THESIS

CHARACTERIZATION AND CONTROL OF INTUMESCENCE DEVELOPMENT AND LEAF EXPANSION FOR *CAPSICUM ANNUUM* PRODUCTION IN CONTROLLED ENVIRONMENTS

Submitted by

Courtney Christine Dunbar

Department of Horticulture and Landscape Architecture

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Master's Committee:

Advisor: Joshua Craver

Chad Miller
Meagan Schipanski

ABSTRACT

CHARACTERIZATION AND CONTROL OF INTUMESCENCE DEVELOPMENT FOR *CAPSICUM ANNUUM* PRODUCTION IN CONTROLLED ENVIRONMENTS

Capsicum annuum (pepper) is an emerging crop for controlled environment production that is susceptible to intumescence. Intumescence is a physiological disorder characterized by unrestricted cellular elongation causing protruding lesions, ultimately leading to epidermal rupture. While the causative factor for this disorder remains unknown, water stress is commonly implicated, and end-of-day (EOD) lighting strategies have been identified as a potential strategy for mitigating intumescence development. However, the timing of pepper leaf area expansion and intumescence development as well as appropriate lighting strategies for their control have yet to be determined. The objectives of this work were to 1) determine the timing of leaf area expansion to better inform EOD or pre-dawn (PD) lighting applications for intumescence suppression; 2) quantify the impact of low-intensity lighting applications on pepper leaf morphology; and 3) determine the role of water stress in the occurrence of intumescence development for pepper. Pepper 'Pot-a-Peño', 'Spicy Jane', and 'California Wonder' were grown in 15-cm pots in a common greenhouse environment. For Objective 1, three weeks after transplant, one uniform leaf on each plant was tagged and plants were evaluated twice a day (0700 and 1900) for one week. For Objective 2, plants were subjected to 30-minute lighting treatments at an intensity of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for two weeks provided at either EOD with blue (EOD_B; 447 nm), red (EOD_R; 659 nm), or far-red light (EOD_{FR}; 734 nm), or PD with blue light (PD_B; 447 nm). For Objective 3, plants were either maintained at 70% container capacity

(control) or allowed a single dry down event reaching 40% container capacity prior to watering event. Intumescence development was visually monitored twice a day (0900) and (1700) during the water stress event. For Objective 1, leaf area expansion was greater at nighttime (NT) (1900-0700) compared to the daytime (DT) (0700-1900) with NT accounting for 66%, 57%, and 59% of total leaf area expansion for 'California Wonder', 'Pot-a-Peño', and 'Spicy Jane', respectively. For Objective 2, responses to lighting treatments were cultivar specific. For example, after two weeks, imaged leaf area was lowest under EOD_B for 'Pot-a-Peño' and greatest under EOD_{FR} for 'Spicy Jane'. For Objective 3, intumescence development was observed on all cultivars subjected to water stress post returning to container capacity, with no incidence of the disorder for control plants. These results will help in the prediction of intumescence development for pepper produced in controlled environments and inform decisions regarding the timing of possible suppression methods to control this disorder.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Controlled Environment Agriculture and Intumescence

Controlled environment agriculture (CEA) has been expanding globally at an accelerated rate; the number of CEA operations almost tripled from 1998 to 2019 in the United States (US), and continued growth of this sector is expected (Dohlman et al. 2024). Production in a controlled environment (e.g., greenhouse, vertical farm) allows for the control and fine-tuning of environmental conditions during crop production compared to the field. The production of specialty crops in controlled environments, in particular, increased 56% between 2009 to 2019 (Dohlman et al. 2024). This rapid expansion is due in part to the invention of light-emitting diodes (LEDs) in the early 1960's, which have since been implemented in controlled environment systems allowing multiple benefits compared to traditional lighting sources [e.g., high-intensity discharge (HID) lamps] including greater spectral control and reduced energy use (Nemali 2022). Ultimately, these advancements allow improved control over the quantity and quality of light provided throughout production as well as the benefit of cost savings due to an improved lamp efficacy. These technological advances enable year-round food production in CEA systems, versus conventional farming systems that are subject to typical production season cycles and the unpredictability of weather conditions. While conventional farming methods are critical for sustained production of staple crops [e.g., corn (*Zea mays*), soybean (*Glycine max*), rice (*Oryza sativa*)] in the US, the reduction of farmable land, increased occurrence of extreme weather events, and increased labor costs have led to the consideration of alternative systems, such as CEA, to manage food securities (World Food and Agriculture 2023). The incorporation of specialty food crops can benefit farmers by diversifying their revenue, providing flexibility in

choosing climate-appropriate species, and maintaining yields with fewer resources (Kim 2016). CEA remains a small sector compared to conventional farming but allows for more control and protection against adverse weather conditions for high value specialty crops. CEA production of high value specialty crops includes species such as tomato (*Solanum lycopersicum)*, cucumber (*Cucumis sativus*), lettuce (*Lactuca sativa*), strawberry (*Fragaria* × *ananassa*), basil (*Ocimum basilicum*), and pepper (*Capsicum annuum*) (Dohlman et al. 2024). Staple food crops, such as rice and wheat (*Triticum aestivum*), are not economically feasible for indoor production in part due to high input costs and a relatively low market price (e.g., US$0.40 kg^{-1} for rice and wheat; Pattison et al. 2018). While production in controlled environments has a high inherent cost, specialty crops have proven to be sustainable and profitable. For example, the US fresh market price in 2018 was reported \$35 kg^{-1} for leafy microgreens, \$12 kg^{-1} for lettuce, and \$8 kg^{-1} for tomatoes (Pattison et al. 2018). Peppers are an emerging high value crop in CEA which is gaining popularity due to its nutritional, culinary, and cultural value (USAID 2014).

As mentioned previously, there are many challenges with the shift from field production to CEA including the high initial capital investment for the construction of these facilities (Wei et al. 2020) as well as operating costs pertaining to sustained production and a lack of skilled labor. Due to the closed nature of CEA, some diseases and disorders become magnified in controlled environments when compared to conventional farming (Singh et al. 2020). Previous literature has mentioned common CEA disorders including blossom end rot, fruit cracking, curved fruits, fruit zippering, and leaf roll (Savvas et al. 2008), oedema (Bewley 1934), and intumescence (Sorauer 1922).

Intumescence is a physiological disorder affecting many specialty crop species that has primarily been documented to occur in controlled environments (Sorauer 1922). Intumescence

development is characterized by the swelling of palisade parenchyma and spongy parenchyma cells, which eventually rupture the epidermal layer (Lang and Tibbitts 1983). Intumescence has been reported to develop on stems, both the abaxial and adaxial surfaces of leaves, and flower buds (Sorauer 1922). While this disorder has been documented for over 140 years, the mechanism of action is not well understood. Previous research has suggested a variety of causative factors for intumescence development including high humidity, low transpiration rate, light quality (spectrum), light intensity, calcium supply, and/or mechanical damage (Pinkard et al. 2006). This review will detail historical and current events regarding intumescence injury and how it continues to impact producers.

1.2 History, Anatomy, and Nomenclature

1.2.1 History and background

The term intumescence was first documented by German botanist and plant pathologist Paul Sorauer in his book Manual of Plant Diseases in the late 1800s (Sorauer 1922). This was later translated to English in 1922. His description of intumescence structures was reported to be “knot-like” or “pustule-like" protrusions that tend to occur in groups. His detailed microscopic observations of the protrusions led to the understanding that this disorder involves the upper palisade parenchyma and spongy parenchyma cells (Sorauer 1922). He noticed the palisade parenchyma and spongy parenchyma cells continue to elongate and eventually push through and rupture the epidermal layer. Sorauer (1922) also noted that chloroplasts tend to disappear as the cells become elongated. This observation was supported by Atkinson (1893), using the term oedema (nomenclature described below), who noted that while the concentration of chlorophyll in the cell did not change, the grains were further separated in their distribution within the enlarged cell. Intumescence has been observed and described in a variety of ways on multiple

plant species. Since the turn of the 20th century, there have been reports of intumescence development in over 14 plant families and 80 species (Pinkard et al. 2006; Sita et al 2024).

1.2.2 Anatomy and physiology

Understanding the anatomical and physiological basis of intumescence is essential for developing effective mitigation strategies in sensitive crops like tomato and pepper. Older studies conducted by Atkinson (1893) and Sourar (1922) provide foundational basics for describing the structure of intumescence lesions primarily in tomatoes. La Rue (1933) later expanded research of this disorder focusing on poplars, but more clarification is still needed due to the various ways intumescence presents itself on different species.

Anatomically, intumescence lesions originate primarily in the inner tissues of the leaf, particularly the palisade parenchyma and spongy mesophyll layers (Sorauer 1922). These cells become hypertrophied, losing their structural uniformity, and begin to exert pressure on the overlying epidermis causing characteristic protrusions, often leading to rupture or separation (Eisa and Dobrenz 1971; Suzuki et al. 2020). Early stages of development are typically marked by green or whitish bumps that become mounded until epidermal rupture, these bumps can occur along the leaf vein and or interveinally (Craver et al. 2014a; Rud 2009). In terms of location, lesion development on tomato 'Maxifort' and ornamental sweet potato (*Ipomoea batatas*) 'Blackie' occurred primarily on the adaxial surface of the leaf but occurred on the adaxial and abaxial side of the leaf for bat-faced cuphea (*Cuphea llavea*) 'Tiny Mice' (Craver et al. 2014a).

1.2.3 Nomenclature Differences

Differing descriptions of protruding structures has led to a difference in nomenclature when describing this disorder. Some of the terms utilized in the literature include excrescences (Hahn et al. 1920), genetic or leaf tumors (La Rue 1933), edema (Morrow and Tibbitts, 1988),

and oedema (Atkinson 1893; Craver et al. 2014a). Each of these names have been used in attempts to classify this disorder on a variety of plant species (Pinkard et al., 2006) (Table 1.)

The abundance of names has led to confusion regarding the appropriate terminology for this disorder. Lang and Tibbitts (1983) suggested the term intumescence as appropriate during their study with cultivated tomato (*Lycopersicon esculentum* Mill.) 'Oxheart'. The use of the term intumescence was further supported by Williams et al. (2015) with a description of the disorder as being marked by the elongation of epidermal cells and rapid expansion of palisade parenchyma cells that rise above the laminar surface. This is in contrast to the term oedema, whereby hypertrophied mesophyll cells form watery blister-like lesions associated with excess water accumulation (Lang and Tibbitts 1983; Williams et al. 2015). Ji Hye Yun et al. (2024) used the term intumescence when researching this disorder in grafted pepper 'Sunhan Gilsand' and 'Nokkwang' as well as tomato 'TY Haruakki' and 'TTM-130' placed in growth chambers under LED lighting. Cruz et al. (2023) also used the term intumescence when evaluating compact peppers grown indoors. With "intumescence" typically associated with the disorder developing on species within the Solanaceae family, this term will be used, as appropriate, for the remainder of this review.

1.3 Causative Factor

Since the early 1890s, possible causative factors of intumescence injury have been a continuous topic of research across various plant families. Following the initial observations, there have been reports of intumescence injury in over 14 plant families and more than 80 species (Table 1).

1.3.1 Genetics

Genetic predisposition has been reported to play a role in plant susceptibility to intumescence. Suarez et al. (2023) observed that tomato 'Maxifort', 'Camaro', and 'Patio' showed differing intumescence development under the same greenhouse conditions. For example, tomato 'Maxifort' showed the highest incidence of intumescence over time compared to 'Camaro', whereas 'Patio' developed the least incidence showing differences in cultivar susceptibility to the disorder. Researchers using quantitative trait loci (QTL) mapping in tomato recombinant inbred line (RIL) populations found that specific QTLs associated with chromosome 1 influence how different cultivars respond to environmental stressors such as light and humidity (Prinzenberg et al. 2022). Prinzenberg (2022) also found the RIL population derived from less susceptible parent lines had less intumescence development themselves. Modern genomic tools, such as transcriptomics and CRISPR-based gene editing, may offer promising approaches to identifying and manipulating genes responsible for intumescence resistance. This cultivar-specific response shows interest in the potential for breeding programs aimed at developing intumescence-resistant varieties for CEA production. As more species are studied under controlled environment conditions, the documentation of susceptible and tolerant genotypes will become more robust, facilitating targeted cultivar development.

1.3.2 Humidity and Excess water

High humidity environments limit transpiration by reducing the amount of water vapor escaping the stomata, leading to water accumulation in plant tissues. This buildup of internal pressure can cause cells to stretch and burst, particularly in young, rapidly expanding leaves (Retana-Cordero et al. 2022).

Atkinson (1893) noticed a disorder involving epidermal cells of tomato that became enlarged and would easily separate and fall off when the plants were grown in a humid greenhouse, which he associated with oedema. Dale (1901) conducted field experiments with *Hibiscus vitifolius* to identify a cause for intumescence development by enclosing various parts of the plant within colored glass or by growing the entire plant in a pit covered with glazing. Their results further supported the involvement of high humidity condition with intumescence development (Dale 1901). Lang and Tibbits (1983) found callus-like masses on two species of tomato, cultivated tomato 'Oxheart', and wild type tomato (*Lycopersicon hirsutum*) 'PI LA 1625', when exposed to 30% relative humidity, but lesions were less obvious than those exposed to higher relative humidities of 80% or 92%.

Hydroponic systems can also contribute to intumescence development due to the abundance of water availability. Suarez et al. (2023) showed that tomato 'Maxifort' and 'Patio' grown in indoor hydroponic systems developed more lesions than those grown in soil, attributing the difference to reduced transpiration and increased root zone moisture. More research is needed to understand the full interaction between hydroponic production and intumescence development.

1.3.3 Temperature

High tempeatures (>30 °C) affect plant physiology by increasing metabolic rates and altering water movement through transpiration (Erickson and Markhart 2001). For CEA, elevated temperatures paired with high humidity reduces the vapor pressure deficit (VPD), this reduction impedes transpiration and results in excess water accumulation within leaf tissues (Jones 1993). This internal pressure builds up and contributes to cellular hypertrophy, leading to the characteristic of swelling and blistering seen in intumescences (Erickson and Markhart

2001). Early research into the mechanisms of intumescence found tomato plants exposed to elevated temperatures during the day showed higher incidence of intumescence injury (Lang and Tibbitts 1983). In a more recent study involving tomato 'CF Rinka 409', observations of structural damage caused by intumescence was more severe when plants were subjected to both a warmer temperature and high humidity (Suzuki et al. 2020). Retana-Cordero et al. (2022) also noted elevated temperatures in combination with specific light spectra and high humidity increased intumescence severity. Eisa and Dobrenz (1971) studied the effects of high temperature (30 °C) and high relative humidity (82-93%) in eggplant (*Solanum melongena*) and concluded these environmental conditions are an essential component in predisposing plants to intumescence development.

1.3.4 Water deficit

Under normal conditions, cell expansion and turgor pressure are tightly regulated by water uptake (Hsiao and Acevedo 1975). When plants experience water deficit, reduced turgor pressure can lead to abnormal cellular expansion or disruption of epidermal cell integrity (Munns et al. 2000; Pantin et al. 2011). In recent research on potted hot peppers 'Pusajuala' and 'Ghotki' and bell pepper 'Green Wonder' and 'PPE-311', drought conditions were initiated when the plant was maintained at 35% field capacity, whereas normal conditions were maintained at 65% field capacity, marking thresholds for pepper water needs (Mahmood et al. 2021). Drought stress also induces a complex hormonal response, including elevated levels of abscisic acid (ABA), which regulates stomatal closure to prevent water loss. However, this regulation can inadvertently lead to heat stress and buildup of reactive oxygen species (ROS), which further damage plant tissues (Mahmood et al. 2021). Such oxidative stress may contribute to intumescence lesions, due to oxidative imbalances influencing cell wall changes and cellular

proliferation (Mahmood et al. 2021). Molla et al. (2023) observed significant variability in physiological responses to drought stress between Ethiopian red pepper 'Local' and 'Markofana', suggesting cultivar-specific leaf anatomy may influence susceptibility to water stress. Additionally, Miyama and Yasui (2021) conducted experiments on tomato 'Momotaro Peace' and 'Reiyo' and found that intumescence incidence varied significantly depending on the cultivar under different watering regimes. Their study included a dry condition of 50% relative humidity and no irrigation, and a wet condition of 90% relative humidity with sub irrigation; they found cells are more likely to rupture when water potential increases after a shift from dry to wet conditions, demonstrating a significant role of water status for intumescence development (Miyama and Yasui 2021). Water deficit often occurs with other stress factors such as high irradiance or imbalanced light spectra. Recent studies have shown that plants grown under UV-deficient or high blue light conditions are more prone to intumescence formation when also experiencing water stress (Eguchi et al. 2016b; Williams et al. 2016). Pantin et al. (2011) emphasized that the control of leaf expansion shifts from metabolic (driven by the availability of carbohydrates) to hydraulic (driven by water potential and transpiration rates) regulation under drought. These shifts may disrupt leaf growth patterns in susceptible cultivars and possibly promote the formation of intumescences. Hoffmann et al. (2015) demonstrated that pepper plants acclimated to high blue light showed less vulnerabilities to water deficit, suggesting target use of light quality can improve plant performance under stress conditions.

