected plants were collected in 2018 from a field in Tallassee, AL and grown under greenhouse conditions. CLRDV infections were maintained long-term with yearly aphid transmission to infect new plants, according to methods of Heilsnis et al. 2022. Plants were tested every spring to confirm infection status, using RT-PCR (Mahas et al. 2022) before use in experiments.

To transmit CLRDV to seedlings in the screenhouse, one to two true leaf cotton seedlings were infested with viruliferous aphids that had a 72-hour acquisition access period on CLRDV-infected plants. Plants were infested based on the availability of aphids. In 2021 plants were infested with five viruliferous aphids each at seven and ten days after planting. In 2022 plants were infested with 20-40 viruliferous aphids 12, 15, and 18 days after planting; after each 72-hour inoculation access period plants were sprayed with M-pede (Gowan, Yuma, AZ) to remove

present aphids before re-infesting. Each year a total of 60 plants were infested with viruliferous aphids, 48 plants were grown as healthy controls, and 12 plants that had been exposed to nonviruliferous aphids served as an 'aphid only' control. All plants were sprayed with 1.02 L/ha flupyradifurone on June 4, 2021, and June 19, 2022 to eliminate aphids from the screenhouse. Although an incubation period of six to eight weeks is best for detecting all CLRDV infections of U.S. isolates (Mahas et al. 2022) early infections can be detected beginning 30 days after inoculation (Galbieri et al. 2010). CLRDV testing was performed on plants infested with viruliferous aphids approximately 30 days after the first inoculation access period on June 28, 2021, and July 7, 2022, using RT-PCR (Mahas et al., 2022). Due to a low number of initial infections detected during early testing both years, cotton plants were reinfested with viruliferous aphids. Plants in the CLRDV-infected treatments were reinfested with 20 viruliferous adult aphids per plant on July 12, 2021, and August 5, 2022. Plants were then sprayed with insecticide to kill aphids July 14, 2021, and August 8, 2022, as described above. All plants were tested for CLRDV infection on September 7, 2021, and September 9, 2022, after cut-out to determine infection status used for data analyses.

End of season data was collected to characterize differences in plant growth and yield loss between healthy and CLRDV infected plants. Plant mapping was conducted at cut out on September 29, 2021, and September 27, 2022, and included recording the first fruiting node, total number of nodes, presence or absence of all boll positions, and plant height. Lint was hand-picked on November 12, 2021, and October 22, 2022, from each plant separately. Seed cotton was ginned using a 10-saw tabletop cotton gin (Dennis Manufacturing Co., Inc.), lint was weighed for each plant, and seeds were collected and counted using a Uline Economy Counting scale (Uline, Pleasant Prairie, WI). To obtain dried root weight, roots were harvested and

washed from each pot December 3 to December 19, 2021, and from November 8 to November 18, 2022. Soil from each pot was sifted through sieves constructed of a wooden frame (36.83cm W x 48.90cm L), with 0.635cm mesh hardware cloth (Garden Zone, Summerville, SC) secured to the bottom. Roots were washed in the sieve to remove soil and placed individually into paper bags. Roots were then dried in a 60 °C SC-400 shelf oven (Grieve Corporation, Round Lake, Illinois).

Data were analyzed using GLIMMIX procedure in SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). Means comparisons were performed between CLRDV infected and healthy plants using Tukey's method at the P =0.05 level. In preliminary analyses the aphid control was compared to the healthy (aphid-free) control; data from these two control groups were pooled for final analysis because aphid infestations did not result in significant differences in any variables measured. Separate analyses were conducted for each year. The main effect of infection was examined in different analyses for each variable measured: lint yield, seed count, plant mapping variables, and dried root weight. The sample size of healthy and CLRDV-infected plants was 60 and 45 in 2021 and 60 and 12 in 2022, respectively.

Results

Lint Yield and Seed Count

A significant reduction in yield was observed in CLRDV-infected plants compared to healthy plants in both years (Fig 19). In 2021, there was a 26% yield loss in the infected treatment, with healthy plants yielding 16.32 g of lint per plant, and infected plants yielding 12.01 g of lint per plant on average. In 2022, there was roughly 41% yield loss in the CLRDV-infected plants, with healthy plants yielding 6.32 g of lint per plant, and infected plants yielding 3.72 g of lint per

plant on average (Fig 19). There was also a significant reduction in the number of seeds produced in infected plants compared to healthy plants in both years of the study. Seed count was reduced by 23% in 2021, and by 36% in 2022 (Fig 20).

