The influence of overwatering, underwatering, and waterlogging on the growth of kale (Brassica oleracea var. acephala)

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The influence of overwatering, underwatering, and waterlogging on the growth of kale (*Brassica oleracea* var. *acephala*)

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The Intergovernmental Panel on Climate Change has predicted that there will be increases in precipitation and heat-induced drought events globally. Information on kale response to waterlogging is minimal. The purpose of this project was to identify the response of kale to three treatments of water stress: underwatering, overwatering, and waterlogging. Plant pigments analyzed displayed a varied response to underwatering and overwatering, with concentrations changing with maturity but with reductions and no changes, respectively, at full harvest maturity. Glucosinolate concentrations were also influenced by maturity and increased under waterlogging and underwatering but no differences with overwatering. Overall, water stress to any degree is not ideal for kale during production, but despite yield reductions, underwatering led to increases among phytonutrients, but increases are apparent and do not equate to increased absorption when consumed.
DEDICATION

I would like to dedicate this to three people in my life, whom without them, I would not be here and do it. First, to Pamela Matney, who treated me as a son to her and welcomed me into her household and family when I needed motherly guidance and reprimands ripened with the perfect amount of love. Whether I am six feet away or 6000 miles away in another country that you do not know I was in until you see pictures, I know you’ll always be willing to listen to my challenges and offer me the motherly advice I need to continue moving forward.

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CHAPTER I
LITERATURE REVIEW

1.1 Background

Water has long been recognized for its importance and vital role in human civilizations and agriculture to meet basic needs. Many factors influence accessibility to freshwater sources, but a global analysis of population distance to freshwater by Kummu et al. (2011) identified that more than 50% of the population lives less than 3 kilometers from surface freshwater. Before industrialization, humans needed proximity to water sources for agriculture, drinking, navigation, and accessibility until efficient and advanced groundwater pumping during the 20th century influenced accessibility and population growth (Fang and Jawitz 2019). This has led to strains on water sources due to high levels of urbanization and agricultural water use, while climactic changes, industrialization, and contamination of water sources have also vastly limited the available water in some areas (Fang and Jawitz 2019, Pradeep 2009).

The development of infrastructures has allowed the extraction and deliverance of water further away from sources in more efficient and effective manners but has become overly expensive or infeasible, having physical, economic, and ecological limitations (Fang and Jawitz 2019). In some cases, the overuse of water sources has led to the depletion of water sources, like that of the Central Valley in California and the High Plains Aquifers in the central United States, limiting available water for human use and agriculture (Fang and Jawitz 2019). The depletion of groundwater is not an isolated event and impacts freshwater availability and even results in
irreversible damages like contamination, lack of agriculture, and lack of drinking water (IPCC 2019). Effective and efficient management of water sources is necessary to ensure adequate and safe water for both human consumption and agricultural production. Depletion of groundwater is not the only cause of water-related problems in modern agriculture.

The Intergovernmental Panel on Climate Change (IPCC) has identified major shifts in the Earth’s climate leading to two of many major trends occurring: 1) a greater frequency and duration of heat events leading to drought, and 2) increases in heavy precipitation events creating waterlogging (IPCC 2019). Both trends yielded opposite results displaying the complex nature of regional variability like that identified by Kundzewicz et al. (2014). Kundzewicz et al. (2014) analyzed flooding on both the global and regional scales displaying that increases in heavy precipitation events are likely, but regional and sub-regional variations exist. In the United States, when comparing the first half to the second half of the 20th-century, it was observed that a wide variation in annual precipitation and seasonal precipitation existed (Easterling et al. 2017). Easterling et al.’s (2017) findings over the 20th-century comparison displayed that an increase in annual precipitation existed for most of the continental United States, with some decreases in the Southeast, and widespread declines in the Western U.S.

Looking at a specific sub-region within Mississippi, precipitation data gathered from a weather station located at the North Mississippi Research and Extension Center in Verona, MS identified that over the past 20 years (2001 to 2021), there was a gradual increasing trend in precipitation (Figure 1.1) (Delta 2022). When compiling the data further and looking at the number of days with precipitation (Figure 1.2) and the mean precipitation per event (Figure 1.3), it can be identified that the mean amount of precipitation during each event did not change much over the 20 years of data (Delta 2022). Only the number of days with precipitation increased
slightly (Delta 2022). While this data does not completely align with the IPCC’s predictions, it
does align with Easterling et al.’s (2017) and Kundzewicz et al.’s (2014) observations.

Another contributor to weather variations is due to El Niño and La Niña. Both events are
caused by the movement of bodies of water in the Pacific Ocean, influencing air movement
across the United States (NOAA 2021). Both influence weather conditions in the winter months
in the Southern United States, with El Niño leading to wetter conditions and La Niña causing
warmer/dryer conditions. Comparing the Oceanic Niño Index (ONI) from the 1990s to the
present times, it is observed there are major El Niño and La Niña trends, and when comparing
the trends of the past 20 years to the total precipitation received at the North Mississippi
Research and Extension Center (Figure 1.1), the annual variations coincide with the ONI (Figure
1.4). Despite the complexity of analyzing weather trends, overall precipitation is increasing
along with the number of days with precipitation and is a cause for concern for farmers. Despite
increasing precipitation trends, precipitation is only one of many factors that are influencing crop
production.

The anthropogenic emission of greenhouse gases while increasing global atmospheric
CO₂ concentrations is also increasing global temperatures and other deleterious effects like the
degradation of the ozone layer (Kakani et al. 2003; Prasad et al. 2017). It is well known that as
temperature increases, plants demand greater water as temperature alongside light helps to drive
transpiration, evaporation, and evapotranspiration. However, climate change prediction models
that only characterize one factor without comprising other factors are quite variable as displayed
by Lobell and Burke (2008). Lobell and Burke (2008) identify that temperature uncertainties can
vastly change model predictions. Yet, it is essential to consider this, among other factors, as not
only can the temperature impact crops be tested, but its testing can also be used to better model short-term changes.

If we look solely at the weather data collected from Verona, specifically the average precipitation per month over the past 20 years (Figure 1.4), we can observe little variation over the entire year. Mathematically, the average monthly rainfall is 109 ± 17 mm (4.3 ± 0.7 inches), which is not a large variability per month. However, crops’ water use efficiency dramatically changes throughout the year and is influenced by temperature, light, carbon dioxide concentration, hormone signals, stomata density, and water availability (Chaerle et al. 2005; Hatfield and Dold 2019). As temperature and light increase, so does the water demand of the plant to combat the increases in transpiration necessary to maintain the water balance in the plant (Hatfield and Prueger 2015). If the average rainfall per month is consistent throughout the year, there may be sufficient rainfall to supply crops in late spring to early summer and once again in fall. However, when summer temperatures occur, deficits will begin to occur as crop demand surpasses rainfed availability, and as winter temperatures occur, an overabundance of rain will occur, leading to waterlogging. In Mississippi, most agronomical and horticultural crops are not grown during the winter due to low temperatures, but these waterlogging events are not limited to only winter but also occur in late fall and early spring. During the summer, producers supplement limited rainfall using various irrigation methods to ensure sufficient water availability for crops (Kebede et al. 2014).

Irrigation of crops has been occurring for hundreds of years, and some of the earliest documented research in the United States occurred during the 1860s, with the Hatch Act establishing research stations in the West focused on assessing water requirements for crops (Jensen 1968). Water requirements were vastly studied over the next 50+ years. However, it was
apparent time plants require no absolute value, but more relative values as numerous other environmental factors impacted water needs (Briggs and Shantz 1913; Jensen 1968). Over many seasons, Briggs and Shantz studied various factors and their influence on the water use of plants, identifying the most influential factor being solar radiation (Jensen 1968). With a relative and non-absolute requirement of water, it is considered the most limiting abiotic factor impacting growth, development, and plant productivity (McElrone et al. 2013).

McElrone et al. (2013) state that less than five percent of all water absorbed by a plant’s roots is retained by the plant and used for growth and development, a value that was also observed by Briggs and Shantz (1913) and by other researchers during early studies (Jensen 1968). Water is vital for plants due to the role it plays in nearly every plant process. Water is used during photosynthesis to produce vital sugars while also ensuring the hydration of plant tissues while stomata are open to collect CO₂, to carry water-soluble nutrients from the soil into plants, aiding in the dispersal of nutrients and sugars throughout the plant, acting as a solution for metabolic processes, and many other processes (McElrone et al. 2013; Araus 2020). Plant’s sessile nature makes it very important for plants to adapt and overcome adverse weather conditions to survive, and while the IPCC predicts the two extremes –flooding and drought – to occur, it is important to understand that overwatering and underwatering of plants can happen to any degree in between the extrema. However, nearly all literature and studies on plants subjected to water stress focus on the extreme sides, with a vast lack of literature on slight variations.

1.2 Water Stress

An overabundance of water leads to waterlogging and flooding conditions. While these two terms are used interchangeably, they are not the same. Kaur et al. (2020) identify clearly that flooding refers to the complete submergence of all or part of the plant, while waterlogging (or
soil waterlogging) refers to the saturation of the soil pores with water. Both variations are detrimental to plant growth leading to anoxic conditions and plant death, but plant adaptation does exist for waterlogging and flooding (Pan et al. 2021). One of the most notable examples of plant adaptation and survival to flooding is in rice (*Oryza sativa*). While not all rice acts the same, in deep water cultivars, the expression of gene families has been intensively studied as the activation of two genes (SNORKEL1 and SNORKEL2) often leads to rapid stem-elongation (Sasidharan and Mustroph 2011). The rapid stem elongation and changes in internal structure allow the stems to act as “snorkels” allowing oxygen to reach lower parts of the plants overcoming the hypoxic conditions plants are subjected to (Sasidharan and Mustroph 2011, Fukao et al. 2019, Pan et al. 2021). Hypoxic conditions also play a role in regulating ethylene response factors being identified in *Arabidopsis thaliana* (Licausi et al. 2010), which was also identified to aid tolerance in *Zea mays* (He et al. 1996), and *Rumex palustris* (Cox et al. 2011), among other plants.

Like flooding, plants subjected to waterlogging stress must overcome the hypoxic conditions exposed to their root zones. Some plants like *Spartina alterniflora*, a salt marsh grass, have adapted and have aerenchyma tissues throughout their entire tissue enabling oxygen movement from their leaves into the roots and allowing oxygenation of the rhizosphere (Teal and Kanwisher 1966). Adaptation of plants to waterlogging has long been explored and observed across many species including Sitka spruce and lodgepole pine (Coutts 1978), broad beans (Evans and Ebert 1960), rice (Green and Etherington 1977), mustards (Greenwood and Goodman 1971), and wheat (Malik et al. 2001) among other plants. Plant response to both flooding and waterlogging is to oxygenate impacted tissues to overcome hypoxia, with the same fate for both if plants are unable to adapt: death. The hypoxic conditions plants are faced with lead to
widespread impacts in plants including decreased photosystem impacts and reductions, cellular metabolic damage to proteins, lipids, pigments, DNA, and photosystem II, reductions across morphological parameters, and increased transcription (Ashraf 2012, Casierra-Posada and Cutler 2017, and Lee et al. 2014).

Unfortunately, both flooding and waterlogging are widespread events, with flooding affecting 17 million km\(^2\) of land annually and waterlogging estimated to affect 10 to 12\% of all agricultural areas (Voesenek and Sasidharan 2013, Shabala 2011). While control of flooding conditions requires large-scale operations and may not be feasible, the impact of waterlogging has been lessened greatly by using genetics, engineering, and agronomic changes (Manik et al. 2019). While genetic changes rely on breeding in adaptive mechanisms, engineering and agronomical changes can both coincide with controlling water movement through the soils through reductions in soil compaction, improving drainage with tiling, utilizing raised bed production allowing water to drain into furrows away from plants, among many other techniques (Singh 2018, Hussain et al. 2018, Manik et al. 2019).

Drought is estimated to impact 40\% of the world’s population as estimated by the World Health Organization (WHO 2022). Based on the predictions by the IPCC, the impact of drought is only estimated to worsen before human intervention can begin mitigating the damages (IPCC 2019). While agriculture production in areas facing drought is unlikely to be productive due to the constraints and need for water by plants to survive and produce food, production in water-deficient areas is still possible. Irrigation knowledge and practices have improved over time, and the use of micro-irrigation is one method that can be used to meet plant's needs; however, maladaptation of systems can cause salinization of soil and can quickly deplete available water (IPCC 2019). Plant adaptation and response to drought stress have been researched, and four
basic response mechanisms have been identified: drought avoidance, drought escape, drought
tolerance, and drought recovery (Ilyas et al. 2021). While each of the four drought response
mechanisms has been documented and described well, they are namely focused on overcoming
varying aspects of the damage caused by drought stress. A decrease in water availability leads to
stomatal closure, decreasing CO$_2$ intake and nutrient uptake, which limits numerous
physiological processes and reduces energy production (Caudle and Maricle 2012, Joshi et al.