1.3.5 Light

Plants perceive light across a range of wavelengths including ultraviolet (UV), blue (400-500 nm), green (500-600 nm), red (600-700 nm), and far-red (700-800 nm). Plant growth is driven by the light reactions of photosynthesis, ultimately resulting in the generation of

carbohydrates enabling the building blocks for cell walls and organs (Hart 1988; Lopez and Runkle 2017). The wavelengths that directly contribute to photosynthesis are termed photosynthetically active radiation (PAR; 400-700 nm) (Lopez and Runkle 2017). Light intensity is measured by determining the number of photons within a square meter per second ($\mu mol \cdot m^{-2} \cdot s^{-1}$) (Lopez and Runkle 2017). Photosynthetic photon flux density (PPFD; $\mu mol \cdot m^{-2} \cdot s^{-1}$) provides a measurement of light intensity within the PAR wavelength range. The daily light integral (DLI; $mol \cdot m^{-2} \cdot d^{-1}$) can further be determined by integrating PPFD over a 24-h period (Lopez and Runkle 2017). While red and blue wavelengths of light have the highest efficiencies for driving photosynthesis, wavelengths both within and outside of the PAR spectrum have been found to impact plant physiology and metabolism (Lopez and Runkle 2017).

In addition to driving photochemistry, light serves as a signal to the plant which drives changes in plant growth and development. There are three classes of photoreceptor proteins that are part of the pigment system plants use to gather information regarding the light environment around them (Kendrick et al. 1993). The three classes include: phytochromes, which primarily absorbs red and far-red light to signal a multitude of plant responses including flowering and "shade avoidance"; cryptochromes, which primarily absorb UV-A (320-400 nm) and blue light signaling de-etiolation, flowering, and circadian oscillation; and phytotropins, which absorb UV-A, blue, and green light regulating phototropism, the movement of chloroplasts, and stomatal opening (Cope et al. 2014). As plant responses to light quality are often species-specific, manipulation of these spectra has also shown to be a tool in reducing intumescence development.

1.3.5.1 Ultra-violet

Emerging technologies in CEA lighting allow for dynamic control over the light spectrum, offering a potential tool for mitigating intumescence. However, balancing light quality

for both growth optimization and lesion prevention remains a challenge, especially within multi-species cultivation systems. Historically, light quality is suggested to be one of the factors involved in the development of intumescence. Typical greenhouse construction involves glazing materials with UV-blocking properties to protect equipment, plant material, and greenhouse laborers from exposure (Both and Faust 2017). Since intumescence development is unique to production in controlled environments, one proposed causative factor for this disorder has been the absence of UV light, particularly UV-B (280-320 nm), as a result of greenhouse glazing materials. Lang and Tibbitts (1983) found intumescence was prevented in tomato 'Oxheart' and wild type tomato 'PI LA 1625' grown under fluorescent UV lamps when housed in Plexiglas boxes that allowed the transmission of UV-B; in contrast, the same tomato cultivars grown in Plexiglas boxes that blocked UV-B radiation developed severe intumescence injury. Further, Craver et al. (2014b) observed the development of intumescences on ornamental sweet potato 'Ace of Spades' in a UV-deficient greenhouse environment, but the disorder was suppressed when supplemental UV-B was provided at an intensity of approximately 0.8 $W \cdot m^{-2}$ (Craver et al. 2014b). Similarly, Eguchi et al. (2016a) noted the inclusion of a small amount of UV-B (0.23 $mmol \cdot m^{-2} \cdot s^{-1}$; 0.33% of PPFD) emitted from sole-source cool white fluorescent lamps likely prevented the development of intumescence in tomato 'Beaufort' seedlings when compared to the same plants grown in the absence of UV-B and far-red light. The absence of the UV-B and far-red wavelengths also resulted in morphological abnormalities in tomato seedling 'Beaufort' such as excessive stem elongation, while the inclusion of these wavelengths was shown to facilitate the suppression of intumescence formation and maintain desired morphological traits (Eguchi et al. 2016a).

1.3.5.2 Red and Far-red light

Red and far-red light are detected by phytochromes, primarily phytochrome B which is comprised of a protein that has two interconvertible forms: a red absorbing inactive form (P_r) and a far-red absorbing active form (P_{fr}) (Hernandez and Kubota 2017). These interconvertible forms change depending on the ratio of red to far-red light (R:FR). P_r absorbs red light and results in the conversion to P_{fr}, while the reverse occurs as P_{fr} absorbs far-red light (Jishi 2024). The proportion of phytochrome in the active P_{fr} form is an indicator for biological responses and is commonly referred to as phytochrome photostationary state (PSS; P_{fr}/P_{Total}) (Jishi 2024). A low PSS ratio (0.10/0.50) would typically be experienced under a canopy and is considered high in the far-red spectrum (Hernandez and Kubota 2017). A low PSS commonly results in the promotion of shade-avoidance responses, this response has generally been characterized by stem elongation, leaf expansion, and biomass accumulation as well as provide critical signaling in the regulation of flowering, dormancy, and seed germination (Hernandez and Kubota 2017). Whereas a high PSS ratio (0.75/0.89) would typically be experienced in full sun and be considered high in the red spectrum (Hernandez and Kubota 2017). A high PSS commonly results in the suppression of the previously described shade avoidance responses (Casal 2013). CEA has taken advantage of the physiological responses to red and far-red lighting to implement strategies to promote desired traits. One of those strategies is the use of end-of-day (EOD) lighting. EOD red and far-red applications can extend the photoperiod, promote stem elongation (which is desired during cut flower and rootstock production), regulate the timing of flowering, as well as mitigate physiological disorders such as intumescence (Eguchi et al. 2016a; Hernandez and Kubota 2017). For example, Morrow and Tibbits (1988) found an application of far-red light at 10 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was effective in reducing intumescence development to 1% of a leaf disc

surface area in wild tomato 'PI LA 1625' when provided immediately following exposure to red light (80 μmol·m^{-2}·s^{-1}) compared to red light alone.

1.3.5.3 Blue Light

Blue wavelengths of light play a critical role in photomorphogenic responses such as stomatal regulation affecting CO_2 diffusion and water relations, stem elongation, and phototropism (Massa et al. 2008). Blue light is detected by cryptochromes signaling de-etiolation, restriction of cellular expansion (at high light intensities), flowering, and circadian oscillation (Cope et al. 2014). Blue light also stimulates cellular expansion patterns and cell division, specifically within the mesophyll tissues of leaves, leading to packed palisade mesophyll cells and enhanced development of spongy mesophyll cells ultimately resulting in thicker leaves (Dou and Niu 2020). Photomorphogenic responses to monochromatic blue light are species-specific. For example, when cabbage (*Brassica campestris*), lettuce, and radish (*Raphanus sativus*) were grown under sole-source blue light, plants were all shorter and more compact compared to plants grown under mixed spectra lighting including red wavelengths (Hernandez and Kubota 2017). This is in contrast to responses in cucumber and pepper, where increased stem elongation and leaf area was observed under monochromatic blue light (Hernandez and Kubota, 2017). Cope et al. (2014) found in pepper 'California Wonder', blue light decreased leaf area expansion regardless of increasing intensities (200 μmol·m^{-2}·s^{-1} or 500 μmol·m^{-2}·s^{-1}). In the same study, stem length increased alongside increasing blue light percentage (12-92% PPFD) in pepper 'California Wonder', which was the opposite response when compared to lettuce 'Waldmann's Green' and radish 'Cherry Belle' where stem length decreased as the percentage of blue light increased (Cope et al. 2014). CEA production systems increasingly leverage blue light to manipulate crop morphology, particularly for leafy greens,

tomatoes, and peppers where compactness and quality are critical traits that directly affect future profits (Benke and Tomkins 2017; Gómez et al. 2019). The addition of blue light to EOD lighting has shown to decrease the incidence of intumescence injury in tomato plants. For example, Eguchi et al. (2016a) studied tomato 'Beaufort', a cultivar highly susceptible to intumescence, and found the use of a high intensity blue light (75% of PPFD) during the photoperiod under sole-source lighting followed by a small dose of EOD far-red light (5.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$) applied for 3.3 minutes contributed an additional 5% suppression of intumescence injury without negative influences on growth and morphology when compared to the use of low intensity blue light (10% of PPFD) .

As discussed, plants responses to various combinations of light spectrum within CEA production are species-specific. Specifically, peppers exhibit responses to blue light that are in contrast to other species (Cope et al. 2014; Hernandez and Kubota 2017), highlighting the need for further research into their specific responses to blue light as well as blue lights impact to mitigate intumescence injury.

1.4 Importance of Peppers

Pepper cultivation, including both sweet (e.g., bell peppers) and hot varieties (e.g., jalapeños, habaneros), plays a vital role in US agriculture. In 2021, U.S. producers harvested over 31,000 acres of bell peppers and more than 10,000 acres of hot peppers, yielding a combined production value over $536 million (Lawrence 2022). The market itself for specialty and spicy peppers has grown significantly in recent decades. Per capita consumption of hot peppers in the US rose from just over three pounds in 1980 to more than seven pounds by 2020 (Woods and Thornsbury 2021).

Despite its economic importance and consumer demand, domestic production has experienced a decline. Between 2000 and 2015, bell pepper acreage in the U.S. dropped from 62,080 to 40,900 acres, largely due to increased competition from imports, regulatory changes, and labor shortages (Biswas et al. 2018). Between 2000 and 2015, US fresh pepper imports increased from 436 million pounds to 1.42 billion pounds, with Mexico accounting for nearly 87% of these imports by 2015 (Biswas et al. 2018). In light of these changes, US pepper growers have faced higher production costs and reduced yields, making it increasingly difficult for domestic producers to compete with imported peppers (Leskovar 2014). With much of the US demand currently being met by imports, this growing popularity presents an opportunity for domestic producers to expand into high-value niche markets.

While field production of peppers occurs during the warmer summer months, environmental stressors, such as drought and heat, have adversely affected US pepper production, especially in the southwest (Leskovar 2014). To overcome the challenges associated with open-field cultivation, many producers are turning to CEA systems. Greenhouse-grown peppers offer significant advantages in terms of yield stability, pest control, and year-round production. These systems allow for precise management of irrigation, light, and nutrients, resulting in higher productivity per unit area (Gómez et al. 2019). Production of sweet peppers in controlled environments is especially prevalent in arid and semi-arid regions such as the southwestern US, where protected cultivation allows for improved water use efficiency and climate control (Hadad et al. 2020). In addition, research into vertical farming and LED lighting systems are being tested for indoor pepper production to further minimize land use and resource inputs (Dou and Niu 2020). Due to the high initial cost of CEA facilities, pepper production in the US remains limited. However, as CEA technologies continue to advance and become more

affordable, the supply of high valued peppers via controlled environment production is expected to increase in order to meet the domestic demand (Berholtz et al. 2023; Gómez et al. 2019).

Table 1. List of reported causation factors resulting in intumescence injury including the plant family and species investigated, alternative terms used in reference to intumescence development, and lesion location. Adapted from Pinkard et al. 2006 and Sita 2024.

Causation Factor	**Family**	**Species**	**Lesion Name**	**Lesion Location**
Mechanical injury	*Asteraceae*	*Hieracium venosum* L.	Intumescence	–
	Brassicaceae	*Brassica oleracea capitata* L.	Intumescence	Leaf
	Brassicaceae	*Brassica oleracea capitata*	Intumescence	Leaf
Chemical injury	*Brassicaceae*	*Brassica oleracea botrytis*	Intumescence	Leaf
	Euphorbiaceae	*Ricinus communis* L.	Tumor	Stem
	Solanaceae	*Solanum tuberosum*	Warts, Intumescence, Lesion	Leaf, Stem
Nutrient status	*Geraniaceae*	*Pelargonium hortorum* Ait.	Oedema	Leaf, Petioles
	Salicaceae	*Populus spp.*	Intumescence	Leaf
	Solanaceae	*Solanum tuberosum*	Warts, Intumescence, Lesion	Leaf, Stem
Hormones (including ethylene)	*Salicaceae*	*Populus grandidentata* Bull	Intumescence	Leaf
	Salicaceae	*Populus tremuloides* Michx.	Intumescence	Leaf
	Solanaceae	*Solanum tuberosum*	Warts, Intumescence, Lesion	Leaf, Stem
	Solanaceae	*Lycopersicon hirsutum* Humb. *and* Ponpl.	Neoplasm, Tumor	Leaf, Stem
Genetics	*Geraniaceae*	*Pelargonium hortorum* L' Her	Oedema	Leaf, Petioles
	Solanaceae	*Lycopersicon hirsutum*	Neoplasm, Tumor	Leaf, Stem
	Solanaceae	*Solanum melongena* L.	Oedema	Leaf, Petioles
Insect injury	*Caryophyllaceae*	*Dianthus sp.*	Oedema	Leaf

	Myrtaceae	*Eucalyptus regnans* F. Muell	Intumescence	Leaf
	Salicaceae	*Populus tremula* L.	Intumescence	Leaf
Air quality and airborne factor(s)	*Myrtaceae*	*Eucalyptus spp.*	Intumescence	Leaf
	Salicaceae	*Populus spp.*	Intumescence	Leaf
	Solanaceae	*Lycopersicon sp.*	Oedema, Tumor, Neoplasm, Intumescence	Leaf
	Solanaceae	*Lycopersicon spp.*	Oedema, Tumor, Neoplasm, Intumescence	Leaf
	Solanaceae	*Solanum tuberosum*	Warts, Intumescence, Lesion	Leaf, Stem
Light quality	*Malvaceae*	*Hibiscus vitifolius* L.	Intumescence	Fruit, Flower sepals, Leaf, Petioles, Stem
	Solanaceae	*Solanum sp.*	Oedema, Tumor, Neoplasm, Intumescence	Leaf, Petioles, Stem
	Solanaceae	*Lycopersicon esculentum* Mill.	Oedema, Tumor, Neoplasm, Intumescence	Leaf, Stem
	Solanaceae	*Lycopersicon spp.*	Oedema, Tumor, Neoplasm, Intumescence	Leaf
	Solanaceae	*Lycopersicon hirsutum*	Neoplasm, Tumor	Leaf, Stem
Light availability	*Geraniaceae*	*Pelargonium xhortorum*	Oedema	Leaf, Petioles
	Geraniaceae	*Pelargonium sp.*	Oedema	Leaf, Petioles
	Malvaceae	*Hibiscus vitifolius* L.	Intumescence	Fruit, Flower sepals, Leaf, Petioles, Stem
	Salicaceae	*Populus tremula*	Intumescence	Leaf
	Solanaceae	*Lycopersicon sp.*	Oedema, Tumor, Neoplasm, Intumescence	Leaf
	Solanaceae	*Solanum tuberosum*	Warts, Intumescence, Lesion	Leaf, Stem

