Tolerance of Young Blackberries to a Selection of Preemergence Herbicides and Rates

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Abstract

Limited preemergence herbicides are registered for new blackberry (Rubus subgenus Rubus) plantings. This greenhouse experiment was designed to investigate the effects of a broad selection of preemergence herbicides at multiple rates on blackberry transplants. Screening was initiated August 2021 and repeated March 2022 in Fayetteville, AR, USA in a greenhouse at the Milo J. Shult Agricultural Research and Extension Center. 'Ouachita' blackberry plugs were transplanted into utility pots that contained field soil and growth media treated with preemergence herbicides. Following transplanting, plant heights were measured from the substrate to the highest apical meristem of 25 representative plants. Initial blackberry plant heights were 13.5 cm and 9.2 cm in 2021 and 2022, respectively. A total of twenty-five treatments were evaluated, consisting of 12 preemergence herbicides at 1× and 2× field rates and one nontreated control. Herbicide treatments included diuron, flumioxazin, halosulfuron, indaziflam, mesotrione, napropamide, oryzalin, pendimethalin, rimsulfuron, S-metolachlor, simazine, and sulfentrazone, applied to substrate in containers at their respective 1× or 2× field rates. Data were collected on plant height, blackberry injury ratings, internode length, leaf chlorophyll content, and destructive harvest including leaf count, leaf dry biomass, and aboveground dry biomass. Specific leaf area and leaf area to dry matter ratio were calculated. When observed, plant injury tended to increase from 7 days after treatment (DAT) until 42 DAT. Greater injury levels were observed in response to treatment with mesotrione at 1x (78%) and 2× rate (90%), halosulfuron at 1x (58%), halosulfuron at 2x (68%), and diuron at 2x (73%). Injury from diuron was rate-dependent, with the 1× rate causing

relatively low injury (19%). At both 1× and 2× rates, flumioxazin, indaziflam, napropamide, *S*-metolachlor, and pendimethalin treatments exhibited similar responses to the nontreated control.

Introduction

A national survey of blackberry (*Rubus* subgenus *Rubus*) growers identified weed control as a key limitation for production, particularly among southern stakeholders (Worthington 2021). Unfortunately, limited registered herbicides exist for blackberry production, and there is relatively little interest on the part of chemical companies in securing new labels for this specialty crop. Pesticide registrants often see negligible value in registering pesticides for use in specialty crops due to low return on investment and liability risk (Gast 2008). This disinterest is largely due to the low acreage and limited market opportunity these crops offer compared to agronomic crops. Herbicide discovery for specialty crops is often the byproduct of investigation of chemical use for agronomic crops (Gast 2008). Chemical company consolidations have also resulted in reduced investigation into new agrichemical development (Gast 2008). Registration of additional herbicide chemistries could reduce the risk of herbicide resistance among weed populations by allowing growers to rotate herbicide active ingredients (Norsworthy et al. 2012; Mitchem and Czarnota 2023).

There are relatively few broadcast, post-emergence herbicide options for blackberry production; therefore, preemergence herbicides are critical for weed control. Annual applications of registered preemergence herbicides are a standard practice to manage weeds in blackberry production (Mitchem and Czarnota 2023). Standard recommendations to maximize yields and profitability are to maintain a minimum area free of weeds centered on the blackberry crowns, called a weed-free strip width measuring 0.9 m for new plantings when blackberry plants are still small and 1.2 m for established plantings as canes develop and need more space for production (Meyers et al. 2014; Meyers et al. 2015; Fernandez et al. 2023; Basinger et al. 2017). Newly-transplanted blackberry plants are less competitive than established blackberry plants, making them more sensitive to weed interference; thus, it is critical to assess which preemergence herbicides are safe for use on young blackberry plants.

A further limitation on preemergence herbicides used in blackberries is the age of the crop and fruit-bearing status. Some herbicides are labeled for use in only in established plantings while others are not similarly restricted. To be considered established, a planting must be in the ground at least 365 days. Some herbicide labels are further restricted to plantings that have been established for two to three years (Gowan Company LLC 2017; Valent U.S.A. LLC 2021). Given the limited herbicide options for blackberry plantings, the objective of this trial was to assess the response of newly planted blackberries to a broad selection of preemergence herbicides at two rates in a greenhouse setting.

Materials and Methods

A greenhouse trial was initiated Aug 31, 2021, and repeated Mar 3, 2022, at the University of Arkansas System Division of Agriculture Milo J. Shult Agricultural Research and Extension Center in Fayetteville, AR, USA (lat. 36.09962 °N, long. 94.17194 °W). The trial was arranged as a randomized complete block design with 12 preemergence herbicides applied at both 1× and 2× of recommended field rates (Table 1). All treatment combinations were replicated five times in each trial run, and a nontreated control receiving no herbicide was included in each replication.

Of the selected herbicides for investigation, flumioxazin, mesotrione, napropamide, oryzalin, and sulfentrazone are currently labeled for use in newly planted blackberry plantings (FMC Corporation 2020; Syngenta Crop Protection LLC 2018; United Phosphorus, Inc. 2012, 2014; Valent U.S.A. LLC 2021). Diuron is not labeled for use in blackberries, with the exception of a California-specific registration (Alligare LLC 2019). The formulation of pendimethalin used in this trial, Prowl[®] H2O (3.8 lb/gal pendimethalin; BASF Corporation, Research Triangle Park, NC, USA), is not labeled for use in blackberries (BASF Corporation 2022); however, another product with the same concentration of pendimethalin, Satellite HydroCap[®] (3.8 lb/gal pendimethalin; United Phosphorus, Inc., King of Prussia, PA, USA) is labeled for surface application prior to transplanting blackberries (UPL NA, Inc. 2022). *S*-metolachlor is labeled for use in blackberries under a section 24(c) special local need label in Oregon and North Carolina (Syngenta Crop Protection LLC 2021, 2023a). Further, three of the herbicides (indaziflam, halosulfuron, and rimsulfuron) are only registered for use in blackberries established for one or more years (Bayer Cropscience 2022; Corteva Agriscience 2021; Gowan Company LLC 2017). Simazine is labeled for use in blackberries but includes two restrictions: do not apply when fruit is present, and a restriction to reduce the application rate by half if the plants are six months of age or younger (Syngenta Crop Protection LLC 2023b). Of these labeled products, napropamide, simazine, and oryzalin are recommended for use in blackberries for all growth stages (Burgos et al. 2014, Mitchem and Czarnota 2023). Flumioxazin, indaziflam, mesotrione, and rimsulfuron are labeled and recommended for use in established plantings (Mitchem and Czarnota 2023).

