Evaluation of Chemical Seed Treatment to Reduce Injury Caused by Preemergent Herbicides on Direct-seeded Turnips and Collard Greens

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Abstract. Poor competitive ability and limited herbicide options make weed management of *Brassica* crops difficult. Growers often adopt the use of transplants, which is less efficient in terms of time, material, and labor when compared with direct seeding, resulting in higher prices per unit. Seed treatment with protective compounds could decrease crop injury from preemergent (PRE) herbicides making it profitable to direct-seed *Brassica* plants for production. Research was conducted to evaluate the ability of three candidate safeners [24-epibrassinolide, melatonin, and ascorbic acid (AsA)] to reduce injury caused by four herbicides (*S*-metolachlor, pyroxasulfone, halosulfuron, and mesotrione) applied PRE on the collard green cultivar Top Bunch and turnip cultivar Purple Top White Globe. Two independent greenhouse trials were conducted at the Clemson University Coastal Research and Education Center in Charleston, SC. Visual injury of the treated plants was evaluated weekly and dry mass was collected 21 days after treatment. Seed treatment did not reduce injury efficiently caused by pyroxasulfone, halosulfuron, and mesotrione; all doses were lethal for both crops. However, collard seeds treated using melatonin and AsA had 66% and 54% less injury caused by *S*-metolachlor at 514 g·ha⁻¹ a.i., respectively. On turnips, melatonin was the only treatment that reduced the *S*-metolachlor damage on seedlings, with 43% less injury than untreated seedlings. Plant injury and plant weight correlated significantly for both *Brassica* crops. The reduction in injury caused by *S*-metolachlor when seeds were treated with melatonin and AsA validated those compounds' protective ability. Seed treatment with melatonin could be combined with PRE applications of *S*-metolachlor to overcome the low weed competitive ability of these species early in the season.

South Carolina ranked first nationally in the production of collard (*Brassica oleracea* var. *viridis*) and turnip greens (*Brassica rapa* var. *rapa* L.) greens (U.S. Department of Agriculture, 2019). Weed management for vegetables in the southeastern United States can be challenging as a result of high levels of rainfall, warm summers, and mild winters that facilitate aggressive weed species germination and infestation (Singer et al., 2013). According to Van Wychen (2019), common lambsquarter (*Chenopodium album* L.), nut-sedge (*Cyperus* spp.), pigweed (*Amaranthus* spp.), and ragweed (*Ambrosia* spp.) are the most troublesome weeds present in leafy greens fields. *Brassica* seedlings are poor competitors with weeds early in the season as a result of their small stature, shallow root system, and thin canopy (Yu et al., 2018).

The critical weed-free period for direct-seeded cole crops is typically 2 to 4 weeks (Miller and Hopen, 1991). Inefficient weed management during the early stages of crop growth can result in major yields losses resulting from the competition for nutrients, water, sunlight, and space.

There are limited herbicide options available for *Brassica* growers (Kemble, 2017). Controlling weeds PRE in turnips and collards is often limited to dimethyl tetrachloroterephthalate applied as a shallow preplant-incorporated application or a broadcast application to the soil surface at time of seeding, followed by hand-weeding. Growers often transplant these crops to decrease the labor required for mechanical weeding. However, Muragi and Sajjan (2019) reported that transplanting lowers production efficiency in terms of time, material, and labor compared with direct seeding. Early-season weed control is the primary challenge for the establishment of *Brassica* crops in direct-seeded production. The use of protective compounds as seed treatment could improve crop tolerance to injury from PRE herbicides, making it feasible to direct-seed *Brassica* crops for production.

