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EVALUATION OF PLANT HORMONES ON HERBICIDE SAFETY IN SWEETPOTATO
(*Ipomoea batatas* L.)

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the
Degree Master of Science
Plant and Environmental Science

by
Giovanni Antoniaci Caputo
August 2021

Accepted by:
Dr. Matthew Cutulle, Committee Chair
Dr. Lambert McCarty
Dr. Jeffrey Adelberg
Dr. Phillip Wadl

ABSTRACT

Sweetpotato [*Ipomoea batatas* L. (Lam)] growers need new technologies to manage weeds as current strategies rely on combinations of pre-emergent (PRE) or cultivation, which do not provide season long weed control. A label expansion of postemergence (POST) herbicides would be extremely beneficial to farmers and could improve weed management in sweetpotato.

Three studies were conducted at the Clemson University Coastal Research and Education Center from January 2019 to November 2020 to investigate the ability of plant hormones and ascorbic acid (AsA) to improve sweetpotato tolerance to applications of POST herbicides. An in vitro study tested bentazon injury on three different sweetpotato cultivars in an in vitro environment. The most tolerant cultivar was used in an additional experiment, where exogenous melatonin was added into the media to evaluate its ability to reduce herbicide damage. A dose-response study of two sweetpotato cultivars to increasing rates of bentazon and mesotrione was conducted. Additionally, the ability of plant hormones and AsA to reduce herbicide injury when added to the tank-mix with those two herbicides was investigated. Furthermore, the interaction between POST herbicides and additional compounds to control troublesome weeds commonly found in sweetpotato production was evaluated. A field trial study was conducted in two locations in the state of South Carolina to investigate bentazon injury, yellow nutsedge (*Cyperus esculentus* L.) control, and final yield, with or without the addition of plant hormones.

The first study identified the sweetpotato cultivar ‘Beauregard’ as having greater tolerance to bentazon applications when compared to the other cultivars. Additionally, melatonin reduced plant injury caused by bentazon. Specifically, melatonin incorporated at 0.1 and 1mM resulted in 32% less injury than bentazon alone and 50% more plant mass when melatonin was added to protected plants from herbicide oxidation.

The greenhouse experiment highlighted the ability of melatonin, 24-Epibrassinolide and AsA to reduce bentazon and mesotrione injury in ‘Beauregard’ and ‘Covington’ sweetpotato. Beauregard treated with bentazon and mesotrione had injuries levels of 63% and 76%, respectively. Tank-mixing herbicides with melatonin had 7% and 48% less injury compared to herbicides alone. Additionally, the compounds did not reduce control of yellow nutsedge from bentazon and control Palmer amaranth (*Amaranthus palmeri* L.) from mesotrione.

In field trials tank mixing bentazon with plant hormones was evaluated. At both locations, bentazon + plant hormones resulted in 50% and 74% more yield of marketable roots than bentazon alone and the non-treated check, in Cameron and Charleston, respectively. For herbicide injury, bentazon applied alone reduced the final yield by 50% in Cameron and 79% in Charleston, compared to tank-mix of bentazon + plant hormones.

These studies suggest plant hormones and ascorbic acid reduce sweetpotato injury to POST mesotrione and bentazon applications. These compounds did not reduce yellow nutsedge and Palmer amaranth control.

DEDICATION

I dedicate this thesis to my wife, Tatielen de Fatima Marafao Caputo. She has been a constant source of support and encouragement during the challenges of graduate school and life. I am genuinely thankful for having you in my life.

To my parents Mr. Marco Aurelio Caputo and Mrs. Shirley Antoniaci Caputo, for all their love and teachings. To my brother and sister-in-law that deserve my thanks as well. And finally, to my niece Larrisa Beatriz Caputo, that during her brief journey on earth, brought joy and love to us.

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I would also like to express my gratitude to The Agricultural Society of South Carolina. The society played a critical role in funding this project.

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CHAPTER ONE

Literature Review

Sweetpotato origin and botany

Sweetpotato [*Ipomoea batatas* (L.) Lam] originates from southern Central America to the north–west of Latin America. (Hawkes, 1989). West Indies explorers introduced it to North America and initial sweetpotato production activities were documented in Virginia (1648), Carolinas (1723), and New England (1764) (Smith et al., 2009). According to the Department of Statistics of the Food and Agriculture Organization (FAO)(2011), world production is 104 million tons. The highest production is from China, Africa, and the Americas, with 80%, 16%, and 3%, respectively. The crop is mainly grown for homestead food and feed use and sale to local markets for fresh consumption.

Linnaeus first described sweetpotato in 1753 using the binomial system as *Convolvulus batatas* (Huaman, 1992). In 1791, Lamarck re-classified the species within the genus *Ipomoea* based on stigma shape and the surface of pollen grains, changing the name to its current form, *Ipomoea batatas* (L.) Lam. (Huaman, 1992). The internationally accepted version of the common English name is now the one-word spelling ‘sweetpotato’ (Lewthwaite, 2004), rather than the older version ‘sweet potato’. The rationale behind this was to distinguish it from the tuberous potato (*Solanum tuberosum* L.) (Lewthwaite, 2004)

Sweetpotato is a dicotyledonous plant from the family Convolvulaceae. Sweetpotato is herbaceous and generally cultivated as an annual with a relatively short production season, generally between 3-4 months. The stems' length varies between 0.5 and 5 m long depending on the variety (Martin, 1985). Leaves are up to 15 cm long and pointed at the tip, are spirally

arranged, and have long petioles of 5-30 cm. Leaf shape is either cordate, entire, or lobed. Flowers can be violet or white and are hermaphroditic but rarely self-fertile. The corolla is 2.5 - 5 cm long. The plant has two types of roots: the reserved root, the one with commercial interest, and absorbent roots, responsible for water and nutrients uptake (Soares et al. 2002).

The tubers form where the roots curve downwards after growing horizontally. Tuber number and size and the color of the skin and flesh vary according to cultivars. Their weight usually is between 0.2 and 3 kg, and their number from two to five or more per plant (Miranda 2005). The number of tuberous roots is a major yield component. Root tubers can have different colors, such as white, yellow, orange, purple, or brown skin surface, and the flesh is white, yellow, orange, red, or purple. Most cultivars attain maximum storage root yield in 12 to 22 weeks after transplanting. The vegetative stage can be between 4 months or as much as 12, depending on the variety and the climate (Ravi and Indira, 1999). Conventionally sweetpotato is propagated using stem cutting fragments 20-30 cm long with 3-5 nodes. Planting material composes of vines, sprouts, and root cuttings. Plant density per hectare is depending on cultivar, but it usually requires 400,000-1,250,000 vines (Du Plooy et al., 1988).

In the US, the most grown sweetpotato cultivar is Covington. This cultivar was developed in 1997 by the North Carolina Department of Agriculture and Consumer Service's (NCDA&CS) Central Crops Research Station in Clayton, Johnston County, NC. 'Covington' leaves are cordate to triangular in shape with no to slight lobing. Flowers can sporadically mature throughout the season in response to environment. The corolla is composed of five fused petals that form a funnel with a pentagonal limb. Storage roots of 'Covington' are oblong, to elliptic. Storage root skin color varies from light to medium rose and flesh color is a uniform orange. This cultivar exhibits resistance to southern rootknot (*Meloidogyne incognata* Kofoid and White)

nematode and to fusarium wilt or stem rot caused by *Fusarium oxysporum* Snyder and Hansen. With high-yield characteristics, ‘Covington’ typically performs better in the southeast leading to it being one of the world's most popular cultivars. In the state of North Carolina, this cultivar has been reported to yield 40ton ha⁻¹, where 56% of those tubers are classified as No 1’s (Yencho et al. 2008).

US sweetpotato production – A weed science perspective

In the US, sweetpotato production has increased since the last Census, reaching an estimated value of \$654 million in 2018 (USDA 2019a). Most of the production is located in the southern region. A small number of states are responsible for most of sweetpotato produced in the US (USDA 2019b) including North Carolina, Louisiana, Mississippi, California, and South Carolina, respectively. Growers' primary concern in sweetpotato production is related to weed management.

The Southeastern region of the US has a unique environment. The area is highly influenced by the strength and position of the Atlantic subtropical high and moisture-laden air from the Gulf of Mexico. Those influences are responsible for the generation of heatwaves and significant rainfall events. Additionally, the region receives heavy rainfall from mid-latitude systems tracking in from the west during winter and early spring (Kunkel et al., 2013). As a consequence, the annual precipitation rates for the region are high. The state of South Carolina has a yearly precipitation average above the national mean, ranging from 1000 to 2000 mm per year (South Carolina Department of Natural Resources), making the management of insects, weeds, and pathogens challenging.

Weed infestation can be influenced by abiotic factors, impacting weed germination and plant development (Singer et al., 2013). Gardarin et al. (2010) reported a correlation between soil temperature and soil water potential on weed germination where the base temperature of spring-emerging species was highly correlated with the high mean soil temperature. Weeds can negatively impact vegetable production, making their management crucial for agricultural production (Utstumo et al., 2018). Weeds directly compete with the crop for light, water, nutrients, space, and other environmental factors.

Additionally, weeds are responsible for harboring crop pests and plant pathogens. Numerous estimations of the total crop yield losses from weeds exist, however this varies among investigators. Parker and Frayer (1975) reported weeds could cause up to 14% losses on yield. A more recent study analyzed crop production in the U.S. and estimated weeds cause \$19 billion from yield loss (Bridges, 1992).

The negative effect of weeds on sweetpotato production is well documented. Palmer amaranth (*Amaranthus palmeri* S. Wats) is one of the most common and troublesome weeds in sweetpotato production. Meyers et al. (2010) reported this weed could reduce the percentage of jumbo no. 1 and marketable sweetpotato yield. Large crabgrass (*Digitaria sanguinalis* L. Scop) is a common weed found in sweetpotato fields (Webster, 2010). Basinger et al. (2019) reported that season-long competition from large crabgrass reduced sweetpotato total yield by 35% to 76%. Yellow nutsedge (*Cyperus esculentus* L.) is another problematic weed in sweetpotato production (Saha et al., 2019). Meyers and Shankle (2015) observed sweetpotato yields can be reduced by 80% due to competition from yellow nutsedge. Additionally, yellow nutsedge shoots can grow through the sweetpotato, reducing their market value.

Currently, weed management for sweetpotatoes is largely dependent on between-row cultivation, mowing, and hand removal. While herbicide options are limited in sweetpotato. To achieve reasonable control of weeds using herbicides, farmers must have a well-planned herbicide program. Preemergence (PRE) herbicides are applied to the soil before the weed emerges. PRE herbicides typically do not provide season-long control of weeds. These herbicides typically control emerging weeds, alleviating stress on the early crop by reducing competition for water, nutrients, and light. In contrast, herbicides labeled for postemergence (POST) control of weeds are applied after weed germination and can be used later into the season. However, no products are currently available to control broadleaves weeds and sedges POST in sweetpotato production, making weed management challenges in this crop.

Meyers et al. (2013) noted the limited options for selective POST herbicides available in sweetpotato, which lead to increased tillage and the use of non-selective herbicides in directed applications. Herbicides providing POST control of broadleaves weeds are solely labeled for between-row applications due to the risk of damaging leaves contacted during application.

Injury caused by herbicides sprayed in sensitive crops can negatively impact the yield. Injury caused by *S*-metolachlor on sweetpotato was reported by Meyers (2012), where applications immediately after transplanting resulted in decreased no. 1 and marketable sweetpotato yields and decreased storage root length to width ratio. Cutulle et al. (2020) reported a lower tolerance of 'Centennial' sweetpotato to *S*-metolachlor in a hydroponic scenario. At the same time, Lindley et al. (2020) highlighted injuries and plant stunting in two sweetpotato cultivars treated with bicyclopyrone, with an antagonistic relationship of vine length and plant dry mass, according to higher herbicide doses. The effect of bentazon on sweetpotato was reported by Motsenbocker and Monaco (1991). They highlighted the different levels of tolerance

between cultivars and bentazon's capacity to cause moderate to high injury. Caputo et al. (2020) reported significant injury from bentazon applied in-vitro in three sweetpotatoes cultivars. Among the cultivars, 'Beauregard' generally exhibited greater tolerance to herbicides. The tolerance of cultivars to clomazone applications was evaluated by Porter (1990), who reported minor injuries on 'Beauregard'. Similar results were reported by Cutulle et al. (2020), who documented that 'Beauregard' had better tolerance to *S*-metolachlor when compared to 'Centennial' sweetpotato in a hydroponics assay. Wadl et al. (2020) also reported the tolerance of 'Beauregard' to clomazone.

Safener mechanisms

Ideally, a safener would improve crop tolerance to POST herbicides without reducing activity in the target weed (Davies and Caseley 1999). The first reported safener was discovered by accident when tomato plants exhibit an antagonistic interaction between the herbicide 2,4-D and the herbicide 2,4,5-T (Hoffman, 1953). Safener mechanisms in plants include (1) competition for the binding site with herbicides; (2) regulating the herbicide uptake and/or transportations; (3) enhancement of herbicide detoxification; and (4) combinations of the above mechanisms (Hatzios and Hoagland, 1989).

Several compounds appear to have a very specific crop-herbicide-safener interaction, suggesting they might counteract herbicides at the site of absorption or action (Gray and Joo, 1976). The ability of safeners to cause an alteration on herbicide uptake and/or translocation is specific between compounds and related to local herbicide entry (Ezra et al. 1982). This can be direct, where there is a competition for binding sites or antagonistic interaction between herbicide and safener. The mechanism by which safeners increase plants' ability to detoxify xenobiotics is much less specific. The protective ability is provided by enhancing the expression

of genes encoding herbicide-metabolizing essential enzymes, such as the glutathione S-transferases (GSTs), cytochrome P450 monooxygenases (P450s), for the herbicide detoxification process (Hatzios, 1989; Riechers et al., 2005).

Herbicide detoxification is an internal process leading to the inactivation of the herbicide, which happens in three phases. Phase I, also known as a conversion, is catalyzed by cytochrome P450s enzymes. The process starts with a chemical modification on the herbicide active ingredient, such as oxidation, reduction, hydrolysis, oxygenation, or hydroxylation, when functional groups (OH, NH₂, COOH) are introduced, making the molecule more hydrophilic, and thus less phytotoxic (Yuan et al., 2007). In Phase II, the modified herbicide molecule from the previous phase is conjugated with endogenous substrates, such as sugars, amino acids, or Glutathione (GSH), increasing the herbicide molecule solubility and reducing toxicity. The main enzymes responsible for conjugation with GSH are the glutathione *S*-transferases (GSTs) (Eerd et al., 2003). In phase III, the metabolites derived from Phase II are actively transported to the vacuole by ATP binding cassette (ABC) transporters (Yuan et al., 2007).