Each experimental unit was a 1 gal plastic container (6 ¼ inches wide at top, 5 ¼ inches wide at bottom, 6 ¼ inches height) filled with a 1:1 v/v ratio of Roxana silt loam field soil from the University of Arkansas Vegetable Research Station, Kibler, AR, USA (lat. 35.37907 °N, long. 94.23318 °W) and general use potting soil (PRO-MIX BX Mycorrhizae, Pro-Mix, Quakertown, PA, USA). The field soil was sourced from an untreated site at the Vegetable Research Station that had not been sprayed with herbicides for over twenty years. The resultant soil and media mixture had a pH of 6.2, electrical conductivity of 281 μ mho·cm⁻¹, and 4.2% organic matter.

Prior to treatment, filled containers were thoroughly watered, allowed to settle and drain to field capacity. Herbicide treatments were applied to prepared containers using a compressed air powered spray chamber calibrated to deliver 20 gal/acre at 1 mph and fitted with two tapered-edge flat fan 1100067 nozzle tips (TeeJet® Technologies, Glendale Heights, IL, USA) placed 50 cm apart. 'Ouachita' blackberry plugs (Agri-Starts, Apopka, FL, USA) from 72-cell trays were transplanted 6 cm deep into the 1 gal utility pots within 24 hours after herbicide application. At the time of transplanting, blackberries were 13 and 9 cm in height for 2021 and 2022 runs, respectively. Care was taken to displace as little substrate as possible to allow for a more accurate representation of root uptake of the preemergence herbicides. This procedure simulates root uptake of herbicides of blackberry plugs transplanted into herbicide-treated field, rather than simulating root uptake of preemergence herbicides by shielded transplants using impermeable grow tubes or LDPE-coated milk/juice cartons. Herbicide application to the substrate is particularly important for herbicides with a known potential for phytotoxicity with foliar exposure on young plants (e.g., simazine, sulfentrazone, diuron, flumioxazin).

Potted plants were then placed in the greenhouse on tables and randomized within each replication. Plants were watered twice weekly to field capacity, limiting excessive drainage. Plants were fertilized once each week with 4 fl oz of prepared solution containing 3.0 g·L⁻¹ of a 24N–3.5P–13.3K soluble fertilizer (Sta-Green Plant Food; Parker Fertilizer Company, Inc., Dallas, TX, USA). Visual injury was rated at 7, 14, 21, 28, 35, and 42 days after treatment (DAT). Injury ratings were recorded on a 0 to 100% scale, with 0 indicating no injury and 100 indicating dead plants. Total canopy reduction (i.e., stunting, reduced leaf size) were included in injury ratings in addition to necrotic or chlorotic leaf surfaces. Chlorophyll content, internode length, and plant height were recorded at 14 and 42 DAT. Chlorophyll content was measured on a representative leaflet of the youngest fully expanded leaf of each plant using Soil Plant Analysis Development (SPAD) chlorophyll meter (SPAD-502Plus, Konica Minolta, Ramsey, NE, USA). Internode length was recorded between the first and second nodes proximal from the apical meristem using an electronic digital caliper (CID Bio-Science Inc., Camas, WA, USA). Plant height was measured from the substrate to the highest apical meristem. Destructive harvest was conducted at 42 DAT, and data were collected on leaf area and leaf count. Leaf area was determined using a leaf area scanner (LI-3100C Area Meter, LI-COR® Biosciences, Lincoln, NE, USA). At termination of the study, leaves and stems were harvested separately, oven-dried (Laboratory Oven, Blue M Electric

Company, New Columbia, PA, USA) at 63°C for 4 days and weighed using a laboratory balance (BP61S – Sartorius, Goettingen, Germany). Total aboveground biomass (dry weight) was recorded as the sum of leaf biomass and stem biomass from each container. Specific leaf area (SLA) was calculated as the ratio of leaf area to leaf biomass, and leaf area to dry matter ratio (LADMR) was calculated as the leaf area to total aboveground biomass ratio.

All data were subjected to ANOVA as a randomized complete block design using the GLIMMIX procedure in SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA). Main effects of herbicide, rate, and herbicide by rate interaction were treated as fixed effects, while block (nested in trial) and trial were treated as random effects. Data were checked for heteroscedasticity by reviewing residual plots in SAS, and means were separated using Tukey's Honest Significant Difference (HSD) multiple comparisons adjustment (α = 0.05). Nontreated containers served as a reference for injury ratings and were excluded from ANOVA when analyzing the effect of herbicide and rate on blackberry injury. For plant height, internode length, leaf chlorophyll content, and biomass, nontreated containers were excluded from the initial means separation analyses because the nontreated control applied to both factors (rate and herbicide) and could not be appropriately accommodated in the model. Instead, data from nontreated containers were included in a subsequent ANOVA using a Dunnett's procedure to determine whether each treatment combination was significantly different (α = 0.05) from the nontreated control.