Group 15 herbicides or very-long-chain fatty acid biosynthesis inhibitors such as *S*-

metolachlor and pyroxasulfone would be beneficial to use in direct-seeded *Brassica* crops if injury could be reduced with a seed treatment. *S*-metolachlor controls important weeds present in leafy greens fields, such as pigweed (*Amaranthus* spp.), carpet weed (*Mollugo verticillate* L.), sedges, and many others annual grasses and broadleaves (Wallace et al., 2020; Wyenandt et al., 2019). In annual grasses, *S*-metolachlor is absorbed by shoot tissues as they grow through the treated soil (Lebaron et al., 1988), and general symptoms are characterized by malformed and twisted seedlings in which leaves are tightly rolled in the whorl and cannot unroll properly (Senseman and Armbrust, 2007). In broadleaf weeds, root absorption is an important pathway for uptake of group 15 herbicides (Lebaron et al., 1988), and symptoms typically consist of crinkled leaves with a heart-shaped appearance (Senseman and Armbrust, 2007). Pyroxasulfone herbicide provides good efficacy on both grass and broadleaf weed species, with excellent selectivity in important row crops. Like other very-long-chain fatty acid herbicides, pyroxasulfone has little effect on germination and potentially inhibits shoot elongation of the germinated seed (Tanetani et al., 2009). Pyroxasulfone was evaluated because it has a lower environmental use rate and binds to more elongase enzymes compared with *S*-metolachlor. Cutulle et al. (2019) observed yellow nutsedge suppression when pyroxasulfone was applied at 89 g·ha⁻¹ a.i.; however, they reported significant levels of injury when sprayed over broccoli 'Emerald Crown' in Charleston, SC.

Halosulfuron is a Weed Science Society of America group 2 herbicide. It controls susceptible annual broadleaf weeds by inhibiting acetolactate synthase enzymes. Halosulfuron controls nutsedge when applied to the soil or on the foliage (Soltani et al., 2018). Talbert et al. (2005) reported the low tolerance of turnip and collard to halosulfuron applied preplant incorporated, where the injury was greater than 95% for both crops, when herbicide was applied at 110 g·ha⁻¹ a.i.

Another herbicide that could be beneficial to *Brassica* production is mesotrione, which acts by inhibiting 4-hydroxyphenylpyruvate dioxygenase in plants. This herbicide would represent a new alternative for weed management in *Brassica* crops if crop injury can be reduced during application. Mesotrione is a very effective in controlling problematic broadleaf weeds (Sutton et al., 2002). Felix et al. (2007) reported intense damage caused by soil residues after a 1-year application in two different sites, where cabbage, tomato, bell pepper, and cucumber had yield reduced to levels between 18% and 60% as a result of herbicide injury.

Compounds with the potential to improve plant tolerance to herbicide applications that enhance the herbicide detoxification process could be an important tool for weed management in direct-seeded *Brassica* crops. The plant hormones melatonin and brassinosteroids have been associated with the upregulation of cytochrome P450 and glutathione *S*-

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transferase (GST), which are essential for phases I and II of the herbicide detoxification process (Zhou et al., 2015). Mandal et al. (2018) showed that the exogenous application of melatonin directly impacts genes involved in stress response and increases cytochrome P450 activity in watermelon (*Citrullus lanatus* L.). Caputo et al. (2020) reported an improvement in sweetpotato [*Ipomoea batatas* (L.) Lam] tolerance to bentazon resulting from exogenous melatonin uptake in vitro. 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). Although AsA has no direct involvement in the herbicide detoxification process, it plays an essential role in stress perception and stress signaling responses in plants, having subcellular distribution according to stress source (Zechmann, 2011). In maize (*Zea mays* L.), seeds treated with 0.25 mM AsA showed reduced toxicity when imbibed with glyphosate at 3.6 mg·L⁻¹ at the seedling stage, with 15% more dry weight than glyphosate alone (Sacała and Roszak, 2018). Compounds that increase enzymes involved in herbicide metabolism, or sequester ROS have the potential to mitigate herbicide injury in crops (Parker, 1983).

The objective of our study was to characterize the tolerance of direct-seeded turnip greens and collard greens to PRE treatment of *S*-metolachlor, pyroxasulfone, mesotrione, and halosulfuron with and without seed treatments of melatonin, 24-epibrassinolide, or AsA.