As aforementioned, the varying degrees of drought response mechanisms are targeted at
overcoming the challenges imposed by water stress. The first stage of response is stomatal
closure which occurs to decrease transpiration and preserve the water that is inside the plant
(Ilyas et al. 2021). However, the closing of stomata interrupts the movement water into and
throughout the plant and the movement of the essential biochemicals and nutrients that rely on
water transpiration to aid in distribution throughout the plant (De Micco and Aronne 2012). The
closure of the stomata also directly impacts photosynthetic within the plant (Medrano et al. 2002;
Martin et al. 2014). Plant’s adaptation to drought stress regarding the stomata and structure has
been shown to range from having decreased stomata, increasing cuticle thickness, folding of the
lamina, increased trichome density, formation of sunken stomata, or stomatal crypts (De Micco

Under limited CO$_2$ concentrations, continued photosynthesis often leads reactive oxygen
species (ROS) formation due to the accumulation of reduced electrons in the photosynthetic
apparatus (Basu et al. 2016). Numerous mechanisms have been identified to help the damage
caused by drought on the photosynthesis system, including dissipating thermal energy, utilizing
the xanthophyll cycle to absorb excess light, scavenging ROS, and the use of the water-water
cycle to absorb photons or sequester electrons (Demmig-Adams and Adams 1996; Niyogi 1999; Demmig-Adams and Adams 2006; Ilyas et al. 2021).

Another strategy of plants to overcome drought stress is increased root density and length. However, Vadez (2014) identified that having “deep and profuse rooting” is necessary but not always advantageous in increasing drought tolerance as there is not a direct correlation between the density and water absorption capabilities among the roots. Increased root length and density is a response of *Helichrysum petiolare* to drought stress (Caser et al. 2016). Yet, Vadez (2014) does identify that specific traits of roots, namely xylem size and abundance, cortical aerenchyma, and root cell numbers, help to alter the hydraulic properties that can confer tolerance.

Surprisingly, plant response to both extremes of water stress results in a similar proliferation of events despite having different starting mechanisms. Drought-faced plants encounter decreases in cellular turgidity, which reduces carbon dioxide intake and photosynthesis, increasing the generation of reactive oxygen species and photorespiration, disrupting enzyme processes, and enabling the accumulation of solutes that lead to toxicity (Joshi et al. 2016, Ilyas et al. 2021). The anoxic conditions faced by plant roots during waterlogging cause closure of stomata, which leads to internal water deficits, reducing carbon dioxide intake and photosynthesis, and generating reactive oxygen species that lead to cellular damage like that observed in drought-impacted plants (Caudle and Maricle 2012, Parent et al. 2008).

### 1.3 Kale Background

Kale (*Brassica oleracea* var. *acephala*) is a leafy green vegetable, with some cultivars for use as ornamental production grown extensively in containers (McAvoy 1994). The United States Department of Agriculture’s National Agricultural Statistics Service reported that 15,325
acres (6,202 hectares) grew kale in 2017, which is up from 3,760 acres (1,524 hectares) in 2000 (USDA, 2017). The rise in kale production and consumption is in share to it containing high mineral nutrients (Ca, Mg, P, K, and Fe), which are necessary for the human diet (Mills and Jones, 1996; Kopsell et al. 2005). Kale also contains pronounced levels of vitamins A, B-6, C, and K (Pathirana et al., 2017). Kale also contains significant secondary metabolites like carotenoids and glucosinolates which are nutritionally important in the diet (Coolong et al. 2013). Apart from kale’s significant concentration of mineral nutrients and secondary metabolites, kale contains substantial concentrations of beneficial polyphenolics and is a low-calorie food (Björkman et al. 2011; Red et al. 2020).

Carotenoids are yellow-orange pigments produced by many plants, algae, fungi, birds, crustaceans, insects, and among others, which aid in conferring oxidative stress resistance, and, when consumed by animals like humans, can also confer similar antioxidative and anti-inflammatory responses (Kopsell et al. 2007; Langi et al. 2018). Apart from aiding in oxidative-stress management, the oxidized cleavage products of carotenoids, known as apo-carotenoids, are essential in the production of retinol, also known as Vitamin A, that are essential in eyes and help to reduce age-related macular degeneration (Nisar et al., 2015; Mordi et al., 2020). Of all the vegetable crops, studies have shown that kale contains the greatest concentration of carotenoids, namely lutein and β-carotene (Holden et al. 1999; USDA 2002).

Glucosinolates are sulfur and nitrogen-containing compounds derived from amino acids which confer the iconic bitterness of consumed crops in Order Capparales (Halkier and Gershenzon, 2006; del Carmen Martínez-Ballesta et al., 2013). Glucosinolates are nutritionally important as their derivative products have been shown to offer anti-carcinogenic properties. Under normal conditions, glucosinolates are stable within plant tissues, but when plants are
subjected to abiotic stresses like drought, waterlogging, temperature changes, and biotic stresses like herbivory, they are rapidly degraded into derivatives to be used as a defensive mechanism to aid in plant survival (Halkier and Gershenzon, 2006; del Carmen Martínez-Ballesta et al., 2013).

Apart from having high mineral nutrient concentrations, low caloric value, and rich in vitamins and secondary metabolites, kale is a vastly understudied crop in terms of nutritional comparability to other leafy green vegetables and requires more research in many different facets of its production and consumption (Tavarajah et al., 2016; Hahn et al. 2022). Some of the earliest research on kale was published in the 1950s observing the self-incompatibility of some kale varieties (Thompson 1957; Thompson and Howard 1959) with later research by Warne (1961) studying juvenility and flowering and by Thompson (1963) observing the effect that waxy and non-waxy kale plants had on the incidence of cabbage aphids. From the 1960s to the 1990s, much research on kale focused on crop improvement ranging from intraspecific crosses (Brown et al. 1990), inbreeding and hybridization of kale (Johnston 1963, Johnston 1965), and investigating the genetics (Mackiewicz 1973; Mackay et al. 1975; Keller and Armstrong 1981) and notable compounds (Kaul et al. 1980; Chweya 1985). While further studies on genetics continued, in the late 1980s, transgenic work began on ornamental kale (Hosoki et al. 1989) moving into the turn of the century with an increase in ornamental kale production, growth controls, and access to new varieties (Bingham 1991; Whipker et al. 1994).

Kale has been recognized as an important crop in the Southeastern United States since the 1990s when the demand from consumers increased and the need for crop improvement for summer production began (Farnham and Garrett 1996). Beginning in the early 2000s, kale studies began focusing more on the nutritional components and how the identified compounds were absorbed when consumed, highlighted by Kurilich et al. (2003). In the mid-to-late 2000s,
researchers continued the identification of these nutritiously favorable compounds varied from different cultigens (Kopsell DA et al. 2004, Kopsell DE et al. 2005), how various abiotic factors like temperature (Lefsrud et al. 2005), light durations (Lefsrud and Kopsell 2006), irradiance (Lefsrud et al. 2006), and the influence of light-emitting diodes (LEDs) (Lefsrud et al. 2008).

Liu et al. (2012) identified that kale could be grown at a lower fertility rate when using sub-irrigation practices maximizing dry mass production in comparison to overhead irrigation which kale, which offered little to no improvement in increasing fertility. Liu et al.’s (2012) results display that kale performs better in containerized or hydroponic production and has lower nutrient demands when irrigating substrate and not foliage which may be due to the waxy cuticle in most Brassicas which impedes foliar absorption. Besides tolerating lower fertility, kale growth was not impacted when subjected to 24-hour flooding with simulated seawater, although photosynthetic reductions did occur (Sun et al. 2015). Furthermore, the absorption of mineral nutrients in kale can be significantly greater and yield no noticeable differences in size, observed when using biosolids (Broderick and Evans 2017), but may be related to the variation in mineral nutrient concentration observed when kale matures (Waterland et al. 2017). Like Broderick and Evans’ (2017) results, Yoder and Davis (2020) also observed that utilizing different organic fertilizers did not affect the growth of kale, only observing natural varietal differences.

Kale’s relative ability to manage climactic variations is evident in Coolong et al.’s (2013) organic leafy green trial. Compared to other leafy greens, kale yield was very uniform over the different growing seasons despite being subjected to adverse weather conditions during the first season (Coolong et al. 2013). However, the weather is still suspected to influence phytohormones and compounds, as observed in most other plants. Ku and Juvik (2013) identified that during their study on methyl jasmonate, differences experienced between the two years of
their study were attributed to weather differences among control plants due to a reduction in precipitation by 44 percent but similar growing degree days between the years. Ku and Juvik (2013) hypothesized that endogenous jasmonate levels, which have been previously shown to accumulate during drought stress, diminished the effect of exogenous methyl jasmonate applications.

Alternative cropping use for kale and additional studies in combination with other crops has been more recently investigated. Wang et al. (2017) investigated how the use of allelopathic compounds produced by Brassicaceae species *Sinapis alba* and *Brassica juncea* hinder the germination of various greens, including kale. Fortunately, not all crops grown around kale have allelopathic reactions. The use of intercropping kale enabled an increase in arthropod diversity while reducing the natural aphid populations on crop leaves when intercropped with cilantro (*Coriandrum sativum* L.) and parsley [*Petroselinum crispum* (Mill.) Nym.] (de Araújo Hendges et al. 2018). Yet the use of kale as an intercrop was found to not be viable in blackberries grown in high tunnels due to temperature requirement differences among crops (Cormier et al. 2020), but when growing kale in high tunnels alone, greater harvest yields existed for plants sown later in the year attributed to more favorable environmental conditions (Heyduck, VanLeeuwen, and Guldan 2020). Outside of edible kale grown under high tunnels, ornamental kale for use as cut flowers has been identified to be a viable production method for local marketing when grown in high tunnels to extend production season (O’Connell 2018).

Cultivation of kale in controlled environments has been focused on the influence of irradiance and LED lighting on its growth and phyto-nutrition. Compared to other leafy green production, kale is better suited for production under low irradiance levels, which will be advantageous in greenhouse production during winter with lower light levels or in facilities
where irradiance is supplied solely through lighting (Baumbauer et al. 2019; Erwin and Gesick 2017). However, varietal differences do exist due to genetic variation among interspecific hybrids and low carbon dioxide levels can significantly reduce overall production more than low irradiance (Erwin and Gesick 2017). Kale growth under LED lighting has not been as impacted by different light treatments. The use of blue monochromatic lighting has been shown to influence hypocotyl elongation in kale microgreens without impacting the germination rate (Kong et al. 2019). While varying light treatments affected the hue color of cotyledons, height, and cotyledon area, there were no yield or quality reductions in appearance when grown under fluorescent and 15% blue:85% red lighting among kale (Ying et al. 2020). Red-pigmented lettuces (anthocyanins) were also shown to be less responsive to light treatments (Ying et al. 2020), while kale pigments (chlorophylls and carotenoids) were unaffected by light treatments (Ying et al. 2021). Unfortunately, other studies have identified that red kale and purple basil’s chlorophyll concentrations were increased under Blue-Red LED light treatments with greater blue concentrations (Dou et al. 2020), which displays the complex nature of LED lighting.

While the literature reviewed does not encompass all the research that has been conducted on kale, the research that has been conducted and presented is highly focused on only a handful of areas. Of the research that has been conducted on kale concerning water stress, studies have focused on different horticultural Brassica oleracea var. alboflabra (Chinese Broccoli or Chinese Kale), which is grown for its broccoli-like florets versus Brassica oleracea var. acephala (kale and collards) grown for their leaves. Only a few studies regarding kale and water stress, like that conducted by Pathirana et al. (2017) focus on a few morpho-physiological and phyto-nutritional aspects, leaving large areas needing to be studied in-depth.
To improve the production, genetic, and nutritional areas of kale, it is essential to improve our understanding of kale to decrease production losses. Overall, the predicted increases in precipitation and heat-induced drought events anticipated to occur by the IPCC (2019) display a major challenge when kale’s physiological and nutritional responses of kale to water stress-related events are relatively unknown. The purpose of these studies is to investigate the responses of kale to various water-stress-related events, including underwatering, overwatering, and waterlogging conditions.
1.4 Figures

Figure 1.1 The annual precipitation per year from 2001 to 2021 for Verona, Mississippi collected at the North Mississippi Research and Extension Center.

Missing years represent significant values missing for years.

Figure 1.2 The number of days with precipitation from 2001 to 2021 for Verona, Mississippi collected at the North Mississippi Research and Extension Center.