Results

Injury. Depending on herbicide active ingredients, plant injury symptoms manifested as chlorosis, bleaching, necrosis, leaf deformation, or general stunting (Figure 1). Because injury ratings were visually assessed relative to a nontreated control, stunting in response to herbicides and rates may not have been initially apparent, but later ratings revealed higher injury levels due to the difference in growth relative to the nontreated control. Thus, blackberry injury symptoms, where present, tended to worsen with time. At 7, 14, and 21 DAT, no significant interaction of herbicide × rate was observed (P =

0.86, 0.71, 0.25 for 7, 14, and 21 DAT, respectively), so the main effect of herbicide was pooled across both 1× and 2× rates is presented (Table 2). Initial blackberry injury was relatively minor across all treatments, with 11 of the 12 herbicide treatments causing ≤3% injury at 7 DAT (Table 2). The greatest injury (7%) at 7 DAT was observed in mesotrione-treated blackberries, exhibiting minor bleaching symptoms on both old- and newly-formed leaves. For many treatments, injury increased over time with mesotrione, halosulfuron, and diuron (2× rate) treatments where initial injury at 7 and 14 DAT was mild (between 0 and 16%), but became severe (58 to 90%) by 42 DAT (Table 2, Figure 1). At 28, 35, and 42 DAT the interaction of herbicide × rate was observed to be significant (P = 0.0011, <0.0001, and <0.0001 for 28, 35, and 42 DAT, respectively), and herbicides are presented separately by rate (Table 2). While the least square means of the 1× rates were numerically lower than the 2× rates for each herbicide, only the 1× and 2× rates of diuron fell into separate statistical groupings according to Tukey's HSD (Table 2). Therefore, herbicide active ingredient was a stronger determinant of blackberry injury rather than herbicide rate. Blackberries treated with flumioxazin, indaziflam, napropamide, *S*-metolachlor, and pendimethalin exhibited injury levels ≤6% throughout the trial even at 2× rates.

Plant Height. Plant height was similar among treatments at 14 DAT, but differences in height were observed at 42 DAT (Table 3). The interaction of herbicide × rate was not significant (P = 0.88 and 0.67 for 14 and 42 DAT, respectively), so the main effect herbicide pooled across both 1× and 2× rates is presented. Relative to the nontreated controls (46 cm) at 42 DAT, plants treated with mesotrione and halosulfuron were much shorter, measuring 12.4 and 13.6 cm, respectively; however, means separation with Tukey's HSD showed that these treatments were not significantly shorter than plants treated with diuron, rimsulfuron, and sulfentrazone, which measured 24.9, 22.3, and 25.7 cm, respectively. A Dunnett's test determined that plants treated with mesotrione, halosulfuron, diuron, rimsulfuron, and sulfentrazone than the nontreated control at 42 DAT (Table 3). Reduced height likely resulted from combinations of leaf bleaching, chlorosis, and necrosis which could reduce

overall plant growth. All other treatments, including oryzalin, simazine, flumioxazin, indaziflam, napropamide, *S*-metolachlor, and pendimethalin did not stunt the blackberry plants relative to the nontreated control (Table 3).

Internode Length. The herbicide × rate interaction effect on internode length was not significant (P = 0.92 and 0.39 for 14 and 42 DAT, respectively); therefore, the herbicide means are presented averaged across rates. At 14 DAT internode length did not differ between herbicide treatments, and none of the treatments differed according to Tukey's multiple comparison's adjustment nor from the nontreated control (5.5 cm) according to a Dunnett's procedure (Table 3). At 42 DAT, relative to the nontreated control (39.2 cm), shortened internode lengths were most evident in mesotrione-, halosulfuron-, and sulfentrazone-treated plants which measured 1.7, 6.0, and 17.0 cm, respectively. At 42 DAT, internode lengths of blackberries treated with mesotrione, diuron, rimsulfuron, halosulfuron, and sulfentrazone were reduced and significantly different from the nontreated control. The same herbicides that reduced internode length also reduced plant heights (Table 3), indicating that height reduction was due, at least in part, to shortening of internodes ("stacking") rather than fewer nodes per plant, although nodes per plant were not counted.

Leaf Chlorophyll Content (SPAD). At 14 DAT, the herbicide × rate interaction effect on leaf chlorophyll content as measured by SPAD was not significant (P = 0.27); thus, herbicide means are presented averaged across both 1× and 2× rates. At 14 DAT, mesotrione- and halosulfuron-treated blackberries exhibited reduced leaf chlorophyll content as measured by SPAD readings relative to the nontreated control (42.0). SPAD readings were lowest in mesotrione-treated plants (26.6) and also significantly reduced in halosulfuron-treated plants (35.4) at 14 DAT (Table 3). This is expected because mesotrione is an inhibitor of HPPD (hydroxyphenyl pyruvate dioxygenase), a key enzyme that facilitates chlorophyll synthesis. At 42 DAT, the herbicide × rate effect was significant (P = 0.03) for SPAD readings; therefore, means are presented separately for rate and herbicide. Blackberries treated with mesotrione

at 1× and 2× rates, and with diuron at the 2× rate reduced SPAD readings, measuring 21.9, 26.2, and 26.5, respectively; and each was significantly different from the nontreated control (45.3) SPAD reading (Table 3). At 42 DAT, 21 of the 24 treatment combinations (herbicide and rate) did not differ from the nontreated control regarding leaf chlorophyll content as measured by SPAD.