Materials and Methods

Seeds of the turnip cultivar Purple Top White Globe (Johnny's Selected Seeds, Fairfield, MA) and collard green cultivar Top Bunch (Johnny's Selected Seeds) were treated with solutions of melatonin (Alfa Aesar, Ward

Hill), 24-epibrassinolide (MCE Med Chem Express, Monmouth Junction, NJ), and AsA (TCI America, Portland, OR). A water solution was prepared for each compound, at 1 M concentration. Compounds were weighed using a Mettler Toledo scale (TLE303E, SNR B705-644588, Langacher, 448606, Greifensee, Switzerland) and were stirred using an ultrasonic homogenizer (model CL-18; FisherBrand, Waltham, MA) at 100 W for 5 min for complete solubilization. The proportion of solution applied was 0.4 mL·g⁻¹ seed. After treatment, seeds were vortexed (Standard Vortex Mixer; VWR, Atlanta, GA) at 3000 rpm for 5 min and were then cooled at 4 °C dry for 24 h. To prevent photodegradation, seeds were protected from light during the entire procedure.

Pots 500 cm³ in volume were filled with Yonges loamy sand-type soil from an organic field that was previously cover-cropped with Italian ryegrass (*Lolium multiflorum* Lam.) and sun hemp (*Crotalaria juncea* L.) at Clemson's Coastal Research and Education Center, Charleston, SC (lat. 32.793239° N, long. -80.068812° W). Herbicide treatments were applied immediately after seeding using a research track sprayer (Generation 4; DeVries Manufacturing, Hollandale, MN), with a water carrier volume of 200 L·ha⁻¹ through 8002EV8 nozzles (Tee jet; Spraying Systems Co., Roswell, GA).

Herbicide treatments consisted of *S*-metolachlor (Dual Magnum; Syngenta Crop Protection, LLC, Greensboro, NC) applied at rates of 514, 1028, and 1542 g·ha⁻¹ a.i.; pyroxasulfone (Zidua, BASF, Houston, TX) at rates of 90, 180, and 270 g·ha⁻¹ a.i.; halosulfuron (Sandea; Gowan Company, Yuma, AZ) at rates of 35, 70, and 105 g·ha⁻¹ a.i.; and mesotrione (Callisto; Syngenta Crop Protection, LLC, Greensboro, NC) applied at rates of 114, 228, and 342 g·ha⁻¹ a.i.

The experiment followed a full factorial design, totaling 52 treatments per crop. It was

arranged in a randomized complete block design and included two independent tests with four replications each. The experiments were conducted from 10 July to 7 Aug. 2019 and 20 Aug. to 17 Sept. 2019. Plants were maintained 21 d in the greenhouse under natural light at 28 °C and 65% relative humidity. Visual injury was rated on a 0% to 100% scale (0 = no injury, 100% = plant death) at 7, 14, and 21 d after treatment (DAT). At the end of the experiment, plants were collected and dried to constant mass. All data were subjected to analysis of variance using mixed-model methodology in JMP (version 14; SAS Institute, Cary, NC). Herbicide treatment, seed treatment, and their interactions were considered fixed effects whereas replication was included as a random effect. No treatment × experimental run interaction occurred for herbicide injury and plant dry mass; therefore, data from both runs were combined.

Results

Seed treatment in the absence of an herbicide did not cause injury in either crop. For both crops, seed treatment did not reduce injury caused by pyroxasulfone, halosulfuron, and mesotrione, whereas the damage caused by PRE applications caused plant death. However, for *S*-metolachlor applications, we observed an improvement in seedling tolerance to PRE applications resulting from seed treatments. In collards, we observed 83% injury to plants without seed treatment from *S*-metolachlor at 514 g·ha⁻¹ a.i. at 21 DAT (Fig. 1). Seed treatment with melatonin or AsA reduced the injury observed on seedlings by 66% and 54%, respectively. For *S*-metolachlor at 1028 g·ha⁻¹ a.i., melatonin and AsA improved tolerance to the herbicide. Seed treatment with AsA or melatonin reduced seedling injuries by 66% and 44%, respectively. Seeds treated with 24-epibrassinolide resulted in lower numerical injury to

