ABSTRACT

Title of Thesis: THE UTILITY OF SAFLUFENACIL ON

GLYPHOSATE-RESISTANT HORSEWEED AND ITS EFFECT ON SELECT SOYBEAN

VARIETIES

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Architecture

Glyphosate-resistant (GR) horseweed [Conyza canadensis (L.) Cronq.] is a major weed in soybean [Glycine max (L.) Merr.] production across the United States. Saflufenacil is a new herbicide labeled for control of GR horseweed in soybean. Due to sensitivity concerns, applications are restricted to 30 days preplant (DPP) on coarse-textured soils with less than 2% organic matter (OM). The utility of saflufenacil tank-mixes on GR and glyphosate-susceptible (GS) horseweed was evaluated in the greenhouse. Saflufenacil at 25 g ai ha⁻¹ tank-mixed with glyphosate at 874 g ae ha⁻¹ resulted in better control of GR and GS horseweed than either product applied alone. In field studies, saflufenacil tank-mixes were applied 30, 15, and 0 DPP to soybean on a coarse-textured and a medium-textured soil. Saflufenacil applied at 50 g ai ha⁻¹ caused a 15-30% reduction to yield and yield components when applied 15 and 0 DPP on the coarse-textured soil.

THE UTILITY OF SAFLUFENACIL ON GLYPHOSATE-RESISTANT HORSEWEED AND ITS EFFECT ON SELECT SOYBEAN VARIETIES

By

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Thesis submitted to the Faculty of the Graduate School of the University of Maryland, College Park, in partial fulfillment of the requirements for the degree of Master of Science 2012

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Chapter 1. Background Literature

Glyphosate Resistance in Weeds

The herbicide glyphosate [N-(phosphonomethyl)glycine] was first commercially available to farmers in the United States (US) in 1974 (Monsanto 2012). Glyphosate is a non-selective herbicide that inhibits 5-enolpyruvylshikimate-3-phospate (EPSP) synthase in plants and is classified as a Group 9 herbicide by the Weed Science Society of America's herbicide mechanism of action classification system (WSSA 2007).

The WSSA mechanism of action classification system was developed in 1997 to help growers readily and easily identify a herbicide's site of action (Mallory-Smith and Retzinger 2003). Herbicides with the same site of action are given the same group number. In 2001, the US Environmental Protection Agency (EPA) used this classification system to establish labeling guidelines to aid in herbicide resistance management (Mallory-Smith and Retzinger 2003). Currently, herbicides sold in the US contain recommendations on the label that caution against using multiple herbicides with the same WSSA group number in a given crop to help prevent herbicide resistance in weeds.

From its commercial release until 1995, glyphosate was primarily used for burndown weed control prior to planting with various crops (Young 2006). In 1996, Glyphosate-resistant (GR) soybeans were commercially released in the US. This technology allowed in-crop, over-the-top applications of glyphosate on soybean (Monsanto 2012). Due to the broad-spectrum weed control offered by glyphosate, growers rapidly adopted this technology which allowed a novel, effective, economical, and easy-to-use postemergence (POST) weed control program in soybean (Green and Owen 2011). The rapid adoption of this technology is reflected by the

increase in glyphosate use on total soybean hectares from 2.5 million kg active ingredient (ai) year⁻¹ in 1995 to 30 million kg ai year⁻¹ in 2002 (Young 2006). The average number of glyphosate applications over the same time period increased from 1 to 1.4 applications year⁻¹ which reflects the increase in POST-use of the herbicide (Young 2006). Over this time period, glyphosate became the dominant herbicide used in soybean. In 2002, glyphosate was applied to 79% of US soybean hectares compared to the second-most used herbicide, pendimethalin, which was applied on only 9% of soybean hectares (Young 2006).

The broad-spectrum weed control offered by glyphosate allowed growers to save money on their weed control program by utilizing glyphosate to control all of their weeds. This change in herbicide programs is reflected by the decline in the number of active ingredients being applied to at least 10% of soybean hectares; from 11 in 1995 to one (glyphosate) in 2002 (Young 2006). The sole reliance on glyphosate for weed control in no-till and conventional systems led to two unintentional consequences; a shift of weed species commonly found in fields, and the evolution of resistance to glyphosate in several weed species. The weed species shift is due in part to inconsistent control of certain weeds such as horseweed [Conyza canadensis (L.) Cronq.], common lambsquarters (Chenopodium album L.), and morningglory (Ipomoea) species (Mithila et al. 2011). Glyphosate resistance was first documented in 1996 in rigid ryegrass (Lolium rigidum Gaudin) in Australia (Heap 2012). Four years later, horseweed was the first GR weed documented in the US. It was also the first known specie that evolved resistance to glyphosate in GR cropping systems (VanGessel 2001). The number of GR weed species in the US has increased in conjunction with an increase in GR crop hectares. In the year 2000, when the first GR weed was discovered in the US, approximately 60% of soybean hectares were planted to GR soybean. During the 2011 growing season, 92% of soybean hectares were planted to GR soybean and the number of documented GR weeds in the US was 13 (Mithila et al. 2011; Soteres 2012).

The increasing number of GR weed species is not isolated to the US. As of April 2012 there were 21 documented GR species around the world (Heap 2012). The rate of evolution of GR weeds has also increased with the global adoption of GR crops. From 1996, when GR crops were commercially released and the first GR weed was identified, to 2000, three GR weed species were identified. From 2001 to 2005, an additional 9 GR species were documented. From 2006 to 2011, 9 additional GR species were documented (Heap 2012). This exponential increase of GR species over a short time seems to indicate that the rate of new GR weed discoveries will not subside in the near future.

On the Delmarva Peninsula, horseweed remains the only documented GR weed (Heap 2012). Since the initial discovery in 2000, all 13 counties on the peninsula have reported cases of GR horseweed biotypes (R.L. Ritter personal communication). Biotypes are populations within a species that have a distinct genetic variation (WSSA 2007). When glyphosate is no longer a viable option for horseweed control, there are limited POST options remaining in soybean (Davis and Johnson 2008). Due to the widespread nature of the GR biotypes, a better understanding of the biology of this weed is necessitated.

Horseweed

Biology and Ecology

Horseweed is a member of the Asteraceae family and is known under several synonyms such as marestail, stickweed, and Canada fleabane. Horseweed has historically been classified as a winter annual that germinates in the late summer or early fall, overwinters in a rosette form,

then bolts (elongates) in the spring and reaches maturity that summer (Regehr and Bazzaz 1979). Certain fall-germinating biotypes have shown no dormancy requirement with the ability to germinate directly following seed shed from the mother plant (Buhler and Owen 1997). However, numerous populations of horseweed have been identified that germinate in the spring months and bolt without first going through a rosette stage (Regehr and Bazzaz 1979; Davis and Johnson 2008). Horseweed seed have been observed germinating 11 months of the year (all except February) under field conditions (Main et al. 2006). Nandula et al. (2006) conducted extensive research regarding environmental factors that affect horseweed germination. Optimal temperature for germination was 24° C daytime temperature coupled with 20° C nighttime temperature. These temperatures reflect the average high temperatures during the spring and autumn months in the Mid-Atlantic region. Horseweed seed exhibited best germination in pH solutions of 6-7, which are typical pH ranges in soybean fields. Horseweed was also found to have optimal germination when seed remained on top of the soil surface. Seed that were buried 0.25 cm deep exhibited a 94-97% reduction in germination when compared to seed on the surface. No germination was observed at planting depths of 0.5 cm and deeper. The combination of these environmental conditions indicates that horseweed germination is well suited for no-till fields in both the fall and spring months. This is illustrated by the fact that on the Delmarva Peninsula, up to 50% of horseweed plants germinate during the spring months (R.L. Ritter personal communication).

Mature horseweed plants have been found to produce more than one million seed plant⁻¹ (Davis et al. 2009c). Plants in direct competition with soybean have been shown to produce 72,000 seed plant⁻¹ (Davis and Johnson 2008). These seed all have a small achene and pappus which allow the seed to be readily wind-dispersed. The movement of horseweed seed has been

documented in several studies. It has been observed that seed released from taller horseweed plants travel farther than seed released from shorter plants (Dauer et al. 2006). Seed have been collected over 100 meters downwind from a mature plant located in corn (*Zea mays* L.) fields (Regehr and Bazzaz 1979). This illustrates horseweed's ability to move within fields and migrate into neighboring fields. Furthermore, experiments have found horseweed seed aloft in the planetary boundary layer (Shields et al. 2006). Environmental models used by these authors indicated that seed in the planetary boundary layer could potentially move 550 km in a single wind event prior to landing at a new location. This movement of horseweed seed, coupled with the fact that horseweed is primarily self-pollinated, allows the possibility of certain biotypes spreading long distances in only a few generations (Smisek 1995).

There are proactive measurements that growers can utilize to prevent the establishment of horseweed populations in their fields. Crop rotation can play a significant role in managing horseweed population levels. Late-season horseweed escapes are more frequent in fields with soybean-soybean crop rotation when compared to a corn-soybean rotation (Davis et al. 2009b). The wide selection of herbicides for corn weed control helps to manage horseweed population levels within a field in a corn-soybean rotation. Standing corn plants can also serve as a protective barrier from wind-blown seed originating from adjacent fields. Rotation is also important since horseweed emerges at greater numbers in soybean residue compared to residue from corn (Main et al. 2006). If corn is being planted into the soybean residue, the utility of corn herbicides can help eliminate those emerged plants and help reduce the number of seed in the seedbank. This is pertinent on the Delmarva Peninsula because many fields are planted to soybean every year. The reasons behind this lack of crop rotation are that many of the soils in the region are coarse-textured, drought-prone soils, and that soybean is a more drought-tolerant crop

than corn (VanGessel 2001). Combine the fact that many of the soybean fields in the region are no-tilled, conditions are ideal for horseweed population growth and distribution.

Growers that practice soybean-soybean rotations are more likely to find GR horseweed populations in their fields (Davis et al. 2009b). The authors speculated that this is most likely due to sole reliance on glyphosate for weed control. Davis et al. (2009a) found that weed control programs that utilized glyphosate as a burndown treatment followed by a POST application of glyphosate led to as high as 13.5 more horseweed plants m⁻² than programs that utilized a residual herbicide in the burndown program over a 2-year period. However, horseweed populations were denser in fields that utilized herbicides other than glyphosate for POST treatments due to poor control of both GR and glyphosate-susceptible (GS) horseweed plants by these alternative herbicides. When applied POST, glyphosate would still fully control the GS populations, thus decreasing overall horseweed density. This trend would likely be reversed over time as GR populations would increase and become dominant in those fields.