P450s and GST enzymes are essential in the herbicide detoxification process. P450s have been reported to modulate multiple metabolic pathways in plants. Many of them are responsible for improving the plant defense system against biotic and abiotic factors (Xu et al. 2015), although their oxidative and reductive capacity make these stand out on the herbicide detoxification pathway. Dubleman et al. (1997) reported the ability of the safener furilazole to enhance P450 activation, resulting in the de-esterification of halosulfuron-methyl to halosulfuron acid in corn seedlings. Plant GSTs are a superfamily of multifunctional enzymes responsible for catalyzing the conjugation of electrophilic xenobiotic substrates with GSH (Dixon et al. 2010). Multiple studies noted GSTs could increase plant immune response and increase plant tolerance

to multiple biotic and abiotic factors, such as salinity (Yang et al. 2014), pathogen attack (Chen and Singh, 2002), drought (Rakhra et al. 2015), and wounding (Vollenweider et al. 2000).

Conjugation of herbicides with GST is a common step in herbicide metabolism, where the rate of conjugation is related to the tolerance of a plant to an herbicide. Safeners could improve GST content and activity, enhancing conjugation with herbicide molecules, increasing the rate of metabolism of the herbicide into non-toxic metabolites. Ye et al. (2019) reported a 29% increase of GST enzyme activity in maize plants treated with safener, reducing the damage caused by tribenuron-methyl.

Numerous herbicide modes of action (MOA) are related to the generation of reactive oxygen species (ROS) in plants (Caverzan et al. 2019). ROS are molecules derived from oxygen species, such as super peroxide anion, peroxide, hydrogen peroxide, hydroxyl radical, and hydrogen ion. Under normal environmental conditions, ROS molecules are responsible for cell signaling. These signals are essential for plant growth, development, response to biotic and abiotic environmental stimuli, and programmed cell death (Bailey-Serres and Mittler 2006). However, the overproduction of ROS can lead to a state of oxidative stress. The enhanced production of ROS from environmental stresses can threaten cells by causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death pathway, and leading to the death of the cell (Cheng and Song 2006). Compounds with antioxidant abilities are related to mitigating ROS overproduction (Mittler et al. 2004).

Potential safener compounds

Melatonin (*N*-acetyl-5-methoxytryptamine) was identified in 1958 in the bovine pineal gland (Maronde, 2007). For decades, it was considered an exclusive compound for animals, but

previous research showed the presence of this hormone in plants (Dubbels et al., 1995). In the melatonin biosynthetic pathway, the first enzymatic reaction product is tryptamine. Tryptamine is synthesized by tryptophan decarboxylase (TDC) in plants. After synthesis, tryptamine is converted to serotonin by tryptamine 5-hydroxylase (T5H). Two enzymes, arylalkylamine *N*-acetyltransferase (AANAT) and acetylserotonin methyltransferase (ASMT) are responsible for converting serotonin to melatonin (Fujiwara, 2010).

Melatonin has multiple functions in plants. It can act as a growth promoter, similar to how auxin indolyl-3-acetic acid (IAA) behaves. Melatonin induces the active growth of hypocotyls in micro-molar concentrations while having an inhibitory effect at high concentrations (Hernandez, 2004). At low concentrations, melatonin can act as a growth promoter or growth inhibitor (Hernandez, 2005). Chen (2009) reported a stimulatory effect of 0.1 mM melatonin in mustard (*Brassica juncea* L.) while a 100 mM concentration had an inhibitory impact. A similar melatonin concentration-dependent study was described in red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) (Posmyk, 2008) and canary grass (*Phalaris canariensis* L.) (Hernandez, 2005). Melatonin can also induce root primordial formation in plants, resulting in new adventitious or lateral roots. This rhizogenic effect has been confirmed in lupin (*Lupinus polyphellus* L.) (Arnao, 2014), cucumber (*Cucumis sativus* L.) (Zhang, 2013), rice (*Oryza sativa* L.) (Park, 2012), and pomegranate (*Punica granatum* L.) (Sarrou, 2014).

Other functions of melatonin include enhancement of photosynthesis by increasing the chlorophyll content and CO₂ uptake. Applications of 0.1mM melatonin to cherry trees (*Prunus avium* L.) increased chlorophyll production while higher application melatonin was inhibitory (Sarropoulou et al., 2012). The ability of melatonin relates to environmental stress, acting directly as an effective antioxidant, decreasing levels of reactive oxygen species, and detoxifying

various chemical contaminants (Arnao 2014). Turk (2014) suggested melatonin increases plant resistance to cold stress in wheat (*Triticum aestivum* L.) seedlings by directly scavenging ROS and modulating redox balance. Mandal et al. (2018) noted external applications of melatonin could directly impact the genes involved in biotic and abiotic stress response. They demonstrated that melatonin could increase cytochrome P450 activity in watermelon (*Citrullus lanatus* L.). In that same study, powdery mildew (*Podosphaera xanthii* Schtdl. U. Braun & S. Takam) disease severity significantly decreased when melatonin was applied exogenously. Caputo et al. (2020) reported an improvement in sweetpotato tolerance to bentazon due to exogenously melatonin applications in an in-vitro environment.

Another group of compounds widely related to improving plant tolerance are Brassinosteroids (BRs). These plant phytohormones are distributed throughout the plant kingdom. In the 1980s, these compounds started to be identified as plant hormones (Mandava, 1988). The essential role of BRs was demonstrated when BR-deficient and BR-insensitive mutant plants were identified. These plants expressed many growth defects, including dwarf phenotypes, dark green leaves, delayed flowering, male sterility, and photomorphogenesis in darkness (Clouse et al. 1996). BRs perception occurs at membrane-localized receptors. Downstream cytosolic regulators transduce BR-mediated signals to the nucleus, where they activate the transcription of BR-responsive genes that drive cellular growth (Plnas-Riverola et al., 2019).

The first evidence that BRs could improve plants' resistance to several environmental stress ware reported by Ikekawa and Zhao (1991). 24-epibrassinolide enhanced the yield of wheat, corn, tobacco (*Nicotiana tabacum* L.), watermelon, and cucumber. Xia et al. (2009) described cucumbers treated with BR as more tolerant to temperature stress. Kuleava et al.

(1991) noted segments of barley (*Hordeum vulgare* L.) leaves pre-incubated in BR had significantly less salt induced injury. Increased water uptake and membrane stability and higher carbon dioxide and nitrogen assimilation rates were observed in BRs treated plants (Sairan,1994). Khripach et al. (2000) noted potato plants sprayed with BR solution, exhibited less incidence of *Phytophthora infestans* (Mont.) de Bary than plants treated with standard fungicides. Interestingly, doses of 24-epibrassinolide at 0.1 mM reduced multiple insecticides and fungicides residues, such as carbofuran, phoxin, omethoate, and others in different crops. According to Zhou et al. (2015), BR induced stress tolerance is preceded by increased NADPH Oxidase and elevated H₂O₂ levels. Finally, BR improves the plant defense system by increasing chlorophyll contents, which ultimately increases photosynthetic capacity, enhances antioxidant system capacity, increases enzymatic activity, and upregulates stress response genes [superoxide (SOD), peroxide (POD), catalase (CAT), glutathione reductase (GR) and ascorbate peroxide (APX)].

Another compound that could reduce herbicide injury is AsA. In plants, AsA is related to beneficial influence on multiple physiological processes such as plant defense against oxidization, co-factor of key enzymes, plant cell division, cell expansion, growth and development, and senescence (Horemans et al. 2000). Though AsA has no direct involvement in the herbicide detoxification process, it plays a role in environmental stress perception and stress signaling responses in plants (Zechmann, 2011). AsA has a crucial role in removing H₂O₂ via AsA-GSH cycle, where ascorbate peroxidase APX utilizes ascorbate as a specific electron donor to reduce H₂O₂ to water. The ability of ascorbate to lose or donate electrons is an antioxidant property. One of the first reports of AsA antioxidant ability was by Conklin et al. (1996), where mutant plants with low AsA were more sensitive to sulfur dioxide and ultraviolet

radiations. Cell cytoplasm constitutes the most abundant ascorbate pool, which is vital in stress perception, redox homeostasis, and subsequent regulation of oxidative stress and plant physiological biochemical responses under different abiotic stresses (Pignocchio et al. 2006). The reduced state of ascorbate and the oxidized state of dehydroascorbate act as signals regulating the interaction between plant and stresses and confer resistance to stresses (Parson and Fry 2010). Improvement of ascorbate content in plants increases plant stress tolerance.

Multiple investigations reported exogenous applications of AsA increased plant tolerance to abiotic stress such as salinity stress in canola (*Brassica napus* L.) (Raza et al., 2013), tomato (*Solanum lycopersicum* L.) (Shalata and Neuann, 2001), *Abelmoschus esculentus* (Bybordi, 2012), *Hordeum vulgare* (Agami, 2014), and drought stress in canola (Shafiq et al., 2014). The authors suggested exogenous ascorbic acid consistently reduced salt-induced accumulation of lipid peroxidation molecules and reduced plant mortality. The ability of ascorbic acid to reduce damage caused by herbicide applications has been reported. Finckh and Kunert (1985) noted peroxidation by oxyfluorfen applications was more intense in plant species with low AsA concentration. Namet Alla and Hassan (2006) saw similar results in corn, where the decreases in AsA contents are accompanied by high inductions of lipid peroxides and carbonyl groups by atrazine applications. The accumulation of antioxidant compounds in plant chloroplast leads to protection against herbicide damage.

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CHAPTER TWO

In Vitro Safening of Bentazon by Melatonin in Sweetpotato (*Ipomoea batatas*)

Abstract

Weed competition is a main factor limiting sweetpotato [*Ipomoea batatas* (L.) Lam] production. Yellow nutsedge (*Cyperus esculentus* L.) is a problematic weed to control due to its ability to quickly infest a field and generate high numbers of tubes and shoots. Compounding this is the lack of a registered herbicide for selective postemergence control of it. Research was conducted to evaluate bentazon dose response of two sweetpotato cultivars and one advanced clone and to determine if the plant hormone melatonin safens sweetpotato tolerance to bentazon. Bioassays using Murashige and Skoog (MS) media supplemented with melatonin (0.232 g a.i./L and 0.023 g a.i./L) and bentazon (0.24 g a.i./L) were conducted. Beauregard was the most tolerant cultivar and tolerated dosages of bentazon two-times higher to those causing the same injury with other cultivars. MS media containing melatonin and bentazon had less injuries and higher plant mass than plants treated with bentazon alone. These results indicate normal sweetpotato injury caused by bentazon may be reduced by melatonin.

Introduction

Sweetpotato is an economically important crop in the United States; in 2017, its worth was more than \$733 million (U.S. Department of Agriculture, 2018), with a total planted area over 60,000 ha throughout the United States (U.S. Department of Agriculture, 2019). Sweetpotato is transplanted using vegetatively propagated stem tip cuttings (slips) and requires ≈2 to 6 weed-free weeks to maximize yield (Harrison and Jackson, 2011a; Smith et al., 2009). The impact of weed competition on yield has been well documented. For example, *Cyperus*

esculentus L. reduces yield by up to 80% (Meyers and Shankle, 2017) while Meyers et al. (2010) reported a 36% to 81% reduction in marketable yield of Beauregard and Covington sweetpotato cultivars from Palmer amaranth (*Amaranthus palmeri* S. Wats) interference.

Because relatively few herbicides are registered for sweetpotato, chemical weed management is challenging (Harrison and Jackson, 2011b). Flumioxazin, S-metolachlor, and clomazone are PRE herbicides registered for application in sweetpotato that control troublesome weeds, such as *Amaranthus palmeri* and annual grasses (Kemble, 2017; Meyers et al., 2010). Currently, a lack of postemergent (POST) herbicides exists to control *Cyperus esculentus* in sweetpotato (Webster, 2010). A potential herbicide that could control or suppress yellow nutsedge is bentazon. The range of tolerance to bentazon in multiple sweetpotato cultivars has been reported by Motsenbocker and Monaco (1991). However, the safety data from this study warrant further exploration of bentazon tolerance in more cultivars with alternative strategies to reduce bentazon injury in sweetpotato. One possible means to increase POST herbicide options for sweetpotato is the incorporation of an herbicide safener, which, ideally, would protect sweetpotato from POST herbicides while not sacrificing weed control (Parker, 1983). Numerous studies of monocot species suggest safeners increase the activity of cytochrome P450s, resulting in increased tolerance to multiple herbicide modes of action through conjugation and metabolism of the herbicide molecule (Hatzios 1991). Furthermore, Dubbleman et al. (1997) recorded the capacity of the safener furilazole to enhance P450 activation, resulting in the de-esterification of halosulfuron-methyl to halosulfuron acid in corn seedlings. A plant hormone shown to increase cytochrome P450s and potentially sequester reactive oxygen species (ROS) in broadleaf vegetable crops is melatonin (N-acetyl-5-methoxytryptamine).

Arnao (2014) highlighted the antioxidant capacity of melatonin, neutralizing ROS, reactive nitrogen species (RNS), and detoxify various chemical contaminants as a response to environmental stress. Abiotic or biotic stresses (e.g., low temperatures, plant competition, and chemical applications) can impact the photosynthetic rate and increase the production of ROS. Increasing ROS production can result in lipid peroxidation of membranes, DNA damage, and inactivation of various enzymes (Cheng and Song, 2006; Foyer and Noctor, 2003). Turk et al. (2014) suggested melatonin can enhance plant resistance to cold stress in wheat (*Triticum aestivum* L.) seedlings by directly scavenging ROS and by modulating redox balance and other defense mechanisms. A tissue culture experiment conducted by Erland et al. (2019) reported exogenous melatonin was taken up by a specific transport mechanism. That mechanism involved active internal transport, which dispersed melatonin as a response to environmental stresses, resulting in the accumulation of this antioxidant substance in endodermal cells. Mandal et al. (2018) noted external applications of melatonin could directly impact the genes involved in biotic and abiotic stress response, and they demonstrated that melatonin could increase cytochrome P450 activity in watermelon (*Citrullus lanatus* L.). In that same study, powdery mildew (*Podosphaera xanthii* Schltdl. U. Braun & S. Takam) disease severity significantly decreased when melatonin was applied exogenously. Transgenic rice (*Oryza sativa* L.) that overexpressed melatonin, contained lower levels of H₂O₂ when treated with butafenacil, thus, confirming a cellular increase in the melatonin level in plants results in resistance to oxidative stress (Park et al., 2013).