Leaf Biomass and Total Aboveground Biomass. The interaction effect of herbicide × rate on leaf biomass was not significant (P = 0.72); thus, the main effect herbicide is presented (Table 4). Leaf biomass was reduced in plants treated with mesotrione (0.4 g), diuron (1.3 g), rimsulfuron (1.3 g), and halosulfuron (0.5 g), each producing significantly less leaf biomass compared to the nontreated control (3.9 g) (Table 4). The remaining eight herbicide treatments did not reduce leaf biomass relative to the nontreated control. For total aboveground biomass, the interaction effect of herbicide × rate was significant (P = 0.04); therefore, means were analyzed separately by rate foreach herbicide (Table 4). At the 1× rate, total aboveground biomass was reduced in blackberry plants treated with mesotrione (1.5 g), halosulfuron (1.8 g), oryzalin (6.4 g), and rimsulfuron (3.1 g), relative to the nontreated control (Table 4). At the 2× rate, total aboveground biomass was significantly reduced in blackberry plants treated with seven of the 12 selected herbicides (Table 4). At the 2× rate, plants treated with diuron, simazine, and sulfentrazone exhibited reductions in total aboveground biomass relative to the nontreated control; however, biomass was not significantly different from the nontreated controls at the 1× rate of these herbicides (Table 4).

Leaf Number and Leaf Area. Reductions in leaf number indicate a developmental delay while reductions in leaf area indicate reduced photosynthetic area of the plant, which could be due to fewer leaves or smaller leaves. The interaction of herbicide × rate was not significant on leaf number (P = 0.13) and leaf area (P = 0.21); thus, only the main effect of herbicide is presented in Table 5. At 42 DAT, the nontreated plants had 26 leaves and a 1173 cm² leaf area. Mesotrione and halosulfuron treatments reduced leaf number to 10 per plant and reduced leaf area to 59 cm² and 125 cm², respectively. Plants treated with mesotrione and halosulfuron had the lowest leaf number while mesotrione-, halosulfuron-, and rimsulfuron-treated plants had the lowest leaf area (Table 5). Mesotrione and halosulfuron were the only herbicides that significantly reduced leaf number relative to the nontreated control. Interestingly, plants treated with pendimethalin had significantly more leaves (37) and, consequently, higher leaf area (1603 cm²) relative to the nontreated control (26 and 1173 cm²). Mesotrione- (59 cm²), diuron- (518 cm²), rimsulfuron- (352 cm²), halosulfuron- (125 cm²), and oryzalin-treated (737 cm²) plants exhibited significantly lower leaf area compared to the nontreated control (1173 cm²) (Table 5).

Specific Leaf Area and Leaf Dry Matter Ratio. For specific leaf area, the interaction of herbicide × rate was significant (P = 0.03); thus, herbicide means are presented by rate (Table 5). The mesotrione treatment caused the lowest SLA among all herbicides at the 1× rate and was significantly different than the nontreated control, with 59% reduction in SLA. The decrease in SLA indicates that leaves from mesotrione-treated plants require more biomass per unit of leaf area, meaning plants require more leaf biomass to produce an equivalent leaf surface area of those treatments with higher SLA values. However, means separation by Tukey's HSD showed little separation between SLA means across all herbicides and rates (Table 5).

The LADMR is the ratio of leaf area (square centimeters) to total aboveground biomass (grams), so a reduced LADMR indicates that a plant canopy area is diminished, with fewer or smaller leaves while an increased LADMR indicates more leaves or increased surface area per leaf, relative to the total aboveground biomass of the plant. A greater LADMR is indicative of increased resource allocation to leaves rather than stems. For LADMR, the interaction of herbicide × rate was significant (P < 0.001); thus, herbicide means are presented by rate. The LADMR of nontreated blackberries was 136 (Table 5). At 1× rates, mesotrione- and halosulfuron-treated plants exhibited the lowest LADMR values, which were significantly lower than the nontreated control (Table 5). Plants treated with mesotrione, diuron, and halosulfuron at the 2× rate had the lowest LADMR values, which were significantly lower than the nontreated control. Generally, LADMR did not differ between 1x and 2x rates of the same herbicide. However, the LADMR of plants treated with 2x rate of diuron was reduced by 64% compared to the nontreated control whereas the 1× rate did not differ from the nontreated control (Table 5). The LADMR of 19 of 24 treatment combinations (herbicide × rate) were similar to the nontreated check.

Discussion

These findings are a helpful demonstration of blackberry growth and injury responses for a selection of herbicides at 1× or 2× rates when grown in a controlled environment. Application of the herbicides directly to media prior to transplanting allowed for characterization herbicidal activity by root uptake. Each of these herbicides is reported to be absorbed by roots when applied to soil (González-Delgado and Shukla 2020; Shaner 2014). It is important to contextualize these findings from a greenhouse trial with container-grown blackberries as distinct from a field trial assessing the same herbicide applications, given the disparities in environment, rainfall dynamics and the composition and volume of native soils. Further, each herbicide in this trial has a distinct soil adsorption coefficient for soil and organic matter that may have been exaggerated in this trial, given the high organic matter of potting mix included in the substrate blend. Thus, it is critical to review the literature for previous field trialing data or generate new field data, using these findings only as a guide for which herbicides to investigate.

For the commercially registered herbicides, our observations are consistent with commercial recommendations, especially regarding caution not to apply mesotrione until they have been established for 1 or more years (Mitchem and Czarnota 2023). The lack of injury in response to pendimethalin and *S*-metolachlor is consistent with field trials of established 'Marion' blackberries, where no injury or yield reduction was observed in response to the 1× and 2× rates of pendimethalin and *S*-metolachlor rate (1.41 kg a.i.·ha⁻¹) similar to the current 1× rate (Peachey 2012). Our findings are

consistent with the findings of Meyers et al. (2015) who reported no yield reduction in established 'Navaho', 'Ouachita', and 'Arapaho' blackberries with similar rates of flumioxazin, oryzalin, simazine, and S-metolachlor. Indaziflam has also previously been shown to inflict no injury to established, field-grown 'Apache' blackberries in the field (Grey et al. 2021).