There have been documented cases of GR biotypes of horseweed that are more competitive than GS biotypes within the same field (Shrestha et al. 2010). In California, GR biotypes experienced earlier and more rapid stem elongation and the ability to reach maturity 3-4 weeks earlier than GS biotypes. Given that herbicides are generally less effective on larger horseweed plants, this gives the GR biotype a better chance at surviving a burndown application prior to crop planting. The GR biotype also accumulated more biomass whether precipitation was adequate for normal growth or under water-stress conditions. In a follow-up study, Alcorta et al. (2011) reported that when in competition with crops, the GR biotype grew taller than the GS biotype. The taller height in the GR biotypes would allow seed from those plants to spread further than seed from the GS biotype. These studies are unique in that the GR biotype in this

ecosystem showed no apparent fitness cost (the idea that if a plant allocates more resources towards defense mechanisms, fewer resources are left for growth and reproduction) for possessing the ability to survive glyphosate treatments (Bergelson and Purrington 1996; Alcorta et al. 2011). The ability of some GR horseweed populations to quickly grow and reproduce indicates that poor control of these populations will lead to widespread migration of GR biotypes.

Historical Control Measures

Horseweed is typically not a weed problem in conventionally tilled fields. A spring disking treatment has been shown to completely eliminate populations containing 15-50 cm tall horseweed plants (Brown and Whitwell 1988). Peak emergence of spring-germinating horseweed populations is typically in the months of April and May (Main et al. 2006). Delaying spring tillage until peak germination has served as a suitable means for controlling horseweed populations in conventionally tilled fields. Many Maryland farmers do not plant soybean until mid-to-late May, so the practice of disking a few days prior to planting has historically controlled horseweed populations in conventional plantings.

Conventional tillage is becoming a limited option for weed management in Maryland.

The state government has set goals to greatly increase no-till hectares within the state as part of its Chesapeake Bay Restoration Plan (Anonymous 2009). In 2011, 78.7% of Maryland soybean hectares were no-tilled, with an additional 13.8% in other conservation tillage practices.

Combined, conservation tillage accounted for 92.5% of Maryland soybean hectares (USDA NASS 2012). With many growers eliminating or severely restricting their conventional tillage regimen, herbicides are increasingly relied on to prepare the seedbed and control weeds. Several

predominantly used burndown programs have met variable success in fields where horseweed is the dominant weed.

Prior to the evolution and spread of GR horseweed, sole use of glyphosate was documented to provide complete control of horseweed prior to crop planting (Bruce and Kells 1990). A single application of paraquat [1,1'-dimethyl-4,4'-bipyridinium ion; (WSSA Group 22)] has shown variable control of horseweed plants prior to planting (Bruce and Kells 1990; Moseley and Hagood 1990). Paraquat + 2,4-D [(2,4-dichlorophenoxy)acetic acid; (WSSA Group 4)] has also shown inconsistent control, with 2,4-D alone outperforming the combination of the two herbicides in some studies (Moseley and Hagood 1990). Growth regulators like 2,4-D have not shown the consistency of control that glyphosate exhibits on GS horseweed plants. In fact, populations of horseweed have been identified that exhibit a 2-fold tolerance to 2,4-D and a 3-fold tolerance to dicamba [3,6-dichloro-2-methoxybenzoic acid; (WSSA Group 4); Kruger et al. 2010].

Glufosinate [2-amino-4-(hydroxymethylphosphinyl)butanoic acid; (WSSA Group 10)] is another herbicide used for burndown treatments prior to planting. While this herbicide has provided excellent control (>90%) of horseweed in Maryland (Ritter and Ikley 2009), previous research has shown poor control around soybean planting. Lack of efficacy from glufosinate has been attributed to low temperatures at time of application. Control of horseweed with glufosinate has been recorded as low as 33% for preplant applications (Owen et al. 2009). Wilson et al. (1985) found that low temperatures for 7 days after application (DAA) of glufosinate led to reduced horseweed control on the Delmarva Peninsula.

Cases of poor efficacy for these popular burndown applications necessitate the need for either new products or tank-mixes that provide better horseweed control. However, horseweed

populations have exhibited resistance to several modes of action currently on the market (Heap 2012).

History of Herbicide Resistance

The first reported case of horseweed showing resistance to herbicides was in 1980 when horseweed populations in Japan were shown to be resistant to the bipyridiliums (WSSA Group 22), paraquat and diquat [6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium ion], which inhibit photosystem I (Heap 2012). Resistance to this class of chemistry was first reported in North America (Canada) in 1993 (Heap 2012). Studies have shown that certain horseweed populations can withstand paraquat applications at rates over four times the recommended label rate and continue to grow and produce seed (Smisek et al. 1998). Populations showing resistance to paraquat were first reported in Delaware in 2003 (Heap 2012). Given horseweed seed's ability to migrate long distances, it is conceivable that several growers across the Delmarva Peninsula might already have the Group 22-resistant horseweed biotypes on their fields.

In 1981, horseweed populations in France were confirmed to have resistance to the triazine herbicides (WSSA Group 5) which inhibit photosystem II. In 2002, a population of horseweed was identified in the US (Michigan) that was resistant to the triazine herbicides and herbicides in WSSA Group 7 (the ureas and amides), which also inhibit photosystem II (Heap 2012). In 1993, horseweed were found in Israel that exhibited resistance to Acetolactate Synthase (ALS)-inhibiting herbicides (WSSA Group 2). It was not until 2001 that a population with such resistance was found in the US, in Ohio (Heap 2012). The last known group of herbicides that horseweed has evolved resistance to was the glycines (WSSA Group 9), in 2000, in the state of Delaware (VanGessel 2001). As of April 2012, 20 states and five different

countries have confirmed horseweed populations with resistance to Group 9 herbicides (Heap 2012). Populations in Ohio have shown cross-resistance to both glyphosate and ALS-inhibiting herbicides (Davis et al. 2009c). These populations are no longer controlled by the most effective POST herbicides on the market, leaving growers little control options after planting (Davis and Johnson 2008).

Glyphosate-Resistant Horseweed

With GR horseweed being widespread and difficult to control, several experiments have been conducted to try to understand the levels of resistance in GR biotypes. VanGessel et al. (2009) found that sensitivity of GR horseweed to glyphosate was dependent on growth stage. They found that horseweed in the seedling and rosette stages were more susceptible to glyphosate than plants in the bolting stage of growth. Sequential applications of glyphosate provided control to the point that no yield loss was observed in soybean; however, the surviving horseweed plants grew to maturity and produced seed. This reproduction event has implications for future management of horseweed populations. Feng et al. (2004) found that glyphosate resistance is inherited as a dominant trait. When they bred GS horseweed plants with GR horseweed plants, the progeny were also GR. Smisek (1995) reported that cross-breeding is limited among horseweed plants. Since glyphosate resistance is inherited as a dominant trait, the spread of GR biotypes can be enhanced when cross-breeding does occur.

Since the first reported case of GR horseweed, scientists have attempted to isolate the resistance mechanisms in horseweed populations. Feng et al. (2004) concluded that differential spray retention by the leaves and differential spray absorption did not contribute to glyphosate resistance. The authors did find that glyphosate was more readily translocated to the roots of

susceptible plants when compared to resistant plants. They also concluded that sensitivity of the leaf tissue to glyphosate was reduced in resistant plants, but they were unable to identify the mechanism for this reduced sensitivity. Previous research had found an increase in EPSP synthase concentration in GR horseweed plants, which allows the plant to sacrifice enough of the enzyme to metabolize glyphosate while continuing normal growth functions (Mueller et al. 2003). Based on shikimate accumulations in leaf tissue, the authors concluded that EPSP synthase was not insensitive to glyphosate, but that the overproduction of the enzyme allowed whole-plant resistance to occur.

Several scientists continued to focus on the translocation of glyphosate within horseweed plants. Findings showed that glyphosate was more readily translocated acropetally from the point of treatment rather than basipetally from leaf tissue to the roots in GR plants (Dinelli et al. 2006). This allowed existing leaves to receive most of the phytotoxic effect of the chemical while new leaves grew from the crown of the plant. More recently, Ge et al. (2010) discovered that glyphosate molecules were sequestered in the vacuoles of GR horseweed plants. They were able to track the glyphosate molecules inside the plant tissue as they were transferred to the vacuole. This provided the first clear evidence of a glyphosate-resistance mechanism in horseweed. This resistance mechanism proved to be temperature-dependent as glyphosate was more readily sequestered in the vacuole in warm temperatures. When glyphosate was applied to cold-acclimated GR horseweed, plant response was similar to that of GS biotypes (Ge et al. 2011). While this shows promise of controlling GR horseweed that emerge in autumn, spring emerging plants are often exposed to warm temperatures prior to herbicide application. The common practice of applying a burndown treatment a few days prior to soybean planting does not afford

the ability to spray all horseweed plants in cold temperatures to take full advantage of circumventing this GR mechanism.

Currently there are six different mechanisms of resistance to glyphosate known for all GR weed species. Four of these mechanisms have not been reported in GR horseweed. GR Italian ryegrass [Lolium perenne L. ssp. multiflorum (Lam.) Husnot] biotypes have shown reduced absorption of glyphosate when compared to GS biotypes (Nandula et al. 2008). A mutated EPSP synthase that is not inhibited by glyphosate has been found in some GR Italian ryegrass biotypes (Jasieniuk et al. 2008). Recently, sourgrass [Digitaria insularis (L.)] Mez ex Ekman) was reported to metabolize glyphosate as a resistance mechanism (De Carvalho et al. 2011). A novel mechanism of glyphosate resistance in giant ragweed (Ambrosia trifida L.) has been reported but is not fully understood. Foliar tissue that has been exposed to glyphosate exhibits rapid necrosis that inhibits translocation to untreated plant tissue (Gaines 2012).

Research has shown that independent evolution of resistance to glyphosate in horseweed is very likely and has happened on at least four separate occasions (Yuan et al. 2010). The authors examined the genetic profile of several GR horseweed populations from across the US and determined that at least four separate biotypes had never cross-bred, leading to speculation of multiple evolutionary events. This could explain how GR horseweed has been found across North America as well as on three other continents. However, regional expansion of GR populations cannot be ruled out. Growers need to be alert to the potential of horseweed escapes in adjacent fields as the seed from these plants can be readily established in neighboring fields. More consistent control measures are needed to assure farmers of adequate burndown control of horseweed prior to planting soybean.