The identification and selection of bentazon herbicide tolerant lines would be beneficial for managing weeds in sweetpotato. In vitro methods are efficient for screening stress tolerance in different plants because they require lower resources and materials than field trials (Cutulle et

al., 2020; Sakhanokho and Kelley, 2009). Rajasekaran et al. (2005) reported the efficiency of tissue culture to analyze cotton (*Gossypium arboreum* L.) plant interactions with multiple antifungal compounds. Cutulle et al. (2009) described the in vitro technique as the most accurate assessment for evaluating resistance to mitotic-inhibiting herbicides on annual bluegrass (*Poa annua* L.). A significant limitation to herbicide programs in sweetpotato is the lack of registered POST herbicides that control *Amaranthus* spp. and yellow nutsedge. Therefore, expanding POST herbicide options would provide growers with more flexibility in their weed management program. Bentazon is a photosystem II inhibiting herbicide with activity on *Cyperus* spp. and would benefit growers if the label were expanded to include sweetpotato.

Understanding the interactions between melatonin and bentazon herbicide in sweetpotato may lead to improvements in weed management. Therefore, the objectives of this study were to: 1) determine the effects of the bentazon rate on sweetpotato clones and 2) characterize the response of 'Beauregard' to bentazon and exogenous applications of melatonin.

Material and Methods

Cultivar dose response

A study was conducted at the Clemson University Coastal Research and Education Center in Charleston, SC, to screen sweetpotato cultivars for bentazon sensitivity. Sweetpotato accessions were obtained from in vitro cultures from the U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS), and U.S. Vegetable Laboratory (USVL) in Charleston, SC. Two sweetpotato cultivars (Beauregard and Covington) and an advanced clone (USDA-09-130) from the USDA ARS and USVL sweetpotato breeding program were included.

For in vitro cultures, Murashige-Skoog basal media (Murashige and Skoog, 1962) was adjusted to a pH of ≈ 5.7 . Bentazon (Basagran, 440 g a.i./L; Redeagle International LLC, Lakeland, FL) was added to the media at increasing logarithmic rates from 0, 0.1, 1, and 10.0 mM and solidified with 4 g/L of phytagar. Subsequently, 40 mL of medium was added to culture tubes (25 × 150 mm; Durex borosilicate glass; VWR International, Radnor, PA) and autoclaved at 121 °C.

When the source plantlets developed, two to three node shoot tip cuttings were excised aseptically and transferred to the media. Cultures were maintained at 25 °C during a photoperiod of 16 h of light and 8 h of dark with a light intensity of $74 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ provided by fluorescent tube lights. The experiment was a randomized complete block with four replications and was repeated twice. Visual ratings were taken 7, 14, and 21 d after transfer (DAT) using a scale ranging from 0% to 100% (0 = no injury; 100% = plant death). Vessel tubes were sealed with parafilm until the end of the experiment due to the contamination risk.

Melatonin safener study

A melatonin safener study was conducted with sweetpotato accessions provided from the same location as the previous study. Beauregard was the most tolerant cultivar from the bentazon screening study. It was selected to determine if melatonin further reduced injury from bentazon. Media preparations, growing conditions, and experimental design were the same as previously described. Bentazon was added to the media at 0 or 1 mM, and melatonin (Alfa Aesar, Ward Hill, MA) was incorporated in the media at concentrations of 0, 0.1, or 1.0 mM. Plants were weighed using a Mettler Toledo scale (TLE303E, SNR B705644588; Langacher, Greifensee, Switzerland) 21 DAT. Visual ratings were obtained at 7, 14, and 21 DAT using a scale ranging from 0% to 100% (0 = no injury; 100% = plant death).

Data analysis

All data were subjected to an analysis of variance using mixed model methodology in JMP (version 14; SAS Institute, Cary, NC). During the herbicide rate trial, the cultivar and interaction between the cultivar and herbicide rate were considered fixed, and replication was considered random. A logistic five-parameter equation was used to determine the herbicide dose-response for each cultivar (Gottschalk and Dunn, 2005):

$$y = c + \frac{(d - c)}{\{1 + \text{Exp}[a(\log b) - \log(\text{Herbicide})]\}^f}$$

where y is the herbicide dose necessary to cause the predicted injury, c is the asymptote for low doses, d is the asymptote for high doses, a is the slope parameter, b is the inflection point, and f is the symmetrical power. The upper asymptote is the point of the growth curve that represents the maximum of the parameter measured. The lower asymptote is the point of the growth curve that represents the minimum of the parameter measured (Paine et al., 2012).

Results

Cultivar dose-response

The injury rate increased as the herbicide concentration increased, and no treatment × experimental run interaction occurred for herbicide injury; therefore, data from both experiments were combined. The cultivar, herbicide concentration, and their interaction had significant effects (Table 2.1). Differences between cultivars at 10 mM of bentazon did not occur because all plants exhibited 100% injury, as shown in Fig. 2.1. At 1 mM of bentazon, all cultivars exhibited a similar response, and injury rates reached 75%, 77%, and 84% for Beaugard, USDA-09-130 and Covington, respectively. Decreasing the concentration to 0.1 mM bentazon resulted in ≈75%

overall injury to ‘Covington’ and USDA-09-130; however, Beauregard was significantly more tolerant, with only 41% injury.

Melatonin safener study

The addition of exogenous melatonin to the media decreased herbicide injury 21 DAT (Fig. 2.2) Without melatonin, bentazon at 1 mM caused 83% injury on Beauregard plants. Comparatively, the injury from herbicide and melatonin supplementation were $\approx 51\%$ for both rates of melatonin (0.1 mM and 1 mM). The reduction was statistically different ($F_{2,2} = 5.4349$ and $P < 0.01$) (Table 2.3, Fig.2.2), however, injuries from bentazon and melatonin supplementation were statistically equal. Plant biomass was also affected by the herbicide (Table 2.4); the final biomass of the untreated control was 1.47 g, whereas the final biomass of plants treated with bentazon was 0.4 g (Fig. 2.3).

Discussion

Cultivar dose-response

Our results are consistent with those of previous studies of herbicide tolerance in sweetpotato. In a clomazone screen of sweetpotato germplasm conducted by Harrison and Jackson (2011b), Beauregard had ≈ 10 -times greater tolerance than sensitive cultivars. Additional experiments with halosulfuron POST demonstrated Beauregard experienced less storage root injury than Covington (Dittmar et al., 2013). These results demonstrated the inherent tolerance of Beauregard to xenobiotics and, when coupled with applications of melatonin, they could increase herbicide detoxification and reduce bentazon injury (Hatzios and Burgos, 2004).

In contrast to the study by Motsenbocker and Monaco (1991), our results highlighted the low tolerance of sweetpotato to bentazon. This difference could be explained by the quick

absorption of the herbicide; according to Mine et al. (1974), the herbicide incorporated in media could act similar to flooded-water herbicide applications, and was quickly available, explaining the fast and aggressive injury caused to plantlets. Bentazon was quickly absorbed through roots or shoots and translocated to leaves, presenting injury 6 to 9 DAT. Because of the small size of the plantlets, they did not have a concentration of carbohydrates necessary to protect themselves from the depletion of the substrate caused by bentazon-inhibited photosynthesis.

Melatonin safener study

Bentazon competes with plastoquinone for the binding site on the D1 protein, thus blocking electron transport from photosystem II. This inhibition of photosynthesis and oxidative stress is followed by cell damage (Dat et al., 1998; Han and Wang, 2002). In agreement with previous reports (Diebold et al., 2004; Herrmann et al., 2017; Lima et al., 2018), our results indicated the bentazon could interfere with plant development, causing severe injury and reducing root and foliar growth.

Under stress conditions, plants usually generate higher levels of ROS, which subsequently induce peroxidation of membrane lipids and oxidative damage (Kar, 2011; Munné-Bosch and Peñuelas, 2003). Melatonin is a plant growth regulator that improves photosynthetic efficiency in higher plants under stressful conditions (Jiang et al., 2016; Yin et al., 2013; Zhao et al., 2015). One molecule of melatonin may eventually scavenge 10 molecules of radicals (Tan et al., 2007), thus reducing the content of ROS and alleviating oxidative damage induced by excessive ROS accumulation (Meng et al., 2014). Wei et al. (2014), reported the capacity of an exogenous melatonin application to upregulate genes associated with stress pathways. This interaction with ROS indicates melatonin is an essential component of the redox system. Our study suggests exogenous applications of melatonin could increase the ability of a plant to

detoxify herbicides, possibly by reducing the ability of the active herbicide to interact with the plant target site and mitigate the damage caused by oxidative stress from ROS. These results complement those of previous experiments in which melatonin was reported to reduce oxidative damage in plants under stressful abiotic conditions such as salt and cold (Fan et al., 2015; Li et al., 2012; Zhou et al., 2016).

Conclusion

In this investigation, we demonstrated that ‘Beauregard’ is significantly more tolerant to bentazon at a concentration of 0.1 mM compared with ‘Covington’ and USDA-09-130. Given the lack of an effective herbicide labeled for the control of broadleaf weeds and sedges POST on sweetpotato, the use of melatonin could enable the use of bentazon for sweetpotato weed management. The relatively high injury rate caused by the herbicide, even when melatonin was present, does not invalidate the potential for melatonin to be developed into a commercial safener. More physiological- and biochemical-based research is necessary to gain a better understanding of the interactions between herbicides and melatonin on plant metabolism. Furthermore, greenhouse and field trials are necessary to characterize the interaction of melatonin and bentazon in conditions more relevant to those of commercial settings. Ideally, these trials could generate data to promote label expansion of a much-needed POST herbicide for *Cyperus esculentus* control in sweetpotato. Finally, future research will focus on the potential antagonism of melatonin on herbicide-driven weed control.

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CHAPTER THREE

Impact of Tank Mixing Plant Hormones with Bentazon and Mesotrione on Sweetpotato Injury and Weed Control

Abstract

Successful weed management is essential to maximize sweetpotato [*Ipomoea batatas* (L.) Lam] production. Currently, there are no selective postemergent (POST) herbicides registered in sweetpotato to suppress broadleaves and nutsedge species. Expansion of bentazon and mesotrione herbicide labels to include sweetpotato would be beneficial for growers. Two experiments were conducted, the first evaluated the dose-response of sweetpotato cultivars ‘Beauregard’ and ‘Covington’ to bentazon and mesotrione when melatonin, 24-epibrassinolide, or ascorbic acid (AsA) were included in the tank-mix. The second evaluated the efficiency of bentazon and mesotrione in control of yellow nutsedge and palmer amaranth when different doses of melatonin, 24-epibrassinolide, or AsA were added to each herbicide. In the first experiment, when treated with herbicide alone, ‘Beauregard’ exhibited injury levels lower than ‘Covington’ for both herbicides. No injury was observed when plants were treated with plant hormones or AsA alone. At the lowest dose of bentazon and mesotrione, the addition of compounds in tank-mix significantly reduced percent injury and increased plant tolerance, requiring higher doses of herbicide to cause 10%, 20%, and 30% injury. In the second experiment, the addition of plant hormones and AsA in tank-mix had no-antagonistic effect on herbicide efficiency, exhibiting similar levels of injuries as herbicides application alone. These results suggest that the use of plant hormones and AsA could improve sweetpotato tolerance to POST applications of bentazon and mesotrione without reducing herbicide efficiency.

Introduction

Sweetpotato production in the US has increased by 38% from 2012 to 2017, reaching an estimated value of \$654 million in 2018 (USDA 2019a). Louisiana, California, Mississippi, North Carolina, and South Carolina are largest producers of sweetpotato in the US (USDA 2019b). One of the biggest challenges for sweetpotato growers is weed control. Weeds interfere with plant growth and compete for limited water, light, and nutrients (Harrison and Jackson 2011a). Yellow nutsedge (*Cyperus esculenus* L.) and Palmer amaranth (*Amaranthus palmeri* S.Wats.) can significantly reduce sweetpotato yield. Meyers and Shankle (2015) reported yellow nutsedge densities of 5 to 90 shoots/m² in planted sweetpotato resulted in yield losses of 18% to 80%. Smith et al. (2020) reported a reduction of 95% in marketable sweetpotato yields after season-long Palmer amaranth interference.

In season weed management for sweetpotatoes relies on herbicides, between-row cultivation, mowing, and hand removal. However, chemical control is limited to 10 registered herbicides (Kamble, 2020). The POST herbicides labeled for sweetpotato are the grass herbicides clethodim, fluazifop, and sethoxydim. Herbicides for POST control of broadleaf weeds are only labeled for between-row application, risking damage to sweetpotato leaves during application. There are no selective POST herbicides registered to control yellow nutsedge and Palmer amaranth in sweetpotato (Webster, 2010). One possible solution to increase POST herbicide options for sweetpotato is to evaluate safener compounds with the potential to reduce non-target herbicide injury while maintaining efficient weed control (Parker, 1983). Hatzios (1991) noted certain safeners could increase the activity of cytochrome P450s (P450), resulting in greater tolerance to multiple herbicide modes of action through conjugation and metabolism of the herbicide molecule.

Some herbicides can induce the overproduction of reactive oxygen species (ROS) as a secondary effect, leading to oxidative damage in essential tissues (Caverzan et al., 2019). Compounds with the ability to increase P450 activity and sequester ROS overproduction have the potential to promote greater stress tolerance in plants. Expanding POST herbicide options would provide growers with more flexibility in their weed management program. Mandal et al. (2018) noted melatonin's exogenous application could directly impact genes involved in biotic and abiotic stress response and melatonin could increase cytochrome P450 activity in watermelon (*Citrullus lanatus* L.). In the same study, powdery mildew (*Podosphaera xanthii* Schltdl. U. Braun & S. Takam) disease severity significantly decreased when melatonin was applied exogenously. Caputo et al. (2020) reported an improvement in sweetpotato tolerance to bentazon due to exogenous melatonin uptake in an in vitro environment.

Brassinosteroids (BR) are plant hormones that improve plant stress resistance (Szekeres et al., 1996; Mussig and Altmann, 1999). According to Zhou et al. (2015), BR induced stress tolerance is preceded by increased NADPH oxidase and elevated H₂O₂ levels. BR can upregulate cytochrome P450, Glutathione S-transferase (GST), hydrolase, and oxidoreductase genes. Another compound potentially reducing herbicide injury is ascorbic acid. Though ASA has no direct involvement in the herbicide detoxification process, it plays an essential role in stress perception and stress signaling responses in plants (Zechmann, 2011). Maize (*Zea mays*) seeds treated with ASA showed reduced toxicity to glyphosate applied at the seedling stage (Sacała and Roszak 2018).

A significant limitation to weed management programs in sweetpotato is the lack of registered POST herbicides for *Amaranthus* spp. and yellow nutsedge control. Expanding POST herbicide options would provide growers with more flexibility in their weed management

program. Understanding the interactions between melatonin, 24-epibrassinolide, Ascorbic acid, with the herbicides bentazon and mesotrione in sweetpotato may lead to improvements in weed management.

