ABSTRACT

ALDRIDGE, RYAN BRUCE. Tolerance of Southern Highbush Blueberry (*Vaccinium corymbosum*) to Saflufenacil Herbicide (Under the direction of Dr. Katherine M. Jennings).

Blueberry is an important crop in North Carolina, worth nearly $78 million in 2015. Although several herbicide options are available for POST broadleaf weed control in blueberries, relatively few are actually used by growers. Saflufenacil is a protoporphyrinogen IX oxidase (PPO) inhibitor registered by BASF Chemical Company in 2009 for PRE and POST control of broadleaf weeds in certain agronomic and tree fruit crops. Greenhouse and field studies were conducted to determine tolerance of Southern highbush blueberry to saflufenacil.

Greenhouse studies were conducted in Research Triangle Park, NC to determine the effect (visual injury, height, growth rate, chlorophyll content, dry weight) of soil applied saflufenacil (0, 50, 100, 200, and 400 g ai ha^{-1}) on three Southern highbush blueberry (*Vaccinium corymbosum*) cultivars and one rabbiteye blueberry (*Vaccinium ashei*) cultivar. Visual injury (purpling/reddening of foliage, some leaf abscission) was most severe at 28 DAT, with rabbiteye blueberry (‘Columbus’) being less sensitive to saflufenacil than the Southern highbush blueberry cultivars. Visual injury ranged from 0 to 4, 3 to 10, 6 to 24, and 13 to 43% with saflufenacil at 50, 100, 200, and 400 g ai ha^{-1} across all cultivars respectively at 28 DAT. Height, growth rate, chlorophyll and dry weight did not differ among treatments.

Field studies were conducted in Burgaw, NC to determine tolerance of immature (2 to 4 yr after transplant) blueberry plants and mature blueberry plants (8+ yr after transplant) to saflufenacil herbicide applied pre-budbreak or during vegetative growth. Treatments included three rates of saflufenacil (50, 100, 200 g ai ha^{-1}), glyphosate alone, glufosinate alone, glyphosate + saflufenacil, glufosinate + saflufenacil, and hexazinone applied POST directed to bushes. Visual injury consisted of necrotic lesions and crinkling on low hanging branches and young sprouts. Visual injury across studies was inconsistent, but generally low (≤10%). No consistent treatment effects on plant growth or fruit yield were observed. Results from both greenhouse and field studies suggest that saflufenacil applied at commercial use rates (50 g ai ha^{-1}) is safe to Southern highbush blueberry and rabbiteye blueberry.

Tolerance of Southern Highbush Blueberry (*Vaccinium corymbosum*) to Saflufenacil Herbicide

by
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A thesis submitted to the Graduate Faculty of
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BIOGRAPHY

Ryan grew up in a small town in the mountains of North Carolina. His father grew Fraser fir Christmas trees as a side business, where Ryan was involuntarily "compelled" to help on the family farm. There he learned basic agronomic principles such as pest control, fertilization, pruning, and planting. He vowed to never spray weeds again so long as he lived (foreshadowing?). Working in trees inspired him to learn more about how plants grow and why they work, so he decided to pursue a horticulture degree at NC State University. Ryan gained a new perspective on the complexities of plant growth and development, and found that he readily connected with his weed science class taught by Dr. Alan York. During the class one of the lab sections visited the crop protection group at BASF. Little did he know that one day he would be on the other side of the tour, giving some of the same demonstrations he saw that day to future weed science students, farmers, and distributors.

Upon degree completion, a temporary position opened in the herbicide research and development group at BASF Chemical Company. Ryan jumped on the opportunity. It was in that office at BASF that Ryan met his eventual wife, Sara. They created a chemistry all of their own that went beyond herbicide and fungicide formulations. Ryan worked as a contractor for two years until a full-time position within BASF opened up as an Ag Biologist. Shortly thereafter Ryan decided to go back to school part time in an attempt to earn a Masters degree while maintaining his duties at BASF. After several years of juggling both work and school, Ryan and Sara welcomed a baby boy, Benjamin Ryan Aldridge, into their lives. Ryan looks forward to teaching/boring Ben about weed science principles in the future.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my committee members (Dr. Katie Jennings, Dr. David Monks, and Dr. Wesley Everman) for all of their support, feedback, and patience. Their willingness to adapt to my needs as a part time graduate student with additional responsibilities outside the scope of my academic career is very much appreciated.

I would also like to thank Dr. Cavell Brownie for her tremendous support in analyzing data and statistical analysis. Her input about how best to interpret data, and experience in SAS code programming were instrumental in the scope of the entire project.

My wife Sara has been continually supportive and accommodating throughout the graduate school experience. My sometimes long days and late nights have often transferred to her in the form of additional responsibilities in my absence. She has always had an open ear to hear my problems, an open mind to offer suggestions or to give her perspective, and an open mouth to voice her support, encouragement, and love.

BASF Corporation, my management, and associated colleagues have generously supported funding for tuition, provided facilities and equipment for experiments, and allowed me to have flexible scheduling as classes, field work, and thesis writing have required my time away from work. Being able to approach my BASF colleagues about their expertise in herbicides, agronomy, and experimental setup/design has been invaluable during my corporate career, graduate project, and beyond.

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Chapter 1

Tolerance of Southern Highbush Blueberry (*Vaccinium corymbosum*) to Saflufenacil Herbicide in Greenhouse Culture

Ryan B. Aldridge, Katherine M. Jennings, David W. Monks, and Wesley J. Everman

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Abstract

Greenhouse studies were conducted in Research Triangle Park, NC to determine the effect of soil applied saflufenacil (0, 50, 100, 200, and 400 g ai ha^{-1}) on three Southern highbush blueberry (*Vaccinium corymbosum*) cultivars and one rabbiteye blueberry (*Vaccinium ashei*) cultivar. Plants were evaluated for visual injury, height, growth rate, chlorophyll content, and dry weight. Visual injury (purpling/reddening of foliage, and some leaf abscission) was most severe at 28 DAT, with rabbiteye blueberry ('Columbus') being less sensitive to saflufenacil than highbush blueberry. Visual injury ranged from 0 to 4, 3 to 10, 6 to 24, and 13 to 43% with saflufenacil at 50, 100, 200, and 400 g ai ha^{-1} across all cultivars respectively at 28 DAT. For highbush blueberry, O'Neal displayed the greatest tolerance, followed by Legacy and New Hanover (approximately equal). Height, growth rate, chlorophyll and dry weight did not differ among treatments. These studies indicate that

blueberry tolerance to saflufenacil is acceptable for further testing at commercial use rates for field use.