Saflufenacil: a New Herbicide

Protoporphyrinogen Oxidase-Inhibiting Chemistry

Diphenylether herbicides were commercially released in the 1960's (Duke et al. 1991). This class of herbicides was the first with a new mode of action known as protoporphyrinogen oxidase (PPO)-inhibiting herbicides (WSSA Group 14). While many studies were conducted throughout the 1960's and the 1970's on this group of herbicide, it was not until the late 1980's that the exact mode of action was truly understood. Though herbicidal activity resulted in symptoms of rapid bleaching and desiccation, which are similar to paraquat and other bipyridiliums, it was discovered that photosynthesis was not necessary for this class of herbicides to control susceptible weed species, as is the case with the bipyridilium herbicides. Commonly used herbicides, such as lactofen [(±)-2-ethoxy-1-methyl-2-oxoethyl 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate] and acifluorfen [5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid], were soon found to inhibit protoporphyrinogen IX oxidase (Protox), the enzyme which converts protoporphyrinogen IX to protoporphyrin IX (Proto). Inhibiting this enzyme also inhibits the synthesis of both chlorophyll and heme. Heme acts as a regulator of the porphyrin pathway, so inhibiting its production leads to uncontrolled levels of Proto to accumulate in plant cells (Duke et al. 1991). High levels of Proto are dangerous since Proto generates singlet oxygens when exposed to light. When these singlet oxygens are concentrated in plant tissue, lipid peroxidation will initiate. Once lipids and proteins are oxidized, loss of chlorophyll and carotenoids results in leaky membranes and desiccated cells and organelles (WSSA 2007). Duke et al. (1991) first reported that high levels of Proto found in the leaves of susceptible plants were strongly correlated to the ensuing herbicidal damage.

PPO-inhibiting chemistry has been used with mixed success in soybean. Sulfentrazone [N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]phenyl]methanesulfonamide] and flumioxazin [2-[7-fluoro-3,4-dihydro-3-oxo-4-(2-propynyl)-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3(2H)-dione] are two PPO-inhibiting herbicides that have been evaluated in preemergence (PRE) applications in soybean. Niekamp et al. (1999) reported excellent control on many large-seeded broadleaf weeds with both herbicides. Others have found variable control levels with sulfentrazone on broadleaf weed species. However, when sulfentrazone was utilized as a PRE treatment prior to a POST application of glyphosate, weed control was excellent (Dirks et al. 2000). One issue that has been reported with PPO-herbicides is high levels of injury on soybean. Stand count reductions up to 73% and yield reductions of 50% have been reported when using these herbicides PRE at labeled rates (Taylor-Lovell et al. 2001).

Other PPO-inhibitors have been utilized mainly in POST programs in soybean.

Acifluorfen exhibits excellent control of morningglory species that are often difficult to control POST in soybean (Barker et al. 1984). Fomesafen [5-[2-chloro-4-(trifluoromethyl)phenoxy]-N-(methylsulfonyl)-2-nitrobenzamide] has shown excellent control of morningglory species, common ragweed (Ambrosia artemisiifolia L.), common lambsquarters, and jimsonweed (Datura stramonium L.); all of which are typically difficult to control POST in soybean (Bailey et al. 2003). Lactofen is another PPO-inhibitor that has shown higher levels of control on larger broadleaf weeds than either fomesafen or acifluorfen (Wesley and Shaw 1992). Although acifluorfen, lactofen, and fomesafen have exhibited greater control of broadleaf weeds in soybean compared to other POST herbicides, soybean injury is an issue with these products.

Studies found that injury ranged from 20-30% for acifluorfen and lactofen, while fomesafen injury was typically less than 10% (Higgins et al. 1988).

There are currently four different weed species resistant to PPO-inhibiting herbicides (Heap 2012). There are currently two known mechanisms of resistance to this mode of action. The ability to overexpress genes that lead to the formation of Protox has neutralized the herbicidal activity of acifluorfen (Lermontova and Grimm 2000). In that study, tobacco (*Nicotiana tabacum* L.) that was resistant to PPO-inhibiting herbicides had Protox concentrations five times higher than concentrations in tobacco plants that were susceptible to the herbicides. Recently, waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] populations in Illinois, Kansas, and Missouri have been identified that contain a mutation to the gene PPX2L that confers resistance to PPO-inhibiting herbicides in the Protox enzyme (Leet et al. 2008; Thinglum et al. 2011). Since horseweed has not developed resistance to this mode of action, a PPO-inhibiting herbicide that controls horseweed with no injurious effects on soybean would be a vital tool in controlling GR horseweed populations.

Saflufenacil Label

Until 2010, a new PPO-inhibiting herbicide had not been released in the 21st century in the US. That year the herbicide saflufenacil [N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)pyrimidinyl)benzoyl-N-isopropyl-N-methylsulfamide] was commercially released by BASF Crop Protection (Grossman et al. 2011). The herbicide is sold in several formulations, all under the brand name: KixorTM. The product is being marketed for burndown and residual PRE applications for broadleaf weed control in several crops including

soybean (Leibl et al. 2008). Grossman et al. (2010) confirmed that saflufenacil exhibits the same physiological changes in plants that are caused by other PPO-inhibiting herbicides.

Saflufenacil is currently offered under four product names in agronomic crops: Sharpen [saflufenacil], OpTill [saflufenacil + imazethapyr [2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid]; (WSSA Group 2)], Verdict [saflufenacil + dimethenamid-P [(RS) 2-chloro-N-(2,4-dimethyl-3-thienyl)-N-(2-methoxy-1methylethyl)acetamide]; (WSSA Group 15)], and OpTill Premium Residual Option (PRO) [OpTill co-packaged with dimethenamid-P]. Sharpen and Verdict labels support application in both corn and soybean, while OpTill and OpTill PRO are labeled for use in soybean only. All products are restricted to burndown and PRE applications. When used in a burndown treatment it is recommended to tank-mix saflufenacil with a methylated seed oil (MSO) at 1% volume-tovolume (v/v) plus ammonium sulfate (AMS) at 1-2% weight-to-volume (w/v) (Anonymous 2008). In soybean, saflufenacil should only be applied at a rate of 25 g ai ha⁻¹. This is equivalent to using Sharpen at 25 g ai ha⁻¹, OpTill at 95 g ai ha⁻¹, and Verdict at 245 g ai ha⁻¹. Application of saflufenacil is restricted to 30 days preplant (DPP) on coarse-textured soils with less than 2% organic matter (OM). In 2011, the Sharpen label was amended to allow an application rate of 37.5 g ai ha⁻¹ in soybean with an additional 14 DPP restriction that stacks on top of previous label restrictions (Anonymous 2012). BASF has claimed that the saflufenacil treatments are effective as PRE treatments prior to POST applications of glyphosate in a two-pass system (Westberg et al. 2008).

Utility of Saflufenacil

Several laboratory studies have been conducted to measure saflufenacil absorption and translocation in plants. Saflufenacil has been documented to have less than 20% of the chemical absorbed by plant foliar tissue when applied alone (Frihauf et al. 2010b). Absorption will exceed 80% when saflufenacil is tank-mixed with a surfactant (Ashigh and Hall 2010). Furthermore, saflufenacil absorption has been shown to increase when tank-mixed with a surfactant plus a formulation of glyphosate that includes its own surfactant. This increased absorption has been attributed to the high surfactant load. Saflufenacil tank-mixed with an unformulated glyphosate product plus a surfactant resulted in similar absorption as saflufenacil alone tank-mixed with a surfactant (Ashigh and Hall 2010). No studies have been published that evaluated the absorption of saflufenacil in plants when tank-mixed with both a MSO and AMS. These products are the recommended tank-mix partners for saflufenacil application (Anonymous 2008).

In translocation studies, saflufenacil has shown limited mobility in the phloem. This is uncharacteristic of other PPO-inhibiting herbicides (Grossman et al 2011). This movement is attributed to the weak-acid side chain of the molecule which is unique to saflufenacil compared to other PPO-inhibitors (Grossman et al. 2011). The majority of saflufenacil movement is in the xylem. This suggests that in burndown treatments, adequate spray coverage of the whole plant is necessary to ensure satisfactory weed control.

Several researchers have examined the utility of saflufenacil in burndown vs. PRE treatments. Saflufenacil is more readily absorbed by foliar tissue of plants rather than root tissue. Studies suggest that plants are 100-fold more sensitive to foliar-applied saflufenacil than having their roots exposed to the herbicide (Grossman et al. 2011). This preliminary study explains why

saflufenacil performs better in burndown applications rather than PRE applications under field conditions.

Saflufenacil exhibited rapid injury symptoms on susceptible plants in several field and laboratory experiments with injury symptoms becoming visible in less than 4 hours in most cases (Frihauf et al. 2010a). The enhanced weed control offered by foliar-applied saflufenacil has led to experiments where the chemical is applied POST to winter annual broadleaf weeds in winter wheat (*Triticum aestivum* L.) to reflect the current practice of tank-mixing herbicides with nitrogen upon wheat green-up in late winter. Good to excellent control of many winter annual broadleaf weeds was observed. However, injury to the wheat crop was deemed commercially unacceptable when compared to other herbicides currently on the market (Frihauf et al 2010a). These studies further point to saflufenacil being best utilized as a burndown or PRE treatment since unacceptable levels of injury can occur to crops if used in POST applications.

The majority of published saflufenacil studies have been conducted in corn. Corn has exhibited exceptional tolerance to saflufenacil. Corn is able to restrict saflufenacil translocation from treated leaves to other parts of the plant due to rapid metabolism of the herbicide into its initial metabolites, [uracil-ring-N-demethylated] and [side-chain-N-dealkylated metabolites] (Grossman et al. 2011). These metabolites contribute very little to Protox inhibition, further explaining crop safety. This rapid metabolism seems to be the best explanation of tolerance in plant species that exhibit reduced sensitivity to saflufenacil.

Moran et al. (2011a) found that safety of saflufenacil applications to corn was enhanced by tank-mixing sodium-bentazon [3-(1-methylethyl)-(1<u>H</u>)-2,1,3-benzothiadiazin-4(3<u>H</u>)-one 2,2-dioxide; (WSSA Group 6)] with the herbicide. The authors found that tank-mixing saflufenacil with sodium-bentazon resulted in half the crop injury than saflufenacil applied alone in 4-leaf

corn at a rate of 150 g ai ha⁻¹ (twice the labeled rate for corn). These tank-mixes have not been reported in other crops to evaluate potential enhanced-safety with sodium-bentazon or other safener products.