Missing years represent significant values missing for years.
Figure 1.3  The mean precipitation per rainfall event from 2001 to 2021 for Verona, Mississippi collected at the North Mississippi Research and Extension Center. Missing years represent significant values missing for years.

Figure 1.4  The mean precipitation per month from 2001 to 2021 for Verona, Mississippi collected at the North Mississippi Research and Extension Center.
1.5 References


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Mackiewicz TH. 1973. Gametogenesis, embryo-sac development and pollen grain morphology in Brassica oleracea var. capitata L. x B. oleracea var. acephala DC hybrid-as compared with the parental forms. Genetica polonica. 14(1).


CHAPTER II
WATERLOGGING EFFECTS ON KALE CAROTENOIDs, CHLOROPHYLLs, AND GLUCOSINOLATES DURING THE JUVENILE STAGE

This thesis chapter is based in full on the previously published article listed below. The permission from co-authors was received to use the work listed below in my thesis. The original publication is available at www.actahort.org.


2.1 Abstract

Sustainably increasing the global food supply in the face of climate change is the utmost important issue that faces the worldwide population. With a changing climate, more prevalent episodes of regional damage due to precipitation and temperature extremes will limit agricultural productivity and may affect the nutritional values of crops. Increasing yields and nutritional values will require implementing novel approaches in gene discovery and plant breeding. The increasing occurrences of extreme precipitation events due to climate change, when combined with the increased global demand for nutritionally rich foods, places pressure on producers to meet demands. Despite being nutritionally rich and increasing in popularity among consumers, the physiological reactions of kale to waterlogging conditions are unknown. The purpose of this study was to determine the effects of short-term waterlogging on juvenile kale plants and to identify changes in key nutritional metabolites. Plants harvested after six days of waterlogging demonstrated a 13% decrease in plant biomass when compared to non-waterlogged plants.
Waterlogged kale plants also exhibited a decrease in carotenoid concentrations of β-carotene, lutein, zeaxanthin, neoxanthin, and chlorophyll A and chlorophyll B concentrations. Elevated levels of total glucosinolates existed for waterlogged kale plants compared to non-waterlogged plants. Of the nutritionally important glucosinolates, elevated concentrations of raphanin, sinigrin, nasturtiin existed for waterlogged plants, and these have been linked to carcinogen-blocking enzymes. Similarly, there were also increased levels of progoitrin and epiprogoitrin which have been linked to health problems such as goiterism. Thus, waterlogging has been shown to decrease the overall nutritional value of kale by decreasing carotenoids and both chlorophylls while marginally increasing nutritionally important glucosinolates.

2.2  Introduction

Kale is a leafy green and is consumed because of its high nutritional values for Ca, Mg, P, K, and Fe (Kopsell et al., 2004a). Leafy greens in the Brassicaceae family also contain high levels of secondary metabolites like carotenoids and flavonoids that are linked to improving human health, making crops like kale attractive to consumers (Coolong et al., 2013). With the high nutritional value and linkage between secondary metabolites with improving human health, kale offers the advantage of being a nutritious food source globally. However, with the changing climate, the predicted increase in precipitation events, and the need for producers to plant crops earlier in the year to increase production, understanding the response of kale to waterlogging is necessary to ensure the nutritional content and yield of the crop is maintained (IPCC, 2014).

2.3  Objectives

This experiment sought to identify:

1. The effect juvenile waterlogging has on biomass.
2. The effect juvenile waterlogging has on the concentration of carotenoids, chlorophylls and glucosinolates.

2.4 Methods and Materials

2.4.1 Plant Material and Growth Conditions

Seeds of Kale ‘Starbor’ were sown into 3.5-inch pots (T.O. Plastics, Clearwater, MN) filled with soilless media (Pro-Mix BX Soilless Media, Premier Tech Horticulture, Quebec, Canada) incorporated with a 15N-9P-12K controlled-release fertilizer (Osmacote plus; Scotts Miracle Gro, Maryville, OH) at a rate of 8.66 kg·m⁻³. Seeds were germinated in a plant growth chambers (Model E-41L2; Percival Scientific, Perry, IA) at 24°C with 12-hour diurnal cycle and thinned to one (1) plant per pot after seven (7) days. Plants were grown for 14-days prior to being divided into two treatment groups with four (4) replications of 12 plants in each and placed in 11L Rubbermaid containers (Rubbermaid Inc., Atlanta, GA) for each treatment. On day 15, one treatment group was waterlogged using tap water and plants from both treatment groups continued their growth in the growth chambers adjusted to a 22/18 °C Day/Night cycle and 12-hour photoperiod. After 6 days, plants were harvested, with fresh mass being measured and stored in a -80°C freezer until processing. Samples were lyophilized using a FreeZone 2.5-L Freeze Dryer (Labconco Corp., Kansas City, MO) for 60 hours to determine the dry mass and for analysis preparation.

2.4.2 Carotenoid and Chlorophyll Extraction and Analysis

The extraction of carotenoids and chlorophyll from leaf tissue was conducted as described by Kopsell et al. (2004) and analyzed using procedures of Emenhiser et al. (1996) and
Davies and Köst (1988) with modifications as follows. In brief, 800 μL of H2O, 800 μL of Ethyl 8’-apo-β-carotenoate (Carotenature, Münsingen, Switzerland), and 2.5 mL tetrahydrofuran were added to ground freeze-dried tissue samples. Each sample was ground ~20 times using a drill press in Size 22 Potter-Elvehjem Tissue Grinders (DWK Life Sciences, Millville, New Jersey). Ground samples were centrifuged for five (5) minutes at ~500 gn and the supernatant was decanted into a graduate vial with penny stopper stored on ice. The pellet was rehydrated with 2.0 mL tetrahydrofuran, resuspended, and re-ground 3 times until the pellet was colorless. After complete extraction, supernatant vials were placed on an N-evaporator (Organomation, Berlin, Massachusetts) and the sample volume was reduced to 0.5 mL. Next, samples were brought up to 5.0 mL using Acetone, vortexed, then filtered using a 0.2 μM polytetrafluoroethylene filter into 2 mL sample vials. All chemicals and consumables used were sourced from Fisher Scientific if not specified (Fisher Scientific International, Inc., Hampton, New Hampshire).

Pigment separation was done using an Agilent 1200 Series HPLC with Photodiode Array Detector (Agilent Technologies, Palo Alto, California). Data was collected, recorded, and integrating using ChemStation Software (Agilent Technologies). Chromatographic separations were achieved using an analytical scale 5 μm, 200 Å polymeric C30 reverse-phase column [(4.6mm i.d. x 250 mm),(ProntoSIL, MAC-MOD Analytical Inc., Chadds Ford, PA). The column was equipped with a guard cartridge (4.0 mm i.d. x 10 mm) and holder (ProntoSIL), maintained at 60° C in the thermostatic column compartment. A 5 μL injection from each sample was used with a dual-reagent, gradiented mobile phase for chromatographic separations with Reagent A containing Methanol and Triethylamine, and Reagent B as Tert-butyl Methyl ether. A concentration gradient will be used starting at 99:1 and increasing to 89:11 before reversion back to the original concentrations of Reagent A: Reagent B, respectively. The eluted compounds
were detected at 453, 652, and 665 nm with reference wavelengths of 800 nm and 100 nm reference bandwidth. Peak assignment for individual pigments was performed by comparing retention times and line spectra from the photodiode array detector utilizing external standards in the mobile phase.

2.4.3 Glucosinolate Extraction and Analysis

Extraction and separation of glucosinolates was performed according to Raney and McGregor (1990) with modifications as described by Barickman et al. (2014) using an 1100 Series HPLC (Agilent Technologies) measured at 230 nm after separation utilizing a 250×4.6 mm i.d., 5-μm Luna C18 reverse-phase column (Phenomenex, Inc., Torrance, CA, USA). In brief, 2.0 mL methanol, 1.0 mL 1mM benzyl-glucosinolate solution, and 0.1 mL of 0.6 M barium-lead acetate were added ground, lyophilized leaf tissue. Samples were shaken for 1 hour and spun on a centrifuge for 10 minutes at 20°C at 2000 rcf. From the resultant supernatant, 0.5 mL were added to a 0.3 mL A25 Sephadex column and washed with 1.8 mL 67% methanol, 1.6 mL H₂O, and 0.9 mL of 0.02M pyridine-acetate. Samples were vacuum extracted until all solvent was removed, after which 50 μL of purified sulfatase were added to each column, capped, and left to incubate at room temperature overnight. Afterwards, columns were eluted with 0.9 mL of H₂O into crimp-top sample vials. To analyze, 25 μL was injected and allowed to separate using a mobile phase consisting of H₂O and acetonitrile. Peak separation, integration, and compound identification were done in ChemStation software (Agilent Technologies) and compared against known compounds.
2.4.4 Statistical Analysis

The experiment was a randomized complete block design with four replications of two waterlogging treatments and 12 plants in a factorial arrangement. In total, 96 plants (4 replications x 2 waterlogging treatments x 12 plants) were utilized to perform statistical analysis. SAS (version 9.4; SAS Institute, Cary, NC) was used to perform statistical analysis of the data. Replicated values of all morphological and photosynthetic parameters measured in this study were analyzed using a one-way analysis of variance of the generalized linear mixed model (PROC GLIMMIX) to determine the effects of waterlogging treatments. Fisher’s Least Significant Difference tests at $p \leq 0.05$ were employed to test the differences among treatments for measured parameters. The standard errors of the mean were calculated using the pooled error term from the ANOVA table. Diagnostic tests were conducted to ensure that treatment variances were statistically equal before pooling.

2.5 Results and Discussion

Non-waterlogged plants had significantly higher fresh mass and dry mass when compared to waterlogged plants (Table 2.1). The dry mass of waterlogged plants had 13% less mass than that of non-waterlogged plants. The dry mass to fresh mass ratio for waterlogged plants was significantly higher than non-waterlogged plants. Reductions in plant fresh and dry mass are common occurrences of non-tolerant species being seen in rapeseed (Wollmer et al., 2017), Chinese kale, and Bok Choy (Issarakraisila et al., 2007).

The increase in the dry to fresh mass ratio of waterlogged plants may be due to reduced growth from the waterlogging treatments limiting stomatal conductance and reducing cell expansion. Stomatal closure is a common response to waterlogging of plants in previous studies (Ashraf 2012; Lee et al. 2014) and noted in studies among other brassica crops (Issarakrasilia et
al., 2007; Lin et al. 2015). Stomata closure has been linked with reductions in photosynthesis among different species (Else et al. 2009; Smethurst and Shabala 2003; Araki et al. 2012) but never disrupting photosynthesis completely. Thus, waterlogged plants were able to continue accumulating metabolites and photosynthetic products in plant tissues contributing to the increased dry mass of plant tissue.

The concentrations of neoxanthin, zeaxanthin, lutein, chlorophyll a, chlorophyll b, and beta-carotene of the waterlogged treatment were lower when compared to the control treatment (Table 2.2). The decrease in carotenoid and chlorophyll concentrations may be the result of the downregulation of genes associated with key photosynthetic pathways. For example, Lee et al. (2014) observed in rapeseed (Brassica napus L.) seedlings, when waterlogged for 3 and 6 days, the key photosynthetic pathway that regulates Rubisco expression significantly reduced, decreasing carbon dioxide fixation and inhibiting carotenoid and chlorophyll concentrations. Similarly, Nabloussi et al. (2019) saw similar decreases in chlorophyll a content among different cultivars studied when rapeseed was subjected to waterlogging at various stages (directly after sowing, after emergence, at rosette, and floral bud stage) when compared to non-waterlogged plants.

Of the 11 glucosinolates analyzed, those with significant concentration differences were all elevated in plant samples subjected to waterlogging compared to the control (Table 2.3). Those that were increased from waterlogging are nutritionally important, like sinigrin (allyl-glucosinolate) which degrades to allyl-isocyanate, offering anti-carcinogenic properties. Furthermore, nutritionally unfavorable glucosinolates also increased, such as progoitrin (2(R)-hydroxy-3-butenyl glucosinolate) and epiprogoitrin (2(S)-hydroxy-3-butenyl glucosinolate), that can cause goiter in animals among other harmful effects (Halkier and Gershenzon 2006).
Previous studies have indicated that water-stress may play a role in glucosinolate concentration. However, variations do occur between brassica crops and individual glucosinolates (Khan et al., 2010; Khan et al., 2011; Xu et al. 2015). Khan et al. (2010) identified that water stress could be a major factor influencing glucosinolate accumulation in broccoli (Brassica oleracea var. italica) from their experiment, which also correlates with the results of our study. Brassicas utilize glucosinolates as a defense mechanism against herbivory and damage due to abiotic factors like water stress (Martínez-Ballesta et al., 2013). Additionally, one study has shown that glucosinolates may act as substrates for myrosinases upon ABA signaling, inhibiting K+ channels, and causing stomatal closure, a typical response of plants to waterlogging (Zhao et al. 2008). The role that glucosinolates may play in affecting stomata may ultimately lead to increases in their accumulation in plant tissues, but additional research is needed to study this in-depth.