	Vitaceae	*Vitis vinifera* L.	Intumescence	Leaf
Temperature	*Araceae*	*Philodendron hastatum* Schott.	Intumescence	Stem
	Balsaminaceae	*Impatiens fulva* Nutt.	Intumescence	–
	Brassicaceae	*Brassica oleracea capitata*	Intumescence	Leaf
	Geraniaceae	*Pelargonium xhortorum*	Oedema	Leaf, Petioles
	Malvaceae	*Hibiscus vitifolius*	Intumescence	Fruit, Flower sepals, Leaf, Petioles, Stem
	Moraceae	*Ficus elastica* Roxb.	Intumescence	–
	Solanaceae	*Lycopersicon sp.*	Oedema, Tumor, Neoplasm, Intumescence	Leaf
	Solanaceae	*Solanum melongena* L.	Oedema	Leaf, Petioles
	Solanaceae	*Solanum sp.*	Oedema, Tumor, Neoplasm, Intumescence	Leaf, Petioles, Stem
Excess water/humidity	*Acanthaceae*	*Aphelandra porteana* Morel	Intumescence	–
	Acanthaceae	*Ruellia formosa* Andr.	Intumescence	–
	Balsaminaceae	*Impatiens fulva*	Oedema	–
	Brassicaceae	*Brassica oleracea capitata*	Intumescence	Leaf
	Geraniaceae	*Pelargonium xhortorum*	Oedema	Leaf, Petioles
	Geraniaceae	*Pelargonium sp.*	Oedema	Leaf, Petioles
	Malvaceae	*Hibiscus vitifolius*	Intumescence	Fruit, Flower sepals, Leaf, Petioles, Stem
	Moraceae	*Ficus elastica*	Intumescence	–
	Myrtaceae	*Eucalyptus spp.*	Intumescence	Leaf
	Pinaceae	*Pinus virginiana*	Excrescences	Root
	Pinaceae	*Pinus sylvestris*	Excrescences	Root
	Pinaceae	*Picea rubens*	Excrescences	Root

	Pinaceae	*Pinus rigida*	Excrescences	Root
	Pinaceae	*Pinus resinosa*	Excrescences	Root
	Pinaceae	*Pinus ponderosa*	Excrescences	Root, Stem
	Salicaceae	*Populus grandidentata*	Intumescence	Leaf
	Salicaceae	*Populus spp.*	Intumescence	Leaf
	Solanaceae	*Lycopersicon sp.*	Oedema, Tumor, Neoplasm, Intumescence	Leaf
	Solanaceae	*Solanum melongena*	Oedema	Leaf, Petioles
	Solanaceae	*Solanum tuberosum*	Warts, Intumescence, Lesion	Leaf, Stem
Other unknown causative factors	*Fabaceae*	*Phaseolus vulgaris*	Intumescence	Stem
	Fabaceae	*Senna multiglandulosa*	Intumescence	–
	Fabaceae	*Cassia floribunda*	Intumescence	–
	Fabaceae	*Vicia faba*	Intumescence	Pods
	Fabaceae	*Pisum sativum*	Intumescence, Tumor	Pods
	Fabaceae	*Vigna unguiculata* L. Walp.	Edema	Leaf
	Pinaceae	*Pinus coulteri*	Excrescences	Root
	Pinaceae	*Pinus banksiana*	Excrescences	Root
	Pinaceae	*Pinus caribaea*	Excrescences	Root
	Pinaceae	*Pinus strobus*	Excrescences	Root
	Pinaceae	*Pinus monticola*	Excrescences	Root
	Pinaceae	*Pinus excelsa*	Excrescences	Root
	Pinaceae	*Picea canadensis*	Excrescences	Root
	Pinaceae	*Picea mariana*	Excrescences	Root
	Pinaceae	*Picea pungens*	Excrescences	Root
	Pinaceae	*Abies balsamea*	Excrescences	–

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CHAPTER 2. CHARACTERIZATION AND TIMING OF LEAF EXPANSION FOR THREE *CAPSICUM ANNUUM* CULTIVARS

2.1 Abstract

Intumescence is a physiological disorder affecting many plant species grown in controlled environments. Intumescence is characterized by unrestricted cellular elongation causing protruding lesions, ultimately leading to epidermal rupture. The impacts of this disorder to the industry involve reduced yield, loss of productivity, and reduced ornamental value. Intumescence has been studied for over 140 years, but the mechanism of action and developmental stages are still not well understood. This study was conducted to determine the timing of leaf area expansion in pepper (*Capsicum annuum*) to better inform mitigation strategies for intumescence development. Three industry relevant pepper cultivars, 'Pot-a-Peño', 'Spicy Jane', and 'California Wonder' were transplanted into 15-cm pots and grown in a common greenhouse environment. Upon initiating the experiment, one uniform, newly developed leaf from each plant was tagged and photos were collected twice daily (0700 and 1900 HR) for 7 d. Image analysis was conducted using ImageJ and anatomical measurements such as area, perimeter, width, and length were recorded. Generally, leaf area expansion was greatest during the night compared to the day for all three cultivars. For example, average leaf expansion over the course of the 7d data collection period was 46%, 34%, and 94% greater during the night compared to the day for 'Spicy Jane', 'Pot-a-Peño', and 'California Wonder', respectively. These results will help in the prediction of intumescence development and informing decisions and techniques for suppression of this disorder.

2.2 Introduction

Pepper (*Capsicum annuum*) consumption is increasing across the US; this includes both hot and bell pepper varieties. For example, the consumption per capita of hot peppers rose from three pounds in 1980 to over seven pounds in 2020 (Lillywhite and Tso 2021). Despite the growing consumer demand, nearly 87% of peppers are currently imported from Mexico to the US (Biswas et al. 2018). Peppers are considered a high value crop within controlled environment agriculture (CEA) and can provide an opportunity for domestic growers to expand into a high demand niche market. CEA can provide significant advantages to pepper production by facilitating year-round production, targeted pest control, and stable yields (Kim 2016). These systems provide precise management of irrigation and nutrition, lighting applications, and temperature control, resulting in higher productivity per area (Gòmez et al. 2019). However, CEA does face challenges including the amplification of some disorders compared to conventional farming due to the closed nature of controlled environments (Singh et al. 2020). Previous research on crops grown in controlled environments, particularly solanaceous species, has noted common disorders such as fruit cracking, curved fruit, blossom end rot, and intumescence (Savvas et al. 2008; Sorauer 1922). Intumescence, like other disorders previously mentioned, can substantially reduce the marketable value of the affected crop.

Intumescence is a physiological disorder characterized by the unregulated elongation and expansion of spongy mesophyll and parenchyma cells (Lang and Tibits 1983; Williams 2016). Normal cellular elongation is part of an essential biological process that enables the plant to grow in shape and size (Hart 1988). As leaves grow, the dynamic interplay of turgor pressure, cell wall loosening, and environmental conditions such as light and humidity plays a role in determining both the rate and extent of expansion (Kirkham 2014; Oguchi et al. 2018). However,

these processes can become dysregulated, possibly resulting in intumescence development. Recent investigations have highlighted the susceptibility of rapidly expanding leaf tissues to intumescence, suggesting a close relationship between the physiological regulation of leaf growth and the onset of this disorder (Miyama and Yasui 2021; Suzuki et al. 2020). For example, rapid cellular expansion in young tomato (*Solanum lycopersicum*) (Suzuki et al. 2020) and pepper (Hoffmann et al. 2015) leaves under high humidity or suboptimal light conditions may disrupt normal cell wall formation and promote cellular rupture or proliferation. Pantin et al. (2011) described leaf expansion as being controlled by either metabolic or hydraulic regulation and suggested that the expansion predominantly occurs during the night when transpiration is low and turgor pressure can be maintained. In a greenhouse study analyzing leaf growth of soybean (*Glycine max* 'Gallec'), Mielewczik et al. (2013) found that maximal growth was observed at night compared to the day. This response is further supported by Volkenburgh (1999) who emphasized that leaf expansion often peaks during the night due to favorable water balances. Miyama and Yasui (2021) conducted research using tomato cultivars 'Momotaro Peace', 'CF House Momotaro', and 'Reiyo' which supports the possibility of intumescence development corresponding with large changes in water potential. Their results found if ambient humidity and soil moisture increases suddenly, water absorption temporarily exceeded the transpiration rate, resulting in cellular rupture in all three cultivars (Miyama and Yasui 2021). Water status plays an essential role in the development and expansion of leaves; this delicate balance can be disrupted leading to morphological changes and intumescence development in newly expanding leaves.

In attempts to mitigate intumescence development, previous research has suggested using end-of-day (EOD) lighting applications (Eguchi et al. 2016a; Eguchi et al. 2016b). Currently, the

use of EOD lighting includes specific spectra of light to manipulate physiological or morphological traits based on the desired outcomes (e.g., compactness, breaking dormancy, flower regulation) (Lopez and Runkle 2017). Since EOD lighting applications have been found to alter leaf morphology, it may be crucial to coincide EOD lighting with leaf expansion in order to suppress intumescence development.

To better understand the timing of intumescence development, identifying when spongy mesophyll and parenchyma cells elongate and expand to drive leaf expansion in pepper is crucial. The objective of this experiment was to determine the timing of accelerated cellular expansion in pepper leaves by comparing leaf expansion rates occurring during the day and night. This study was intended to inform potential intumescence mitigation strategies for pepper production in CEA. We hypothesize that pepper leaves will have a higher rate of expansion during the night compared to the day based on previous research.

2.3 Materials and Methods

2.3.1 Plant Materials and Greenhouse Environment

Pepper cultivars 'Pot-a-Peño', 'Spicy Jane', and 'California Wonder' were evaluated during the summer of 2024 based on their susceptibility to intumescence development. On 11 July 2024, seeds were sown into 128-cell trays using commercial soilless germination media (Berger BM2 Seed Germination; Berger, Saint-Modeste, Quebec City, CA) and covered lightly with vermiculite (Sun Gro® Horticultural Vermiculite Premium Grade Medium A-2 Coarse; Sun Gro®, Agawam, MA, USA). Trays were fertigated daily using Jack's water-soluble fertilizer (Jack's 13N–0.9P–10.8K Plug LX; J.R. Peters, Inc., Allentown, PA, USA) providing 75 $mg \cdot L^{-1}$ nitrogen. pH and electrical conductivity (EC) of the fertilizer solution were confirmed using a handheld meter (Bluelab Combo Meter, Bluelab, Tauranga, New Zealand). Trays were

germinated under fitted plastic domes for two weeks. Upon germination, humidity domes were removed. Seedlings were transplanted into 15-cm plastic pots on 1 Aug 2024. Transplants were fertigated every other day using a water-soluble fertilizer (Jack's 21N–2.2P–16.7K; J.R. Peters, Inc., Allentown, PA, USA) providing 150 $mg \cdot L^{-1}$ nitrogen per industry recommendation. Seed trays and transplants were grown and maintained in a greenhouse located at the Colorado State University Horticulture Center in Fort Collins, CO, USA. Greenhouse environmental conditions were controlled using a VeriStep dynamic control system (Wadsworth Control System, Arvada, CO, USA) with a temperature set point of 25/18 °C (day/night). Air temperature, relative humidity (RH), photosynthetic photon flux density (PPFD), and carbon dioxide (CO_2) concentration were monitored and logged every 10 min using an aspirated datalogger (Guardian SM-600; Apogee® Instruments, Logan, UT, USA), with average values reported in Table 2. Two replicates of five plants per cultivar (N=10) were conducted simultaneously, blocked by bench located within the same greenhouse. Within each bench, plant positions were randomly rotated daily to ensure uniformity.

2.3.2 Data Collection

On 16 Aug 2024, one uniform leaf (no less than 2 cm in length) at the 4th node of the apical meristem, measured from the base, was tagged on each plant for imaging. Nondestructive imaging started on 20 Aug 2024 and ended the morning of 27 Aug 2024. Five plants from each species per replication were observed twice daily (0700 and 1900 HR) for 7 d, with photos of the adaxial surface of tagged leaves collected during each observation using a digital camera (Hero12 Black; GoPro, San Mateo, CA, USA) affixed with a 15X lens (15X Macro Lens; QKOO, China) connected to a 15 cm × 23 cm white clipboard via a gooseneck clamp (Suptig Jaws Flex Clamp Gooseneck Mount; Suptig, Shenzhen, China) to maintain a fixed position. To

gently secure the leaf in a fixed position, a clear sleeve (73 × 122 mm matte sleeve; GameGenic Ingenious Supplies, Essen, Germany) was affixed to the clipboard. The camera lens was centered upon the middle of the card sleeve and positioned 15 cm from the clipboard surface. Photos were analyzed using ImageJ software (US National Institutes of Health, Bethesda, MD, USA) to determine leaf length (cm), width (cm), perimeter (cm), and area (cm^2). Calculations to determine change in leaf area expansion occurring during the day (Daytime; DT) were conducted by deducting the current days PM values from the AM values (e.g., $PMDay_1 – AMDay_1$= DT 1), while change in leaf area expansion occurring during the night (Nighttime; NT) was calculated by deducting the current days AM value from the previous day's PM value (e.g., $AMDay_2 – PMDay_1$ = NT 1). For a detailed description of image capture and processing, see Appendix A.

2.3.3 Statistical Analysis

The experiment was conducted using a randomized complete block design, with time of day (DT or NT) serving as a treatment factor, bench as a blocking variable, and plant number as a random variable. Data analysis for each replication was combined and represented in the data reported. The effects of DT and NT were compared by analysis of variance (ANOVA) with R and RStudio [R version 4.3 (R Core Team 2023)] as well as jamovi [jamovi version 2.6 (The jamovi project, 2025)], using Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

2.4 Results

California Wonder – While average leaf area increased consistently over the course of the 7 d data collection period for pepper 'California Wonder' during both DT and NT (Fig. 1), leaf area expansion was generally greater during NT compared to DT (Fig. 2). Specifically, leaf area expansion during NT was greater than DT on days 1-4. While results were not significant, leaf area expansion was also greater during NT compared to DT on days 5 ($P = 0.052$) and 6 ($P =$

0.425). Similarly, average leaf length, width, perimeter, and area over the 7 d data collection period was greatest during NT compared to DT (Table 3). For example, average leaf area expansion for ‘California Wonder’ was 94% greater during NT compared to DT over the 7 d data collection period.

Pot-a-Peño – Similarly, average leaf area increased consistently over the duration of the 7 d data collection period for ‘Pot-a-Peño’ during both DT and NT (Fig. 3), with leaf area expansion being greater during the NT compared to DT (Fig. 4). Specifically, leaf area expansion during the NT was greater than DT on days 3 and 4 (Fig. 4). While results were not significant, leaf area expansion was also greater during the NT compared to DT on days 1 ($P = 0.069$), 2 ($P = 0.262$), 5 ($P = 0.066$), and 6 ($P = 0.125$). Similarly, average leaf length, width, perimeter, and area for the 7 d data collection period were greatest during the NT compared to DT (Table 3). For example, average leaf area expansion for ‘Pot-a-Peño’ was 34% greater during the NT compared to DT over the 7 d data collection period.

Spicy Jane – Consistent with the other two cultivars, ‘Spicy Jane’ leaf area increased consistently throughout the 7 d data collection period for during both DT and NT (Fig. 5), with the greatest leaf area expansion occurring predominantly during the NT compared to DT (Fig. 6). Specifically leaf area expansion during the NT was greater than DT on days 1, 3, 4, and 5 (Fig. 6). Although results were not significant, leaf area expansion was also greater on days 2 ($P = 0.136$), and 6 ($P = 0.724$). Similarly, average leaf length, width, perimeter, and area for the 7 d data collection period were greatest during the NT compared to DT (Table 3). For example, average leaf area expansion for ‘Spicy Jane’ was 46% greater during the NT compared to the DT over the 7 d data collection period.

2.5 Discussion

Although there is much debate over causative factors, intumescence development is consistently observed on rapidly expanding leaves. Leaf expansion is a continual process until the leaf reaches a mature state; as the leaf reaches maturity, the growth rate slows or even stops, which could help to explain why rapidly expanding leaves have been shown most susceptible to intumescence development while mature leaves are generally free from symptoms (Eisa and Dobrenz 1971). Suzuki's (2020) research with tomato 'CF Rinka 409' found intumescence injury had the highest severity in developing leaves, low severity in fully developed leaves, and little to no development in young leaves with slower rates of expansion. La Rue (1933) found similar results while researching intumescence development on poplar leaves, observing very young leaves did not show any susceptibility to intumescence development until reaching about half their final mature size. Due to leaf maturity being a seemingly important factor in susceptibility to intumescence development, immature rapidly expanding leaves were chosen to be tagged for image analysis and development observations in the present study.