Fig. 1. Percent injury 21 d after transplant of 'Top Bunch' collards sprayed with *S*-metolachlor (three concentrations listed on x-axis) for the four treatment groups: 1) control plants, 2) melatonin, 3) 24-epibrassinolide, and 4) ascorbic acid. 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 ($P < 0.05$). Error bars show the SE of the mean. 24-Epi, 24-epibrassinolide; AsA, ascorbic acid; Mel, melatonin.

Fig. 2. Plant dry mass 21 d after transplant of 'Top Bunch' collards sprayed with *S*-metolachlor (three concentrations listed on x-axis) for the four treatment groups: 1) control plants, 2) melatonin, 3) 24-epibrassinolide, and 4) ascorbic acid. 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 ($P < 0.05$). Error bars show the SE of the mean. 24-Epi, 24-epibrassinolide; AsA, ascorbic acid; Mel, melatonin.

collards from *S*-metolachlor at 514 g·ha⁻¹ a.i.; however, this reduction was not significant at $P < 0.05$. No differences were observed between treatments for the highest dose of *S*-metolachlor.

The improved herbicide tolerance from the seed treatments resulted in a significant increase in plant weight. When *S*-metolachlor was applied at 514 g·ha⁻¹ a.i., the average weight of seedlings without treatment was 0.06 g (Fig. 2). For the same herbicide dose, seed treatment using melatonin, 24-epibrassinolide, and AsA resulted in plant weight 12×, 8×, and 10× greater than plants without seed treatment. When herbicide was applied at 1028 g·ha⁻¹ a.i., we observed a greater plant

weight resulting from seed treatment, where all the three compounds resulted in ≈3.5× more plant mass than untreated seedlings. At the highest dose of *S*-metolachlor, melatonin and 24-epibrassinolide treatments resulted in greater plant weight than untreated seedlings, weighing 3× more than untreated seedlings.

On turnip greens, we observed a lower injury caused by *S*-metolachlor at 514 g·ha⁻¹ a.i. on seeds treated with melatonin (Fig. 3). At 21 DAT, the injury observed on seedlings without treatment was 91%, whereas melatonin-treated seedlings had 43% less injury. Seed treatment using 24-epibrassinolide and AsA resulted in a lower injury rating; however, this reduction was not significant at $P < 0.05$. When

S-metolachlor was applied at 1028 and 1542 g·ha⁻¹ a.i., no differences in herbicide injury were observed between treatments, where the herbicide caused injuries between 82% and 99%, respectively. The results for turnip dry mass indicated that melatonin, 24-epibrassinolide, and AsA can reduce the herbicides' harmful effects on plant weight at the lowest dose of *S*-metolachlor tested (Fig. 4). The average weight of untreated plants was 0.03 g, whereas for seed treatments using melatonin, 24-epibrassinolide, and AsA, the weight was 5.5×, 2.5×, and 4.5× greater, respectively. No differences were observed among treatments when *S*-metolachlor was applied at 1028 and 1542 g·ha⁻¹ a.i.

Fig. 3. Percent injury 21 d after transplant of 'Purple Top White Globe' turnip greens sprayed with *S*-metolachlor (three concentrations listed on x-axis) for the four treatment groups: 1) control plants, 2) melatonin, 3) 24-epibrassinolide, and 4) ascorbic acid. 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 ($P < 0.05$). Error bars show the SE of the mean. 24-Epi, 24-epibrassinolide; AsA, ascorbic acid; Mel, melatonin.

