EFFECTS OF COLORED SHADE NET, IRRIGATION LEVEL, AND NITROGEN FERTILIZATION ON ORGANIC JALAPEÑO PEPPER (*Capsicum annuum* L.): PLANT PHYSIOLOGY, FRUIT YIELD, AND QUALITY

by

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ABSTRACT

Little or no information is available regarding the effects of colored shade nets, different irrigation levels, and the interaction between nitrogen and shading in jalapeño pepper. This study investigated the impacts of colored shade nets, irrigation levels, and nitrogen and shading effects on plant physiology, fruit yield, and the quality of organic jalapeño pepper in Tifton, GA. Using colored shade nets reduced the root zone temperature and light quantity, which enhanced plant vegetative growth. However, regarding fruit yield, shade nets did not improve the marketable fruit number and weight in jalapeño pepper. In addition, shade nets had either no or inconsistent effects between the two seasons on the fruit quality, such as water loss rate, total phenolic contents, total flavonoids, copper-reducing antioxidant capacity and Trolox equivalent antioxidant capacity, capsaicin, and dihydrocapsaicin of jalapeño pepper. Different N fertilizer levels did not significantly impact leaf gas exchange, fruit yield, or quality under shade nets. Nitrogen levels of 179 to 358 $kg \cdot ha^{-1}$ increased fruit yield and reduced the fruit water loss rate in unshaded conditions. Responses of the jalapeño pepper were inconsistent between years 1 and 2 in terms of plant biomass, leaf gas exchange, fruit yield, and quality because of variations in soil moisture, soil temperature, and evapotranspiration. Considering fruit yield and quality of jalapeño pepper, an irrigation level of 75% crop evapotranspiration can effectively save water. The shading net did not enhance jalapeño pepper fruit yield. Under open-field conditions, jalapeño pepper treated with 179 to 358 $kg \cdot ha^{-1}$ N (from organic fertilizer) resulted in the highest fruit yield. However, inconsistent responses between the two growing seasons while using different colored shade nets and irrigation levels require further investigation.

INDEX WORDS: Abiotic stress, leaf gas exchange, shade nets, jalapeño pepper, organic agriculture

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DEDICATION

This work is dedicated to my family and friends for their love, support, and encouragement.

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INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Capsicum includes various species, such as *Capsicum annuum, Capsicum frutescens, Capsicum chinense, Capsicum baccatum,* and *Capsicum pubescens,* native to temperate, subtropical, and tropical regions of the Americas (Shirasawa et al., 2013). Each species comprises many cultivated varieties, each with its characteristics in terms of fruit size, shape, color, flavor, and pungency (Ibiza et al., 2012). Jalapeño pepper (*C. annuum*) is a popular chili pepper cultivar widely used in various cuisines around the world. It is medium-sized pod-type fruit, typically 5-9 cm, with a thick and fleshy texture and distinctive conical or cylindrical shape with rounded tips.

The cultivation of vegetables in open field conditions faces many challenges caused by environmental factors, both abiotic and biotic (Rouphael et al., 2018). Abiotic stresses, including salinity, water-related issues such as drought and flooding, high temperatures, mineral imbalances or toxicities, are further exacerbated by the impact of climate change (Raza et al., 2019; Semida et al., 2013; Suzuki et al., 2014).

While hot peppers thrive in warm conditions, excessively high temperatures exceeding 32°C can hinder plant growth and yield. Extreme heat can cause disturbances in the usual physiological and biochemical functions of plants, diminish the number of flowers and fruit set, and compromise the overall quality of the fruit (Dodd et al., 2000; Rylski and Spigelman, 1986). Shading is commonly employed in Mediterranean countries like Israel and Morocco as a viable solution to mitigate the detrimental effects of excessive light and heat stress. (Demotes-Mainard et al., 2016; Tanny et al., 2014).

Proper water management is crucial for crops like peppers, which are sensitive to both water deficit and water surplus. Overwatering can lead to oxygen deprivation within the root zone, causing anaerobic conditions that are detrimental to plant health. Insufficient water restricts leaf expansion and reduces the photosynthetic capacity of the plant. Striking the right balance in watering practices is essential for optimizing the growth and productivity of pepper crops. (Saddiq et al., 1985). The impact of water status on crops is often clear through changes in vegetative growth and development. Chili pepper plants were reported to respond to water stress by restricting their rate of leaf area development, limiting dry matter production (Beese et al., 1982)

Besides, it is necessary to understand the relationship between nitrogen availability, the amount of photosynthetically active radiation (PAR) reaching the canopy, and its implications on crop morphology, anatomy, physiology, and yield. High light intensity and low nitrogen fertilizer adversely affected plant growth, chlorophyll content, anthocyanin concentrations, photochemical capacity, and anti-oxidative metabolism in *Arabidopsis thaliana* (Cohen et al., 2019). Different plant species may have different responses to light and N fertilizer fluctuations, so a balanced nitrogen supply and optimum PAR are essential for favorable plant development (de Oliveira et al., 2019). Optimum nitrogen fertilizer application and light interaction at the canopy level are essential for proper plant growth and fruit yield in chili pepper (Islam et al., 2018).

The objectives of the studies are to evaluate the effects of colored shading, organic nitrogen fertilization and shading, irrigation plant growth and physiology, and fruit yield and quality in organic jalapeño pepper

1.2 Literature Review

Light quality and quantity

Different characteristics of light, such as wavelengths, intensity, duration, and direction, can affect plant growth, physiology, and biochemistry (Chen et al., 2004). Responses of plants to light differ based on the environment, location, season, genotype, and cultivation practices (Kozai, 2016). The light spectrum is divided into three regions: Ultraviolet (100-400 nm), visible light (400-700), and far-red (700-800 nm) (Chen and Chory, 2011). The UV spectrum comprises three categories: UV-A (320-400 nm), UV-B (280-320 nm), and UV-C (<280 nm) (Jenkins, 2014). Visible light is categorized into violet (380-450 nm), blue (450-500 nm), green (500-570 nm), yellow (570-590 nm), and red (630-770 nm). The highest energy is got in the lowest wavelength of light. High light intensities can cause heat stress in leaves, resulting in enzyme deactivation and photo-inhibition (Yamori, 2016). Therefore, light manipulation is vital for improving the quantity and quality of plant products.

Photo-selective Nets

Colored mulches at the soil level and shading nets above the plants are used for manipulating the light that reaches the crop plants. Conventionally, black shading nets were mainly used to protect from intense sunlight. However, recently colored shading nets have often been used for crop improvement and production (Ben-Yakir et al., 2012). Shading nets are less expensive compared to the greenhouse and high tunnels and can provide manipulated light spectrum. Shading nets protect against heavy rain, too much sun, and pests while extending the harvesting season (Stamps and Chandler, 2008). Photo-selective nets can selectively modify specific components of solar radiation passing through the net holes unaffected (Shahak, 2012). This modification primarily affected the UV, PAR, and NIR wavelength ranges (Al-Helal and Abdel-Ghany, 2010; Castellano et al., 2008). Different colors of shade nets are available in the market, such as blue, gray, pearl, red, white, and yellow. Colored shade cloths can absorb the UV, red, and far-red spectral regions while enriching the spectral region of their respective colors. For example, red shade cloth can absorb UV, blue, and green spectral regions while enriching the far and far-red spectral regions. The pearl shade net is designed to scatter the light higher than other shade net colors (Rajapakse and Shahak, 2008). Colored nets can elevate temperature and are more wind resistant than black nets (Arthurs et al., 2013). Using a proper shade net helps to control vegetative growth, such as leaf size, branch length, and plant height. Shading nets, whether used in open-field production or in protected system, offer the flexibility to make microclimate adjustments according to the specific needs of different plants during various growth stages, seasons, and growing conditions.(Kalcsits et al., 2017; Mupambi et al., 2018).

Effects of colored shade nets on plant physiology, yield, and fruit quality

Reduction of transmitted solar radiation in greenhouses using colored shade nets increases crop productivity by about 40% by reducing the air, soil, and canopy temperatures and transpiration rate while increasing the water use efficiency (WUE) (Ahemd et al., 2016). Shading can be used both in summer and winter to change the microclimate either by reducing the radiation or by insulating it (Willits, 2001). Shading nets decrease light quantity and change light quality, which ultimately changes other environmental conditions (Smith, 1982) Ferreira et al. (2014) reported use of the reflective shade nets with a 40% shading factor reduced the PAR and solar radiation by 48.3% and 46.3%, respectively, without changing the temperature and RH when compared to open field conditions. While using different colored shading nets, such as red, blue, pearl, and black nets with a 50% shading factor, showed that PAR was reduced mostly under the black nets. Also, they reported high air temperature and wind resistance in colored shade nets than in black nets (Arthurs et al., 2013). However, relative humidity is often higher under the nets than outside because of the high transpiration rate of the crop and the reduction of the wind speed (Elad et al., 2007; Meena et al., 2014). Al-Helal and Abdel-Ghany (2010) reported that nets with bright colors (i.e., white, orange) showed higher transmittances than those with dark colors (black, green, and blue). Therefore, they suggested nets with a high porosity and bright color to cover agricultural structures under diffused radiation under cloudy or overcast weather conditions.

Hot pepper grown under 30% shade produced the tallest plant and largest leaf area compared to 80% shade and open field conditions (Lee et al., 2014). Retamales et al. (2006) found that high bush blueberry cultivars under black shade nets reduced PAR, which impacted the vegetative growth (increased internode length, shoot length, leaf area) compared to the open field. Kitta et al. (2014) studied the leaf gas exchange and photosynthetic light acclimation in sweet pepper leaves in Central Greece in the open field and three colored shade nets (pearl, white, and green) with different shading intensities and reported similar net photosynthesis in both shaded and unshaded leaves. Pearl nets, compared to unshaded and black nets, improved plant growth and morphology in different vegetable plants, such as pepper, tomato, lettuce, and cabbage (McElhannon, 2007).

The ideal shading level for most fruits and vegetables is 20-40% because a higher shading level above 40% reduces photosynthesis (wang Ma and Cheng, 2004). Traditional black shading nets replaced with colored shading nets (red, yellow, or pearl nets) of similar shading factors resulted in 15%-40% higher fruit production in different bell pepper cultivars (Shahak et al., 2008). Similarly, colored nets (green, red, and blue) exceeded 52.5% marketable fruit yield in the black net to 132.8% in the beige net. Unshaded conditions resulted in higher non-commercial fruits (Ayala-Tafoya et al., 2015). Also, Santana et al. (2012) evaluated the effect of photoselective blue and red screens on yellow and red sweet peppers' productivity compared to open fields and reported increased quality fruit yield in photoselective nets. Elad et al. (2007) studied the effect of different colored shading nets in two bell pepper cultivars (‘Vergasa’ and ‘Romans’) and found an increased number of fruits under nets than in open field conditions. (Ilic et al., 2012) studied the effect of colored shade nets such as pearl and red nets with 40% PAR transmissivity and blue and black with 50% PAR transmissivity on the productivity of tomato crops in Serbia. They reported increased tomato production by 35% in shading nets compared to open field cultivation. Kittas et al. (2015) studied the effects of four shade nets (black-40% and 49%, green- 34%, green and black mixed- 40%) and found reduced cracking disorder by 50% and increased marketable tomato fruit yield by about 50% compared to unshaded conditions. Shading greenhouse tomato did not affect the rate of fruit production within 3 weeks of application, but after more than 6 weeks, yield was reduced by 30% in 50% shade density (Gent, 2008).

Many health-promoting bioactive compounds, such as capsaicinoids, ascorbic acid, carotenoids, and flavonoids, are abundant in hot pepper (Srinivasan, 2005). Those bioactive compounds vary with cultivar and growing environment (Martí et al., 2011; Ziino et al., 2009). Under transparent plastic film, Semida et al. (2013) reported that Iceberg lettuce ‘Dublin’ showed a response to low night temperature by increasing secondary metabolites (total phenolic and flavonoid contents). Under pearl and black nets, Alkalia-Tuvia et al. (2014) found increased carotenoid content in two red cultivars of bell pepper during storage and shelf-life simulation compared to unshaded conditions. Selahle et al. (2015) reported that red and yellow sweet pepper fruits produced under the black net accumulated higher β-carotene, lower total phenolic contents, and deep red and orange color after storage. In other studies, pearl netting increased fruit firmness, ascorbic acid content, and antioxidant but reduce post-harvest water loss in sweet pepper (Kong et al., 2013; Mashabela et al., 2015) and tomatoes (Tinyane et al., 2013).

Effect of water stress during plant growth, fruit yield, and quality

Because of climate change effects, there is a reduction in freshwater availability due to decreased precipitation and increased evapotranspiration (Lee et al., 2018) which can create a potential threat to crop production. Qin (2021) stated that global agricultural production consumes about 70% of freshwater, and 23% and 27% global population are exposed to high and extremely high-water scarcity. Therefore, it is necessary to apply water-saving techniques that can increase the productivity of crops in water-limited areas by increasing water use efficiency (WUE).

The amount of water necessary for the crop depends upon the soil type, temperature, humidity, wind, and light (Sezen et al., 2007). Typically, vegetable crops require more frequent irrigation and more water per unit of dry weight produced than many agronomic crops (Dukes et al., 2010; Howell, 2001). Therefore, an adequate amount of water supply throughout the growing period is needed for the higher yield of the crop (Dorji et al., 2005; González-Dugo et al., 2007). Different plants respond and adapt differently to water stress through various morphological, biochemical, physiological, and molecular aspects, resulting in drought avoidance or drought escape (Basu et al., 2016). Water stress reduces the synthesis of chlorophyll and the activity of some enzymes; it also reduces the biomass of the plants, the leaf area, and the photosynthetic activity. High water stress (deficit) is more sensitive to expansive growth than carbon assimilation. Under water stress, leaf water potential, leaf area index, and photosynthetic rate significantly decrease in bell pepper (Delfine et al., 2001)

Excessive water also inhibits root growth and microbial activities and reduces leaf expansion and photosynthesis (Saddiq et al., 1985). During the flowering stage, excessive irrigation can cause flower loss and poor fruit setting, and rotting of fruit during the ripening stage (Doorenbos and Kassam, 1979). Qu et al. (1999) studied the effect of water-logging on apple trees and found that short-term water-logging increases leaf surface area and long-term water-logging can cause disorganization of leaf epidermis and stomatal closure. In cotton, water logging resulted in a reduction in stem elongation, shoot mass, root mass, and leaf number (Christianson et al., 2010). In pepper, judicious irrigation water management is essential for open field and greenhouse conditions because of its high sensitivity to water stress because of high transpiration from leaf surfaces (González-Dugo et al., 2007; Showemimo and Olarewaju, 2007).

Compared to traditional irrigation methods, drip irrigation is a viable option to improve water use efficiency. Antony and Singandhupe (2004) found that drip irrigation at 100% cumulative pan evaporation (CPE) was adequate for bell pepper var. California Wonder in plant height, root length, and fruit yield. However, Padrón et al. (2015) found the highest fruit weight at 75% ETc and better fruit diameter and length. Also, Fernández et al. (2005) found that water deficit did not decrease the number of fruits but increased unmarketable fruit due to smaller-sized fruit and more fruit with sunscald and BER incidence in bell pepper. Wang et al. (2019) studied nitrogen and irrigation rates in greenhouse tomatoes. They found that fruit yield (t/ha) was not significantly different between 80 and 100% ETo at 180 and 250 kg N/ha. Díaz-Pérez and Hook (2017) studied different irrigation level effects on bell pepper. They indicated that 130% ETc decreased marketable fruit yield by 24% compared to 100% ETc, which showed the adverse effects of excessive irrigation. Shabbir et al. (2020) studied the leaf morphology of tomatoes under full irrigation (100% ETc) and deficit irrigation (75% ETc). They found that leaf area, leaf area index, and specific leaf area were highest under full irrigation than under deficit irrigation. Deficit irrigation in citrus reduced the ratio of sugars to acids, delayed the ripening of the fruit, and reduced the fruit size and yield (Metochis, 1989). In greenhouse-grown bell pepper, the average yield from the 100% available water content (AWC) was highest than 40% and 20% but not different from the yield obtained from 60% and 80% AWC, which implies that water stress below 60% AWC creates an clear decrease in crop yield (Ihuoma and Madramootoo, 2019).

In some crop species, moderate water deficits can change the flavor of the fruits, i.e., fruit quality. Moderate water stress during early and late growth impact berries fruit color and biochemical compounds (Fereres and Villalobos, 2016). Phytochemicals such as flavonoids and groups of phenolics, help in stress amelioration, plant growth, and development (Cardenas-Manríquez et al., 2016; Nakabayashi et al., 2014). External factors, such as water availability, can influence capsaicin content, flavonoids, and phenolic content. Low irrigation frequency increased the content of vitamin C, total carotenoids, soluble solids content, and pH in green peppers (Marín et al., 2009). Leskovar et al. (2004) reported having a positive effect on watermelon quality, such as SSC, lycopene, and vitamin C, by applying regulated deficit irrigation. In hot pepper 'Battle', vitamin C content decreased under deficit irrigated conditions (Ahmed et al., 2014). The pungency levels of pepper fruits are affected by temperature, light, and fertilization (Arce-Rodríguez and Ochoa-Alejo, 2017). As previous research showed that capsaicin biosynthesis in hot pepper fruits competes with an active accumulation of soluble phenolics and lignin content (Hall et al., 1987), it is expected that water stress might affect the pungency level by altering the balance of this competition for capsaicin synthesis. Capsaicinoid accumulation in fruit depends on the stage of development and size of the fruit (Estrada et al., 1999; Fayos et al., 2019). Capsaicinoid levels in some hot pepper cultivars with small fruit sizes are less affected by water stress (Phimchan et al., 2012), but in the Habanero cultivar, it increased under severe water stress (Ruiz-Lau et al., 2011).

Effects of light and nitrogen on plant growth and fruit quality

Nitrogen is an important nutrient required for the growth, development, and production of secondary metabolites in plants (Verma and Shukla, 2015; Waterman and Mole, 2019). The direct link between the availability of N and radiation use efficiency highlights the central role of this nutrient in crop production (Gastal et al., 2015). Nitrate is the predominant form of nitrogen available to most plants grown under normal field conditions (Robbins, 1937). Based on the N assimilation capacity of plants as ammonium or nitrate, light availability can limit the regulation of enzymes such as Nitrate Reductase (Beevers and Hageman, 1980). Light and N regulate the photosynthesis and synthesis of active compounds in most vegetable crops. Balanced N supply helps to favor plant development during fluctuations in light intensities (Demšar et al., 2004).

In commercial varieties of *Capsicum chinense* (Biquinho and Habanero), N fertilization showed remarkable differences in terms of fruit set and size, sink-source ratio, and leaf morphology (de Ávila Silva et al., 2019). Also, increased N supply reduced, but shading increased specific leaf area (SLA) in both peppers. In marigold (*Tagetes erecta* L.), low and medium N levels and high light stimulated the synthesis of essential oils in leaves (Peralta-Sánchez et al., 2020). However, Marchese et al. (2008) found higher amounts of essential oils in different herbs, such as dill (*Anethum graveolens*), sage (*Salvia officinalis*), and pariparoba (*Pothomorphe umbellate*) when grown under shaded conditions. Similarly, shading increased the nutritional value of forage by increasing the N concentration in St. Augustine's grass *(Stenotaphrum secundatum*), Brazilian grass (*Axonopus compressus*), and Kikuyu grass (*Pennisetum cladestinum*) (Samarakoon et al., 1990). Pan et al. (2016) studied the effects of different shading systems and N rates in rice (*Oryza sativa*) in root morphology, nutrient accumulation, and photosynthetic parameters. They found that double shading reduced the total nutrient accumulation and decreased rice yields. Likewise, they did not find significant interaction effects between nitrogen and shading on the photosynthetic rate, transpiration rate, and total root length and volume of rice. Plants adapt to light and nutrient availability by modifying the leaf metabolism and biomass allocation. In lettuce (*Lactuca sativa*

L. var. Youmaicai), low N supply and high light intensity increased the leaf dry biomass accumulation due to an imbalance between carbon and nitrogen ratios In grapevines, high N and low light increased vegetative growth but favored the incidence of fungal diseases in leaves and fruit resulting in a lower number of berries per bunch (Smart, 1985).

Conclusion

Therefore, it is important to study jalapeño pepper fruit under limited light intensity, i.e. under shade nets, limited water availability, and optimum N in shading conditions to anticipate the impacts of climate change on its growth, physiology, fruit yield, and quality. It is crucial to understand the threshold limit for water, temperature, nutrient, and light for increasing the production of jalapeño pepper.

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CHAPTER 2

SHADE NETS CAN INCREASE PLANT GROWTH BUT NOT FRUIT YIELD IN ORGANIC JALAPEÑO PEPPER (*Capsicum annuum* L.).

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ABSTRACT

Shade nets can be an effective strategy to reduce adverse climate impacts. Using colored shade nets on horticultural crops, especially vegetables, has become popular because of the net's spectral properties. Colored shade nets can reduce the light intensity and change the spectral bands of light, thus affecting crop quality, yield, and biosynthesis of metabolites. Little or no information is available regarding the effects of different colored shade nets on jalapeño pepper (*Capsicum annuum* L.) plant growth, physiology, and yield. This study aimed to investigate the impact of colored shade nets on plant growth and the fruit yield of jalapeño pepper. The treatments were black, red, silver, and white nets (40% shade factor) and unshaded as control. The results showed that the red net changed the light quality by shifting light intensity towards red and far-red wavelength regions. Differences in mean air temperature between shade net treatments were minimal. However, mean soil temperatures were lower under shade nets than in unshaded conditions. Plant stem diameter, height, and leaf chlorophyll content increased under shade nets than in unshaded conditions. There were no significant differences in foliar nutrients between shade treatments in both seasons. The highest transpiration and stomatal conductance were observed in unshaded and white shade nets. Other parameters such as net photosynthesis, electron transport rate, intercellular CO_2 concentration, and water use efficiency were not significantly different between the treatments except photosystem II efficiency (PhiPS2), which was reduced under unshaded conditions. In both years, total marketable and non-marketable yields were not significantly different between shade nets and unshaded conditions. However, in 2019, the total yield decreased with the increasing incidence of bacterial leaf spot disease. Overall, shade nets enhanced plant vegetative growth but not fruit yield and productivity in jalapeño pepper.

Keywords: Colored shade nets, heat stress, jalapeño pepper, organic system.

2.1 INTRODUCTION

Plants routinely need to adapt to changes in their environment, including changes in light quantity, quality, and duration (Fankhauser et al., 1999a; Ouzounis et al., 2015). Photoreceptor pigments allow plants to sense these changes and trigger the necessary morphological and physiological responses (Franklin and Quail, 2010). Because of global climate change, there is an increasing trend in air temperatures, solar radiation, and atmospheric CO_2 concentrations, challenging current vegetable production methods (Hatfield et al., 2011). Therefore, applying cost-effective, environment-friendly, and sustainable technology is essential for improving vegetable production.

Recently, innovative technologies such as shading nets have been used to alter the temperature and relative humidity and protect crops against insects, pathogens, hail, and heavy rainfall (Ben-Yakir et al., 2012; Stamps, 2009). Photo-selective shade nets can modify the light quality by altering light diffusion, reflectance, transmittance, and absorbance in the ultraviolet (UV) (100-400nm), photosynthetically active radiation (PAR) (400-700nm), and near-infrared (NIR) (760-1500 nm) wavelength ranges (Al-Helal and Abdel-Ghany, 2010; Castellano et al., 2008). Colored shade nets, in particular, have been of interest in recent studies, as they might offer unique plant responses compared to commonly used black shade nets (Díaz-Pérez and John, 2019; Ilic et al., 2017; Ilic et al., 2015).

Jalapeño pepper (*Capsicum annuum*) is a medium-sized pod-type fruit (5-10 cm long) that can be consumed as either green or ripe. Most U.S. commercial jalapeño pepper supply is grown in New Mexico, Texas, California, and on many small farms throughout the Southwest as a niche crop (Araujo et al., 2015). The total hot pepper production in the U.S. decreased from $135 million in 7,325 to $100 million in 4,653 ha (USDA-NASS, 2021). In the southern U.S., summer and fall production accounts for much of the supply of jalapeño peppers. Although hot peppers require warm conditions for better growth, temperatures above 32°C can limit plant growth and yield. High temperatures reduce flower numbers, fruit set, quality, interference with normal physiological and biochemical processes, and the nutritional quality of field-grown peppers (Dodd et al., 2005; López-Marín et al., 2011). Growers use shading technology in Mediterranean countries where hot summers provide an unfavorable environment for plant growth and reduce crop quality (Demotes-Mainard et al., 2016; Tanny et al., 2014).

In recent years in the U.S., the application of shade on horticultural crops is becoming popular, especially in protected structures such as high tunnels and greenhouses and in open field conditions in tree fruits such as apples, citrus, and grapes to reduce the loss due to abiotic and biotic stresses and to improve postharvest shelf life (Boini et al., 2021; Martínez-Lüscher et al., 2017). However, vegetable production in the open-field with shading nets is uncommon. The ideal shading level for most fruits and vegetables is 20%-40% because photosynthesis begins to decrease above a 40% shade level (Maughan et al., 2017). Further, shade values above 40% may cause flower drop and reduce fruit set in some crops (Rylski and Spigelman, 1986). Traditional black shading nets replaced with colored shading nets (red, yellow, or pearl) of similar shading factors resulted in 15%- 40% increased fruit production in different bell pepper (*Capsisum annuum*) cultivars (Shahak et al., 2009). Santana et al. (2012) evaluated the effect of photo-selective blue and red screens on the productivity of yellow and red sweet peppers compared to open-field. They reported increased fruit yield and quality in photoselective nets than in unshaded conditions.

There is an increased demand for jalapeño pepper production as the consumption rate has increased compared to other hot peppers in the U.S. (Burden and Huntrods, 2012; Lillywhite et al., 2013). Although bell pepper and jalapeño pepper belong to the same species (*Capsicum* *annum)*, their growth requirements may differ. There is limited information on the physiology and production of jalapeño pepper (Johnson and Decoteau, 1996; Rho et al., 2020). There are no studies on the effect of colored shading nets on organic jalapeño pepper. Therefore, this study aims to understand the growth, physiological responses, and yield of organic jalapeño pepper in the southeast U.S. during the spring-summer season.

2.2 MATERIALS AND METHODS

2.2.1 Experimental site, design, and treatments

Two field trials were conducted in the Spring-Summer of 2019 and 2021 at the Organic Horticulture Farm, University of Georgia, Tifton, GA. The soil was sandy loam with a pH of 6.5. In 2019, transplants were purchased from Veazey plant farm, Tifton, GA and planted in the field on 10 May However, in 2021, jalapeño pepper 'Compadre' (untreated seed) (Clifton seed co, Moultrie, GA) was grown for six weeks in a greenhouse using polystyrene 400-cell (2.5 x 2.5 cm cell) using commercial potting mixture Sunshine #1 (Sunshine Growers, Vancouver, BC). Seedlings were transplanted to an organically certified field (29 April 2021) on raised beds with two rows of plants per bed (45 cm distance between rows) and 30 cm separation between plants within the row. Each bed was 6.1 m long and contained 30 plants. Before laying mulch, the soil was fertilized with nitrogen (N), P_2O_5, and K_2O at 358 kg/ha, 286 kg/ha, and 215 kg/ha, respectively, using 5:4:3 organic poultry fertilizer (Symphony Organic Fertilizer, Virginia). Drip irrigation tape was placed 5 cm deep in the center of each bed (Ro-Drip; Roberts Irrigation Products, Inc., San Marcos, CA). The bed was covered with white-on-black (2019) and black (2021) plastic mulch (RepelGro; ReflecTek Foils, Inc., Lake Zurich, IL). Plants were irrigated when the cumulative crop evapotranspiration (ETc) reached about 1.27 cm.

The experimental design was a randomized complete block with four replications and five treatments of shade nets (black, red, silver, white, and unshaded as control). According to the manufacturer, the net shade factor was 40% (Green-tek, Janesville, WI). Shade nets were installed one month (11 June 2019 and 29 May 2021) after transplanting into the field using a north-south orientation. Each shade net was 2.7 m wide, 2 m high, and 6.8 m long. Shade nets were suspended above the jalapeño pepper plants by metal fence posts and PVC pipe in tunnel-shaped structures, leaving the sides of the structures uncovered.

2.2.2 Microenvironment

Air temperatures were measured periodically (hourly) during the seasons (2019 and 2021) with a temperature sensor inside a datalogger (WatchDog 1650, Spectrum Technologies, Inc., Aurora, IL, USA). Root zone temperature (RZT) at a depth of 10 cm was measured with copper-constantan thermocouples (Model 107, Campbell Scientific, Logan, UT) connected to a data logger (CR10X and CR1000X; Campbell Scientific, Logan, UT). The RZT measurements started on 18 June 2019 and 7 June 2021 until the end of the season. The thermocouples were placed midway between plants. Light quantity was determined as the amount of light transmitted [Photosynthetic photon flux density (PPFD); 400-700 nm, ultraviolet (UV); (380-400 nm), blue (B); (400-500 nm), green (G); (500-600 nm), red (R); (600-700), far-red (FR); (700-780 nm). Light readings were taken between 12:00-13:00 HR using a spectrometer (LI-180, LI-COR, Lincoln, NE) on sunny and clear days in the mid-section above the plant canopy at two points. Regarding light quality transmittance, spectral ratios of light quantity at different spectra were calculated as B/G, B/R, G/R, R/FR, and PPFD/UV.

2.2.3 Plant growth and leaf chlorophyll content

Plant height and stem diameter were measured (five plants per plot) weekly, starting two weeks after placing shading nets. At the final harvest, two randomly selected plants from the middle section of each plot were cut down at the soil level to determine the top plant dry weight. The leaf area was measured using a leaf area meter (LI-3000C, LI-COR Lincoln, NE). Specific leaf weight, calculated as the ratio between leaf dry weight and leaf area, is an indicator of leaf thickness. Leaf chlorophyll index (CI) was measured with a chlorophyll meter (SPAD-502 Plus, Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA). Three leaves per plot were collected on 1 July and 20 July 2021 and analyzed individually for chemical composition. Carotenoids and chlorophyll (*Chl*) *a* and *b* were determined spectrophotometrically in fresh leaves. Leaf discs (2 cm^2) were collected from leaf interveinal areas, placed into tubes, and extracted in 1-1.5 mL ethanol (100% v/v) for 24 h. Pigment concentrations were calculated from the absorbance values of the extract at 470, 649, and 664 nm using the following equations (Lichtenthaler and Wellburn, 1983):

Chlorophyll a	C_a (µg/ml)	$C_a = 13.36A_{664} - 5.19A_{649}$
Chlorophyll b	C_b (µg/ml)	$C_b = 27.43A_{649} - 8.12A_{664}$
Carotenoids	C_{x+c} (µg/ml)	$C_{x+c} = (1000A_{470} - 2.13Ca - 97.63C_b)/209$

2.2.4 Leaf mineral nutrients

Leaf samples (25-30 fully developed leaves from new growth) were collected from five plants in the middle section of the plot. Leaf samples were dried at 70°C for 2 d to constant weight and analyzed for mineral nutrient concentration at the commercial laboratory (Waters Agricultural Laboratories, Inc., Camilla, GA).

2.2.5 Leaf gas exchange

Leaf gas exchange measurements (net photosynthesis (An), intercellular CO_2 (Ci), stomatal conductance (g_s), transpiration (E), water use efficiency (WUE) calculated as An/E, photosynthetically active radiation (PAR), and quantum yield of PSII (PhiPS2) were done with a portable photosynthesis system (LI-COR 6400XT equipped with an integrated 6400-40 leaf chamber fluorometer; LI-COR, Inc., Lincoln, NE, USA). The airflow rate was at 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$on the reference side. The CO_2 concentration was 400 $\mu mol \cdot mol^{-1}$ with a CO_2 mixer and a CO_2 tank. Ambient light tracking was used as a source of light for measurements. Measurements were conducted in developed plants on clear days at 1200 to 1500 HR using two developed and fully exposed leaves per plot. Measurements were done on 26 June and 11 July 2019 and on 15 and 25 June, and 27 July 2021.