Plant Mapping

There was a significant reduction in plant height in CLRDV infected plants compared to the healthy plants in 2021, but not in 2022 (Table 12). Differences observed in the total number of nodes were not consistent between years. In 2021 CLRDV infected plants had fewer nodes, but more were observed in 2022. Retention of first position bolls was significantly reduced in infected plants in both years, but differences at other positions were either not statistically significant or consistent between both years. The sum of bolls at all positions were significantly reduced in CLRDV infected plants both years.

Root Weight

There was no significant difference in dried root weight in either year (Fig 21). Dried root weight was 14.68 g per plant and 13.51 g per plant in CLRDV negative and CLRDV positive treatments, respectively. Dried root weight was 6.71 g per plant and 5.83 g per plant in CLRDV negative and CLRDV positive treatments in 2022 (Fig 21).

Screenhouse Environmental Conditions

The environment inside of the screenhouse was notably hotter and more humid compared to the environment outside of the screenhouse (Fig 22). A weather station at the Auburn Regional Airport was used to compare temperature and dewpoint data from inside the screenhouse to the outside environment, starting from when the cotton was placed in the screenhouse until the end of September (July to September in 2021 and June to September in 2022). There was a significant increase in temperature inside of the screenhouse compared to the outside

environment in both years (Fig 22A), and there was a significant increase in dewpoint in the screenhouse in both years as well (Fig 22B).

Discussion

This study was conducted to determine if yield loss occurred in CLRDV infected cotton plants grown in heat stress conditions. To date, CLRDV yield losses measured in experimental settings have been variable due to environmental factors that were not measured in the study (Chapter 2 of thesis). Because measurements collected from research plots are comprised of infected and healthy plants data from small plots may not have been able to capture variation between healthy and infected plants (Chapter 2 of thesis, Mahas et al 2022). Significant yield loss occurred in both years of the study along with reduced first position bolls, total number of bolls, and seed set. This is consistent with other reports of a reduced boll set present in CLRDV-infected plants (Brown et al. 2020). Other poleroviruses are known to cause a reduction in root weight (Stevens et al. 2004; Stevens and Hallsworth 2003), and although a numerical reduction was observed, there were no significant differences.

There were no obvious visual differences between healthy and infected plants in this study. Symptoms were rated on a scale of one to five, with one indicating the least severity, and five indicating the most severity. Symptoms rated included cupping of leaves, reddening of stem, reddening of leaf veins, reddening of petioles, bronzing of the leaf, leaf tenting, leaf drooping, rugosity, and stunted plant growth (Avelar et al. 2019; Brown et al. 2020; Edula et al. 2023). The majority of symptoms were not rated above a severity of one in either year, and there were no significant differences between the healthy and CLRDV infected plants in any analyses (data not shown).

The environment of the screenhouse was much hotter and more humid than the outside environment. The optimum temperature for cotton is reported to be between 74.3 and 89.6°F (Burke et al. 1988). Temperatures above this can cause a reduction in lint yield and seed production (Constable and Bange 2015; Gao et al. 2021; Pettigrew 2008). Daytime temperatures in the screenhouse reached over 100° F several days both years and reached as high as 118°F in 2022 (data not shown). We are not able to state how much the high heat environment itself contributed to yield loss because it was not possible to include a comparable 'normal environment' treatment; any insect-free, rain exclusion cages we had access to raised the temperature of the environment.

This is the first study in the U.S. to document a consistent yield loss associated with CLRDV in more than one year of a multi-year study. These results provide support that heat stress is one environmental component that interacts with CLRDV to produce yield loss outcomes, even when visual signs of disease are not apparent. More research is needed to determine whether additional CLRDV-host-environment interactions reduce cotton yield.