Fig. 4. Plant dry mass 21 d after transplant of 'Purple Top White Globe' turnip greens sprayed with *S*-metolachlor (three concentrations listed on x-axis) for the four treatment groups: 1) control plants, 2) melatonin, 3) 24-epibrassinolide, and 4) ascorbic acid. 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 ($P < 0.05$). Error bars show the SE of the mean. 24-Epi, 24-epibrassinolide; AsA, ascorbic acid; Mel, melatonin.

Discussion

Our results highlight the lower tolerance of turnip and collard greens to PRE applications of *S*-metolachlor, pyroxasulfone, halosulfuron, and mesotrione. Reis et al. (2017) reported 80% injury caused by 240 g ha⁻¹ a.i. of *S*-metolachlor applications before cabbage transplanting (*Brassica oleracea* var. *capitata*). When *S*-metolachlor was applied to direct-seeded *Brassica* crops, Harrison et al. (1998) reported similar results in collards, in which a greenhouse experiment demonstrated that *S*-metolachlor reduced plant emergence and caused severe injury levels between 60% and 80%. Norsworthy and Smith (2005) reported divergent results from ours; their turnip plants had less than 15% injury when *S*-metolachlor was applied PRE at 450 g ha⁻¹ a.i., and collards had less than 10%. These findings suggest that the compounds used could have a protective effect on *Brassica* seedlings from damage by *S*-metolachlor. Plant injury and plant weight correlated significantly ($P < 0.0001$), with $r^2 = 0.87$ and $r^2 = 0.875$ for turnip and collard greens, respectively.

The reduction of plant injury shown in these trials demonstrates the potential of melatonin and AsA to improve collard and turnip tolerance to PRE *S*-metolachlor applications, particularly at lower herbicide concentrations. In vegetable crops, *S*-metolachlor is typically labeled to spray after crop transplantation or is applied over the top after the crop reaches 3 inches tall (Kemble, 2021); thus, weeds present will not be controlled without costly hand-weeding. The use of *S*-metolachlor PRE would provide a weed-free environment at the most critical period, giving the crop an advantage over weeds.

Crop tolerance can be attributed to the ability of plants to metabolize herbicide by detoxification reactions, preventing the accumulation

of active herbicide reaching phytotoxic levels. Cottingham and Hatzios (1992) suggest that enhanced metabolism driven by GSTs is the mechanism of tolerance to *S*-metolachlor in corn hybrids. Melatonin improved turnip and collard tolerance to *S*-metolachlor, possibly increasing cytochrome P450 or GST activity, and speeding up the herbicide detoxification process (Mandal et al., 2018). Caputo et al. (2020) reported that external application of melatonin reduced bentazon damage on sweetpotato [*Ipomoea batatas* L. (Lam)] plants. In addition, a secondary effect of *S*-metolachlor is related to oxidative damage resulting from ROS overproduction. We believe that AsA may reduce injury by scavenging ROS produced by *S*-metolachlor's secondary effects, protecting seedlings from oxidative damage (Sacała and Roszak, 2018). The high levels of injury observed on seedlings were expected because the plants are less hardy. Thus, the significant decrease in injury observed with some of the treatment combinations relative to *S*-metolachlor alone is promising.

The lower competitive ability of *Brassica* plants with weeds results in the majority of growers adopting transplanting over direct seeding. The adoption of seed treatments used in this study could enable direct *Brassica* seeding, which would reduce operational costs and improve herbicide application strategies. The protective ability of melatonin seed treatment and AsA could enhance the number of PRE herbicides registered in turnip and collard greens. In conclusion, this study demonstrated an interaction between seed treatment using potential safeners and PRE herbicides related to turnip and collard green injury. The reduction of injury caused by *S*-metolachlor when seeds were treated with melatonin and AsA validated those compounds' protective ability in turnip and collard greens. Our

results could be used to support the use of direct seeding if an expanded label of *S*-metolachlor is obtained. In addition, field trials would be necessary to characterize the interaction of PRE herbicides with safeners in commercial settings. Ideally, these field trials could generate data to promote label expansion of a much-needed PRE herbicide for *Brassica* crops.

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