2.2.6 Fruit yield

In 2019, jalapeño pepper fruits were harvested and graded as marketable and unmarketable without separating them based on fruit length. However, in 2021, jalapeño pepper fruit was graded based on the fruit length as marketable: large (7-10 cm) and medium (5-7cm). Anthracnose (*Colletotrichum acutatum*) infected, pepper weevil (*Anthonomus eugenii*) infested, and less than 5 cm long fruits were categorized as unmarketable (Jovicich et al., 2007). The number and weight of marketable and non-marketable fruit per plant were determined. Average individual fruit weight was derived mathematically from the marketable fruit weight and number. Four harvests were in 2019 (4 July to 23 July) and 2021 (17 June to 21 July).

2.2.7 Data analysis

Data were analyzed using a generalized linear model of one-way analysis of variance (ANOVA) of the means of the different treatments using R Statistical Software (Team, 2023) . When the ANOVA model showed significant treatment effects, a within-group separation was performed using Tukey's HSD test at 0.05% (Tukey, 1977). Regression analysis and graphs were prepared using the SigmaPlot 14 software (Systat Software, San Jose, CA). Data were analyzed separately per year because the plastic mulch color was different in 2019 and 2021.

2.3. RESULTS

2.3.1 Microenvironment

All shading net treatments had reduced incoming light intensity (PPFD) compared to the unshaded control. The PPFD was lowest with black (962 µmol m^{-2} s^{-1}) and red nets (1053 µmol m^{-2} s^{-1}) and highest in unshaded conditions (2151 µmol m^{-2} s^{-1}) (Table 2.1). UV was highest in the unshaded and lowest in black and red shade nets. Blue and green light intensity was highest in the unshaded and lowest in the red shade net. Red and far-red light intensities were highest in the following order: Unshaded > White, Red > Silver > Black shade nets. The blue/green ratios (B/G) were the highest in the red and the lowest in the white shade nets. The B/G ratio was similar among black, silver, and unshaded treatments. The lowest blue/red (B/R) and green/red (G/R) ratios occurred under red netting. Similarly, red/far-red ratios (R/FR) were the highest in unshaded and lowest under red nets. The PPFD/UV ratios were highest in the white shade net and were similar among the other four treatments. Spectral analysis showed that black, silver, and white nets were wavelength neutral and reduced the sunlight by the same amount over the entire visible spectrum (neutral nets) (Figure 2.2). In contrast, the red nets altered the spectral light distribution (photo-selective nets). They reduced light transmission in the blue and green spectra but increased the light transmission in the red and far red spectra. The results showed that light quantity depended on the net shading percentage, while light quality depended on net color.

In both 2019 and 2021, there was no significant difference in minimum air temperatures between treatments (Table 2.2 & 2.3). In 2019, the mean and maximum air temperature was highest in the white shade net (30.4°C and 41.5°C) and lowest under unshaded conditions (28.4°C and 33.9°C) (Table 2.2). The minimum soil temperature was highest in the unshaded (28°C) and lowest in black (26.7°C) and red (26.6°C) shade nets. Mean soil temperature was highest in the unshaded conditions (39.5°C) and lowest in black (28.5°C) and silver (28.6°C) shade nets. Maximum soil temperature was highest in unshaded, white, and red shade nets and lowest in black and silver shade nets. In 2021, the mean air temperature was highest under the red net (27.9°C) and lowest under the silver net (26.6°C), while the maximum air temperature was highest under the white net (43.9°C) and lowest under the silver net (33.6°C). Minimum, mean, and maximal soil temperatures were highest in unshaded conditions and lowest in black and silver nets.

2.3.2 Plant growth and chlorophyll content

Plants grown under black, red, and silver shade nets were significantly taller than in white shade nets and unshaded conditions in the 2019 and 2021 growing seasons (Figure 2.4 A & B). In 2019, stem diameter was not significantly different between treatments, but in 2021, unshaded conditions resulted in the lowest stem diameter compared to shade nets (Figure 2.4 C & D). There were no significant differences in top plant dry weight, leaf area, specific leaf weight, and normalized chlorophyll index in 2019. (Table 2.4). In 2021, there were no significant differences in top plant dry weight, specific leaf weight, CI, and normalized chlorophyll index. However, the leaf area was lower in unshaded conditions than under shade nets. Leaf chlorophyll a, b, and total chlorophyll were highest in the black shade net and lowest in unshaded conditions. However, leaf carotenoids were not different between shading nets and unshaded conditions (Table 2.5).

2.3.3. Leaf mineral nutrients

In 2019, shading treatments had no significant influence on the leaf mineral concentrations except Fe, which had increased concentration in the black net (Table 2.6). In 2021, foliar N was highest in silver net and unshaded conditions, and lowest in white net (Table 2.7). The Zn content was highest in silver net and lowest in unshaded conditions. The remaining nutrient concentrations were not significantly different between treatments.

2.3.4 Leaf gas exchange

In 2019, An, Ci, ETR, and WUE, were not significantly different between treatments (Table 2.8). The PAR ranged from 1881 $\mu mol.m^{-2}. s^{-1}$ in the unshaded treatment to 963.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the black shade net. The quantum yield of photosystem-II (PhiPS2) was the lowest in the unshaded treatment, but there were no significant differences between shade nets. Transpiration (E) was highest in white shade net and unshaded conditions. Stomatal conductance was highest in the white shade net, and the leaf temperature was lowest in the black shade net.

2.3.5 Fruit yield

In 2019, marketable and non-marketable fruit numbers and weight were not different among treatments. However, individual fruit weight was highest in the black shade net and lowest under unshaded conditions (Table 2 9). In 2021, there were no significant treatment differences in the number and weight of large-sized fruits. However, medium-fruit number and weight were highest in the unshaded and lowest in black and silver shade nets. The total marketable fruit number and yield per plant were not significantly different among the treatments (Table 2.10). Small fruit number and weight were highest in the unshaded and white shade net and lowest in the black shade net (Table 2.11). Fruit infected with anthracnose and pepper weevil and total non-marketable weight and number of fruits per plant were not significantly different among treatments. Total or marketable yields were not affected by shade treatment in 2019 because of bacterial leaf spot disease in foliage. In 2019, the total marketable yield decreased with the increasing incidence of bacterial leaf spot disease (Figure 2.5). However, in 2021, when there was not any incidence of bacterial leaf spot disease in jalapeño pepper, the total marketable yield and total yield increased with increasing light intensity (PPFD) (Figure 2.7).

2.4 DISCUSSION

2.4.1 Microenvironment

Colored shade nets cause significant changes in light quality and quantity. Irrespective of their color, shade nets can reduce the incidence of solar radiation reaching the plant canopy under the nets (Kalcsits et al., 2017). Shade nets have different transmission, diffusion, and reflection properties based on the net color and thread density. In our study, only transmitted light was measured. The red shade net was photo-selective, while the black, silver, and white were neutral shade nets. Photo-selective shade nets can change the spectral light composition by shifting the percentage transmittance of PAR towards the wavelength of the net’s particular (Arthurs et al., 2013). Our results for PAR under different shade nets are consistent with Arthurs et al. (2013) that even with the same shading factor (50% as reported by the manufacturer), black shade net reduced PAR the most compared to red, pearl, and blue nets while reduced least under the red nets. Also, nominal shading factor designations do not precisely determine actual PAR under the shade net. Reduction in PAR mainly depends upon the net type, color, mesh size (Middleton and McWaters, 2002), and architecture of the net installation.

In our experiment, the differences between treatments for average air temperatures were smaller than when comparing RZT. There are contradicting reports about the effect of shade nets on air temperature. Arthurs et al. (2013) reported an increase in air temperature under shade nets compared to unshaded. In contrast, Kalcsits et al. (2017) found no significant differences in air temperature under shade nets compared to unshaded conditions. Zhang et al. (2022) found that shade nets decreased air temperatures by 2.08 to 2.19°C compared to the unshaded condition in tea plants. Similarly, Mohawesh et al. (2022) found a significant reduction in air temperature by 3-5°C under black nets (50% shading intensity) than in white and green shade nets compared to unshaded conditions. Díaz-Pérez and John (2019) studied similar colored shade nets as in the present study and found a minimal reduction in air temperature (0.1- 0.2°C) under shade nets compared to unshaded conditions. In the present study, shade nets were supported by tunnel-shaped structures in east-west oriented rows, which protected the south-north facing side and top of the row. A reason for contradicting reports on the air temperature shift under the shading nets might be the height and direction of placing shading nets over the plant canopy. Tanny et al. (2008) also found the significant role of the height of the shade nets above the plant canopy in reducing air temperature as they found the same radiation but higher air and leaf temperatures in 2m-high screen houses compared to those that were 4m tall. However, in another study, an increase in height from 4 to 6 m did not change the mean air temperature and relative humidity (Teitel et al., 2017). In the current study, even though PPFD was different between the treatments, we did not find any relationship between air temperature and PPFD.

2.4.2 Plant growth

Increased growth indices such as plant height and leaf area under shade nets are adaptive plant responses as plants can activate the phototropic mechanism to improve the light-capturing capacity (Goyal et al., 2016; Smith, 2000). Similar to the current study, Kumar et al. (2013), Kitta Evangelini et al. (2014), and Díaz-Pérez and John (2019) reported that shaded plants were taller than those exposed to full sunlight. Also, Ilic et al. (2017) found a high leaf area index in lettuce under shade nets compared to unshaded conditions. In the present study, bacterial leaf spot disease in 2019 might have resulted in a reduction in the plant height, leaf area, and total plant biomass than in 2021. Similarly, Awad-Allah et al. (2021) also found a significant reduction in plant height, number of branches, and number of leaves per plant in sweet pepper infected with bacterial leaf spot disease.

Microclimate modifications such as temperature, light quality, quantity, and humidity affect plant chlorophyll content (Bourque and Naylor, 1971; Huang et al., 2017). Shaded leaves have enhanced chlorophyll content (a and b) to maximize light capture efficiency under low light intensity, increase the light-harvesting complex, and enlarge the antenna in PS-II (Horton, 2012; Ruban, 2015). Liu et al. (2020) observed increased chloroplasts and thylakoid compactness under shading in tea leaf plastids. Similarly, fresh leaves' increased high chlorophyll content is related to the duration and degree of shading rather than leaf age and shading season (Sano et al., 2018). Hikosaka and Terashima (1996) found a high *Chl a/b* ratio with increasing irradiance. However, in the present study, the *chl a/b* ratio was unaffected by the shade net treatments. *Chl a* is directly related to photosynthesis, while *chl b* is mainly related to PSII for trapping diffused light (Field et al., 2013). Our results agreed with Ilic et al. (2015) that total chlorophyll content is highest in black shade nets compared to unshaded conditions.

2.4.3 Leaf mineral content

There is limited information about the mineral nutrient uptake and accumulation of vegetable crops under shade nets. Shading nets did not significantly affect the leaf nutrient concentration in the present study (shade level between 30% and 50%). Díaz-Pérez (2013) also found minor changes in leaf macro-nutrients (N, K, Ca, and Mg) concentrations in bell pepper at 30% and 47% shade levels. However, Colonna et al. (2016) reported that leafy vegetables at the time of harvest accumulated more N, K, Ca, and Mg under low light intensity. Zhao and Oosterhuis

(1998) also showed that field cotton plants accumulated more leaf nutrients (N, P, S) under 40% shading than in unshaded conditions. In greenhouse bell pepper, leaf micro and macronutrients were not significantly different between no-netting and netting (30%) treatments (Gálvez et al., 2020). In the present study, although there was increased chlorophyll content in the shaded leaves, leaf mineral nutrients did not significantly differ between the shade treatments. In a study with 50% and 79% shading in perennial wall rocket (*Diplotaxis tenuifolia* L.-D.C.) leaf K, P, Ca, and Mg decreased with shading, while N, S, and Na were not significantly different between unshaded and shaded treatments (Caruso et al., 2020).

2.4.4 Leaf gas exchange

According to Ashraf (2001), leaf gas exchange varies depending on the environmental conditions and the crop being studied. The leaf-gas exchange measurements helped in evaluating plant responses under different shade nets. In Georgia, the maximum daily PPFD during midsummer is about 1500-2200 μmol $m^{-2}s^{-1}$. In field-grown solanaceous vegetable crops such as tomatoes (Kaiser et al., 2018) and bell pepper (Kabir et al., 2022), maximum net photosynthesis acclimated at about 50% to 75% light availability to full sunlight. There was similar net photosynthesis, net photosynthesis, intercellular CO2, electron transport rate, transpiration, and water use efficiency in the open field and 30% shade (Kabir et al., 2022). Also, Valladares and Niinemets (2008) found that shade-tolerant species maintain higher photosynthetic capacity and adequate growth rate under low light intensity compared to intolerant species (John and James, 2003; Syvertsen and Smith, 1984). Kitta E. et al. (2014) also found that sweet peppers grown under 13% and 34% white shade nets and 36% green shade nets maintained similar net photosynthesis as in unshaded conditions, irrespective of shading intensity and net types because of physiological acclimatization. Consistent with our results, Campany et al. (2016) found that the unshaded leaves had higher g_s and E than in shaded leaves. It might be because of the variation in size of stomata between shaded and unshaded conditions, as larger sized stomata open slowly than small sized (Drake et al., 2013; Fanourakis et al., 2015).

2.4.5 Fruit yield

In the current study, the total fruit number and yield in 2019 were affected by bacterial leaf spot disease incidence in foliage. The disease appeared the second week after transplanting and reduced plant growth (height, diameter, and leaf area) and fruit yield. One reason for the acute disease incidence in 2019 might be seed contamination, as transplants were purchased from a nursery. Robinson et al. (2006) found a curvilinear relationship between temperature and *X. campestris* pv. *vitians* infectivity on lettuce where lesion number per leaf reduced below 15 °C and at 30 °C. We also observed a decreased incidence of bacterial leaf spot disease when the temperature increased beyond 30°C (Figure 2.6). Regarding the fruit yield loss because of disease infection in foliage, Dougherty (1978); Pohronezny and Volin (1983) also found decreased marketable fruit weight in tomatoes because of defoliation and early-stage infection. Moreover, Jenkins (1963) also found decreased bell pepper production with increased bacterial leaf spot. Similarly, in another study, infected plants had reduced bell pepper fruit number and weight (Awad-Allah et al., 2021).

In 2021, total fruit weight (g/plant) increased quadratically, and total marketable yield increased linearly with increasing light levels (PPFD) (Figure 7). Similarly, Gent (2007), working with different shading intensities, found the total yield of tomatoes decreased linearly with increasing shade., In contrast to the jalapeño pepper in the present study, Díaz-Pérez et al. (2020) found decreased bell pepper yield under unshaded conditions but no differences between colored shade net treatments. Rylski and Spigelman (1986) observed increased sweet pepper production at 26% shading compared to unshaded conditions. Kabir et al. (2022) found increased bell pepper marketable yield at 30% shading (≈1400 μmol m^{-2} s^{-1}), equivalent to photosynthesis's light saturation point. In red bell pepper, 52% of the unshaded fruits were damaged due to sun scald disorder (Day, 2010). In eggplant, 21%-30% shading resulted in a higher marketable fruit yield than the open field. López-Marín et al. (2022) found that high radiation reduced marketable yield due to increased sunscald in bell pepper fruits. In bell pepper, Kabir et al. (2022) found maximum net photosynthesis, ETR, and WUE at 19% shade in bell pepper. The meta-analysis of yield responses of fruit vegetables at different levels of shade suggested that yield increased at 20% shading (Laub et al., 2022). Using a photovoltaic greenhouse in tomato plants showed that both total and marketable yield decreased with increased shading percentage (López-Díaz et al., 2020) due to a reduction in PAR for the crop canopy.

2.5 CONCLUSIONS

Colored shade nets changed microclimate, including light quality, quantity, and air and root zone temperature. Red shade nets changed the light spectral composition being photo-selective, while black, silver, and white being neutral shade nets only changed light quantity. Colored shade nets enhanced plant height, stem diameter, leaf area, and chlorophyll content in jalapeño pepper compared to the unshaded control. A 30%-40% reduction in PPFD did not consistently reduce the leaf gas exchange variables, such as net photosynthesis and stomatal conductance. Colored shade nets did not improve fruit weight and number in jalapeño pepper. The results provide new insight into the growth behavior and effectiveness of shading in vegetative growth rather than in the reproductive stage in jalapeño pepper.

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Tables and Figures

Table 2.1 Total PPFD, UV, Blue, Green, Red, and Far-Red light intensity and spectral ratios of transmitted light under black, red, silver, and white shade nets and unshaded in organic jalapeño pepper in Spring-Summer 2021, Tifton, GA.

	Light Intensity (µmol m^{-2} s^{-1})							Light Quality (ratios)				
Shade nets	PPFD	UV	Blue (B)	Green (G)	Red (R)	Far-Red (FR)	Reduced PPFD (%)	B/G	B/R	G/R	R/FR	PPFD/UV
Black	962 d[z]	24 c	263 d	341 d	358 d	263 d	55	0.77 b	0.73 a	0.95 a	1.36 ab	40.5 b
Red	1053 d	26 c	221 e	281 e	551 b	444 b	51	0.79 a	0.4 c	0.5 b	1.23 d	40.6 b
Silver	1217 c	29 b	329 c	433 c	455 c	343 c	43	0.77 b	0.72 a	0.95 a	1.32 bc	41.3 b
White	1534 b	31 b	402 b	544 b	588 b	448 b	29	0.74 c	0.68 b	0.93 a	1.31 c	48.9 a
Unshaded	2151 a	53 a	587 a	764 a	800 a	577 a	--	0.76 b	0.73 a	0.95 a	1.38 a	40.4 b
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 2.2 Average seasonal air temperature (Tair) and root zone temperature (RZT) under different colored shading nets in organic jalapeño pepper in Spring-Summer 2019, Tifton, GA.

	Tair (°C)			RZT (°C)		
Shade nets	Minimum	Mean	Maximum	Minimum	Mean	Maximum
Black	23.2	29 c[z]	36.6 c	26.7 d	28.5 d	30.6 b
Red	22.9	29.9 b	39.4 b	26.6 d	29.5 c	33.1 a
Silver	23	29.8 b	40.2 b	26.9 c	28.6 d	30.6 b
White	22.9	30.4 a	41.5 a	27.3 b	29.9 b	33.2 a
Unshaded	22.9	28.4 d	33.9 d	28 a	30.5 a	33.4 a
P-value	0.529	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 2.3 Average seasonal air temperature (Tair) and root zone temperature (RZT) under different colored shading nets in organic jalapeño pepper in Spring-Summer 2021, Tifton, GA.

	Tair (°C)			RZT (°C)		
Shade nets	Minimum	Mean	Maximum	Minimum	Mean	Maximum
Black	22.8	27.3 ab	34.3 cd	25.1 e	26.7 d	29.2 c
Red	22.9	27.9 a	39.3 b	25.7 c	27.3 c	29.2 c
Silver	22.9	26.6 b	33.6 d	25.5 d	26.8 d	28.7 d
White	23	27.2 ab	43.9 a	25.8 b	27.6 b	29.9 b
Unshaded	22.9	27.5 ab	35.7 c	26.7 a	28.9 a	31.5 a
P-value	0.843	0.021	<0.001	<0.001	<0.001	<0.001

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 2.4 Effect of colored shading nets on average above ground plant dry weight, leaf area, specific leaf weight, chlorophyll index (CI), and normalized CI in organic jalapeño pepper in Spring-Summer of 2019 and 2021, Tifton, GA.

Year	Shade nets	Above-ground dry weight (g $plant^{-1}$)	Leaf area (cm^2/leaf)	Specific leaf weight (g m^{-2})	CI	Normalized CI
2019	Black	75.2	14.65	40.85	47.23 ab[z]	1.33
	Red	70.3	15.64	50.46	51.2 ab	1.19
	Silver	62.5	16.62	47.08	54.67 a	1.22
	White	80.2	14.15	58.56	54.15 a	0.96
	Unshaded	65.2	13.14	79.84	42.72 b	0.80
	P-value	0.669	0.398	0.068	0.011	0.197
2021	Black	123.9	24.41 a	46.09	38.46	0.88
	Red	125.6	24.09 a	52.04	33.80	0.66
	Silver	125.2	24.36 a	49.42	39.12	0.83
	White	102.8	21.78 a	60.4	41.78	0.66
	Unshaded	107.5	16.22 b	61.95	40.28	0.70
	P-value	0.295	<0.001	0.115	0.299	0.328

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 2.5 Chlorophyll a (*Chl a*) and b (*Chl b*), carotenoids (car), and total chlorophyll (Total *chl (a+b)* in leaves of organic jalapeño pepper grown under different colored shading nets in Spring-Summer of 2021, Tifton, GA.

Shade nets	*Chl a* (µg/mL)	*Chl b* (µg/mL)	car (µg/mL)	Total *chl (a+b)* (µg/mL)
Black	7.68 a	3.07 a	1.95	10.75 a
Red	6.79 ab	2.79 ab	1.75	9.58 ab
Silver	7.36 a	2.90 ab	1.9	10.26 ab
White	6.89 ab	2.69 ab	1.83	9.59 ab
Unshaded	6.35 b	2.57 b	1.76	8.92 b
P-value	0.0017	0.054	0.13	0.003

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 2.6 Foliar nutrients (macro and micro) concentrations of organic jalapeño pepper collected at the mid-season fruiting stage under different colored shading nets in Spring-Summer 2019, Tifton, GA.

Shade nets	Macronutrients (%)						Micronutrients (ppm)				
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Black	4.36	0.36	3.85	1.5	0.45	0.56	38	62	105 a[z]	49	47
Red	4.39	0.36	3.91	1.39	0.42	0.57	34	46	94 b	47	43
Silver	4.41	0.34	3.83	1.46	0.46	0.55	33	44	96 ab	46	40
White	4.15	0.36	3.87	1.48	0.46	0.57	35	45	91 b	45	41
Unshaded	4.09	0.37	3.68	1.43	0.43	0.55	41	56	88 b	50	44
P-value	0.1101	0.898	0.754	0.822	0.819	0.961	0.584	0.573	0.0029	0.514	0.104

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 2.7 Foliar nutrients (macro and micro) concentrations of organic jalapeño pepper collected at the late-season fruiting stage under different colored shading nets in Spring-Summer 2021, Tifton, GA.

Shade nets	Macronutrients (%)						Micronutrients (ppm)				
	N	P	K	Ca	Mg	S	B	Cu	Fe	Zn	Mn
Black	2.78 ab[z]	0.5	2.8	0.87	0.31	0.35	33	12	148	46 ab	66
Red	2.84 ab	0.48	2.76	0.85	0.29	0.35	33	13	127	44 ab	89
Silver	2.88 a	0.48	2.94	0.79	0.31	0.36	31	13	136	47 a	67
White	2.71 b	0.54	2.85	0.85	0.3	0.36	35	13	139	44 ab	70
Unshaded	2.90 a	0.5	2.74	0.77	0.3	0.36	34	13	159	42 b	83
P-value	0.013	0.497	0.511	0.44	0.744	0.76	0.348	0.881	0.933	0.037	0.833

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 2.8 Net photosynthesis (An), intercellular CO_2 (Ci), transpiration (E), electron transport rate (ETR), stomatal conductance (g_s), leaf temperature (LeafT), photosynthetically active radiation (PAR), PSII efficiency (PhiPS2), and Water use efficiency (WUE), in organic jalapeño pepper in Spring 2019, Tifton, GA.

Shade nets	An ($\mu mol.m^{-2}.s^{-1}$)	Ci ($\mu mol.mol^{-1}$)	E ($mmol.m^{-2}.s^{-1}$)	ETR ($\mu mol.m^{-2}.s^{-1}$)	g_s ($mol.m^{-2}.s^{-1}$)	Leaf T ° C	PAR ($\mu mol.m^{-2}.s^{-1}$)	PhiPS2	WUE ($\mu mol.mmol^{-1}$)
Black	21.9	295.8	9.8 b[z]	137.9	0.48 b	32.5 b	963.2 d	0.34 a	2.4
Red	24.9	295.7	10.4 ab	148.9	0.46 b	33.8 a	1135.6 bc	0.30 a	2.2
Silver	23.7	300.2	9.8 b	135.9	0.46 b	33.5 a	1075.6 c	0.29 a	2.2
White	25.6	304.3	11.6 a	154.2	0.61 a	32.9 ab	1198.8 b	0.30 a	2.2
Unshaded	23.9	302	12.1 a	146.9	0.54 ab	33.8 a	1881 a	0.18 b	2.0
P-value	0.226	0.829	0.0003	0.362	0.019	0.003	<0.0001	<0.0001	0.118

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 2.9 Marketable weight, number, individual fruit weight, non-marketable weight, and fruit number per plant in organic jalapeño pepper under different shading nets in Spring-Summer 2019, Tifton, GA.

	Marketable			Non-marketable (Anthracnose and BER)		Total	
Shade Nets	N	Wt. (g/plant)	Individual Fruit Wt. (g)	N	Wt. (g/plant)	N	Wt. (g/plant)
Black	14.7	371	26.2 a[z]	0.15	3.8	14.9	374.8
Red	14.9	320	21.9 ab	0.28	6.1	15.2	326.1
Silver	16.1	363	22.3 ab	0.11	2.9	16.2	365.9
White	19.1	423	21.8 ab	0.1	1.9	19.2	424.9
Unshaded	17.3	303	17.5 b	0.04	0.9	17.3	303.9
P-value	0.587	0.426	0.0153	0.328	0.269	0.633	0.448

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 2.10 Marketable weight (Wt.), number (N), individual fruit weight, and total yield per plant in organic jalapeño pepper under different shading nets Spring-Summer 2021, Tifton, GA.

Shade	Large		Medium		Total marketable		Individual Fruit	Total yield	
nets	N	Wt.	N	Wt.	N	Wt.	Wt.	N	Wt.
	(per plant)	(g/plant)	(per plant)	(g/plant)	(per plant)	(g/plant)	(g)	(per plant)	(g/plant)
Black	3.5	116.4	6.4 b[z]	151.5 b	10	267.9	26.5	19.8	410.0
Red	2.9	98.6	7.4 ab	181.5 ab	10.5	284	26.3	21.4	454.3
Silver	3.5	113.3	6.3 b	154.3 b	10	270.4	26.9	20.7	445.1
White	3.1	102.5	8.1 ab	198.6 ab	11.2	301.1	26.3	27.7	568.9
Unshaded	3.3	101.4	11.0 a	264.7 a	14.4	366.1	24.3	29.4	635.1
P-value	0.98	0.985	0.024	0.043	0.303	0.571	0.062	0.073	0.13

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 2.11 Number and weight of non-marketable fruit of organic jalapeño pepper under different shading nets Spring-Summer 2021, Tifton, GA.

Shade nets	Small		Anthracnose		Weevil infested		Total non-marketable fruit	
	N	Wt. (g/plant)	N	Wt. (g/plant)	N	Wt. (g/plant)	N	Wt. (g/plant)
Black	4.4 b	83.7 b	0.33	9.4	5.1	49	9.8	142.2
Red	7 ab	127.6 ab	0.49	12.4	3.6	34.3	11.1	174.2
Silver	6.8 ab	128.5 ab	0.43	9.9	3.7	38.6	11	177.5
White	11.4 a	204.7 a	0.76	17.9	4.3	45.3	16.5	267.8
Unshaded	12.6 a	234.7 a	0.39	7.9	2	26.3	15	268.9
P-value	0.007	0.008	0.489	0.667	0.824	0.927	0.832	0.914

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Figure 2.1 Shade nets installed in the arch-shaped structure for jalapeño pepper plants in Tifton GA.

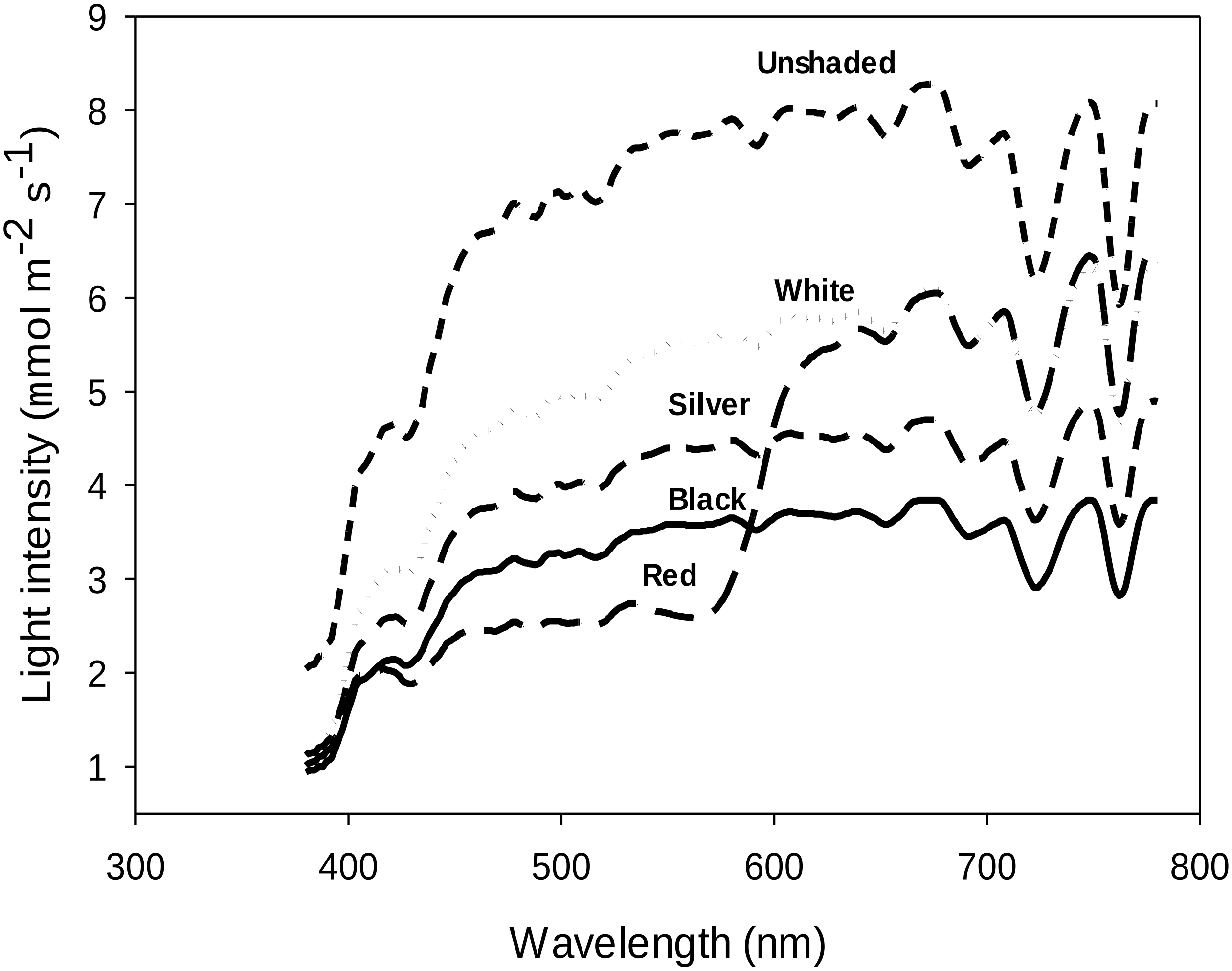

Figure 2.2 Light spectrum distribution under different colored shade nets at 380-780 nm wavelengths in organic jalapeño pepper in Spring-Summer 2021, Tifton, GA.