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Plant Mapping								
Infection	**Plant Height (inches)**	**Total Nodes**	**1st position bolls**	**2nd position bolls**	**3rd position bolls**	**4th position bolls**	**5th position bolls**	**Total number of bolls**
2021								
CLRDV -	40.01(0.39) a	17.88(0.12) a	5.82 (0.17) a	1.90 (0.18) a	0.12 (0.06) b	0.00 (0.01) a	0.00 (0.02) a	7.83 (0.31) a
CLRDV +	36.85(0.45) b	17.47(0.14) b	4.47 (0.20) b	1.44 (0.21) a	0.31 (0.07) a	0.02 (0.01) a	0.04 (0.02) a	6.29 (0.35) b
Significance of Main Effects								
	$F_{1,103}$ =28.36, *P*= 0.000*1*	$F_{1,103}$ = 5.26, *P*= 0.0238	$F_{1,103}$ = 27.09, *P*= 0.0001	$F_{1,103}$ = 2.77, *P*= 0.0992	$F_{1,103}$ = 4.59, *P*= 0.0346	$F_{1,103}$ = 1.34, *P*= 0.2501	$F_{1,103}$ = 2.74, *P*= 0.1011	$F_{1,103}$ = 10.94, *P*= 0.0013
2022								
CLRDV -	47.62(0.69) a	19.13(0.34) b	4.85 (0.17) a	0.20 (0.07) a	0.02 (0.02) a	N/A	N/A	5.07 (0.17) a
CLRDV +	50.54(1.54) a	21.42(0.76) a	3.83 (0.38) b	0.17 (0.16) a	0.00 (0.03) a	N/A	N/A	4.00 (0.37) b
Significance of Main Effects								
	$F_{1,70}$ =3.01, *P*= 0.0870	$F_{1,70}$ = 7.57, *P*= 0.0075	$F_{1,70}$ = 5.96, *P*= 0.0171	$F_{1,70}$ = 0.04, *P*= 0.8489	$F_{1,70}$ = 0.20, *P*= 0.6579	N/A	N/A	$F_{1,70}$ = 6.88, *P*= 0.0107

Table 12: The average (±standard error) of plant height, total number of nodes, retention of first position, second, third, fourth, or fifth boll position, or the total sum of bolls in all positions were compared between CLRDV infected and healthy plants. Data were analyzed separately for each year and variable using GLIMMIX procedure in SAS (version 9.4). Means comparisons were performed using Tukey's method at *P*=0.05. N/A indicates no bolls were present at a particular boll position.

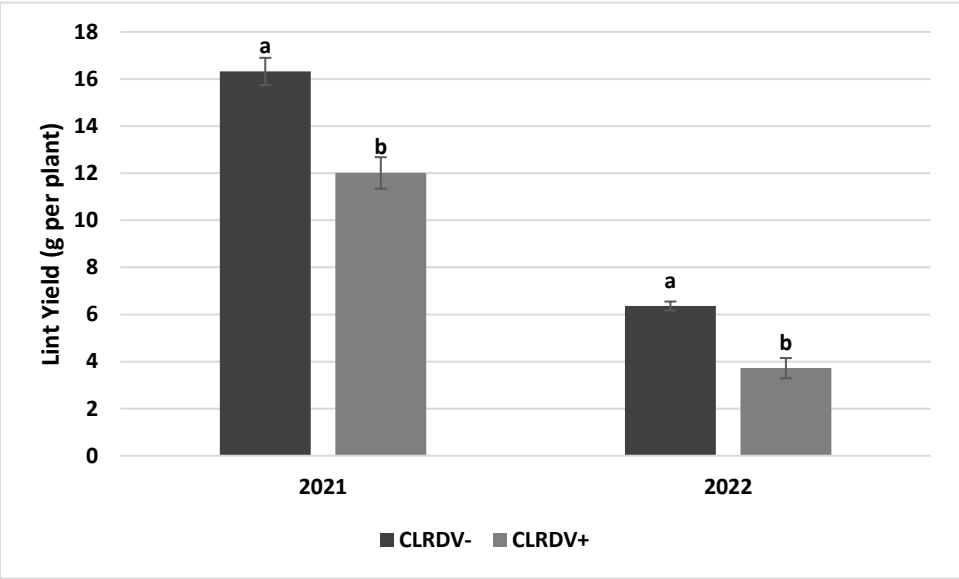

Figure 19: The average (±standard error) of lint yield (g per plant) in 2021 and 2022 for CLRDV infected and healthy plants. Means comparisons among treatments were conducted separately for each year and performed using Tukey's method at *P*=0.05.