2.6 Conclusion

The impact of waterlogging on kale plants can lead to decreased plant yields. Thus, waterlogging can elicit variations in nutritionally important plant biochemicals, like plant pigments and glucosinolates. Further studies are necessary to identify the influence of how short-term waterlogging during juvenile stages affects mature stages of plant growth at harvest to identify if the variation in plant biochemicals is temporary or will lead to further variations in nutritional content.
### 2.7 Tables

Table 2.1  Fresh mass, dry mass, and dry mass to fresh mass ratio of kale plants subjected to non-waterlogged (control) and waterlogged treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FM(^a) (g)</th>
<th>DM (g)</th>
<th>DM/FM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.88 a</td>
<td>1.01 a</td>
<td>9.29 b</td>
</tr>
<tr>
<td>Waterlogged</td>
<td>7.94 b</td>
<td>0.88 b</td>
<td>11.14 a</td>
</tr>
</tbody>
</table>

\[ P\text{-Value}^{b,c} \quad *** \quad * \quad *** \]

Values in column followed by the same letter are not different at \( \alpha = 0.05 \), according to Tukey’s LSD.

\(^a\) FM – Fresh Mass; DM – Dry Mass; DM/FM – Dry Mass to Fresh Mass (Dry Mass ÷ Fresh Mass \times 100)

\(^b\) *, *** indicate significant at \( P \leq 0.05 \), significant at \( P \leq 0.001 \), respectively.

\(^c\) The standard error of the mean was: FM – 0.41; DM – 0.04; DM/FM – 0.21
Table 2.2  Carotenoid and chlorophyll concentrations of kale leaf tissue samples subjected to non-waterlogging (control) and waterlogged treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Neo</th>
<th>Viol</th>
<th>Anth</th>
<th>Zea</th>
<th>Lut</th>
<th>ChlB</th>
<th>ChlA</th>
<th>Bcar</th>
<th>Total Chl</th>
<th>Total Xan</th>
<th>ZA/ZAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.509 a</td>
<td>0.349 a</td>
<td>0.032 a</td>
<td>0.066 a</td>
<td>1.648 a</td>
<td>1.915 a</td>
<td>17.523 a</td>
<td>0.909 a</td>
<td>19.438 a</td>
<td>0.447 a</td>
<td>0.219 a</td>
</tr>
<tr>
<td>Waterlogged</td>
<td>0.456 b</td>
<td>0.349 a</td>
<td>0.032 a</td>
<td>0.055 b</td>
<td>1.518 b</td>
<td>1.652 b</td>
<td>15.061 b</td>
<td>0.800 b</td>
<td>16.712 b</td>
<td>0.435 a</td>
<td>0.199 a</td>
</tr>
</tbody>
</table>

P-value\textsuperscript{d,e}  *  ns  ns  **  *  **  **  **  **  ns  ns

Values in column followed by the same letter are not different at $\alpha=0.05$, according to Tukey’s LSD.

\textsuperscript{a} ZA/ZAV does not have any units

\textsuperscript{b} Neo – Neoxanthin; Viol – Violaxanthin; Anth – Antheraxanthin; Zea – Zeaxanthin; Lut – Lutein; ChlB – Chlorophyll B; Chl A – Chlorophyll A; Total Chl – Total Chlorophylls; Total Xan – Total Xanthophylls; ZA/ZAV – Xanthophyll Ratio.

\textsuperscript{c} ZA/ZAV is a ratio of Zea and Anth to the Total Xanthophylls (Sum of Zea, Anth, and Viol)

\textsuperscript{d} ns, *, ** indicate non-significant, significant at $P \leq 0.05$, significant at $P \leq 0.01$, respectively.

\textsuperscript{e} The standard error of the mean was: Neo - 0.014; Vio – 0.0112; Anth – 0.002; Zea – 0.002; Lut – 0.041; ChlB – 0.066; ChlA – 0.761; Bcar – 0.025; Total Chl – 0.82553; Total Xan – 0.013; ZA/ZAV – 0.007.
Table 2.3  Glucosinolate concentrations of kale leaf tissue samples subjected to non-waterlogging (control) and waterlogged treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Progoitrin&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Epiprogoitrin</th>
<th>Raphanin</th>
<th>Sinigrin</th>
<th>Brassicin</th>
<th>Nasturtiin</th>
<th>Aliphatic</th>
<th>Aromatic</th>
<th>Indole</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.064&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.062&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.021&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.042&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.047&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.202&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.231&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.230&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.377&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.838&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Waterlog</td>
<td>0.125&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.124&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.031&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.058&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.508&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.727&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.384&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.760&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.857&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

P-Value<sup>b,c</sup> *** *** * *** *** *** *** * ***

Values in column followed by the same letter are not different at α=0.05, according to Tukey’s LSD. Non-significant glucosinolates analyzed were not included in the table. Non-significant glucosinolates were Glucoiberin, Glucosinalbin, Glucosinalbin, Glucobarbarin, and Neo-glucobrassicin.

<sup>a</sup> Progoitrin – Glucoprogoitrin; Epiprogoitrin – Glucoepiprogoitrin; Raphanin – Glucoraphanin; Sinigrin – Sinigrin; Brassicin – Glucobrassicin; Nasturtiin – Gluconasturtiin

<sup>b</sup> *, **, *** indicate significant at P ≤ 0.05, significant at P ≤ 0.01, significant at P ≤ 0.001, respectively.

<sup>c</sup> The standard error of the mean was: Iberin – 0.005; Progoitrin – 0.009; Epiprogoitrin – 0.007; Raphanin – 0.005; Sinigrin – 0.004; Sinalbin – 0.003; Napin – 0.004; Barbarin – 0.005; Brassicin – 0.136; Nasturtiin – 0.050; Neo-Brassicin – 0.082; Aliphatic – 0.016; Aromatic – 0.049; Indole – 0.143; Total – 0.166.
2.8 References


CHAPTER III

OVERWATERING AND UNDERWATERING ALTERS MORPHOLOGICAL, PHOTOSYNTHETIC, AND NUTRITIONAL COMPONENTS ON KALE ‘WINTERBOR’

This thesis chapter is based in full on the previously published article listed below. The permission from co-authors was received to use the work listed below in my thesis. The original publication is available at https://www.sciencedirect.com/journal/scientia-horticulturae.


3.1 Abstract

Kale is a leafy green that contains nutritiously favorable secondary metabolites, like glucosinolates and carotenoids, and important mineral nutrients like Ca, Mg, P, K, and Fe. Limited information is available on how small changes in irrigation applications using sensor-based irrigation affects plant growth. In this study, ‘Winterbor’ kale was grown in containers with soilless media using sensor-based irrigation schemes applying three treatments – overwatered, optimal, and underwatered – in the greenhouse at the North Mississippi Research and Extension Center, Verona, MS, USA. Overwatering and underwatering treatments resulted in smaller morphological features when compared to optimally treated but did not vastly differ from each other. Fresh mass of kale was the greatest for optimally treated plants, with the underwatered treatment having reduced mass but greater than that of the overwatered treatment. Concentrations for mineral nutrients, glucosinolates, and pigments changed alongside of gas exchange and chlorophyll fluorescence measurements all having temporal and treatment variations. Apparent nutritional content of kale leaves was significantly reduced for overwatering
and underwatering treatments compared to optimal treatments yet underwatered treatments had little to no impact on favorable secondary metabolites and mineral nutrients.

3.2 Introduction

The importance of water to plants has long been recognized by farmers, observed by the strong connection between ancient civilizations originating near freshwater sources to supply crops and meet their most basic needs (Pradeep 2009). Still today, farmers and researchers continue to investigate how water impacts plants. The focus most recently has been on how moisture extremes affect plants due to the current and predicted challenges faced, as organizations like the Intergovernmental Panel on Climate Change (IPCC) model the major shifts in the Earth’s climate (IPCC 2019). The IPCC (2019) has identified two major global trends occurring: 1) an increased frequency and duration of heat-related events that are causing droughts across many regions, and 2) the increase in the severity of heavy precipitation events that lead to waterlogging conditions.

Using agronomic, engineering, and genetic advances and practices, producers can reduce the negative impacts caused by waterlogging (Manik et al. 2019). While micro-irrigation can be used to conserve water during droughts, maladaptation of systems is causing salinization of soil and ground-water depletion (IPCC 2019). There is a delicate balance in micro-irrigation between the amount of water to apply and when to meet the crop’s needs (Vera et al. 2021). Using soil moisture sensors is one way to accurately determine and quantify the current water status of soil to manage micro-irrigation systems proactively. Many different soil moisture sensors and dataloggers are also available, enabling growers to identify what is best for them.

Vera et al. (2021) summarize the major volumetric water content (VWC, also known as volumetric soil water content) sensors currently available for use. These sensors use
electromagnetic sensors measuring the permittivity of the soil, but do not directly measure water content, requiring calibration to correlate a known volume of water with the electrical output of that sensor (Vera et al. 2021). When appropriately deployed, sensors can accurately measure VWC and be used in parallel with automatic irrigation systems to maintain optimal moisture levels, reducing total irrigation needs compared to manual irrigation practices (Vera et al. 2021; Dukes et al., 2010).

Soil or substrate moisture level is also highly variable in containerized production systems being identified as species-specific and not correlated with estimated transpiration or leaf area (Daniels et al., 2012). Additional considerations must also be weighed as sensors require substrate-specific calibrations based on mixes (Rhie and Kim 2017), understanding the high porosity of soilless substrates (Belayneh et al. 2013), and understanding the container’s influence on optimal moisture level and their stability (Flax et al. 2018). When growers understand all the components that influence moisture in their system, sensors can be deployed alongside automatic irrigation systems. Automated systems can also be implemented in other production systems outside of the field, such as those using protected culture practices in high tunnels, greenhouses, or vertical growth systems. Although the prevalence of flooding and drought stress in protected environments is unlikely, variations like overwatering and underwatering can still arise and be problematic (Carillo et al. 2020).

Kale (Brassica oleracea var. acephala) is a leafy green vegetable that is widely consumed due to its high nutritional content for the major mineral nutrients – Ca, Mg, P, K, and Fe (Kopsell et al. 2005) and its high levels of nutritionally critical secondary metabolites including carotenoids and glucosinolates (Coolong et al. 2013). According to the United States Department of Agriculture National Agricultural Statistics Service, in 2000 there were 3,760
acres (1524 hectares) of kale grown. In contrast to 2017, the production area had increased to 15,325 acres (6202 hectares), displaying the overall consumer demand (USDA, 2017). Kale plants also perform well when grown in containers, as ornamental cultivars are often produced extensively in containers for marketing to consumers as a fall crop alongside ornamental cabbage and mums (McAvoy 1994). Coolong et al. (2013) identified that kale production was relatively stable in field trials during adverse weather conditions displaying that it may be more resilient than other leafy greens. Yet there is a severe lack of kale research on plant response to abiotic stress.

One challenge that kale shares among other members of the Order Capparales are the iconic sulphury-bitterness occurring in plants resultant of glucosinolates production (del Carmen Martínez-Ballesta et al. 2013). Under normal growth conditions, glucosinolates are relatively stable but rapidly degrade under stressful conditions acting as a defensive mechanism (del Carmen Martínez-Ballesta et al. 2013; Halkier and Gershenzon 2006). While these derivatives are bitter, they also confer anti-carcinogenic properties when consumed (del Carmen Martínez-Ballesta et al. 2013; Halkier and Gershenzon 2006). Previous research demonstrated that short-term waterlogging of kale plants impacts the concentration of nutritionally important glucosinolates (Brazel et al. 2021). However, waterlogging is not necessarily common in container production due to the high porosity of soilless mixtures.

To combat the challenge of protecting plants from environmental changes, preserving nutritional content, and balancing the water requirements for plants grown in soilless media, understanding how to utilize sensor-based irrigation is essential to meet the consumer demand for kale. Therefore, the focus of this study was to identify how small changes in irrigation
thresholds of container-grown kale impact the mineral nutrient, secondary metabolites, and morphology.

3.3 Objectives

The purpose of this experiment is to identify:

1. The effect that small water stress variations have on the mass and morphological parameters.
2. Water stress affects on the concentrations of carotenoids, chlorophylls, glucosinolate and mineral nutrient concentrations.
3. The effects that water stress has on the photosynthetic and fluorometric parameters of photochemistry.