Leaf expansion in all three cultivars was greatest during NT compared to DT, with NT accounting for 66%, 57%, and 59% of total leaf area expansion for 'California Wonder', 'Pot-a-Peño', and 'Spicy Jane', respectively. This is consistent with previous research conducted by Mielewczik (2013), whereby leaf expansion in soybean was evaluated using a marker-tracking time-lapse camera which revealed greater leaf expansion occurred during the night compared to the day. These results are also supported by earlier research demonstrating that both metabolic and hydraulic processes vary between day and night and influence growth rates, with metabolic control dominating in early development and hydraulic constraints becoming more pronounced as leaves mature (Pantin et al. 2011; Volkenburgh 1999). For example, in *Arabidopsis*, Pantin et

al. (2011) reported that metabolic-driven expansion in young leaves was most apparent at night, when favorable water balance supported cell enlargement. However, this nocturnal expansion could be limited by restricted starch reserves relative to the high carbon demand for growth. These authors found that as leaves continue to develop, the main constraint shifted to hydraulics, with reduced daytime turgor increasingly restricting expansion. This hydraulic limitation was associated with a reduced capacity of the leaf's water transport system to supply growing tissues under conditions of high evaporative demand. At both developmental stages, nighttime expansion tended to be more favorable due to improved water balance, which allowed partial recovery from daytime turgor loss (Pantin et al. 2011).

While not statistically analyzed, there were also clear differences in habit and leaf size between cultivars. The most prominent difference being that pepper 'California Wonder' is a bell pepper variety with a mounded habit while pepper 'Pot-a-Peño' and 'Spicy Jane' are both hot pepper varieties. Pepper 'Pot-a-Peño' and 'Spicy Jane' are bred for a compact habit and proliferation of smaller leaves, respectively, leading to a lower magnitude of response in terms of leaf area expansion. While not included in the present study, these differences between cultivars should be considered.

This study confirms the timing in which pepper leaves expand, providing a basis for intumescence research among upcoming pepper cultivars of interest to CEA production. Since cellular expansion primarily occurred during NT, it is possible that this is a window of opportunity for the suppression of intumescence development in susceptible species. Thus, mitigation strategies could be timed to coincide with periods of rapid cellular expansion. Recognizing the timing of cellular expansion, growers can potentially expand their current use of

EOD or pre-dawn lighting applications to mitigate intumescence development in susceptible pepper cultivars.

Table 2. Mean ± SD daily light integral (DLI), temperature, relative humidity, and CO_2 concentration across two experimental replications (benches) during the day (0700-1900 HR) and night (1900-0700 HR) from 20 Aug 2024 – 27 Aug 2024.

	DLI	Temperature	Humidity	CO_2
	$mol \cdot m^{-2} \cdot d^{-1}$	° C	%	$\mu mol \cdot mol^{-1}$
Day	19.2 ± 4.6	24.9 ± 0.4	48.5 ± 3.5	475.3 ± 5.2
Night	–	22.3 ± 1.0	51.1 ± 8.3	502.6 ± 10.2

Table 3. Least square means of daily leaf area, length, width, and perimeter during the daytime (DT; 0700 – 1900 HR) and nighttime (NT; 1900 – 0700 HR) for individual leaves of pepper (*Capsicum annuum*) 'California Wonder', 'Pot-a-Peño', and 'Spicy Jane' collected over a 7 d data collection period from 20 Aug 2024 – 27 Aug 2024 for two experiment replicates (N = 10).

	Area (cm^2)	Length (cm)	Width (cm)	Perimeter (cm)
California Wonder				
DT	4.68[z]	0.51	0.39	1.49
NT	9.07	1.01	0.61	2.80
Sig.	***[y]	***	**	***
Pot-a-Peño				
DT	2.27	0.40	0.22	1.04
NT	3.04	0.59	0.27	1.40
Sig.	NS	*	NS	NS
Spicy Jane				
DT	1.40	0.31	0.16	0.78
NT	2.04	0.42	0.20	1.06
Sig.	**	NS	NS	NS

[z]Mean values are based on single leaves selected from ten plants across two experimental replications.

[y]NS, *, **, *** indicate non-significance or significance between DT and NT within a cultivar at $P \leq 0.05$, 0.01, and 0.001, respectively.

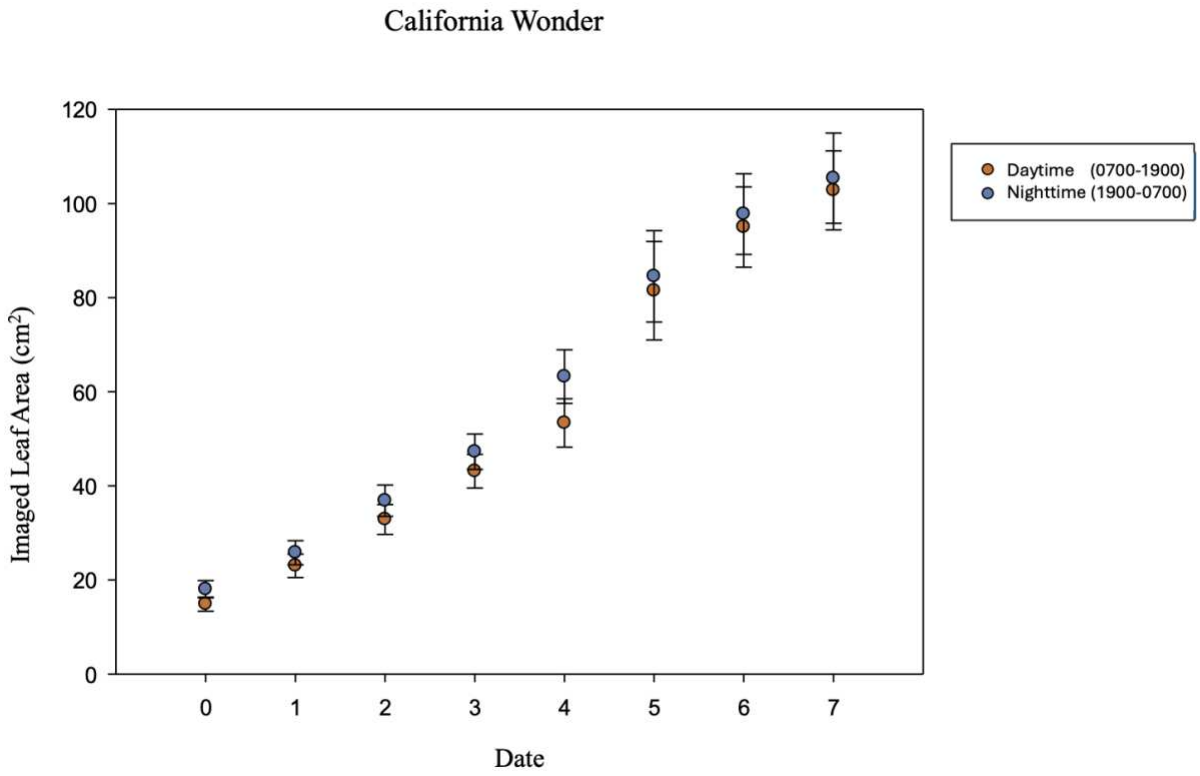

Figure 1. Imaged leaf area for individual leaves of pepper (*Capsicum annuum*) 'California Wonder' measured daily over a 7 d data collection period for two experiment replicates (N = 10). Values represent the average leaf area for plants measured during the daytime (0700-1900 HR) in orange circles and nighttime (1900-0700 HR) in blue circles. Error bars indicate ± standard error of the mean.

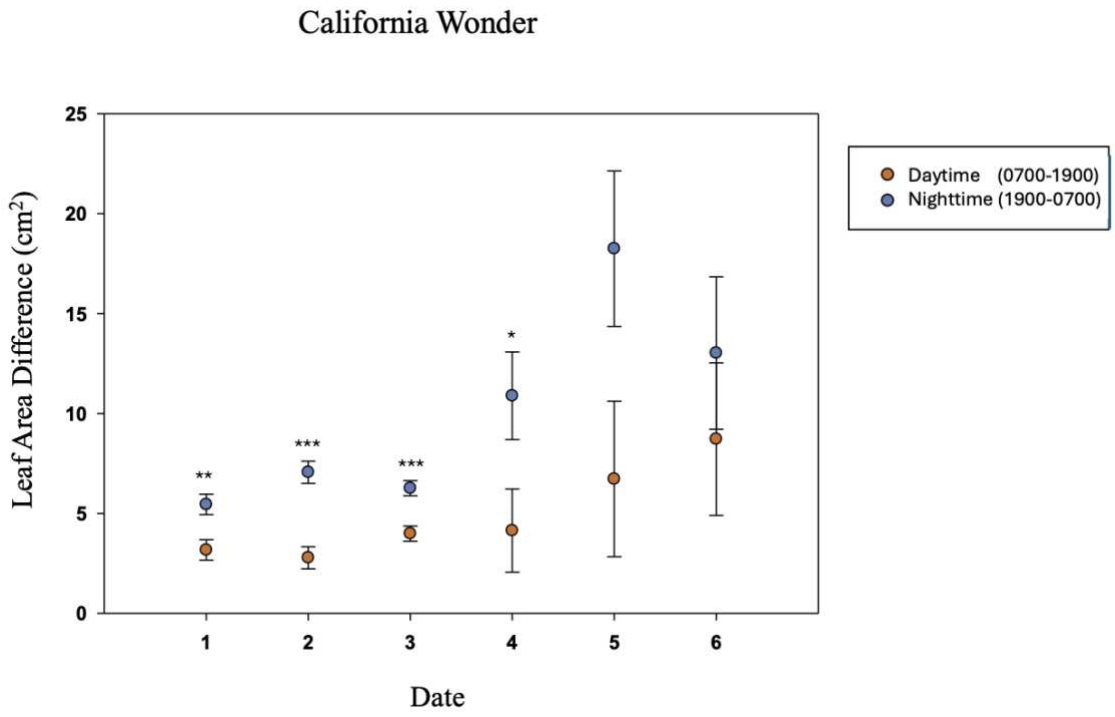

Figure 2. Calculated leaf area difference (cm^2) of individual imaged leaves to determine change in leaf area expansion occurring during the day (Daytime; DT) (0700-1900 HR, in orange circles) and night (Nighttime; NT) (1900-0700 HR, in blue circles) for pepper (Capsicum annuum) 'California Wonder' on specific dates throughout a 7 d data collection period for two experiment replicates (N = 10). Daytime leaf area difference was calculated by deducting the current days PM values from the AM values (e.g., $PMDay_1 - AMDay_1$= DT 1), while nighttime leaf area difference was calculated by deducting the current days AM value from the previous day's PM value (e.g., $AMDay_2 - PMDay_1$ = NT 1). Error bars indicate ± standard error of the mean, with *, **, *** indicating significance at $P \leq 0.05$, 0.01, and 0.001, respectively, between day and night for the specified date intervals.

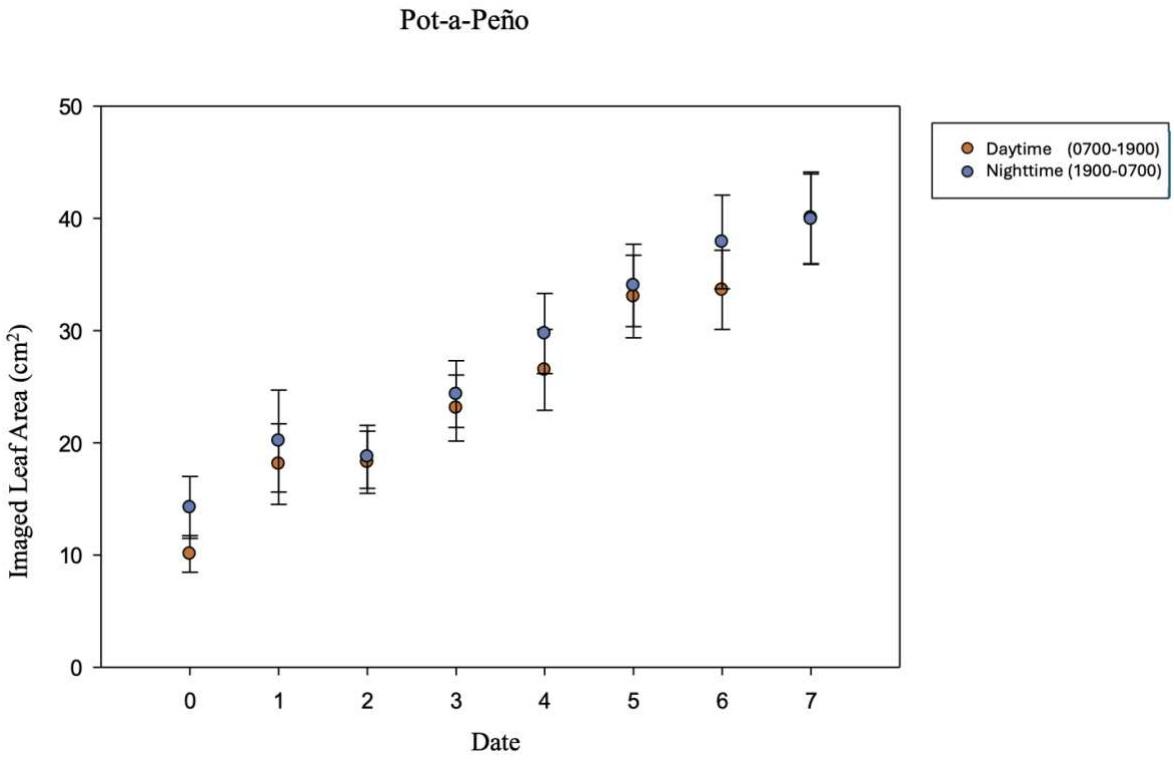

Figure 3. Imaged leaf area for individual leaves of pepper (*Capsicum annuum*) 'Pot-a-Peño' measured daily over a 7 d data collection period for two experiment replicates (N = 10). Values represent the average leaf area for plants measured during the daytime (0700-1900 HR) in orange circles and nighttime (1900-0700 HR) in blue circles. Error bars indicate ± standard error of the mean.

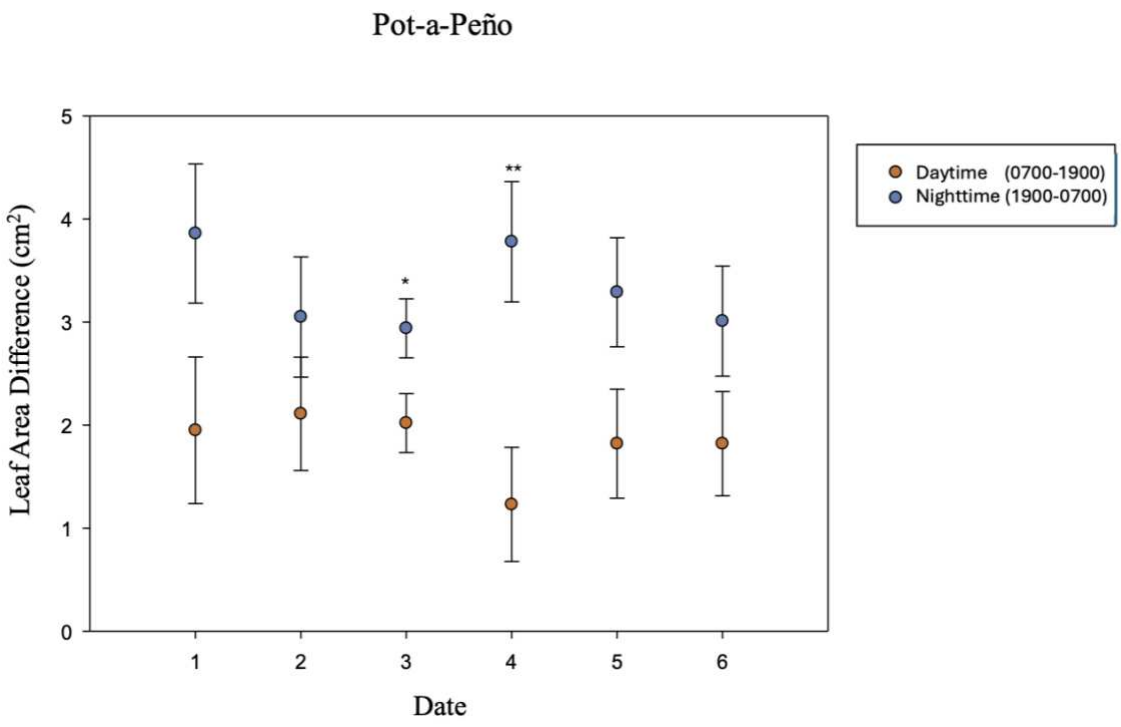

Figure 4. Calculated leaf area difference (cm^2) of individual imaged leaves to determine change in leaf area expansion occurring during the day (Daytime; DT) (0700-1900 HR, in orange circles) and night (Nighttime; NT) (1900-0700 HR, in blue circles) for pepper (*Capsicum annuum*) 'Pot-a-Peño' on specific dates throughout a 7 d data collection period for two experiment replicates (N = 10). Day leaf area difference was calculated by deducting the current days PM values from the AM values (e.g., $PMDay_1 - AMDay_1 = DT\ 1$), while night leaf area difference was calculated by deducting the current days AM value from the previous day's PM value (e.g., $AMDay_2 - PMDay_1 = NT\ 1$). Error bars indicate ± standard error of the mean, with *, **, *** indicating significance at $P \leq 0.05$, 0.01, and 0.001, respectively, between day and night for the specified date intervals.