This study's objectives were to: (1) characterize the dose response of 'Beauregard' and 'Covington' to bentazon and mesotrione, in addition and absence of exogenous applications of melatonin, ascorbic acid, or 24-epibrassinolide; and (2) characterize the response of yellow nutsedge and Palmer amaranth to bentazon and mesotrione, in addition and absence of exogenous applications of melatonin, ascorbic acid, or 24-epibrassinolide.

Material and Methods

Sweetpotato greenhouse dose response screening

Experiments were conducted in a greenhouse, located at the Clemson Coastal Research and Education Center in Charleston, South Carolina (32.794608, -80.068361), utilizing sweetpotato cultivars 'Beauregard' and 'Covington'. Slips of both cultivars were obtained from New Sprouts Organic Farm (Black Mountain, North Carolina, 28711, US) and transplanted into pots with a volume of 500 cm³ filled with organic, native soil collected from an experimental field at the same research station. POST treatments consisted of bentazon applied at 514, 1028, and 1542 g ai ha⁻¹ (Basagran 4, RedEagle International LLC, 5143 S Lakeland Drive, Lakeland, FL, 33183, US), or mesotrione at rates of 300, 600, and 900 g ai ha⁻¹ (Callisto, Syngenta Crop Protection, LLC, Greensboro, NC, 27419, US) tank mixed with melatonin (100g ai ha⁻¹) (Alfa Aesar, Ward Hill, MA, 01835), 24-epibrassinolide (100g ai ha⁻¹) (MCE MedChemExpress, Monmouth Junction, NJ, 08852) or Ascorbic Acid (100g ai ha⁻¹) (TCI America, Portland, OR, 97203). Each safener compound was applied without herbicide and a nontreated control was included. The experiment was arranged in a randomized complete block design with four

replications and was repeated twice in space. Treatments were applied 21 days after transplant. At the time of the application, plants measured 10 cm in height and had 3-5 true leaves. Plants were maintained 28 days after treatment in the greenhouse under natural light at 28°C and 65% relative humidity. They were evaluated based on height and visual injury on a 0 to 100% scale (0 = no injury to 100% = plant death), at 7, 14, 21, and 28 days after treatment. Plant dry mass was collected at the end of the experiment.

Weed antagonism greenhouse screening

Experiments were conducted in a greenhouse, located at the Clemson Coastal Research and Education Center, Charleston, South Carolina, to evaluate the bentazon control of yellow nutsedge and mesotrione control of Palmer amaranth when different rates of safeners were added in the tank-mix. Seeds were sewn into 500 cm³ pots containing the same proportion of sand and growing medium (Farfard® Growing Mix 3B, Sungro Horticulture, 770 Silver Street, Agawam, MA). Treatments were applied 2 weeks after planting. At the time of application, Palmer amaranth plants were approximately 5 cm in height with 3 true leaves, while yellow nutsedge plants were 10 cm in height with 4 true leaves.

Treatments included POST applications of bentazon at 0 and 514 g ai ha⁻¹, and mesotrione at 0 and 300 g ai ha⁻¹. Herbicides were then applied alone, or tank mixed with melatonin at 0, 10, 50, 100, and 500 g ai ha⁻¹, 24-epibrassinolide at 0, 10, 50, 100, and 500 g ai ha⁻¹ or ascorbic acid at 0, 10, 50, 100, and 500 g ai ha⁻¹, for a total of 26 treatments per weed specie. The experiment was arranged in a randomized complete block design with four replications and repeated twice in time. Plants were maintained for 28 days after treatment in the greenhouse under natural light at 28°C and 65% relative humidity and were evaluated based on height measurements and visual injury on a 0 to 100% scale (0 = no injury to 100% = plant

death), at 7, 14, 21 and 28 days after treatment. Plant dry mass was collected at the end of the experiment.

Treatments preparation and application

Melatonin, 24-epibrassinolide, and ascorbic acid were weighed using a Mettler Toledo scale (TLE303E, SNR B705644588, Langacher, 448606, Greifensee, Switzerland), and stirred using an ultrasonic homogenizer (FisherBrand, Model CL-18, Serial no 2019070469, Waltham, MA, 02451) at 100w for 5 min for complete solubilization. All treatments were applied utilizing a research track sprayer (Generation 4, Serial number SB8-270, DeVries Manufacturing, Hollandale, MN), with a water carrier volume of 200 L·ha⁻¹ through 8002EV8 nozzles (Teejet; Spraying Systems Co., Roswell, GA).

Data analysis

All data were subjected to ANOVA using mixed model methodology in JMP (version 14, SAS Institute, Cary, NC). With herbicide concentration, variety trial run was analyzed as a fixed effect and replication was considered random. Trial run did not have an effect on dependent variables, thus trial runs are pooled together. A logistic five-parameter equation was used to determine the herbicide dose-response for each cultivar (Gottschalk and Dunn, 2005).

$$y = c + \frac{(d-c)}{(1+\text{Exp}(a(\log(b)-\log(\text{Herbicide})))^f)}$$

where y is the herbicide dose necessary to cause the predicted injury, c is the asymptote for low doses, d is the asymptote for high doses, a is the slope parameter, b is the inflection point, and f is the symmetrical power. The upper asymptote is the point of the growth curve

representing the maximum of the parameter measured. The lower asymptote is the point of the growth curve representing the minimum of the parameter measured (Paine et al., 2012).

Results

Sweetpotato greenhouse dose response screening

No differences in plant injury for both cultivars were observed between treatments when bentazon rate was greater than 1028g ai ha⁻¹. When bentazon was applied at 514 g ai ha⁻¹, injury on Beauregard and Covington plants was 63% and 86%, respectively (Fig 3.1). For Beauregard, tank mixing bentazon and melatonin reduced percent injury to 6%, while AsA, in association with herbicide, reduced injury to 38%. The lowest dose of bentazon caused 85% injury in Covington plants, however, when tank-mix with AsA or melatonin, injury observed was 48% and 57%, respectively. Applications containing 24-epibrassinolide with the herbicide did not reduce percent injury in either cultivar.

Mesotrione applied at 300 g ai ha⁻¹ caused 78% and 91% injury to Beauregard and Covington, respectively (Fig 3.2). All treatments receiving doses above this rate were not significantly different for percent injury. For Beauregard, all three safener compounds used reduced percent injury to plants, compared to an herbicide applied alone. The percent injury observed for treatments containing 300 g ai ha⁻¹ in a tank mix with melatonin, 24-epibrassinolide, or AsA in Beauregard plants was 48%, 58%, and 50%, respectively.

Herbicide projections were calculated to determine the dose of bentazon and mesotrione required to cause 10%, 20%, and 30% of injury for each cultivar when applied alone or in tank-mix with safeners (Table 3.1). Tank mixing melatonin, 24-epibrassinolide, or AsA with mesotrione increased Covington plants' herbicide tolerance, requiring a dose 6X higher to cause

the similar damage vs. herbicide applications alone. However, safeners did not reduce bentazon injury in Covington. The dose of mesotrione necessary to cause 30% injury in Beauregard was 72 g ai ha⁻¹ while the application associated with melatonin, 24-epibrassinolide or AsA increases the dose to 169, 148 and, 194g ai ha⁻¹, respectively. For bentazon, the combination of herbicide and melatonin results in an improvement in plant tolerance, requiring a dose of 819 g ai ha⁻¹ to cause 30% injury.

Weed antagonism greenhouse screening

No injury was observed for yellow nutsedge plants treated exclusively with additional compounds. When bentazon was applied at 514 g ai ha⁻¹, 96% injury was observed 3 weeks after treatment (WAT) (Fig 3.3). The addition of melatonin, 24-epibrassinolide or AsA had no statistically difference in injury between herbicide treatments with or without safeners. Differences also were not observed between the multiple ranges of additional compounds associated with bentazon.

A similar response was observed in Palmer amaranth plants. No dose of melatonin, 24-epibrassinolide, or AsA caused harmful effects on plants at 3 WAT. Mesotrione caused stunting on Palmer amaranth plants, with 96% injury when applied alone (Fig 3.4). No antagonistic effect was observed due addition of melatonin, 24-epibrassinolide or AsA into tank-mix.

Discussion

Sweetpotato greenhouse dose response screening

Bentazon symptoms were observed 3 DAT and included yellowing (chlorosis) of leaf tissue followed by death (necrosis) of the tissue, beginning around the leaf margins and progressing towards the center. Similar symptoms were observed in other susceptible crops (Han

and Wang 2002; Willians and Nelson 2014; Nosratti et al. 2017). Bentazon is a POST herbicide group 6 photosystem II inhibitor that competes with plastoquinone for the binding site on the D1 protein, blocking electron transport from photosystem II. This blockage induces secondary effects in several metabolic pathways, such as ROS production (Macedo et al., 2008; Pospíšil, 2009). When bentazon was applied at 540 g ai ha⁻¹, the percent injury observed in Beauregard and Covington was 64% and 87%, respectively. A similar response was reported by Caputo et al. (2020), where Beauregard plants had a greater tolerance to bentazon added into the media in an in vitro environment. The reduction in percent plant injury when bentazon was tank-mixed with melatonin or AsA could be associated with an improvement in herbicide tolerance and antioxidant activity. Melatonin is related to improving cytochrome P450 and Glutathione S-transferase (GST), while AsA is associated with activation of GST and Glutathione (GS), critical molecules for the herbicide detoxification process (Arnao and Hernández-Ruiz 2014; Soares et al. 2016). Additionally, the ability to scavenge radical molecules makes them play a prominent role in the antioxidant defense network of plants, crucial as protectors against oxidative stress (Asensi-Fabado and Munné-Bosch 2010; Arnao and Hernández-Ruiz 2014).

Mesotrione applications inhibit the HPPD enzyme (p-hydroxyphenylpyruvate dioxygenase), a precursor to plastoquinone and tocopherols in oxidative degradation of chlorophyll and photosynthetic membranes (Grossmann and Ehrhardt, 2007). Mesotrione is an indirect inhibitor of phytoene desaturase, plastoquinone works as a cofactor for phytoene desaturase, the first step in the carotenoid synthesis pathway. The lack of tocopherols also contributes to plant death by decreasing ROS's buffering capacity (Matringe et al., 2005). Initial stages of symptoms were observed in 5-7 DAT, starting with reduced leaf pigmentation and progressing to total chlorosis of leaves and necrosis. For Covington, the only improvement on

herbicide tolerance was observed when melatonin was tank-mixed with mesotrione. Conversely, for Beauregard, all compounds used increased plant tolerance to the lowest herbicide dose. However, no improvement was observed when doses were greater than 300 g ai ha⁻¹ (Fig 3.2). 24-epibrassinolide is a member of the Brassinosteroids. This class of plant hormone has been related to upregulated pesticide metabolism. Zhou et al. (2015) reported an intrinsic capacity of 24-epibrassinolide applications in co-upregulate genes related to pesticide detoxification, reducing residues of organophosphorus residues, organochlorine, and carbamate pesticides by 30–70% on multiple crops.

Herbicide projections show higher toxicity of mesotrione than bentazon to sweetpotato, requiring lower doses to cause 10, 20, and 30% injury 3 WAT (Table 3.1). Cultivars had different tolerance responses to herbicides. For both herbicides alone and all injury levels, dose predictions were higher for Beauregard than Covington. Caputo et al. (2020) reported a predicted dose of bentazon 2.5x higher than Beauregard to cause the same injury level in an in-vitro environment. In a clomazone screen of sweet potato germplasm conducted by Harrison and Jackson (2011b), Beauregard had ~10x greater tolerance than sensitive cultivars. POST experiments with halosulfuron demonstrated Beauregard experienced less storage root injury than Covington (Dittmar et al., 2013).

The use of safeners in a tank mix with herbicide had a positive effect on plant tolerance. In some cases, the injury observed was high even using safeners, however the dose prediction data could provide important information to design a chemical weed management with low injury to sweetpotato plants.

Weed antagonism greenhouse screening

Mesotrione and bentazon are herbicides labeled to control Palmer amaranth and yellow nutsedge in various crops, respectively, so herbicide-alone treatments were expected to have a high percentage of injury. Antagonism is the negative relationship between two or more compounds, resulting in less than the predicted effect of each herbicide applied separately (Merritt et al. 2020). The risk of using non-crop specific safeners in tank-mix is that while it increases the tolerance of non-target plants, it may reduce the herbicide efficiency on targeted weeds. To minimize that, herbicide applications must follow the technical recommendations to reach full weed control. In our experiments, we applied the treatments when plants had the maximum height recommended on the label. No reductions in chemical efficiency were observed when safeners were added into tank-mix with herbicides, reaching the same levels of weed control of herbicide applications alone.

Conclusion

This study demonstrated the successful use of bentazon and mesotrione plus safeners in sweetpotato. The lower percent injury observed in sweetpotato plants when safeners were added into the herbicide application demonstrates those compounds' protective ability. A common concern when using non-specific safeners is the antagonist interaction with an herbicide, which would reduce weed control. When following the label guidelines to apply the proper dose at maximum plant height, the efficiency to control Palmer amaranth and yellow nutsedge by mesotrione and bentazon, respectively, was not reduced by any safener dose added. Additional physiological and biochemical based research would be necessary to gain a better understanding of the interactions between herbicides and melatonin, 24-epibrassinolide, and AsA on plant metabolism. Field trials are necessary to characterize the interaction of mesotrione and bentazon with safeners in commercial settings. Ideally, these trials could generate data to promote label

expansion of a much-needed POST herbicide for yellow nutsedge and Palmer amaranth control in sweetpotato.

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CHAPTER FOUR

BENTAZON IN TANK-MIX WITH PLANT HORMONES IMPROVES WEED MANAGEMENT IN SWEETPOTATO (*Ipomoea batatas* L.)

Abstract

Weed management is essential to maximize yield in sweetpotato [*Ipomoea batatas* (L.) Lam] production. Currently, there is a lack of herbicides registered in sweetpotato to suppress nutsedge species. Yellow nutsedge (*Cyperus esculentus* L.) is a problematic weed to control due to its ability to quickly generate high numbers of tubers and shoots. Expanding the bentazon label to include sweetpotato would be beneficial for growers as this would be able to control yellow nutsedge during the season. Experiments were conducted to evaluate interactions between bentazon and plant hormones on weed control and sweetpotato crop safety. Treatments included a non-treated check, melatonin (100 g ai ha⁻¹), brassinosteroids mixture (BSM) (1000g ai ha⁻¹), bentazon (200g ai ha⁻¹), bentazon + melatonin, bentazon + BSM, and bentazon + melatonin + BSM. The experiment was conducted at two locations in South Carolina. Evaluations consisted of percent yellow nutsedge control, percent sweetpotato injury, and sweetpotato yield. Results indicate bentazon applied alone had a stunting effect on sweetpotato plants, reducing the final yield by 50% in Cameron, SC and 79% in Charleston, SC compared to tank-mix of bentazon + plant hormones. The addition of plant hormones mixed with bentazon reduced sweetpotato injury by 50% without impacting weed control. Also, yield from plants treated with bentazon and plant hormones was 50% and 74% greater in Cameron and Charleston, respectively, than the non-treated check. These results indicate the use of plant hormones could improve sweetpotato tolerance to postemergent applications of bentazon without reducing weed control.