Research has shown that a PRE application of the product Verdict at 735 g ha⁻¹ (the labeled rate for corn) provided excellent control of many common broadleaf weeds found in corn. However, Verdict applied at 245 g ha⁻¹ (the labeled rate for soybean) did not control large-seeded broadleaf weeds at a commercially acceptable level when applied PRE (Moran et al. 2011b). This study shows that saflufenacil may not be effective as a PRE treatment in soybean to achieve control of problematic weed species.

Few papers have been published that evaluated the efficacy of saflufenacil on GR horseweed. Those that have looked at this issue have all involved horseweed populations that are established in cotton (*Gossypium hirsutum* L.). Owen et al. (2011) found that horseweed control exceeded 90% when saflufenacil was applied at 25 g ha⁻¹ between 21 and 0 DPP. This control exceeded that of two other PPO-inhibitors commonly used in cotton; flumioxazin and fomesafen. In unpublished data, the authors found residual control of horseweed with saflufenacil was shorter than other PPO-inhibitors. The authors felt that applications made prior to 21 DPP would result in horseweed escapes that would compete with the cotton crop. This supports the claim by Westberg et al. (2008) that saflufenacil mixtures are best utilized as PRE treatments for a preferred POST program.

Studies have also shown that when saflufenacil was applied at 25 g ha⁻¹ and tank-mixed with glyphosate, the efficacy on both GR and GS horseweed was improved when compared to either product applied alone (Mellendorf et al. 2008; Waggoner et al. 2011). Waggoner et al.

(2011) found that saflufenacil tank-mixed with glyphosate showed improved control of GR horseweed over other tank-mixes of glufosinate + saflufenacil or paraquat + saflufenacil.

There is currently only one published paper that evaluates the utility of saflufenacil in soybean. Soltani et al. (2010) evaluated saflufenacil applied PRE at rates of 100 and 200 g ha⁻¹. This study was conducted on soils that all contained greater than 3% OM. The highest rate of visual injury was 22% at the 200 g ha⁻¹ rate (which is eight times the labeled rate). This only resulted in a yield reduction of less than 5% compared to the weed-free control. However, soil types used in that study are not reflective of soils that are typically found on the Delmarva Peninsula. The purpose of this research was to evaluate the utility of saflufenacil on horseweed populations in Maryland and the effect of saflufenacil on soybean grown in coarse-textured soils with less than 2% OM, that are typically found in the coastal plains states.

Chapter 2. Greenhouse Experiments to Evaluate Saflufenacil Tank-Mix Efficacy on Horseweed

Introduction

Prior to saflufenacil's commercial release in 2010, BASF advertised the product's utility on GR weeds, particularly horseweed (Anonymous 2008). It was stated that the rapid plant death provided by saflufenacil results in complete burndown within 4 DAA. Independent studies have confirmed both the time and level of control stated in the technical brochure (Owen et al. 2011; Frihauf et al. 2010a). Saflufenacil was identified as a good tank-mix partner with glyphosate to enhance efficacy on other difficult to control weeds. Glyphosate increases control of grass species that saflufenacil does not easily control. The rapid burndown offered by saflufenacil tank-mixes provides growers the opportunity to plant sooner than other glyphosate-based burndown programs.

Saflufenacil is labeled for control of horseweed up to 15 cm tall. Horseweed height in the Mid-Atlantic often exceeds 30 cm at soybean planting (Moseley and Hagood 1990). The goal of this experiment was to apply saflufenacil tank-mixes to bolting horseweed at three different horseweed heights. However, after failure of the plants to bolt under greenhouse conditions, the experiment was amended to apply treatments to three different rosette sizes.

Materials and Methods

Seed Collection

Seed were collected August 2010 and August 2011, when horseweed plants reached maturity, from two different horseweed populations in Maryland. Seed were collected from a confirmed GR population at the Wye Research and Education Center (WREC) located in Queenstown, MD. Seed were also collected from a confirmed GS population at the Central Maryland Research and Education Center (CMREC) located in Beltsville, MD. Seed were stored in a dark freezer at 0° C until their use in the experiments.

Plant Growth and Greenhouse Conditions

Seed were placed on the surface of a commercial potting mix (Sunshine Container Potting Mix: 75-85% Canadian Sphagnum Peat Moss; 15-25% perlite, dolomite, and limestone) in 6-cell packs measuring 4.5-cm x 6.75-cm x 5-cm. The cell packs were placed in a misting room where day/night temperatures were 25° C/21° C with a 13-hour photoperiod supplemented by high-pressure sodium lamps. Irrigation was supplied by overhead misting nozzles that simulate light rainfall conditions. After 4 weeks of growth, individual cells were transplanted into 15-cm diameter x 10.5-cm pots and thinned to three plants pot⁻¹. The pots were transferred to a new room where temperature and photoperiod remained the same as previously described but irrigation was now supplied by an automated drip-tape system which irrigated twice daily. This process was repeated twice at 2-week intervals to establish three planting dates. Plants remained in the greenhouse for an additional 8, 10, and 12 weeks based on planting date to allow the plants to reach three different rosette sizes.

Treatments and Timings

The experiment had five treatments established in a randomized complete block (RCB) split-plot design with three treatment replications within blocks. The whole plot examined four different herbicide treatments, along with an untreated check. The split-plot factor in the experiment was horseweed biotype (GR vs. GS). Plants were blocked according to the size of horseweed rosettes.

Horseweed plants were grown to three different rosette sizes categorized as:

- (i) Large at 8.25 cm tall
- (ii) Medium at 4.5 cm tall
- (iii) Small at 2 cm tall

The five treatments were:

- (i) Untreated control
- (ii) Potassium salt of glyphosate (formulated as Roundup PowerMAX) at 874 g acid equivalent (ae) ha⁻¹ + MSO at 1% v/v + AMS at 2% w/v
- (iii) Saflufenacil at 25 g ai ha⁻¹ + MSO at 1% v/v + AMS at 2% w/v
- (iv) Saflufenacil at 25 g ai ha⁻¹ + glyphosate at 874 g ae ha⁻¹ + MSO at 1% v/v + AMS at 2% w/v
- (v) Pre-packaged mix of Saflufenacil + imazethapyr at 95 g ai ha $^{-1}$ + MSO at 1% v/v + AMS at 2% w/v

Plants were moved outside the greenhouse for treatment application. Applications were made with a handheld CO₂-pressurized backpack sprayer with six TeeJet SS8004 nozzles (Spraying Systems Co., Wheaton, IL) spaced 51 cm apart. Applications were made with a carrying volume of 168 Liters ha⁻¹ (L ha⁻¹), at a pressure of 138 kilopascals (kPa), with a travel

speed of 4.8 kilometers hour⁻¹ (kph). The boom was held 51 cm over horseweed canopy. After application, pots were returned to the greenhouse and reattached to the irrigation system where the plants remained for 4 weeks.

Control Ratings

Treatment effect was measured using a visual control rating on a scale of 0 (no control) to 100 (complete plant death). Visual ratings were taken at intervals of 7, 14, and 28 DAA. After the final visual assessment at 28 DAA, plants were clipped at the soil surface and fresh weight measurements were taken. Fresh weight was measured for all plants in a pot then divided by the number of plants pot⁻¹ to get average fresh weight plant⁻¹. After fresh weights were measured, plants were dried in a VWR forced air dryer (VWR International, Radnor, PA) at 35°C over a 3-day period. After that period, plant dry weight was measured and calculated as average weight plant⁻¹. The study was repeated once.

Data Analysis

Data were subjected to the MIXED procedure of the Statistical Analysis Software (SAS) 9.2 software (SAS Institute, Cary, NC). Treatments were treated as fixed effects while both block and treatment x block interaction were treated as random effects. Fisher's Least Significant Differences (LSD) were calculated at the 0.05 level to compare means when overall F-test was significant. Data from both studies were pooled due to no interaction between them. There was a significant difference between GR and GS biotypes for all data collected, so GR and GS biotype analyses were performed separately.

Results and Discussion

Visual Rating

For both the GR and GS horseweed biotypes, the overall F-Test was significant at the 0.05 level for visual rating at all three rating intervals. There was no interaction between blocks, so all data were pooled. For the 7 and 14 DAA ratings in the GR biotype, the treatment containing saflufenacil + glyphosate provided higher control than all other treatments (Table 2.1). At 28 DAA, there was no longer a difference between saflufenacil + glyphosate and glyphosate. This was due to regrowth from the crown that was observed between 14 and 28 DAA in the saflufenacil + glyphosate treatment.

At 7 DAA, saflufenacil provided better control of GR horseweed than glyphosate (Table 2.1). This is further evidence for the rapid action of saflufenacil within plants. In ratings taken following the 7 DAA rating, the control level of the saflufenacil-only treatment and the pre-packaged mix of saflufenacil + imazethapyr declined. This decline in control was due to extensive regrowth by plants that received these two treatments. By 28 DAA, all treatments containing saflufenacil experienced regrowth. This regrowth is likely due to the fact that only the apical meristem and existing leaves came in contact with the herbicide. For plants in the rosette form, the apical meristem protects the other growing points from dangers that can affect the exposed plant tissue (Gurevitch et al. 2006). These other meristem tissues are generally inactive due to hormones produced by the apical meristem that inhibit proliferation of these cells. This is known as apical dominance (Gurevitch et al. 2006). If the apical meristem is injured, apical dominance is broken, and the plant induces hormones that allow the axillary meristem tissue to rapidly produce new foliage. Since saflufenacil translocation is mainly restricted to the xylem in plants, the herbicide likely did not translocate downward past the apical meristem and toward the

roots. This suggests that after saflufenacil broke apical dominance, the protected meristem tissue was able to initiate rapid regrowth.

In contrast, the glyphosate treatment never experienced regrowth in either biotype (Tables 2.1 and 2.2). This can be attributed to glyphosate's slow mode of action which allows the herbicide to translocate to most parts of the plant before plant injury reaches levels that inhibit translocation (Grossman et al. 2011).

In the GS biotype, there was no regrowth from any treatment until after the 14 DAA rating (Table 2.2). As expected, glyphosate alone provided much higher control when compared to the GR biotype (Tables 2.1 and 2.2). The most noteworthy difference between treatments in the GS biotype is the level of control between glyphosate and saflufenacil + glyphosate at 7 and 14 DAA. At 7 DAA, the tank-mix of saflufenacil + glyphosate provided better control of GS horseweed when compared to glyphosate alone (Table 2.2). By 14 DAA, the control offered by both treatments was comparable. This reinforces the hypothesis that saflufenacil tank-mixes provide more rapid burndown of plants than glyphosate alone.