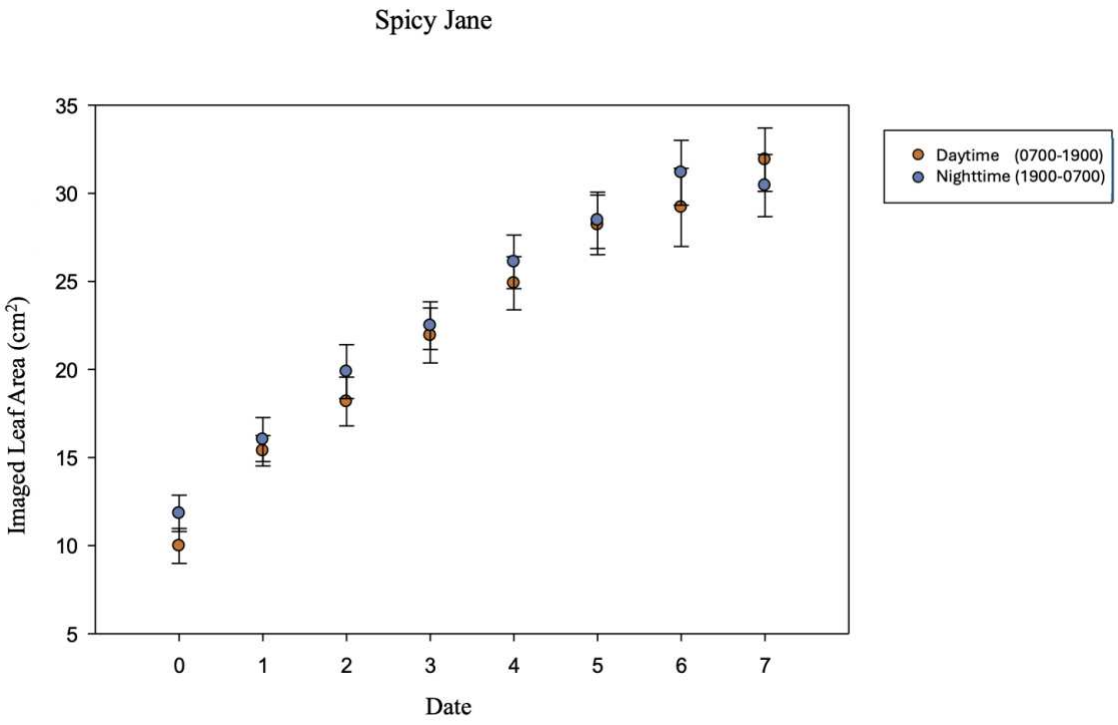

Figure 5. Imaged leaf area for individual leaves of pepper (*Capsicum annuum*) 'Spicy Jane' measured daily over a 7 d data collection period for two experiment replicates (N = 10). Values represent the average leaf area for plants measured during the daytime (0700-1900 HR) in orange circles and nighttime (1900-0700 HR) in blue circles. Error bars indicate ± standard error of the mean.

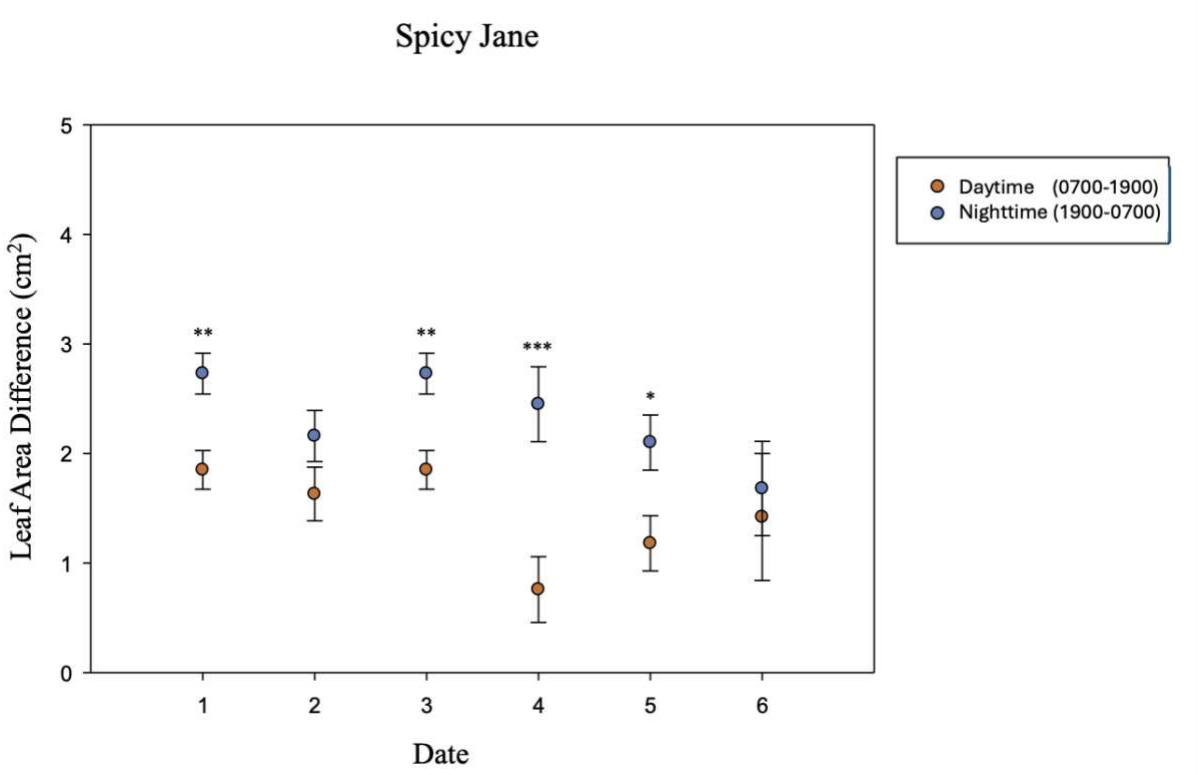

Figure 6. Calculated leaf area difference (cm^2) of individual imaged leaves to determine change in leaf area expansion occurring during the day (Daytime; DT) (0700-1900 HR, in orange circles) and night (Nighttime; NT) (1900-0700 HR, in blue circles) for pepper (*Capsicum annuum*) 'Spicy Jane' on specific dates throughout a 7 d data collection period for two experiment replicates (N = 10). Day leaf area difference was calculated by deducting the current days PM values from the AM values (e.g., $PMDay_1 - AMDay_1$= DT 1), while night leaf area difference was calculated by deducting the current days AM value from the previous day's PM value (e.g., $AMDay_2 - PMDay_1$ = NT 1). Error bars indicate ± standard error of the mean, with *, **, *** indicating significance at $P \leq 0.05$, 0.01, and 0.001, respectively, between day and night for the specified date intervals.

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CHAPTER 3: THE EFFECT OF END-OF-DAY AND PRE-DAWN LIGHTING STRATEGIES ON MORPHOLOGY AND INTUMESCENCE DEVELOPMENT FOR THREE *CAPSICUM ANNUUM* CULTIVARS

3.1 Abstract

Intumescence is a physiological disorder characterized by hypertrophy and hyperplasia of epidermal and palisade parenchyma cells occurring on leaves, petioles, and stems of multiple plant species. Whether blue, far-red, or ultraviolet light are implicated, end-of-day (EOD) lighting strategies have been proven effective at controlling intumescence. Especially for emerging crops with limited research on production strategies (e.g., pepper), EOD lighting is a potentially cost-effective means to control intumescence, which may otherwise limit adaptability of these crops for controlled-environment production systems. With the use of low-intensity EOD and pre-dawn (PD) LED lighting applications as a means of providing a cost-effective option to alleviate crop losses from intumescence injury in susceptible pepper cultivars, this study was aimed at characterizing morphological changes occurring during EOD or PD light treatments. Three pepper (*Capsicum annuum*) cultivars 'California Wonder', 'Pot-a-Peño', and 'Spicy Jane' were all grown in a common greenhouse setting. Five plants from each cultivar were allocated to one of four light treatments, comprised of three EOD treatments consisting of blue (EOD_B), red (EOD_R) and far-red light (EOD_{FR}) as well as one pre-dawn blue light treatment (PD_B). Plants in each lighting treatment were subjected to a 30-minute duration at an intensity of ~25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ supplied at either EOD or PD depending on their assigned treatment group. While there was no intumescence development throughout the duration of this study, there were changes to plant morphology. Specifically, total leaf dry mass, stem dry mass, total dry mass,

and compactness were all greatest for pepper ‘California Wonder’ grown under PD_B. While peppers ‘Pot-a-Peño’ and ‘Spicy Jane’ did not have any differences in total leaf dry mass, stem dry mass, total dry mass, or compactness, height differences were observed whereby plants were taller under EOD_R compared to PD_B. The relevance of cultivar-specific morphological responses to light quality should be taken into account when using either EOD or PD lighting strategies to mitigate intumescence injury.

3.2 Introduction

Plants use light as a form of energy for photosynthesis, converting water and carbon dioxide into sugars, but light is also used as a signal regulating morphology and physiology (Lopez et al. 2017). The specific wavelengths of light detected by plants that drive photosynthetic activity are traditionally referred to as photosynthetically active radiation (PAR; 400-700 nm). While wavelengths outside of the PAR range [e.g., UV-A and UV-B (300-400 nm), far-red (700-800nm)] are not generally considered photosynthetically active, they do elicit other plant responses including changes to plant morphology and pigmentation (Hernandez and Kubota 2017). In controlled environment agriculture (CEA), where electric lighting supplements or replaces sunlight, growers must carefully balance light spectrum, intensity, and timing to optimize production. The introduction of light-emitting diode (LED) technologies has transformed CEA production by offering spectral precision, lower energy costs, and longer fixture lifespans compared to traditional high-pressure sodium or fluorescent lamps (Both and Faust 2017; Massa et al. 2008). These innovations allow for the targeted application of blue, red, and far-red light to manipulate photosynthetic efficiency, plant architecture, and reproductive development. The growing popularity and cost reduction of tunable lighting systems and continuous advancements in LED technologies have allowed growers the ability to manipulate

the lighting environment to promote desired outcomes such as biomass accumulation (Massa et al. 2008) and the promotion or inhibition of flowering via photoperiod manipulation (Runkle et al. 2017) in ways that were not possible with earlier lighting systems. This ability to manipulate light quality and quantity has also revealed a possible link between the spectral environment and the occurrence of physiological disorders.

One disorder of rising interest within CEA is intumescence. Intumescence is characterized by the hypertrophy and hyperplasia of cells, typically the palisade parenchyma and spongy mesophyll, which erratically elongate resulting in the rupture of the epidermal layer and the formation of crystalized or wart-like lesions on leaves, stems, or petioles (Sita et al. 2024; Sorauer 1922). These lesions reduce photosynthetic capacity resulting in reduced yield, leaf abscission when severe, as well as diminished marketability of ornamental crops (Sita et al. 2024). Intumescence has been observed in many crop species grown in CEA such as tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), pepper, and potato (*Solanum tuberosum*), as well as ornamental species such as cuphea (*Cuphea llavea*) and ornamental sweet potato (*Ipomoea batatas*) (Craver et al. 2014; Lang and Tibbits 1983; Suzuki et al. 2020). Observations occurring in pepper are of rising interest due to their high consumer demand and relatively high market value in CEA (Gómez et al. 2019). Given the increasing importance of pepper production in controlled environments, management strategies to reduce intumescence are essential for their success in CEA production.

Greenhouse end-of-day (EOD) lighting applications are typically characterized as low-intensity electric light provided at the end of the photoperiod and are commonly used to regulate flowering. Recent studies suggest that lighting strategies, particularly EOD applications, offer a promising tool for managing intumescence injury. Massa et al. (2008) demonstrated that

maintaining at least 15% blue light of total irradiation suppressed intumescence in cowpea (*Vigna unguiculata*), but lesions did develop when blue light was maintained at less than 15% total irradiation. Eguchi et al. (2016b) found intumescence injury was suppressed in tomato cultivars 'Beaufort' and 'Maxifort' when EOD far-red light was provided in addition to a sole-source lighting environment consisting of 10% blue (400-500 nm) and 90% red (600-700 nm) light. Additionally, supplementation with UV-B has been shown to reduce intumescence development in ornamental sweetpotato 'Ace of Spades' and tomato 'Beaufort' when compared with plants grown under UV-B–blocking films or UV-B–deficient lighting conditions (Craver et al. 2014b; Kubota et al. 2017). In greenhouse production where ambient solar radiation cannot be easily controlled and often diminishes spectral manipulation via LEDs, EOD light strategies are a practical means to modify plant responses by providing a targeted light spectra when solar radiation is no longer a factor. Thus, EOD lighting presents the potential for growers to apply low-intensity spectral treatments at specific times of day to mitigate intumescence injury without significantly altering energy costs.

However, designing effective EOD lighting strategies is essential as these applications often influence plant morphology through a variety of photoreceptor proteins. For example, red and far-red light regulate the active and inactive forms of phytochromes, which in turn controls shade avoidance responses typically associated with stem elongation and leaf expansion (Hernandez and Kubota 2017). The use of EOD far-red lighting in CEA has been successful in manipulating plant morphology, including the induction of increased hypocotyl elongation in tomato rootstock for grafting purposes (Chia and Kubota 2010). Specifically, Chia and Kubota (2010) found that EOD lighting with a low ratio of red to far-red light (R:FR) enhanced hypocotyl elongation in tomato rootstock seedlings 'Aloha' and 'Maxifort' as compared to a

control receiving no EOD far-red lighting. On the other hand, blue light can be perceived by cryptochromes, which play a role in regulating stomatal function, leaf thickness, restriction of cellular expansion at high intensities, de-etiolation, and circadian oscillation (Dou and Niu 2020; Massa et al. 2008). These responses are highly dynamic and sensitive to both spectrum and timing; thus, manipulating the light environment at either EOD or pre-dawn (PD) may offer pepper producers a means to suppress physiological disorders, such as intumescence, while also providing lighting inputs to manipulate crop architecture, flowering, and yield positively. While further research is needed, EOD lighting provides a valuable tool for both scientific and commercial applications.

Although pepper is an important global crop, its cultivation in controlled environments is limited by challenges such as intumescence injury and variable morphological responses to lighting strategies (Cope et al. 2014; Hernandez and Kubota 2017). Therefore, the objective of this experiment was to quantify the effects of low-intensity, monochromatic EOD and PD lighting applications on the morphology and intumescence development of three commercially relevant pepper cultivars ‘California Wonder’, ‘Pot-a-Peño’, and ‘Spicy Jane’. By quantifying changes in leaf morphology, this research aims to identify a cost-effective lighting strategy that incorporates both appropriate spectrum and timing to reduce the incidence of intumescence injury while maintaining desirable growth characteristics for CEA pepper production.