Introduction

In the US, sweetpotato production has increased since the last survey, reaching an estimated value of \$654 million in 2018 (USDA 2019a). A small number of states are responsible for most of the sweetpotato produced in the US (USDA 2019b). A major challenge for sweetpotato growers is weed management. Weeds interfere with plant growth and compete for limited water, light and nutrients (Harrison and Jackson 2011a). Yellow nutsedge is a common weed in vegetables crops and negatively impacts sweetpotato yield. Meyers and Shankle (2015) noted yellow nutsedge densities of 5 to 90 shoots m⁻² in planted sweetpotato resulted in yield losses of 18% to 80%. In season weed management for sweetpotatoes relies on herbicides, between-row cultivation, mowing and hand removal. However, the chemical control is currently limited to 10 registered herbicides (Kamble, 2020). Herbicides labeled for control of weeds postemergence in sweetpotato are products that exclusively control grasses species. Herbicides for postemergence (POST) control of broadleaf weed species are just labeled for between-row use and risk damage to leaves contacted during application. There are no selective POST herbicides registered to control yellow nutsedge in sweetpotato (Webster, 2010). One possible means to increase POST herbicide options for sweetpotato is to evaluate compounds having the potential to reduce herbicide injury while maintaining efficient weed control (Parker, 1983). Hatzios (1991) divulged certain safeners increase the activity of cytochrome P450s, resulting in greater tolerance to multiple herbicide modes of action through conjugation and metabolism of the herbicide molecule.

Some herbicides can induce the overproduction of reactive oxygen species (ROS) as a secondary effect, leading to oxidative damage in essential tissues (Caverzan et al. 2019). Compounds with the capacity to improve cytochrome P450 activity and sequester ROS

overproduction have the potential to promote a greater tolerance to stress in plants. Mandal et al. (2018) reported melatonin's external application could directly impact the genes involved in biotic and abiotic stress response. They demonstrated melatonin could increase cytochrome P450 activity in watermelon (*Citrullus lanatus* L.). In the same study, powdery mildew, caused by *Podosphaera xanthii* (Castagne) U. Braun & N. Shishkoff, significantly decreased when melatonin was applied exogenously. While Caputo et al. (2020) reported in an in vitro environment, melatonin improved sweetpotato tolerance to bentazon, reducing visual injury and resulting in a greater plant weight, compared to herbicide alone. Brassinosteroids (BR) are a group of plant hormones noted to improve a plant's tolerance to stress (Szekeres et al. 1996; Mussig and Altmann, 1999). According to Zhou et al. (2015), BR can upregulate Glutathione S-transferase genes (GSTs). Studies have reported the ability of GSTs to improve herbicide tolerance (Li et al., 2017).

A significant limitation to weed management programs in sweetpotato is the lack of registered POST herbicides for yellow nutsedge control. Therefore, expanding POST herbicide options would provide growers with more flexibility in their weed management program. Bentazon is a POST herbicide that inhibits photosystem II by competing with plastoquinone for the binding site on the D1 protein (Zhou et al., 2016). Keller et al. (2020) reported bentazon reduced the production of yellow nutsedge tubers per plant, minimizing the risks of weed infestation. Understanding the interactions between melatonin and BR with bentazon in sweetpotato may lead to improvements in weed management. Therefore, this study's objective was to evaluate field response of treatments containing plant hormones and bentazon in tank-mix for sweetpotato cultivar Bayou Belle.

Material and Methods

Slip propagation beds were prepared in a commercial field (33.5360886; -80.6588085) in Cameron, South Carolina, soil type Norfolk loamy sand, and at Clemson Coastal Research and Education Center (32.789824, -80.059851), in Charleston, South Carolina, with a Yonges loamy sand soil. Five days before the transplant, fields were treated with sodium salt of fomesafen (Reflex, 22.8 g ai L⁻¹, Syngenta US, Greensboro, NC, 27409, US) at 135 g ai ha⁻¹ and paraquat (Gramoxone, 30.1 g ai L⁻¹, Syngenta US, Greensboro, NC, 27409, US) at 300 g ai ha⁻¹. Bayou Belle sweetpotato slips were provided by the USDA, ARS, US Vegetable Laboratory, Charleston, South Carolina. Slips were healed for 5 days in a high tunnel and transplanted on May 08 and June 18 at the commercial field and research station field, respectively. Plots were 1m x 3m long, containing 2 rows per plot, distance between beds was 50 cm and plants was 30 cm, for a total of 20 plants per plot. Additionally, 500 kg ha⁻¹ of 15-0-15 fertilizer (Vertigro 15-0-15, Vertigro, Summerfield, FL, 34491, US) was applied 28 days after transplant.

Treatments included POST application of bentazon (200 g ai ha⁻¹) (Bentazon 4, Red Eagle International, Lakeland, FL, 33803, US), melatonin (100 g ai ha⁻¹) (Alfa Aesar, Ward Hill, MA, 01835, US), vitazyme (1000g ai ha⁻¹) (Vitazyme Organic Fertilizer, Vital Earth Resources Inc., Gladewater, TX, 75647, US) alone or tank-mixed, and a nontreated check (Table 4.1). Vitazyme is an organic fertilizer containing a brassinoesteroids mixture (BSM) at 87 mg L⁻¹, according to the manufacture. The experiment was arranged in a randomized complete block design with four replications.

All treatments were applied 21 days after transplanting using a 200 L ha⁻¹ backpack sprayer (Bellspray Inc., Opelousa, LA, 70570, US) equipped with 8002VS nozzles pressurized to 275 kPa (Teejet Technologies, Tifton, GA, 31794, US). At the time of application, average plant

height was 15 cm, and 3 to 4 leaves, per plant. Plant injury was evaluated visually on a 0 to 100% scale (0 = no injury to 100% = plant death) plus yellow nutsedge control on a 0 to 100% scale (0= no weed control to 100%= total weed free) at 1, 3, 6, and 9 weeks after treatment (WAT). Vines were removed 105 days after planting using a gas string trimmer (Model Stihl FS 91-R, Stihl USA, Virginia Beach, VA, 23452, US) and storage roots were harvested with a single row potato digger (Model D-10M, U.S. Small Farm Equipment, Co., Worland, WY, 82401, US). At the end of the experiment, storage roots were hand graded as jumbo (>8.9 cm in diam), number (no.) 1 (>4.4 cm but <8.9 cm), or canner (>2.5 cm but <4.4 cm) grades (USDA 2005). Total marketable yield was calculated as the sum of jumbo, no 1 and canner grades. All data were subjected to ANOVA using mixed model methodology in JMP (version 14, SAS Institute, Cary, NC, 27513-2414, US).

Results

Cameron, South Carolina Trail

At the Cameron, SC location, plots exclusively treated with plants hormones did not show any signs of injury. All treatments containing bentazon, alone or in tank-mix with plant hormones had similar levels of plant injury at 1WAT (Fig 4.1). However, at 3WAT and 6WAT, injury observed in plants exclusively treated with bentazon was twice to plants treated with combinations of bentazon + plant hormones. Plants treated with the tank-mix of bentazon and plant hormones recovered quicker from the initial injury caused by the herbicide. Treatments containing only herbicide resulted in $\approx 50\%$ more injury at 3WAT, and 18% of injury 6WAT, while tank-mix of herbicide + plant hormones did not present injury on week 6. No difference in injury was observed in plants treated with melatonin, BSM, or melatonin + BSM, through time. At 9 WAT, bentazon injury had dissipated.

Yellow nutsedge control was similar between all treatments containing bentazon at 1WAT. Plots treated exclusively with bentazon were not as effective in controlling yellow nutsedge emergence throughout the season, resulting in a 30% greater weed population. No antagonist interaction was observed for nutsedge control between melatonin, BSM and bentazon, resulting in excellent levels of weed control during the experiment (Fig 4.2).

In Cameron SC, Bayou Belle in the non-treated check yielded 14,733, 13,325, 4,842 ,and 28,060 kg ha⁻¹ of jumbo, no 1, canner, and marketable storage roots, respectively (Table 4.2). Jumbo yield was similar among all treatments. The average yield of no 1 and total marketable storage roots, was \approx two times greater when bentazon was tank mixed with melatonin + BSM than in non-treated check or bentazon applied alone. Plants treated with bentazon alone yielded 76% more cannery than the tank-mix between bentazon + plant hormones treatments. All treatments containing plant hormones in tank-mix with herbicide had similar yield.

Contrasts were performed to determine the effect of bentazon and plant hormones on sweetpotato injury (Table 4.3). We observed that plant hormones add into tank-mix would reduce plant injury at 3 and 6 WAT by \approx 50%, compared to bentazon alone. However, such treatments did not affect injury at 1 and 9 WAT.

Charleston, South Carolina Trail

In Charleston, SC, at 1WAT, all treatments containing bentazon, alone or in tank-mix, had similar levels of injury on sweetpotato plants. Faster recovery in herbicide injury was noted when bentazon was applied in a tank mix with melatonin, BSM, or melatonin + BSM, having no statistically difference between those three treatments (Fig 4.3). At 3 WAT, plants treated with herbicide alone had injuries of 51%, whereas the association with melatonin, BSM, and

melatonin + BSM treatments had injuries levels reduced to 18%, 26% and, 10%, respectively. At 6 WAT, treatments containing additional compounds and bentazon had no visible injuries, while plants sprayed with bentazon alone had 43% injury. Afterwards, herbicide injury was not observed in any treatment.

Control of yellow nutsedge was not reduced when bentazon was tank-mixed with the plant hormones 1 WAT (Fig 4.4). Yellow nutsedge infestation become more intense in plots treated with just bentazon at 3 and 6 WAT, than plots treated with bentazon + plant hormones. Those plots had 22% and 52% more yellow nutsedge plants at 3 and 6 WAT, compared to tank-mix treatments.

In Charleston, SC, the non-treated check yielded 167, 1,417, and 1.583 kg ha⁻¹ of no 1, canner, and marketable storage roots, respectively (Table 4.4). No jumbos were harvested in Charleston. The yield of no.1 storage roots was similar between all treatments. Treatments containing bentazon in tank-mix with additional compounds yielded similar for marketable storage roots, while herbicide alone had a yielded only 26% of that same grade. For total marketable storage roots, bentazon with melatonin + BSM had approximately 4x the yield observed with the non-treated check, bentazon alone, and BSM. However, yield differences were not observed between the different compounds in tank-mix with herbicide. Contrast tests illustrate that tank-mixing plant hormones with bentazon reduced herbicide injury in sweetpotato (Table 4.5).

Discussion

Weed competition is one of the major factors in limiting yield potential of sweetpotato. The lack of herbicide options for POST sedge control contributes to yield loss. In this study, the

addition of plant hormones tank-mixed with bentazon reduced herbicide damage on sweetpotato plants. Percent injury of plants treated with tank-mix were 50% lower than the plants treated with bentazon alone at both locations. Injury symptoms were observed 5 to 7 days after treatment, that included yellowing (chlorosis) of leaf tissue followed by death (necrosis) of the tissue, beginning around the leaf margins and progressing towards the center. In Cameron, SC, injury 1WAT was similar between all treatments containing herbicide. However, injury was reduced quicker in plants treated with a tank-mix of herbicide + plant hormones. Injury observed in plants exclusively treated with bentazon at 3 and 6 WAT was twice the value observed in plants treated with bentazon + plant hormones. A similar trend was observed in Charleston, SC, with a quicker reduction in herbicide injury observed in plants treated with bentazon + plant hormones (Fig 3). At 6 WAT, plots treated with only bentazon had injury levels of 41%, while plots containing plant hormones and bentazon had fully recovered. The susceptibility of sweetpotato to bentazon was reported by Caputo et al. (2020), where three cultivars exhibited significant levels of injuries to the herbicide incorporated into Murashige and Skoog basal medium.

Bentazon application induces secondary effects in several metabolic pathways, such as ROS production (Macedo et al., 2008; Pospíšil, 2009). As reported by Zhou et al. (2015), melatonin and BRs are related to improving plant stress tolerance by activating cytochrome p450, glutathione, and glutathione S-Transferase, essential for the herbicide detoxification process. Additionally, the ability to scavenge radical molecules makes them play a prominent role in the antioxidant defense network of plants, crucial as protectors against oxidative stress (Asensi-Fabado and Munné-Bosch, 2010; Arnao and Hernández-Ruiz, 2014). Furthermore, melatonin has antioxidant properties, preventing possible oxidative damages caused by bentazon. Reports suggest that melatonin can act as a ROS scavenger by activating antioxidant enzymes ROS

detoxification to maintain the equilibrium of the cellular ROS at a low level (Reiter et al., 2000; Shi et al., 2015; Xia et al., 2020).

Yellow nutsedge control was similar between treatments at 1 WAT. This suggests the interaction between bentazon and the tank-mix compounds did not result in an antagonist interaction, maintaining the herbicide efficiency. However, plots exclusively treated with bentazon had superior number of remaining yellow nutsedge plants, about 40%, at 3 and 6 WAT, on both locations. (Fig 4.2 and 4.4). As part of weed management on sweetpotatoes, plant architecture plays an important role on tolerance to weed interference (La Bonte et al. 1999). Harrison and Jackson (2011a) noted weed interference in the first 3 to 4 weeks after transplant could negatively impact sweetpotato yield, and cultivars with spreading habits were less competitive, requiring a higher period of weed-free interval. In our studies, duration of bentazon injury in plants lasted much of the growing season. This negatively affected plant growth, stunting plant development and reducing the plant's competitive potential, resulted in greater weed infestation. Concurrently, melatonin and BSM reduced the negative impact of bentazon on plant structure, providing greater plant development and weed suppression.

At both locations, the tank-mix of bentazon + melatonin + BSM resulted in greater yield of marketable storage roots than bentazon alone and the non-treated check control. According to Smith et al. (2019), 'Bayou Belle' cultivar had an average yield of 21,857 and 35,172 kg ha⁻¹ of no 1 and total marketable, respectively in Louisiana. A similar yield was observed in Cameron, SC, where no differences were observed for jumbo, no 1, canner, and total storage roots grades between treatments containing bentazon in tank-mix with plant hormones (Table 4.2). However, in Charleston, SC, the yield observed was lower than expected for the cultivar. Despite the treatment effect on the yield of canners' yield and total marketable storage roots, the highest yield

obtained was the total marketable root storage, treatment using bentazon + melatonin + BSM that yielded only 4,333 kg ha⁻¹.