Early and rapid growth is critical for weed competition and plant development, particularly in first year plantings; so the observed reductions in plant heights in response to herbicide treatments would likely make crops less weed-competitive. The internode length of blackberries has been shown to vary by cultivar and in response to prohexidione-Ca, a plant growth regulator (Johns 2022). Results from this trial indicate that preemergence herbicides can alter internode length and thus the stature and architecture of the plant and its canopy may also be affected. However, the effect of the majority of selected herbicides in this study on internode length did not differ from the nontreated control (Table 3).

Although flumioxazin did not cause significant reduction of leaf chlorophyll content in the present blackberry trial, Saladin et al. (2003) reported a negative impact on photosynthesis and a reduction in foliar chlorophyll and carotenoids content when flumioxazin was applied to young grapes (*Vitis vinifera*). Flumioxazin-treated plants exhibited slight interveinal necrosis in leaves which would account for some injury and localized reductions in leaf chlorophyll content, although neither was different from the nontreated control (Table 2, Table 3). Leaf chlorophyll content was most affected by herbicide treatments that caused bleaching (mesotrione) and plant death. Because mesotrione is a carotenoid biosynthesis inhibitor (HPPD inhibitor) and disrupts synthesis of chlorophyll, it is not surprising that this treatment is most prominent in the response variable that measures chlorophyll content were taken on the newest fully expanded leaf of blackberries, it is possible that diuron, a photosystem II inhibitor that exhibits symptoms primarily in old leaves (Shaner 2014), would not have registered based

on this measurement. It is also possible that the potential risk of flumioxazin was underestimated because injury can occur on sensitive species as a result of rain-splashed soil onto foliage (United Phosphorus, Inc. 2014), and soil splashing did not occur in this greenhouse trial.

At the 2× rate, plants treated with diuron, simazine, and sulfentrazone exhibited reductions in total aboveground biomass, which indicates a rate-dependent response for total aboveground biomass for these particular herbicides. The difference in response between the 1× and 2× rates of diuron, simazine, and sulfentrazone demonstrate the necessity for proper calibration and adherence to product labels in order to avoid exceeding the 1× field rate and incurring avoidable damage to plants. These findings reinforce the importance of applying a reduced rate of simazine (1/2× rate for established blackberries) on first year blackberries, which is the current commercial recommendation (Syngenta Crop Protection LLC 2023b; Mitchem and Czarnota 2023).

Reductions in leaf number were observed in response to mesotrione and diuron, two of the more highly injurious herbicides (Table 2, Table 5). Interestingly, penidmethalin-treated plants were observed to have increased leaf number and leaf area, relative to the nontreated control. This divergence from the expected pattern may be an example of hormesis, where plants were stimulated to increase growth. Hormesis is a dose-response phenomenon where otherwise inhibitory substances can stimulate plant growth at low rates and has specifically been observed in black-grass (*Alopecurus myosuroides*) treated with pendimethalin (Metcalfe et al. 2017; Belz and Duke 2014).

Of the chemical compounds investigated mesotrione, halosulfuron, and diuron (2× rate) treatments incurred the highest injury levels in blackberry and the greatest reductions in plant growth. Though mesotrione is labeled for use in blackberries, it is appropriately recommended for use in plants established 1 or more years (Mitchem and Czarnota 2023). While the greenhouse screening indicated indaziflam caused no injury for newly planted blackberries, field data in true soils is necessary to assess the crop safety before any serious consideration is given to expanding the indaziflam herbicide label to include newly planted blackberries. The 24(c) labeling for *S*-metolachlor in several states is supported by these findings and may be worth exploring for other states where alternative herbicides are not available. Flumioxazin, napropamide, oryzalin, pendimethalin, and simazine treatments sustained little or no damage and corroborated their labeling designations and field use (Burgos et al. 2014; Meyers et al. 2015). Of the herbicides tested, it was observed that indaziflam and diuron may have additional utility beyond current labels or recommendations. Despite the current restrictions and recommendations, young plants exposed to those indaziflam and diuron did not exhibit dramatic reductions in plant height or total aboveground biomass. Though, plants exposed to diuron at the 2× rate exhibited unacceptable levels of injury, which poses a risk in the event of improper calibration or spray pattern overlap.

Some important context for this research is the loss of some blackberry herbicide options either from regulation or discontinued production of the herbicide. A recent discontinuation of oryzalin has occurred with little explanation, and the commercial product Surflan® (4 lb/gal oryzalin; United Phosphorus, Inc., King of Prussia, PA, USA), is no longer available for purchase (Neal 2021). A further limitation looms as a US Environmental Protection Agency (USEPA) interim report discloses the intended discontinuation of diuron for use in food crops (USEPA 2022). Thus, the limited herbicide options for blackberry weed control are becoming more limited with the loss of two previously-registered herbicides. Successful blackberry production without weed interference will be reliant on fewer chemistries and may necessitate the implementation of integrated weed management strategies. This characterization of blackberry responses to soil-applied herbicides in greenhouse conditions supports current commercial herbicide recommendations, reinforces the importance of proper calibration with potentially injurious chemicals, and offers insight into which herbicide chemistries could be considered for assessment for use in the field.