3.3 Materials and Methods

3.3.1 Plant Materials and Greenhouse Environment

Seeds of hot pepper cultivars ‘Pot-a-Peño’ and ‘Spicy Jane’ and bell pepper cultivar ‘California Wonder’ were sown on 11 July 2024 in 128-cell trays using a commercial soilless germination media (Berger BM2 Seed Germination; Berger; Saint-Modeste, Quebec City, CA),

then covered lightly with vermiculite (Sun Gro® Horticultural Vermiculite Premium Grade Medium A-2 Coarse;. Sun Gro®, Agawam, Massachusetts, USA) and placed under fitted plastic domes for two weeks until germination occurred. Seedling trays were irrigated daily using Jack's water-soluble fertilizer (Jack's 13N–0.9P–10.8K Plug LX; J.R. Peters, Inc., Allentown, PA) supplying 75 $mg \cdot L^{-1}$ N. The water-soluble nutrient concentration as well as pH and electrical conductivity (EC) were monitored using a handheld pH/EC meter (Bluelab Combo Meter; Bluelab, Tauranga, New Zealand). Seedlings were transplanted into 15-cm standard plastic pots on 1 Aug 2024 and were irrigated as needed using a water-soluble fertilizer (Jack's 21N–2.2P–16.7K; J.R. Peters, Inc., Allentown, PA) supplying 150 $mg \cdot L^{-1}$ N. All stages of pepper production were conducted in a greenhouse located at the Colorado State University Horticulture Center located in Fort Collins, CO, USA. Greenhouse environmental conditions were controlled and monitored using a VeriStep dynamic control system (Wadsworth Control System, Arvada, CO, USA) with a day/night temperature setpoint of 25/21 °C. Air temperature (°C), relative humidity (RH), photosynthetic photon flux density (PPFD), and carbon dioxide (CO_2) concentration were monitored using aspirated dataloggers (Guardian SM-600; Apogee® Instruments, Logan, Utah, USA), with data reported in Table 4.

3.3.2 Lighting Treatments

Beginning on 22 August 2024, five plants from each cultivar were subjected to one of four 30-minute lighting treatments at an intensity of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for two weeks provided at either EOD (2030 HR) with blue (EOD_B; peak of 447 nm), red (EOD_R; peak of 659 nm), or far-red light (EOD_{FR}; peak of 734 nm), or pre-dawn (PD; 0500 HR) with blue light (PD_B; peak of 447 nm). All lighting treatments were provided by tunable LED fixtures (Elixia; Heliospectra, Gothenburg, Sweden) suspended ~1 m above 1 × 1.5 m bench sections in the greenhouse

surrounded by opaque polyethylene sheeting to prevent light contamination from adjacent treatments. For EOD treatment applications, plants were moved under the appropriate tunable LED fixture at ~2015 HR and returned to a randomized bench location in the greenhouse at ~2100 HR. For the PD treatment applications, plants were moved at ~2100 HR and returned to a randomized bench location in the greenhouse the following morning at ~0700 HR. Light intensity was measured at the beginning of the experiment at canopy height using a handheld spectrometer (MS-100; Apogee® Instruments, Logan, Utah, USA), with average ± SD for each treatment reported in Table 5.

3.3.3 Leaf Imaging

Leaf imaging was conducted prior to treatment initiation (Day 0) and subsequently on Days 7 and 14 at ~0900 HR using a digital camera (Hero12 Black; GoPro, San Mateo, California, USA) affixed with a 15X lens (15X Macro Lens, QKOO, China) attached to a 15 × 23 cm white clipboard via a preconfigured gooseneck clamp (Suptig Jaws Flex Clamp Gooseneck Mount; Suptig, Shenzhen, China) to maintain a fixed position. For each plant, one uniform leaf at the 4th node of the apical meristem was tagged (no less than 2 cm in length) for imaging of the adaxial leaf surface to evaluate changes in leaf area in response to EOD and PD treatments. The tagged leaf was gently secured in a fixed position on the clipboard via a clear sleeve (73 × 122 mm matte sleeve; GameGenic Ingenious Supplies, Essen, Germany). The camera lens was centered upon the middle of the card sleeve and positioned 15 cm above the clipboard surface. Image analysis of photographed leaves was conducted using ImageJ software (ImageJ, US National Institutes of Health, Bethesda, Maryland, USA), the same image processing steps outlined in Chapter 2 and the Appendix were utilized for this experiment. Calculations to determine weekly changes in imaged leaf area expansion (ILAE) were calculated

by deducting Day 0 from Day 7 values ($Day_7 – Day_0 = LAE_7$) or from Day 14 values ($Day_{14} – Day_0 = LAE_{14}$).

3.3.4 Destructive Data Collection

Destructive data collection for all plants occurred on 6 Sep 2024. Data collection included recording plant height measured from the base of the plant at soil level to the highest point of the apical meristem. Tagged leaves were imaged before being removed at the node. The remaining leaves were removed at the node to be counted as well as flowers and developing fruit were removed and counts were recorded. Tagged leaves, stems, and non-tagged leaves of each plant were separated and dried at 70 °C to determine the dry mass of each using an analytical microbalance (Analytical Balance ME54E; Mettler Toledo Ltd, Columbus, OH, USA). Imaged leaf mass area was determined by dividing the individual leaf dry mass by the imaged leaf area (ILMA; $mg \cdot cm^{-2}$). Calculations to determine plant compactness were calculated by dividing stem dry mass by plant height ($g \cdot cm^{-1}$).

3.3.5 Statistical Analysis

The experiment was conducted using a randomized complete block design, with lighting treatments serving as factors and bench as a blocking variable. Replications were combined in the analysis, and effects of lighting treatment were compared by analysis of variance (ANOVA) with R and RStudio [(Version 4.3) (R Core Team 2023)] as well as jamovi [jamovi version 2.6 (The jamovi project, 2025)], with pairwise comparisons conducted using Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

3.4 Results

No incidence of intumescence was observed for any of the cultivars under the lighting treatments provided.Thus, the focus of the results will be on the morphological impacts of EOD and PD lighting strategies.

California Wonder –

No differences were observed for Week 1 (data not shown) or Week 2 ILAE for 'California Wonder' (Fig. 7). Similarly, no difference in plant height or ILMA were observed (Table 6). TLDM, SDM, TDM, and compactness were all greatest for 'California Wonder' grown under PD_B (Table 6). For example, TLDM was 26% and 21% greater under PD_B compared to EOD_{FR} and EOD_B, respectively. Similarly, compactness was 37% and 41% greater under PD_B compared to EOD_{FR} and EOD_B, respectively (Table 6). While no difference in number of flowers was observed for 'California Wonder', plants grown under PD_B produced the greatest number of fruits (Table 6).

Pot-a-Peño –

No differences were observed for Week 1 (data not shown) or Week 2 ILAE for 'Pot-a-Peño' (Fig. 8). Additionally, no difference in TLDM, SDM, TDM, compactness, or ILMA were observed (Table 6). However, height was greatest for Pot-a-Peño' under EOD_R, with an 11% increase compared to PD_B (Table 6). While no difference in the number of flowers was observed between treatments for Pot-a-Peño, plants grown under EOD_B produced the greatest number of fruits (Table 6).

Spicy Jane -

Similar to 'California Wonder' and 'Pot-a-Peño', no differences were observed for Week 1 (data not shown) or Week 2 ILAE for 'Spicy Jane' (Fig. 9). TLDM, SDM, TDM, compactness,

and ILMA were also similar across lighting treatments (Table 6). Plant height for 'Pot-a-Peño was greatest under EOD_R, with a 9% increase compared to PD_B (Table 6). No difference in the number of flowers or fruits between lighting treatments was observed for 'Spicy Jane' (Table 6).

3.5 Discussion

Typical responses to red and far-red light include increased stem elongation and leaf expansion, thinner leaves, and biomass accumulation, as well as playing key roles in flowering regulation, dormancy, and seed germination (Hernandez and Kubota 2017). Benefits to using EOD_{FR} are currently being implemented in CEA; for example, increasing stem elongation in cut flower production of chrysanthemums (*Chrysanthemum indicum*) and hypocotyl elongation of tomato rootstock (Hernandez and Kubota 2017). In terms of leaf expansion, increased percentages of far-red light in particular result in increases for many species, such as tomato and lettuce, by influencing a specific phytochrome-mediated response termed 'shade avoidance' (Casal 2013; Hernandez and Kubota 2017). However, no differences in ILAE were observed for any of the three cultivars in the present study. The lack of differences could be influenced by the duration of this study being limited to two weeks. However, with the primary focus for this experiment being the active development of individual pepper leaves, the shorter duration was appropriate. While results were not significant, ILAE was greatest under EOD_{FR} for 'Spicy Jane', which would be expected based on typical responses to far-red light as a signal for shade avoidance (Fig. 9).

On the contrary, while results were not significant, ILAE was greatest under EOD_B and PD_B for 'California Wonder' and 'Pot-a-Peño', respectively (Fig. 7 and 8). Typical plant responses to blue light include de-etiolation, restriction of cellular expansion, circadian oscillation, and flowering (Cope et al. 2014; Hernandez and Kubota 2017). Blue light can also

stimulate cellular expansion and cell division in the mesophyll tissues resulting in thicker leaves (Dou and Niu 2020). While the increase in ILAE for 'California Wonder' under EOD_B is in contrast to some historical expectations, this response coincides with research conducted by Hernandez and Kubota (2016) and Cope et al. (2014) who found that cucumber (*Cucumis sativus*) 'Cumlaude' and pepper 'California Wonder' experienced increased leaf area under monochromatic blue. Further, experiments conducted by Fan et al. (2013) and Cope et al. (2014) found that cabbage (*Brassica oleracea*) 'Te Ai Wing' and radish (*Raphanus* sativus) 'Cherry Belle' did not display this same leaf area increase under monochromatic blue light, supporting species-specific light quality responses. Cope et al. (2014) further supports these responses, as increased stem elongation was observed under monochromatic blue light for pepper 'California Wonder' compared to broad-spectrum light treatments. ILAE for 'Pot-a-Peño' being highest under PD_B and lowest under EOD_B may suggest timing is critical for blue light applications for this cultivar. With the inconsistent responses observed with ILAE, further research is warranted to clarify how the timing of EOD blue light influences morphology and species-specific responses.

While PD_B suppressed stem elongation in all three cultivars, differences were only significant for 'Pot-a-Peño' and 'Spicy Jane'. Blue light has been shown to suppress plant elongation responses. For example, Claypool and Leith (2021) found that pepper 'Ace' seedlings all displayed reduced height when grown under a spectrum containing blue light compared to seedlings grown without the inclusion of these wavelengths. Conversely, previous research by Cope et al. (2014) found that stem length increased alongside an increasing percentage of blue light (12-92%) for pepper 'California Wonder'. A key difference is the use of EOD lighting in a greenhouse for the present study while Cope et al. (2014) conducted experiments in growth

chambers using sole-source lighting. Sole-source lighting facilitates customization of light quality and intensity allowing for fine-tuned manipulation of photomorphogenesis, but achieving the same outcome with EOD lighting in the greenhouse is much more difficult due to the abundance of natural light during the day masking subtle responses to EOD treatments (if applied prior to the end of the photoperiod) as well as general use of low intensities for EOD applications (Percival and Craver 2023). Similarly, compactness was greatest for plants grown under PD_B, but only for 'California Wonder'. Similar trends for 'California Wonder' were observed regarding biomass accumulation (TLDM, SDM, and TDM) under PD_B. The trends observed regarding PD_B suggest timing is an important factor in controlling photomorphogenesis for these pepper cultivars. While pepper responses to EOD_B and PD_B appear to be species-specific, further research may illustrate applications whereby low-intensity blue light could prove useful as a non-chemical means of controlling elongation growth.

EOD_B and PD_B treatments positively impacted fruit development in 'California Wonder' and 'Pot-a-Peño' when compared to EOD_R and EOD_{FR} treatments, with EOD_{FR} resulting in the lowest number of fruits produced in all three cultivars. Although lighting slightly influenced fruit number, there were no significant differences to flower development across the three cultivars. Due to the limited timeframe and scope of this experiment as well as inconsistent trends across cultivars, further research is needed to fully understand the impacts of EOD and PD light on flower and fruit set for peppers.

3.6 Conclusion

While results from the present study suggest that EOD or PD lighting strategies may assist in promoting desirable photomorphogenic traits, such as increased compactness, responses appear to be species- and cultivar-specific. Additionally, the lack of intumescence development

on any of the cultivars throughout the experiment raises additional questions regarding causative factors for this disorder and whether lighting strategies may be useful in suppression. Therefore, future research should evaluate EOD and PD lighting strategies under active intumescence development to determine possible suppression mechanisms as well as increase the duration of lighting treatments to more fully evaluate possible impacts on pepper morphology and fruit quality.

Table 4. Mean ± SD daily light integral (DLI), temperature, relative humidity, and CO_2 concentration across two experimental replications (benches) during the day (0700-1900 hr) and night (1900-0700 hr) from 22 Aug 2024 - 06 Sept 2024.

	DLI	Temp.	Humidity	CO_2
	$mol \cdot m^{-2} \cdot d^{-1}$	° C	%	$\mu mol \cdot mol^{-1}$
Day	18.9 ± 4.7	24.5 ± 0.5	45.1 ± 4.6	471.5 ± 7.8
Night	–	21.4 ± 1.1	48.4 ± 7.6	500.8 ± 11.6

Table 5. Mean ± SD photon flux density ($\mu mol \cdot m^{-2} \cdot s^{-1}$) of blue (400-500 nm), red (600-700 nm), or far-red (700-800 nm) end-of-day (EOD) or pre-dawn (PD) lighting treatments provided by tunable LED fixtures (Elixia; Heliospectra, Gothenburg, Sweden).

Light Treatment	Blue	Red	Far-red
EOD	25.0 ± 5.1	24.9 ± 3.6	25.4 ± 3.2
PD	25.0 ± 5.1	-	-

Table 6. Least square means for total leaf dry mass (TLDM), stem dry mass (SDM), total dry mass (TDM), height, compactness, imaged leaf mass area (ILMA), number of fruits, and number of flowers for pepper (Capsicum annuum) 'California Wonder', 'Pot-a-Peño', and 'Spicy Jane' plants subjected to one of four 30-minute lighting treatments at an intensity of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for two weeks provided at either EOD (2030 HR) with blue (EOD_B), red (EOD_R), or far-red light (EOD_{FR}), or pre-dawn (PD; 0500 HR) with blue light (PD_B).

Cultivar	Lighting Treatment	TLDM (g)	SDM (g)	TDM (g)	Height (cm)	Compactness ($g \cdot cm^{-1}$)	ILMA ($mg \cdot cm^{-2}$)	Fruit Number	Flower Number
California Wonder	EOD_B	5.3[z] b[y]	2.4 b	7.7 b	45.9	0.051 b	2.88	0.1 b	3.2
	EOD_R	5.7 ab	3.1 ab	8.8 ab	51.0	0.061 ab	2.91	0 b	5.2
	EOD_{FR}	5.1 b	2.6 b	7.7 b	48.1	0.052 b	2.87	0 b	3.9
	PD_B	6.5 a	3.7 a	10.2 a	50.9	0.072 a	2.89	0.8 a	5.1
Pot-a-Peño	EOD_B	6.2	3.9	10.1	50.6 ab	0.077	3.18	5.40 a	11.3
	EOD_R	5.5	3.7	9.3	52.4 a	0.072	2.92	4.70 ab	9.8
	EOD_{FR}	5.7	3.6	9.3	51.1 ab	0.070	3.13	2.70 b	10.8
	PD_B	5.8	3.4	9.2	47.4 b	0.071	3.08	3.30 ab	9.7
Spicy Jane	EOD_B	5.9	4.1	10.0	52.1 ab	0.079	2.87	11.30	20.6
	EOD_R	5.6	4.3	9.9	54.2 a	0.079	2.64	8.30	23.5
	EOD_{FR}	5.7	4.0	9.6	52.2 ab	0.076	2.62	7.30	21.5
	PD_B	5.8	3.8	9.6	49.9 b	0.076	2.82	10.30	19.1

[z]Mean values are based on ten plants across two experimental replications.