Soil characteristics had a significant influence on the final yield. The Charleston field consists of Yonges loamy sand (fine loamy mixed, thermic Albaqualf), this type of soil is characterized as very deep, poorly drained, moderately permeable and consists of thick loamy sediments. For August and September, Charleston had precipitation above normal levels (National Weather Service, 2021). These rain events, associated with soil characteristics caused an accumulation of water for long periods. A three year trial evaluated the yield of 'Bayou Belle' grown in Charleston, SC the yield observed was 6,846, 3.150, 12.879 kg ha⁻¹ of total storage roots for 2010, 2011 and 2012, respectively (Jackson, 2011). During 2011 in Charleston, precipitation events were similar to 2020, where July, August and September had slightly above average rain and on concentrated days, resulting on periods of soggy soil. Sweetpotato is highly vulnerable to flooding stress (Lin et al. 2006) as indicated in previous research, where the yield of storage roots in sweetpotato was reduced by 57% when flooded at midseason (Roberts and Russo, 1991).

In conclusion, this investigation demonstrated that plant hormones tank-mixed with bentazon can reduce herbicide injury in sweetpotato. The lower injury observed, and the significantly higher yield demonstrated melatonin and BSM improved sweetpotato tolerance to bentazon. Simultaneously, the tank-mix of melatonin, BSM and bentazon did not reduce herbicide efficiency and resulted in a greater yellow nutsedge control. Finally, future experiments should be performed in multiple locations with multiple cultivars to generate data to that can be used to promote label expansion of bentazon, which is a much-needed POST herbicide for yellow nutsedge control in sweetpotato. Our study is the first to provide critical baseline data on reduced

crop injury and increased yield of bentazon when tank-mixed with plant hormones to provide effective control of yellow nutsedge in sweetpotato.

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TABLES

Table 2.1: Test of the effects of treatment factors and their interactions on injury to three cultivars of sweetpotato 3 weeks after treatment

Source	df	SS	F-ratio	P>F
Cultivar	2	2467.41	9.4805	0.0002*
Herbicide	3	130048.67	333.12224	<0.0001*
Cultivar*Herbicide	6	4130.93	5.2907	0.0001*

df, degrees of freedom; SS, sum of squares

*, ***, Significant at $P < 0.001$

Table 2.2 Estimated concentrations of bentazon (mM) required to cause 10%, 20%, or 30% visual injury to ‘Beauregard’, ‘Covington’, and USDA-09-130 sweetpotato slips 3 weeks after treatment

Cultivar	10%	20%	30%
Beauregard	0.0178	0.0419	0.0710
Covington	0.0073	0.0157	0.0252
USDA 09_130	0.0053	0.0115	0.0188

Table 2.3 Test of the effects of treatment factors and their interactions on injury caused to sweetpotato slips of cultivar Beauregard 3 weeks after treatment

Source	df	SS	F-ratio	P>F
Herbicide	1	38919.485	145.899	<0.0001*
Melatonin	2	2055.689	3.8531	0.0297***
Herbicide*Melatonin	2	2899.6	5.4349	0.0083

df, degrees of freedom; SS, sum of squares

*, *** Significant at P<0.001 or 0.05, respectively

Table 2.4 Test of the effects of treatment factors and their interactions on the weight of sweetpotato plantlets of cultivar Beauregard 3 weeks after treatment

Source	df	SS	F-ratio	P>F
Herbicide	1	7.5369	17.1780	0.0002*
Melatonin	2	0.5945	0.6775	0.5137
Herbicide*Melatonin	2	0.4430	0.5049	0.6075

df, degrees of freedom; SS, sum of squares

* Significant at $P < 0.001$

Table 3.1 Estimated dose (LD) of bentazon and mesotrione (g ai ha⁻¹) required to cause 10%, 20%, or 30% visual injury to ‘Beauregard’ and ‘Covington’ sweetpotato plants with and without various safeners 3 weeks after treatment

CULTIVAR	HERBICIDE	SAFENER	R ²	LD ₁₀ (10% Injury)	LD ₂₀ (20% injury)	LD ₃₀ (30% of injury)
Beauregard	Bentazon	None	0.86	274.22	336.37	379.28
		Melatonin	0.90	572.10	719.31	819.51
		24-Epi	0.71	140.04	286.40	387.85
		AsA	0.81	174.27	350.09	469.42
	Mesotrione	None	0.95	17.45	37.83	71.97
		Melatonin	0.90	49.27	105.75	169.27
		24-Epi	0.90	53.22	101.41	148.31
		AsA	0.89	74.01	137.42	194.33
Covington	Bentazon	None	0.97	114.93	167.77	203.03
		Melatonin	0.82	139.22	206.14	258.44
		24-Epi	0.95	127.42	157.93	208.26
		AsA	0.86	117.71	170.40	207.91
	Mesotrione	None	0.98	11.71	14.57	23.77
		Melatonin	0.92	45.99	111.68	155.75
		24-Epi	0.85	33.69	102.46	129.72
		AsA	0.85	41.54	95.89	134.78

Table 4.1. Treatments applied 21 days after transplant, herbicide and additional compound applied on broadcast on sweetpotato field.

Herbicide (g ai ha⁻¹)	Additional compound (g ai ha⁻¹)
Untreated	untreated
Bentazon (200)	----
---	Melatonin (100)
---	BSM (1000)
Bentazon (200)	Melatonin (100)
Bentazon (200)	BSM (1000)
Bentazon (200)	Melatonin (100) + BSM (1000)

Table 4.2. Effect of Bentazon and additional compounds applied 21DAT, on the final yield of sweetpotato cultivar Bayou belle, Cameron, SC.

Bentazon (g ai ha⁻¹)	Additional compound	Jumbo	No 1	Jumbo + No 1	Canner	Total marketable
----- Kg ha⁻¹ -----						
	Non-treated check	14,733 a	13,325 b	28,058 bc	4,841 ab	28,063 b
514	None	11,275 a	11,950 b	23,225 c	7,616 a	24,741 b
0	Melatonin (100 g ai ha ⁻¹)	22,275 a	13,416 b	35,691 abc	2,716 ab	39,408 ab
0	BSM (1L ai ha ⁻¹)	23,634 a	20,958 ab	44,592 ab	3,641 ab	48,243 ab
514	Melatonin (100 g ai ha ⁻¹)	20,825 a	21,033 ab	41,858 abc	2,033 b	45,500 ab
514	BSM (1L ai ha ⁻¹)	21,066 a	23,375 ab	44,442 abc	1,066.70 b	45,508 ab
514	Melatonin (100 g ai ha ⁻¹) + BSM (1L ai ha ⁻¹)	22,333 a	27,658 a	49,991 a	2,175.50 b	52,167 a

Total marketable is the aggregate of Jumbo, No 1, and canner grades Means with different letters are significantly different according to Tukey's multiple range tests ($P < 0.05$).

BSM, Brassinosteroids mixture; DAT, days after transplant.

Table 4.3. Contrast tests of percent injury to determine how additional compounds affect bentazon injury on sweetpotato plants in Cameron, SC.

Contrasting factors	Metric	Estimate	Significance
No additional compound (-1) vs Melatonin (+0.33), BSM (+0.33), Melatonin + BSM (+0.33)	Herbicide injury 1WAT	0.4291	NS
No additional compound (-1) vs Melatonin (+0.33), BSM (+0.33), Melatonin + BSM (+0.33)	Herbicide injury 3WAT	9.3258	**
No additional compound (-1) vs Melatonin (+0.33), BSM (+0.33), Melatonin + BSM (+0.33)	Herbicide injury 6WAT	11.1901	**
No additional compound (-1) vs Melatonin (+0.33), BSM (+0.33), Melatonin + BSM (+0.33)	Herbicide injury 9WAT	0.9684	NS

Brassinosteroids mixture, BSM; Weeks after treatment, WAT

** Significant at the 0.01 probability level.

‡ NS, nonsignificant at 0.05 probability level.

Table 4.4. Effect of Bentazon and additional compounds applied 21 DAT, on the final yield of sweetpotato cultivar Bayou belle, Charleston, SC.

Bentazon (g ai ha⁻¹)	Additional compound	No 1	Jumbo + No 1	Canner	Total marketable
-----Kg ha ⁻¹ -----					
Non-treated check		166 a	166 a	1,416 bc	1,583 bc
514	None	250 a	250 a	666 c	916 c
0	Melatonin (100 g ai ha ⁻¹)	250 a	250 a	1,833 abc	2,083 abc
0	BSM (1L ai ha ⁻¹)	83 a	83 a	1,583 abc	1,666 bc
514	Melatonin (100 g ai ha ⁻¹)	500 a	500 a	3,083 a	3,583 ab
514	BSM (1L ai ha ⁻¹)	583 a	583 a	2,916 ab	3,500 ab
514	Melatonin (100 g ai ha ⁻¹) + BSM (1L ai ha ⁻¹)	1333 a	1333 a	3,000 ab	4,333 a

Total marketable is the aggregate of Jumbo, No 1, and canner grades. Means with different letters are significantly different according to Tukey's multiple range tests ($P < 0.05$).

BSM, Brassinosteroids mixture; DAT, days after transplant.

Table 4.5. Contrast tests of injury means to determine how additional compounds affect bentazon injury on sweetpotato plants in Charleston, SC.

Contrasting factors	Metric	Estimate	Significance
No additional compound (-1) vs Melatonin (+0.33), BSM (+0.33), Melatonin + BSM (+0.33)	Herbicide injury 1WAT	5.8000	NS
No additional compound (-1) vs Melatonin (+0.33), BSM (+0.33), Melatonin + BSM (+0.33)	Herbicide injury 3WAT	29.4329	***
No additional compound (-1) vs Melatonin (+0.33), BSM (+0.33), Melatonin + BSM (+0.33)	Herbicide injury 6WAT	26.3817	***
No additional compound (-1) vs Melatonin (+0.33), BSM (+0.33), Melatonin + BSM (+0.33)	Herbicide injury 9WAT	0.9234	NS

Brassinosteroids mixture, BSM; Weeks after treatment, WAT

*** Significant at the 0.001 probability level.

‡ NS, nonsignificant at 0.05 probability level.

FIGURES

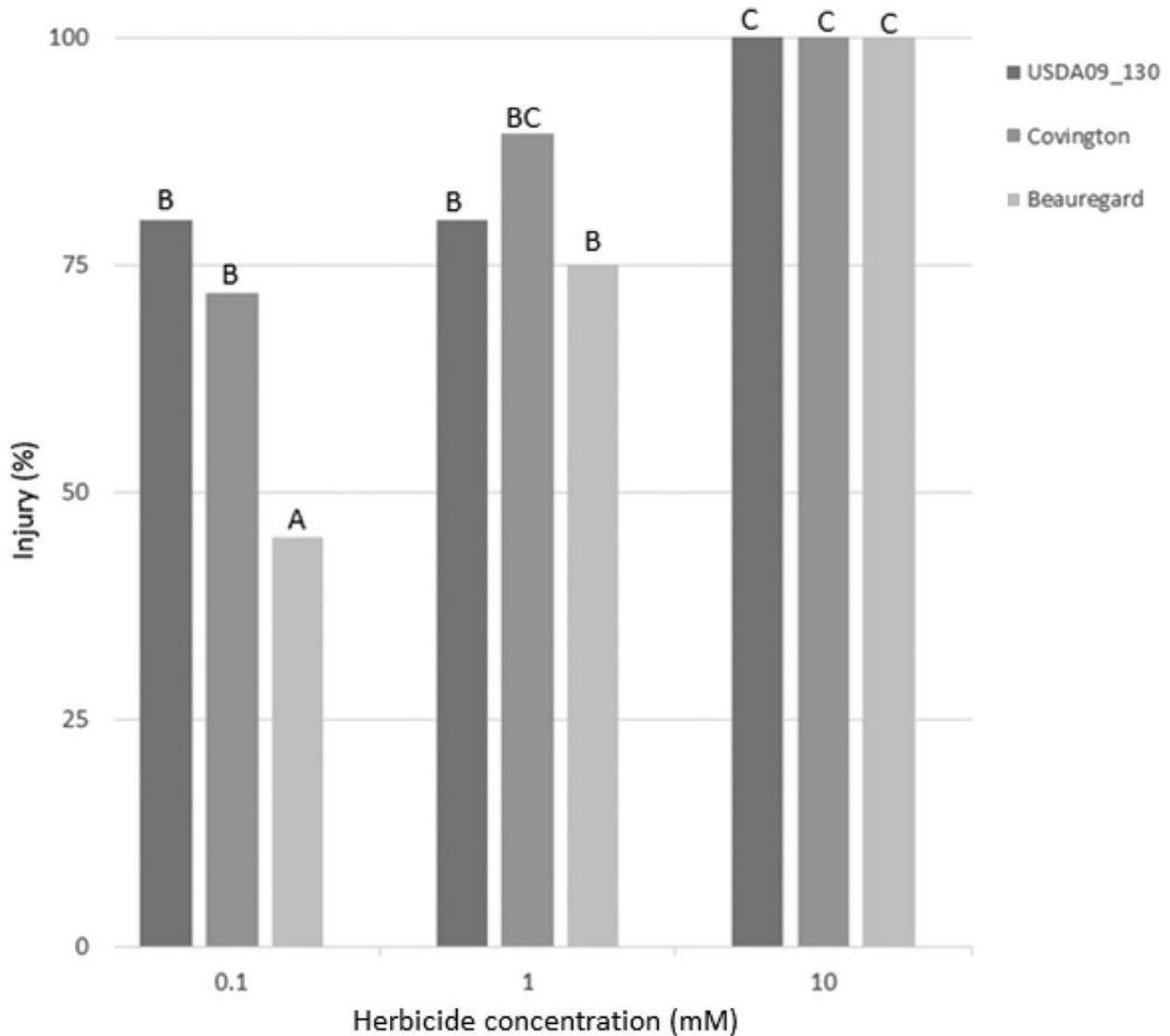


Figure 2.1. Percent injury at 21 d after treatment of sweetpotato cultivars ‘Beauregard’, ‘Covington’, and USDA-09-130 caused by three bentazon concentrations incorporated into Murashige and Skoog (MS) basal media. Injury intervals range from 0% to 100% (0 = no injury; 100% = plant death). Values are the averages of four replicates. Means with different letters are significantly different according to Tukey’s multiple range tests ($P < 0.05$).