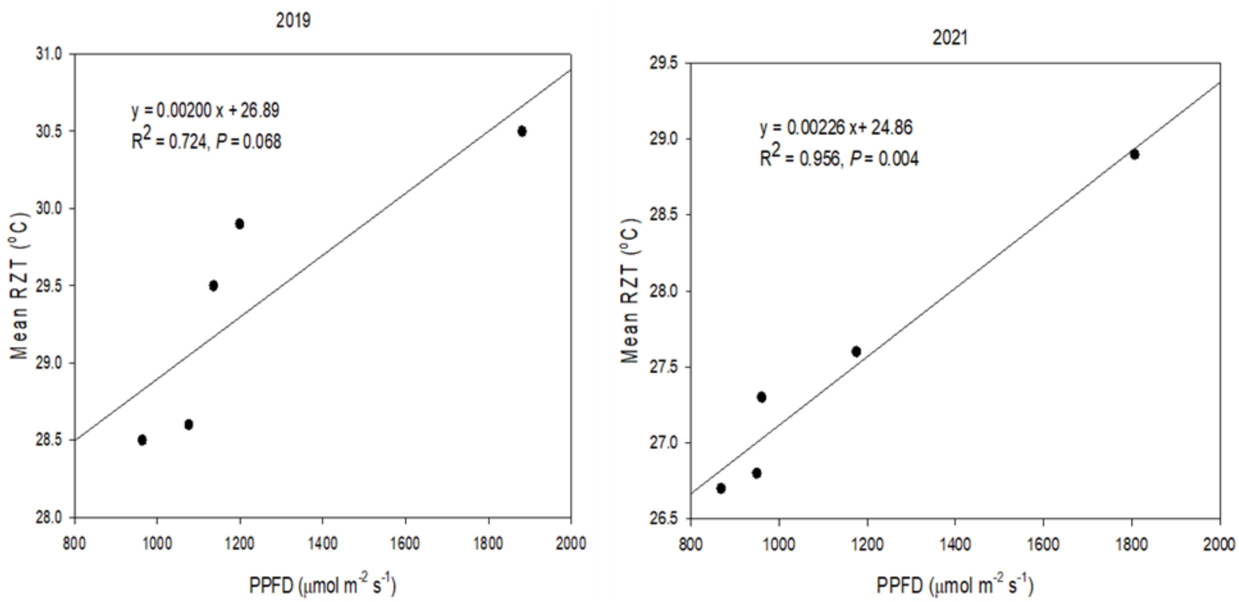

Figure 2.3 Mean RZT as affected by mid-day PPFD under different colored shade nets in organic jalapeño pepper in Spring-Summer 2019 and 2021, Tifton, GA.

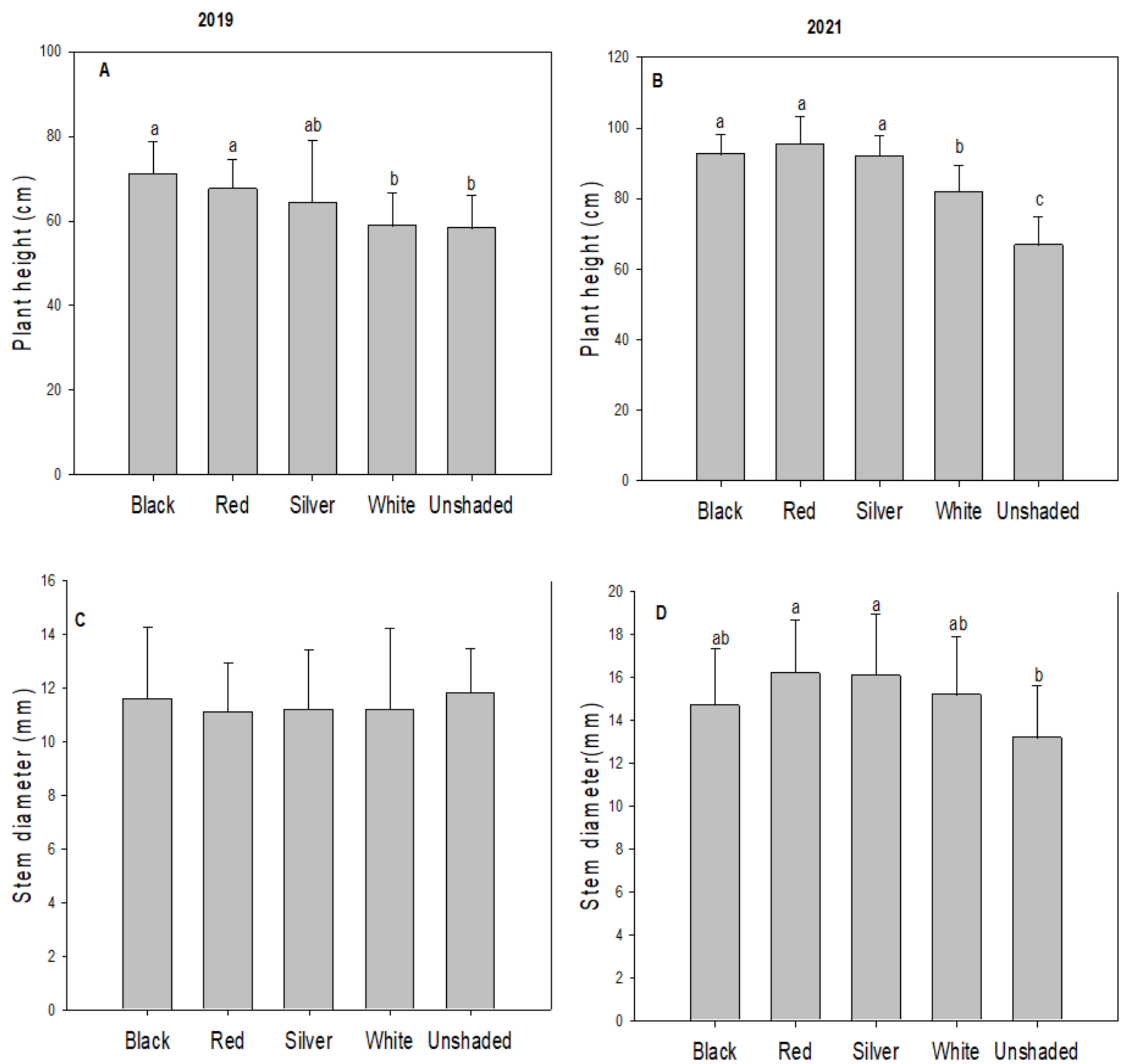

Figure 2.4 Plant height [2019 (A) and 2021 (B)] and stem diameter [2019 (C) and 2021 (D)] under colored shading nets of organic jalapeño pepper in Spring-Summer, Tifton, GA. Vertical bars indicate standard errors. Different letters on the top of the columns within each figure represent a significant difference between the treatments indicated by Tukey's HSD test at $p \leq 0.05$.

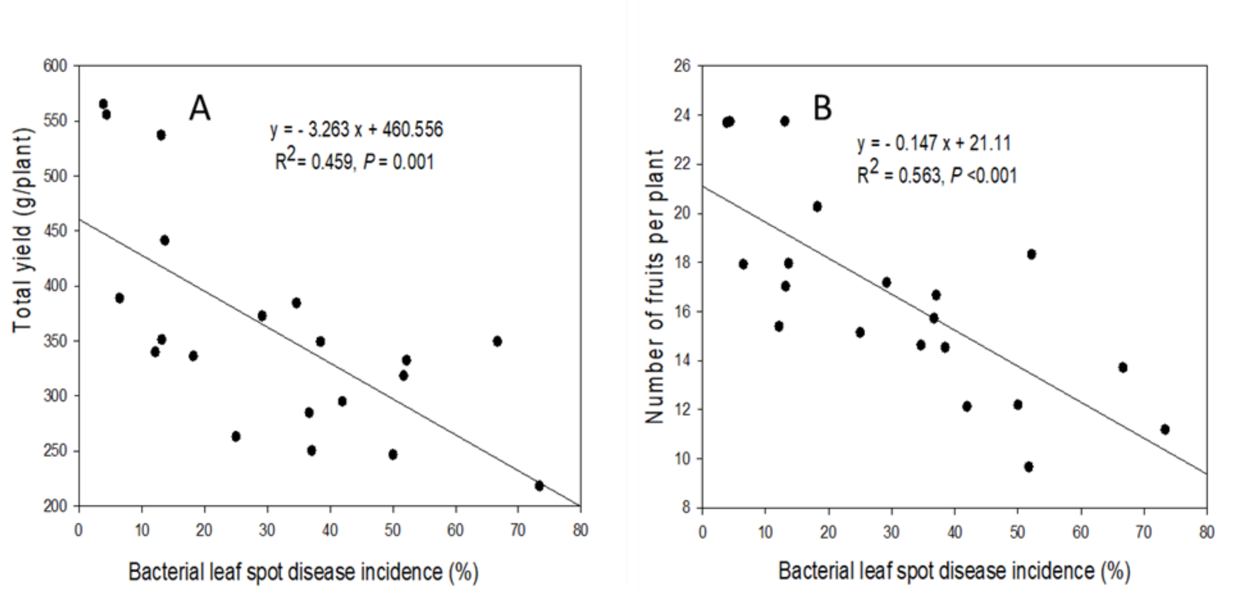

Figure 2.5 Total yield per plant (A) and fruit number per plant (B) as affected by bacterial leaf spot incidence [*Xanthomonas campestris* pv. *vesicatoria* (Xcv)] under different colored shading nets in organic jalapeño pepper in Spring 2019, Tifton, GA.

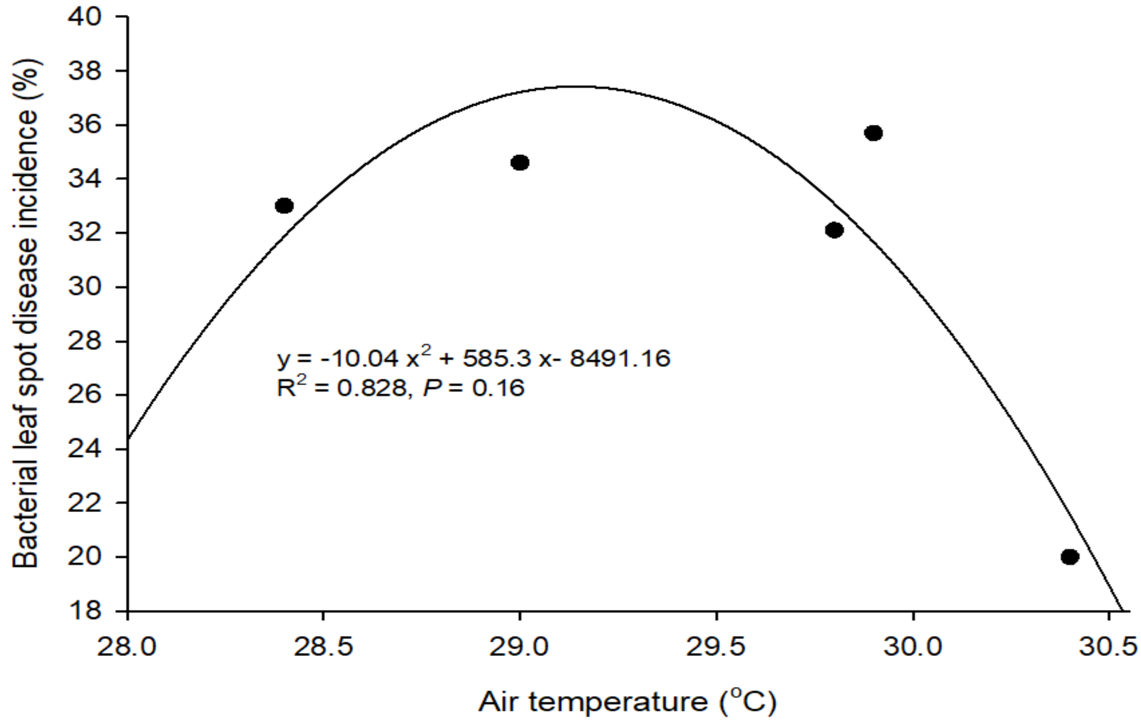

Figure 2.6 Relationship between bacterial leaf spot disease incidence and air temperature in organic jalapeño pepper in Spring-Summer 2019, Tifton, GA.

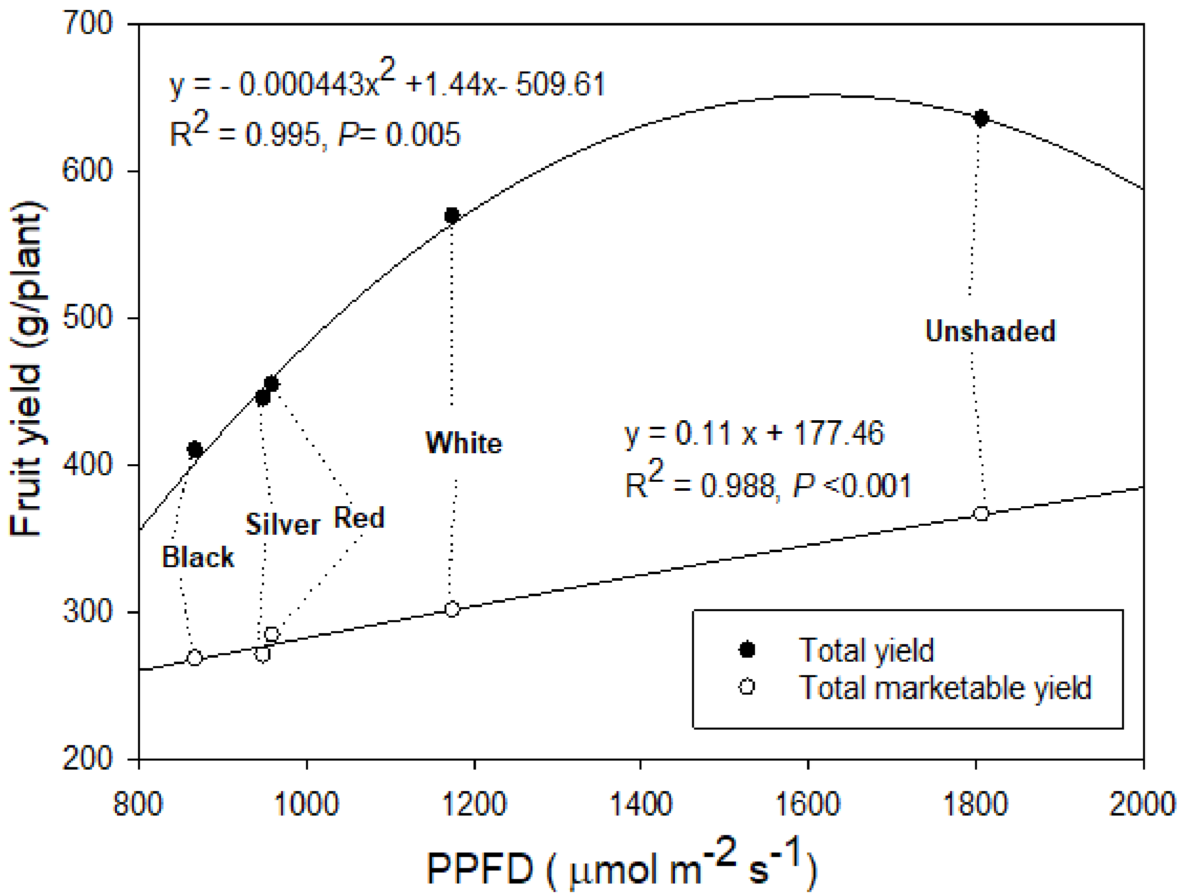

Figure 2.7 Marketable and total yield per plant as affected by PPFD under different colored shading nets in organic jalapeño pepper in Spring-Summer 2021, Tifton, GA.

CHAPTER 3

INFLUENCE OF COLORED SHADING NETS ON FRUIT QUALITY ATTRIBUTES OF ORGANIC JALAPEÑO PEPPER (*Capsicum annuum* L.)

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ABSTRACT

Jalapeño pepper (*Capsicum annuum* L.) is rich in nutraceuticals, such as capsaicinoids, carotenoids, phenols, flavonoids, and antioxidants. Due to global warming issues, research is carried out on protected agriculture technologies, such as colored shading nets. Shade nets modify the light quantity and quality, reaching the plant canopy. This study aimed to understand the effect of colored shade nets on fruit mineral concentration, surface color, water loss rate, and bioactive compounds of organic jalapeño pepper. The experiment was conducted in Tifton, GA, during the spring-summer of 2019 and 2021 in a randomized complete block design with four replications and five shade net treatments [black, red, silver, and white nets (40% shade factor), and unshaded as a control]. Results showed no effect of shade nets on the fruit mineral concentration of jalapeño pepper. Regarding fruit surface color, Lightness (L*) and Chroma (C*) value were highest in the unshaded treatments and lowest in the black shade nets. While hue angle was consistently high under black shade nets due to less PPFD and root zone temperature. Fruit water loss rate, total phenolic content (TPC), total flavonoids (TF), antioxidant capacity [Cupric Reducing Antioxidant Capacity (CUPRAC), Trolox Equivalent Antioxidant Capacity (TEAC)], and capsaicinoids (capsaicin, dihydrocapsaicin, and SHU) responded differently among shade nets treatments over two growing seasons. A strong and positive correlation existed between root zone temperature and TPC, TF, and CUPRAC. However, photosynthetic photon flux density (PPFD) was unrelated to TPC, TF, CUPRAC, TEAC, and capsaicin. In conclusion, colored shade nets had either no or inconsistent effects between two seasons on the fruit quality of jalapeño pepper.

Keywords: jalapeño pepper, colored shade nets, bioactive compounds.

3.1 INTRODUCTION

The *Capsicum* genus comprises the five most widely cultivated species, namely *C. annuum* L., *C. frutescens*, *C. chinense* Jacq., *C. baccatum* L., and *C pubescens* Ruiz & Pav. These species play a significant role in global production, contributing 48 million metric tons, which can be used as spices and vegetables (FAO, 2022). Peppers, in particular, are excellent sources of phytochemicals, such as vitamins, carotenoids, flavonoids, phenolic acids, and pungent capsaicinoids (Raffo et al., 2006), all of which are essential components of the human diet (Jayaprakasha et al., 2012). Production of such phytochemical can be influenced by the cultivar, maturity stage, growing environment, and post-harvest handling techniques (Chirinos et al., 2007; Shotorbani et al., 2013). To cope with abiotic and biotic stresses, leaves, flowers, and fruits contain antioxidant compounds that can serve as a defense mechanism (Hasanuzzaman et al., 2013; León-Chan et al., 2017).

Jalapeño pepper (*C. annum*) is the most commonly consumed hot pepper in the United States (Lillywhite et al., 2013) in fresh and processed form (pickles, salsa, and powder) (DeWitt and Bosland, 2009; Vera-Guzmán et al., 2017). They are excellent sources of antioxidants and bioactive compounds, reducing the risk of cancer and cardiovascular diseases (Alvarez-Parrilla et al., 2012; Cervantes-Paz et al., 2014). Pepper production in open field conditions primarily begins in March/April and extends until frost arrives, predominantly in Southern Georgia (Coolong et al., 2019; Díaz-Pérez and Hook, 2017). Fruit quality can be adversely reduced during summer production when temperatures surpass 35 °C. Protected agriculture technologies such as shade nets are employed to cope with these challenges and maintain fruit quality and yield (Caruso et al., 2020; Díaz-Pérez et al., 2020). Photo-selective nets that modify the quality of light are commonly used in bell pepper production in Europe, South Africa, and Middle Eastern countries (Fallik et al., 2008; Mashabela et al., 2015). These nets relatively increase the proportion of scattered light and modify the spectral bands of light in a particular wavelength (Shahak et al., 2008), allowing the deeper penetration of scattered light into the plant canopies (Castellano et al., 2008). They improve fruit color and firmness, delay ripening, and reduce post-harvest decay (Goren et al., 2011; Selahle et al., 2015). By adjusting the spectral light, such as red, blue, and UV, it is possible to enhance the bioactive compounds in fruits and vegetables, mitigating tissue damage caused by high light stress(Grace, 2005; Saewan and Jimtaisong, 2013).

When different varieties and types of Capsicum fruit (hot and sweet) are grown under shade nets of varying colors, the content of bioactive compounds and vitamins can vary significantly. For example, during post-harvest storage, pearl shade nets (40% shading) with a high spectral R/FR ratio and blue light (400-500 nm) increased antioxidant activity and ascorbic acid levels compared to black shade nets in sweet pepper varieties such as ‘Vergasa,’ ‘HTSP-3’, and ‘Celaya’ (Kong et al., 2013; Selahle et al., 2015). In another study, yellow shade nets (21% shading) increased the flavonoid content, while pearl nets increased the total phenolic content in HTSP-5 sweet pepper at harvest (Mashabela et al., 2015). The highest capsaicinoid content was observed under 70% shading in ‘Bhut Jolokia’ and 50% in ‘Akanee Pirote’ (Jeeatid et al., 2017). A controlled environment with low light intensity and high relative humidity increased capsaicinoid production in hot pepper hybrids (*Capsicum chinense* Jacq). The total capsaicinoid content was highest under colored shading nets (white, red, and green) compared to the unshaded treatment in F1 hybrid pepper cultivars like 'Star Flame' and 'Fire Flame(Zsuzsa et al., 2017).

A previous study found that reducing light intensity does not necessarily result in lower content of bioactive components in fruits. The information about the impacts of shade nets on jalapeño pepper on microclimate, plant growth, physiological responses, and fruit yield are reported in a companion study. However, the impact of shade nets on the bioactive compounds, fruit color, and the water loss rate of jalapeño peppers remains uncertain. Therefore, the present study aims to compare the effects of colored shading nets on fruit color, weight loss rate, and bioactive compounds (total phenols, flavonoids, antioxidants, and capsaicinoids) in organic jalapeño peppers.

3.2 MATERIALS AND METHODS

3.2.1 Experimental site, design, and treatments

Two field trials were conducted in the spring-summer of 2019 and 2021 in certified organic land at the Horticulture Farm, University of Georgia, Tifton, GA. Jalapeño pepper 'Compadre' (untreated seed) was grown for six weeks in a greenhouse using polystyrene 400-cell (2.5 x 2.5 cm cell) using commercial potting mixture Sunshine #1 (Sunshine Growers, Vancouver, BC). The soil was sandy loam with a pH of 6.5. Seedlings were grown on raised beds with two rows of plants per bed (45 cm distance between rows) and 30 cm separation between plants within the row. The planting dates were 10 May 2019 and 29 April 2021. Before laying mulch, the soil was fertilized with nitrogen (N), P_2O_5, and K_2O at 358 kg/ha, 286 kg/ha, and 215 kg/ha, respectively, using 5:4:3 organic poultry fertilizer (Symphony Organic Fertilizer, Virginia). Drip irrigation tape was placed 5 cm deep in the center of each bed (Ro-Drip; Roberts Irrigation Products, Inc., San Marcos, CA). The bed was covered with white-on-black (2019) and black (2021) plastic mulch (RepelGro; ReflecTek Foils, Inc., Lake Zurich, IL). Plants were irrigated when the cumulative crop evapotranspiration (ETc) reached about 1.27 cm. The experimental design was a randomized complete block with four replications and five treatments of shade nets (black, red, silver, white, and unshaded as control). According to the manufacturer, the net shade factor was 40% (Green-tek, Janesville, WI). Shade nets were installed one month (11 June 2019 and 29 May 2021) after transplanting in the field using a north-south orientation. Each shade net was 2.7 m wide, 2 m high, and 6.8 m long. Shade nets were suspended above the jalapeño pepper plants by metal fence posts and PVC pipe in tunnel-shaped structures, leaving the sides of the structures uncovered.

3.2.2 Microenvironment

Air temperature was measured with a temperature sensor inside a datalogger (WatchDog 1650 mini dataloggers; Spectrum Technologies, Inc., Aurora, IL, USA). Root zone temperature (RZT) at a depth of 10 cm was measured with copper-constantan thermocouples (Model 107, Campbell Scientific Logan, UT) connected to a data logger (CR-10X and CR 1000X; Campbell Scientific, Logan, UT). The RZT measurements started on 18 June 2019 and 7 June 2021 until the end of the season. The thermocouples were placed midway between plants. Light quantity was determined as Photosynthetic photon flux density (PPFD) with the quantum sensor of a portable photosynthesis system [LI-COR 6400XT (LI-COR, Inc., Lincoln, NE)] on clear sunny days at 1200-1400 HR Eastern Standard Time.

3.2.3 Fruit mineral concentration

In 2019 and 2021, five mature green fruits from each plot were cut into halves and dried in an oven at 70°C for 2-3 days to constant weight. Mineral nutrient analysis was determined by a commercial laboratory (Waters Agricultural Laboratories, Inc. Camilla, GA).

3.2.4 Fruit color

Fruit skin color was measured (three readings per fruit) from the middle section of the mature green fruits (five per plot) with CR-400 (8mm aperture, D65 illuminant) handheld colorimeter (Konica Minolta, Ramsey, NJ). The instrument was calibrated with white standard reflector plates before use. The color was measured as L* [from 0 (white) to 100 (black)], a* [from -a (green) to +a (red)], and b* [from -b (blue) to +b (yellow)] (McGuire, 1992; Ornelas-Paz et al., 2013). The a* and b* values were converted to hue angle (h) and chroma (C*), following the procedures of Topuz et al. (2009):

$h = 180 + \arctan(b^*/a^*)$, [$a^*<0$, $b^*>0$].

$C^* = (a^2 + b^2)^{1/2}$.

3.2.5 Water loss rate (WLR)

The fruit water loss rate was measured by placing fruits on a table (20 fruits per treatment) in a controlled temperature room [21 °C and vapor pressure deficit (VPD) of 1.36 kPa]. Fruit WLR was measured gravimetrically by weighing individual fruit daily for 7 d. The fruit WLR was calculated as:

$$\text{WLR } (\%\text{ loss d}^{-1}) = (\Delta FW/FWo)\,(100/t)$$

Where ΔFW is the change in fruit weight (FW) (g), *t* is the period (day) between two consecutive FW determinations, FWo is initial FW at the beginning of the weighing period. Mean values of WLR were calculated for individual fruit from measurements made over seven days (keeping period). The mean FWo used were 22.7 g and 29.9 g (unshaded), 23.1 g and 30.3 g (black), 24.5 g and 30.5 g (red), 22.2g and 32.9 g (silver), and 22 g and 31.9 g (white) in 2019 and 2021, respectively.

3.2.6 Bioactive compounds

Three fruits per plot were taken to the laboratory and cut into halves immediately after harvest. Samples were extracted by grinding and soaking them in 95% ethanol for 2 d at room temperature. Homogenized samples were refrigerated at 4 °C until starting the analysis. Each sample extract was analyzed in triplicate for each assay.

Total phenolic content (TPC) was determined following the Folin-Ciocalteu method (Derakhshan et al., 2018; Singleton et al., 1999). The samples of 5 µL were added 1195 mL of water, 75 µL of Folin-Ciocalteu reagent, and 225 µL of Na_2CO_3 solution. After incubating at 40 °C for 30 minutes under dark conditions, TPC was determined at 765 nm using a spectrophotometer (Beckman Coulter, Brea, CA, USA), and data were expressed as gallic acid equivalents (GAE) in mg. L^{-1}. The standard curve was prepared using gallic acid solution (0-500 mg. L^{-1}).

Total flavonoid content (TFC) was determined using the aluminum chloride method (Gu et al., 2019) and expressed as quercetin equivalents (µg/mL). 20 µL samples were mixed with 100 µL 10% $AlCl_3$, 20 µL 1M KoAC, and 940 µL H_2O. TFC was determined after 30 min at room temperature, reading the reaction mixture at 415 nm, using a spectrophotometer (Beckman Coulter, Brea, CA, USA).

Two different assays determined antioxidant capacity: Trolox Equivalent Antioxidant Capacity-2,2′-diphenyl-1-picrylhydrazyl (TEAC-DPPH) (Liao et al., 2012) and Cupric reducing antioxidant capacity (CUPRAC) (Apak et al., 2008) methods. Trolox was used as the standard for the calibration curve. Trolox was used as the standard for the calibration curve in all assays. For the TEAC-DPPH assay, a 20µL sample was mixed with 80µL 100% MeOH and 1mL of freshly prepared 200 µM DPPH in 80% MeOH. After 30 min at room temperature, TEAC-DPPH was measured at 520 nm and expressed in µM Trolox. CUPRAC was determined using a 20 µL sample mixed with 80 µL of 80% EtOH, 0.4 mL of 7.5 mM neocuproine (Nc) in 96% EtOH, 0.34 mL of H_2O, 0.4 mL of 100 mM $CuCl_2$ prepared in H_2O, and 0.4 mL of 1M ammonium acetate (pH-7). Mixtures were thoroughly mixed and let to stand for 30 minutes at room temperature before absorbance at 450 nm was measured and expressed in µM Trolox.

The capsaicinoid was estimated by UV-VIS spectrophotometer. The absorbance was determined using a 20 µL sample mixed with 980 µL of 100% EtOH at a wavelength of 290 nm.

The capsaicin and dihydrocapsaicin concentrations were calculated using a standard curve of capsaicin and dihydrocapsaicin and were expressed as µg/g fresh weight of the jalapeño pepper and converted into Scoville Heat Unit (SHU) (Davis et al., 2007; Domínguez-Martínez et al., 2014; González-Zamora et al., 2015).

3.2.7 Data analysis

Data were analyzed using a generalized linear model of one-way analysis of variance (ANOVA) using R Statistical Software (Team, 2023). When the ANOVA model showed significant treatment effects, a within-group separation was performed using Tukey's HSD test at 0.05%. Regression analysis and graphs were prepared using the SigmaPlot 14 software (Systat Software, San Jose, CA). Pearson correlation analysis determined the relationships between bioactive compounds and environmental factors, such as light and root zone temperature.

3.3. RESULTS

3.3.1 Microenvironment

Seasonal air temperature, soil temperature, relative humidity, and PPFD for the 2019 and 2021 seasons are shown in Tables 3.1 and 3.2

3.3.2 Fruit mineral concentration

Shading treatments did not significantly influence macro and micro fruit mineral concentrations in both years except Cu (Table 3.3 & 3.4). Cu was found to be highest in red shade nets and lowest in unshaded conditions in 2021 (Table 3.4).

3.3.3 Fruit color

In 2019, the fruit L* value was highest in the white shade net and unshaded treatment and lowest under black and silver shade nets (Figure 3.1). The hue angle was highest in black and silver shade nets and lowest in red, white, and unshaded treatments. Chroma value was highest in unshaded and red and lowest under a black shade net. In 2021, the L* value was highest in unshaded and lowest in silver shade. The hue angle was highest in the black and lowest under the red shade net, and the chroma value was highest in the unshaded and lowest under the black and silver shade nets. Fruit color was related to soil temperature. Fruit L* and chroma value increased while hue angle decreased with increasing mean root zone temperature under shade nets (Figure 3.2).

3.3.4 Water loss rate (WLR)

In 2019, the weight loss rate was lowest in silver shade nets and the highest in black and red nets. However, in 2021, the weight loss rate was highest in silver but lowest in unshaded conditions (Figure 3.3).

3.3.5 Bioactive compounds

In 2019, total phenolic content (TPC) and total flavonoids (TF) were highest in the unshaded treatment (Figure 3.4). However, the shade net treatments did not influence CUPRAC and TEAC-DPPH. In 2021, TPC and CUPRAC were highest in unshaded conditions. However, TF was unaffected by shade net treatments, and TEAC-DPPH was the highest in the silver shade net and the lowest in the red shade net.

In 2019, capsaicin and SHU were the highest in unshaded and red shade net treatments and lowest in black shade net (Table 3.5). Dihydrocapsaicin was highest in unshaded and lowest in the black shade net However, in 2021, capsaicin, dihydrocapsaicin, and SHU were highest in the white shade net and lowest in the red shade net.

3.3.6 Correlation analysis of PPFD, RZT, and bioactive compounds

There was a significant and positive correlation between RZT and TPC (r = 0.81), RZT and TF (r = 0.97), and RZT and CUPRAC (r = 0.9), suggesting that the bioactive compounds increase with increasing RZT (Table 3.6). Similarly, TPC was positively correlated with TF (r = 0.87). PPFD was not correlated with TPC, CUPRAC, TEAC, and capsaicin. TPC and TF showed a strong and significant correlation with the CUPRAC method, but not the TEAC method. Capsaicin showed a negative correlation with TPC, TF, and CUPRAC.

3.4 DISCUSSION

3.4.1 Fruit mineral concentration

Shade nets did not affect fruit mineral nutrient concentration in the present study. There are few reports on the effect of shading on fruit mineral nutrition compared to leaf mineral elements. Kabir et al. (2022a) found an increased concentration of macro-and micronutrients, except B, with increased shading intensity in bell pepper. Similarly, bell pepper N, P, and K increased with increasing shade levels Díaz-Pérez (2018). In another study, Villalobos-Soublett et al. (2021) found shading nets decreased Ca, Mg, and Zn mineral nutrient concentration in petiole at the flowering stage in grape vines.