Plant Weights

For the GR horseweed biotype, the overall F-test was not significant for both fresh and dry plant weight. Even when using orthogonal contrasts to compare means for both fresh and dry weight, only saflufenacil + glyphosate was different from the untreated control (Table 2.3). As expected, glyphosate did not reduce fresh or dry weight. Saflufenacil and saflufenacil + imazethapyr did not reduce horseweed fresh or dry weight (Table 2.3). This can be attributed to the rapid regrowth by the plants. There were no differences between treatments for both fresh and dry weights of GR horseweed plants (Table 2.3). That only saflufenacil + glyphosate

reduced plant weight compared to the untreated check confirms previous reports of more effective horseweed control when tank-mixing the two herbicides than with either product applied alone (Mellendorf et al. 2008). This is unique from previous reports that tank-mixes of glyphosate + PPO-inhibitors cause reduced efficacy for both herbicides (Ashigh and Hall 2010). Starke and Oliver (1998) speculated that rapid necrosis by fomesafen and sulfentrazone inhibited the ability of glyphosate to translocate, causing reduced efficacy. Compared to other PPO-inhibiting herbicides, saflufenacil inhibits Protox slowly. This allows both saflufenacil and glyphosate to translocate prior to tissue destruction, explaining the synergism between the herbicides (Grossman et al. 2011).

For the GS biotype, the overall F-test was significant for both the fresh and dry weights (Table 2.4). Both treatments containing glyphosate reduced GS horseweed fresh and dry weight 80% and 72%, respectively (Table 2.4). As with the GR biotype, neither saflufenacil nor saflufenacil + imazethapyr reduced horseweed fresh or dry weight. This is attributed to plant regrowth. Both treatments containing glyphosate resulted in a reduction in fresh and dry weight compared to the treatments without glyphosate. These data confirm that despite the rapid activity of saflufenacil, there is no antagonism between saflufenacil and glyphosate for controlling GS horseweed.

Control of both GR and GS horseweed with saflufenacil in this study was inconsistent with control data from field trials (Owen et al. 2011; Waggoner et al 2011). It is worth noting that the rosette sizes achieved in these experiments are much larger than those found under field conditions (Davis and Johnson 2008). The conditions in the greenhouse allowed the plants to grow in an ideal environment, with no competition and without stresses typically found in the field. This potentially allowed the rosettes to grow to larger sizes without triggering internode

elongation. The bolting requirements of horseweed are still largely unknown (Davis et al. 2009c). The size of these horseweed plants suggests large stores of carbohydrates which could allow the rapid regrowth observed following the breaking of apical dominance.

Table 2.1. Glyphosate-resistant (GR) horseweed control^a 7, 14, and 28 days after application (DAA) in greenhouse studies.

Treatment	Rate ^b	7 DAA	14 DAA	28 DAA
	g ha ⁻¹		<u></u> %	
Untreated	0	0	0	0
Glyphosate	874	11	35	37
Saflufenacil	25	35	32	20
Saflufenacil + glyphosate	25 + 874	61	67	57
Saflufenacil + imazethapyr ^c	95	24	21	17
$LSD_{0.05}$		23	25	25

^a Control is expressed on a scale of 0 (no control) to 100 (complete control).

^b Rates for glyphosate are expressed in ae whereas all other herbicides are expressed in ai.

^c Pre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

Table 2.2. Glyphosate-susceptible (GS) horseweed control^a 7, 14, and 28 days after application (DAA) in greenhouse studies.

Treatment	Rate ^b	7 DAA	14 DAA	28 DAA
	g ha ⁻¹		% ———	
Untreated	0	0	0	0
Glyphosate	874	33	86	88
Saflufenacil	25	31	36	23
Saflufenacil + glyphosate	25 + 874	77	88	81
Saflufenacil + imazethapyr ^c	95	33	38	27
$LSD_{0.05}$		17	18	17

^a Control is expressed on a scale of 0 (no control) to 100 (complete control).

^b Rates for glyphosate are expressed in ae whereas all other herbicides are expressed in ai.

^c Pre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

Table 2.3. Fresh and dry weights of glyphosate-resistant (GR) horseweed in greenhouse studies.^a

Treatment	Rate ^b	Fresh weight	Fresh weight	Dry weight	Dry weight
	g	mg	% untreated ^c	mg	% untreated
	ha ⁻¹	plant ⁻¹		plant ⁻¹	
Untreated	0	4713.9	100	745.0	100
Glyphosate	874	3230.0	69	571.1	77
Saflufenacil	25	3367.2	72	617.2	83
Saflufenacil +	25 +	2284.5	48	419.9	56
glyphosate	874				
Saflufenacil + imazethapyr ^d	95	3792.2	80	596.1	67
LSD _{0.05} ^e		1871.7	40	311.8	42

^a Weights were taken 4 weeks after application.

^b Rates for glyphosate are expressed in ae whereas all other herbicides are expressed in ai.

^c Weights expressed as percent weight of the untreated check.

^d Pre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

^e Means compared using orthogonal contrasts.

Table 2.4. Fresh and dry weights of glyphosate-susceptible (GS) horseweed in greenhouse studies.a

Treatment	Rate ^b	Fresh weight	Fresh weight	Dry weight	Dry weight
	g ha ⁻¹	mg plant ⁻¹	% untreated ^c	mg plant ⁻¹	% untreated
Untreated	0	4359.5	100	689.2	100
Glyphosate	874	868.3	20	194.4	28
Saflufenacil	25	4118.9	94	546.8	79
Saflufenacil + glyphosate	25 + 874	867.2	20	195.0	28
Saflufenacil + imazethapyr ^d	95	3406.1	78	551.1	80
$LSD_{0.05}$		2222.3	51	282.9	41

 ^a Weights were taken 4 weeks after application.
 ^b Rates for glyphosate are expressed in ae whereas all other herbicides are expressed in ai.
 ^c Weights expressed as percent weight of the untreated check.
 ^d Pre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

Chapter 3. Field Experiments to Evaluate the Effect of Saflufenacil Application Timing and Rates on Soybean

Introduction

Due to sensitivity concerns expressed by BASF, saflufenacil applications in soybean are restricted to 30 DPP on coarse-textured soils with less than 2% OM (Anonymous 2012). The company conducted in-house screening programs to examine the effect of the herbicide on soybean plants. During screening, many soybean varieties were found to be tolerant to the herbicide regardless of soil type. However, some soybean varieties were found to be sensitive to herbicide applications made within 30 DPP on coarse-textured soils that contained less than 2% OM. When the herbicide was commercially released in 2010, BASF had a master list of tolerant and sensitive soybean varieties (BASF personal communications). Rather than allocate resources to an extensive screening program to update the list to reflect new varieties constantly entering the soybean market, BASF decided to restrict application intervals on coarse-textured soils containing less than 2% OM.

The 30 DPP restriction interval adversely affects Maryland growers for a number of reasons. Previous research has shown that saflufenacil residual can start to break down after 21 days (Owen et al. 2011). Without appropriate tank-mix partners, this leads to a probability of broadleaf weeds becoming reestablished in the field up to 1 week prior to crop planting. This defeats the purpose of a good burndown program. More worrisome is the probability of more GR horseweed plants becoming established in the crop. Many of the best in-crop herbicides for controlling GR horseweed are ALS-inhibiting herbicides (Kruger et al. 2009). This would lead to growers likely utilizing ALS-inhibiting herbicides as a rescue treatment; exposing the horseweed population to selection pressure from this group of herbicides. Kruger et al. (2009) documented

populations of horseweed in Ohio that are cross-resistant to glyphosate and ALS-inhibiting herbicides. Similar populations have been confirmed on the Delmarva Peninsula (R. L. Ritter personal communication). The ability to apply saflufenacil 0 DPP would help reduce the number of applications containing ALS-inhibiting herbicides to alleviate selection pressure for evolution of ALS-resistant horseweed.

Given the well-established GR horseweed populations on the Delmarva Peninsula that can germinate through the month of May, combined with the coarse-textured soils that contain less than 2% OM, further investigations into the effect of saflufenacil on soybean would be beneficial. This experiment was designed to evaluate both sensitive and tolerant soybean variety performance on both a coarse-textured and medium-textured soil when subjected to saflufenacil burndown programs within the 30 DPP label restriction.

Materials and Methods

Sites

Experiments were conducted in 2010 and 2011 at the Wye Research and Education Center (WREC) located in Queenstown, MD. The site was chosen for its Mattapex-Butlertown silt loam soil [fine-loamy, 5.9 cation exchange capacity (CEC), 2.0% OM]. Experiments were also conducted in 2010 and 2011 at the Central Maryland Research and Education Center (CMREC) located in Beltsville, MD. This site was chosen for its Evesboro-Downer complex soil (loamy-sand, 4.8 CEC, 1.3% OM).

Soybean Varieties

Both saflufenacil -sensitive and -tolerant varieties were chosen for each location to match maturity groups commonly grown in the region. At WREC, Pioneer 94M80 (Pioneer Hi-Bred, Johnston, IA) was the sensitive variety while Asgrow 4703 (Monsanto Company, St. Louis, MO) was the tolerant variety. At CMREC, Pioneer 93Y80 was the sensitive variety while Pioneer 93Y70 was the tolerant variety.

Treatments and Management

Experiments contained four herbicide treatments each applied to independent plots at 30, 15, and 0 DPP; resulting in 12 independent treatments. Experiments used a Randomized Complete Block design with three replications.

The herbicide treatments were:

- (i) Potassium salt of glyphosate (formulated as Roundup PowerMAX) at 874 g ae ha $^{-1}$ + MSO at 1% v/v + AMS at 2% w/v
- (ii) Glyphosate at 874 g ae ha⁻¹ + saflufenacil at 25 g ai ha⁻¹ + MSO at 1% v/v + AMS at 2% w/v
- (iii) Glyphosate at 874 g ae ha $^{-1}$ + saflufenacil at 50 g ai ha $^{-1}$ + MSO at 1% v/v + AMS at 2% w/v
- (iv) Glyphosate at 874 g ae ha $^{-1}$ + the pre-packaged mix of saflufenacil + imazethapyr at 95 g ai ha $^{-1}$ + MSO at 1% v/v + AMS at 2% w/v

Plots measured 3.05 m wide x 6.1 m long. Applications were made with a handheld CO₂-pressurized backpack sprayer with six TeeJet SS8004 nozzles spaced 51 cm apart. Applications

were made with a carrying volume of 168 L ha⁻¹, at a pressure of 138 kPa, with a travel speed of 4.8 kph. The boom was held 51 cm over the canopy of existing foliage.