Nomenclature: saflufenacil; highbush blueberry, *Vaccinium corymbosum*, rabbiteye blueberry, *Vaccinium ashei*

Key words: Injury, cultivar, perennial crop, leaf abscission, reddening foliage

Introduction

Blueberry is an important crop in North Carolina, worth nearly $78 million in 2015 (USDA 2016). Nationally, North Carolina ranks seventh in production in the U.S., with blueberry grown on 2779 ha in 2012 (USDA 2012a). Blueberry has a very diverse genetic background, with highbush (*Vaccinium corymbosum*), rabbiteye (*Vaccinium ashei)*, and lowbush (*Vaccinium angustifolium*) commonly grown in North America (Die and Rowland 2013, Delaplane and Mayer 2001). Although blueberry is indigenous to North America, highbush blueberry has only been commercially cultivated for approximately 80 years (Demchak 2012). Southern highbush (95% of North Carolina total ha) and rabbiteye (5% of North Carolina total ha) blueberry are grown commercially in North Carolina (Roberts 2009). Soil properties are predominantly responsible for the disproportionate amount of southern highbush blueberry produced within the region relative to rabbiteye blueberry (B. Cline, personal communication), as well as a limiting factor for expansion of commercial ha within the state (Strik and Yarborough 2005). These unique properties include pH between 4 to 5, well drained but moist soil (typically with a shallow water table between 35 and 75 cm), and organic matter content greater than 2% (Krewer et al. 2007). Roberts (2009) noted that rabbiteye blueberries tend to be distributed throughout North Carolina, and tolerate a wider range of soil conditions, as well as increased heat and drought tolerance as compared to Southern highbush cultivars (Krewer et al. 2007).

Weeds are common pests in North Carolina blueberry, with weed populations consisting of a mixture of both annual and perennial weeds (Roberts 2009). Weeds compete with plants for water, nutrients, sunlight, and space, often resulting in yield and quality loss (Sciarappa 2004). Weeds may serve as alternate hosts for insects and diseases, and produce a favorable environment for vertebrate pests such as voles (Hancock and Retamales 2012). As with other long term perennial crops, blueberry fields have a tendency to harbor difficult to control perennial weeds in mature plantings (Meyers 2012, Monaco 2002, Roberts 2009). Some research has indicated that after solely relying on chemical control to manage weeds, a weed shift favoring perennial weed species can take place (Triplett et al. 1972, Tworkoski et al. 2000). Alternately, some research has found very little shift in weed spectrum after 8 years of a continual herbicide program (Lapointe 2001).

Several options exist for managing weeds in blueberry fields. These options include mowing, cultivation, mulching, chemical control, and hand weeding (B. Cline, personal communication; Monaco 2002). Although several herbicide options are available for POST broadleaf weed control in blueberries [seven unique modes of action according to the North Carolina Agricultural Chemicals Manual (2016)], relatively few are actually employed by growers. North Carolina growers have depended on the same selection of POST herbicides in blueberry for many years. The emergence of herbicide resistant weeds in agronomic and vegetable cropping systems has proven reliance on a limited number of herbicides can be detrimental to overall weed control.

Saflufenacil, also known as BAS 800H, is a protoporphyrinogen IX oxidase (PPO) inhibitor registered by BASF Chemical Company in 2009. Saflufenacil has PRE and POST activity on broadleaf weeds (Ashigh 2010). Saflufenacil POST at 6 g ai ha^{-1} reduced biomass of 5 weed species an average of 90% (GR90) in a greenhouse (Geier et al 2009). When applied PRE across the same 5 species, 9 g ai ha^{-1} reduced weed density by 90% (DR90). Jhala et al. (2013) found saflufenacil to be complementary with indaziflam and/ or glufosinate in a citrus orchard environment. Tank mixes of saflufenacil and glufosinate were more effective than glyphosate alone, and the addition of indaziflam to the mixture of saflufenacil plus glufosinate provided longer residual control and increased spectrum of weeds controlled. Saflufenacil has no grass control, so tank mixes with an appropriate graminicide is necessary in the presence of grass weed pressure.

Blueberry fields are often heavily infested with annual and perennial weeds and growers would benefit from having a new registration for a herbicide with PRE and POST activity on weeds that is safe to blueberry. Thus, a greenhouse study was conducted to determine the tolerance of blueberry cultivars to saflufenacil herbicide.

Materials and Methods

Greenhouse studies were conducted at the BASF research facility in Research Triangle Park, NC (35.88°N, 78.86°W) in 2012. Woody rooted cuttings of southern highbush ‘Legacy’, ‘New Hanover’, and ‘O’Neal’ and rabbiteye ‘Columbus’ blueberry were dug from a commercial blueberry nursery in Ivanhoe, NC. Cuttings were transplanted into 18.5 cm diameter pots containing 1.75 L of coarse sand (Sands and Soils, Durham NC), described as ’white coarse sand’ by the supplier. Sand had 0.1% organic matter, pH 5.8, and CEC 1.2 cmol kg^{-1}. Transplant occurred September 9, 2011 for run 1, and February 23, 2012 for run 2. Plants were fertilized once per wk with a blend of water soluble fertilizers (’Jacks Professional’, JR Peters INC, Allentown, PA) that delivered 228 ppm nitrogen, 23.7 ppm potassium, 194 ppm phosphorous, and 1.85 ppm iron. Approximately 2 wk after transplanting, ammonium sulfate was applied at 372 kg ha^{-1} (1 g per pot) to adjust soil pH down into an appropriate range (approximately pH 4.8) for blueberry growth.

Treatments consisted of five rates [0, 50 (1x registered field rate in tree, nut, and vine cropping systems), 100, 200, and 400 g ai ha^{-1}] of saflufenacil (Treevix^{TM} herbicide, BASF Corporation, Agricultural Products Center, 26 Davis Drive, Research Triangle Park, NC 27709) applied when blueberry reached approximately 30 cm height when transplants had 15 to 25 cm new growth. Treatments were applied directly to soil surface in an aqueous solution at 10 mL per pot distributed evenly across the soil surface using a serological pipettor (‘PipetAid’ Drummond Scientific Company, Broomall, PA). Plants were watered as needed, typically once per d in order to minimize leachate. Saucers were placed under each pot to capture any leachate draining out of pots and then reintroduced back into the pot to ensure continuous herbicide exposure throughout the experiment. Greenhouse conditions were 14 h photoperiod at 28C d/24C night. Supplemental lighting was triggered when ambient light fell below approximately 350 µmol m^{-2} sec^{-1}, and was provided by high pressure sodium lamps. The study was conducted twice in time.

The experimental design was a randomized complete block with 5 replications. Visual injury [scale of 0 (no injury) to 100% (death)], and height were determined at 7, 14, 28, and 56 d after treatment (DAT) (Frans et al. 1986). Height was measured from the tip of the longest stem to the soil surface. Growth (change in height) was determined over three periods (7 to 28 DAT, 29 to 56 DAT, and 7 to 56 DAT) by subtracting early height values from late height values. Chlorophyll content was determined using a SPAD meter (Konika Minolta model # SPAD-502, Ramsey, NJ), with 5 subsamples per plant taken at random. Destructive harvest of all blueberry plants was conducted 56 DAT. At harvest soil was removed from roots by gentle shaking followed by a steady stream of water applied through a garden hose with attached 1000PL water breaker (Dramm Corporation, Manitowoc, WI). Samples were placed in paper bags and placed in a drying oven at 60 C for approximately one wk and then whole plant dry wt was measured.