Similarly, shaded cotton plants (63% shading) had significantly higher K, Ca, and B but lower Zn concentrations in floral buds than open-field cotton bolls (Zhao and Oosterhuis, 1998). In another study, shade levels between 0%-50% did not affect N, P, K, Ca, Mg, and S concentrations in potato tubers (Schulz et al., 2019). Chen et al. (1997) found decreased Ca^{2+} uptake in apples and kiwifruit when sunlight incidence was reduced by 40%. However, de Freitas et al. (2013) found increased uptake of fruit Ca^{2+} in apples, with a 50% reduction in PAR. In dried figs, shading increased the concentration of N, P, Ca, and Mg (Jokar et al., 2021). In the present study, the reason for the similar fruit mineral concentration among shade net treatments showed that jalapeño fruit might be able to translocate nutrients irrespective of light and RZT changes associated with the presence of shade nets.

3.4.2 Fruit surface color

Fruit skin color in pepper varies according to cultivar, maturity, and growing conditions (Barrera et al., 2008; Nagle et al., 1979). Fruit color is represented either in L*a*b* or L*C*H* models. A hue angle (H*) ranges from 0º to 360°, here 0° (or 360°) represents maximum redness, 90° maximum yellowness, 180° maximum greenness, and 270° maximum blueness (Scalisi et al., 2022). Typically, hue angle indicates changes in color during pepper ripening because of enzymatic degradation of chlorophyll and an increase in carotenoid biosynthesis (Barrera et al., 2008).

In the present study, colored shade net treatments significantly affected fruit skin color values. The hue angle ranged from 125º to 130º as peppers were harvested at the matured green stage. Increased hue angle in black and silver nets represented a high chlorophyll content in peppers and delayed ripening (Hirst et al., 1990). Unshaded conditions resulted in less hue angle but high L* and chroma values, probably because of high carotenoid concentration. Ilic et al. (2017) found reduced carotenoid concentration in bell pepper fruits grown in unshaded conditions compared to shade nets (red, black, pearl, and blue). Díaz-Pérez et al. (2020) also found high a* but less L* and b* values in bell pepper under black and silver shade nets compared to unshaded conditions. Furthermore, Gómez et al. (1998) found a darker color in greenhouse-grown paprika pepper because of the reduced light transmission.

3.4.3 Water loss rate (WLR)

Lownds et al. (1994) discovered that the primary factor restricting the post-harvest shelf life of peppers is the water loss rate. There is only a little research related to the water loss rate of jalapeño pepper (Jansasithorn et al., 2014). In the present study, after the 7-d keeping period, jalapeño pepper had about 7% to 8% fruit water loss. About 50% of fruit were marketable.

Another study also found that 7% weight loss should be considered the maximum permissible water loss for nine cultivars of different types of pepper (Bell, New Mexican, Jalapeño, Serrano, and Yellow wax) (Lownds et al., 1994). In the current study, shading net treatments had no consistent effects on postharvest water loss rate per day (%) based on two year's study. Similarly, Kabir et al. (2022b) also found inconsistent effects of shading nets on bell pepper's water loss rate when different intensities of shade nets were used. In another study by Díaz-Pérez (2014), no effects of shade nets on the postharvest water loss rate in bell pepper were observed. Usually, water loss in fruits occurs through stomata and lenticels on the fruit surface, but peppers lack stomata (Lownds et al., 1993), so fruit transpiration occurs either through the pedicel and calyx or via the cuticle and cracks on the fruit skin (Banks and Nicholson, 2000; Díaz-Pérez et al., 2007).

Smith et al. (2006) conducted a study showing differences in epicuticular wax levels among four bell pepper cultivars. However, no clear correlation trend between wax levels and water loss rate was evident. In contrast, both Lownds et al. (1993) and Maalekuu et al. (2004) found a negative correlation between the epicuticular wax content with water loss rate in three different pepper [‘Keystone’ (bell type), ‘NuMex R Naky’ (New Mexican type), and ‘SantaFe Grande’ (yellow wax type)]. On the other hand, Parsons et al. (2012) found a positive correlation between fruit water loss and the cuticular membrane in peppers, where higher amounts of terpenoids were associated with higher water loss rates. Although the present study did not evaluate the cuticular properties of jalapeño peppers, there is a possibility of variation in cuticle thickness and wax constituents because of the different amounts and quality of light transmitted through different shade nets.

3.4.4 Bioactive compounds

Bioactive compounds, including phenols, flavonoids, and antioxidant properties, play a significant role in protecting vegetables against heat and light stress (Raúl et al., 2017; Toscano et al., 2019). This two-year study observed inconsistent responses regarding total flavonoids, TEAC-DPPH, CUPRAC, and capsaicinoids. Although total phenolic contents were consistently highest in unshaded conditions compared to shade nets in both years, in 2019, the total phenols and flavonoid content values were higher than in 2021. This increase may be attributed to a bacterial leaf spot (*Xanthomonas campestris* pv. *vesicatoria*) in 2019, which could have stimulated the production of phenols and flavonoids as a defense mechanism against pathogens. Mikulic-Petkovsek et al. (2013) also found higher levels of phenolic contents (1.5-1.7 times) in diseased fruits compared to healthy ones in pepper. Similar findings have been reported in other studies, showing that higher total phenolic and flavonoid contents are a response to fungal, bacterial, viral, and insect-pest attacks (Koc and ÜSTÜN, 2011; Schovánková and Opatová, 2011). However, TPC and TF in 2019 were unrelated to bacterial leaf spot disease (data not shown).

Previous research showed that pearl shade nets had the highest ascorbic acid content and had antioxidant capacity in bell pepper (Goren et al., 2011; Kong et al., 2013). Baby spinach showed the highest flavonoid concentration under shade nets (Bergquist et al., 2007), while lettuce displayed the highest flavonoid in unshaded treatment with no difference in TPC between shade nets and unshaded treatments Ilić et al. (2017a). Angmo et al. (2021) found similarities in phenolic and flavonoid contents in bell pepper grown under open field and greenhouse conditions.

In the present study, capsaicinoid content in two years differed in colored shade net treatments. Fruit capsaicin, dihydrocapsaicin, and SHU were higher in 2021 than in 2019, even though the same methods were used for quantification. Most of the previous research used HPLC for capsaicinoid determination (Al Othman et al., 2011; Antonious and Jarret, 2006; Naves et al., 2019). However, a UV-vis spectrophotometer was used for the current study, and this method has a high possibility of interference from other compounds in the extract. Correlation analysis also showed that high TPC and CUPRAC could significantly reduce the SHU in the jalapeño fruits. We also observed higher root zone temperatures, total phenols, flavonoids, and CUPRAC in 2019 than in 2021. In addition, Gonzalez-Zamora et al. (2013) found decreased capsaicinoids level in *C. annuum* peppers such as jalapeño and De Arbol) but increased accumulation in Guajillo and Serrano peppers at high temperature conditions However, Johnson et al. (2021) and Sora et al. (2015), when quantified capsaicin in Habanero pepper (*C. chinense*) using HPLC, found a strong positive correlation with total phenolic contents. TEAC-DPPH antioxidant assay had a very weak correlation with TPC and TF in the current study. Moreover, Hamed et al. (2019) also found a poor correlation between TPC and TF with DPPH antioxidant assay and no correlation with SHU.

3.5 CONCLUSIONS

Colored shade nets modified root zone and air temperature, light quantity, and light quality reaching the plant canopy of jalapeño pepper. The present study, however, showed inconsistent effects on the fruit quality of jalapeño pepper in terms of mineral concentration, water loss rate, and bioactive compounds. Fruit surface color lightness (L*) and chroma (C*) increased while Hue angle (H*) decreased under unshaded conditions than in shade nets. In both years, total phenolic contents in fruits were higher in unshaded conditions, showing that phenolics provide a protective mechanism against heat and light stress. Overall, the study suggests that the impact of colored shade nets on the fruit quality of jalapeño pepper is uncertain and may vary depending on environmental factors and growing conditions. Further research is needed to understand the underlying mechanisms and optimize shade net strategies for jalapeño pepper production.

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Tables and figures

Table 3.1 Air temperature (Tair), root zone temperature (RZT), and photosynthetic photon flux density (PPFD) under colored shading nets in organic jalapeño pepper in Spring-Summer 2019, Tifton, GA.

Shade nets	Tair (°C)			RZT (°C)			PPFD ($\mu mol.m^{-2}s^{-1}$)
	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Mean
Black	23.2	29 c	36.6 c	26.7 d	28.5 d	30.6 b	983.5 dz
Red	22.9	29.9 b	39.4 b	26.6 d	29.5 c	33.1 a	1123.2 bc
Silver	23	29.8 b	40.2 b	26.9 c	28.6 d	30.6 b	1079.6 c
White	22.9	30.4 a	41.5 a	27.3 b	29.9 b	33.2 a	1210.5 b
Unshaded	22.9	28.4 d	33.9 d	28 a	30.5 a	33.4 a	1927 a
P-value	0.529	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.001

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 3.2 Air temperature (Tair), root zone temperature (RZT), and photosynthetic photon flux density (PPFD) under colored shading nets in organic jalapeño pepper in Spring-Summer 2021, Tifton, GA.

	Tair (°C)			RZT (°C)			PPFD ($\mu mol.m^{-2}s^{-1}$)
Shade nets	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Mean
Black	22.8	27.3 ab^z	34.3 cd	25.1 e	26.7 d	29.2 c	867.0 d^z
Red	22.9	27.9 a	39.3 b	25.7 c	27.3 c	29.2 c	970.7 c
Silver	22.9	26.6 b	33.6 d	25.5 d	26.8 d	28.7 d	958.2 c
White	23	27.2 ab	43.9 a	25.8 b	27.6 b	29.9 b	1190.8 b
Unshaded	22.9	27.5 ab	35.7 c	26.7 a	28.9 a	31.5 a	1881.8 a
P-value	0.843	0.021	<0.001	<0.001	<0.001	<0.001	<0.001

^{z}Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 3.3 Fruit nutrient (macro and micro) concentrations of organic jalapeño pepper at the mature green stage under colored shading nets in Spring-Summer of 2019, Tifton, GA.

Shade nets	Macro nutrients (%)						Micro nutrients (ppm)				
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Black	2.2	0.5	3.1	0.19	0.2	0.25	13.8	13.8	55.8	16.5	33
Red	2.1	0.5	3	0.18	0.2	0.25	14.8	12.5	57	16.5	32.8
Silver	2.2	0.5	3.1	0.18	0.2	0.24	13.8	12.8	54.8	15.5	31.8
White	2.1	0.5	3	0.18	0.2	0.25	14.5	13	52	15.8	31.3
Unshaded	2.1	0.5	3	0.2	0.2	0.26	15	13.3	57.8	20.3	35
P-value	0.572	0.877	0.483	0.517	0.975	0.758	0.746	0.41	0.773	0.642	0.24

Table 3.4 Fruit nutrient (macro and micro) concentrations of organic jalapeño pepper at the mature green stage under colored shading nets in Spring-Summer of 2021, Tifton, GA.

Shade nets	Macro nutrients (%)						Micro nutrients (ppm)				
	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Black	2.4	0.48	3.2	0.16	0.2	0.26	14.8	11 ab[z]	64	19.5	34.3
Red	2.5	0.47	3.2	0.15	0.2	0.26	13.8	11.25 a	63	19.3	35
Silver	2.5	0.46	3.1	0.14	0.2	0.26	14	10 ab	63	19	35.3
White	2.3	0.46	3.1	0.14	0.2	0.24	15.3	10.5 ab	60	18.5	31.5
Unshaded	2.2	0.45	3	0.14	0.2	0.24	15.3	9.5 b	53	22.8	30.8
P-value	0.092	0.235	0.436	0.325	0.702	0.104	0.193	0.034	0.09	0.732	0.188

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 3.5 Capsaicin (C), dihydrocapsaicin (DHC), and Scoville heat unit (SHU) of organic jalapeño pepper collected at mature green stage under colored shading nets in Spring-Summer of 2019 and 2021, Tifton, GA.

Shade nets	2019			2021		
	Capsaicin (μg/g)	Dihydrocapsaicin (μg/g)	SHU	Capsaicin (μg/g)	Dihydrocapsaicin (μg/g)	SHU
Black	598.2 b[z]	292.9 b	14,180.7 b	1446.7 ab	978.6 ab	38,804.8 ab
Red	927.7 a	231.3 ab	23,214 a	1362.3 b	894.9 b	36116.2 b
Silver	896.2 ab	499.3 ab	22,328.7 ab	1508.6 ab	1031.4 ab	40,639.4 ab
White	800.2 ab	416.9 ab	19,479.8 ab	1675.7 a	1149 a	45,194.1 a
Unshaded	1050 a	612.9 a	26,607.4 a	1626.4 ab	1093.7 ab	43,521.1 ab
P-value	<0.0001	0.0036	0.0016	0.0403	0.0432	0.0417

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 3.6 Pearson correlation coefficients (r) between PPFD, root zone temperature (RZT), total phenolic contents (TPC), total flavonoids (TF), antioxidant activities, and capsaicin of organic jalapeño pepper collected at mature green stage under colored shading nets in Spring-Summer of 2019 and 2021, Tifton, GA.[z]

	RZT	PPFD	TPC	TF	CUPRAC	TEAC
PPFD	0.68*					
TPC	0.81**	0.27				
TF	0.97**	0.62	0.87**			
CUPRAC	0.9**	0.47	0.94**	0.91**		
TEAC	-0.25	0.09	-0.24	-0.26	-0.02	
SHU	-0.59	0.1	-0.88**	-0.62	-0.72 *	0.42

[z]TPC (Total phenolic content expressed as mgGAE/L), TF (Total flavonoids expressed as quercetin equivalent mg/mL), CUPRAC, and TEAC (Cupric reducing and Trolox equivalent antioxidant capacities expressed as µM), and Scoville heat unit (SHU). Values with * and ** are significant at $P < 0.05$ and $P < 0.01$, respectively

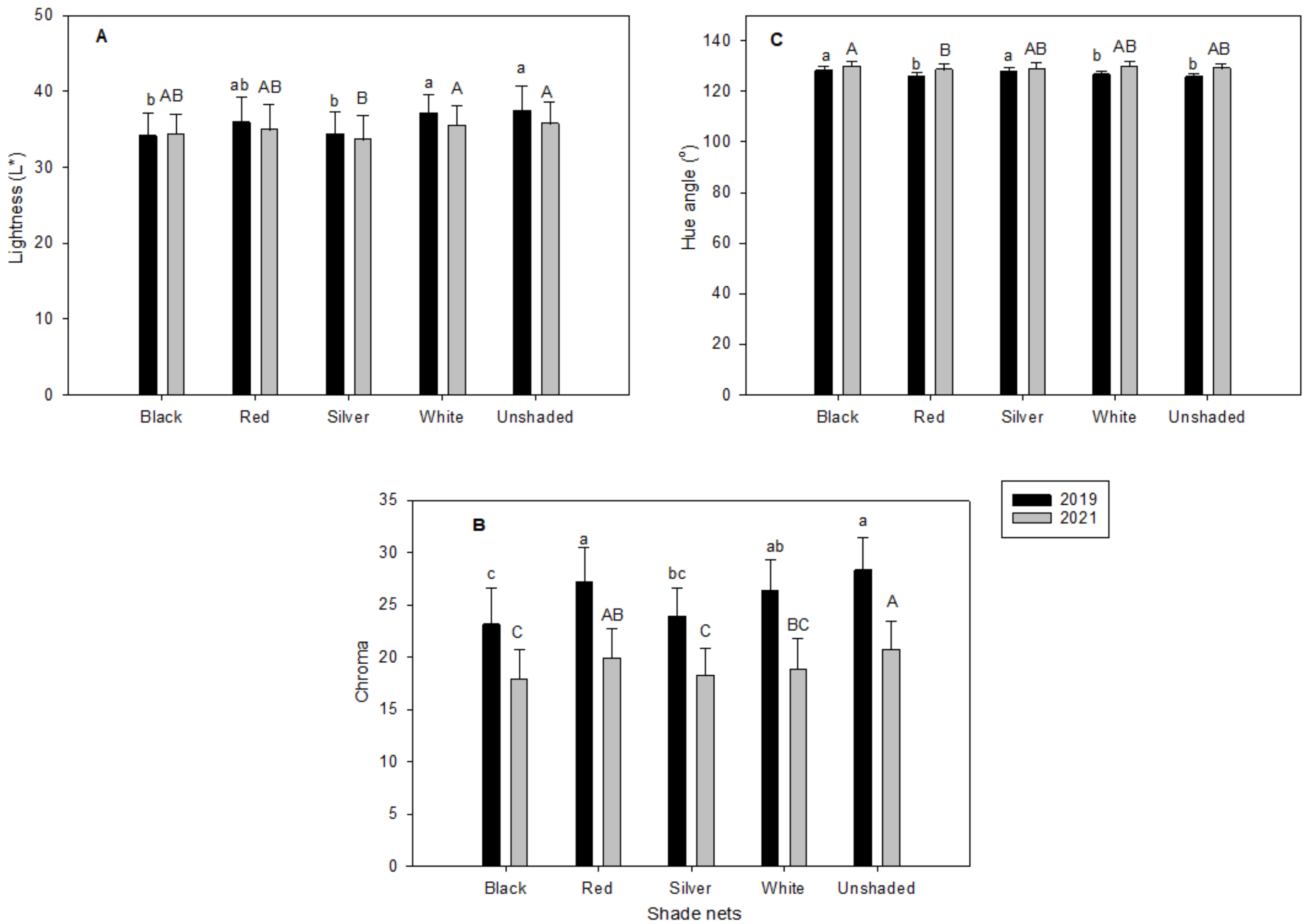

Figure 3.1 Fruit surface color of green mature organic jalapeño pepper [Lightness*(A), Chroma (B), and Hue angle (C)] grown under colored shade nets, Tifton, GA, Spring-Summer 2019 and 2021. Each bar represents the mean of 4 replications ± standard error, and different le letters at the top represent mean separation by the Tukey HSD test ($p < 0.05$).

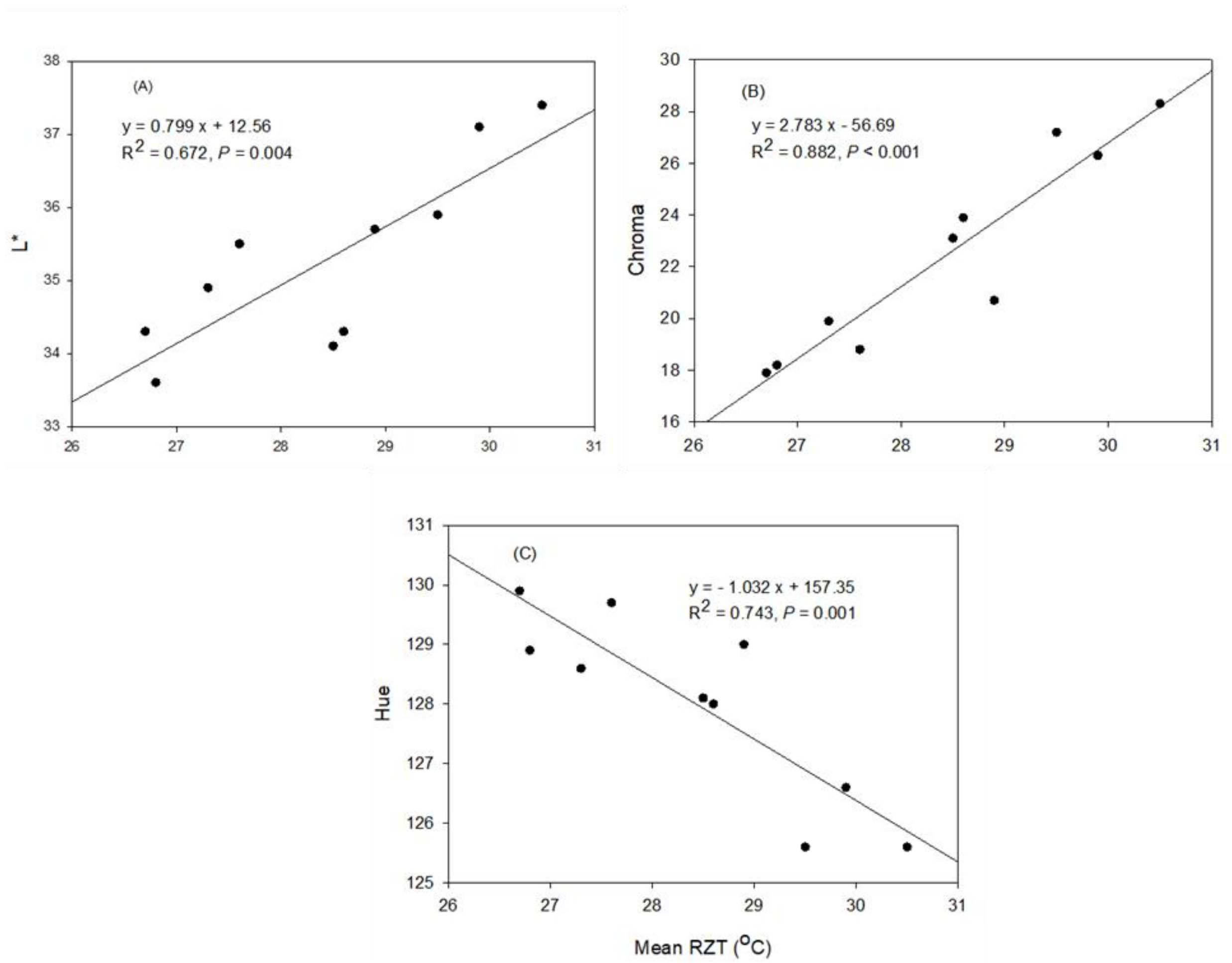

Figure 3.2 Relationships between L*(A), chroma (B), and hue angle (C) and mean root zone temperature (RZT) in organic jalapeño pepper under colored shade nets (black, red, silver, white, and unshaded) in Spring-Summer 2019 and 2021. Fruit were harvested at the mature green stage, Tifton, GA.

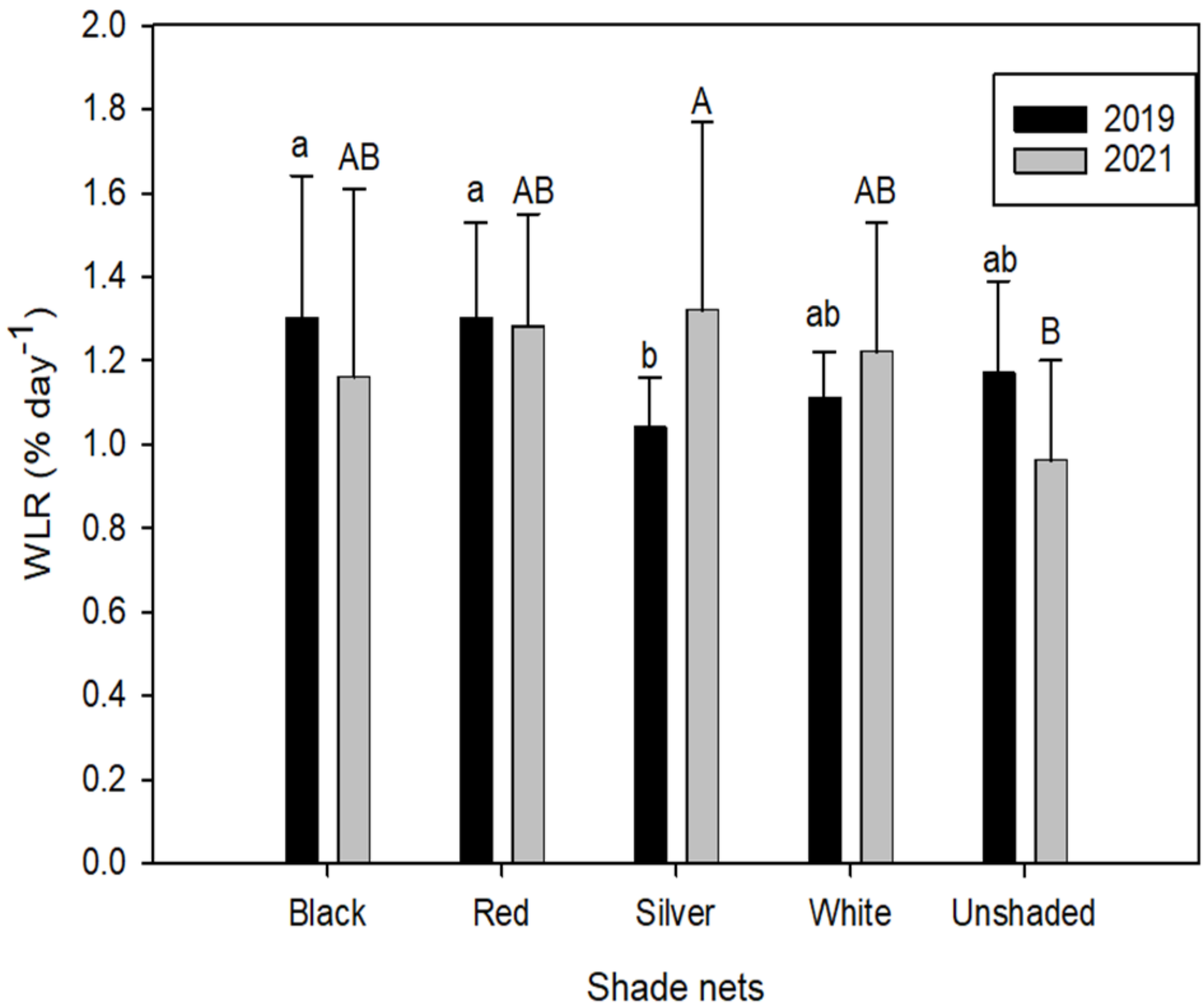

Figure 3.3 Postharvest weight loss rate of marketable mature green organic jalapeño pepper fruit grown under colored shade nets (black, red, silver, white, and unshaded) in Spring-Summer 2019 and 2021, Tifton Ga. Each bar represents the mean of 4 replications ± standard error, and different letters at the top represent mean separation by the Tukey HSD test ($p < 0.05$). Fruits were kept at 21°C (vapor pressure deficit of 1.36 kPa) for 7d.

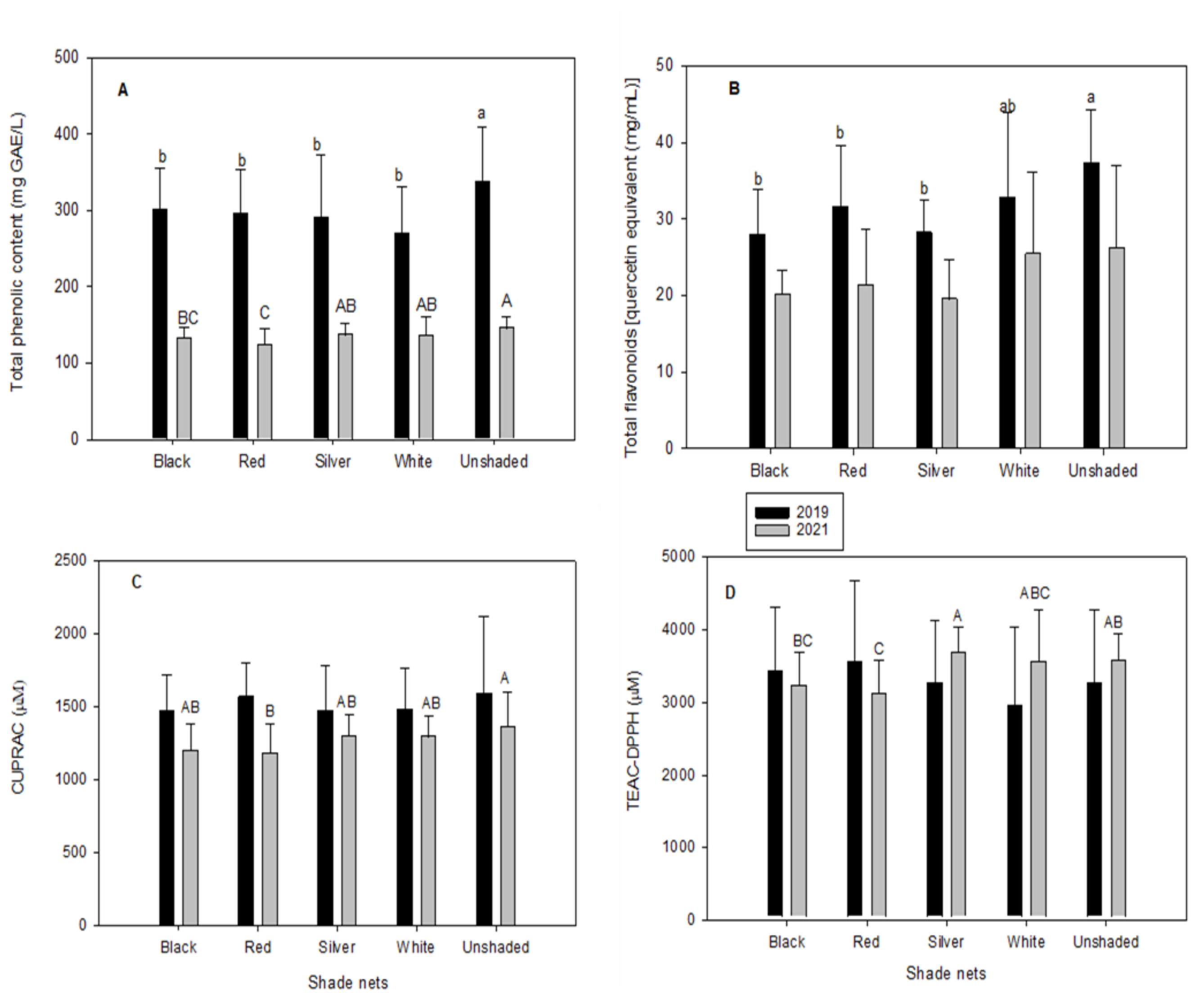

Figure 3.4 Total phenolic content (TPC) and total flavonoids (TF), cupric reducing antioxidant capacity (CUPRAC), Trolox Equivalent Antioxidant Capacity-2,2′-diphenyl-1-picrylhydrazyl (TEAC-DPPH) in organic jalapeño pepper under colored shade nets in Spring- Summer 2019 and 2021. Fruit harvested at the mature green stage, Tifton, GA. Each bar represents the mean of 4 replications ± standard error. Different letters at the top of the bar represent mean separation by the Tukey HSD test ($p < 0.05$).

CHAPTER 4

PLANT PHYSIOLOGY AND FRUIT YIELD AND QUALITY OF ORGANIC JALAPEÑO PEPPER (*Capsicum annuum* L.) AS INFLUENCED BY ORGANIC NITROGEN FERTILIZATION LEVEL AND SHADING

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ABSTRACT

Vegetable production has been studied extensively with nitrogen fertilization and shading. However, reports quantifying the effects of N and shading on the growth and productivity of organic jalapeno pepper still need to be included. This study aimed to assess leaf and fruit mineral concentration, leaf gas exchange, fruit yield, and fruit quality of jalapeño pepper as affected by organic N fertilization levels and shading. The experimental design was a split-plot randomized complete block design, with shading (shaded vs. unshaded) as the main plot and four organic N fertilization levels (0, 179, 358, and 716 $kg \cdot ha^{-1}$ N) as split plots. Shading reduced mean and minimum root zone temperature (RZT), maximum air temperature, mean leaf temperature, and light intensity (PPFD) compared to unshaded conditions. Leaf phosphorus (P), boron (B), copper (Cu), N, sulfur (S), and iron (Fe) increased while magnesium (Mg) and zinc (Zn) decreased with increasing N fertilization levels. Fruit N, potassium (K), calcium (Ca), S, Cu, Fe, manganese (Mn), and Zn increased, while Mg and B decreased with N levels. Shading increased concentrations of leaf iron (Fe) and fruit P, Ca, and Cu. Leaf net photosynthesis (Pn), electron transport rate (ETR), and photosystem-II efficiency (PhiPS2) increased while internal CO_2 (Ci) decreased with increasing N fertilization. In addition, leaf g_s and E increased quadratically with increasing N levels with optimum g_s and E values at 358 $kg \cdot ha^{-1}$ N. Shading did not impact leaf gas exchange processes except for increased photosystem-II efficiency PhiPS2. Marketable fruit yield, length, diameter, fresh weight, and dry weight increased quadratically with increasing N levels. Shading reduced medium-sized fruit yield and all categories of cull yield compared to unshaded conditions. Total flavonoids (TF) decreased with increasing N levels, while shading reduced total phenolic contents (TPC). In conclusion, shading utilization and organic nitrogen fertilization beyond 358 $kg \cdot ha^{-1}$ N were unsuitable for producing organic jalapeño pepper.