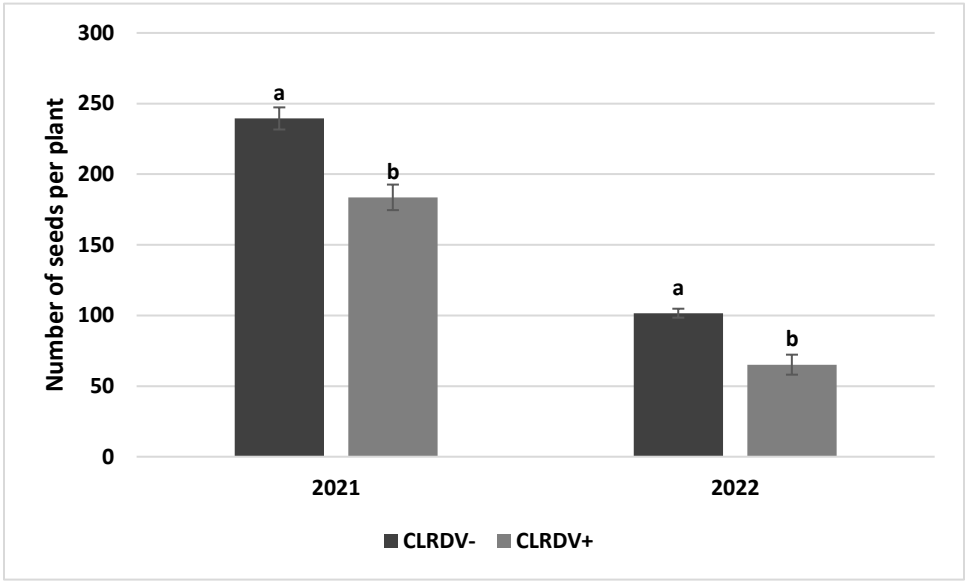

Figure 20: The average (±standard error) of seed count (number of seeds per plant) in 2021 and 2022. Means comparisons among treatments were conducted separately for each year and performed using Tukey's method at *P*=0.05.

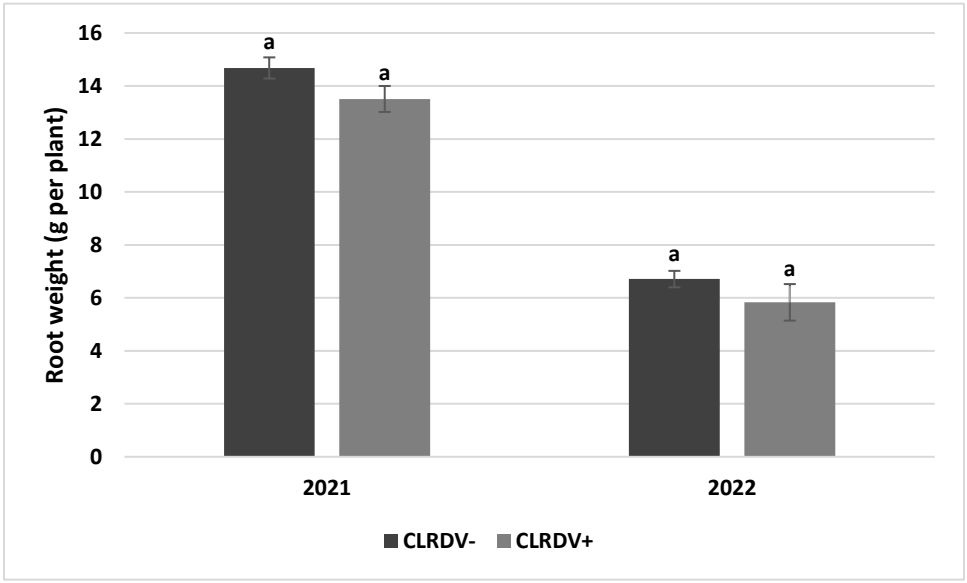

Figure 21: The average (±standard error) of dried root weight (g per plant) in 2021 and 2022. Means comparisons among treatments were conducted separately for each year and performed using Tukey's method at *P*=0.05.