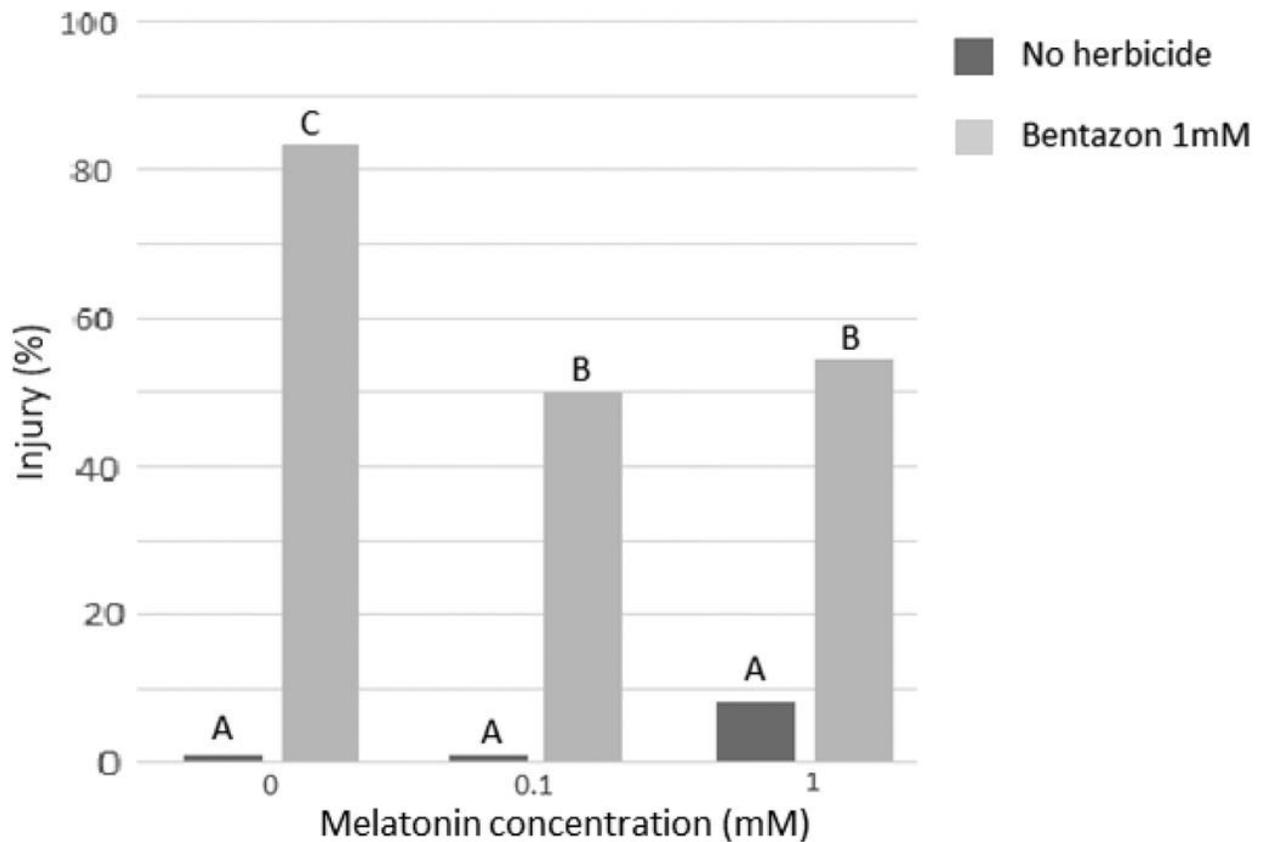


Figure 2.2. Percent injury of Beauregard sweetpotato by bentazon and melatonin incorporated in Murashige and Skoog (MS) media at 21 d after treatment. Injury intervals ranged from 0% to 100% (0 = no injury; 100% = plant death). Values are the averages of four replicates. Different letters indicate significant differences according to Tukey's multiple range tests ($P < 0.05$).

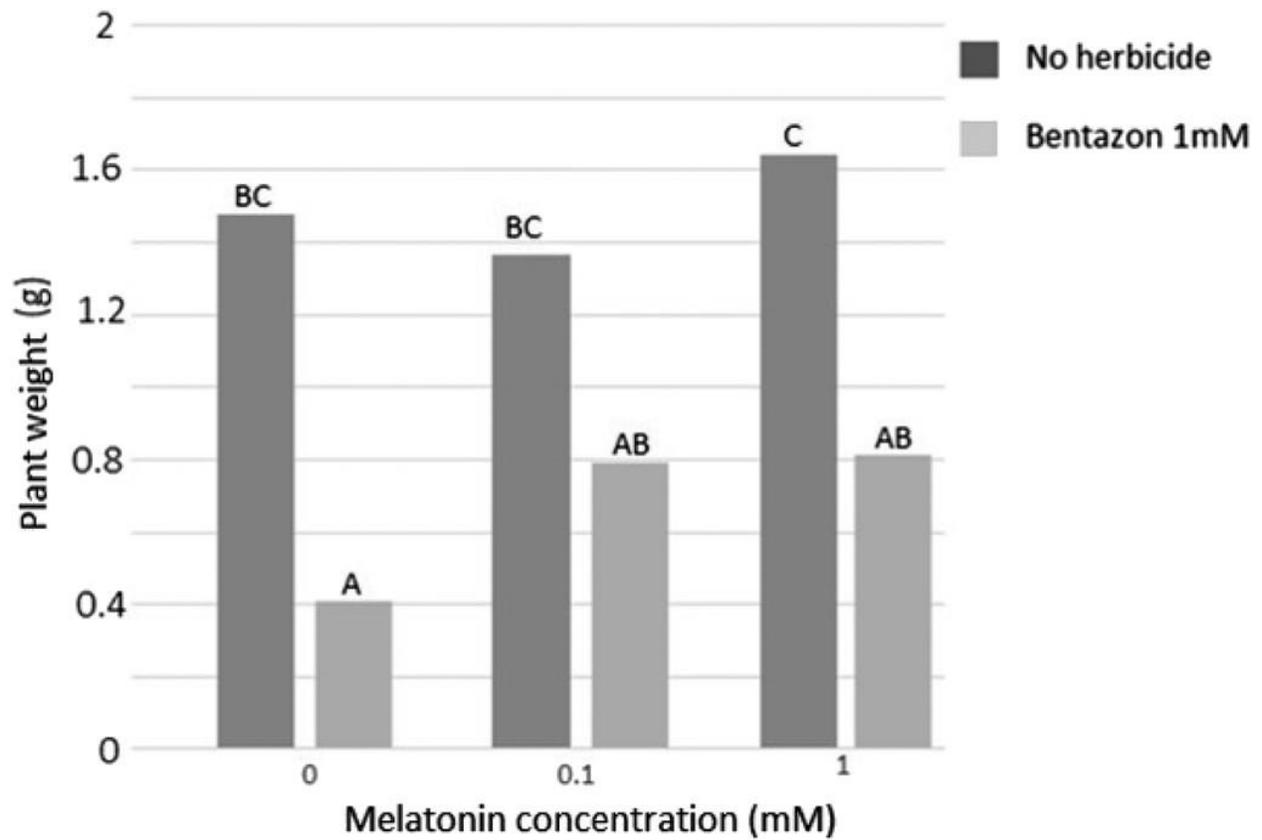


Figure 2.3. Weight of Beauregard sweetpotato plants in Murashige and Skoog (MS) basal media incorporated with bentazon and melatonin at 21 d after treatment. Values are the averages of four replicates. Different letters indicate significant differences according to Tukey's multiple range tests ($P < 0.05$).

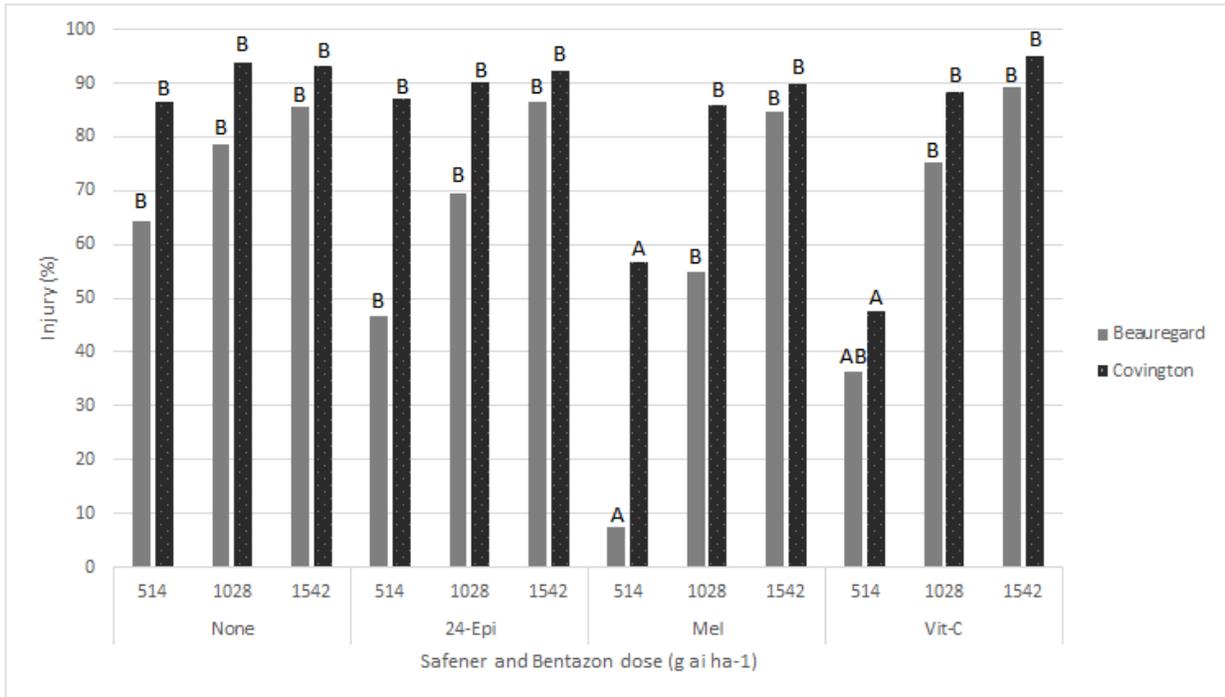


Figure 3.1. Percent injury at 21 d after treatment of sweetpotato cultivars ‘Beauregard’, ‘Covington’, to three doses of bentazon applied alone or in tank-mix with melatonin, Ascorbic acid or 24-Epibrassinolide. Injury intervals range from 0% to 100% (0 = no injury; 100% = plant death). Values are the averages of four replicates. Means with different letters are significantly different according to Tukey’s multiple range tests ($P < 0.05$).

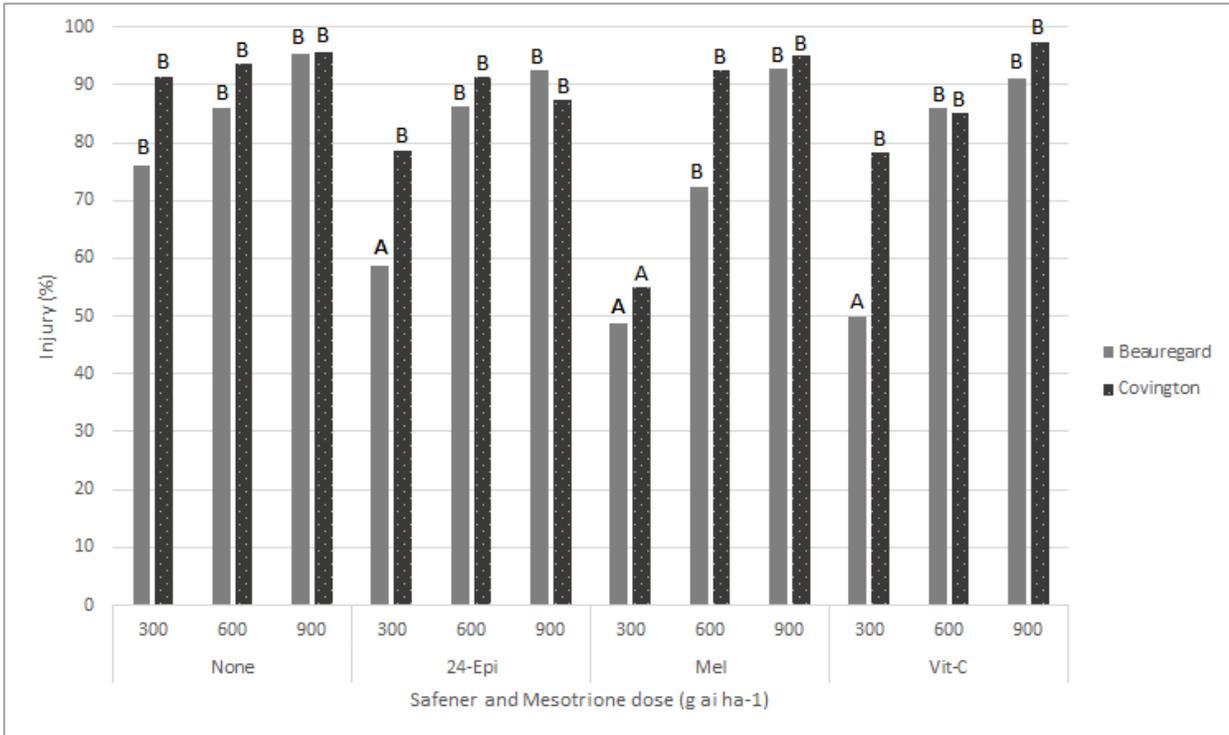


Figure 3.2. Percent injury at 21 d after treatment of sweetpotato cultivars ‘Beauregard’, ‘Covington’, to three doses of mesotrione applied alone or in tank-mix with melatonin, Ascorbic acid or 24-Epibrassinolide. Injury intervals range from 0% to 100% (0 = no injury; 100% = plant death). Values are the averages of four replicates. Means with different letters are significantly different according to Tukey’s multiple range tests ($P < 0.05$).