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Common Name	Trade Name	Rate (g∙	ha⁻¹ a.i.) ⁱ	Manufacturer	Manufacturer Location
		1×	2×		
Oryzalin	Surflan [®]	4,483	8,967	United Phosphorous, Inc.	King of Prussia, PA, USA
Napropamide	Devrinol [®] DF-XT	4,483	8,967	United Phosphorous, Inc.	King of Prussia, PA, USA
Pendimethalin	Prowl [®] H2O	3,363	6,725	BASF	Research Triangle Park, NC, USA
S-metolachlor	Dual Magnum [®]	1,597	3,194	Syngenta Crop Protection, LLC	Greensboro, NC, USA
Flumioxazin	Chateau®	210	420	Valent U.S.A., LLC	San Ramon, CA, USA
Mesotrione	Callisto®	158	315	Syngenta Crop Protection, LLC	Greensboro, NC, USA
Simazine	Princep [®]	1,233	2,466	Syngenta Crop Protection, LLC	Greensboro, NC, USA
Diuron	Diuron 80-DF	1,569	3,138	Alligare, LLC	Opelika, AL, USA
Halosulfuron	Sandea®	53	105	Gowan Company, LLC	Yuma, AZ, USA
Rimsulfuron	Matrix®	70	140	Corteva [™] Agriscience	Indianapolis, IN, USA
Indaziflam	Alion®	50	101	Bayer	St. Louis, MO, USA
Sulfentrazone	Zeus [®] XC	211	420	FMC Corporation	Philadelphia, PA, USA

Table 1. Herbicides tested in the greenhouse experiment on newly planted blackberries in Fayetteville, AR, USA in 2021 and 2022.

ⁱ1 g·ha⁻¹ = 0.0143 oz/acre.

	7 DAT ⁱⁱ	14 DAT	21 DAT	28 DAT		35 DAT		42 DAT	
Herbicide ⁱⁱⁱ	Combined	Combined	Combined	1×	2×	1×	2×	1×	2×
Visual injury rating (%) ^{iv}									
Mesotrione	7 a	16 a	36 a	68 ab	73 a	75 ab	86 a	78 ab	90 a
Diuron	0 b	3 c	14 bc	11 de	50 abc	13 c	68 ab	19 de	73 ab
Halosulfuron	3 ab	10 b	22 b	34 cd	45 bc	58 b	59 a	58 bc	68 ab
Sulfentrazone	1 b	3 c	7 cd	12 de	16 de	17 c	21 c	25 de	34 cd
Oryzalin	0 b	0 c	1 d	2 e	2 e	2 c	12 c	4 e	24 de
Rimsulfuron	1 b	0 c	2 d	4 e	4 e	8 c	10 c	18 de	19 de
Simazine	0 b	2 c	6 cd	1 e	17 de	2 c	21 c	0 e	17 de
Flumioxazin	0 b	0 c	2 d	0 e	7 e	0 c	5 c	1 e	6 de
Indaziflam	0 b	0 c	1 d	0 e	1 e	0 c	2 c	1 e	4 e
Napropamide	0 b	1 c	4 cd	1 e	1 e	0 c	0 c	1 e	1 e
S-metolachlor	0 b	0 c	0 d	0 e	0 e	0 c	1 c	0 e	0 e
Pendimethalin	0 b	0 c	0 d	0 e	0 e	0 c	0 c	1 e	0 e
P-value ^v	<0.001	<0.001	<0.001	<0.001		<0.001		<0.001	

Table 2. Visual injury ratings of young blackberries in response preemergence herbicides applied to soil-substrate mix at 1× and 2× rates in 2021 and 2022 greenhouse trials in Fayetteville, AR, USA. Blackberries were transplanted into substrate following herbicide applications within 24 hours.¹

ⁱMeans were separated using Tukey's Honest Significant Difference at a α =0.05 significance level and means followed by the same letter are not

1 significantly different. Means were compared by date (DAT) within each parameter.

ⁱⁱAbbreviation: DAT, days after treatment, 1× indicates the selected field rate for a herbicide, 2× indicates twice the field rate for a herbicide,

3 Combined indicates the means pooled across both 1× and 2× herbicide rates.

4 ⁱⁱⁱHerbicide rates (g·ha⁻¹a.i.): mesotrione (1× = 158, 2× = 315); diuron (1× = 1,569, 2× = 3,138); halosulfuron (1× = 53, 2× = 105); sulfentrazone (1×

5 = 211, 2× = 420); oryzalin (1× = 4,483, 2× = 8,967); rimsulfuron (1× = 70, 2× = 140); simazine (1× = 1,233, 2× = 2,466); flumioxazin (1× = 210, 2× =

- 6 420); indaziflam (1× = 50, 2× = 101); napropamide (1× = 4,483, 2× = 8,967); S-metolachlor (1× = 1,597, 2× = 3,194); pendimethalin (1× = 3,363, 2×
- 7 = 6,725). Conversion: $1 \text{ g} \cdot \text{ha}^{-1} = 0.0143 \text{ oz/acre.}$
- 8 ^{iv}Injury ratings were assessed visually on a 0 to 100% scale with 0 indicating a healthy plant and 100% indicating total plant death.
- 9 "Herbicide and rate effects were tested for any interaction effect (α =0.05). Where no significant herbicide × rate effect was detected, the main
- 10 effect herbicide is reported with rates combined. In cases where a significant herbicide × rate effect was detected; rates are presented as
- 11 separate column.