[y]Means sharing lettering are not statistically different using Tukey's honestly significant difference at $P \leq 0.05$.

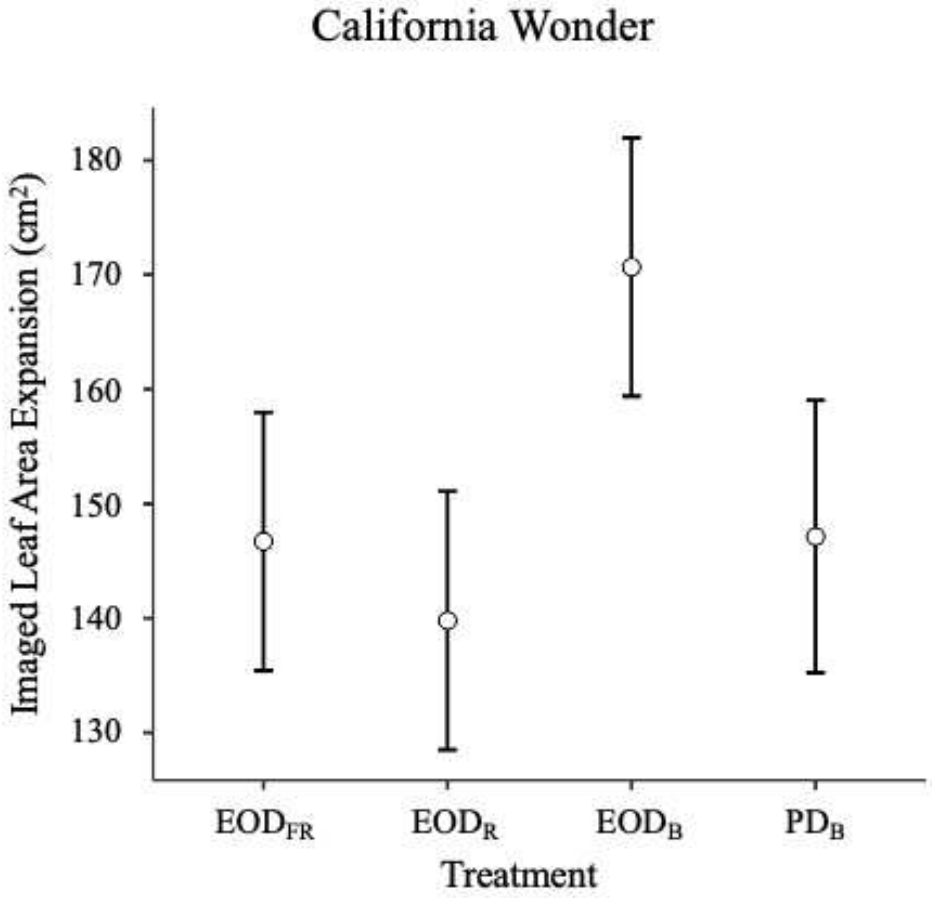

Figure 7. Imaged leaf area expansion (ILAE; cm^2) for pepper (*Capsicum annuum*) 'California Wonder' plants subjected to one of four 30-minute lighting treatments at an intensity of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for two weeks provided at either EOD (2030 HR) with blue (EOD_B), red (EOD_R), or far-red light (EOD_{FR}), or pre-dawn (PD; 0500 HR) with blue light (PD_B). Means with no lettering are not statistically different using Tukey's honestly significant difference at $P \leq 0.05$.

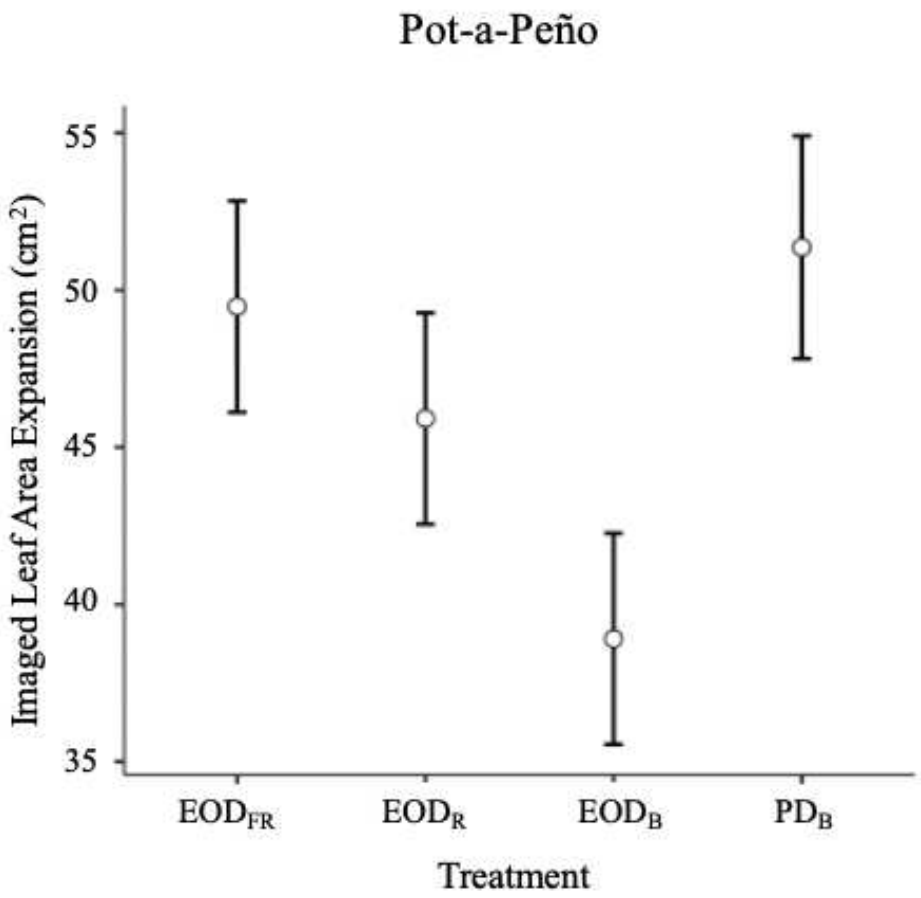

Figure 8. Imaged leaf area expansion (ILAE; cm^2) for pepper (*Capsicum annuum*) 'Pot-a-Peño' plants subjected to one of four 30-minute lighting treatments at an intensity of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for two weeks provided at either EOD (2030 HR) with blue (EOD_B), red (EOD_R), or far-red light (EOD_{FR}), or pre-dawn (PD; 0500 HR) with blue light (PD_B). Means with no lettering are not statistically different using Tukey's honestly significant difference at $P \leq 0.05$.

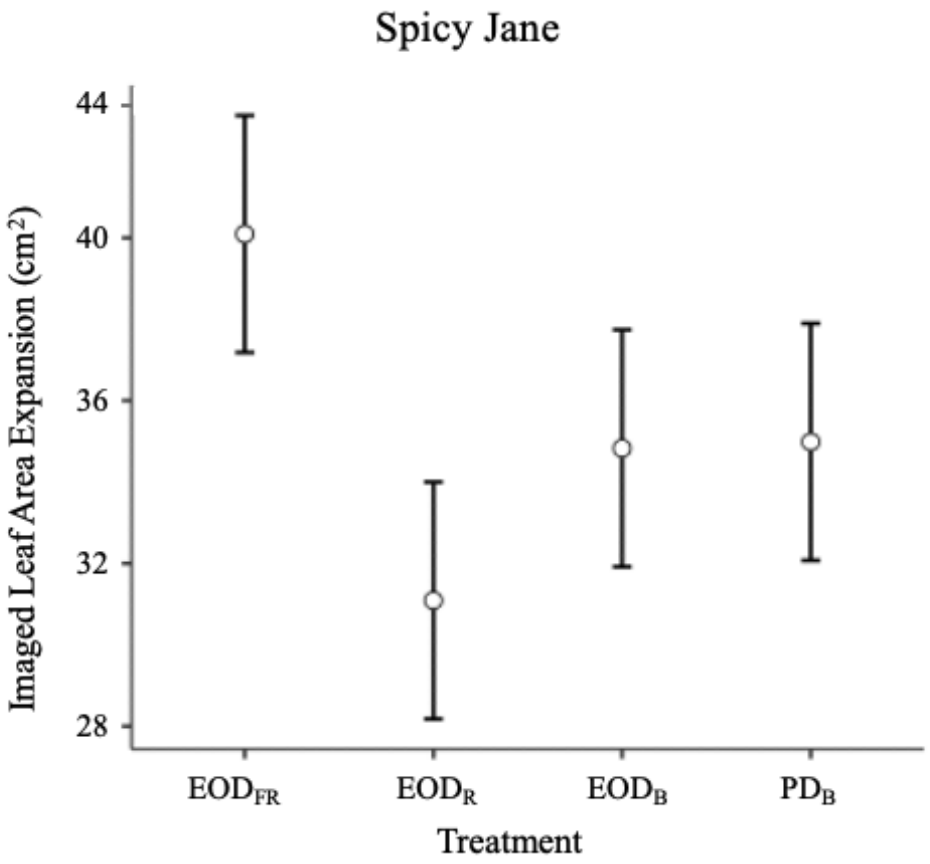

Figure 9. Imaged leaf area expansion (ILAE; cm^2) for pepper (*Capsicum annuum*) 'Spicy Jane' plants subjected to one of four 30-minute lighting treatments at an intensity of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for two weeks provided at either EOD (2030 HR) with blue (EOD_B), red (EOD_R), or far-red light (EOD_{FR}), or pre-dawn (PD; 0500 HR) with blue light (PD_B). Means with no lettering are not statistically different using Tukey's honestly significant difference at $P \leq 0.05$.

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APPENDIX A – IMAGEJ ANALYSIS FOR LEAF EXPANSION

Details of ImageJ Image Analysis for Leaf Expansion

Steps of image capture and processing are as follows:

1. Open ImageJ and import the file/image
    a. Using the “File” tab go to “Open” an image and select the desired photo to be used to set the scale, a secondary window will appear with the selected image
2. Use the “Straight line” tool to draw a line on an object of a known size (e.g., a ruler)
3. Go to “Analyze” then scroll to “Set Scale”
    a. Enter in the distance measured with the “Straight Line” tool
    b. Enter the desired measurement units
    c. Check the box “Global” (this will apply the same scale to all the images until the program is closed), then hit “OK”
4. Isolating the tagged leaf
    a. Choose “File” > “Open” and choose the photo for analysis
    b. Use the “Rectangle” tab to capture the leaf to be analyzed. Select the smallest rectangle around the leaf
    c. Choose “Image” > “Crop”, this will isolate the leaf to be analyzed
5. Change the image to a binary image (black and white)
    a. Choose “Process” > “Binary” > “Make Binary”
    b. Use the “Paintbrush Tool” to remove large clusters of pixels that are not part of the leaves.

c. Go to “Process” > “Noise” > “Remove Outliers” or Despeckle” to remove small specks that are not part of the leaves. This step can be skipped when the image has a clean background.

6. Using the “Wand (tracing) Tool” select the area of the leaf to be measured. The selected area will be outlined with a yellow line and ensure only the leaf is captured.
    a. Go to “Analyze” > “Measure” and another window labeled “Results” will open with the area and perimeter measurements present in the units previously set when setting the scale
7. Next choose the “Straight Line” tool and select two points, one at the end of the petiole and one at the tip of the leaf, to capture the length of the leaf
    a. The tool will draw a straight line between the two points (the petiole and the tip of the leaf)
    b. Choose “Analyze” > “Measure”, the measurement will populate in the “Results” window
8. Next choose the “Straight Line” tool and measure the width of the leaf by choosing the widest points of the leaf margins
    a. The tool will draw a straight line between the two points (the widest point of the leaf margin)
    b. Choose “Analyze” > “Measure”, the measurement will populate in the “Results” window
9. Once the desired measurements are captured for that photo, document the results for leaf and save the image as a .TIFF file

APPENDIX B – DROUGHT STRESS INITIATES INTUMESCENCE DEVELOPMENT FOR THREE CULTIVARS OF *CAPSICUM ANNUUM*

B.1 Introduction

Pepper (*Capsicum annuum*) production has recently expanded to controlled environments as a means of producing high quality edible and ornamental peppers year-round. However, balancing of production inputs, such as water and fertilizer, is critical to ensure quality and maximize yield. For many cultivars, production guidelines can be sourced directly from the breeding company and are typically published annually alongside new cultivar introductions [e.g., PanAmerican 2023 Seed Product Information Guide (PanAmerican 2023)]. These guidelines detail the pertinent information needed to successfully produce a crop from propagation to finishing. For example, detailed watering guidelines outline stage-specific recommendations for pepper, assigning a level and a visual/tactile matrix to describe the desired moisture content (PanAmerican 2023). Using this guide, substrate moisture content ranges from Level 1: Dry (substrate color – very light brown or gray; substrate feel when squeezed – no moisture is detected in substrate; substrate structure – dusty and freely scatters when blown) to Level 5: Saturated (substrate color – brown-black, glistening with water; substrate feel when squeezed – water runs freely out of substrate; substrate structure – substrate has a semi-liquid consistency) (PanAmerican 2023). For peppers, this production guide recommends maintaining moisture content between Levels 4 and 2 during the root establishment phase, reducing to between Levels 3 and 1 up to flowering, and finally maintaining between Levels 4 and 2 throughout flowering (PanAmerican 2022). Thus, it is common for pepper production to incorporate periods of limited water availability, often imposing drought stress.

Previous research has found water status to play a critical role in photosynthetic rates, flower and fruit development, as well as physiological disorders such as intumescence (Lang and Tibbits 1983; Mahmood et al. 2021; Miyama and Yasui 2021). Pepper can be susceptible both to water deficit and excess, primarily during flowering, with impacts to yield being of greatest concern for producers. In a study conducted by Ou et al. (2011), six pepper species (*Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum chinese*, *Capsicum frutescens* L., *Capsicum pubescens*, and wild type *Capsicum baccatum*) were subjected to waterlogging events lasting between one to three days which resulted in decreased photosynthetic rate and reduced recovery time of photosynthetic rate and stomatal conductance to normal levels once drainage of excessive water occurred and regular irrigation resumed for all six species. In terms of water limitations, pepper cultivars 'Pusajuala', 'Ghotki', 'Green Wonder', and 'PPE-311' exposed to drought stress (35% field capacity) during flowering decreased reproductive growth and overall pungency compared to a control (65% field capacity), facilitating the development of appropriate irrigation thresholds for pepper (Mahmood et al. 2021).

Previous research has identified a probable link between intumescence development and water status (Lang and Tibbits 1983; Miyama and Yasui 2021). Intumescence is a physiological disorder characterized by hypertrophy and hyperplasia of epidermal and palisade parenchyma cells on leaves, petioles, and stems of multiple plant species when produced in a controlled environment (Morrow and Tibbitts, 1988; Wetzstein and Frett, 1984). Excess water has been observed to cause intumescence development on tomato (*Solanum lycopersicum*) (Atkinson, 1893) and potato (*Solanum tuberosum*) (Douglas, 1907) as well as other specialty crops. Rapid changes in water status have also been linked to this disorder; for example, Miyama and Yasui (2021) reported tomato 'Momotaro Peace', a cultivar shown to be susceptible to drought,

experienced the highest severity of intumescence injury when subjected to a reduced soil moisture content (~64%) followed by irrigation to field capacity compared to other cultivars in the study. These authors proposed that sudden increases in soil moisture content during periods when xylem pressure potential is low (indicative of drought) may result in water absorption temporarily exceeding transpiration rate, likely leading to cellular rupture and the characteristic injuries associated with intumescence (Miyama and Yasui 2021).