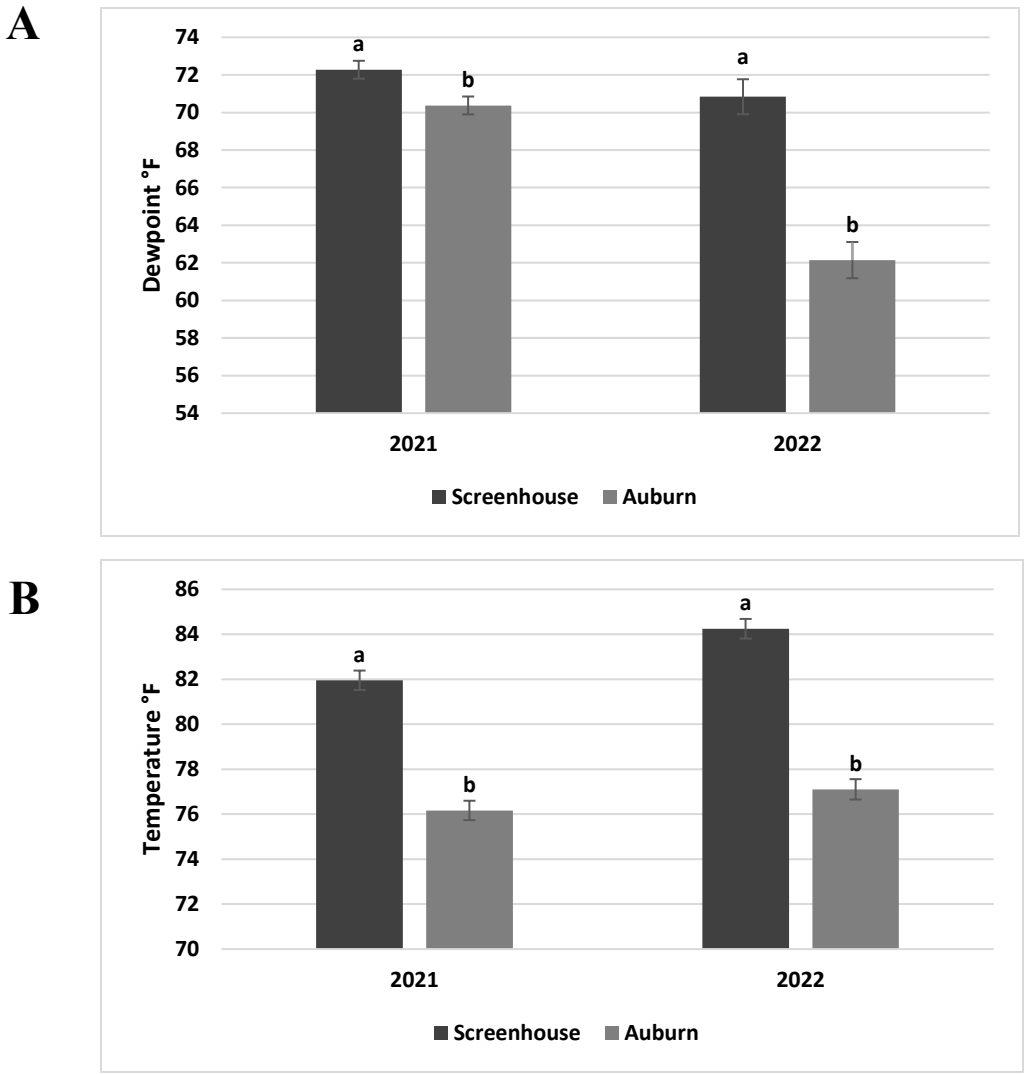

Figure 22: Average monthly temperature from June through September in (A) 2021 and 2022, and average monthly dewpoint from June through September in (B) 2021 and 2022. Means comparisons among variables were conducted separately for each year and performed using Tukey's method at P=0.05.