Keywords: jalapeño pepper, nitrogen, shade net, bioactive compounds

4.1 INTRODUCTION

Nitrogen is an essential plant nutrient. It is a component of amino acids, proteins, nucleic acids, and chlorophyll content (Ohyama, 2010). Plant N status is crucial in balancing vegetative and reproductive growth (Warner et al., 2004). The relationship between N fertilization and light intensity may be important for optimizing crop productivity and upholding sustainable practices (Spiertz, 2009).

Soil N availability is limited by adverse environmental conditions such as low light intensity, water deficit, and salinity. Insufficient and excessive negatively affect vegetable production. In addition, excessive N fertilization may cause N leaching and reduced fruit secondary metabolites and vitamins (Yasuor et al., 2013). Excessive N supply often leads to increased vegetative growth at the expense of fruit and root development in vegetable crops (Castellanos et al., 2011; Stefanelli et al., 2010).

Plants can adapt and survive in diverse environmental conditions by modifying their morpho-physiological characteristics (Lambers et al., 1990). The significance of using protective shade cloth in field crops (vegetables and fruits) has risen to reduce the impacts of high light and temperature stress resulting from global warming (Ilic et al., 2012; Martínez-Lüscher et al., 2017; OmbÓDi et al., 2015; Rekha et al., 2014). Optimization of light and N fertilization is required for plants to ensure growth, marketable yield, dry matter production, and production of metabolites (Arcel et al., 2023). The light level for maximum net photosynthesis in jalapeño pepper has not been determined. However, Kabir et al. (2022a) studied the light response curve under open field and shaded conditions in bell pepper and found that maximum net photosynthesis was at 1239 µmol m^{-2} s^{-1} in the open field and 1198 µmol m^{-2} s^{-1} under 47% shade net. High light intensity combined with low N fertilizer reduced the growth and productivity of plants (Du et al., 2021). In addition, a balanced nutrient supply tends to overcome negative impacts from adverse light intensities in plants (de Oliveira et al., 2019). In tomatoes, Hernández et al. (2019) found a decreased concentration of β-carotene in shading conditions with a high rate of N. In blueberries 'Duke', the anthocyanin concentration and fruit size decreased.

Most studies on bell pepper focused on the effects of N and light intensity on plant growth and yield, especially in controlled environments. However, no studies are on the interactive effects of N fertilization and shading under field conditions. Nitrogen use for bell pepper range from 180 to 250 $kg{\cdot}ha^{-1}$ in the Southeastern United States (Coolong et al., 2019; Mayorga-Gómez et al., 2020). However, there are no studies on the organic fertilization of jalapeño pepper either in open-field (unshaded) or under shaded conditions. This study aimed to assess leaf gas exchange, yield components, and fruit quality of jalapeño pepper as affected by organic N fertilization level and shading.

4.2 MATERIALS AND METHODS

4.2.1 Experimental site, design, and treatments

The experiment was conducted in the spring-summer of 2021 at the Organic Horticulture Farm, University of Georgia, Tifton, GA. The soil is a Tifton loamy sand series (0% to 2% slope). The soil had a pH of 6.2 with 158, 114, and 1237 $kg{\cdot}ha^{-1}$ of phosphorous (P), potassium (K), and calcium (Ca), respectively, before applying fertilizer. Jalapeño pepper 'Compadre' (untreated seed) was grown for six weeks in a greenhouse using polystyrene 400-cell (2.5 x 2.5 cm cell) using commercial potting mixture Sunshine #1 (Sunshine Growers, Vancouver, BC). Seedlings were transplanted to an organically certified field (6 May 2021). Plants were grown on raised beds with two rows of plants per bed, with a 45cm distance between rows and a 30 cm separation between plants within the row. Drip irrigation tape was placed 5 cm deep in the center of each bed (Ro-Drip; Roberts Irrigation Products, Inc., San Marcos, CA). The bed was covered with white-on-black plastic mulch (RepelGro; ReflecTek Foils, Inc., Lake Zurich, IL). Plants were irrigated when the cumulative crop evapotranspiration (ETc) reached about 1.27 cm.

The experiment was laid out in a split-plot design with four replications. The main plot was shading (shaded and unshaded), and the subplot was organic N level (0, 179, 358, and 716 $kg \cdot ha^{-1}$ N applied as organic fertilizer). The main plot was a 20 m long bed, and the split-plot was 10 m long. Shading was applied using a black shade net (Green-tek, Janesville, WI). Shade nets were installed one month (7 June 2021) after transplanting in the field using a north-south orientation. Each shade net was 2.7 m wide, 2 m high, and 6.8 m long. Shade nets were suspended above the jalapeño pepper plants by metal fence posts and PVC pipe in tunnel-shaped structures, leaving the sides of the structures uncovered. The organic fertilizer was applied manually three days before transplanting on 3 May 2021. The fertilizer was Organic Poultry Fertilizer [5:4:3:8 (N: P_2O_5: K_2O: Ca) (Symphony Organic Fertilizer, Virginia)]. Of the 5% N, 3.5% was water-insoluble (slow-release form).

4.2.2 Microenvironment

Air temperature was measured with a temperature sensor inside a datalogger (WatchDog 1650 mini dataloggers; Spectrum Technologies, Inc., Aurora, IL, USA). Soil temperature was measured with soil temperature sensors (Spectrum Technologies) connected to the same data logger. Soil temperature sensors were placed 10 cm deep within the row, midway between the two plants. The datalogger collected hourly data. Light quantity was determined three times as the amount of light transmitted [Photosynthetic photon flux density (PPFD)] between 12:00-13:00 HR using a spectrometer (LI-180, LI-COR, Lincoln, NE) on sunny and clear days in the mid-section just above the plant canopy at two points on 29 June, 15 July, 8 August 2021.

4.2.3 Leaf, fruit, and soil mineral nutrients

Samples of 25-30 fully developed, healthy leaves from new growth and five fruits cut into halves from each plot were collected. Leaf and fruit samples were dried at 70 °C for 2 d to constant weight and analyzed for mineral nutrients (N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn). Soil samples (10-15 cm depth) were collected at the end of the experiment from the middle sections of each plot and dried for 2-3 days at 70 °C. Analyses of soil and plant mineral nutrients were conducted by a commercial laboratory (Waters Agricultural Laboratories, Inc., Camilla, GA).

4.2.4 Leaf gas exchange

Leaf gas exchange measurements [net photosynthesis (Pn) stomatal conductance (g_s), transpiration (E), intercellular CO_2 (Ci), electron transport rate (ETR), and photosystem-II efficiency (PhiPS2)] were done with a portable photosynthesis system (LI-COR 6400XT equipped with an integrated 6400-40 leaf chamber fluorometer; LI-COR, Inc., Lincoln, NE, USA). The airflow rate was 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on the reference side. The CO_2 concentration was set at 400 $\mu mol \cdot mol^{-1}$ with a CO_2 mixer and a CO_2 tank. The ambient light-tracking setting was used for leaf gas exchange measurements. Measurements were conducted on clear days at 1200 to 1500 HR (25 June, 11, and 18 Aug.) on well-exposed, healthy, and developed leaves of mature plants.

4.2.5 Fruit yield

Jalapeño pepper fruits were harvested and graded as marketable or cull. Fruit length was used as a grading criterion. Marketable included ‘large’ (7-10 cm long) and ‘medium’ (5-7 cm long) fruit. Cull included ‘small’ (<5 cm length) fruit, fruit with blossom-end rot (BER), fruit with anthracnose (*Colletotrichum acutatum*) rot, and fruit with damage by pepper weevil (*Anthonomus eugenii*). Fruits were harvested (mature green) on 6 and 27 July 2021.

4.2.6 Water loss rate (WLR) and firmness

Fruit WLR was measured by placing fruit on trays (20 fruits per treatment) and kept in a controlled-temperature room (21 °C, vapor pressure difference 1.36 kPa) for seven days (keeping period). Fruit water loss was measured gravimetrically by weighing individual fruit daily. The WLR was determined as a daily percent weight loss of the fruit with respect to the weight the day before each measurement. The mean initial fruit weights (FW_o) were: shaded [27.42 g, 33.15 g, 36 g, and 35.85 g] and unshaded [24.57 g, 33.21 g, 34.85 g, and 28.8 g], respectively, at 0, 179, 358, and 716 $kg \cdot ha^{-1}$N.

The fruit WLR was calculated as follows:

WLR (% loss d^{-1}) = ($\Delta FW/FW_o$) x (100/t)

Where, ΔFW is the change in fruit FW (g); t is the period (day) between two consecutive fruit FW determinations; and FW_o is fruit FW at the beginning of the weighing period. Mean values of WLR were calculated for individual fruit from measurements.

In the same fruit, firmness was measured daily using a hedonic scale (1 = spongy soft; 2 = soft; 3 = firm soft; 4 = moderately firm; 5 = firm) (Díaz-Pérez et al., 2007).

4.2.7 Bioactive compounds

Three fruits per plot were taken to the laboratory and cut into halves immediately after harvest. Samples were extracted by grinding and soaking them in 95% ethanol for 2 d at room temperature. Homogenized samples were refrigerated at 4 °C until starting the analysis. Each sample extract was analyzed in triplicate for each assay.

Total phenolic content (TPC) was determined following the Folin-Ciocalteu method (Derakhshan et al., 2018; Singleton et al., 1999). The samples of 5 µL were added 1195 mL of water, 75 µL of Folin-Ciocalteu reagent, and 225 µL of Na_2CO_3 solution. After incubating at 40 °C for 30 minutes under dark conditions, TPC was determined at 765 nm using a spectrophotometer (Beckman Coulter, Brea, CA, USA), and data were expressed as gallic acid equivalents (GAE) in mg. L^{-1}. The standard curve was prepared using gallic acid solution (0-500 mg. L^{-1}).

Total flavonoid content (TFC) was determined using the aluminum chloride method (Gu et al., 2019) and expressed as quercetin equivalents (µg/mL). 20 µL samples were mixed with 100 µL 10% $AlCl_3$, 20 µL 1M KoAC, and 940 µL H_2O. TFC was determined after 30 min at room temperature, reading the reaction mixture at 415 nm, using a spectrophotometer (Beckman Coulter, Brea, CA, USA).

Two different assays determined antioxidant capacity: Trolox Equivalent Antioxidant Capacity-2,2′-diphenyl-1-picrylhydrazyl (TEAC-DPPH) (Liao et al., 2012) and Cupric reducing antioxidant capacity (CUPRAC) (Apak et al., 2008) methods. Trolox was used as the standard for the calibration curve. Trolox was used as the standard for the calibration curve in all assays. For the TEAC-DPPH assay, a 20µL sample was mixed with 80µL 100% MeOH and 1mL of freshly prepared 200 µM DPPH in 80% MeOH. After 30 min at room temperature, TEAC-DPPH was measured at 520 nm and expressed in µM Trolox. CUPRAC was determined using a 20 µL sample mixed with 80 µL of 80% EtOH, 0.4 mL of 7.5 mM neocuproine (Nc) in 96% EtOH, 0.34 mL of H_2O, 0.4 mL of 100 mM $CuCl_2$ prepared in H_2O, and 0.4 mL of 1M ammonium acetate (pH-7). Mixtures were thoroughly mixed and let to stand for 30 minutes at room temperature before absorbance at 450 nm was measured and expressed in µM Trolox.

The capsaicinoid was estimated by UV-VIS spectrophotometer. The absorbance was determined using a 20 µL sample mixed with 980 µL of 100% EtOH at a wavelength of 290 nm. The capsaicin and dihydrocapsaicin concentrations were calculated using a standard curve of capsaicin and dihydrocapsaicin and were expressed as $\mu g \cdot g^{-1}$ fresh weight of the jalapeño pepper and converted into Scoville Heat Unit (SHU) (Davis et al., 2007; Domínguez-Martínez et al., 2014; González-Zamora et al., 2015).

4.2.8 Data analysis

Data were analyzed with linear mixed models in which the main plot (shaded or unshaded) and subplots (N levels) were fixed effects and replicate blocks were treated as random effects using R Statistical Software (Team, 2022). When the ANOVA model showed significant treatment effects, a within-group separation was performed using the LSD test at 0.05% for shading and interaction of N level and shading. Regression analysis determined the linear and quadratic relationship of measured variables with N levels. Graphs were prepared using the SigmaPlot 14 software (Systat Software, San Jose, CA.

4.3 RESULTS

4.3.1 Microenvironment

Shading did not influence the maximum root zone temperature (RZT) but reduced the mean root zone temperature (RZT) by 1.3 °C and minimum RZT by 1.4 °C compared to the unshaded conditions (Table 4.1). Mean and minimum air temperatures were not affected by shading. However, shading reduced the maximum air temperature by 1.3 °C and the leaf temperature by 0.9 °C. In addition, photosynthetic photon flux density (PPFD) was about 50% higher in unshaded treatments than in shaded conditions.

4.3.2 Leaf, fruit, and soil nutrient concentration

Leaf P, B, and Cu decreased, whereas Mg and Zn increased with N levels (Table 4.2). Other foliar nutrients, such as N, S, and Fe, decreased quadratically with increasing N levels. Leaf K and Ca were unaffected by N levels. Fruit N, K, Ca, S, Cu, Fe, Mn, and Zn concentrations increased while Mg and B decreased with N levels (Table 4.3). Shading did not affect macro-and micronutrients except leaf Fe and fruit P, Ca, and Cu, which were highest in shaded compared to unshaded conditions.

Overall, here were no interactions between N levels and shading for leaf and fruit nutrients, except for leaf S and Fe and fruit Fe and Zn (Table 4.2 & 4.3). Leaf S and Fe in unshaded conditions decreased with increasing N levels up to 358 $kg \cdot ha^{-1}$, while in shaded conditions, S and Fe increased above 179 $kg \cdot ha^{-1}$ N (Figure 4.1). Fruit Fe and Zn increased with increasing N levels under shading. However, in unshaded conditions, both nutrients increased with N level, reaching maximal values at 179 $kg \cdot ha^{-1}$ N (Figure 4.2).

Soil nitrate-N, P, K, and Ca increased with increasing N fertilization (Table 4.4) while shading had no effects on these soil nutrients.

4.3.3 Leaf gas exchange

Leaf Pn, ETR, and PhiPS2 increased, while Ci decreased with increasing N levels (Table 4.5). Leaf g_s and E increased quadratically, reaching maximum values at 358 $kg \cdot ha^{-1}$ N. Shading did not affect any leaf gas exchange parameters (Pn, g_s, Ci, ETR, and E), except PhiPS2, which was reduced under unshaded conditions. There were no N x shading effects for the leaf gas exchange parameters except g_s, which was unaffected by the N level in shaded plants and increased with the N level in unshaded plants (Figure 4.3).

4.3.4 Fruit yield

Large-sized and total marketable fruit number and weight and individual fruit weight increased quadratically with N levels (Table 4.6). Small-sized fruit number and weight decreased quadratically with increasing N levels, while the incidences of anthracnose and weevil increased with increasing N levels. Unshaded treatment doubled the medium-sized fruit number and weight compared to shade conditions. Shading reduced the number and weight of small-sized and total cull and the number of fruits with anthracnose and weevil damage. There were N x shading interactions for the large-sized fruit weight and total cull fruit number and weight. Large-sized fruit weight was highest at 358 $kg \cdot ha^{-1}$ N and lowest at 0 $kg \cdot ha^{-1}$ N in unshaded conditions (Figure 4.4). However, the cull fruit number and weight were highest at 179 and 358 $kg \cdot ha^{-1}$ N in unshaded conditions.

4.3.5 Water loss rate (WLR) and firmness

Fruit length, diameter, fresh weight, and dry weight increased quadratically with increasing N levels, while firmness, water content, and WLR were unaffected by N levels (Table 4.7). Fruit under shaded conditions had the highest water content and WLR but the lowest dry weight and firmness than in unshaded conditions. However, fruit growth measured as length, diameter, and fresh weight was unaffected by shading. Nitrogen and shading had no interactive effects on fruit characteristics (length, diameter, fresh weight, dry weight, firmness, water content, and WLR). Fruit WLR decreased with increasing keeping time. However, fruit firmness was constant for the first two days and, after day 3, decreased with increasing keeping time (Figure 4.5 A & B).

4.3.6 Bioactive compounds

Total flavonoids decreased with increasing N levels, while other bioactive compounds (TPC, CUPRAC, TEAC, C, and DHC) were unaffected (Table 4.8). Fruit TPC was reduced under shaded conditions, while the rest of the bioactive compounds were unaffected. N x shading had only significant effects on TPC. In unshaded conditions, TPC increased from 0 to 179 kg/ha^{-1} N and declined onwards, while in shaded treatments, TPC was similar between N levels (Figure 4.6).

4.4 DISCUSSION

4.4.1 Microenvironment

Our results were consistent with previous reports that showed that shading reduces the light intensity, leaf, and root zone temperature (Díaz-Pérez, 2013; Díaz-Pérez and John, 2019; Kittas et al., 2015; Mohotti and Lawlor, 2002). Also, dos Reis and Pereira (2022) found a reduction in infrared radiation in black shade nets (compared to unshaded conditions), which may contribute to lowering the air temperature under shading. Mean and minimum air temperatures did not differ between shaded and unshaded conditions in the current study. However, shade net reduced maximum air temperature by 1.3 °C compared to unshaded conditions. Other studies also found either no or minimal effects of shade nets on air temperature (Díaz-Pérez and John, 2019; Kittas et al., 2015). Fully covered shade-house with 50% shade cloth in Central Florida reduced maximum daily air temperature by 0.4 °C compared to ambient conditions (Arthurs et al., 2013). In this study, midday times were preferred for PPFD measurements because of the most perpendicular angle of the incidence of solar radiation. Morales et al. (2018) also found the highest light intensity at noon.

4.4.2 Leaf and fruit mineral concentration

In our study, leaf P, B, and Cu decreased with increasing N levels. However, N, K, S, and Fe were high at both low (0 kg·ha^{-1}) and high (716 kg·ha^{-1}) but reduced at medium levels (179 and 358 kg·ha^{-1} N). Most fruit nutrients increased with increasing N levels except B and Mg, which decreased. Also, in soil, N, P, K, and Ca also increased with increasing N levels. In a previous study, high-bush blueberry had increased foliar N with increasing N supply in hydroponic (Yañez-Mansilla et al., 2015) and field (Ehret et al., 2014) productions. Mészáros et al. (2021) found high

N, P, and Fe nutrients in apple (*Malus domestica* Borkh) leaves with low crop load. However, in our study, total yield did not correlate with foliar, fruit, and soil nitrogen concentrations (data not shown).

According to the sufficiency ranges of macro-and micronutrients in bell pepper leaves, nutrients were within the sufficiency ranges except for N in the current study (Jones et al., 1991). Based on that report, the sufficiency range of N is 4%-6% during early fruit set in bell pepper leaves.

Abul-Soud et al. (2014) found that shading plants might create a conducive microenvironment for the absorption of nutrients by reducing soil temperatures and light intensity. However, in the current study, applying shade nets increased the concentrations of a few mineral elements (leaf Fe and fruit P, Ca, and Cu) compared to unshaded treatments. Previously, El-Gizawy et al. (1992) and Liu et al. (2003) showed that foliar N, P, and K levels increased under shaded plants than in unshaded conditions in tomatoes. However, Caruso et al. (2020) found no difference in the leaf mineral composition of the perennial wall rocket (*Diplotaxis tenuifolia* L.-D.C.) plants under shaded and unshaded conditions. Similarly, Mohawesh et al. (2022) also found no significant difference in major leaf mineral elements (N, P, K) at 12 weeks after transplanting.

4.4.3 Leaf gas exchange

Nitrogen is the major factor controlling the activity of Rubisco (Evans, 1989), an essential enzyme for photosynthesis. We observed increased leaf Pn, ETR, and PhiPS2 with increasing organic N fertilizer levels. However, leaf g_s and E increased with increasing N levels up to 358 $kg \cdot ha^{-1}$ and then decreased at 716 $kg \cdot ha^{-1}$. Previous studies also found increased g_s with low to medium N fertilizer levels and then decreased with high N in Mongolian oak plants (*Quercus mongolica* Fish. ex Ledeb) (Zhu et al., 2020) and field-grown maize (*Zea mays* L.) (Isla et al., 2016). In addition, Han et al. (2021) also observed decreased stomatal conductance with increased N levels in chili peppers (*C. annuum* L.) when fertilizer levels from 0 to 1200 kg·ha^{-1} were used. The reduction of g_s at high N in the current study might be due to decreased rubisco activation of leaves because of high N content (Cheng and Fuchigami, 2000).

In the current study, we observed similar leaf gas exchange in both shaded and unshaded conditions. These results might be due to the acclimatization capacity of jalapeño pepper, even at low light intensity. Kitta et al. (2014) also found similar Pn, E, and g_s in bell pepper plants under shaded and unshaded conditions. In Georgia (USA), Kabir et al. (2022a) found that light saturation point was 1229 µmol m^{-2} s^{-1}, with no increase in photosynthesis above that light level

There were N x shading effects only on g_s. In unshaded conditions, g_s increased with increasing N levels up to 358 kg·ha^{-1} and decreased with 716 kg·ha^{-1}. However, in shaded conditions, g_s were similar across different N levels. A previous study found that small-sized stomata responded more quickly to environmental change, allowing leaves to attain high g_s under unfavorable conditions (Hetherington and Woodward, 2003). In this study, we did not evaluate the stomatal size and density, but previous studies reported variation in stomatal sizes at different light intensities (Onwueme and Johnston, 2000) and nitrogen levels (Otoo et al., 1989).

4.4.4 Fruit yield

Large-sized and total marketable fruit yield (both number and weight) increased with increasing N levels up to 358 kg·ha^{-1} and then decreased at a high N level (716 kg·ha^{-1}). Rodríguez et al. (2020) also found increased yield in sweet pepper from 64 to 516 kg·ha^{-1} and remained constant beyond 516 kg·ha^{-1} due to high N leaching. In our study, 179 and 358 kg·ha^{-1} N produced similar total marketable numbers and yields due to similar medium-sized fruit numbers and weight. Other researchers have also recommended 280 kg·ha^{-1} N, but with 133% of the recommended irrigation on sandy soil in Florida (Simonne et al., 2006) and 252 kg·ha^{-1} N for drip irrigated bell pepper on clay loam soils in California (Hartz et al., 1993).

Shading reduced the number and weight of medium-sized fruits, while large-sized and total marketable yield were similar between shaded and unshaded treatments. Similarly, Kabir et al. (2022b) found decreased yield in bell pepper above 40% shading. The decreased fruit yield with shading showed that jalapeño pepper could not produce sufficient assimilates because of reduced light intensity. A similar result was observed from the meta-regression of fruit trees and vegetables, showing that 20% to 30% shading increases marketable yield (Laub et al., 2022).

4.4.5 Water loss rate (WLR) and firmness

In this study, N level did not affect fruit WLR, water content, and firmness. Warner et al. (2004) also found similar fruit firmness, size, and soluble solids in tomatoes (*Lycopersicon esculentum* Mill.) at different N fertilization levels. In cabbage (*Brassica oleracea* L.), the highest N rate of 391 kg·ha^{-1} resulted in the highest postharvest weight loss (33.7%) and shriveling of cabbage heads compared to lower N rates (Gonzaga et al., 2021). In pear (*Pyrus communis* L.), high N levels also decreased pulp firmness when fruits were kept at ambient conditions for seven days after 90 days of refrigeration (Sete et al., 2019). Another study found that fruit firmness was related to water content and solute metabolism-regulating cell turgor (Vicente et al., 2007). In this study, fruit WLR and firmness decreased with increasing keeping days.

In the current study, shaded conditions resulted in the highest fruit WLR and water content and the lowest dry weight and firmness than in unshaded conditions. Similarly, Díaz-Pérez (2014) and Díaz-Pérez et al. (2020) also found a higher WLR in shaded fruit than in unshaded conditions. In concert with the present study, fruit water content (%) in northern high-bush blueberry plants (*Vaccinium corymbosum*. cv. Elliott) was high in shaded than in unshaded fruit (Lobos et al., 2013). Due to the lack of stomata in the fruit epidermis, water loss in pepper fruit occurs primarily through the cuticle (Blanke and Holthe, 1997; Díaz-Pérez et al., 2007). The pepper cuticle is composed of lipids, wax, and polysaccharides; these compounds provide a barrier to water loss (Ball, 1997; Yeats and Rose, 2013). Also, previous studies found that temperature, RH, and light intensity changes altered the cuticle's amount, composition, and thickness (Domínguez et al., 2012; Hull et al., 1975; Tattini et al., 2005). Reed and Tukey (1982) also found high cuticle thickness in Brussels Sprouts (*Brassica oleraceae* L., *Gemmifera* group) leaf at low temperature and light intensity. Differences in water loss rate might be due to differences in cuticular composition between shaded and unshaded fruits. However, in the current study, cuticle composition was not studied. Other environmental factors, such as light intensity, root zone temperature, and day-night temperature difference, also affect fruit water accumulation (Adams et al., 2001; De Koning, 1988).

4.4.6 Bioactive compounds

In the current study, only total flavonoids decreased quadratically with increasing N levels. Cruz et al. (2020) found decreased total phenols and flavonoids with increased N levels (0, 200 ppm, 400 ppm, and 600 ppm) in different cultivars of basil (*Ocimum basilicum* L.). In kiwi berries (*Actinidia deliciosa*), the increased N level in the soil led to a gradual decrease in TEAC and TPC values (Stefaniak et al., 2020). However, Ehret et al. (2014) found total antioxidant (ORAC) levels unaffected by N levels. In concert with this study, Nunez-Ramirez et al. (2011) found no effects of N levels (32, 80, 160, and 320 $kg\cdot ha^{-1}$) in Habanero pepper (*Capsicum chinense* Jacq.). Similar to our results about capsaicin and dihydrocapsaicin, different rates of N and K fertilization also did not affect the capsaicinoids in jalapeño pepper (Johnson and Decoteau, 1996) and Habanero pepper (Medina-Lara et al., 2008). This study's values of capsaicin and dihydrocapsaicin were similar to those reported by Orellana-Escobedo et al. (2013) in dried jalapeño pepper produced in Mexico.

In our study, among the biotic compounds, only TPC was affected by shading. Higher values of TPC in unshaded than in shaded conditions could be attributed to the TPC's protective role against excessive solar radiation (Raúl et al., 2017; Toscano et al., 2019). Similar findings were reported by Ilić et al. (2017a) in lettuce, where the highest flavonoid content was observed in unshaded treatment, while no difference in TPC was observed between shade nets and unshaded treatments. In asparagus, all bioactive compounds (phenol and flavonoids) were highest in unshaded conditions, but shading effect was found in antioxidant activity (Gao et al., 2021).

There was N x Shading interaction in TPC in this study with the highest TPC in unshaded conditions at 179 $kg \cdot ha^{-1}$ while TPC was unaffected by N level under shading. However, in the study conducted by Hernández et al. (2019) in tomatoes with different N levels and shading (40%), a decline in TPC with N levels was observed only under full sunlight conditions. Interestingly, under shading, no significant differences in TPC were observed.

4.6 CONCLUSIONS

In conclusion, this study examined the effects of organic nitrogen fertilization levels and shading on the growth, productivity, and quality of organic jalapeño pepper. Shading reduced root zone and air temperatures, as well as light intensity, compared to unshaded conditions. Leaf and fruit mineral concentrations were influenced by nitrogen levels, with phosphorus, boron, copper, nitrogen, sulfur, and iron increasing, while magnesium and zinc decreasing with increasing nitrogen fertilization. Shading increased leaf iron concentration and fruit phosphorus, calcium, and copper concentrations. Leaf gas exchange parameters such as net photosynthesis, stomatal conductance, and transpiration increased with nitrogen levels, reaching optimal values at 358 kg·ha^{-1} N. Shading had minimal effects on leaf gas exchange except for increased photosystem-II efficiency. Marketable fruit yield, size, and weight increased quadratically with nitrogen levels, while shading reduced medium-sized fruit yield and all categories of cull yield. Total flavonoids decreased with increasing nitrogen levels, while shading reduced total phenolic contents. The study concludes that shading utilization and organic nitrogen fertilization beyond 358 kg·ha^{-1} N were unsuitable for producing organic jalapeño pepper. These findings provide valuable insights for optimizing the growth and productivity of organic jalapeño pepper crops, highlighting the importance of nitrogen fertilization and shading management in organic vegetable production systems.

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Tables and Figures

Table 4.1 Root zone temperature (RZT), air temperature (Ta), leaf temperature (Leaf T), and photosynthetic photon flux density (PPFD under black shade net and unshaded conditions in organic jalapeño pepper, Spring 2021, Tifton, GA.

	RZT (º C)			Ta (º C)			Leaf T (º C)	PPFD (µmol m^{-2} s^{-1})
Treatments	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Mean	Mean
Shaded	31.5	26.7 b[z]	24.2 b	34.4 b	27.4	22.9	34.5 b	837 b a
Unshaded	31.7	28 a	25.6 a	35.7 a	27.6	22.8	35.4 a	1694 a b
P	0.72	<0.0001	<0.0001	0.0273	0.33	0.41	<0.0001	<0.0001

[z]Means followed by the same letter are not significantly different based on the LSD test at $p \leq 0.05$.

Table 4.2 Leaf nutrient concentration [macronutrients (%) and micronutrient (ppm)] in organic jalapeño pepper under shaded and unshaded conditions with different nitrogen fertilization levels, Spring 2021, Tifton, GA.

	Macronutrients (%)						Micronutrients (ppm)			
Treatments	N	P	K	Ca	Mg	S	B	Cu	Fe	Zn
N levels ($kg \cdot ha^{-1}$) [N]										
0	3.15	0.71	3.04	1.41	0.54	0.37	42.3	11.6	63.4	44.3
179	2.73	0.56	2.68	1.36	0.55	0.35	36.5	10.1	53.9	46.3
358	2.94	0.44	2.67	1.35	0.59	0.36	27.6	8.9	56.4	49.3
716	3.61	0.37	2.8	1.38	0.64	0.4	21.1	8.4	64.8	53.9
Significance[x]										
L	0.012	<0.0001	0.37	0.9	0.007	0.056	0.0003	0.004	0.306	0.0003
Q	0.001	<0.0001	0.06	0.82	0.026	0.025	0.001	0.008	0.015	0.002
Shading [S]										
Shaded	3.19	0.56	2.88	1.31	0.56	0.37	32.3	10.2	64.5 a[z]	49.4
Unshaded	3.01	0.47	2.69	1.44	0.61	0.36	30.7	9.1	54.1 b	47.6
P										
S	0.16	0.24	0.11	0.4	0.44	0.38	0.84	0.54	0.023	0.46
N x S	0.08	0.95	0.68	0.35	0.78	0.01	0.98	0.42	0.007	0.64

[x]L = Linear and Q = quadratic regression.

[z]Means followed by the same letter are not significantly different based on the LSD test at $p \leq 0.05$.

Table 4.3 Fruit nutrient concentration [macronutrients (%) and micronutrient (ppm)] in organic jalapeño pepper under shaded and unshaded conditions with different nitrogen fertilization levels, Spring 2021, Tifton, GA.