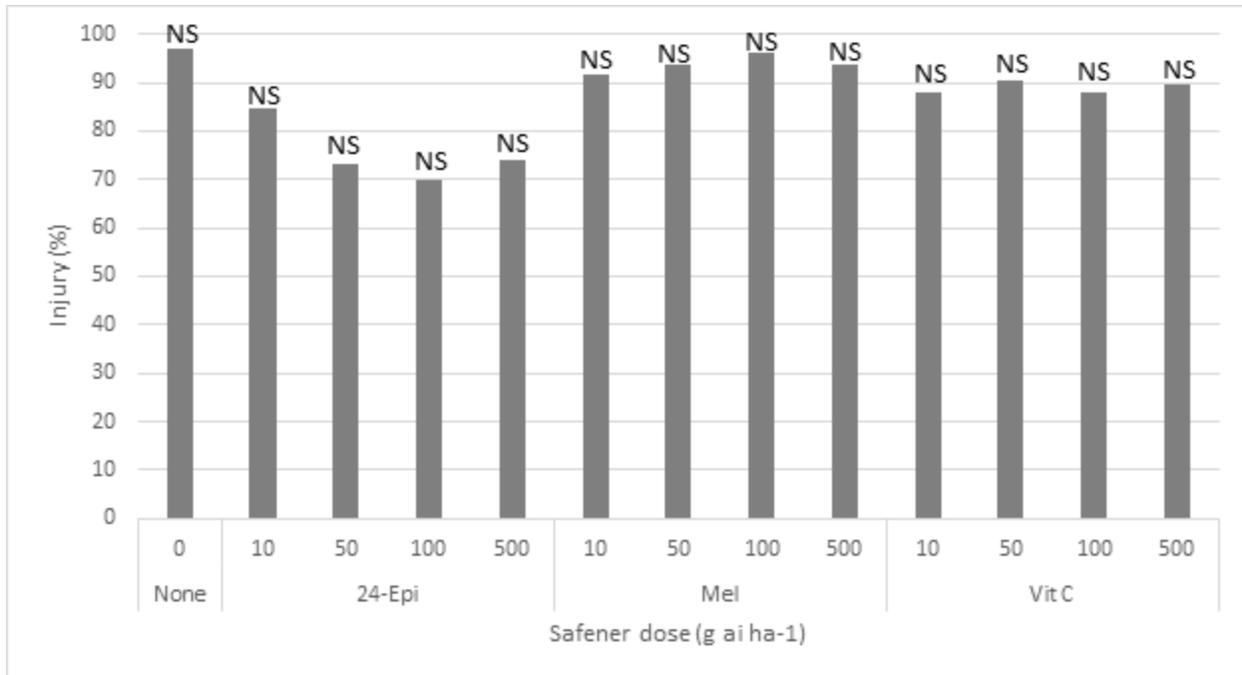


Figure 3.3. Percent injury at 21 d after treatment of Yellow nutsedge to 514g ai ha⁻¹ of bentazon applied alone or in tank-mix with doses of melatonin, ascorbic acid or 24-Epibrassinolide. Injury intervals range from 0% to 100% (0 = no injury; 100% = plant death). Values are the averages of four replicates. Means with different letters are significantly different according to Tukey's multiple range tests ($P < 0.05$). Mel: Melatonin, 24-Epi: 24-Epibrassinolide, Vit-C: ascorbic acid.

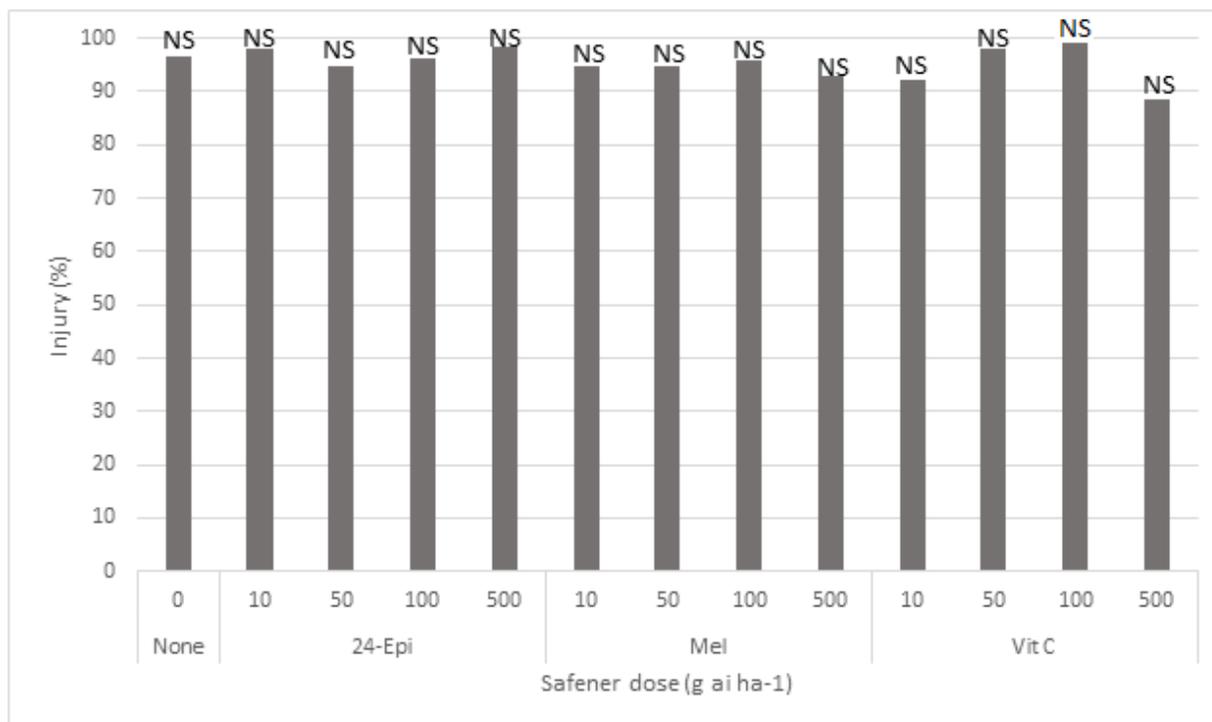


Figure 3.4. Percent injury at 21 d after treatment of Palmer amaranth to 300g ai ha⁻¹ of mesotrione applied alone or in tank-mix with doses of melatonin, ascorbic acid or 24-Epibrassinolide. Injury intervals range from 0% to 100% (0 = no injury; 100% = plant death). Values are the averages of four replicates. Means with different letters are significantly different according to Tukey's multiple range tests ($P < 0.05$). Mel: Melatonin, 24-Epi: 24-Epibrassinolide, Vit-C: ascorbic acid.

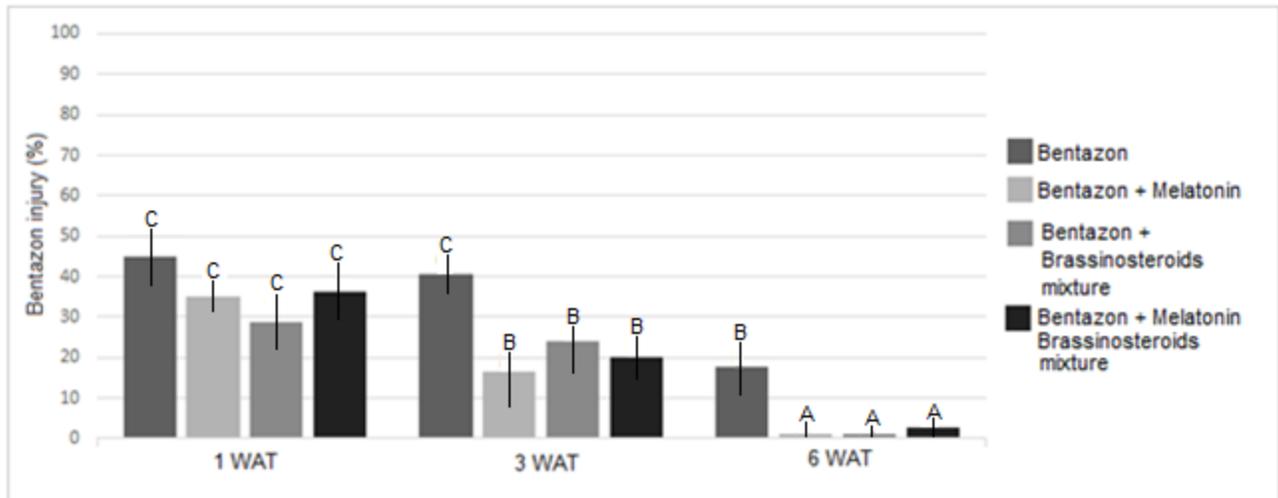


Figure 4.1. Response of sweetpotato cultivar Bayou Belle to applications of Bentazon alone or in tank-mix with Melatonin, BSM or Melatonin + BSM, in Cameron, SC.. Injury intervals range from 0% to 100% (0 = no injury; 100% = plant death). Values are the averages of four replicates. Means with different letters are significantly different according to Tukey's multiple range tests ($P < 0.05$).

BSM, Brassinosteroids mixture; WAT, weeks after transplant.

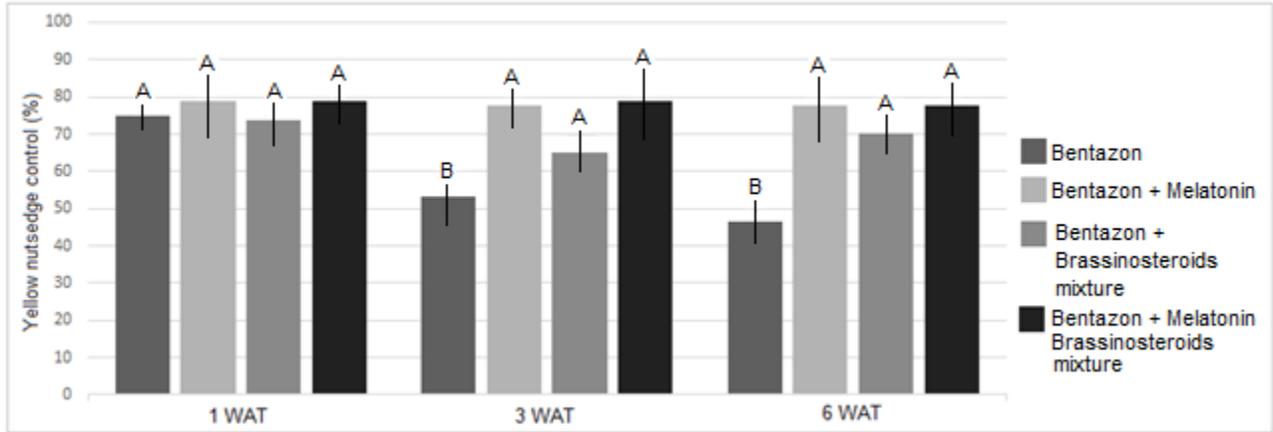


Figure 4.2. Response to applications of Bentazon alone or in tank-mix with Melatonin, BSM or Melatonin + BSM, in Cameron, SC.. Yellow nutsedge control interval range from 0% to 100% (0 = no control; 100% = total control). Values are the averages of four replicates. Means with different letters are significantly different according to Tukey's multiple range tests ($P < 0.05$).

BSM, Brassinosteroids mixture; WAT, weeks after transplant.

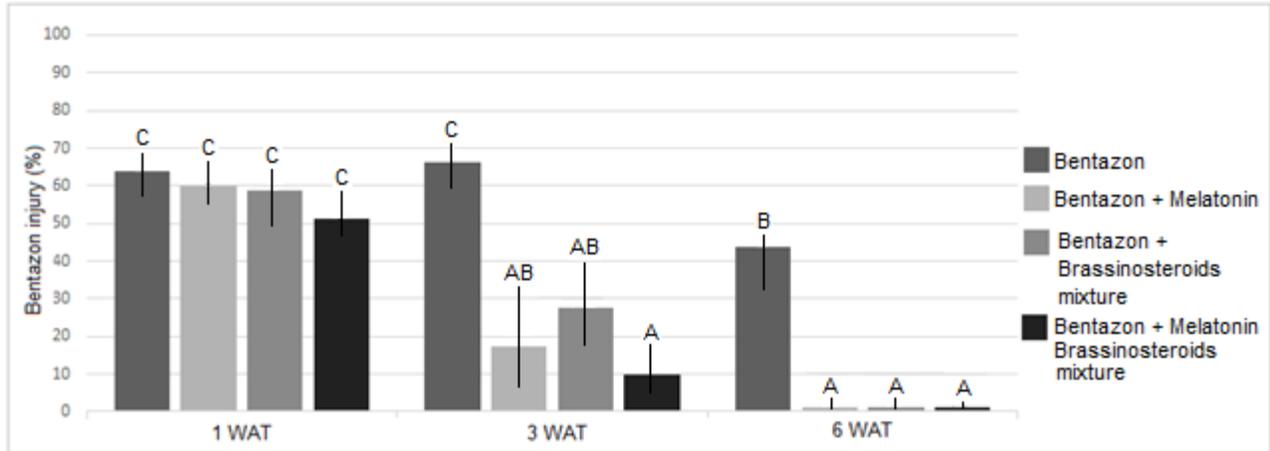


Figure 4.3. Response of sweetpotato cultivar Bayou Belle to applications of Bentazon alone or in tank-mix with Melatonin, BSM or Melatonin + BSM, in Charleston, SC. Injury intervals range from 0% to 100% (0 = no injury; 100% = plant death). Values are the averages of four replicates. Means with different letters are significantly different according to Tukey's multiple range tests ($P < 0.05$).

BSM, Brassinosteroids mixture; WAT, weeks after transplant.

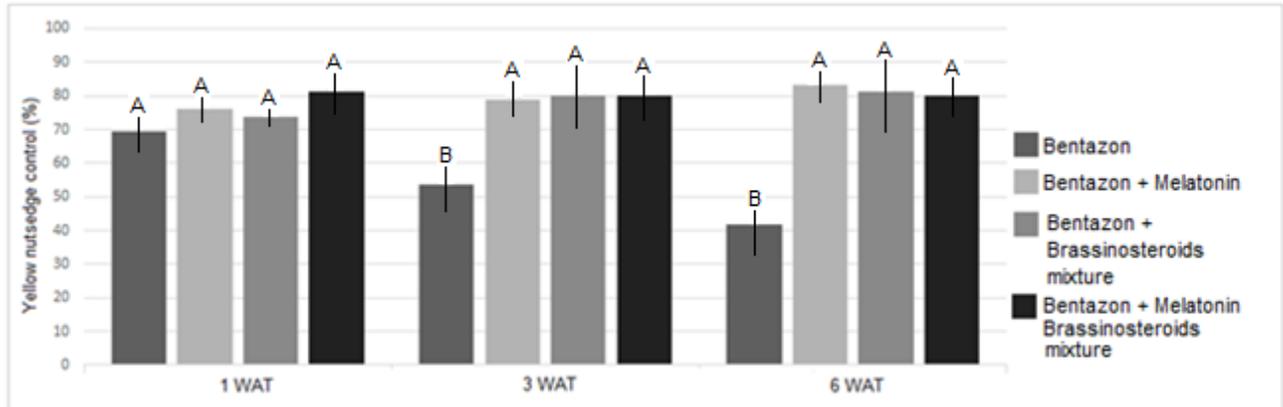


Figure 4.4. Response to applications of Bentazon alone or in tank-mix with Melatonin, BSM or Melatonin + BSM, in Charleston, SC.. Yellow nutsedge control interval range from 0% to 100% (0 = no control; 100% = total control). Values are the averages of four replicates. Means with different letters are significantly different according to Tukey's multiple range tests ($P < 0.05$).

BSM, Brassinosteroids mixture; WAT, weeks after transplant.