3.4 Methods and Materials

3.4.1 Plant Material and Growth Conditions.

The study was conducted between January and March 2020. Kale ‘Winterbor’ seeds were sown into 72 cell trays filled with Propagation Mix (Sun Grow Horticulture, Agawam, MA) and germinated in a greenhouse at the North Mississippi Research and Extension Center, Verona, MS, USA. Seedlings were grown for 28 days before being transplanted into their treatment containers consisting of three-gallon containers filled with Pro-Mix BX soilless medium (Premier Tech Horticulture, Quebec, Canada) and incorporated with a 15N-9P-12K, three to four-month controlled-release fertilizer (Osmacote plus; Scotts, Maryville, OH) at a rate of 5.9 kg·m⁻³.
The environment was maintained at 22/18 °C day/night with a 16-hour photoperiod. High-pressure sodium lights were used to provide supplemental lighting and daylength extension. The average daily light integral was 27.96 ± 1.30 mol·m⁻²·d⁻¹, measured every 10 minutes using quantum sensors (SQ-110, Apogee Instruments, Logan, UT) and a CR1000X datalogger (Campbell Scientific, Logan, UT).

Substrate volumetric water content (VWC) was measured using capacitance sensors (Teros 12; Meter Group, Inc., Pullman, WA), and readings from each sensor were used to compute VWC based on a substrate-specific calibration. Each Teros 12 sensor was inserted into the pot from the side. The three prongs of the sensor were vertically aligned in the center of the container and inserted perpendicular to the substrate level. Irrigation control was managed using the CR1000X data logger and relay driver (SDM-CD16AC controller; Campbell Scientific), administering solenoid valve control. The VWC thresholds for irrigation of 0.35, 0.25, and 0.15 m³·m⁻³, corresponding to overwatered, optimal (control), and underwatered conditions, were programmed into the datalogger to automate irrigation. Each container was irrigated with a dribble ring (Dramm Crop., Manitowoc, WI) connected to a 2 L·h⁻¹ pressure-compensating emitter (Netafim USA, Fresno, CA). Containers were subjected to their irrigation thresholds for 7 days before transplanting to ensure equilibration. The VWC of each experimental unit was checked every 10 minutes, and if the unit dropped below its respective threshold, the corresponding irrigation valve was opened for 10 s, providing 11.1 mL/container only supplying irrigation on a need basis.

3.4.2 Measurements and Analysis

Plants were harvested at 0, 7, 14, and 28 days after transplanting, with 18, 12, 6, and 6 plants harvested at each date, respectively. At each harvest, plants were combined into 6 total
samples, to ensure large enough sample volumes, weighed for fresh mass, and freeze-dried in a FreeZone 2.5 L (Labconco Corp., Kansas City, MO, USA) for 60 hours to determine the dry mass (per plant) and percent dry mass (dry mass/fresh mass*100). After freeze-drying, samples were ground with a mortar and pestle and stored in an ultra-low -80°C freezer before chemical analysis.

### 3.4.3 Morphology

Morphological parameters were evaluated on all plants on day 28, measuring plant height from the substrate surface, the petiole length, leaf width, and leaf length of the 3rd and 4th leaf from the bottom of the plant (being fully expanded), and the total leaf number.

### 3.4.4 Glucosinolate Extraction and Analysis

Extraction and separation of glucosinolates were performed according to Raney and McGregor (1990) with modifications as described by Barickman et al. (2014b) using an 1100 Series HPLC (Agilent Technologies) measured at 230 nm after separation utilizing a 250×4.6 mm i.d., 5-μm Luna C18 reverse-phase column (Phenomenex, Inc., Torrance, CA, USA). In brief, 2.0 mL methanol, 1.0 mL 1mM benzyl-glucosinolate solution, and 0.1 mL of 0.6 M barium-lead acetate were added to the ground, lyophilized leaf tissue. Samples were shaken for 1 hour and spun on centrifuge for 10 minutes at 20°C at 2000 rcf. From the resultant supernatant, 0.5 mL were added to a 0.3 mL A25 Sephadex column, washed with 1.8 mL 67% methanol, 1.6 mL H2O, and 0.9 mL of 0.02M pyridine-acetate. Samples were vacuum extracted until all solvent was removed, after which 50 μL of purified sulfatase were added to each column, capped, and left to incubate at room temperature overnight. Afterwards, columns were eluted
with 0.9 mL of H₂O into crimp-top sample vials. To analyze, 25 μL was injected and allowed to separate using a mobile phase consisting of H₂O and acetonitrile. Peak separation, integration, and compound identification were done in ChemStation software (Agilent Technologies) and compared against known compounds.

### 3.4.5 Carotenoid and Chlorophyll Extraction and Analysis

The extraction of carotenoids and chlorophyll from leaf tissue was conducted as described by Kopsell et al. (2004) and analyzed using procedures of Emenhiser et al. (1996) and Davies and Köst (1988) with modifications as follows. In brief, 800 μL of H₂O, 800 μL of Ethyl 8’-apo-β-carotenoate (Carotenature, Münsingen, Switzerland), and 2.5 mL tetrahydrofuran were added to ground freeze-dried tissue samples. Each sample was ground ~20 times using a drill press in Size 22 Potter-Elvehjem Tissue Grinders (DWK Life Sciences, Millville, New Jersey). Ground samples were centrifuged for five (5) minutes at ~500 g and the supernatant was decanted into a graduate vial with penny stopper stored on ice. The pellet was rehydrated with 2.0 mL tetrahydrofuran, resuspended, and re-ground 3 times until the pellet was colorless. After complete extraction, supernatant vials were placed on an N-evaporator (Organomation, Berlin, Massachusetts) and the sample volume was reduced to 0.5 mL. Next, samples were brought up to 5.0 mL using Acetone, vortexed, then filtered using a 0.2 μM polytetrafluoroethylene filter into 2 mL sample vials. All chemicals and consumables used were sourced from Fisher Scientific if not specified (Fisher Scientific International, Inc., Hampton, New Hampshire).

Pigment separation was done using an Agilent 1200 Series HPLC with Photodiode Array Detector (Agilent Technologies, Palo Alto, California). Data was collected, recorded, and integrated using ChemStation Software (Agilent Technologies). Chromatographic separations were achieved using an analytical scale 5 μm, 200 Å polymeric C₃₀ reverse-phase column
The column was equipped with a guard cartridge (4.0 mm i.d. x 10 mm) and holder (ProntoSIL), maintained at 60°C in the thermostatic column compartment. A 5 μL injection from each sample was used with a dual-reagent, gradiented mobile phase for chromatographic separations with Reagent A containing Methanol and Triethylamine, and Reagent B as Tert-butyl Methyl ether. A concentration gradient will be used starting at 99:1 and increasing to 89:11 before reversion back to the original concentrations of Reagent A: Reagent B, respectively. The eluted compounds were detected at 453, 652, and 665 nm with reference wavelengths of 800 nm and 100 nm reference bandwidth. Peak assignment for individual pigments was performed by comparing retention times and line spectra from the photodiode array detector utilizing external standards in the mobile phase.

### 3.4.6 Mineral Nutrient Extraction and Analysis

The extraction and analysis of plant mineral nutrients were conducted as described by Barickman et al. (2014a) using inductively coupled plasma-mass spectrometry from freeze-dried samples. In brief, tissue was combined with 2 mL of nitric acid and digested in a microwave digestion unit (Ethos, Milestone Inc., Shelton, Connecticut). The temperature was increased to 140°C for 5 min at 1000W and 2000 kPa, followed by a temperature increase to 210°C for 10 min at 1000W and 4000 kPa. Samples were then cooled for 10 minutes and 2000 kPa to 20°C. Of the resultant digest, 100 μL was diluted with 9.9 mL of a 2% HNO3, and 0.5% HCL (v/v) solution prior to analysis. The diluted sample was auto-sampled using an ASX-510 autosampler (CETAC, Omaha, Nebraska) and measured by injecting X in an inductively coupled plasma mass spectroscopy (7500 ICP-MS, Agilent Technologies, Inc., Palo Alto, California).
instrument was optimized daily using a 1 μg L\(^{-1}\) tuning solution containing Lithium Yttrium, Cesium, and Cobalt in 2% HNO\(_3\) matrix.

### 3.4.7 Photosynthesis and Chlorophyll Fluorescence Measurements

Assimilation (A), transpiration (E), and stomatal conductance (\(g_{sw}\)) were measured on the uppermost, fully expanded leaf of plants on days 0, 7, 14, and 28 between 11:00 and 13:00 h. An LI-6800 Portable Photosynthesis System equipped with Multiphase Flash Fluorometer (LI-6800-01A, LI-COR Biosciences, Lincoln, NE) was used with the leave cuvette maintained at 415 ppm CO\(_2\), VPD of 1.5 kPa, 24.5°C chamber temperature, and PPFD of 350 μmol m\(^{-2}\) s\(^{-1}\). Intrinsic Water Use Efficiency (iWUE) was calculated as the ratio of A/\(g_{sw}\). Chlorophyll fluorescence measurements were taken on the same uppermost, fully expanded leaf as gas exchange measurements. Dark-adapted measurements were taken between 3:00 and 5:00 on the same day before light-adapted measurements were taken between 11:00 and 13:00. Measurements were taken as described by Olorunwa et al. (2022) with modifications for dark-adapted measurements as described: dark-adapted minimal fluorescence (\(F_o\)) and dark-adapted maximal fluorescence (\(F_m\)) were estimated using the multi-phase flash protocol on the LI-6800 and used to calculate the dark-adapted variable fluorescence (\(F_v = F_m - F_o\)). Dark-adapted, maximal quantum efficiency was calculated as \(F_v/F_m\). The effective quantum yield of Photosystem II (\(\Phi_{PSII}\)) was calculated as \((1-F_s/F_m')\). Dark-adapted, non-photochemical quenching (NPQ) was calculated as \(((F_m - F_m') / F_m')\). Photochemical quenching (qPr\(F_o\)) was calculated as \((F_m' - F_s) / (F_m' - F_o)\). Alt. Photochemical quenching using light- and dark-adapted measurements (qN\(F_o\)) was calculated as \((F_m-F_m')/(F_m-F_o)\). The electron transport rate (ETR) was calculated as described by Genty et al. 1989.
3.4.8 Experimental Design and Statistical Analysis

Kale plants were arranged in a randomized complete block design with four replications. Within each treatment block, plants were completely randomized based on harvest date with buffer containers between treatments. Statistical analyses were conducted using SAS (version 9.4; SAS Institute, Cary, NC). Biochemical and morphological parameters were analyzed using the PROC GLM analysis of variance (ANOVA) followed by mean separation (Table 3.1). Principal Component Analysis (PCA) was performed using R software (version: 4.1.0, RStudio, Inc., Vienna, Austria).

3.5 Results

3.5.1 Morphology

Between days 0 and 14, there was a steady increase in leaf fresh mass of all plants regardless of treatment (Figure 3.1). Between days 14 and 28, there were differential increases in leaf fresh mass, with the optimal irrigation treatment having the greatest mass (463.5 ± 8.75 g·plant⁻¹), overwatered treatment having the least (256.2 ± 8.78 g·plant⁻¹) and the underwatered treatment being in-between (321.6 ± 8.75 g·plant⁻¹). For percent dry mass, there was an overall decrease across all treatments between days 0 and 14, after which a gradual increase occurred until day 28, with the final percentage for optimal (13.97 ± 0.27 %) being lower than overwatered (14.66 ± 0.27 %) and underwatered (15.12 ± 0.27 %) treatments.

For morphological parameters measured (Figure 3.2), the optimal irrigation treatment was significantly greater than those of the overwatered and underwatered treatments, which were not different from one another, except for the overwatered treatment of the mean leaf length, being slightly less than the underwatered treatment.
3.5.2 Glucosinolates

A slight increase in the concentration of glucoprogoitrin (progoitrin) and glucoraphanin (raphanin) (Figure 3.3) was observed from 0 to 14 days of treatment, after which a large significant increase in concentrations was observed at day 28. The overwatered treatment concentrations of both progoitrin and raphanin did not increase significantly between days 0 and 14, different from the other two treatments. After 14 days, the increase in the overwatered treatment observed by day 28 of both progoitrin and raphanin (11.63 ± 0.64 mg∙g⁻¹ dm; 4.15 ± 0.24 mg∙g⁻¹ dm) was not different from the optimal treatment’s concentration (11.01 ± 0.63 mg∙g⁻¹ dm; 4.19 ± 0.24 mg∙g⁻¹ dm) while the underwatered treatment yielded the greatest concentration (13.72 ± 0.64 mg∙g⁻¹ dm; 5.45±0.24 mg∙g⁻¹ dm).