Data were subjected to ANOVA using SAS PROC GLM (SAS 9.4, SAS Institute, Cary, NC) and PROC MIXED with fixed effect of saflufenacil rate, and random effects of replication, cultivar, run, cultivar x run, cultivar x rate, run x rate and run x cultivar x rate. Data for visual injury percentages were transformed via arcsine transformation, but transformations had no effect on significance, therefore non-transformed data are presented in percentages in order to facilitate easier interpretation. Significant effects were separated using t-tests with LSD and $P \leq 0.05$. Treatment means were linearly regressed via SAS PROC MIXED, excluding untreated controls, with fixed effect of rate and rate x cultivar, and random effect of replication.

Results and Discussion

Blueberry Injury. Predicted injury to blueberry at 7 DAT had a strong linear relationship with saflufenacil rate, with predicted injury increasing as saflufenacil rate increased from 50 to 400 g ha^{-1} (Figure 1.1). Injury was observed as purpling or chlorosis of foliage, and leaf drop and was observed on blueberry as early as 7 DAT. With exception of New Hanover (20%) and Legacy (15%) at 400 g, predicted injury at 7 DAT did not exceed 10% across rates of saflufenacil. Regression modeling indicated that slope estimates for each cultivar were significant with the exception of Legacy. New Hanover displayed the least tolerance (greatest slope value) to saflufenacil. Columbus displayed the greatest tolerance (lowest slope value) to saflufenacil.

By 14 DAT, predicted injury values, as well as slope values for each cultivar increased compared to 7 DAT ratings (Figure 1.2). Modeling indicated that all cultivars had significant slope values. Predicted injury at rates ≤200 g were all below 15%, with the exception of Legacy at 200 g (15%). At 400 g, all Southern highbush cultivars displayed injury greater than 20%, with the rabbiteye cultivar Columbus displaying 13% injury.

O'Neal was less susceptible (slope value of 0.060) to saflufenacil compared to other Southern highbush varieties Legacy and New Hanover (slope values of 0.076 and 0.080, respectively). Columbus displayed the greatest tolerance of all varieties (slope value of 0.035).

Injury was greatest at 28 DAT (Figure 1.3). Relationships remained the same from 14 DAT, with Columbus displaying the highest tolerance (slope value of 0.028), followed by O'Neal (0.082), followed by Legacy (0.115) and New Hanover (0.117). Predicted injury to all blueberry cultivars was less than 5 and 10% for 50 and 100 g ha^{-1}, respectively. Predicted injury at 200 g ranged from 7% (Columbus) to 20% (Legacy), and from 12% (Columbus) to 43% (Legacy) at 400 g.

Plants began to recover by 56 DAT, as injury began to decline as compared to 28 DAT (Figure 1.4). Trends remained the same from previous observations, with Columbus displaying the greatest tolerance to saflufenacil, followed by O'Neal, Legacy and New Hanover. Predicted injury values for blueberry ranged from 0 to 4% at 50 g ha^{-1} saflufenacil, 3 to 8% at 100 g, 6 to 15% at 200 g, and 13 to 30% at 400 g ha^{-1} saflufenacil.

According to Wu and Boyd (2012) commercially acceptable herbicide injury to blueberry is subjective, generally falling between 15 and 20% (15% was used as the standard level in our studies) depending on grower and severity of weed infestation. At 50 g ai ha^{-1} (1x commercial rate in tree fruit crops) saflufenacil, nearly all plants displayed acceptable tolerance in a system designed to exacerbate herbicide response from root uptake. The 100 g ai ha^{-1} (2x commercial rate in some tree fruit crops) rate of saflufenacil caused slight injury, but injury was still within commercially acceptable levels (≤15%) (KM Jennings, personal communication; Wu and Boyd, 2012) for all cultivars. Saflufenacil at up to 400 g (8 times the registered rate in some fruit crops) did not cause any herbicide-induced plant mortality. However, this rate was too injurious to Legacy and New Hanover. Likewise, differential cultivar tolerance has also been observed in field applications of halosulfuron to the cultivar Legacy (KM Jennings, personal communication). Saflufenacil injury developed soon after application and then increased up to 28 DAT, and gradually decreased to 56 DAT. Treatments were applied post-directed with no spray particle drift from applications, so care should be taken to minimize spray drift to blueberry in a field setting. Use of a shielded sprayer is suggested on the Treevix™ tree fruit label (Anonymous 2013).

Height and Growth. Total height or growth rate of blueberry did not differ across herbicide treatments (data not shown). New Hanover (13.4 cm) displayed the fastest growth rate from 7 to 56 DAT followed by O'Neal (12.7 cm), Columbus (12.5 cm), and Legacy (10 cm). However, no differences in growth rate of blueberry cultivars occurred from 7 to 28, 28 to 56, or 7 to 56 DAT.

At all timings a significant varietal effect, as well as a run x cultivar effect for height was observed. New Hanover in the first run was much smaller at saflufenacil application (21.7 cm) compared to other cultivars (Columbus 41 cm, Legacy 38.5 cm, and O'Neal 32.4 cm). Initial plant heights at treatment in the second run were similar for Columbus (37.5 cm), Legacy (33.3 cm), New Hanover (36.1 cm), and O'Neal (36.8 cm).

SPAD. No consistent or significant saflufenacil rate effect was observed at any timing for SPAD meter readings with exception of 400 g ha^{-1} saflufenacil (35.7) compared to the nontreated (39.8) at 28 DAT. At 28 DAT, Columbus had a SPAD reading of 37.0 compared to 40.3 for Legacy. At 56 DAT New Hanover had a lower SPAD reading (39.0) compared to O'Neal (41.8) and Legacy (42.3). Significant run and run x cultivar interaction was observed across all rating dates with SPAD values averaging 4.3 points higher for run 1 than run 2.

SPAD meter readings did not appear to be a good indicator of plant injury. Despite the change in appearance of leaf tissue from green to a red/purple leaf color at higher saflufenacil rates, SPAD readings as an indicator of chlorophyll content appeared to remain the same. Leaves most affected by herbicide application would also have been most prone to desiccation/abscission. Given the non-destructive sampling technique, it would be possible to do more replications per plant, and perhaps focus on a distinct region within each plant (i.e. only sampling new leaves vs old leaves), as chlorophyll levels vary depending on age of leaf tissue as well as N status (Ling et al. 2010).

Whole Plant Dry Weight. Plant weights for each herbicide rate were converted to % nontreated within cultivar (Table 1.1). There were no significant effects for run, cultivar, rate, or cultivar x rate, so data was pooled across runs. Legacy and New Hanover treated with 400 g saflufenacil displayed the lowest weight compared to nontreated, each at 76%. Weight values ranged from 116% to 76% across all cultivars and rates. Generally, blueberry weight from plants treated with saflufenacil was greater than or equal to the nontreated with the exception of 400 g saflufenacil. Leaf number/abscission did not appear to be a factor in final dry weight. Some abscission took place on nearly all plants during the experiments, with slightly more abscission at higher herbicide rates. Future studies could include monitoring leaf number or leaf abscission. In hindsight, perhaps separating blueberries at the crown to get both shoot and root weights might have given more insight into how plants were being effected as saflufenacil can have an inhibitory effect on root growth (Hixson 2008). At harvest there did not appear to be any visual differences in root growth, but recording both shoot and root weights would have served as confirmation of no effect on growth above and below ground.