Experiments at WREC were planted 7 June 2010 and 6 June 2011 at a rate of 520,000 seed ha⁻¹. However, in 2011 the tolerant variety at WREC was inadvertently planted at a rate of 780,000 seed ha⁻¹. Studies at CMREC were planted 1 June 2010 and 31 May 2011 at a rate of 407,000 seed ha⁻¹. All studies were planted 3 cm deep with a Great Plains Solid Stand 10 no-till drill (Great Plains Mfg. Inc., Salina, KS) with 19-cm row spacing. At 4 weeks after planting (WAP), all treatments received an in-crop application of glyphosate at 874 g ae ha⁻¹ + MSO at 1% v/v + AMS at 2% w/v.

Experiments at WREC were harvested on 12 November 2010 and 8 November 2011 using a Massey Ferguson 540 combine (AGCO, Duluth, GA) equipped with a Weigh-Tronix 1080 electronic grain scale (Avery Weigh-Tronix, Fairmont, MN) to measure grain weight plot⁻¹. Experiments at CMREC were harvested on 8 November 2010 and 11 November 2011 using a John Deere 4400 combine (Deere & Company, Moline, IL) equipped with a HarvestMaster HM-401 harvest system (Juniper Systems Inc., Logan, UT) to measure grain weight plot⁻¹. Seed moisture at harvest was measured for all experiments using a DICKEY-john GAC 2100 moisture sensor (Churchill Industries, Minneapolis, MN). Yields were calculated to kg ha⁻¹ and adjusted to 13.0% grain moisture.

Stand Counts and Height Measurements

At 4 WAP, stand counts were taken for each treatment. Four rows plot⁻¹ were randomly selected and plants per 3.05 m of row were counted. These numbers were averaged and calculated to the number of plants ha⁻¹.

After stand counts were taken, six plants plot⁻¹ were randomly selected and flagged. The height of these plants was measured at 4, 7, and 10 WAP. Height measurements were taken from the soil surface to the top of the plant. In 2011, soybean growth stage was also noted when height measurements were taken.

Yield Components

Prior to harvest the individually marked plants were clipped at the soil surface and stored until three yield component factors could be analyzed.

The factors were:

- (i) Number of pods plant⁻¹
- (ii) Number of seed pod⁻¹
- (iii) Average weight seed⁻¹

After counting the number of pods plant⁻¹, seed were threshed using a Swanson Plot Thresher (Swanson Machine Co., Champaign, IL). Total seed plant⁻¹ was counted using a model 750-2 electronic seed counter (International Marketing and Design Co., San Antonio, TX). Total seed plant⁻¹ was used to calculate the number of seed pod⁻¹. The total weight of seed plant⁻¹ was measured and used to calculate weight seed⁻¹. The three yield components were combined to calculate the total yield plant⁻¹.

Data Analysis

Data were analyzed as a RCB design using the MIXED procedure in SAS. When no significant interaction between years was detected, data were combined. Year, block within year,

and treatment x block interactions were treated as random effects. Treatment was classified as a fixed effect. Sites were analyzed separately due to differences in soil types and varieties grown. At CMREC, there were some significant interactions between soybean varieties, so varieties were analyzed separately. At WREC, there were no significant interactions between soybean varieties and herbicide treatments; however, varieties were analyzed separately for clarity. Fisher's protected LSD was calculated at the 0.05 level for mean comparisons when significance of the overall F test was present. Pre-planned orthogonal contrasts were used to compare treatments within their respective application intervals.

Results and Discussion

Stand Count

There were no differences in the 2-year average stand count for the tolerant varieties at both locations as well as the sensitive variety at WREC for all application timings (Tables 3.5, 3.6, and 3.7). For the sensitive variety at CMREC, there were no differences for treatments applied 30 DPP. The glyphosate treatment had fewer plants ha⁻¹ than the treatment containing saflufenacil at 50 g ha⁻¹ when applied 15 DPP (Table 3.8). The 25 g ha⁻¹ treatment had a lower, but not significantly different stand count than the treatment containing saflufenacil at 50 g ha⁻¹ when applied 15 DPP (p=0.0940; Table 3.8). These data show that saflufenacil applied at twice the labeled rate did not reduce stand count when applied 15 DPP, which is within the 30 DPP label restriction.

There were no differences in stand count among 0 DPP treatments for the sensitive variety at CMREC (Table 3.8). However, the weather conditions necessary for herbicide activation varied greatly in 2010 and 2011. In 2010, CMREC received adequate rainfall for activation within 5 days after planting (DAP; Table 3.3). This rainfall event happened prior to

soybean emergence. The overall F test for stand counts was significant for the sensitive variety at CMREC in 2010, so means were separated using Fisher's protected LSD. For the 0 DPP timing in 2010, both saflufenacil at 25 g ha⁻¹ and saflufenacil at 50 g ha⁻¹ treatments caused a reduction in plants ha⁻¹ when measured 4 WAP (Table 3.13). In 2011, the first rainfall event at CMREC occurred 10 DAP (Table 3.4). The first rain event that provided adequate rainfall for herbicide activation took place 6 days later at 16 DAP (Table 3.4). The plants were in the V2-V3 stage of growth at the time of that rainfall. When stand counts were taken 4 WAP in 2011, there were no differences among herbicide treatments (Table 3.13). Thus, reduction in stand count was lessened when the PRE applications of saflufenacil were activated after soybean emergence.

Grossman et al. (2011) found saflufenacil in higher concentrations within plant tissue when the herbicide came in contact with the cotyledons, rather than the root tissue, of broadleaf plants. The authors speculated that foliar absorption of saflufenacil in seedlings led to higher plant injury than root-absorbed saflufenacil. Based on this research, the stand count data suggest that in 2010 the soybean cotyledons were exposed to saflufenacil prior to crop emergence, leading to higher rates of injury. In 2011, only the roots were exposed to the herbicide treatments. The plants were in the V2-V3 stage of growth at time of exposure. It seems the larger soybean plants were able to tolerate the 0 DPP treatments.

Plant Height

There were no differences in height during any measurement interval for both tolerant varieties (Tables 3.9 and 3.11). For the sensitive variety at CMREC, there were no differences among treatments applied 0 DPP (Table 3.12). However, there was a trend that plants exposed to saflufenacil at 50 g ha⁻¹ at 0 DPP were shorter at the 4 and 7 WAP measurements when the

plants were in the V3-V4 and R1-R2 growth stages, respectively. Though not significantly different, at 7 WAP, saflufenacil at 50 g ha⁻¹ caused plants to be 5 cm shorter than all other treatments (Table 3.12).

At WREC at 4 WAP, the plants treated with saflufenacil at 50 g ha⁻¹ were shorter than the plants treated with either saflufenacil at 25 g ha⁻¹ or glyphosate when applied 0 DPP to the sensitive variety (Table 3.10). At the time of measurement, the soybean plants were in the V3-V4 growth stage. Though there were no significant differences, the plants treated with saflufenacil at 50 g ha⁻¹ at 0 DPP were shorter 7 WAP (R1-R2 growth stage) than plants receiving other treatments at 0 DPP. By 10 WAP, when the R3-R4 growth stages had been reached, there were no differences or trends in the data, indicating that the crop outgrew initial stunting. Thus, there can be slight levels of stunting in the vegetative stages of soybean growth in sensitive varieties on both coarse-textured and medium-textured soils but this injury may dissipate as the plants mature.

Soybean Yield and Yield Components

There were no differences in yield or yield components for all application dates for the tolerant variety at WREC (Table 3.5). For the sensitive variety at WREC, there were no differences in yield or yield components for treatments applied 30 DPP (Table 3.6). For treatments applied 15 DPP, there were differences in yield components. The treatment containing saflufenacil at 50 g ha⁻¹ yielded over 10 pods plant⁻¹ greater than other treatments. This resulted in over 4 g plant⁻¹ yield difference between saflufenacil at 50 g ha⁻¹ and other treatments applied 15 DPP despite there being no differences for beans pod⁻¹ or weight seed⁻¹. Though weed control was not evaluated in these experiments, there were morningglory plants present at 4 WAP (when

glyphosate was applied to eliminate weed escapes) that could have affected yield plant⁻¹. The differences in yield plant⁻¹ for saflufenacil at 50 g ha⁻¹ could be attributed to extended residual control offered by the higher rate of saflufenacil (Davis et al. 2010). There were yield no differences between treatments applied 15 DPP. There were no differences in yield or yield components for treatments applied 0 DPP to the sensitive variety at WREC (Table 3.6).

There were no differences in yield for the tolerant variety at CMREC (Table 3.7). Though not significant, yield was lower for saflufenacil at 50 g ha⁻¹ than saflufenacil + imazethapyr when applied 0 DPP (p=0.0791). Saflufenacil applied at 50 g ha⁻¹ resulted in lower, though not significantly different, yield plant⁻¹ than saflufenacil + imazethapyr when applied 0 DPP (p=0.1360). These data suggest that when exposed to high rates of saflufenacil 0 DPP on coarsetextured soils, this variety could experience yield reductions.

There were yield differences in the sensitive variety grown at CMREC (Table 3.8). For the 30 DPP treatments, the treatment containing saflufenacil + imazethapyr yielded more kg grain ha⁻¹ than the glyphosate treatment (Table 3.8). Though weed control was not measured in the field experiments, yellow foxtail [*Setaria pumila* (Poir.) Roemer & J.A. Schultes] plants were present at planting for 30 DPP treatments that did not receive imazethapyr. The yield differences were likely due to the extended residual weed control offered by the combination of saflufenacil and imazethapyr (Westberg et al. 2008).

Saflufenacil applied at 50 g ha⁻¹ at 15 DPP to the sensitive variety at CMREC resulted in fewer pods plant⁻¹ than the glyphosate treatment (Table 3.8). Since there were no differences in beans pod⁻¹ or seed weight, the reason saflufenacil at 50 g ha⁻¹ caused lower yield plant⁻¹ than the glyphosate treatment is attributed to reduced pod count (Table 3.8). This difference in yield plant⁻¹ could be attributed to the reduction in stand count for glyphosate compared to saflufenacil

at 50 g ha⁻¹ when applied 15 DPP (Table 3.8). Weber et al. (1966) found that soybean plants grown in lower densities yield more pods and more grain plant⁻¹ than plants grown in higher populations. Though not significantly different, saflufenacil + imazethapyr caused lower yield plant⁻¹ than glyphosate when applied 15 DPP (p=0.1028). The plant yield data for treatments applied 15 DPP seem to support the current 30 DPP restriction on the saflufenacil label.