For glucoeipiprogoitrin (epiprogoitrin) and sinigrin (Figure 3.3), both the optimal and underwatered treatments increased from 0 to 14 days. The overwatered treatment epiprogoitrin concentration only increased between 7 and 14 days, while the concentration of sinigrin initially decreased from days 0 to 7, then increased from days 7 to 14. For all treatments after 14 days, there was an overall decrease in epiprogoitrin and sinigrin concentration. By day 28, the underwatered treatment yielded the greatest concentration for epiprogoitrin (0.186 ± 0.015 mg∙g⁻¹ dm) and sinigrin (0.109 ± 0.019 mg∙g⁻¹ dm), while the concentrations of the optimal (0.168 ± 0.015 mg∙g⁻¹ dm; 0.066 ± 0.019 mg∙g⁻¹ dm) and overwatered (0.144 ± 0.015 mg∙g⁻¹ dm; 0.063 ± 0.019 mg∙g⁻¹ dm) treatments were slightly lower.

The concentrations of other glucosinolates analyzed can be seen in Appendix Table A.1, but exhibited similar trends seen in Figure 3.3.
3.5.3 **Total Chlorophylls, Lutein, and β-carotene**

Overall, the concentrations of total chlorophylls, lutein, and β-carotene increased from day 0 to day 14, followed by a slight decrease from day 14 to day 28 (Figure 3.4). For total chlorophylls (Chlorophyll A + Chlorophyll B), the underwatered treatment had the smallest concentration decrease by day 28 (9.98 ± 0.46 mg·g⁻¹ dm) but was not different when compared to the optimal (9.33 ± 0.46 mg·g⁻¹ dm) treatment, while the overwatered (6.71 ± 0.46 mg·g⁻¹ dm) treatment had the lowest overall. Similarly, the lutein concentration for the overwatered treatment (585.2 ± 23.8 μg·g⁻¹ dm) was significantly lower than that of the optimal (728.2 ± 23.8 μg·g⁻¹ dm) and the under watered (737.5 ± 23.8 μg·g⁻¹ dm) treatments, not different from one another. For β-carotene, the decrease in the concentration of all pigments resulted in similar trends to that of lutein for the optimal (434.7 ± 20.1 μg·g⁻¹ dm) and underwatered (473.1 ± 20.1 μg·g⁻¹ dm) treatments being greater than the overwatered (340.7 ± 20.1 μg·g⁻¹ dm) concentration on day 28.

Appendix Table A.2 contains the remaining carotenoids and chlorophylls measured, which all exhibited similar trends, as seen in Figure 3.4.

3.5.4 **Mineral Nutrient**

More changes in mineral nutrient concentration were present in the roots than in the shoots of samples as only B and Mo were influenced by treatment in the shoots (Table 3.2) while treatment differences in the roots existed for B, Mo, Mg, P, S, and K (Table 3.3). The concentration of B and Mo exhibited similar trends, with the under watered treatment having the lowest concentrations (9.66 ± 1.42 μg·g⁻¹ dry mass; 1.05 ± 0.30 μg·g⁻¹ dry mass) when compared to the optimal (16.19 ± 1.42 μg·g⁻¹ dry mass; 2.39 ± 0.30 μg·g⁻¹ dry mass) and the overwatered (14.07 ± 1.42 μg·g⁻¹ dry mass; 2.72 ± 0.30 μg·g⁻¹ dry mass) treatments. The treatments also
exhibited similar trends to those in the roots with under watered treatment having lower concentrations than the optimal and over watered treatments for B (4.14 ± 0.43 μg·g⁻¹ dry mass; 5.47 ± 0.43 μg·g⁻¹ dry mass; 5.83 ± 0.43 μg·g⁻¹ dry mass), P (2481.2 ± 193.2 μg·g⁻¹ dry mass; 3350.0 ± 193.2 μg·g⁻¹ dry mass; 3027.8 ± 193.2 μg·g⁻¹ dry mass), K (14001 ± 885 μg·g⁻¹ dry mass; 16996 ± 885 μg·g⁻¹ dry mass; 18320 ± 885 μg·g⁻¹ dry mass), and Mo (0.682 ± 0.127 μg·g⁻¹ dry mass; 1.160 ± 0.127 μg·g⁻¹ dry mass; 1.232 ± 0.127 μg·g⁻¹ dry mass). For Mg, the over watered treatment (2457.8 ± 113.7 μg·g⁻¹ dry mass) had a greater concentration than the under watered treatment (1865.9 ± 113.7 μg·g⁻¹ dry mass), which were both similar to the optimal treatment (2179.5 ± 113.7 μg·g⁻¹ dry mass). Likewise, the S concentration of the over watered treatment (4868.5 ± 259.4 μg·g⁻¹ dry mass) was greater than both the optimal (4120.8 ± 256.4 μg·g⁻¹ dry mass) and the under watered treatment (3588.2 ± 256.4 μg·g⁻¹ dry mass).

### 3.5.5 Photosynthesis and Chlorophyll Fluorescence

The transpiration rate (E) and stomatal conductance (gˢw) of all treatments (Figure 3.5) increased until day 14 and then decreased by day 28 resulting in the over watered treatment having greater rates (0.0041 ± 0.0004 mmol H₂O·m⁻²·s⁻¹; 0.336 ± 0.035 mol·m⁻²·s⁻¹) than that of the optimal (0.0017 ± 0.0004 mmol H₂O·m⁻²·s⁻¹; 0.136 ± 0.035 mol·m⁻²·s⁻¹) and the under watered (0.0020 ± 0.0004 mmol H₂O·m⁻²·s⁻¹; 0.140 ± 0.037 mol·m⁻²·s⁻¹), which were not different from one another. The assimilation rate (A) (Figure 3.5) for all treatments increased until day 14 when the over watered treatment’s rate remained unchanged (14.91 ± 0.67 μmol CO₂·m⁻²·s⁻¹) at day 28 and the optimal (9.79 ± 0.067 μmol CO₂·m⁻²·s⁻¹) and the under watered (11.58 ± 0.69 μmol CO₂·m⁻²·s⁻¹) decreased significantly. The intrinsic water-use efficiency (IWUE, Figure 3.5) slowly increased from day 0 to day 14, after which a dramatic increase occurred for treatments, with the
overwatered treatment (60.17 ± 4.37 μmol · mol⁻¹) having the lowest and the optimal (99.20 ± 4.37 μmol · mol⁻¹) and the underwatered (102.49 ± 4.51 μmol · mol⁻¹) having greater values.

The dark-adapted, maximum quantum yield of photosystem II photochemistry (Fᵥ/Fₘ) (Figure 3.6) exhibited no treatment differences, increasing from day 0 until day 14. No difference existed between day 14 and 28 nor irrigation treatment for Fᵥ/Fₘ. Like Fᵥ/Fₘ, treatment differences did not exist for photochemical quenching (qPᵣₒ) and exhibited differences by date with an overall decrease from day 0 to day 28 (Figure 3.6). The effective quantum yield of photosystem II photochemistry (Φₚₛₛᵣ) exhibited a decline from day 0 to day 28, with the optimal slightly increasing being the greatest by day 7 and then decreasing becoming the lowest (0.294 ± 0.019) by day 28 compared to the overwatered (0.380 ± 0.019) and under watered (0.351 ± 0.019) treatments, insignificant from one another. An opposite trend existed in alternative non-photochemical quenching (qNᵣₒ) with a gradual increase from day 0 to day 28 occurring with optimally treated plants having the lowest by day 7, then becoming the largest value by day 14 and having the greatest by day 28 (0.767 ± 0.0268) compared to overwatered (0.667 ± 0.0268) and underwatered (0.706 ± 0.0268) treatments. Non-photochemical quenching (NPQ) displayed a similar response as qNᵣₒ with a gradual increase from day 0 to day 28, with the optimal treatment having a lower value by day 7 and becoming the greatest by day 28 (1.861 ± 0.075). The under watered treatment had the greatest value on day 7 for NPQ but displayed varied increases on day 14 and day 28 (1.494 ± 0.075), similar to the overwatered treatment (1.346 ± 0.075). The electron transport rate (ETR) elicited a varied reaction based on the day and treatment. The ETR increased to day 7 among all treatments, after which the optimal and the under watered treatments gradually decreased through day 28 (84.18 ± 4.17 μmol·electrons m⁻²s⁻¹).
1; 90.96 ± 4.17 μmol·electrons m\(^{-2}\)s\(^{-1}\), respectively). The ETR of the overwatered treatment fell by day 14 and then increased through day 28 (105.64 ± 4.17 μmol·electrons m\(^{-2}\)s\(^{-1}\)).

### 3.5.6 Principal Component Analysis

Principal component analysis (PCA) of the data displayed correlations between analyzed values for each harvest date (Figure 3.7). As kale plants matured, tighter clusters of related parameters were analyzed for each date, explaining the parameters as a whole and not displaying treatment differences. On Day 0, the individual parameters are scattered, and only a few correlations are shown. The loading plot on Day 7 displays more closely groupings of parameters, including: xanthophyll concentrations (zeaxanthin, violaxanthin, and antheraxanthin) being correlated; remaining pigment concentrations being negatively correlated with \(g_{sw}\) and E; glucosinolate concentrations, shoot fresh mass, shoot dry mass, A, ETR, and \(\Phi_{PSII}\), and \(F_v/F_m\) being inversely correlated from NPQ and \(q_{N_{Fo}}\). By Day 14, the loading plot displayed a strong grouping of all plant pigment concentrations (excluding antheraxanthin) and the dry mass percentage being closely correlated and inversely correlated with xanthophyll ratio, raphanin, sinigrin, progoitrin, shoot fresh mass, shoot dry mass, and \(F_v/F_m\). The photosynthetic parameters on day 14 displayed a similar inverse correlation as ETR, \(\Phi_{PSII}\), \(q_{P_{Fo}}\), and A were opposite of \(q_{N_{Fo}}\) and NPQ, like in Day 7, with \(I_{WUE}\) being opposite of \(g_{sw}\) and E. The PCA analysis on day 28 showed a similar correlation of all plant pigments being all positively correlated excluding zeaxanthin, which was more correlated with shoot dry mass and negatively correlated with E, \(g_{sw}\), and progoitrin. Plant leaf A and \(\Phi_{PSII}\) were closely correlated with \(q_{P_{Fo}}\) and \(F_v/F_m\), and oppositely correlated with NPQ and \(q_{N_{Fo}}\).
3.6 Discussion

Plants subjected to the two extremes of water stress react with similar end results although the mechanisms used by plants to survive to differ. Brassica species respond to drought stress with reductions in photosynthetic performance and plant growth (Pan et al. 2011), reductions of some mineral nutrient contents (Pathirana et al. 2017; Ashraf et al. 2013), and varying concentrations of glucosinolates with lower fresh and dry masses (Shawon et al., 2020). Similarly, waterlogging of Brassica species leads to reductions in plant pigments (Lee et al. 2014; Men et al. 2020; Brazel et al. 2021), reductions in photosynthetic parameters (Issarakraisila et al. 2007; Lee et al. 2014), decreases in plant fresh mass (Issarakraisila et al. 2007; Brazel et al. 2021), and varying concentrations of individual glucosinolates (Khan et al. 2011; Brazel et al. 2021).

When plants are faced with drought, the generation of reactive oxygen species and decreased turgidity leads to accumulation of solutes, toxicity, and enzyme disruption, ultimately causing reduced photosynthesis and water use efficiency, and eventually death if prolonged (Joshi et al. 2016). On the opposite side, when plants are faced with waterlogging, anoxic conditions in the roots cause stomatal closing, reducing CO₂ intake and leading to reductions in photosynthesis and excessive light energy generating reactive oxygen species offering the same damaging effects observed by those caused by drought stress (Caudle and Maricle 2012). With both waterlogging and drought stress ultimately ending up with similar cellular damage within the plants, the results of our study compared to other Brassica studies analyzing mineral nutrient, glucosinolate, pigment concentrations are analogous with one other despite the different severities of stresses imposed in ours.
Overall, underwatering plants led to much lower fresh mass, reductions in plant morphological parameters, decreases in nutritionally essential plant pigments, elevated sulfur content in the roots, and greater photochemical energy wasted compared to optimally irrigated plants. The majority of the results observed do not differentiate from what was observed by the other studies previously mentioned when plants were exposed to waterlogging stress. Mineral nutrients in waterlogging have been shown to be influenced by waterlogging, and only sulfur was observed to be affected by our overwatering treatment (Boem et al. 1996). Zhao et al. 2008 identified that waterlogging stress leads to the upregulation of micro-RNAs that decrease the activity of enzymes and sulfur assimilation, resulting in a lower concentration of sulfur in the plant, yet sulfur concentrations were elevated, thus showing no hindrance to its assimilation in our study (Fukao et al. 2019).