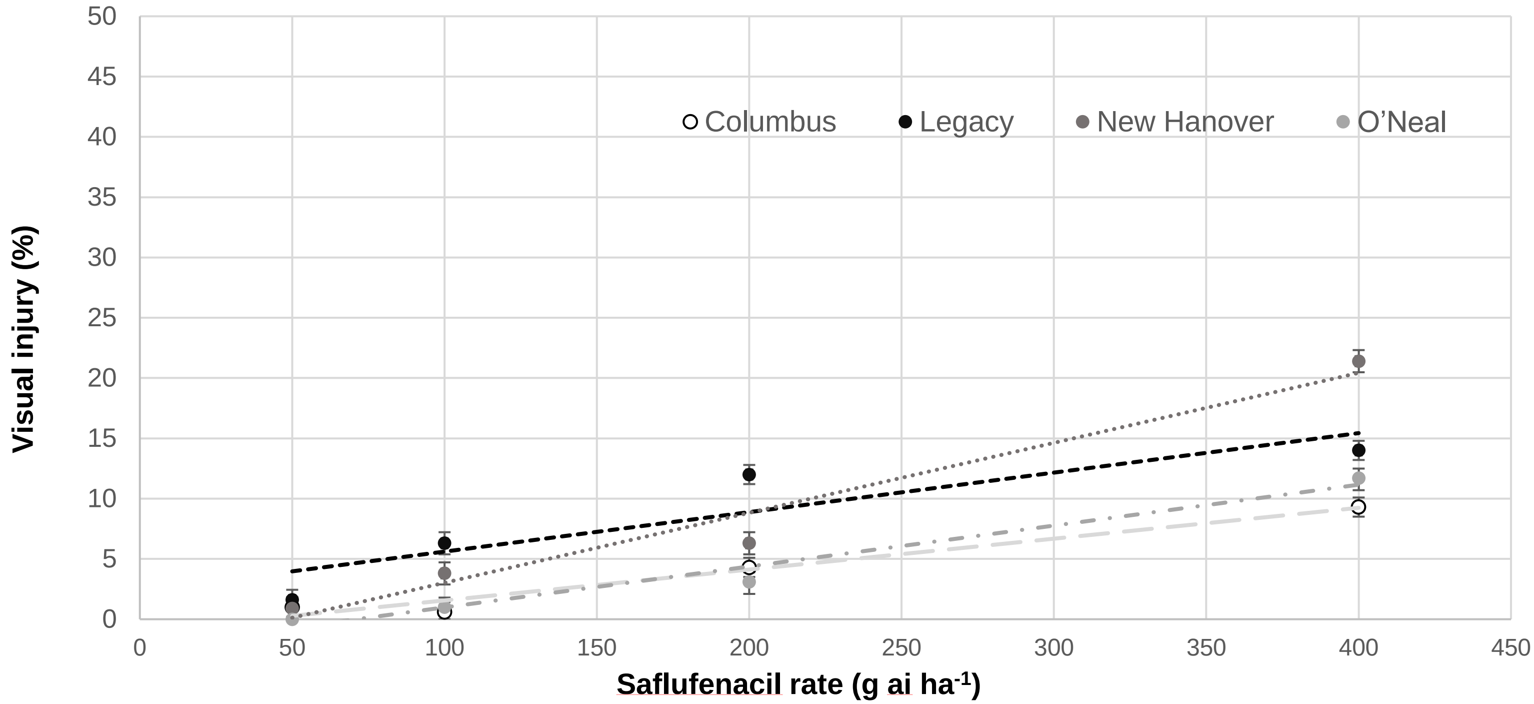

Figure 1.1. Response of blueberry cultivar injury to soil applied saflufenacil rate at 7 DAT. Predicted models for Columbus, O’Neal, Legacy and New Hanover is y = 0.026x - 1.00 (R^2 = 0.97), y = 0.034x - 2.41 (R^2 = 0.972), y = 0.033x + 2.33 (R^2 = 0.812), y = 0.058x – 2.78 (R^2 = 0.966), respectively, where y = visual injury (%) and x = saflufenacil rate (g ai ha^{-1}).