	Macronutrients (%)						Micronutrients (ppm)				
Treatments	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
N levels ($kg \cdot ha^{-1}$) [N]											
0	1.81	0.49	2.63	0.11	0.223	0.21	14.6	9.9	43.3	10.5	24.5
179	2.24	0.47	2.61	0.12	0.213	0.24	12.6	9.9	63.5	12.6	32.9
358	2.38	0.47	2.95	0.14	0.205	0.25	11	10.5	63.1	17.8	34.8
716	2.57	0.5	3.1	0.13	0.207	0.26	11.4	10.9	66.4	17.3	38
Significance[x]											
L	<0.001	0.38	0.0006	0.013	0.013	0.0001	0.0001	0.047	0.0001	0.001	<0.001
Q	<0.001	0.25	0.003	0.011	0.006	0.0002	<0.001	0.14	<0.0001	0.001	<0.001
Shading [S]											
Shaded	2.29	0.51 a[z]	2.88	0.134 a	0.213	0.24	12.3	10.6 a	62.3	14.9	33.8
Unshaded	2.21	0.46 b	2.76	0.118 b	0.213	0.23	12.5	9.9 b	55.9	14.1	31.3
P											
S	0.36	0.007	0.42	0.02	1	0.051	0.74	0.048	0.11	0.69	0.15
N x S	0.29	0.22	0.56	0.08	0.52	0.107	0.45	0.16	0.01	0.11	0.02

[x]L = Linear and Q = quadratic regression.

[z]Means followed by the same letter are not significantly different based on the LSD test at $p \leq 0.05$.

Table 4.4 Soil nutrient concentration [Nitrate-N (ppm), P, K, and Ca (lbs/acre)] under shaded and unshaded conditions with different nitrogen fertilization levels in organic jalapeño pepper, Spring 2021, Tifton, GA.

Treatments N levels ($kg\cdot ha^{-1}$)[N]	Pn ($\mu mol\ m^{-2}\ s^{-1}$)	g_s ($mmolm^{-2}s^{-1}$)	Ci ($\mu mol\ mol^{-1}$)	E ($mmolm^{-2}s^{-1}$)	ETR ($\mu mol\ m^{-2}\ s^{-1}$)	PhiPS2
0	16.4	137.5	173	5.9	110.4	0.23
179	19.7	150.2	154	6.5	121.4	0.25
358	21.8	165.7	151.4	7	131.5	0.27
716	23.2	158.3	126.5	6.7	142.8	0.29
Significance[x]						
L	<0.001	0.034	<0.001	0.052	<0.001	0.009
Q	<0.001	0.016	<0.001	0.026	<0.001	0.03
Shading [S]						
Shaded	18.7	147	159.7	6.1	121.4	0.34 a[z]
Unshaded	22	159	140.9	6.9	132.5	0.18 b
P						
S	0.12	0.41	0.14	0.17	0.38	0.001
N x S	0.051	0.045	0.66	0.06	0.46	0.96

[x]L = Linear and Q = quadratic regression.

[z]Means within the same column followed by the same letter are not significantly different based on the LSD test at $p \leq 0.05$.

Table 4.5 Marketable and cull fruit number and weight per plant in organic jalapeño pepper under shaded and unshaded conditions with different nitrogen levels in Spring 2021, Tifton, GA.

Treatments	Marketable yield/plant							Cull yield/plant								
					Total		Individual									
N levels	Large		Medium		Marketable		Fruit	Small		Anthracnose		Weevil		Total cull		
$(kg\cdot ha^{-1})$[N]	No.	Wt.(g)	No.	Wt.(g)	No.	Wt.(g)	Wt.(g)	No.	Wt.(g)	No.	Wt.(g)	No.	Wt.(g)	No.	Wt.(g)	
0	1.5	57.4	4.5	126.7	6	184.1	30.3	11.6	212.2	0.08	2.1	2.1	29.3	13.8	243.7	
179	4.9	193.4	7.1	217.2	12	410.6	33.3	6.2	147.8	0.18	5	13.5	133.6	19.9	286.4	
358	6.9	263.3	7	217.9	13.9	481.2	33.6	4.3	96.4	0.16	3	8.4	85	12.8	184.4	
716	2.8	108.6	7.2	225.4	9.9	334	35.1	9.2	209.1	0.96	17.2	12.8	109.7	23	336.1	
Significance[x]																
L	0.59	0.57	0.19	0.15	0.24	0.22	0.11	0.61	0.92	0.003	0.009	0.023	0.06	0.13	0.32	
Q	<0.001	<0.001	0.22	0.18	0.003	0.001	0.03	0.016	0.07	0.005	0.018	0.029	0.03	0.26	0.32	
Shading [S]																
Shaded	4.1	151.6	4.4 b	128.3 b	8.4	279.8	33.1	3.8 b	81.3 b	0.1 b	1.8 b	6.1 b	62.2 b	10 b	145.3 b	
Unshaded	4	159.8	8.5 a	265.3 a	12.5	425.1	33	11.8 a	251.4 a	0.6 a	11.9 a	12.3 a	116.6 a	24.7 a	378 a	
P																
S	0.94	0.8	0.016	0.017	0.08	0.07	0.94	0.003	0.003	0.016	0.012	0.004	0.0005	0.0006	0.001	
N x S	0.055	0.006	0.13	0.13	0.09	0.06	0.51	0.07	0.08	0.2	0.33	0.13	0.07	0.005	0.001	

[x]L = Linear and Q = quadratic regression. [z]Means within the same column followed by the same letter are not significantly different based on the LSD test at $p \leq 0.05$.

Table 4.6 Fruit length and diameter, fresh weight (FW), dry weight (DW), firmness, water content, and water loss rate (WLR) of organic jalapeño pepper under shaded and unshaded conditions with different nitrogen levels, Spring 2021, Tifton, GA.

Treatments	Length	Diameter	FW	DW	Firmness	Water content	WLR
N levels ($kg \cdot ha^{-1}$)[N]	(cm)	(cm)	(g)	(g)		(%)	(% d^{-1})
0	7.2	2.6	28.4	2.47	4.3	91.2	1.91
179	7.8	2.8	35.9	2.94	4.5	91.8	1.97
358	8.1	2.8	38.7	3.21	4.4	91.7	1.91
716	7.7	2.7	34.1	2.96	4.3	91.3	1.92
Significance^{x}							
L	0.014	0.18	0.07	0.07	0.46	0.97	0.92
Q	<0.001	<0.001	<0.001	0.004	0.55	0.37	0.97
Shading (S)							
Shaded	7.9	2.8	34.1	2.7 b^{z}	4.2 b	92.1 a	2.3 a
Unshaded	7.5	2.7	34.5	3.1 a	4.6 a	90.9 b	1.6 b
P							
S	0.21	0.12	0.45	0.03	0.009	0.02	0.003
N x S	0.29	0.74	0.67	0.55	0.17	0.29	0.33

^{x}L = Linear and Q = quadratic regression.

^{z}Means within the same column followed by the same letter are not significantly different based on the LSD test at $p \leq 0.05$.

Table 4.7 Total phenols (TPC), total flavonoids (TF), Cupric reducing antioxidant capacity (CUPRAC), Trolox equivalent antioxidant capacity (TEAC), Capsaicin (C), and Dihydrocapsaicin (DHC) in organic jalapeño pepper collected at mature green stage under shaded and unshaded conditions with different nitrogen levels, Spring 2021, Tifton, GA.

Treatments	TPC	TF	CUPRAC	TEAC	C	DHC
N levels ($kg \cdot ha^{-1}$)[N]	mg GAE/L	mg quercetin/mL	µM	µM	µg/g	µg/g
0	142.2	22.7	2491.9	3221	472	244
179	148.7	22.5	2591.5	3156	397	210
358	141.5	19.5	2382.9	2941	400	207
716	137.9	20.4	2453.1	2925	459	238
Significancex						
L	0.24	0.06	0.54	0.33	0.94	0.97
Q	0.37	0.007	0.81	0.59	0.17	0.44
Shading (S)						
Shaded	136.4 bz	19.5	2233.8	2970	437	228
Unshaded	148.8 a	22	2730.2	3151	429	223
P						
S	0.002	0.053	0.09	0.57	0.82	0.82
N x S	0.01	0.65	0.26	0.94	0.25	0.22

[x]L = Linear and Q = quadratic regression.

[z]Means within the same column followed by the same letter are not significantly different based on the LSD test at $p \leq 0.05$.

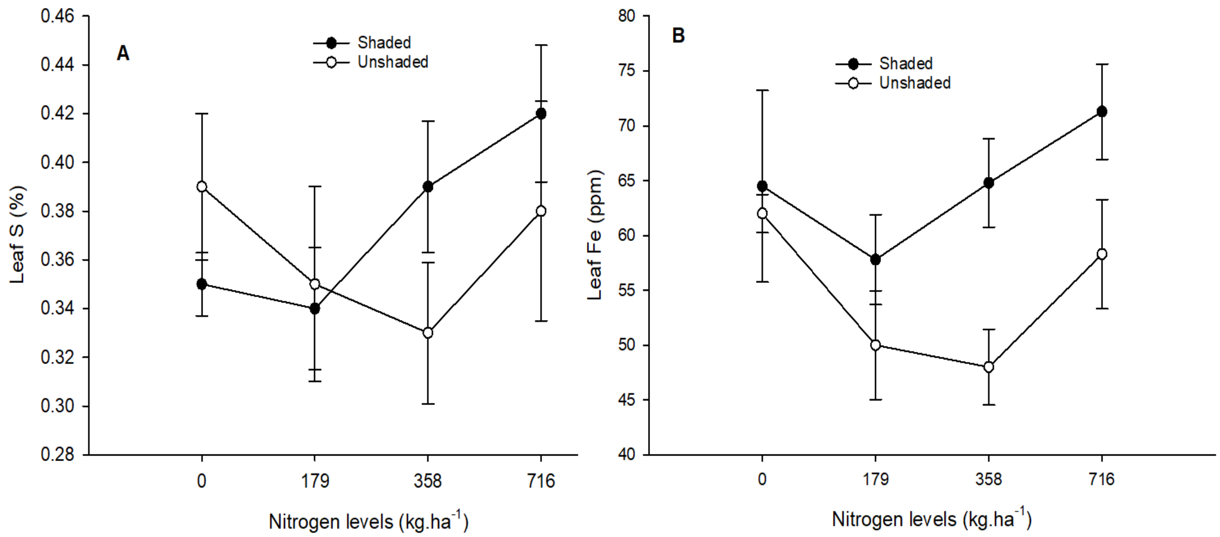

Figure 4.1 Influence of nitrogen fertilization level on leaf S and Fe in organic jalapeño pepper under shaded and unshaded conditions, Spring 2021, Tifton, GA. Values are the means of four replicates, with error bars representing the standard error.

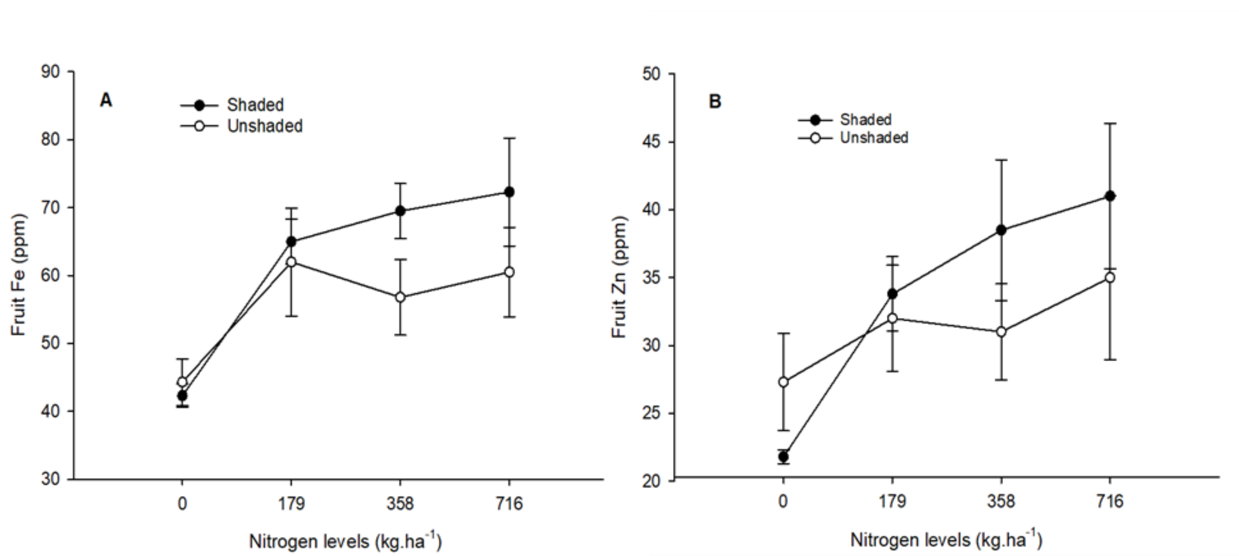

Figure 4.2 Influence of nitrogen fertilization level on fruit Fe and Zn content in organic jalapeño pepper under shaded and unshaded conditions, Spring 2021, Tifton, GA. Values are the means of four replicates, with error bars representing the standard error.

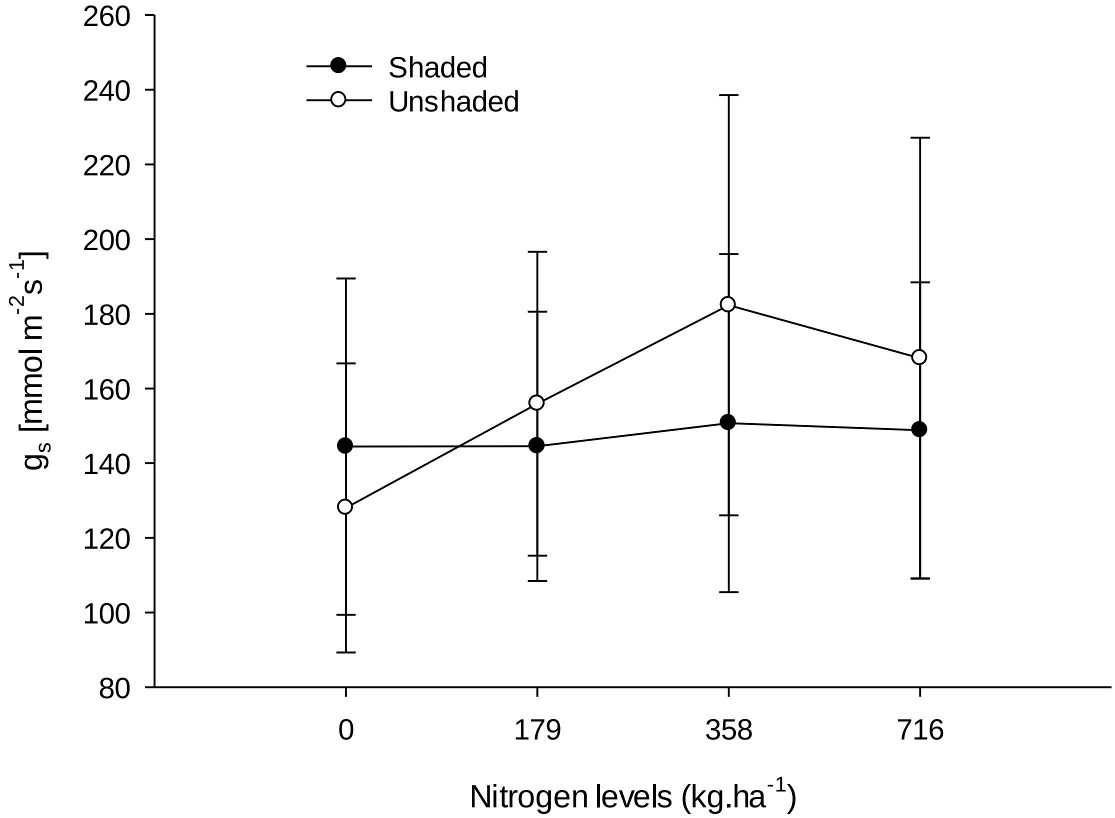

Figure 4.3 Influence of nitrogen fertilization levels in stomatal conductance (g_s) of organic jalapeño pepper under shaded and unshaded conditions, Spring 2021, Tifton, GA. Values are the means of four replicates, with error bars representing the standard error.

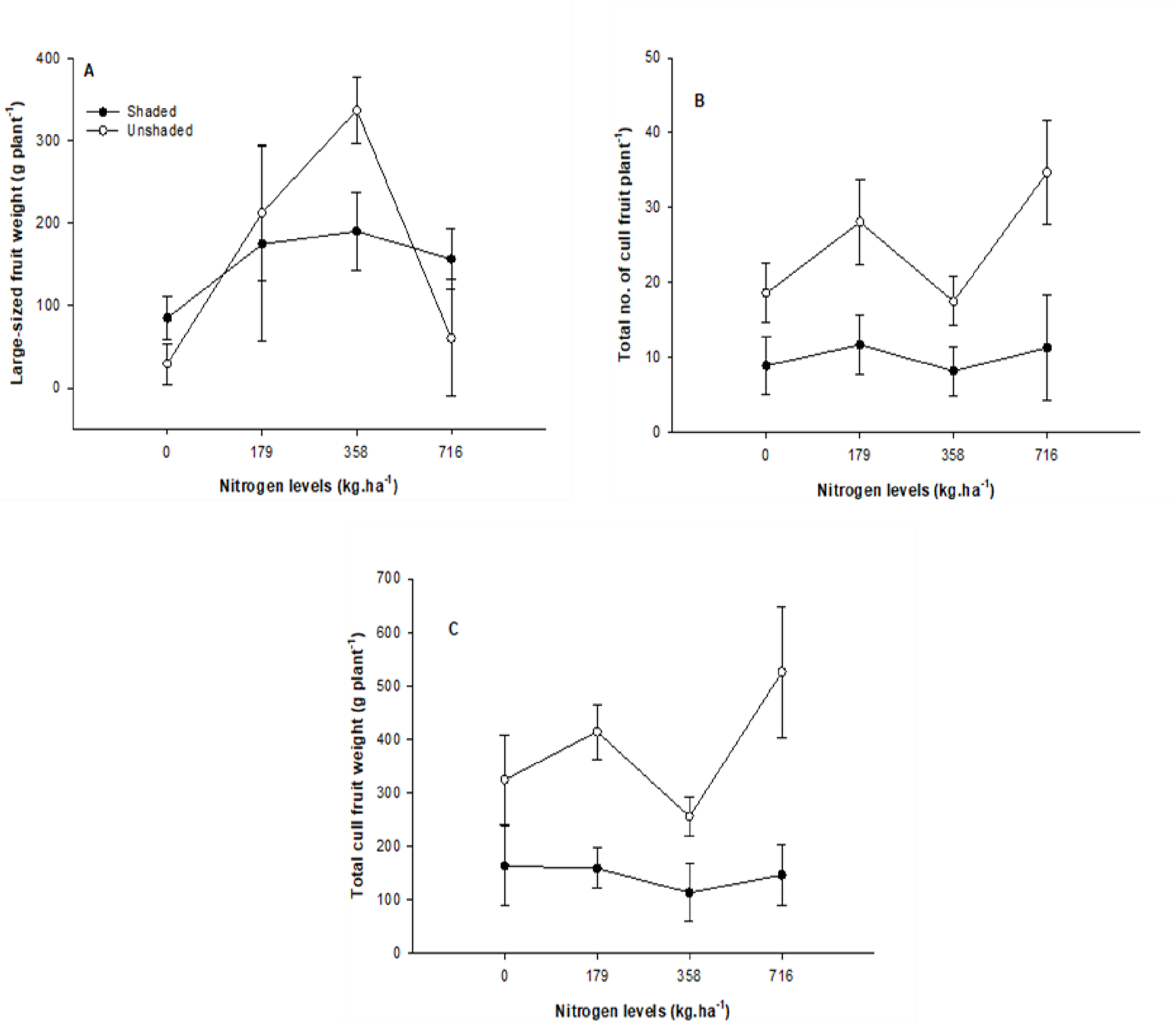

Figure 4.4 Effects of different nitrogen levels on large-sized fruit weight (A), total cull fruit number (B), and total cull weight per plant (C) under shaded and unshaded conditions in organic jalapeño pepper, Spring 2021, Tifton, GA. Values are the means of four replicates, with error bars representing the standard error.

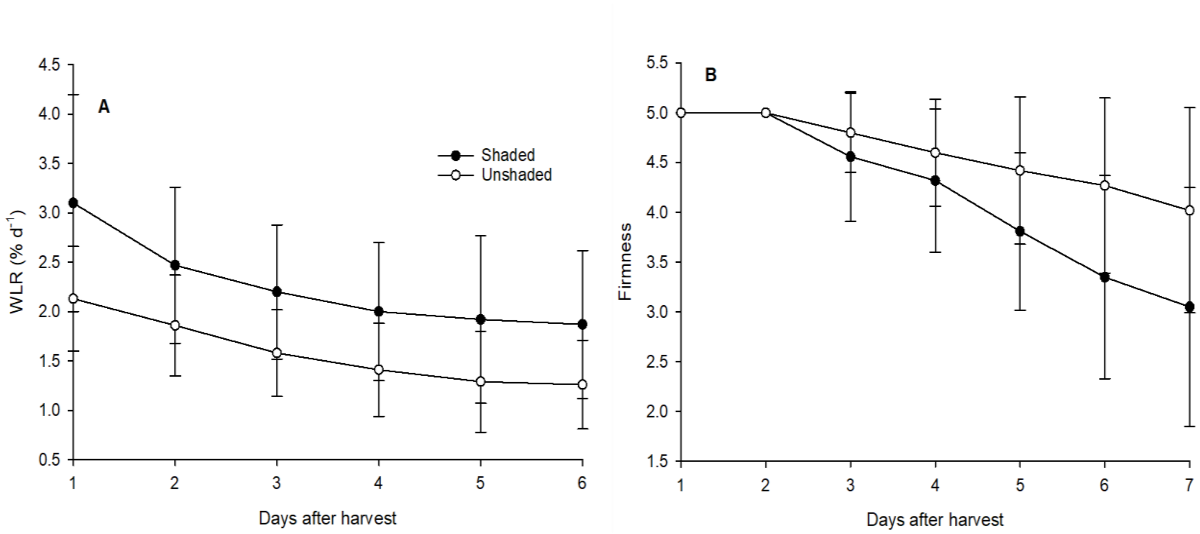

Figure 4.5 Relationship between water loss rate (WLR) (A) and firmness (B) with days after harvest at 21 °C. Fruit firmness was measured hedonically using a 1-5 scale (1=spongy soft, 5=firm). Each value is the mean for four replicates ± standard errors.

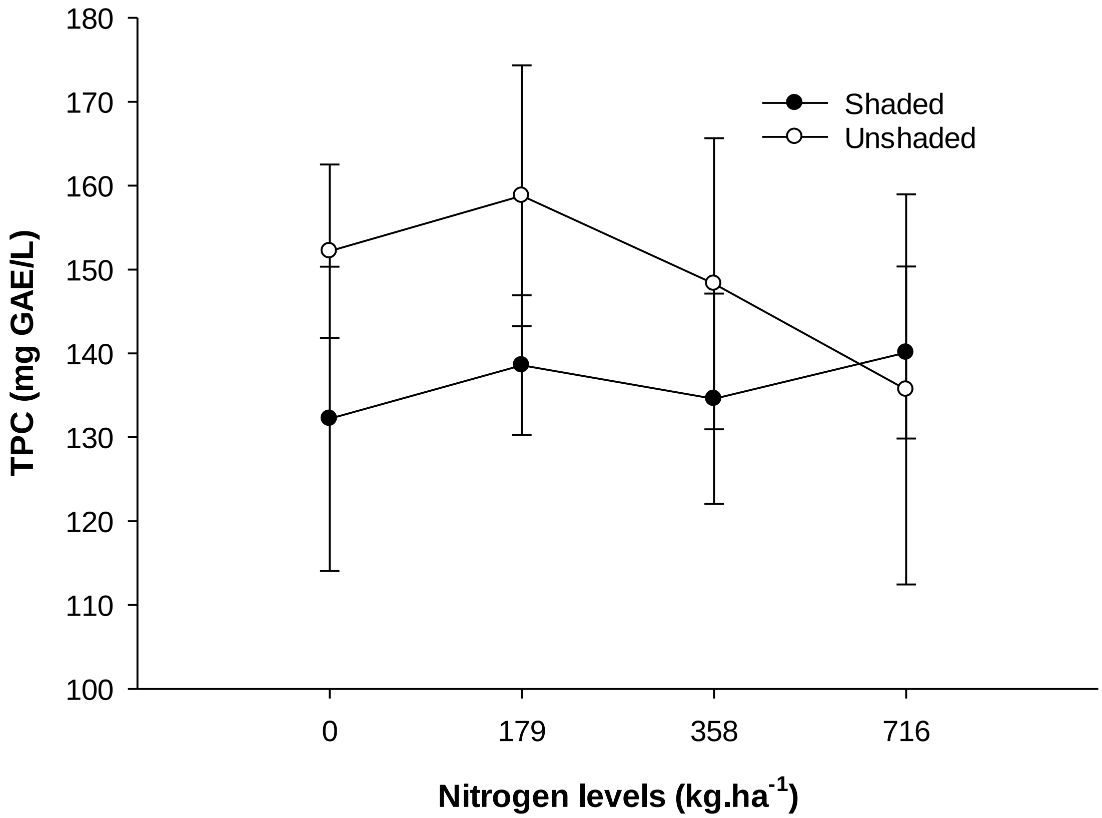

Figure 4.6 Influence of nitrogen fertilization levels under shaded and unshaded conditions in Total phenolic contents (TPC) of the organic jalapeño pepper fruit, Spring 2021, Tifton, GA. Values are the means of four replicates, with error bars representing the standard error.

CHAPTER 5

IRRIGATION LEVEL AFFECTS PLANT GROWTH, PHYSIOLOGY, FRUIT YIELD, AND QUALITY OF ORGANIC JALAPEÑO PEPPER (*Capsicum annuum* L.)

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ABSTRACT

Insufficient information exists regarding the water requirements for jalapeño pepper production. The objective of this study was to assess the impact of varying drip irrigation levels on the growth, physiology, yield, and quality of organic jalapeño peppers. The experiment was conducted over two years, in the Spring-Summer of 2021 (year 1) and 2022 (year 2). using five different drip irrigation levels: 25%, 50%, 75%, 100%, and 150% crop evapotranspiration rate (ETc). Irrigation level did not affect plant dry biomass (leaf and shoot), but the leaf weight ratio decreased with increasing irrigation levels. However, the leaf water potential increased with increasing irrigation levels in both years. In year 1, leaf gas exchange variables showed no response to irrigation level, while in year 2, except photosystem II efficiency (PhiPS2), all other variables increased with irrigation level reaching maximum values at 150% ETc. All leaf and fruit mineral nutrients were unaffected by irrigation level except leaf N, which decreased with increasing irrigation level. In year 1, leaf N, P, K, Mg, B, and Cu and fruit P, K, B, Cu, Fe, and Zn concentrations were higher than in year 2. The number and weight of large-sized fruits increased with higher irrigation levels in year 1. In year 2, increasing irrigation levels resulted in higher numbers and weights of large, medium, and total marketable fruits, with the highest yield observed at 150% ETc. However, in terms of cull yield, only the number and weight of small-sized fruits per plant exhibited a quadratic relationship with the irrigation level, leading to reduced yield at 150% ETc in year 2. Elevating irrigation levels caused a decline in the concentrations of total phenols, flavonoids, cupric-reducing antioxidants, capsaicin, dihydrocapsaicin, and Scoville heat units (SHU). These findings indicated that high irrigation levels inhibit the synthesis of these bioactive compounds. Therefore, optimizing irrigation practices is necessary to achieve high fruit yield while also enhancing the levels of bioactive compounds. According to our study, irrigation

at 75% ETc can be implemented as a regulated deficit irrigation strategy, resulting in comparable marketable fruit numbers and sizes as 100% ETc, while simultaneously increasing bioactive compounds.

Keywords: Deficit irrigation, gas exchange, jalapeño pepper, water stress.

5.1 INTRODUCTION

On a global scale, the availability of water is a significant concern for vegetable production, particularly in regions with hot and dry summers and unpredictable rainfall patterns (Solankey et al., 2021). Climate change intensifies drought events and raises global temperatures (Berg and Sheffield, 2018). Agricultural practices account for approximately 70%-80% of global water resource utilization (Pimentel et al., 2004). To address these challenges, it is crucial to establish innovative irrigation strategies that can maintain optimal fruit yield and quality while minimizing water usage and wastage.

Pepper plants' water requirements may vary depending on species, weather, and soil characteristics. Because of their shallow root system, peppers typically extract 70% to 80% of the required water from the top 30 cm of the soil profile (Dalla Costa and Gianquinto, 2002; Guang-Cheng et al., 2010). One effective technological advancement is regulated deficit drip irrigation, based on crop phenological stages and evapotranspiration, to enhance water use efficiency (WUE) in pepper cultivation (Abdelkhalik et al., 2020; Foday et al., 2012).

With bell pepper (*C. annuum* L.), Abdelkhalik et al. (2020) demonstrated that applying 75% regulated deficit irrigation during fruit setting and harvesting resulted in a minimal loss of marketable yield (-19%) while achieving significant water savings (21%). Similarly, Owusu-

Sekyere et al. (2010) found that a 20% deficit irrigation strategy maintained similar growth, development, and yield in hot pepper (*Capsicum frutescens*). Studies involving different Hungarian hot pepper cultivars and species and ‘Habanero’ subjected to deficit irrigation showed comparable levels of vitamin C and capsaicinoids in fruits compared with those grown under full irrigation supply (Duah et al., 2021). Additionally, Anjum et al. (2012) discovered that drought resistant cultivar ‘Shanshu-2001’, exhibited increased levels of total soluble proteins and proline, along with enhanced activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) at lower irrigation levels. Ćosić et al. (2015) found that deficit irrigation of 20%-30% still produced similar amounts of organic acids, sugar content, and antioxidant capacity in bell pepper. Also, Celebi (2018) also found that 20% deficit irrigation can be viable for drought-stressed environments. In bell pepper, irrigation at 50%- 67% of the crop evapotranspiration (ETc) was sufficient to maximize vegetative growth and fruit yield, while excessive irrigation increased the incidence of blossom-end rot (BER) (Díaz-Pérez and Hook, 2017; Kabir et al., 2021).

It is worth noting that the jalapeño pepper, which belongs to the same group as the bell pepper, has not received extensive research attention. Despite being the most preferred spicy pepper in the United States, comprehensive studies on its specific water requirements are lacking. Therefore, before implementing a deficit irrigation strategy for jalapeno pepper, it is crucial to thoroughly evaluate its performance under various drip irrigation levels based on crop evapotranspiration (ETc) under open field conditions, particularly under plastic mulch. This study aimed to assess the dry biomass, leaf gas exchange, fruit yield, and quality of jalapeño pepper.

5.2 MATERIALS AND METHODS

5.2.1. Experimental site and design

Two experiments were conducted in the spring-summer of 2021 and 2022 at the Organic Horticulture Farm, University of Georgia, Tifton, GA. The soil of the experimental field is sandy loam with a pH of 6.5. Jalapeño pepper 'Compadre' (untreated seed) plants were grown on raised beds with two rows of plants per bed (45 cm distance between rows) and 30 cm separation between plants within the row. The planting dates were 12 April 2021 and 19 April 2022. Before laying mulch, the soil was fertilized with nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) at 358 kg/ha, 286 kg/ha, and 215 kg/ha, respectively, using 5:4:3 organic poultry fertilizer (Symphony Organic Fertilizer, Virginia). Raised beds (4.6 m long x 0.91 m wide, 1.8 m centers) were covered with black plastic film mulches [low-density polyethylene with a slick surface texture, 1.52 m wide and 25 mm thick (RepelGro; ReflecTek Foils, Inc., Lake Zurich, IL)]. During the first 40-45 days, all plants received the same irrigation depth (100% ETc) to establish plants equally. Afterward, one, two, three, four, and six drip irrigation tapes [30-cm emitter spacing and a 16.7-mL·min^{-1} emitter flow (Rivulis Irrigation, San Diego, CA)] were placed 5 cm deep in the center of the bed to apply 25%, 50%, 75%, 100% and 150% the rate of evapotranspiration, respectively. Irrigation treatments were applied following a randomized complete block design with four replications started five weeks after planting (15 May 2021 and 25 May 2022). Irrigation treatments were applied when the cumulative evapotranspiration reached about 1.27 cm. The amount of water applied was based on the 100% ETc treatment and equivalent to the amount of water lost by ETc, with an additional 10%, assuming a 90% irrigation efficiency.