	Height		Internode length		Leaf chlorophyll content		
	14 DAT ⁱⁱ	42 DAT	14 DAT	42 DAT	14 DAT	42	DAT
Herbicide ⁱⁱⁱ	Combined cm ^{iv}		Combined mm ^v		Combined	1×	2×
Mesotrione	11.5	12.4 d*	4.1	1.7 e*	26.6 c*	21.9 c*	26.2 bc*
Diuron	12.6	24.9 bcd*	4.7	20.5 bcd*	38.1 ab	41.7 a	26.5 bc*
Halosulfuron	11.0	13.6 d*	6.8	6.0 de*	35.4 b*	39.8 a	41.1 a
Sulfentrazone	11.2	25.7 bcd*	4.1	17.0 cde*	42.3 ab	42.4 a	44.1 a
Oryzalin	11.7	34.0 abc	6.6	23.4 abc	44.2 a	43.1 a	45.6 a
Rimsulfuron	10.7	22.3 cd*	3.5	22.1 bcd*	42.0 ab	44.9 a	43.9 a
Simazine	12.1	33.0 abc	4.5	29.2 abc	40.1 ab	40.4 a	38.2 ab
Flumioxazin	12.1	44.9 a	7.9	35.5 ab	38.8 ab	43.1 a	44.0 a
Indaziflam	12.1	37.2 ab	7.4	30.9 abc	41.8 ab	42.5 a	42.0 a
Napropamide	12.4	44.0 a	8.7	35.4 ab	43.3 a	44.5 a	42.5 a
S-Metolachlor	12.4	42.5 a	5.4	31.2 abc	43.0 a	41.9 a	39.4 a
Pendimethalin	12.9	46.7 a	8.6	35.5 ab	40.1 ab	45.5 a	42.9 a
Nontreated	11.3	41.6	5.5	39.2	42.0	4	5.3
P-value ^{vi}	0.033	<0.001	0.030	<0.001	<0.001	0.	026

Table 3. Blackberry height to the apical meristem, internode length of the first internode proximal from the apical meristem, and leaf chlorophyll content of the newest fully expanded leaf in response preemergence herbicides applied to soil-substrate mix at 1× and 2× rates in 2021 and 2022 greenhouse trials in Fayetteville, AR, USA. Blackberries were transplanted into substrate following herbicide applications within 24 hours.ⁱ

¹² ⁱMeans were separated using Tukey's Honest Significant Difference at a α =0.05 significance level and means followed by the same letter are not

13 significantly different. Means lacking letters were determined not to be significantly different following a Tukey's multiple comparisons

14 adjustment. Nontreated controls were excluded from analyses for which Dunnett's Procedure was used to compare each treatment combination

15 to the nontreated control. Means followed by an asterisk (*) are significantly different from the nontreated control according to Dunnett's

16 procedure at an α =0.05 significance level. Means were compared by date (DAT) within each parameter.

- ¹⁷ ⁱⁱAbbreviations: DAT, days after treatment; SPAD, Soil Plant Analysis Development chlorophyll meter reading; 1× indicates the field rate for a
- 18 herbicide, 2× indicates twice the field rate for a herbicide, Combined indicates the means pooled across both 1× and 2× herbicide rates.
- 19 ⁱⁱⁱHerbicide rates (g·ha⁻¹a.i.): mesotrione (1× = 158, 2× = 315); diuron (1× = 1,569, 2× = 3,138); halosulfuron (1× = 53, 2× = 105); sulfentrazone (1×
- 20 = 211, 2× = 420); oryzalin (1× = 4,483, 2× = 8,967); rimsulfuron (1× = 70, 2× = 140); simazine (1× = 1,233, 2× = 2,466); flumioxazin (1× = 210, 2× =
- 21 420); indaziflam (1× = 50, 2× = 101); napropamide (1× = 4,483, 2× = 8,967); S-metolachlor (1× = 1,597, 2× = 3,194); pendimethalin (1× = 3,363, 2×
- 22 = 6,725). Conversion: $1 \text{ g} \cdot \text{ha}^{-1} = 0.0143 \text{ oz/acre.}$
- ^{iv}1 cm = 0.3937 inch.
- ^v1 mm = 0.0394 inch.
- vi Herbicide and rate effects were tested for any interaction effect (α =0.05). Where no significant herbicide × rate effect was detected, the main
- 26 effect herbicide is reported with rates combined. In cases where a significant herbicide × rate effect was detected; rates are presented as

27 separate column.

Table 4. Leaf biomass and total aboveground biomass reported from destructive harvest (42 days after treatment) of container-grown blackberry plants in response to in response preemergence herbicides applied to soil-substrate mix at 1× and 2× rates in 2021 and 2022 greenhouse trials in Fayetteville, AR, USA. Blackberries were transplanted into substrate following herbicide applications within 24 hours.^{i,ii}

	Leaf biomass	Total above	eground biomass
Herbicide ⁱⁱⁱ	combined	1×	2×
		g ^{iv}	
Mesotrione	0.4 d*	1.5 g*	1.2 g*
Diuron	1.3 cd*	6.0 b-f	1.8 g*
Halosulfuron	0.5 d*	1.8 g*	1.4 g*
Sulfentrazone	3.2 ab	7.2 a-d	4.9 c-g*
Oryzalin	2.6 bc	6.4 b-f*	4.3 efg*
Rimsulfuron	1.3 cd*	3.1 fg*	3.0 fg*
Simazine	2.9 bc	8.1 a-d	4.4 d-g*
Flumioxazin	3.7 ab	9.1 ab	7.9 а-е
Indaziflam	3.5 ab	7.7 а-е	7.6 а-е
Napropamide	3.8 ab	9.3 ab	8.2 a-d
S-Metolachlor	3.8 ab	8.8 ab	8.3 abc
Pendimethalin	4.6 a	11.0 a	10.6 a
Nontreated	3.9		8.4
P-value ^v	<0.001		0.048

¹Means were separated using Tukey's Honest Significant Difference at a α =0.05 significance level and

29 means followed by the same letter are not significantly different. Nontreated controls were excluded

30 from analyses for which Dunnett's Procedure was used to compare each treatment combination to the

31 nontreated control. Means followed by an asterisk (*) are significantly different from the nontreated

32 control according to Dunnett's procedure at an α =0.05 significance level.

³³ ⁱⁱAbbreviation: 1× indicates the selected field rate for a herbicide, 2× indicates twice the field rate for a

34 herbicide, Combined indicated the means pooled across both 1× and 2× herbicide rates.