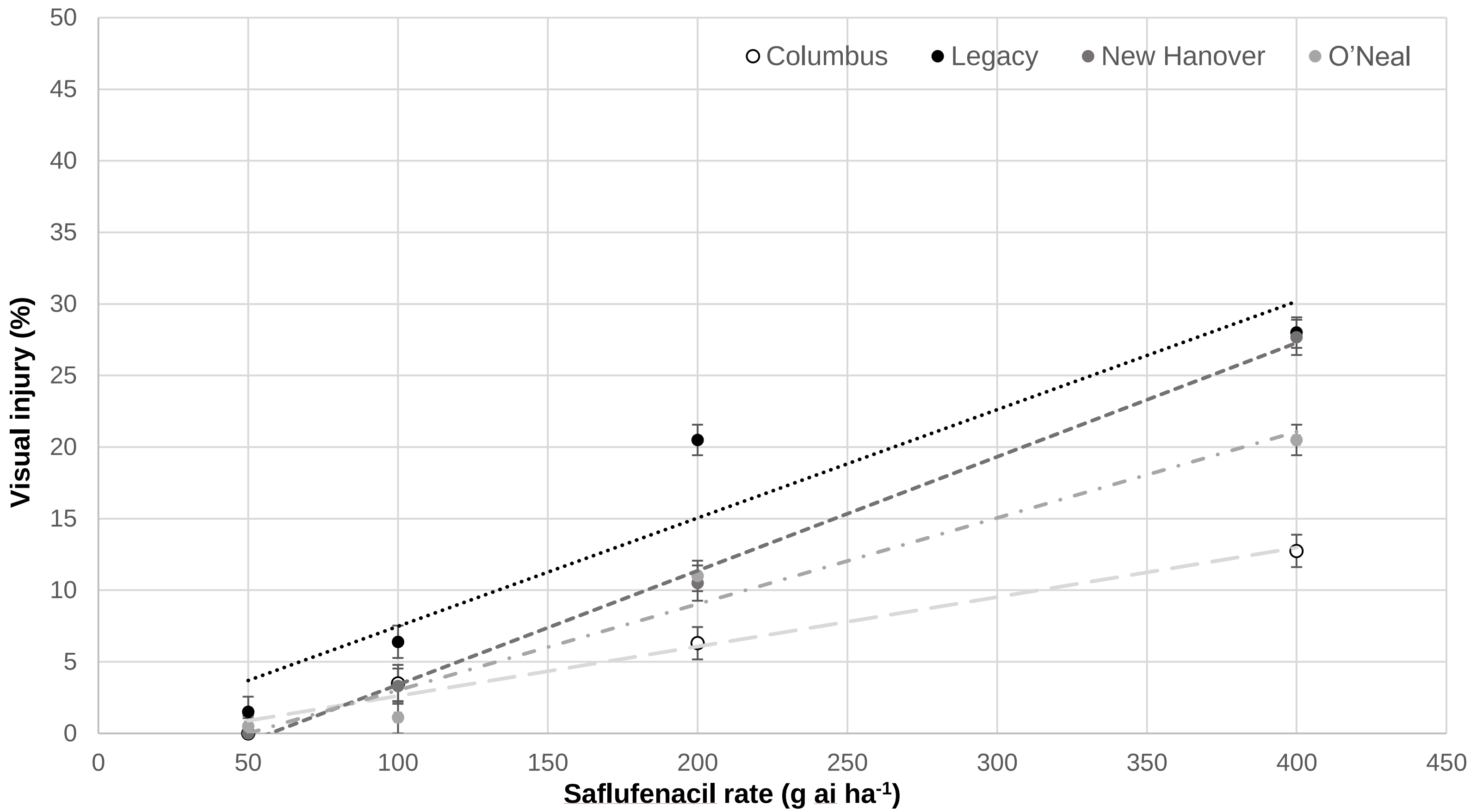

Figure 1.2. Response of blueberry cultivar to injury soil applied saflufenacil rate at 14 DAT. Predicted models for Columbus, O'Neal, Legacy and New Hanover is y = 0.035x -0.84 (R^2 = 0.981), y = 0.060x – 3.01 (R^2 = 0.970), y = 0.076x – 0.09 (R^2 = 0.910), y = 0.080x – 4.56 (R^2 = 0.997), respectively, where y = visual injury (%) and x = saflufenacil rate (g ai ha^{-1}).

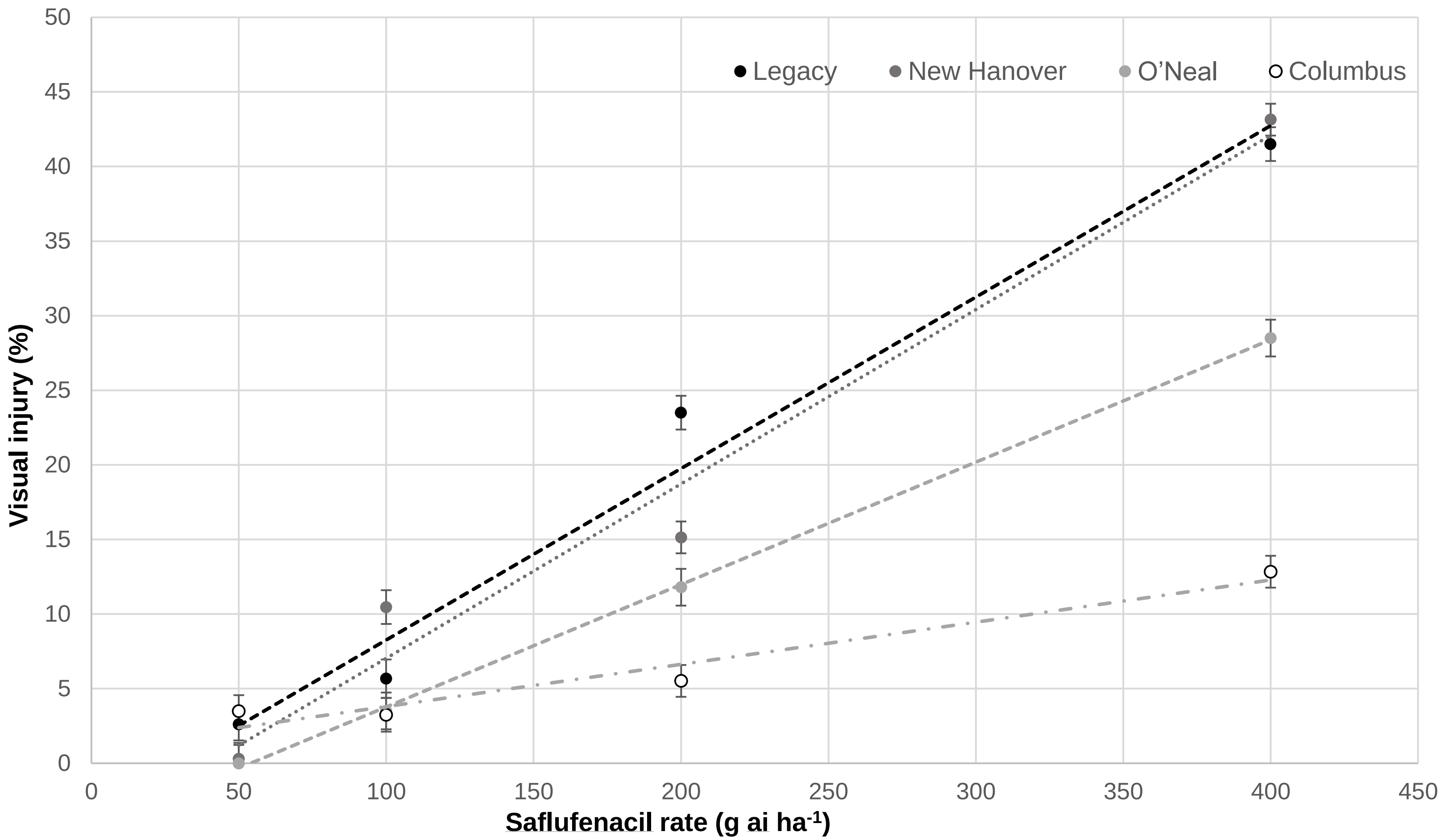

Figure 1.3. Response of blueberry cultivar to injury soil applied saflufenacil rate at 28 DAT. Predicted models for Columbus, O'Neal, Legacy and New Hanover is y = 0.028x + 0.98 (R^2 = 0.949), y = 0.082x – 4.43 (R^2 = 0.999), y = 0.115x – 3.23 (R^2 = 0.977), y = 0.117x – 4.65 (R^2 = 0.974), respectively, where y = visual injury (%) and x = saflufenacil rate (g ai ha^{-1}).