Sulfur-containing secondary metabolites called glucosinolates are one use of sulfur in brassicas. It was observed that overwatering did not lead to differences among glucosinolate concentrations from that of the optimal treatment. Thus, the combination of a lack of glucosinolate production, reduced mass accumulation, and elevated transpiration may lead to the accumulation of sulfur within the roots. In combination with the accumulation of sulfur in the roots, there was also an observed increase in NPQ, or light energy released as heat. Rice plants that are deprived of sulfur and waterlogged have displayed elevated NPQ, but the elevated sulfur concentrations observed in the roots do not display that it was unavailable to the plant (Lunde et al. 2008). Li et al. (2007) displayed in turnip roots that both nitrogen and sulfur supply in the plant growth medium influence nutrient uptake in the plant and also glucosinolate concentration. Thus, the irrigation frequency of the overwatered treatment may have led to the slow-release fertilizer used to release more nutrients, as Groves et al. (1998) observed in container grown
plants and allowed greater uptake of sulfur into the plant. Furthermore, biochemical concentrations were not elevated under waterlogging treatment; the plant would no utilize the sulfur.

Like the overwatering treatment, the underwatering treatment had reductions in fresh mass and among plant morphological parameters compared to the optimally irrigated plants. However, the reduction of fresh mass, was less than that observed by the overwatered treatment, while the plant morphological reductions were equal to or slightly more than overwatered treatments. Different from the overwatered treatment, the underwatered treatment had greater glucosinolate concentrations and decreases in the concentration of mineral nutrients. The observed results of irrigation treatments, like that of the overwatering treatment, do not differ from what would be expected among drought-treated plants as water is essential to plant reactions and in facilitating the movement of nutrients into and throughout the plant. Additionally, the reductions in mineral nutrients were not among Ca, Fe, and Zn, which are notably consumed in kale, and the latter two, which are commonly deficient in human diets, especially in developing countries (Ahmad et al. 2022).

The increase in glucosinolate concentration, while not atypic among drought stress, is more prevalent to observe decreases or no influence on glucosinolate concentration, appearing to be influenced mainly be the severity and duration of water stress (del Carmen Martínez-Ballesta et al. 2013). It was observed that underwatering treatments led to greater concentrations of most glucosinolates, including both nutritionally favorable and unfavorable. Plants utilize glucosinolates as a defense mechanism against herbivory and as prevention of cellular damage under abiotic stress, like water stress (del Carmen Martínez-Ballesta et al. 2013). Glucosinolates have also been linked with enhancing guard cell activity during drought stress by improving
myrosinase activity (Zhao et al. 2008). While glucosinolates have both favorable and unfavorable types, there has not been sufficient evidence to conclude that the unfavorable types are anti-nutritional or are linked with carcinogenic properties (Shapiro et al. 2006; Cartea and Velasco 2007). Thus, increases in glucosinolate concentration caused by underwatering would increase the overall nutritional content of the plants, making consumption more favorable. However, increases in glucosinolate would also increase the bitter taste of the resulting kale and may not be as palatable to consumers (Cartea and Velasco 2007).

Like glucosinolates, chlorophylls and carotenoid pigments are nutritionally essential secondary biochemicals. Former studies have identified that changes in plant nutrition impact pigment concentrations in and as discussed earlier, can be influenced by moisture availability to plants (Kopsell et al. 2007). Both chlorophylls and carotenoids are important in plant and human health as they offer oxidative stress management through scavenging reactive oxygen species; thus, elevated concentrations can be found in plants experiencing oxidative stress, which may result from water stress (Langi et al. 2018; Mordi et al. 2020). However, overwatered plants experienced significant reductions in pigment concentrations, and is an observed trend in plants facing waterlogging in Chinese cabbage (Šola et al. 2021), lucerne (Smethurst and Shabala 2003), and other Brassica species (Lee et al. 2014; Men et al. 2020; Brazel et al. 2021). This decrease in pigment concentrations may result from the inhibition of pigment production, like the downregulation of abscisic acid production, a carotenoid derivative, observed in waterlogging stress (Simpson et al. 2018; Šola et al. 2021).

Decreased concentrations of carotenoids and chlorophylls are a typical response when plants are subjected to drought stress, as observed in mung bean (Batra et al. 2014) and kale (Barickman et al. 2020). However, the carotenoid and chlorophyll concentrations of Chinese
cabbage did not change or increase under drought stress (Šola et al. 2021), which was also observed in maize (Schlemmer et al. 2005) and moringa (Nouman et al. 2018). The increased pigment concentrations observed may be caused by high demand and upregulation in abscisic acid production being used to respond and confer tolerance in response to drought stress (Thompson et al. 2007; Simpson et al. 2018).

Although no treatment differences were identified to be correlated entirely with specific parameters on the PCAs conducted, it was identified that the shift in parameter correlations changed based on harvest date, displaying the influence that maturity has on kale plants. Overlaying the loading and score plots onto a biplot on Day 7 identified that some separation of treatments may be present, with overwatering samples being correlated with elevated dry mass percentage, stomatal conductance and transpiration while oppositely correlated with decreases in pigments, glucosinolates, and other photosynthetic parameters yet some overwatered samples do not follow this observation. Larger groupings of observations on Days 14 and 28 display that there may be some trends from treatments, yet they also display a similar trend as displayed on Day 7 with some non-parallel observations.

The results of our PCA lack sufficient evidence of treatment effects explained by the components may be a result from measured parameters being poor at showing direct trends. El-Nakhel et al. (2020) identified in lettuce that only plant pigments showed a strong correlation with maturity in red lettuce, while green types of lettuce, regardless of age, did not display major differences in concentration. As ‘Winterbor’ is a blue-green kale, the lack of strong reactions by plant pigments in our study to separate treatments may align with those results found by El-Nakhel et al. (2020) who did see changes in pigment concentrations of green lettuce as they matured yet were much less than the changes of pigment concentrations observed within red
lettuce. This observation that the parameters used may be poor at showing the treatment trends in
the PCA analysis may also be due to the treatments applied to the plants being a minimal
influence, but this does not indicate the treatments were less impactful on the plant.

### 3.7 Conclusion

Trends among brassica crops in response to different water stress leads to fluctuations
among mineral nutrients, glucosinolates, photosynthesis, chlorophyll fluorescence, and plant
morphology. While overwatering and underwatering are far from the respective ends of the water
stress spectrum, small changes in water availability have been shown to elicit similar responses
as waterlogging and drought in container-grown kale using sensor-based irrigation. When
overwatering kale, it was observed that the overall nutritional value of the crop was reduced
among leaf tissues compared with optimally treated plants while yielding significantly reduced
consumable fresh mass. Underwatering kale although reducing fresh mass and major decreases
in mineral nutrient content among leaf and root tissues, also led to increases or no impact on
nutritionally favorable secondary metabolites and mineral nutrients.

Although both treatments led to reductions in plant growth, the impacts of overwatering
appear to be equally impactful as underwatering yet may be more nutritionally detrimental to the
final consumable product. However, the dietary availability of the nutritionally critical secondary
metabolites analyzed was not considered. It may further support our findings that overwatered
plants may be less nutritionally rich than underwatered plants. Further studies on small water
stress treatments in kale should consider analyzing if treatments impact the palatability or the
nutritional availability of the product to consumers.
Figure 3.1  The influence of overwatered, optimal, and underwatered treatments on the dry mass percent and fresh mass of kale leaf tissue.

The dry mass percentage (1A) and fresh mass (1B) of kale leaf tissue per plant when subjected to overwatered, optimal, and underwatered irrigation treatments for 0, 7, 14, and 28 days Statistical summary can be seen in Table 3.1.
Figure 3.2  The plant height, petiole length, leaf width, leaf length, and leaf number of kale plants when subjected to overwatered, optimal, and underwatered irrigation treatments after 28 days of treatment.

Statistical summary can be seen in Table 3.1.
Figure 3.3  The concentrations of four glucosinolate extracted from kale leaf tissues when subjected to overwatered, optimal, and underwatered irrigation treatments for 0, 7, 14, and 28 days.

The concentration of glucoprogoitrin (3A), glucoepiprogoitrin (3B), glucoraphanin (3C), and sinigrin (3D). Statistical summary can be seen in Table 3.1.
Figure 3.4  The impact of overwatered, optimal, and underwatered irrigation treatments on chlorophyll and carotenoid concentrations for 0, 7, 14, and 28 days.

The total chlorophyll (4A), lutein (4B), and β-carotene (4C) concentrations of extracted plant pigments from kale leaf tissue. Statistical summary can be seen in Table 3.1.
Figure 3.5  The effect of overwatered, optimal, and underwatered irrigation treatments on plant photosynthetic parameters after 0, 7, 14, and 28 days of treatment.

The transpiration rate (5A), assimilation rate (5B), stomatal conductance (5C), and intrinsic water use efficiency (5D) measured from kale leaves. Statistical summary can be seen in Table 3.1.
Figure 3.6 Fluorometric parameters measured from kale leaves when subjected to overwatered, optimal, and underwatered treatments for 0, 7, 14, and 28 days.

Dark-adapted, maximum quantum yield of photosystem II photochemistry ($F_v/F_m$; 6A) light-adapted, effective quantum yield of photosystem II photochemistry ($\Phi_{PSII}$; 6B), electron transport rate (ETR; 6C), non-photochemical quenching (NPQ; 6D), alternative non-photochemical quenching ($q_{N,Fo}$; 6E), and photochemical quenching ($q_{P,Fo}$; 6F). Statistical summary can be seen in Table 3.1.
Figure 3.7  Biplots of kale plants subjected to overwatered, optimal, and underwatered irrigation treatments after PCA. 
Biplots were of parameters measured on day 0 (7A), day 7 (7B), day 14 (7C), and day 28 (7D).
### 3.9 Tables

**Table 3.1** Statistical summary of the effects of irrigation treatments (Trt), harvest date (Harv), and the interaction (Trt*Harv) on plant morphology, phytonutrients, photosynthesis, and fluorometric parameters of container grown kale.

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Fresh Mass</th>
<th>Dry Mass (%)</th>
<th>Height</th>
<th>Petiole Length</th>
<th>Leaf Width</th>
<th>Leaf Length</th>
<th>Leaf Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trt</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.002</td>
<td>0.0019</td>
<td>0.0120</td>
<td>0.0002</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Harv</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trt*Harv</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phytonutrients</th>
<th>Progoitrin</th>
<th>Epiprogoitrin</th>
<th>Raphanin</th>
<th>Sinigrin</th>
<th>Total Chl</th>
<th>Lutein</th>
<th>β-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trt</td>
<td>0.1327</td>
<td>0.0004</td>
<td>0.0060</td>
<td>0.0876</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Harv</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trt*Harv</td>
<td>0.1790</td>
<td>0.0076</td>
<td>0.0005</td>
<td>0.0004</td>
<td>0.0038</td>
<td>0.0020</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Photosynthesis and Fluorescence</th>
<th>E</th>
<th>gsw</th>
<th>A</th>
<th>I_wue</th>
<th>Fv/Fm</th>
<th>Φ_PSIi</th>
<th>ETR</th>
<th>NPQ</th>
<th>qN_Fo</th>
<th>qP_Fo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trt</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0817</td>
<td>0.1268</td>
<td>0.1270</td>
<td>0.1878</td>
<td>0.2895</td>
<td>0.0048</td>
<td>0.0427</td>
<td>0.9914</td>
</tr>
<tr>
<td>Harv</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Trt*Harv</td>
<td>0.0216</td>
<td>0.0259</td>
<td>0.0002</td>
<td>0.1379</td>
<td>0.1380</td>
<td>0.0445</td>
<td>0.0275</td>
<td>0.0029</td>
<td>0.1037</td>
<td>0.9728</td>
</tr>
</tbody>
</table>

1Dry Mass (%) – Dry Mass Percentage
2Height, Petiole Length, Leaf Width, Leaf Length, and Leaf Number only were taken on Day 28 of treatment.
3Progoitrin – Glucoprogoitrin, Epiprogoitrin – Glucoepiprogoitrin; Raphanin – Glucoraphanin; Total Chl – Total Chlorophylls: Sum of Chlorophyll A and Chlorophyll B.
4E – transpiration rate; gsw – stomatal conductance; A – assimilation; I_wue – intrinsic water use efficiency; Fv/Fm – dark-adapted, maximum quantum yield of photosystem II; Φ_PSIi – light-adapted, effective quantum yield of photosystem II photochemistry; ETR – electron transport rate; NPQ – Non-photochemical quenching; qN_Fo – alternative non-photochemical quenching; qP_Fo – photochemical quenching.
Table 3.2  The mineral nutrient concentrations of boron (B) and molybdenum (Mo) extracted from kale leaf tissues after being subjected to overwatered, optimal, and underwatered irrigation treatments for 28 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (μg·g⁻¹ dry mass)</th>
<th>B</th>
<th>Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overwatered</td>
<td>14.074 a</td>
<td>2.7181 a</td>
<td></td>
</tr>
<tr>
<td>Optimal</td>
<td>16.185 a</td>
<td>2.3876 a</td>
<td></td>
</tr>
<tr>
<td>Underwatered</td>
<td>9.66 b</td>
<td>1.0522 b</td>
<td></td>
</tr>
</tbody>
</table>

P-Value a,b  
***  ***

Values in column followed by the same letter are not different at α=0.05.
Non-significant mineral nutrients in shoots were not included in the table. Non-significant mineral nutrients were magnesium, phosphorus, sulfur, potassium, manganese, iron, copper, and zinc.

a *** indicate significant at P≤0.001.
b The standard error of the mean was: B - 1.418; Mo - 0.297.