35 ⁱⁱⁱHerbicide rates (g·ha⁻¹a.i.): mesotrione (1× = 158, 2× = 315); diuron (1× = 1,569, 2× = 3,138);

36 halosulfuron (1× = 53, 2× = 105); sulfentrazone (1× = 211, 2× = 420); oryzalin (1× = 4,483, 2× = 8,967);

37 rimsulfuron ($1 \times = 70$, $2 \times = 140$); simazine ($1 \times = 1,233$, $2 \times = 2,466$); flumioxazin ($1 \times = 210$, $2 \times = 420$);

- 38 indaziflam (1× = 50, 2× = 101); napropamide (1× = 4,483, 2× = 8,967); S-metolachlor (1× = 1,597, 2× =
- 39 3,194); pendimethalin (1× = 3,363, 2× = 6,725). Conversion: $1 \text{ g} \cdot \text{ha}^{-1} = 0.0143 \text{ oz/acre.}$
- 40 ^{iv}1 g = 0.0353 oz.
- 41 vHerbicide and rate effects were tested for any interaction effect (α =0.05). Where no significant
- 42 herbicide × rate effect was detected, the main effect herbicide is reported with rates combined. In cases
- 43 where a significant herbicide × rate effect was detected; rates are presented as separate column.

Table 5. Leaf number, leaf area, specific leaf area (SLA, ratio of leaf area [square centimeters] to leaf biomass [grams]), and leaf area to dry matter ratio (LADMR, ratio of leaf area [square centimeters] to total aboveground biomass [grams]) reported from destructive harvest (42 days after treatment) of containergrown blackberries in response preemergence herbicides applied to soil-substrate mix at 1× and 2× rates in 2021 and 2022 greenhouse trials in Fayetteville, AR, USA. Blackberries were transplanted into substrate following herbicide applications within 24 hours.ⁱ

	Leaves	Leaf area	SLA ⁱⁱⁱ		LADMR ⁱⁱⁱ	
Herbicide ⁱⁱ	combined	combined	1×	2×	1×	2×
	no	cm² iii				
Mesotrione	10 e*	59 h*	165 def*	107 f*	52 de*	30 e*
Diuron	20 cd	518 efg*	443 abc	147 ef*	150 a	49 de*
Halosulfuron	10 e*	125 gh*	303 b-f	201 c-f*	79 b-e*	60 cde*
Sulfentrazone	27 bcd	966 bcd	401 a-d	324 a-f	133 a	125 ab
Oryzalin	23 bcd	737 def*	386 а-е	313 a-f	140 a	103 a-d
Rimsulfuron	19 d	352 fgh*	306 b-f	320 a-f	114 abc	107 abc
Simazine	28 bc	896 cde	432 abc	367 a-d	122 ab	140 a
Flumioxazin	29 ab	1303 abc	491 ab	556 a	149 a	144 a
Indaziflam	30 ab	1168 a-d	438 abc	433 abc	144 a	140 a
Napropamide	31 ab	1349 ab	445 abc	485 ab	154 a	146 a
S-Metolachlor	30 ab	1283 abc	435 abc	460 ab	137 a	152 a
Pendimethalin	37 a*	1603 a*	498 ab	494 ab	146 a	146 a
Nontreated	26	1173		400		136
P-value ^{iv}	<0.001	<0.001	0.029		<	0.001

⁴⁴ ⁱMeans were separated using Tukey's Honest Significant Difference at a α =0.05 significance level and means followed by the same letter are not

45 significantly different. Nontreated controls were excluded from analyses for which Dunnett's Procedure was used to compare each treatment

46 combination to the nontreated control. Means followed by an asterisk (*) are significantly different from the nontreated control according to

47 Dunnett's procedure at an α =0.05 significance level.

- 48 "Herbicide rates (g·ha⁻¹a.i.): mesotrione (1× = 158, 2× = 315); diuron (1× = 1,569, 2× = 3,138); halosulfuron (1× = 53, 2× = 105); sulfentrazone (1×
- 49 = 211, 2× = 420); oryzalin (1× = 4,483, 2× = 8,967); rimsulfuron (1× = 70, 2× = 140); simazine (1× = 1,233, 2× = 2,466); flumioxazin (1× = 210, 2× =
- 50 420); indaziflam (1× = 50, 2× = 101); napropamide (1× = 4,483, 2× = 8,967); S-metolachlor (1× = 1,597, 2× = 3,194); pendimethalin (1× = 3,363, 2×
- 51 = 6,725). Conversion: 1 g·ha⁻¹ = 0.0143 oz/acre.
- 52 $iii 1 \text{ cm}^2 = 0.1550 \text{ in}^2$. 1 g = 0.0353 oz.
- 53 ^{iv}Herbicide and rate effects were tested for any interaction effect (α =0.05). Where no significant herbicide × rate effect was detected, the main
- 54 effect herbicide is reported with rates combined. In cases where a significant herbicide × rate effect was detected; rates are presented as
- 55 separate column.



- 57 Figure 1. Images of representative blackberry plants at 40 days after treatment with preemergence herbicides applied to soil-substrate mix at 1×
- 58 and 2× rates in 2022 greenhouse trial in Fayetteville, AR, USA. Blackberries were transplanted into substrate within 24 hours following herbicide
- 59 applications. Treatments included (A) nontreated control, (B) halosulfuron at 1× rate (53 g ai·ha⁻¹), (C) diuron at 1× rate (1,569 g·ha⁻¹ a.i.), (D)
- 60 diuron at 2× rate (3,138 g·ha⁻¹a.i.), (E) mesotrione at 1× rate (158 g·ha⁻¹a.i.), (F) mesotrione at 2× rate (315 g·ha⁻¹a.i.), (G) sulfentrazone at 1×
- for rate (211 g·ha⁻¹ a.i.), (H) sulfentrazone at 2× rate (420 g·ha⁻¹ a.i.). Conversion: 1 g·ha⁻¹ = 0.0143 oz/acre.