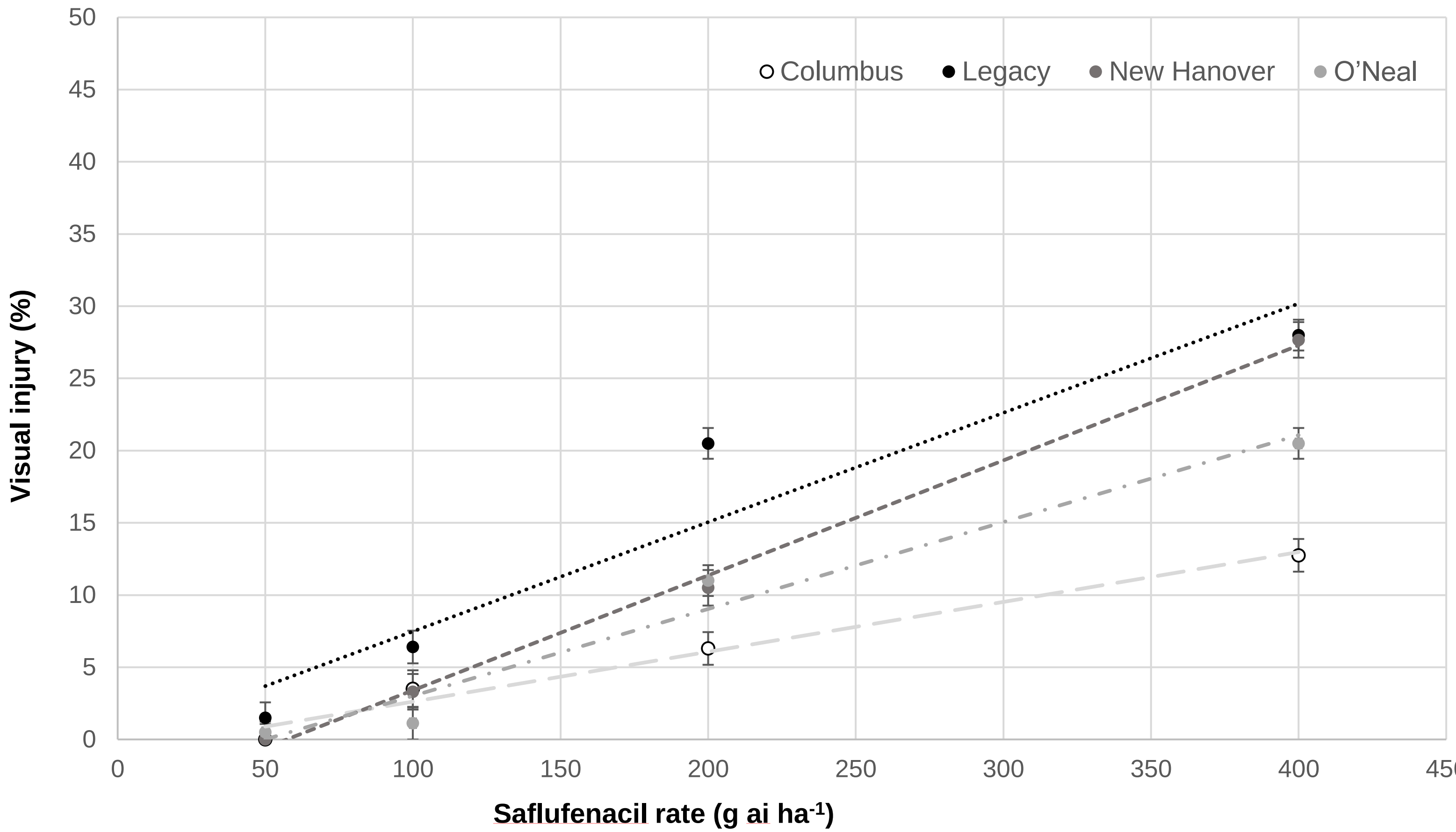

Figure 1.4. Response of blueberry cultivar to injury soil applied saflufenacil rate at 56 DAT. Predicted models for Columbus, O'Neal, Legacy and New Hanover is y = 0.035x - 0.84 (R^2 = 0.981), y = 0.060x – 3.00 (R^2 = 0.970), y = 0.076x – 0.09 (R^2 = 0.910), y = 0.080x – 4.56 (R^2 = 0.997), respectively, where y = visual injury (%) and x = saflufenacil rate (g ai ha^{-1}).

Table 1.1 Final dry weight of saflufenacil treated blueberry cultivars at 56 DAT[a]

	Saflufenacil rate (g ai ha^{-1})					
Rate	**0**	**50**	**100**	**200**	**400**	**Mean[b]**
			Weight (% nontreated)			
Columbus	100	88	94	83	93	**92 a**
Legacy	100	100	112	86	76	**95 a**
New Hanover	100	104	116	115	76	**102 a**
O’Neal	100	88	101	95	88	**94 a**

[a] DAT=d after treatment.
[b] Means within a column followed by the same letter are not statistically different at α=0.05.

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Chapter 2

Effect of Saflufenacil on Southern Highbush Blueberry (*Vaccinium corymbosum*)

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Abstract

Field studies were conducted in Burgaw, NC to determine tolerance of immature (2 to 4 yr old) blueberry plants and mature (8+ yr after transplant) blueberry to saflufenacil herbicide applied pre-budbreak or during vegetative growth. Visual injury across studies did not exceed 11%. Vegetative growth applications tended to display higher visual injury levels compared to pre-budbreak applications. No change in response to saflufenacil was observed as rate increased, and no treatments consistently injured blueberry. Likewise saflufenacil had no effect on plant growth, or fruit yield.

Nomenclature: Saflufenacil; Southern highbush blueberry (*Vaccinium corymbosum*)

Key words: Injury, small fruit, crop tolerance

Introduction

Blueberry is a high value crop in North Carolina, generating nearly $78 million in 2015 (USDA 2016). Although several herbicides are registered for PRE weed control, few herbicides are registered for POST broadleaf weed control in blueberry fields. Seven modes of action may be used for POST broadleaf weed control in blueberries in North Carolina (North Carolina Agricultural Chemicals Manual 2016), although actual utilization by growers are limited to 4 modes of action [EPSP synthase inhibition (glyphosate), glutamine synthatase inhibition (glufosinate), acetolactate synthase inhibition (halosulfuron) and photosystem I inhibition (paraquat)] (KM Jennings, personal communication). Growers have depended on the same limited selection of POST herbicides in blueberry for many years. Repeated use of herbicides with the same mode of action can lead to weed populations that exhibit herbicide resistance (Shaner 2014). The emergence, and now prevalence, of glyphosate resistance should stress the importance of herbicide stewardship for all growers, including blueberry producers. Other options for weed control in blueberry plantings need to be explored for their effectiveness, as well as their potential for commercial adoption.

Hexazinone has been widely used in blueberry fields over the past thirty years. DuPont USA originally registered hexazinone in 1975 (Anonymous 2016). In North Carolina, hexazinone was applied to 71, 72, and 50% of bearing blueberry hectarage in 1991, 2009, and 2011, respectively (USDA 2011). As might be anticipated, after over reliance on hexazinone in blueberry for the past thirty years, herbicide resistance has been documented (Li et al. 2014). Resistance to hexazinone in red sorrel (*Rumex acetosella*) was confirmed in 2013 in a lowbush blueberry field in Nova Scotia. Other herbicide modes of action are needed to reduce the potential for other herbicide resistant weed populations to establish.