5.2.2. Climatic conditions and water management

During the experiment, soil temperature (maximum, average, and minimum) and volumetric water content (VWC) of the soil profile (0-30 cm) were monitored periodically with time domain reflectometry (TDR) sensors (two per treatment; CS-615, Campbell Scientific, Logan, UT) connected to a data logger (CR-1000X; Campbell Scientific). Soil moisture sensors had three metallic 30 cm rods inserted vertically within the row between two plants at mid-section. Daily air temperature (minimum, maximum), evapotranspiration, and rainfall data were collected from a University of Georgia weather station within 1 km of the experimental site.

Seasonal trends of maximum, mean, and minimum soil temperatures (Figures 5.1 A & B), daily rainfall (mm), maximum and minimum air temperatures (Figures 5.2 A & B), and daily evapotranspiration (ETo) (Figure 5.4) for 2021 and 2022 are shown. Trends of soil volumetric water content (VWC) for 2021 and 2022 are shown in Figure 5.3 A & B.

5.2.3. Plant dry biomass and leaf chlorophyll content

At the final harvest, two plants randomly selected from each plot were excised at soil level to determine leaf and stem dry weights after drying at 70 ºC. Leaf weight ratio (LWR) was calculated as the ratio of leaf dry weight by shoot (leaf + stem) dry weight. Three leaves per plot were collected and analyzed individually for chemical composition. Carotenoids and chlorophyll (*Chl*) *a* and *b* were determined spectrophotometrically in fresh leaves. Leaf discs (2 cm^2) were collected from leaf interveinal areas, placed into tubes, and extracted in 1-1.5 mL ethanol (100% v/v) for 24 h. Pigment concentrations were calculated from the absorbance of the extract at 470, 649, and 664 nm using the following equations (Lichtenthaler and Wellburn, 1983):

Chlorophyll a	C_a (µg/ml)	$C_a=13.36A_{664} - 5.19A_{649}$
Chlorophyll b	C_b (µg/ml)	$C_b=27.43A_{649} - 8.12A_{664}$
Carotenoids	C_{x+c} (µg/ml)	$C_{x+c}= (1000A_{470} - 2.13Ca - 97.63C_b)/209$

5.2.4 Mid-day leaf water potential and gas exchange

Mid-day leaf water potential was measured on sunny, clear days between 1100 h and 1500 h Eastern Standard Time with a Pump-Up pressure chamber (Meron et al., 1987) (PMS Instrument Company, 1725 Geary Street SE, Albany, OR) 2 days before irrigations throughout the growing seasons. Measurements were made thrice in 2021 and twice in 2022 on two healthy, well-developed, and sunlit leaves per plot. Immediately before leaf excision, leaves were enclosed within an airtight polyethylene bag. After excision, the bagged leaf was placed inside the metal chamber of the Pump-Up pressure chamber and sealed, leaving only a portion of the petiole outside the chamber. The chamber was pressurized to about 0.05 MPa pressure per stroke until the sap exudate was observed from the petiole cut surface. At this endpoint, leaf potential (bar) was recorded from the manometer gauge.

Leaf gas exchange measurements [net photosynthesis (Pn) stomatal conductance (g_s), transpiration (E), intercellular CO_2 (Ci), electron transport rate (ETR), and photosystem-II efficiency (PhiPS2)] were done with a portable photosynthesis system (LI-COR 6400XT equipped with an integrated 6400-40 leaf chamber fluorometer; LI-COR, Inc., Lincoln, NE, USA). The airflow rate was 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ on the reference side. The CO_2 concentration was set at 400 $\mu mol \cdot mol^{-1}$ with a CO_2 mixer and a CO_2 tank. Photosynthetically active radiation (PAR) was set at 1800 $\mu mol\ m^{-2}\ s^{-1}$. Measurements were conducted in developed plants on clear days at 1200 to 1500 HR using two to three well-developed and fully exposed leaves per plot. Measurements were done on 15 and 25 June, and 14 July 2021, and 22 and 28 June 2022.

5.2.5 Leaf and fruit nutrient concentrations

Samples of 25-30 fully developed, healthy leaves from new growth and five fruits cut into halves from each plot were collected. Leaf and fruit samples were dried at 70 °C for 2 d to constant weight and analyzed for mineral nutrients (N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn). Analyses of leaf and fruit mineral nutrients were performed by a commercial laboratory (Waters Agricultural Laboratories, Inc., Camilla, GA).

5.2.6 Fruit yield

Jalapeño pepper fruits were harvested and graded as marketable or cull. Fruit length was used as a grading criterion for marketable [large (7-10 cm) and medium (5-7 cm)]. Cull fruits were grouped as: 'small' (<5 cm length), had defects due to anthracnose (*Colletotrichum acutatum*), or damage by pepper weevil (*Anthonomus eugenii*) or blossom end rot (BER). Fruits were harvested (mature green) four times from 11 June to 23 July 2021 and 16 June to 22 July 2022.

5.2.7 Bioactive compounds

Three fruits per plot were taken to the laboratory and cut into halves immediately after harvest. Samples were extracted by grinding and soaking them in 95% ethanol for 2 d at room temperature (20-22 °C). Homogenized samples were refrigerated at 4 °C until starting the analysis. Each sample extract was analyzed in triplicate for each assay.

Total phenolic content (TPC) was determined following the Folin-Ciocalteu method (Derakhshan et al., 2018; Singleton et al., 1999). The samples of 5 µL were added 1195 mL of water, 75 µL of Folin-Ciocalteu reagent, and 225 µL of Na_2CO_3 solution. After incubating at 40 °C for 30 minutes under dark conditions, TPC was determined at 765 nm using a spectrophotometer (Beckman Coulter, Brea, CA, USA), and data were expressed as gallic acid equivalents (GAE) in mg. L^{-1}. The standard curve was prepared using a gallic acid solution (0-500 mg. L^{-1}).

Total flavonoid content (TFC) was determined using the aluminum chloride method (Chang et al., 2002; Gu et al., 2019) and expressed as quercetin equivalents (µg/mL). 20 µL samples were mixed with 100 µL 10% $AlCl_3$, 20 µL 1M KoAC, and 940 µL H_2O. TFC was determined after 30 min at room temperature, reading the reaction mixture at 415 nm, using a spectrophotometer (Beckman Coulter, Brea, CA, USA).

Two different assays determined antioxidant capacity: Trolox Equivalent Antioxidant Capacity-2,2′-diphenyl-1-picrylhydrazyl (TEAC-DPPH) (Liao et al., 2012) and Cupric reducing antioxidant capacity (CUPRAC) (Apak et al., 2008) methods. Trolox was used as the standard for the calibration curve. Trolox was used as the standard for the calibration curve in all assays. For the TEAC-DPPH assay, a 20µL sample was mixed with 80µL 100% MeOH and 1mL of freshly prepared 200 µM DPPH in 80% MeOH. After 30 min at room temperature, TEAC-DPPH was measured at 520 nm and expressed in µM Trolox. CUPRAC was determined using a 20 µL sample mixed with 80 µL of 80% EtOH, 0.4 mL of 7.5 mM neocuproine (Nc) in 96% EtOH, 0.34 mL of H_2O, 0.4 mL of 100 mM $CuCl_2$ prepared in H_2O, and 0.4 mL of 1M ammonium acetate (pH-7). Mixtures were thoroughly mixed and let to stand for 30 minutes at room temperature before absorbance at 450 nm was measured and expressed in µM Trolox.

The capsaicinoid was estimated by UV-VIS spectrophotometer. The absorbance was determined using a 20 µL sample mixed with 980 µL of 100% EtOH at a wavelength of 290 nm. The capsaicin and dihydrocapsaicin concentrations were calculated using a standard curve of capsaicin and dihydrocapsaicin and were expressed as µg/g fresh weight of the jalapeño pepper and converted into Scoville Heat Unit (SHU) (Davis et al., 2007; Domínguez-Martínez et al., 2014; González-Zamora et al., 2015).

5.2.8 Data analysis

Analysis of variance (ANOVA) was performed to assess the impact of treatments, year, and the interaction between treatments and year. Additionally, a regression analysis was performed to examine the effects of irrigation levels. Data from all years were pooled if no year x treatment interactions were found. When the ANOVA model showed significant treatment effects, a within-group separation was performed using Tukey's HSD test at 0.05% (Tukey, 1977) All analyses were performed using R Statistical Software (Team, 2023). Graphs were prepared using the SigmaPlot 14 software (Systat Software, San Jose, CA).

5.3. RESULTS

5.3.1 Weather Conditions and soil water content

Cumulative rainfall was 596.6 mm and 265.4 mm; cumulative ETo was 471 mm and 501 mm in 2021 and 2022, respectively (data not shown). Average rates of daily VWC ranged from 0.05 $m^3 \cdot m^{-3}$ at 25% ETc to 0.25 $m^3 \cdot m^{-3}$ at 150% ETc in 2021, while it ranged from 0.02 $m^3 \cdot m^{-3}$ at 25% ETc to 0.15 $m^3 \cdot m^{-3}$ at 150% ETc in 2022 (Figure 5.3 A & B).

5.3.2 Plant Biomass and chlorophyll concentrations

Leaf dry weight, stem dry weight, and total dry weight of mature plants were not influenced by irrigation level (Table 5.1). In contrast, the leaf weight ratio decreased with increasing irrigation level. The LDW, SDW, and TDW were higher in 2022 than in 2021.

In 2021, chlorophyll a and b, carotenoids, and total chlorophyll decreased with increasing irrigation levels (Table 5.2), while irrigation levels did not affect *chl a*, *b*, or total *chl (a+b)* in 2022. Carotenoids increased with increasing irrigation up to 75% ETc and then declined with further increases in irrigation level.

5.3.3 Leaf water potential (LWP) and gas exchange

Leaf water potential (LWP) increased linearly with increasing irrigation levels (Figure 5.5 A and B). LWP ranged from -1.2 MPa to -0.6 MPa in 2021 and from -1.4 MPa to -0.8 MPa in 2022. In 2021, leaf net photosynthesis (Pn), stomatal conductance (g_s), transpiration rate (E) (Figure 5.6 A, C, and E), intrinsic water use efficiency(iWUE) and electron transport rate (ETR)

(Figure 5.7 A & E) were unaffected by irrigation levels in 2021. Photosystem-II efficiency (PhiPS2) decreased with irrigation level (Figure 5.7 C). In 2022, leaf Pn, gs, E (Figure 5.6 B, D, and F), PhiPS2, and ETR (Figure 5.7 D & F) exhibited positive quadratic relationships with irrigation levels. In contrast, iWUE also showed a quadratic but negative relationship with irrigation levels (Figure 5.7 B).

5.3.4 Leaf and fruit nutrient concentrations

Foliar N decreased with increasing irrigation level, whereas the other nutrients were unaffected by irrigation level (Table 5.3). Leaf N, P, K, Mg, S, B, and Cu were higher, while Mn was lower in 2021 than in 2022. There were no irrigation level x year interactions for the foliar nutrients.

The irrigation level affected none of the fruit nutrients (Table 5.4). Fruit N, Ca, and Mg were higher, while P, K, B, Cu, Fe, and Zn were lower in 2022 than in 2021. There were no irrigation level x year interactions for fruit mineral nutrients.

5.3.5 Fruit yield

In 2021, irrigation levels had no significant effect on medium-sized and total fruit number and weight per plant and individual fruit weight (Table 5.5). However, large-sized fruit numbers and weight per plant increased with increasing irrigation levels. In 2022, large, medium, and total fruit number and weight increased with increasing irrigation levels (Table 5.5). Individual fruit weight decreased linearly up to 75% ETc and then increased with irrigation levels. Total fruit weight per plant increased linearly with increasing Pn reaching a peak at 150% ETc (Figure 5.8).

Regarding cull yield, in 2021, the number and weight of fruit damaged by anthracnose decreased with increasing irrigation level (Table 5.6). Number and weight of small-sized and weevil-infested fruit and total cull fruit number and weight were unaffected by the irrigation level.

In 2022, the number and weight of fruit with anthracnose, sunscald, and weevil and total cull fruit yield (number and weight) were unaffected by irrigation level. However, small-sized fruit number and weight increased with increasing irrigation level reaching maximum values at 100% ETc.

5.3.6 Bioactive compounds

Fruit TPC, TF, and CUPRAC values decreased with increasing irrigation levels (Table 5.7). However, TEAC-DPPH was unaffected by irrigation levels. Regarding the effects of the year, the highest total phenols, CUPRAC and TEAC-DPPH, were found in 2022, while TF was found to be the highest in 2021. Fruit capsaicin, dihydrocapsaicin, and SHU values decreased linearly and quadratically in 2021 and 2022 with the increasing irrigation levels (Figure 5.9 A, B, C, D, E, and F) respectively.

5.4 DISCUSSION

5.4.1 Weather conditions and soil water content

Tifton sandy loam soil has a field capacity of 0.18-0.23 m^3 m^{-3} (Liang et al., 2016). Rainfall and evapotranspiration data from two years suggested that low water demand in 2021than in 2022. Most studies on drip irrigation under plastic mulch do not consider rainfall data while scheduling irrigation (Simonne et al., 2006; Zotarelli et al., 2011). However, Zotarelli et al. (2011) found about a 1% increase in VWC in soil when rainfall was about 45-48 mm. In this study, rainfall in 2021 was double of 2022, which explained the probable reason for high VWC in year 2021 than in year 2022 Similar to our results about VWC, Díaz-Pérez and Hook (2017) also observed similar ranges from 6% to 20% at irrigation rates from 33% to 133% ETc.

5.4.2 Plant Biomass and leaf chlorophyll concentration

In this study, the above-ground biomass of the plant (leaf and stem) at the last harvest did not differ between irrigation levels. In eggplant (*Solanum melongena* L.), Díaz-Pérez and Eaton

(2015) found similar plant height and total plant dry biomass at the last harvest. In this study, similar dry weight of leaves and stems may be because full irrigation was applied for 4-5 weeks to establish transplant. High vegetative growth occurs during the early stage of plant development in short-season crops like bell pepper (before 60 Days after planting) (Aidoo et al., 2019). However, the year-effect on plant dry biomass (LDW, SDW, and TDW) was significant, with 50% higher values in 2022 than in 2021. The decreased biomass in 2021 compared to 2022 might be because of the leaching of nutrients due to high cumulative rainfall. The leaching of nitrogen was further explained by leaf N concentration and chlorophyll content. Leaf weight ratio (LWR) represents the proportion of biomass allocated to the leaves relative to the total aboveground biomass. The decrease in LWR with increased irrigation levels in this study suggested a shift in resource allocation toward other plant parts, such as stems and roots.

5.4.3 Leaf water potential (LWP) and gas exchange

Increased leaf water potential (LWP) with increasing irrigation levels in both years in this study showed that plants with low irrigation levels were under drought stress. Kabir et al. (2021) also found in bell pepper an increasing LWP (from -0.6 to -0.2 MPa) with increasing irrigation levels from 33% to 133% of the ETc. In poblano pepper (*C. annuum* cv. Don), Coolong et al. (2012) found poor correlation between mid-day LWP and relative water content to soil water potential when there is enough moisture in the soil. In addition, they observed similar LWP ranges from -0.7 to -2 MPa similar in our study (-0.6 to -1.4 MPa). In bell pepper, Colak (2021) found that, in surface and subsurface drip irrigation, LWP values between -0.89 to -0.95 MPa can be used as indicators for starting irrigation. In our study, we observed drought stressed plants exclusively only at 25% ETc in 2021. However, in 2022, the range extended from 25% to 75% ETc, indicating varying levels of drought stress.

In 2021, Pn, g_s, E, iWUE, and ETR, were unaffected by irrigation levels. However, in 2022, when there was drier period, leaf gas exchange variables increased with increasing irrigation levels except iWUE, an important indicator of plant water use. Such responses observed in our study attributed to the improved water availability in 2021 than in 2022. Most of the previous study also showed that Pn, g_s, E, and ETR decreased in water stressed conditions in many crops (Kabir et al., 2021; Urban et al., 2017; Yan et al., 2016; Yenni et al., 2022).

5.4.4 Leaf and fruit mineral concentrations

The current study revealed no noteworthy variances in the concentrations of most macronutrients and micronutrients in both the leaves and fruits, except for leaf nitrogen. Leaf N levels decreased as irrigation levels increased, and high irrigation rates were likely associated with increased nitrate leaching. In another study, higher leaf Ca, N, P, Fe, Zn, and K concentrations were observed in rainfed than irrigated plants (Delfine et al., 2002). Kabir et al. (2021) found quadratic relationships between fruit macronutrients (excluding Ca^{2+}) and micronutrients with irrigation rate, with nutrient concentrations decreasing at 133% ETc when irrigation rates from 33% to 133% were used. The year significantly affected all leaf and fruit mineral concentrations in this study. Year 1 had a higher nutrient concentration of leaf nutrients (N, P, K, Mg, S, B, and Cu) and fruit nutrients (P, K, B, Cu, Fe, and Zn) than year 2. A higher concentration of most of the nutrients in year 1 might be because of a higher soil water content than in year 2. Végh (1991) also found limited root growth at low soil moisture conditions. However, we did not measure root length and density in this study.

5.4.5 Fruit yield

Irrigation levels had varying effects on fruit yield across the two years of the study. The increased large-sized fruit number, weight, and individual fruit weight with increased irrigation levels (in both years) suggested that adequate soil water availability enhances fruit size. This observation was also supported by Simonne et al. (2006), who found the increased yield of a Fancy premium-grade bell pepper when different irrigation rates [33%, 66%, 100%, and 133% of pan evaporation (Ep)] in Florida conditions. On the other hand, the lack of significant effects on medium-sized and total fruit yield in year 1 may show that soil moisture content at different irrigation levels sufficed to meet the water requirements for overall fruit production of medium-sized pepper. The correlation between total fruit weight and net photosynthesis in year 2 of this study showed the importance of Pn in fruit yield (Figure 5.8). The positive relationship between increasing marketable fruit number and weight with increasing irrigation levels suggested that water availability plays a crucial role in determining fruit yield and size. Similarly, Dalla Costa and Gianquinto (2002) also found the highest marketable yield of a bell pepper at 120% evapotranspiration (ETo) irrigation level than at 40% ETo. Nevertheless, in Georgia conditions, Kabir et al. (2021) observed no significant impact on marketable and non-marketable yields with comparable irrigation rates [33%, 67%, 100%, and 133% ETc].

The cull fruit because of anthracnose, pepper weevil, BER, and small size was unrelated to irrigation levels in this study for both years. A previous study discovered that secondary metabolites act as defense mechanisms against insect infestations by producing toxins that affect insects' growth and developmental processes (Behura et al., 2011; Wouters et al., 2016). However, in our study, the irrigation level did not impact the weevil damage fruit number and weight in jalapeño peppers. In addition, the similarity in the incidence of blossom-end rot among irrigation treatments could be attributed to comparable fruit Ca concentrations across different irrigation levels. Previous research has shown that insufficient Ca and irrigation can accelerate blossom-end rot in tomatoes (Taylor et al., 2004).

5.4.7 Bioactive compounds

In the current study, we observed that increasing irrigation levels led to a decrease in total phenolic content (TPC), total flavonoid content (TF), and cupric-reducing antioxidant capacity (CUPRAC). Similar to our results, Navarro et al. (2010) found increased TPC in 'Clemenules' mandarin citrus when applied to deficit irrigation. Contrary to these findings, Zamljen et al. (2020) have reported higher concentrations of TPC, capsaicin, and dihydrocapsaicin in two species of chilies *C. annuum* L var 'Chili-AS Rot' and *C. chinense* Jacq var 'Naga Morich'. Similarly, Malejane et al. (2018) found that 75% deficit irrigation resulted in the highest quercetin content, while 50% deficit irrigation had the highest Trolox equivalent antioxidant capacity (TEAC) values in leafy lettuce 'Lollo Bionda' and 'Vera'. The observed increase in TPC, TF, and CUPRAC in our study can be attributed to the redox potential of these compounds. During drought stress, plants experience an increase in the production of reactive oxygen species (ROS) that can cause cellular damage. These compounds play a crucial role in neutralizing reactive oxygen species (ROS) and enhancing plant resistance during drought stress (Khazaei et al., 2020; Zheng and Wang, 2001).

The content of fruit capsaicin, dihydrocapsaicin, and Scoville Heat Units (SHU decreased with increasing irrigation levels in 2021, while in 2022, these compounds reached their maximum values at 75% of the crop evapotranspiration (ETc). Differences in the capsaicin, dihydrocapsaicin, and SHU between the two years could be attributed to the higher soil moisture content in 2021 compared to 2022 (Figure 5.3 A and B). However, in both years, the capsaicinoid contents decreased at high irrigation levels. The increased pungency observed under deficit irrigation may be because of hydric stress. Similar findings have been reported in other studies with different hot pepper varieties, including 'Beauty Zest', 'Home Flavor', and 'Hungariana' (Sung et al., 2005), as well as in 'Padrón pepper (*C. annuum* L.) and Habanero pepper (Estrada et al., 1999; Ruiz-Lau et al., 2011). In addition, Sung et al. (2005) observed the highest activity of enzymes such as Phenylalanine ammonia-lyase (PAL), cinnamic acid-4-hydroxylase (C4H), and Capsaicinoid synthetase (CS) responsible for the synthesis of capsaicinoids under deficit irrigation treatment.

5.5 CONCLUSIONS

The study showed that increasing irrigation levels resulted in improved plant water status, as indicated by increased leaf water potential. In 2021, leaf gas exchange variables did not vary between irrigation treatments because of high cumulative rainfall resulting in high soil moisture content. However, in 2022, leaf gas exchange variables showed varying responses to irrigation levels, with increased net photosynthesis, stomatal conductance, and transpiration rate at higher irrigation levels. However, intrinsic water use efficiency decreased with increasing irrigation levels, indicating a trade-off between water use and carbon assimilation. Plant biomass and chlorophyll concentrations were not significantly affected by irrigation levels, suggesting that the overall growth of the plants was not influenced by water availability. However, the leaf weight ratio decreased with increasing irrigation levels, showing a shift in resource allocation. Fruit yield parameters showed contrasting responses to irrigation levels between the two years. In the first year, medium-sized and total fruit yield were unaffected by irrigation levels, while large-sized fruit yield increased with higher irrigation levels. In the second year, all fruit yield parameters increased with increasing irrigation levels, showing the importance of water availability for fruit production. Total phenols, total flavonoids, CUPRAC, and capsaicinoids (capsaicin, dihydrocapsaicin, and SHU) decreased with increasing irrigation levels in both years. Overall, the findings suggest that water availability plays a crucial role in jalapeño pepper production, particularly in terms of fruit yield and quality. Adequate irrigation can enhance fruit size, while deficit irrigation may increase pungency. Marketable fruit yield and bioactive compounds in 75% ETc were comparable to 100%

ETc in 2022. Thus, to save water while maintaining optimum yield and fruit quality, deficit irrigation of 75% ETc can be used. The findings of this study provide valuable insights for farmers and agricultural practitioners in implementing efficient irrigation strategies for jalapeño pepper cultivation in water-limited regions.

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Tables and Figures

Table 5.1 Effect of irrigation levels [percentage of crop evapotranspiration (ETc)] and year on leaf dry weight (LDW), leaf weight ratio (LWR), shoot dry weight (SDW), and total dry weight (TDW) of organic jalapeño pepper plants at the end of harvest period. Spring-Summer 2021 and 2022, Tifton, GA.

Treatments Irrigation levels [I] (%)	LDW (g)	LWR	SDW (g)	TDW (g)
25	45.9	0.4	71.3	117.3
50	54.6	0.38	88.6	143.1
75	52.8	0.36	91.9	144.7
100	49.2	0.34	94.3	143.5
150	50.1	0.35	91.7	141.8
Significance[x]				
L	0.93	0.0003	0.29	0.48
Q	0.88	<0.001	0.36	0.56
Year (Y)				
2021	36.8 b[z]	0.37	62.9 b	99.7 b
2022	64.3 a	0.36	112.2 a	176.4 a
P				
Y	<0.0001	0.16	<0.0001	<0.0001
I*Y	0.96	0.68	0.99	0.98

[x]L = Linear and Q = quadratic regression.

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 5.2 Effect of irrigation rate [percentage of crop evapotranspiration (ETc)] in chlorophyll a (*chl a*) and b (*chl b*), carotenoids (car), and total chlorophyll (Total *chl (a+b)*) contents in leaves of organic jalapeño pepper, Spring-Summer 2021 and 2022, Tifton,GA.

	2021				2022			
Irrigation levels (%)	*Chl a* (µg/mL)	*Chl b* (µg/mL)	car (µg/mL)	Total *chl (a+b)* (µg/mL)	*Chl a* (µg/mL)	*Chl b* (µg/mL)	car (µg/mL)	Total *chl (a+b)* (µg/mL)
25	7.3	2.8	2.3	10.1	5.5	2	1.57	7.5
50	7	2.7	2.2	9.7	5.4	2	1.56	7.4
75	6	2.3	1.9	8.4	6	2.2	1.79	8.2
100	5.8	2.3	1.8	8	6	2.2	1.75	8.2
150	5.6	2.2	2	7.8	5.6	2	1.72	7.6
Significance[x]								
L	<0.0001	0.0004	0.03	<0.0001	0.48	0.5	0.03	0.48
Q	<0.0001	0.0006	0.02	0.0001	0.12	0.09	0.02	0.11

[x]L = Linear and Q = quadratic regression.

Table 5.3 Effect of the year and irrigation level on micro and macro nutrients of organic jalapeno pepper leaves in Spring-Summer 2021 and 2022, Tifton, GA.

Treatments	Macronutrient (%)						Micronutrients (ppm)				
Irrigation levels [I]	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
25	2.43	0.41	2.73	0.212	0.188	0.236	12.75	10.75	59.6	20.37	35.37
50	2.41	0.42	2.67	0.15	0.18	0.235	12.37	10.13	66.9	18.87	36.12
75	2.38	0.42	2.68	0.14	0.173	0.235	12.25	9.9	58.5	16.38	36.75
100	2.37	0.43	2.62	0.135	0.178	0.236	12.62	10.13	61.3	16	37
150	2.26	0.42	2.65	0.136	0.173	0.225	12.12	9.9	54.1	15.25	35.75
Significance[x]											
L	0.01	0.52	0.75	0.11	0.26	0.21	0.79	0.37	0.18	0.22	0.81
Q	0.04	0.64	0.92	0.12	0.45	0.34	0.96	0.57	0.29	0.48	0.59
Year [Y]											
2021	2.4 a[z]	0.47 a	3.15 a	0.175	0.19 a	0.245 a	15.8 a	11.6 a	62.3	12.1 b	35.05
2022	2.3 b	0.37 b	2.18 b	0.134	0.17 b	0.221 b	9.05 b	8.8 b	57.9	24.2 a	37.35
P											
Y	0.025	<0.0001	<0.0001	0.16	0.011	<0.0001	<0.0001	<0.0001	0.25	<0.0001	0.06
I*Y	0.18	0.86	0.36	0.99	0.9	0.37	0.62	0.81	0.3	0.9	0.95

[x]L = Linear and Q = quadratic regression.

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

Table 5.4 Effect of the year and irrigation level on micro and macro nutrients of organic jalapeno pepper fruit in Spring-Summer 2021 and 2022, Tifton, GA.

Treatments	Macronutrient (%)						Micronutrients (ppm)			
Irrigation levels [I]	N	P	K	Ca	Mg	S	B	Cu	Fe	Zn
25	3.33	0.276	2.85	0.98	0.32	0.31	25.3	8.8	56.9	41.4
50	3.32	0.306	3.13	1.07	0.34	0.34	25.3	9.6	65.9	48.4
75	3.19	0.266	2.62	0.98	0.3	0.3	21	7.8	56.5	41.6
100	3.29	0.272	2.7	1.04	0.33	0.31	22.5	7.8	56	41.3
150	3.19	0.276	2.7	1.05	0.33	0.31	22.9	7.4	58	42.6
Significance[x]										
L	0.64	0.74	0.39	0.72	0.86	0.42	0.49	0.15	0.61	0.77
Q	0.89	0.94	0.67	0.93	0.93	0.72	0.62	0.36	0.88	0.95
Year [Y]										
2021	2.7 b[z]	0.34 a	3.26 a	0.84 b	0.27 b	0.31	26.5 a	9.8 a	62.6 a	48.2 a
2022	3.8 a	0.21 b	2.34 b	1.21 a	0.39 a	0.32	20.3 b	6.7 b	54.6 b	38 b
P										
Y	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.73	0.024	0.0003	0.028	0.01
I*Y	0.88	0.47	0.35	0.18	0.053	0.36	0.64	0.72	0.17	0.43

[x]L = Linear and Q = quadratic regression.

[z]Means followed by the same letter are not significantly different based on the LSD test at $p \leq 0.05$.

Table 5.5 Effects of irrigation levels [percentage of crop evapotranspiration (ETc)] on marketable fruit yield in organic jalapeño pepper in Spring-Summer 2021 and 2022, Tifton, GA.

Year	Irrigation level	Large		Medium		Total Marketable		Individual Weight
	(%)	N	Wt. (g/plant)	N	Wt. (g/plant)	N	Wt. (g/plant)	(g/fruit)
2021	25	2.6	68.4	16.8	362	19.3	430.4	22.3
	50	2.5	78.1	13.7	291.2	16.2	369.3	22.8
	75	3.9	112.5	14.2	320.3	18.1	432.8	23.9
	100	3.4	103.2	13.4	308.9	16.8	412.1	24.5
	150	5.3	168.9	16.6	376.6	21.8	545.5	25
	Significance[x]							
	L	0.028	0.028	0.85	0.43	0.19	0.055	0.06
	Q	0.09	0.055	0.084	0.09	0.062	0.056	0.18
2022	25	1.25	31.56	8.33	147.9	9.6	179.4	18.7
	50	2.48	59.77	13.59	221.8	16	281.5	17.6
	75	4.11	91.69	17.67	279.8	21.8	371.5	17
	100	4.3	100	16.63	283.6	20.9	383.6	18.4
	150	10.79	273.6	20.53	344.7	31.3	618.3	19.8
	Significance[x]							
	L	<0.0001	<0.0001	0.0003	0.0003	<0.0001	<0.0001	0.087
	Q	<0.0001	<0.0001	0.0006	0.0009	<0.0001	<0.0001	0.048

[x]L = Linear and Q = quadratic regression.

Table 5.6 Effect of irrigation levels [percentage of crop evapotranspiration (ETc)] on non-marketable (cull yield) fruit yield in organic jalapeño pepper in Spring-Summer 2021 and 2022, Tifton, GA.

Year	Irrigation level	Anthracnose and BER		Small		Sunscald		Weevil		Total cull	
	(%)	N	Wt. (g/plant)	N	Wt. (g/plant)	N	Wt. (g/plant)	N	Wt. (g/plant)	N	Wt. (g/plant)
	25	1.5	32.6	13.5	246.8			5	54.4	20.1	333.7
	50	0.6	11.4	13.7	243.4			4.8	47.9	19.2	302.8
2021	75	0.5	11.4	17.4	302.3	ND	ND	5.2	43.3	23.2	357
	100	0.7	15.6	16.8	299.9			4.3	43.7	21.8	359.1
	150	0.7	20.1	14.7	272.8			6.2	54.1	21.7	347.1
	Significance[x]										
	L	0.13	0.44	0.55	0.5	-	-	0.49	0.98	0.56	0.6
	Q	0.03	0.04	0.39	0.53	-	-	0.59	0.54	0.75	0.84
	25	1.4	21.5	8.5	98.6	3.6	18.2	0.05	0.7	14.7	154.1
	50	2	28.2	8.7	102.4	2.3	34.6	0.4	3.7	14.1	178.2
2022	75	1.1	16.9	14.9	167	0.4	6.9	0.8	10.3	18.4	219.9
	100	1.7	25.3	16.7	210.3	1.8	26.9	0.7	8.3	21.5	279.2
	150	0.9	14.3	7.6	95.2	1.1	19.3	0.2	2.7	10.6	144
	Significance[x]										
	L	0.65	0.81	0.81	0.67	0.12	0.77	0.4	0.48	0.2	0.62
	Q	0.82	0.96	0.03	0.04	0.15	0.96	0.59	0.72	0.42	0.42

[x]L = Linear and Q = quadratic regression.