For the 0 DPP applications to the sensitive variety at CMREC, saflufenacil at 50 g ha⁻¹ yielded more pods plant⁻¹ than glyphosate (Table 3.8). There were no differences among the treatments for other yield components (Table 3.8). Though not significantly different, saflufenacil at 50 g ha⁻¹ did produce a higher yield plant⁻¹ than glyphosate when applied 0 DPP (p=0.0898). The increase in yield plant⁻¹ for saflufenacil at 50 g ha⁻¹ could be attributed to the reduced, though not significantly different, plant population when applied 0 DPP.

In contrast to the plant yield, the treatment containing 50 g ha⁻¹ of saflufenacil yielded fewer kg ha⁻¹ than both glyphosate and saflufenacil + imazethapyr (Table 3.8). This reduction in yield is present despite the different climatic conditions observed around the planting date in 2010 and 2011 (Tables 3.3 and 3.4). When analyzed by year, there are no significant differences, but yield losses follow the same trend where saflufenacil at 50 g ha⁻¹ yielded fewer kg ha⁻¹ than the saflufenacil + imazethapyr (p=0.0642 in 2010; p=0.1758 in 2011) and glyphosate (p=0.0939 in 2010; p=0.1279 in 2011) treatments applied at the same time (Table 3.13). For both 2010 and 2011, yield components follow the trend in the 2-year average where, while not significant, saflufenacil at 50 g ha⁻¹ had a higher yield plant⁻¹ than other treatments. In 2011, the only difference in yield components was that saflufenacil at 50 g ha⁻¹ yielded more pods plant⁻¹ than glyphosate when applied 0 DPP (Table 3.13).

In 2010, the increase in yield plant⁻¹ in plots treated with saflufenacil at 50 g ha⁻¹ could be attributed to reduced stand counts (Table 3.13). The reduction in yield in kg ha⁻¹ suggests that the increase in yield plant⁻¹ did not compensate for the reduced population. However, in 2011, there were no differences or patterns in stand count or any of the yield components that can explain the reduced, though not significant, yields for saflufenacil at 50 g ha⁻¹ (Table 3.13).

Table 3.1. Date of treatment application, planting, and precipitation at the Wye Research and Education Center (WREC) located in Queenstown, MD in 2010.

		WREC	2010	
	May		June	
	Treatment application	Rainfall	Treatment application ^a	Rainfall
Date		(mm)		(mm)
1		0		0
2		0		25.40
3		0		0
4	30 DPP ^b	1.52		6.60
5		0		0
6		0		0
7		0	Plant	2.54
8		0	0 DPP	0
9		0		1.27
10		0		0
11		0		0
12		2.54		0
13		10.67		0
14		0		0
15		0.51		0
16		0		5.84
17		0		0
18		12.70		0
19		1.78		0
20	15 DPP	0		0
21		0		0
22		0		13.21
23		0.51		0
24		0		2.79
25		0		0
26		0		0
27		0		0
28		11.18		10.16
29		0		0
30		0		0
31		0		N/A

^a POST applications made 1 July 2010. ^b Abbreviations: DPP, days preplant; POST, postemergence.

Table 3.2. Date of treatment application, planting, and precipitation at the Wye Research and Education Center (WREC) located in Queenstown, MD in 2011.

		WREC 2	2011	
	May		June	
	Treatment application	Rainfall	Treatment application	Rainfall
Date		(mm)		(mm)
1		1.78		0.51
2		0		0
3		0		0
4		21.84		0
5		0		2.54
6		0	Plant + 0 DPP	5.84
7		0		0
8		0		0
9	30 DPP ^a	0		0
10		0		20.83
11		0		0
12		0		14.22
13		0		0
14		27.69		0
15		1.02		0
16		0		2.03
17		0.51		0
18		4.57		0.51
19		9.65		0
20		0		6.60
21		0		3.81
22		0	POST	0.25
23		0.51		0
	15 DPP	0		0
25		0		0
26		0		0
27		0		0
28		0		3.81
29		0		0
30		0		0
31		0		N/A

^a Abbreviations: DPP, days preplant; POST, postemergence.

Table 3.3. Date of treatment application, planting, and precipitation at the Central Maryland Research and Education Center (CMREC) located in Beltsville, MD in 2010.

		CMREC	2010	
	May		June	
	Treatment application	Rainfall	Treatment application	Rainfall
Date		(mm)		(mm)
1		0	Plant + 0 DPP	0
2		0.25		0
3		14.48		1.78
4		0		0.25
5	30 DPP ^a	0		0
6		0		9.40
7		0		0
3		0		0
•		0		2.54
10		0		0
11		3.56		0
12		34.80		0
13		0		0
14		3.30		0
15		0		0
16		0		11.78
17		8.89		0.25
18		8.38		0
19	15 DPP	0.25		0
20		0		0
21		0	POST	0
22		0.25		4.57
23		23.88		0.25
24		0		0
25		0.51		0
26		0		0
27		7.62		0
28		2.03		25.65
29		0		0
30		0		0
31		0		N/A

^a Abbreviations: DPP, days preplant; POST, postemergence.

Table 3.4. Date of treatment application, planting, and precipitation at the Central Maryland Research and Education Center (CMREC) located in Beltsville, MD in 2011.

		CMREC	2011	
	May		June	
	Treatment application	Rainfall	Treatment application	Rainfall
Date		(mm)		(mm)
1		1.27		0
2	30 DPP ^a	0		0
3		1.02		0
4		16.26		0
5		0		0
6		0.51		0
7		0.25		0
8		0		0
9		0		0
10		0		1.52
11		0		0.25
12		0		2.03
13		0		0.25
14		11.43		0
15	15 DPP	0.25		0
16		0		10.92
17		3.81		3.30
18		6.35		1.78
19		3.56		0
20		0		5.84
21		0		0.25
22		0		0
23		0.51		0
24		0.25	POST	0
25		0.23		0
26		0		0
27		0		0.51
28		0		4.32
29		0		0
				0
30	DI	0		
31	Plant + 0 DPP	0		N/A

^a Abbreviations: DPP, days preplant; POST, postemergence.

Table 3.5. Two-year average soybean yield and yield components for tolerant variety at the Wye Research and Education Center (WREC) located in Queenstown, MD.

Treatment	Rate ^a	Timing ^b	Yield	Population	Pods plant ⁻¹	Beans pod ⁻¹	Seed weight	Yield plant ⁻¹
	g ha ⁻¹		kg ha ⁻¹	Plants ha ⁻¹	no.	no.	mg seed ⁻¹	g plant ⁻¹
Glyphosate	874	30 DPP	2295	593267	28.6	2.6	120.0	8.59
Saflufenacil + glyphosate	25 + 874	30 DPP	2644	613725	33.1	2.4	128.3	10.13
Saflufenacil + glyphosate	50 + 874	30 DPP	2518	642950	29.7	2.5	131.7	9.48
Saflufenacil + imazethapyr ^c + glyphosate	95 + 874	30 DPP	2558	645872	27.3	2.5	125.0	8.41
LSD _{0.05}			NS	NS	NS	NS	NS	NS
Glyphosate Saflufenacil + glyphosate	874 25 + 874	15 DPP 15 DPP	2586 2696	569887 672175	28.6 26.3	2.7 2.5	118.3 126.7	8.61 8.36
Saflufenacil + glyphosate	50 + 874	15 DPP	2330	640027	32.5	2.5	126.7	10.12
Saflufenacil + imazethapyr + glyphosate	95 + 874	15 DPP	2433	692632	31.0	2.5	126.7	9.79
$LSD_{0.05}$			NS	NS	NS	NS	NS	NS
Glyphosate	874	0 DPP	2578	616647	26.8	2.6	125.0	8.41
Saflufenacil + glyphosate	25 + 874	0 DPP	2299	610802	29.9	2.4	131.7	9.33
Saflufenacil + glyphosate	50 + 874	0 DPP	2586	599112	33.1	2.4	135.0	10.47
Saflufenacil + imazethapyr + glyphosate	95 + 874	0 DPP	2586	619570	33.3	2.5	131.7	10.77
$LSD_{0.05}$			NS	NS	NS	NS	NS	NS

^aRates are in ae for glyphosate and ai for all other products.

^bAbbreviations: DPP, days preplant.

^cPre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

Table 3.6. Two-year average soybean yield and yield components for sensitive variety at the Wye Research and Education Center (WREC) located in Queenstown, MD.

Treatment	Rate ^a	Timing ^b	Yield	Population	Pods plant ⁻¹	Beans pod ⁻¹	Seed weight	Yield plant ⁻¹
	g ha ⁻¹		kg ha ⁻¹	Plants ha ⁻¹	no.	no.	mg seed ⁻¹	g plant ⁻¹
Glyphosate	874	30 DPP	2228	394538	40.1	2.4	138.3	13.12
Saflufenacil + glyphosate	25 + 874	30 DPP	2642	429608	35.0	2.5	148.3	12.32
Saflufenacil + glyphosate	50 + 874	30 DPP	2238	400383	36.7	2.5	145.0	12.89
Saflufenacil + imazethapyr ^c +	95 + 874	30 DPP	2441	429608	38.4	2.6	138.3	13.09
glyphosate LSD _{0.05}			NS	NS	NS	NS	NS	NS
Glyphosate	874	15 DPP	2681	406228	37.7	2.6	140.0	13.04
Saflufenacil + glyphosate	25 + 874	15 DPP	2667	377003	38.2	2.6	138.3	12.90
Saflufenacil + glyphosate	50 + 874	15 DPP	2713	382848	48.4	2.5	143.3	17.22
Saflufenacil + imazethapyr + glyphosate	95 + 874	15 DPP	2634	417918	34.0	2.6	153.3	13.10
$LSD_{0.05}$			NS	NS	9.5	NS	NS	3.70
Glyphosate	874	0 DPP	2764	438375	36.3	2.6	143.3	13.48
Saflufenacil + glyphosate	25 + 874	0 DPP	2732	406228	37.7	2.4	146.7	13.11
Saflufenacil + glyphosate	50 + 874	0 DPP	2526	432530	37.5	2.5	151.7	13.88
Saflufenacil + imazethapyr + glyphosate	95 + 874	0 DPP	2464	397460	39.5	2.7	141.7	12.43
LSD _{0.05}		1	NS	NS	NS	NS	NS	NS

^aRates are in ae for glyphosate and ai for all other products.

^bAbbreviations: DPP, days preplant.

^cPre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

Table 3.7. Two-year average soybean yield and yield components for tolerant variety at the Central Maryland Research and Education Center (CMREC) located in Beltsville, MD.