Table 3.3  The mineral nutrient concentrations of boron (B), magnesium (Mg), phosphorus (P), sulfur (S), potassium (K), and molybdenum (Mo) extracted from kale root tissues after being subjected to overwatered, optimal, and underwatered irrigation treatments for 28 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (μg·g⁻¹ dry mass)</th>
<th>B</th>
<th>Mg</th>
<th>P</th>
<th>S</th>
<th>K</th>
<th>Mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overwatered</td>
<td>5.8295 a</td>
<td>2457.8 a</td>
<td>3027.8 a</td>
<td>4868.5 a</td>
<td>18320 a</td>
<td>1.2321 a</td>
<td></td>
</tr>
<tr>
<td>Optimal</td>
<td>5.4671 a</td>
<td>2179.5 ab</td>
<td>3350 a</td>
<td>4120.8 b</td>
<td>16996 a</td>
<td>1.1602 a</td>
<td></td>
</tr>
<tr>
<td>Underwatered</td>
<td>4.1432 b</td>
<td>1865.9 b</td>
<td>2481.2 b</td>
<td>3588.2 b</td>
<td>14001 b</td>
<td>0.6815 b</td>
<td></td>
</tr>
</tbody>
</table>

P-Value a,b  
*  **  ***  **  **  **

Values in column followed by the same letter are not different at α=0.05.
Non-significant mineral nutrients in roots were not included in the table. Non-significant mineral nutrients were calcium, manganese, iron, copper, and zinc.
a *, ** indicate significant at P≤0.05, significant at P≤0.01, respectively.
b The standard error of the mean was: B- 0.433; Mg- 113.72; P- 193.2; S- 259.4; K- 884.6; Mo – 0.127
3.10 References


CHAPTER IV
SUMMARY OF WORKS

4.1 Summary

Predicted changes in climate change by the Intergovernmental Panel on Climate Change (IPCC) are of great concern for global crop production. Predicted changes in precipitation events and heat-induced drought events when combined with regional and subregional variations make it difficult to prepare for climate change even with recent infrastructure development between water deliverance from sources to crops and water dispersal away from crops. Negative consequences have arisen from the over-extraction of water and contamination of water sources due to over-fertilization and run-off. Even efficient and effective manners of irrigation can arise future challenges like salinization of soils and over-accumulation of plant exudates in the rhizosphere.

Regional and sub-regional variation for precipitation changes over the past century is well observed across the United States for precipitation but does not provide enough explanation for the seasonal and annual perturbations in precipitation for sub-regions like Verona, Mississippi. Evidence of increasing precipitation is evident from weather data collected at the North Mississippi Research and Extension Center in Verona. The weather data identifies while the average precipitation per event is not increasing per annum, the increase in annual precipitation is due to an increase in the total number of precipitation events per year. However, year-to-year fluctuations are greatly influenced by El Niño and La Niña and monthly
precipitation, while nearly constant month-to-month in Verona, is influenced by temperature and light incidence.

Kale is a highly nutritious crop, containing pronounced levels of vitamins, mineral nutrients, and secondary metabolites that are essential in the human diet. Early research on kale focused heavily on genetics, insect herbivory resistance, and transgenics moving towards a more specialized focus on nutritional components like pigments, mineral nutrition, and its anti-carcinogenic properties. Most recently, kale research has focused on improving its production, intercropping capabilities, and the influence of LED lighting on its growth and phyto-nutrition. Over the nearly 75 years of cited research, the impact of abiotic stressors on kale production and nutritional content has not been studied intensively, hence the importance of this research.

The results of the studies discussed in this thesis identify that kale’s response to various water stresses does not differentiate far from what is observed among other Brassicaceae crops in other studies. These studies focused on three aspects of water stress: waterlogging is the complete submergence of root systems in water; overwatering is the over-application of water to roots while allowing full drainage and aeration of media; and underwatering is the underapplication of water to roots never allowing significant saturation of media.

Waterlogging plants resulted in a decrease in mass, a decrease in chlorophyll and carotenoid concentrations, and a slight elevation in nutritionally favorable and unfavorable glucosinolate concentrations. These results directly coincide with other studies and display a challenge to producers where temporal waterlogging may happen due to heavy precipitation events or over-irrigation. Although the focus of this project was to identify the impacts during waterlogging, the resultant impact on mass and secondary metabolites displays prolonged consequences to plant health and older stages of growth and development. Future research
should focus on the impact waterlogging juvenile kale has on the later stages of growth and whether plants that are more established have greater tolerance or susceptibility to stress.

Waterlogging, flooding, and drought stress are extreme cases of water-stress events. Yet, modern irrigation and agricultural practices have reduced the frequency of extreme events, but minor fluctuations from optimal moisture levels do happen regularly and are investigated in the second study. While overwatering and underwatering are far from the respective ends of the water stress spectrum, small changes in water availability have been shown to elicit similar responses as waterlogging and drought in container-grown kale using sensor-based irrigation. The response of plants to small changes displays the complex nature of plants and identifies that small differences can cause large impacts on crop production. While it is not ideal to overwater or underwater crops to any degree, the results in the second project display that it may be beneficial to underwater crops being less nutritionally detrimental to the final product. However, the dietary availability of nutritional compounds quantified and evaluated are apparent and not directly correlated, requiring further and more specialized analyses not capable within the facilities of the project. Producers should be concerned with overwatering and underwatering of kale, which is common and easily conducted, in different production systems. Future studies to better understand the impact of small changes in water availability should focus on the palatability and actual nutrient availability to consumers whilst considering how these are impacted by water stress in different production systems.
APPENDIX A

CHAPTER III ADDITIONAL TABLES
Table A.1  The concentration of glucosinolates of kale leaves harvested from overwatered, optimal, and underwatered plants after 0, 7, 14, and 28 days of irrigation treatment.

<table>
<thead>
<tr>
<th>Day</th>
<th>Concentration (mg: g(^{-1}) dry mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iberin</td>
</tr>
<tr>
<td>-----</td>
<td>--------</td>
</tr>
<tr>
<td>Overwatered</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>7</td>
<td>0.010</td>
</tr>
<tr>
<td>14</td>
<td>0.012</td>
</tr>
<tr>
<td>28</td>
<td>0.005</td>
</tr>
<tr>
<td>Optimal</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>7</td>
<td>0.018</td>
</tr>
<tr>
<td>14</td>
<td>0.044</td>
</tr>
<tr>
<td>28</td>
<td>0.021</td>
</tr>
<tr>
<td>Underwatered</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>7</td>
<td>0.031</td>
</tr>
<tr>
<td>14</td>
<td>0.009</td>
</tr>
<tr>
<td>28</td>
<td>0.019</td>
</tr>
</tbody>
</table>

| SE\(^4\) | 0.006 | 0.061 | 0.015 | 0.986 | 0.230 | 0.889 | 1.137 | 1.754 |

| Trt   | 0.0015 | 0.0031 | 0.8623 | <0.0001 | 0.0002 | 0.0467 | <0.0001 | <0.0001 |
| Harv  | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Trt*Harv | 0.0007 | <0.0001 | 0.9893 | 0.0003 | 0.0044 | 0.0225 | 0.0003 | 0.0006 |

\(^1\)Day- represents the concentration of glucosinolates 0, 7, 14, and 28 days of respective irrigation treatment

\(^2\)Iberin – Glucoiberin; Napin – Gluconapin; Barbarin – Glucobarbarin; Brassicin – Glucobrassicin; Neo-Brassicin – Neoglucobrassicin

\(^3\)Total – The sum of glucosinolates including glucoprogoitrin, glucoepiprogoitrin, glucoraphanin, and sinigrin in from Figure 3.3.

\(^4\)SE – The Standard Error of the Mean

Values followed by the same letter within each column are not significantly different from one another.
The concentration of plant pigments of kale leaves harvested from overwatered, optimal, and underwatered plants after 0, 7, 14, and 28 days of irrigation treatment.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>81.87 F</td>
<td>47.49 E</td>
<td>8.74 E</td>
<td>37.55 F</td>
<td>275.08 F</td>
<td>1616.08 G</td>
<td>93.77 F</td>
<td>0.502 A</td>
</tr>
<tr>
<td>7</td>
<td>106.50 F</td>
<td>67.81 ED</td>
<td>13.93 D</td>
<td>40.79 EF</td>
<td>387.66 E</td>
<td>3546.95 F</td>
<td>122.53 EF</td>
<td>0.472 A</td>
</tr>
<tr>
<td>14</td>
<td>294.88 B</td>
<td>207.67 AB</td>
<td>23.99 B</td>
<td>62.31 ABC</td>
<td>927.24 B</td>
<td>9520.22 ABC</td>
<td>293.97 ABC</td>
<td>0.300 B</td>
</tr>
<tr>
<td>28</td>
<td>232.16 C</td>
<td>172.86 BC</td>
<td>24.54 B</td>
<td>54.41 CD</td>
<td>674.37 C</td>
<td>6040.48 D</td>
<td>251.82 D</td>
<td>0.313 B</td>
</tr>
</tbody>
</table>

**Overwatered**

| 0   | 81.87 F | 47.49 E | 8.74 E | 37.55 F | 275.08 F | 1616.08 G | 93.77 F | 0.502 A |
| 7   | 183.62 D | 94.56 D | 18.54 C | 49.00 ED | 631.42 C | 5522.59 DE | 162.10 E | 0.467 A |
| 14  | 335.10 A | 195.89 AB | 26.17 B | 57.56 BCD | 1061.05 A | 10656.24 A | 279.62 BCD | 0.333 B |
| 28  | 291.86 B | 217.35 A | 31.48 A | 66.08 AB | 912.89 B | 8420.64 C | 314.91 AB | 0.327 B |

**Optimal**

| 0   | 81.87 F | 47.49 E | 8.74 E | 37.55 F | 275.08 F | 1616.08 G | 93.77 F | 0.502 A |
| 7   | 143.85 E | 66.11 DE | 17.61 CD | 40.85 EF | 498.75 D | 4491.93 EF | 124.58 FE | 0.490 A |
| 14  | 340.36 A | 159.50 C | 32.52 A | 70.13 A | 1042.08 A | 9725.04 AB | 262.16 CD | 0.486 A |
| 28  | 294.49 B | 227.82 A | 30.46 A | 65.64 AB | 915.65 B | 9063.97 BC | 323.92 A | 0.297 B |

**Underwatered**

| 0   | 81.87 F | 47.49 E | 8.74 E | 37.55 F | 275.08 F | 1616.08 G | 93.77 F | 0.502 A |
| 7   | 143.85 E | 66.11 DE | 17.61 CD | 40.85 EF | 498.75 D | 4491.93 EF | 124.58 FE | 0.490 A |
| 14  | 340.36 A | 159.50 C | 32.52 A | 70.13 A | 1042.08 A | 9725.04 AB | 262.16 CD | 0.486 A |
| 28  | 294.49 B | 227.82 A | 30.46 A | 65.64 AB | 915.65 B | 9063.97 BC | 323.92 A | 0.297 B |

| SE  | 9.88  | 12.68 | 1.38  | 3.56  | 36.25  | 429.12 | 14.6   | 0.027  |
| Trt | <0.0001 | 0.1864 | <0.0001 | 0.1364 | <0.0001 | <0.0001 | 0.1048 | 0.0402 |
| Harv.| <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| Trt*Harv.| 0.0011 | 0.0068 | 0.0071 | 0.0502 | 0.0028 | 0.0037 | 0.0115 | 0.0026 |

1Xan. Ratio does not have units
2Day – harvest date: 0, 7, 14, and 28 days of irrigation treatment
3Neo – Neoxanthin; Viol – Violaxanthin; Anth – Antheraxanthin; Zea – Zeaxanthin; Chl. B – Chlorophyll B; Chl. A – Chlorophyll A; Ttl. Xan – Total Xanthophylls; Xan. Ratio – Xanthophyll Ratio
4Xan. Ratio is calculated as (Zea + Anth / Zea + Anth + Viol)

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5SE – The Standard Error of the Mean
Values followed by the same letter within each column are not significantly different.