Saflufenacil is a protoporphyrinogen IX oxidase (PPO) inhibitor registered by BASF Chemical Company in 2009 (also known as BAS 800H). Saflufenacil is a weakly acidic herbicide in the pyrimidinedione family, and has a pKa level of 4.41 ± .025. Due to its acidic nature, water solubility varies according to solution pH, and was reported as being 14 mg L^{-1} at pH 4, 25 mg L^{-1} at pH 5, and 2100 mg L^{-1} at pH 7 by the EPA pesticide fact sheet (2009). Saflufenacil has a half-life (DT_{50}) of approximately 21 d (Mueller et al. 2014). Soil sorption values (Kd) for saflufenacil have been reported between 0.1 and 2.4 mL g^{-1} (Hixson 2008). Saflufenacil has a sorption coefficient of 4-92 mL g^{-1} when normalized for organic carbon (Koc), but some research indicates that humic matter content is a better indicator of saflufenacil sorption compared to organic matter (Hixson 2008). These sorption coefficients (Kd and Koc) of saflufenacil are generally considered low, although the elevated levels of organic matter and humic matter and relative speed at which saflufenacil is sorbed to organic matter indicates a decreased potential for saflufenacil to be bioavailable after application in blueberry fields. With soil pH at or near pKa, approximately 50% of the saflufenacil molecules will be present in the protonated form, thereby conferring less water solubility, indicating less potential for movement into groundwater via water infiltration. Longer residence time in the upper portion of the soil profile generally leads to faster degradation (Papiernik 2012). However, groundwater contamination is always of concern, especially in situations where a shallow water table exists. Given the physiochemical properties of saflufenacil, coupled with the high organic matter, low pH soils common of highbush blueberry producing areas, bioavailability of saflufenacil after application should be limited (Gannon et al. 2014).

The active ingredient saflufenacil has both PRE and POST activity on broadleaf weeds (Ashigh 2010). Saflufenacil POST at 6 g ai ha^{-1} reduced biomass of 5 weeds by 90% (GR90) in a greenhouse study (Geier et al. 2009). Saflufenacil PRE at 9 g ai ha^{-1} reduced weed density of the same 5 species by 90% (DR90). In the field, adjuvants decreased the rate of saflufenacil POST needed for 90% control (Knezevic et al. 2009). Jhala et al. (2013) found saflufenacil to be complementary with indaziflam and/or glufosinate for weed control in a citrus orchard. Tank mixes of saflufenacil and glufosinate were more effective than glufosinate alone, and the addition of indaziflam to saflufenacil plus glufosinate provided broad spectrum weed control. Saflufenacil provides no grass control, so tank mixes with an appropriate graminicide are necessary in the presence of grass weeds.

Saflufenacil is currently registered in citrus, tree nut, and pome fruit crops, however it is not registered in blueberries. Blueberry growers need additional options for weed control in order to effectively manage herbicide resistance potential, and saflufenacil may be an effective option if found safe and effective in blueberry. Thus, studies were conducted in the field to determine tolerance of Southern highbush blueberry to saflufenacil.

Materials and Methods

Immature blueberry. Field studies were conducted at a commercial blueberry farm near Burgaw, NC (34.60°N, 77.85°W) in 2012 and 2013. Soil was a Murville muck (sandy, siliceous, thermic Umbric Endoaquods) with pH 4.3, 25% organic matter and 13.6 cmol kg^{-1} CEC. Plants were grown on a raised bed, approximately 20 cm above grade. Herbicide treatments were applied either prior to blueberry budbreak (pre-budbreak) or during active vegetative growth. Herbicide treatments consisted of saflufenacil (Treevix®, BASF Corp., Research Triangle Park, NC) applied at 50, 100, or 200 g ai ha^{-1}, saflufenacil at 50 g ai ha^{-1} plus glyphosate (Roundup WeatherMax®, Monsanto Co., St. Louis, MO) at 870 g ae ha^{-1}, saflufenacil at 50 g ai ha^{-1} plus glufosinate (Rely 200®, Bayer CropScience, Research Triangle Park, NC) at 1096 g ai ha^{-1}, glyphosate alone, glufosinate alone, and hexazinone (Velpar L®, DuPont, Wilmington, DE) at 1120 g ai ha^{-1}. Nontreated check plots were included for comparison. Methylated seed oil (1.87 L ha^{-1}) and ammonium sulfate (400 g ha^{-1}) were added to all saflufencil containing treatments. Ammonium sulfate, methylated seed oil, and non-ionic surfactant (0.4 L ha^{-1}) were included with glyphosate alone, glufosinate alone, and hexazinone treatments, respectively. Treatments were arranged in a randomized complete block design with four replications.

Herbicide treatments were applied POST-directed down each side of blueberry row using a CO_2 pressurized backpack sprayer with a two nozzle boom with AIXR 110015 nozzles (Teejet Technologies, Springfield, IL) calibrated to deliver 187 L ha^{-1}. Pre-budbreak treatments, and treatments during active vegetative growth were applied on March 23, 2012 and March 14, 2013, and on May 4, 2012 and May 17, 2013, respectively (Table 2.1). Plots consisted of four bushes spaced 1.2 m apart within row, and 3 m between rows.

Visual blueberry crop injury (scale of 0 to 100% with 0 = no injury and 100 = death) was determined 7, 13, 27, 42, and 55 days after treatment (DAT) pre-budbreak and 7, 14, 21, and 28 DAT for active vegetative growth treatments in 2012 and 2013 (Frans et al. 1986).

Blueberry growth was measured by flagging three branches on a representative plant in each plot on the day of application for vegetative herbicide treatment timings, and approximately 2 wk after pre-budbreak treatments. The change in branch length was determined as the season progressed. All berries (green to fully ripe) were harvested from one bush per plot 55 DAT in 2012 and 2013, respectively. Similar techniques for yield determination have been employed by other researchers (Gupton et al. 1996). Berries were separated into green and blue (mature) berries and weighed separately. A berry was considered mature if >80% of the surface was blue. Ten samples of 100 green and blue berries were weighed and green berry weight was converted to total blue berry weight for each plant harvested (Equation 1).

Calculated blue blueberry weight = (mean blue berry weight/mean green berry weight)* total green berry weight [1]

Mature blueberry. Studies with mature blueberry had identical treatments as the immature blueberry studies except only one herbicide application timing each year (pre-budbreak in 2012, active vegetative growth in 2013) was made. Application conditions were equivalent to those in the immature blueberry study, and parameters measured were similar. Herbicide treatments were applied on March 23, 2012, and May 17, 2013. Due to lack of weed emergence by March 2013, herbicide treatment application was delayed until May 17 to allow weed emergence and subsequent collection of weed control data. However, little to no weed emergence occurred between March and May, at which point the study was initiated to collect crop injury ratings prior to harvest. Ratings were taken 7, 13, 27, 42, and 55 DAT in

2012, 10 and 17 DAT in 2013. Over 95% of berries harvested in the 2013 mature blueberry trial were harvested green to ensure all berries harvested before berry loss or grower harvest.

