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

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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 yellow nutsedge. Research was conducted to evaluate the bentazon dose response of two sweetpotato cultivars and one advanced clone and to evaluate the plant hormone melatonin to determine its ability to safen bentazon post emergence. 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 to evaluate the effect of bentazon on sweetpotato and to determine the interactive response of the Beauregard cultivar to bentazon and exogenous applications of melatonin. Beauregard swas the most tolerant cultivar and required dosages of bentazon that were two-times higher to cause the same injury compared with other cultivars. MS media containing melatonin and bentazon showed fewer injuries and higher plant mass than plants treated with bentazon alone. These results indicate that sweetpotato injury caused by bentazon may be reduced by melatonin.

Sweetpotato [Ipomoea batatas (L.) Lam] is an economically important crop in the United States; in 2017, its worth was more than $733 million (U.S. Department of Agriculture, 2019), and a total area more than 60,000 ha was planted 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 reduces yield by up to 80% (Meyers and Shankle, 2017). However, Meyers et al. (2010) reported a 36% to 81% reduction in marketable yield of Beauregard and Covington sweetpotato cultivars with 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 on sweetpotato that control troublesome weeds, such as Amaranthus palmeri and annual grasses (Kemble, 2017; Meyers et al., 2010). There is a lack of POST herbicides to control Cyperus esculentus in sweetpotato (Webster, 2010). A potential herbicide that is used to evaluate sweetpotato and 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 herbicide safener concepts, which, ideally, would protect sweetpotato from POST herbicides while not antagonizing weed control (Parker, 1983). Numerous studies of monocot species have reported that 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, Dubleman 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 that has been shown to increase cytochrome P450s and potentially sequester reactive oxygen species in broad-leaf vegetable crops is melatonin. Arnao (2014) highlighted the antioxidant capacity of melatonin (N-acetyl-5-methoxytryptamine), which is able to scavenge reactive oxygen species (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 that 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 that 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 that the external application 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) 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 that a cellular increase in the
Table 1. Test of the effects of treatment factors and their interactions on injury to three cultivars of sweetpotato 3 weeks after treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>F ratio</th>
<th>P &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar</td>
<td>2</td>
<td>2467.41</td>
<td>9.4805</td>
<td>0.0002*</td>
</tr>
<tr>
<td>Herbicide</td>
<td>3</td>
<td>130048.67</td>
<td>333.1224</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Cultivar*Herbicide</td>
<td>6</td>
<td>4130.93</td>
<td>5.2907</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

df = degrees of freedom; SS = sum of squares. *Significant at P < 0.001.

Fig. 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).

Table 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.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beauregard</td>
<td>0.0178</td>
<td>0.0419</td>
<td>0.0710</td>
</tr>
<tr>
<td>Covington</td>
<td>0.0073</td>
<td>0.0157</td>
<td>0.0252</td>
</tr>
<tr>
<td>USDA-09-130</td>
<td>0.0053</td>
<td>0.0115</td>
<td>0.0188</td>
</tr>
</tbody>
</table>

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; Sakhahnokho 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 was 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.

Materials 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 x 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 μmol·m⁻²·s⁻¹ provided by fluorescent tube lights. The experiment was a randomized complete block with four replications that 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 that of 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 designs 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...
Results and Discussion

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 1). Differences between cultivars at 10 mM of bentazon did not occur because all plants exhibited 100% injury, as shown in Fig. 1. At 1 mM of bentazon, all cultivars exhibited a similar response, and injury rates reached 75%, 77%, and 84% for Beauregard, 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. Herbicide projections were calculated to determine the concentration of bentazon required to cause 10%, 20%, and 30% of injury for each cultivar (Table 2). Based on these calculations, USDA-09-130 was the most sensitive to bentazon. Covington was more tolerant to bentazon than USDA-09-130 and required a higher dose to reach the same injury threshold. The herbicide dose necessary to cause similar injury to Beauregard slips was twice that of the other cultivars.

Melatonin safener. The addition of exogenous melatonin to the media decreased herbicide injury 21 DAT (Fig. 2). Without melatonin, bentazon at 1 mM caused an injury of 83% on Beauregard plants. Comparatively, the injury from herbicide and melatonin supplementation were both 51% for both rates of melatonin (0.1 mM and 1 mM), as evidenced by the injury caused by the herbicide being 30% lower when melatonin was applied in conjunction. This means the reduction was statistically different (F2,2 = 5.4349 and P < 0.01) (Table 3, Fig. 2). However, the injuries caused by bentazon and melatonin supplementation were statistically equal for both melatonin concentrations. Plant biomass was also affected by the herbicide (Table 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. 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 Motzenbocker 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. 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, thus 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 proves that melatonin is an essential component of the redox system. Our study suggests that 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 edges 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 herbicide effect. Planta Daninha 36:1–10.

Literature Cited