ND = No Data

Table 5.7 Effect of year and irrigation levels [percentage of crop evapotranspiration (ETc)] on total phenols (TPC), total flavonoids (TF), Cupric reducing antioxidant capacity (CUPRAC), and Trolox equivalent antioxidant capacity (TEAC) of organic jalapeño pepper fruits collected at a mature green stage in Spring-Summer 2021 and 2022, Tifton, GA.

Irrigation level (%) [I]	TPC (mg GAE/L)	TF (mg quercetin equivalent/mL)	CUPRAC μM	TEAC-DPPH μM
25	118.6	29.7	1238.2	1818.7
50	115.6	25	1110	1776.8
75	118.7	23.2	1129.7	1853.8
100	109	21.9	1081.2	1726
150	103.3	18.5	1012.9	1658
Significance[x]				
L	0.03	<0.0001	0.012	0.25
Q	0.08	<0.0001	0.038	0.47
Year [Y]				
2021	96 b[z]	25.3 a	964 b	1471.7 b
2022	135.7 a	21.5 b	1313.9 a	2160 a
P				
Y	<0.0001	0.013	<0.0001	<0.0001
I*Y	0.92	0.82	0.28	0.47

[x]L = Linear and Q = quadratic regression.

[z]Means within the same column followed by the same letter are not significantly different based on the Tukey's HSD test at $p \leq 0.05$.

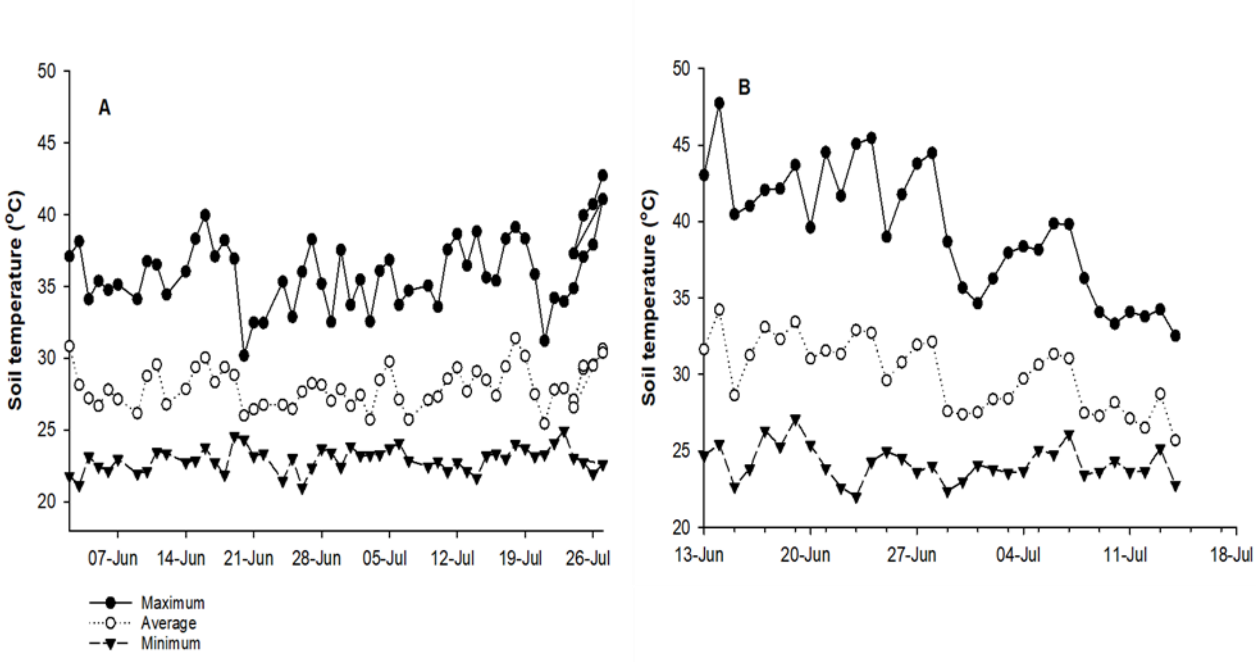

Figure 5.1 Soil temperature (maximum, mean, and minimum) during the experimental period [2021 (A); 2022 (B)] in organic jalapeño pepper, Spring-Summer, Tifton, GA.

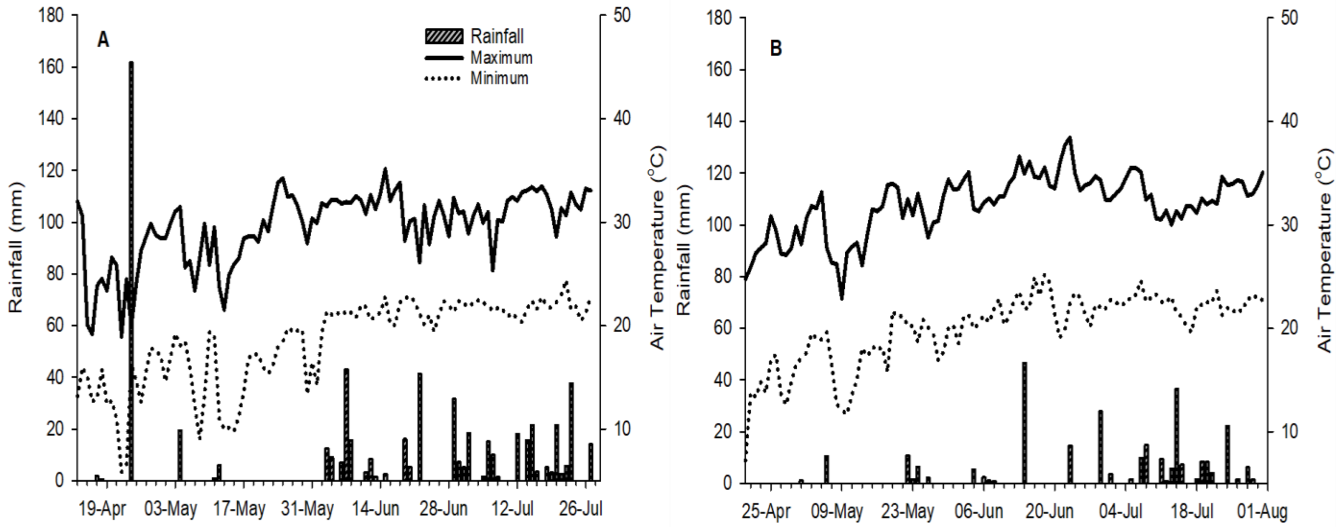

Figure 5.2 Air temperature (maximum and minimum) and rainfall during the experimental period [2021 (A); 2022 (B)] in organic jalapeño pepper, Spring-Summer, Tifton, GA.

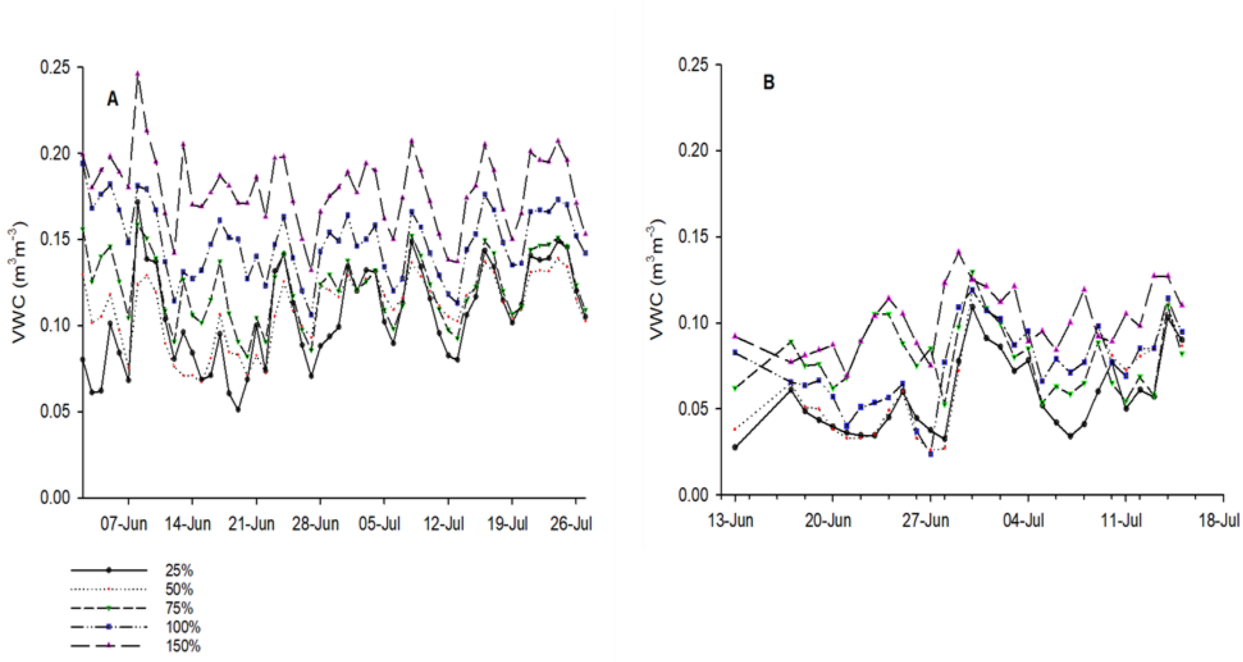

Figure 5.3 Soil volumetric water content (VWC) trend in 2021 (A) and 2022 (B) in organic jalapeño pepper, Spring-Summer, Tifton, GA. Each point is the average of two sensors.

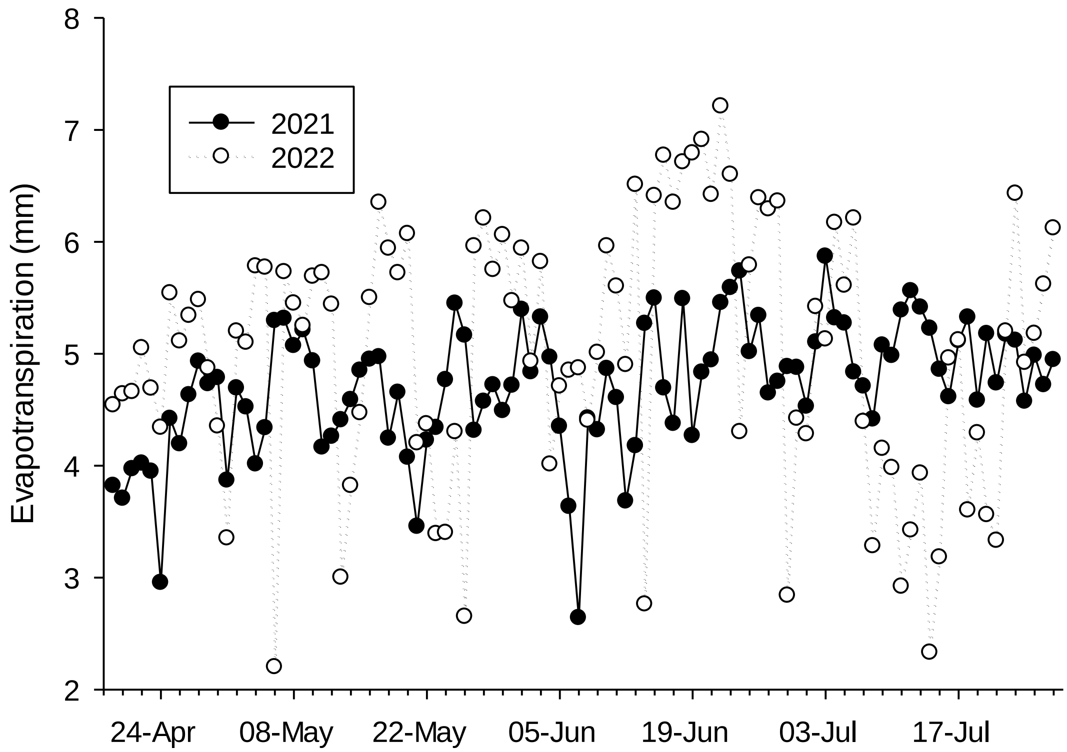

Figure 5.4 Reference evapotranspiration during the experimental period in 2021 and 2022 in organic jalapeño pepper, Spring-Summer, Tifton, GA.

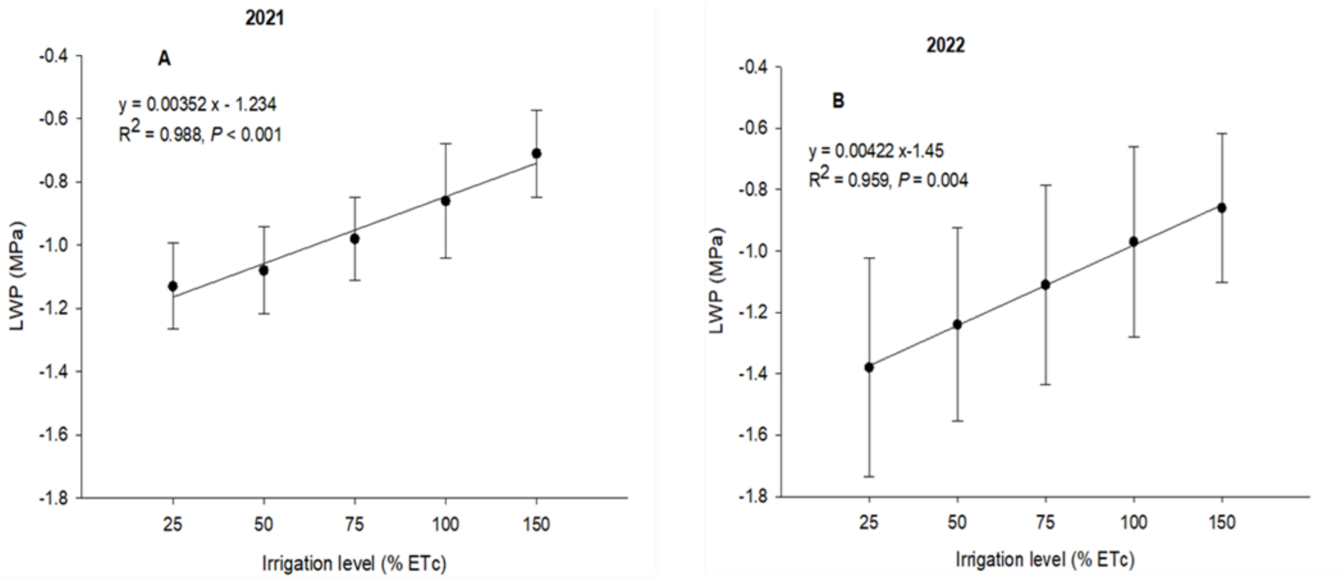

Figure 5.5 Leaf water potential (LWP) in organic jalapeño pepper as a function of irrigation levels (percentage of crop evapotranspiration) in 2021 (A) and 2022 (B). The solid lines were fit by either linear regression. The error bars represent mean ± S.E.

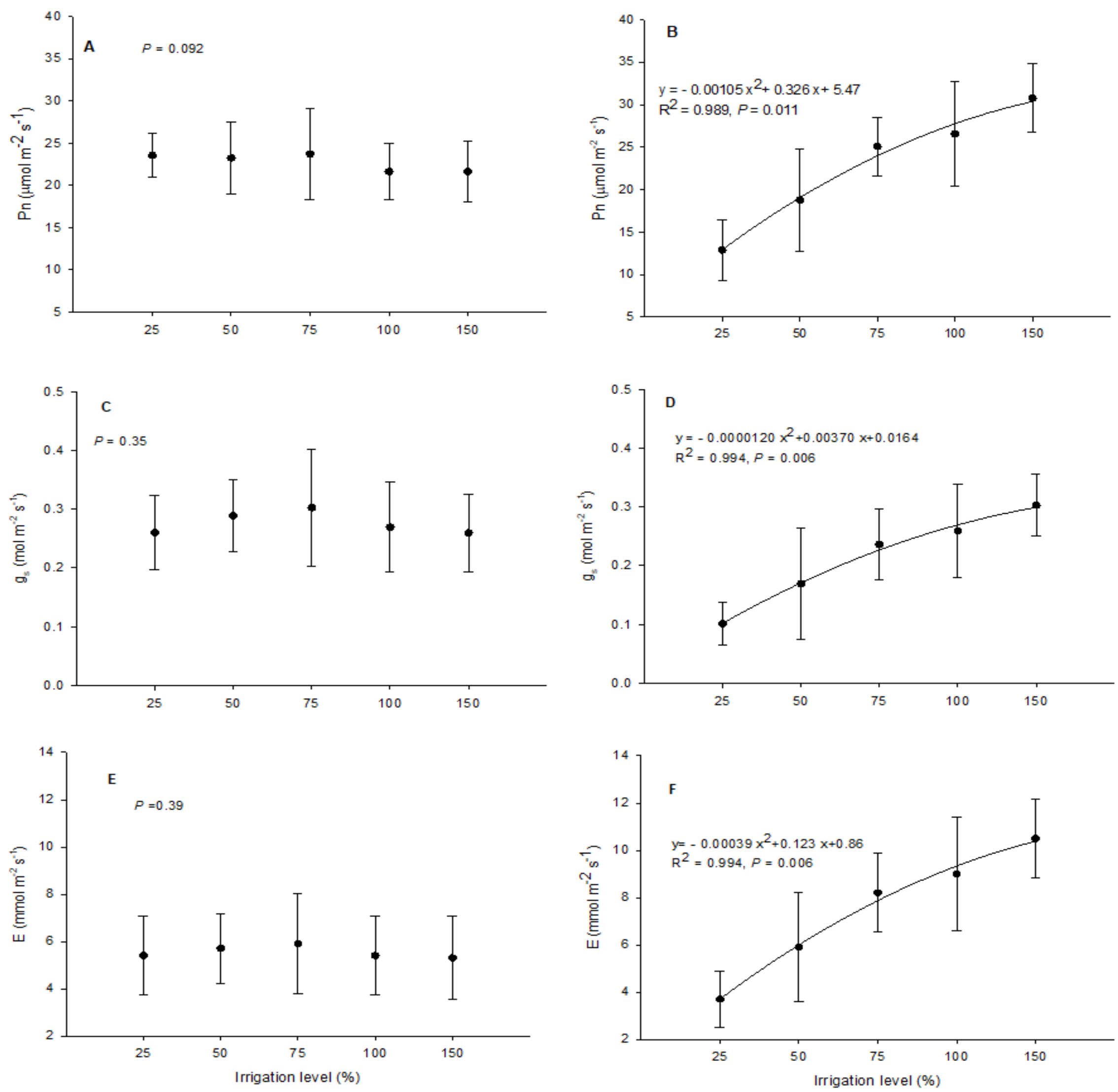

Figure 5.6 Leaf net photosynthesis (Pn) [A, B], stomatal conductance (g_s) [C, D], and transpiration rate (E) [E, F] in organic jalapeño pepper as affected by irrigation levels (percentage of crop evapotranspiration), Spring-Summer of 2021 and 2022, Tifton, GA. The solid lines were fit by either linear regression. The error bars represent mean ± S.E.

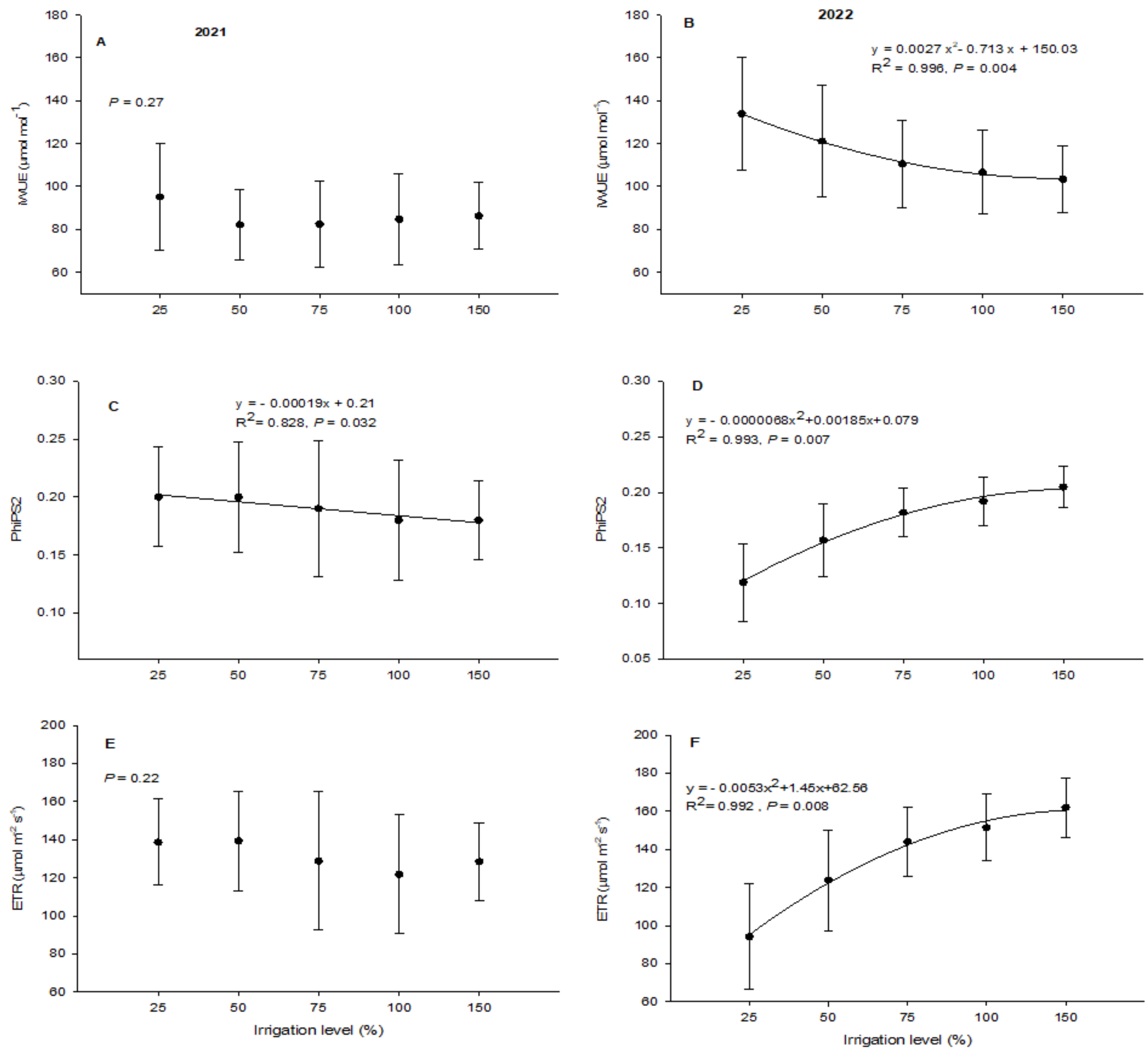

Figure 5.7 Intrinsic Water Use Efficiency (iWUE) [A, B], photosystem II efficiency (PhiPS2) [C, D], and electron transport rate (ETR) [E, F] in organic jalapeño pepper as affected by irrigation levels (percentage of crop evapotranspiration), Spring-Summer of 2021 and 2022, Tifton, GA. The solid lines were fit by quadratic regression. The error bars represent mean ± S.E.

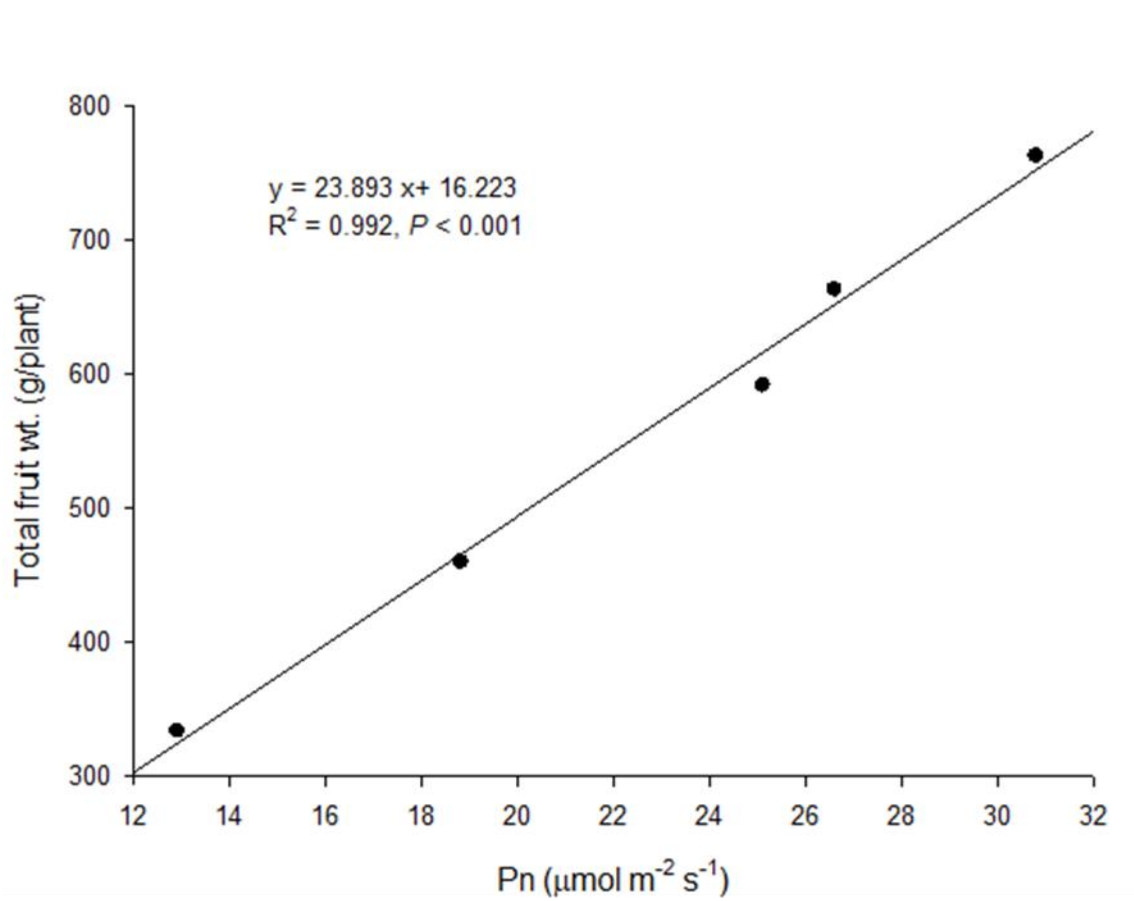

Figure 5.8 Relationship between net photosynthesis (Pn) and total fruit weight (g/plant) in organic jalapeño pepper in Spring-Summer 2022, Tifton, GA.

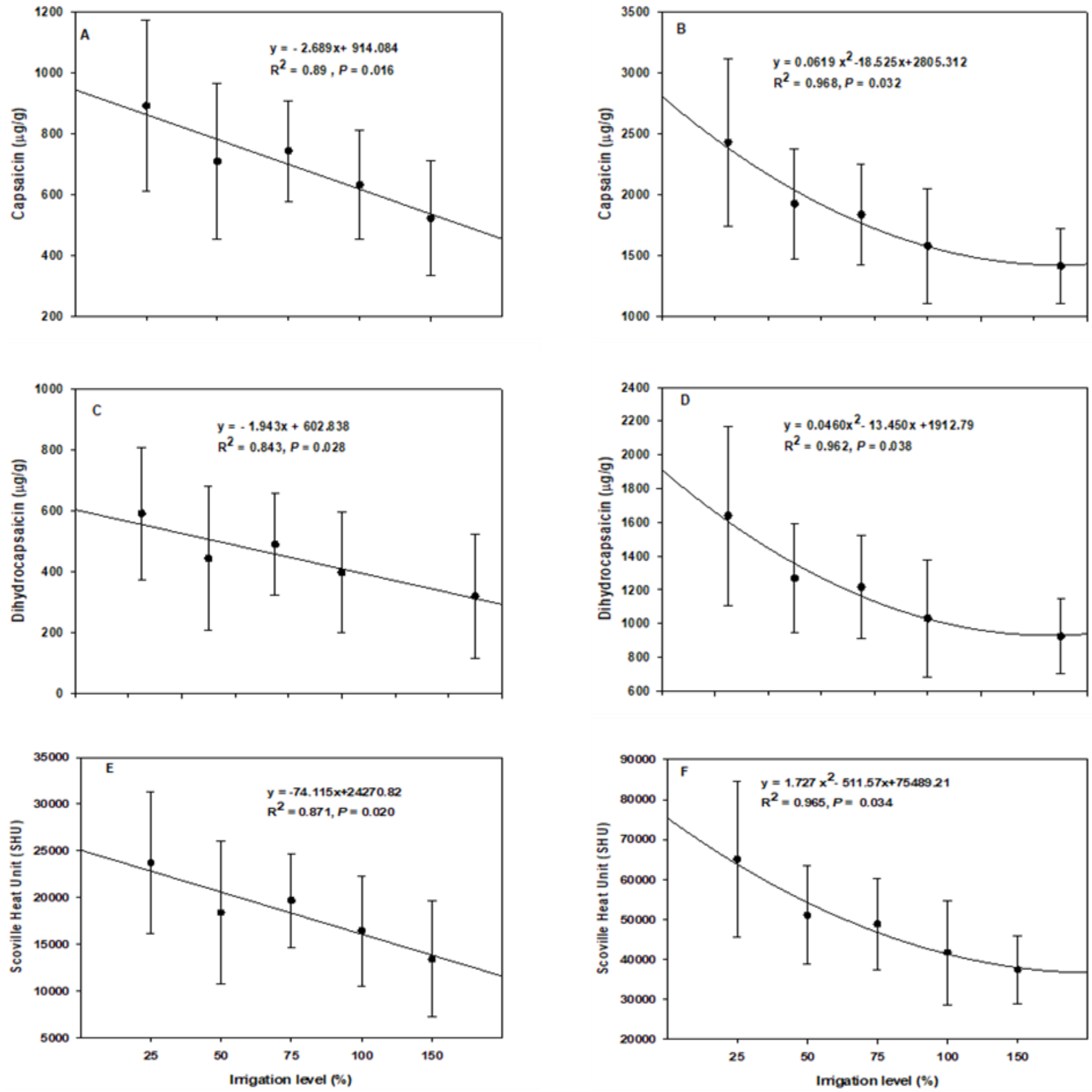

Figure 5.9 Capsaicin, dihydrocapsaicin, and Scoville heat unit (SHU) in organic jalapeño pepper as affected by irrigation levels [percentage of crop evapotranspiration (ETc)] in Spring-Summer of 2021(A, C, and E) and 2022 (B, D, and F), Tifton, GA. Fruit harvested at the mature green stage. The error bars represent mean ± SE.

CHAPTER 6
GENERAL CONCLUSIONS

Using colored shade nets can effectively change the microclimate, including light quality, quantity, and temperature. Using 40% shade nets only enhanced vegetative growth but not fruit yield in organic jalapeño pepper. The shade nets had either no or inconsistent effects on the fruit quality of organic jalapeño pepper across two growing seasons. For optimum production of organic jalapeño pepper, organic fertilizer at 358 $kg \cdot ha^{-1}$ N in unshaded conditions were recommended based on the shade nets and organic fertilization studies. Implementing regulated deficit irrigation at 75% ETc was a viable strategy, as it resulted in comparable marketable fruit numbers and sizes as 100% ETc while simultaneously increasing the concentration of bioactive compounds. Overall, the research contributes to understanding various factors influencing jalapeño pepper cultivation, including shading, organic nitrogen fertilization, and irrigation management. These findings can guide farmers and researchers in making informed decisions to improve cultivation practices, maximize yield, and enhance the quality of jalapeño pepper crops. Further studies are encouraged to explore additional aspects and refine management strategies for sustainable and efficient organic production of jalapeño pepper.