Growing conditions. Growing conditions in 2012 included adequate rainfall (approximately 39 cm from March 1 to June 30), along with moderate temperatures. Some weed pressure was present, but species were not weeds that saflufenacil controls (monocotyledonous and grass species not listed on product label) (Anonymous 2013). Weeds most commonly observed included Carolina redroot (*Lachnanthes caroliniana*), needleleaf rosette grass (*Dichanthelium aciculare*), large crabgrass (*Digitaria sanguinalis*), common pokeweed (*Phytolacca americana*), and Maryland meadowbeauty (*Rhexia mariana*). Saflufenacil produced small "superficial" necrotic areas on grass species and Carolina redroot, but regrowth quickly resumed. Broadleaf weeds were not present uniformly across the study or at levels high enough to get an accurate assessment of saflufenacil control. Approximately 7 d before the second application to immature bushes was applied, the studies were oversprayed with glufosinate POST directed due to high population and low level of control of Carolina redroot with saflufenacil.

The growing season in 2013 was unusually cool and wet during March. Average high temperatures were approximately 2.5 C cooler than normal, and 4.3 C cooler than average minimum temperatures for March. Frost protection was conducted approximately seven times within the first two weeks following early herbicide treatment application at an estimated amount of ~2.5 cm of irrigation per event, coupled with approximately 4 cm natural precipitation (average precipitation is approximately 10 cm). Given the delay in growth due to cooler temperatures, high amount of water infiltration, and high soil OM content (>20%), injury from saflufenacil was slight and variable in the early application timing. Herbicide injury was difficult to discern from freezing injury.

Data were subjected to ANOVA using SAS PROC GLM (SAS 9.4, SAS Institute, Cary, NC) and PROC MIXED with fixed effect of treatment, and random effects of replication, study, replication x study, and application (immature studies). Data for visual injury percentages were transformed via arcsine transformation, but transformations had no effect on significance, therefore non-transformed data are presented in percentages in order to facilitate easier interpretation. Significant effects were separated using t-tests with LSD and $P \leq 0.05$.

Results/ Discussion

Immature blueberry.

Visual Injury. Injury to blueberry from saflufenacil occurred as necrotic lesions and leaf crinkling developing soon after saflufenacil application. An interaction between year x timing x treatment was observed and data are reported appropriate to that interaction. Generally, pre-budbreak applications of saflufenacil did not cause significant injury. In 2012 no injury to blueberry was observed at 7 and 13 DAT and injury at 27 DAT was only 0 to 3% from saflufenacil pre-budbreak treatments. Likewise in 2013 injury to blueberry from saflufenacil pre-budbreak treatment was 0 to 5% across rating dates. Injury was greater when saflufenacil was applied to actively growing blueberry compared to pre-budbreak applications. However observed injury to blueberry when saflufenacil was applied to actively growing blueberry was slight (≤11% in 2012; ≤7% in 2013) and would be considered acceptable by growers (Wu and Boyd 2012).

Growth. No interactions were observed for any factor in the experiment. Blueberry growth from 7 to 28 DAT did not differ between yr, timings or herbicide treatments.

Yield. Commercial blueberry establish for 2 to 3 yr after transplant, and become commercially productive after year 3. In 2012, the immature blueberry study was young, and consequently produced negligible berry yield. In 2013 significance was not observed when comparing treatments, application timings, or treatment x application timing for either total calculated yield, or berry number. Mean yield was 2.60 kg $bush^{-1}$, while nontreated bushes averaged 2.05 kg $bush^{-1}$ (Table 2.2).

Mature bushes.

Visual Injury. An interaction for year x treatment was observed for visual injury. Thus, visual injury data is presented by year. No injury to blueberry was observed 7, 13, and 27 DAT in 2012. When combining 42 and 55 DAT ratings, injury to blueberry was observed in the treatments containing saflufenacil. However, injury in these treatments did not exceed 6%. Blueberry injury was not observed where glyphosate or hexazinone were applied alone. No significant effects were observed for treatment, rating date, or multiple interactions. Blueberry injury from saflufenacil at 10 and 17 DAT did not exceed 10% in 2013. Study was terminated in 2013 after 17 DAT due to bush harvest, plant recovery from visual injury, and no further injury expected.

Growth. Blueberry growth was similar in all treatments both years. The lack of differences in growth among treatments was likely due to lack of injury caused by these treatments.

Yield. A significant trial by herbicide interaction was observed so trials were analyzed individually. Blueberry yield in the herbicide treatments did not differ from each other or from the nontreated in 2012 (Table 2.2). Likewise, in 2013 most treatments were similar to nontreated blueberry. However in 2013 mature blueberry yield was greatest in the hexazinone (5.57 kg) and 50 g ha^{-1} saflufenacil (5.04 kg) treatments. The 50 g saflufenacil treatment had similar yield in 2013 as the grower standard hexazinone. Weeds were scarcely present for duration of the studies, so adverse effects from weed competition on yield should not be considered as a factor in yield disparity.

Overall injury across all studies (both immature and mature) was variable but low (<11%). Although there is not a uniform standard, generally herbicide injury is considered commercially acceptable at levels <15% (Wu and Boyd 2014). Treatments of saflufenacil up to 200 g ai ha^{-1} (4x commercial rate) did not cause injury consistently, and would be deemed commercially acceptable. No treatment effects were observed for blueberry growth in immature or mature blueberry. Yield effects were mixed, but generally yields were no different from nontreated blueberry (with the exception of two treatments which yielded greater than nontreated in mature blueberry in 2013). Based on our studies, saflufenacil should be considered safe to blueberry in regards to injury, especially when precautions are taken to minimize physical spray drift.

Table 2.1 Application timings for immature and mature blueberry tolerance studies in Burgaw, NC in 2012 and 2013.

Treatment stage	**Immature bushes**		**Mature bushes**	
	2012	2013	2012	2013
Pre-budbreak	March 23	March 14	March 23	
Active veg.[a] growth	May 4	May 17		May 17

[a]veg=vegetative

Table 2.2 Effect of herbicide treatments on blueberry yield in Burgaw, NC in 2012 and 2013.

		Yield		
		2012 Mature	2013 Mature	2013 Immature
Treatment	Rate (g ai ha^{-1})	(kg $bush^{-1}$)[a]		
Nontreated		5.37 a	3.54 c	2.05 a
Saflufenacil	50	3.84 a	5.04 ab	3.16 a
Saflufenacil	100	4.30 a	4.16 bc	3.10 a
Saflufenacil	200	4.16 a	4.21 bc	2.76 a
Saflufenacil + glyphosate	50 + 870	3.29 a	3.15 c	2.19 a
Saflufenacil + glufosinate	50 + 1096	5.52 a	3.56 c	3.05 a
Glyphosate	870	4.41 a	4.37 abc	2.90 a
Glufosinate	1096	4.08 a	4.12 bc	2.14 a
Hexazinone	1120	3.34 a	5.57 a	2.81 a

[a] Means followed by the same letter within a column do not differ significantly (α=.05).

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APPENDIX

Appendix A. Photos of nontreated blueberry and blueberry treated with soil applied saflufenacil at 28 DAT in a greenhouse.

A) Nontreated

B) Saflufenacil at 400 g ai ha^{-1}