For crops that are susceptible to intumescence injury, effective watering is a potentially cost-effective means to control intumescence, which may otherwise limit adaptability for controlled-environment production systems. However, current guidelines for production which impose drought stress may facilitate the development of this disorder. We hypothesize that the combination of drought stress followed by a thorough irrigation event will result in intumescence development for susceptible pepper cultivars, thus providing insight on managing a potential environment stimulus for the development of this physiological disorder. Results may lead to a cost-effective strategy for alleviating intumescence injury on susceptible pepper cultivars produced in a controlled environment via appropriate irrigation strategies as well as further the scientific understanding of causative factors and mechanisms pertaining to this disorder.

B.2 Materials and Methods

B.2.1 Plant Materials and Greenhouse Environment

Pepper cultivars ‘California Wonder’, ‘Pot-a-Peño’, and ‘Spicy Jane’, were evaluated during the winter of 2025. On 16 Jan. 2025, seeds of each cultivar were single sown into 128-cell trays using commercial soilless germination media (Berger BM2 Seed Germination; Berger, Saint-Modeste, Quebec City, CA) then covered lightly with vermiculite (Sun Gro® Horticultural Vermiculite Premium Grade Medium A-2 Coarse, Sun Gro®, Agawam, MA, USA). Trays were

irrigated as needed using Jack's water-soluble fertilizer (Jack's 13N–0.9P–10.8K Plug LX; J.R. Peters, Inc., Allentown, PA, USA) providing 75 $mg \cdot L^{-1}$ nitrogen. Nutrient solution pH and electrical conductivity (EC) were monitored using a handheld meter (Bluelab Combo Meter; Bluelab, Tauranga, New Zealand). Seedling trays were germinated under fitted plastic domes for three weeks. Upon germination humidity domes were removed. Seedlings were transplanted into 15-cm standard plastic pots on 6 Feb 2025. Transplants were irrigated every other day using a water-soluble fertilizer (Jack's 21N–2.2P–16.7K; J.R. Peters, Inc., Allentown, PA, USA) providing 150 $mg \cdot L^{-1}$ nitrogen. Seedling trays and transplants were grown and maintained in a greenhouse located at the Colorado State University Horticulture Center in Fort Collins, CO, USA. Greenhouse environmental conditions were controlled using a VeriStep dynamic control system (Wadsworth Control System, Arvada, CO, USA) with an air temperature set point of 22/20 °C (day/night) (Table 1). Air temperature (°C), relative humidity (RH), photosynthetic photon flux density (PPFD), and carbon dioxide (CO_2) concentration were monitored and logged every 10 min using an aspirated datalogger (Guardian SM-600; Apogee® Instruments, Logan, UT, USA), with data compiled in Table 7.

B.2.2 Irrigation Treatments and Data Collection

On 24 Feb 2025, all plants, regardless of treatment, were watered and allowed to drain to container capacity for experiment initiation. Three plants per cultivar were designated to a "well-watered" treatment serving as the control and three plants per cultivar were designated to a "water stress" treatment. Plants in the control were watered as needed to maintain 70% of container capacity, while plants in the water stress treatment were allowed to reach 40% of container capacity prior to an irrigation event. Percentages of container capacity were calculated using a saturation value of 800g measuring the whole plant. Guidance as to appropriate irrigation

regimes for pepper were sourced from Pan American Seed (PanAmerican 2022) for the development of treatment thresholds. Plants were weighed daily at 0900 and 1700 HR using a digital balance (Ohaus Adventurer™ Pro Precision AV4101C, Parsippany, NJ, USA) to determine the timing of irrigation events. Once the water stress treatment reached the pre-determined threshold all plants were watered to field capacity signaling the watering event.

All plants were evaluated twice daily (0900 and 1700 HR) for intumescence development over the course of the 12-d experiment. Special attention was given 2-d prior, and 5-d post the irrigation event for the water stress treatment to capture the timing of intumescence development. Leaves showing signs of intumescence were tagged, indicating the time and date of the observation. Additionally, node number from the apical meristem for tagged leaves was recorded and leaf images collected. Leaf imaging was conducted using a digital camera (Hero12 Black, GoPro, San Mateo, California, USA), affixed with a 15X lens (15X Macro Lens, QKOO, China) connected to a 15 cm × 23 cm white clipboard via a gooseneck clamp (Suptig Jaws Flex Clamp Gooseneck Mount, Suptig, Shenzhen, China) to maintain a fixed static position.

Destructive data was collected on 8 March 2025 and included plant height (measured in cm from the base of the stem to the apical meristem), and number of leaves with/without intumescence injury. The stem of each plant was cut at soil level, and leaves were removed at the node to be counted. All vegetative plant material (tagged leaves, stems, and non-tagged leaves) of each plant were separated and dried at 70 °C for 3 d to determine the dry mass using an analytical microbalance (Analytical Balance ME54E; Mettler-Toledo, LLC, Columbus, OH, USA).

B.2.3 Statistical Analysis

Two replicates of three plants per cultivar per treatment were conducted simultaneously, blocked by bench. Within each bench, plant positions were rotated daily to ensure uniformity. The experiment was a randomized complete block design with irrigation treatment as a factor and bench as a blocking variable. Data analysis for each replication was combined and represented in the data reported. The effects of irrigation treatment were compared by analysis of variance (ANOVA) using jamovi [jamovi version 2.6 (The jamovi project, 2025)] with Tukey's honestly significant difference (HSD) test at $P \leq 0.05$.

B.3 Results and Discussion

All plants in the water stress treatment were allowed to dry down to between 320 – 350g followed by a one-time watering event. The water stress treatment reached the pre-determined threshold on 02 Mar 2025 at the 0900 HR interval, and all plants were watered to container capacity on 02 Mar 2025 at the 1700 HR interval signaling the watering event. The following morning at the 0900 HR observation, intumescence development was observed on 75% of 'Pot-a-Peño' and 'Spicy Jane' plants in the water stress treatment and 100% of 'Pot-a-Peño' and 'Spicy Jane' plants the following day (Fig. 11 and 12), there was no intumescence development observed before the watering event on any plants. Intumescence development was observed on 16% and 43% of the total leaves for 'Spicy Jane' and 'Pot-a-Peño', respectively, over the course of the 12 d experiment (data not shown). For 'California Wonder', intumescence development occurred 3 d post the watering event for the water stress treatment and only impacted 10% of the plants (Fig. 10). For 'California Wonder' plants affected, intumescence development was observed on 25% of the total leaves present over the course of the 12 d experiment (data not shown). Intumescence development occurred between the 4^{th} and 9^{th} node from the apical

meristem for ‘Pot-a-Peño’ and ‘Spicy Jane’ and on the 4th and 5th node for ‘California Wonder’ (data not shown). For all cultivars, plants in the control treatment exhibited no signs of intumescence injury over the course of the 12 d experiment. Based on the data from the present experiment, it is likely that intumescence development for pepper is dependent on cultivar-specific drought tolerance. For example, Miyama and Yasui (2021) reported that tomato ‘Momotaro Peace’ exhibited a significant decrease in xylem pressure potential (i.e., lower drought tolerance) under dry soil moisture conditions and, as a result, developed severe intumescence injury following sudden increases in soil moisture when compared to the more drought-tolerant cultivars ‘Reiyo’ and ‘CF House Momotaro’. While the exact mechanism for intumescence development is still unclear, water stress has been shown to induce hormonal responses regulating stomatal closure to prevent water loss which can lead to the buildup of reactive oxygen species (ROS) damaging plant tissues (Mahmood et al. 2021). This additional oxidative stress may contribute to intumescence injury as characterized by abnormal cellular proliferation and cell wall changes (Mahmood et al. 2021). The presence of intumescence injury can also affect leaf water content. Suzuki et al. (2020) investigated intumescence development on tomato ‘Rinka 409’ under normal [natural ultraviolet (UV) in a glass greenhouse] or low (supplied via fluorescent lamp) UV radiation conditions and reported that plants grown under low UV radiation developed intumescence and reduced water content (86.2%), while those under normal UV radiation showed no signs of the disorder and higher water content (90.88%). Importantly, tomato plants with intumescence injury exhibited a significant reduction in leaf water content compared to plants without injury. Their results suggest leaf water status can be greatly impacted by intumescence development and, as a result, affect the plants’ ability to fully recover from water stress (Suzuki et al. 2020).

While results from the present study show that drought stress may be a causative factor in the development of intumescence for susceptible pepper cultivars, further research is needed to clarify the timing (temporal and developmental) in which intumescence development occurs as well as determining water stress thresholds. These finding could further support pepper production in controlled environments by providing cost-effective irrigation recommendations that promote yield and quality while also preventing intumescence development.

Table 7. Mean ± SD daily light integral (DLI), temperature, relative humidity, and CO_2 concentration across two experimental replications (benches) during the day (0700-1900 HR) and night (1900-0700 HR) from 06 Feb 2025 - 07 Mar 2025.

	DLI	Temperature	Humidity	CO_2
	$mol \cdot m^{-2} \cdot d^{-1}$	° C	%	$\mu mol \cdot mol^{-1}$
Day	13.5 ± 3.5	21.8 ± 1.4	19.1 ± 5.3	596.6 ± 42.9
Night	–	20.9 ± 0.8	22.7 ± 4.3	647.8 ± 51.9

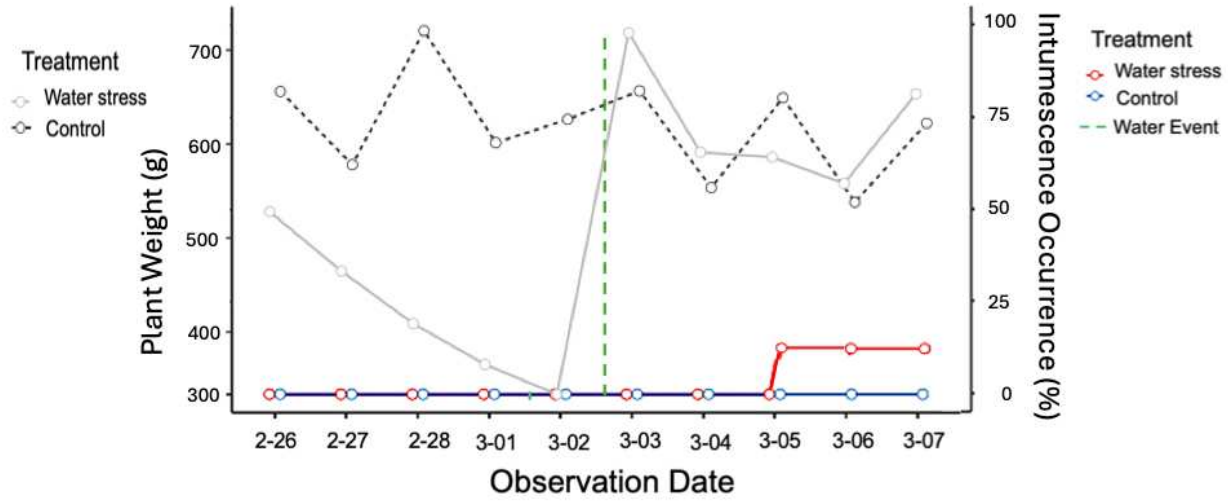

Figure 10. **Left axis:** Plant weight (g) showing the average of six plants per day for pepper (*Capsicum annuum*) 'California Wonder' after either being watered as needed to maintain 70% of container capacity (Control; dashed dark gray line) or allowed to reach 40% of container capacity (Water stress; solid light gray line) prior to an irrigation event (green line). **Right axis:** Percentage of plants showing symptoms of intumescence development for the Control (blue line) or Water stress treatment (red line). All plants were evaluated twice daily (0900 and 1700 HR) for intumescence development over the course of the 12 d experiment.

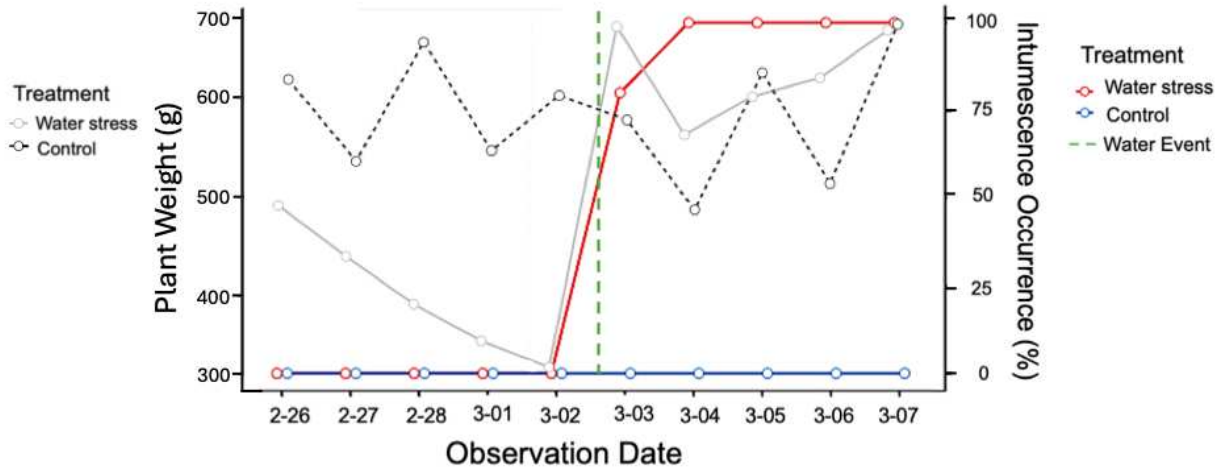

Figure 11. **Left axis:** Plant weight (g) showing the average of six plants per day for pepper (*Capsicum annuum*) 'Pot-a-Peño' after either being watered as needed to maintain 70% of container capacity (Control; dashed dark gray line) or allowed to reach 40% of container capacity (Water stress; solid light gray line) prior to an irrigation event (green line). **Right axis:** Percentage of plants showing symptoms of intumescence development for the Control (blue line) or Water stress treatment (red line). All plants were evaluated twice daily (0900 and 1700 HR) for intumescence development over the course of the 12 d experiment.

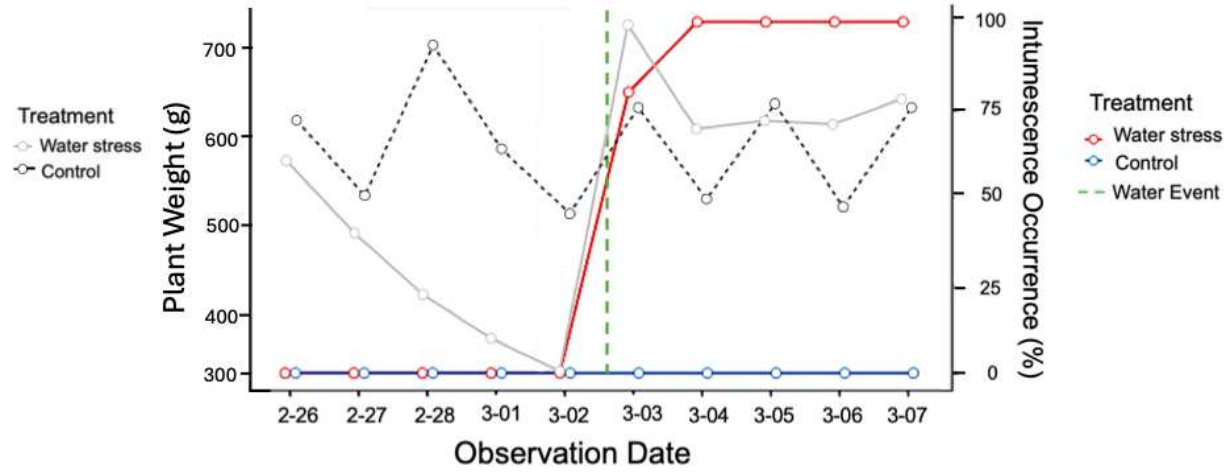

Figure 12. **Left axis:** Plant weight (g) showing the average of six plants per day for pepper (*Capsicum annuum*) 'Spicy Jane' after either being watered as needed to maintain 70% of container capacity (Control; dashed dark gray line) or allowed to reach 40% of container capacity (Water stress; solid light gray line) prior to an irrigation event (green line). **Right axis:** Percentage of plants showing symptoms of intumescence development for the Control (blue line) or Water stress treatment (red line). All plants were evaluated twice daily (0900 and 1700 HR) for intumescence development over the course of the 12 d experiment.

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