Treatment	Ratea	Timing ^b	Yield	Population	Pods plant ⁻¹	Beans pod ⁻¹	Seed weight	Yield plant ⁻¹
	g ha ⁻¹		kg ha ⁻¹	Plants ha ⁻¹	no.	no.	mg seed ⁻¹	g plant ⁻¹
Glyphosate	874	30 DPP	3990	277638	39.8	2.7	158.3	16.72
Saflufenacil + glyphosate	25 + 874	30 DPP	3925	283483	37.6	2.7	153.3	15.34
Saflufenacil + glyphosate	50 + 874	30 DPP	4172	289328	44.9	2.6	156.7	18.73
Saflufenacil + imazethapyr ^c + glyphosate	95 + 874	30 DPP	3739	309785	41.3	2.6	160.0	17.49
$LSD_{0.05}$			NS	NS	NS	NS	NS	NS
Glyphosate	874	15 DPP	3847	295173	36.1	2.6	168.3	15.83
Saflufenacil + glyphosate	25 + 874	15 DPP	3480	303940	34.5	2.6	156.7	13.75
Saflufenacil + glyphosate	50 + 874	15 DPP	4185	268870	41.4	2.6	158.3	17.26
Saflufenacil + imazethapyr + glyphosate	95 + 874	15 DPP	3917	286405	41.8	2.6	158.3	17.67
$LSD_{0.05}$			NS	NS	NS	NS	NS	NS
Glyphosate Saflufenacil + glyphosate	874 25 + 874	0 DPP 0 DPP	3800 3805	312708 298095	33.6 34.9	2.8 2.7	150.0 155.0	14.25 14.70
Saflufenacil + glyphosate	50 + 874	0 DPP	3559	312708	30.4	2.7	156.7	12.67
Saflufenacil + imazethapyr + glyphosate	95 + 874	0 DPP	4281	298095	39.0	2.6	161.7	16.30
LSD _{0.05}	-11	-4 1 -: £	NS	NS	NS	NS	NS	NS

^aRates are in ae for glyphosate and ai for all other products.

^bAbbreviations: DPP, days preplant.

^cPre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

Table 3.8. Two-year average soybean yield and yield components for sensitive variety at the Central Maryland Research and Education Center (CMREC) located in Beltsville, MD.

Treatment	Rate ^a	Timing ^b	Yield	Population	Pods plant ⁻¹	Beans pod ⁻¹	Seed weight	Yield plant ⁻¹
	g ha ⁻¹		kg ha ⁻¹	Plants ha ⁻¹	no.	no.	mg seed ⁻¹	g plant ⁻¹
Glyphosate	874	30 DPP	3359	315630	38.0	2.6	140.0	14.16
Saflufenacil + glyphosate	25 + 874	30 DPP	3782	344855	38.6	2.7	146.7	13.78
Saflufenacil + glyphosate	50 + 874	30 DPP	3539	330242	37.1	2.7	140.0	14.34
Saflufenacil + imazethapyr ^c + glyphosate	95 + 874	30 DPP	4065	342491	38.4	2.8	147.7	15.88
LSD _{0.05}			571	NS	NS	NS	NS	NS
Glyphosate	874	15 DPP	3464	273330	45.2	2.7	149.3	17.86
Saflufenacil + glyphosate	25 + 874	15 DPP	3783	305284	41.9	2.7	139.1	16.24
Saflufenacil + glyphosate	50 + 874	15 DPP	3868	356545	32.1	2.7	145.0	12.26
Saflufenacil + imazethapyr + glyphosate	95 + 874	15 DPP	3914	324397	37.4	2.8	143.3	14.58
LSD _{0.05}			NS	61552	9.2	NS	NS	4.06
Glyphosate Saflufenacil +	874 25 +	0 DPP 0 DPP	3930 3477	330242 336087	34.2 35.6	2.7 2.8	141.7 148.3	13.32 14.21
glyphosate Saflufenacil + glyphosate	874 50 + 874	0 DPP	3338	315630	44.1	2.8	138.3	16.59
Saflufenacil + imazethapyr + glyphosate	95 + 874	0 DPP	3939	341332	36.2	2.6	137.2	14.24
LSD _{0.05}		1	571	NS	9.2	NS	NS	NS

^aRates are in ae for glyphosate and ai for all other products.

^bAbbreviations: DPP, days preplant.

^cPre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

Table 3.9. Two-year average soybean height for the tolerant variety at the Wye Research and Education Center (WREC) located in Queenstown, MD.

Treatment	Ratea	Timing ^b	4 WAP	7 WAP	10 WAP
	g ha ⁻¹		cm	cm	cm
Growth stage ^c			V3-V4	R1-R2	R3-R4
Glyphosate	874	30 DPP	18.3	53.0	82.6
Saflufenacil +	25 +	30 DPP	18.2	54.4	88.1
glyphosate	874				
Saflufenacil +	50 +	30 DPP	18.6	53.1	85.2
glyphosate	874				
Saflufenacil +	95 +	30 DPP	17.7	57.6	89.2
imazethapyr ^d +	874				
glyphosate			3.70		
$LSD_{0.05}$			NS	NS	NS
Clh4-	974	15 DDD	17.2	<i>57</i> 0	01.7
Glyphosate	874 25 +	15 DPP 15 DPP	17.3 17.6	57.8	91.7
Saflufenacil +	25 + 874	13 DPP	17.0	57.9	89.1
glyphosate Saflufenacil +	50 +	15 DPP	17.3	55.4	85.9
glyphosate	30 + 874	13 DFF	17.3	33.4	03.9
Saflufenacil +	95 +	15 DPP	16.7	58.0	90.0
imazethapyr +	874	13 D11	10.7	30.0	70.0
glyphosate	071				
LSD _{0.05}			NS	NS	NS
2.52 0.03					
Glyphosate	874	0 DPP	16.0	54.3	88.4
Saflufenacil +	25 +	0 DPP	17.8	52.9	85.5
glyphosate	874				
Saflufenacil +	50 +	0 DPP	17.6	54.9	87.0
glyphosate	874				
Saflufenacil +	95 +	0 DPP	16.3	55.5	87.8
imazethapyr +	874				
glyphosate					
LSD _{0.05}			NS	NS	NS

^a Rates are in ae for glyphosate and ai for all other products.

^b Abbreviations: DPP, days preplant; WAP, weeks after planting.

^c Growth stage was only measured in 2011.

^d Pre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

Table 3.10. Two-year average soybean height for the sensitive variety at the Wye Research and Education Center (WREC) located in Queenstown, MD.

Treatment	Ratea	Timing ^b	4 WAP	7 WAP	10 WAP
	g ha ⁻¹		cm	cm	cm
Growth stage ^c			V3-V4	R1-R2	R3-R4
Glyphosate	874	30 DPP	19.2	47.9	78.9
Saflufenacil +	25 +	30 DPP	20.1	53.8	87.1
glyphosate	874				
Saflufenacil +	50 +	30 DPP	19.0	53.8	84.9
glyphosate	874				
Saflufenacil +	95 +	30 DPP	19.7	55.6	85.9
imazethapyr ^d +	874				
glyphosate					
$LSD_{0.05}$			NS	NS	NS
Glyphosate	874	15 DPP	18.7	51.3	83.5
Saflufenacil +	25 +	15 DPP	18.4	52.9	84.5
glyphosate	874				
Saflufenacil +	50 +	15 DPP	19.2	51.7	83.6
glyphosate	874				
Saflufenacil +	95 +	15 DPP	19.9	59.1	92.2
imazethapyr +	874				
glyphosate					
LSD _{0.05}			NS	NS	NS
	07.4	0 DDD	10.1	55.0	00.7
Glyphosate	874	0 DPP	19.1	55.2	88.7
Saflufenacil +	25 +	0 DPP	19.2	54.4	90.4
glyphosate	874	0 DDD	17.4	50.5	00.0
Saflufenacil +	50 +	0 DPP	17.4	50.5	88.0
glyphosate	874	0 DDD	10.1	50.0	062
Saflufenacil +	95 +	0 DPP	18.1	52.2	86.3
imazethapyr +	874				
glyphosate			1 7	NO	NG
$LSD_{0.05}$			1.7	NS	NS

^a Rates are in ae for glyphosate and ai for all other products.

^b Abbreviations: DPP, days preplant; WAP, weeks after planting.

^c Growth stage was only measured in 2011.

^d Pre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

Table 3.11. Two-year average soybean height for the tolerant variety at the Central Maryland Research and Education Center (CMREC) located in Beltsville, MD.

Treatment	Ratea	Timing ^b	4 WAP	7 WAP	10 WAP
	g ha ⁻¹		cm	cm	cm
Growth stage ^c			V3-V4	R1-R2	R3-R4
Glyphosate	874	30 DPP	20.8	62.9	80.6
Saflufenacil +	25 +	30 DPP	21.3	67.9	84.7
glyphosate	874				
Saflufenacil +	50 +	30 DPP	22.2	67.6	85.7
glyphosate	874				
Saflufenacil +	95 +	30 DPP	21.0	66.4	85.0
imazethapyr ^d +	874				
glyphosate					
$LSD_{0.05}$			NS	NS	NS
Glyphosate	874	15 DPP	20.8	63.7	81.0
Saflufenacil +	25 +	15 DPP	21.5	61.4	78.4
glyphosate	874	4.5.000		- 10	20.5
Saflufenacil +	50 +	15 DPP	21.7	71.9	89.5
glyphosate	874	1 5 D DD	01.1	60.6	00.1
Saflufenacil +	95 +	15 DPP	21.1	69.6	89.1
imazethapyr +	874				
glyphosate			MC	NG	NG
$LSD_{0.05}$			NS	NS	NS
Clymbogoto	874	0 DPP	21.0	68.9	86.9
Glyphosate Saflufenacil +	25 +	0 DPP	21.6	61.0	80.9
glyphosate	23 + 874	0 DFF	21.0	01.0	80.2
Saflufenacil +	50 +	0 DPP	22.1	63.7	80.9
glyphosate	30 + 874	UDII	44.1	03.7	00.9
Saflufenacil +	95 +	0 DPP	22.6	68.5	88.6
imazethapyr +	874	O DI I	22.0	00.5	00.0
glyphosate	074				
LSD _{0.05}			NS	NS	NS

^a Rates are in ae for glyphosate and ai for all other products.

^b Abbreviations: DPP, days preplant; WAP, weeks after planting.

^c Growth stage was only measured in 2011.

^d Pre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

Table 3.12. Two-year average soybean height for the sensitive variety at the Central Maryland Research and Education Center (CMREC) located in Beltsville, MD.

Treatment	Ratea	Timing ^b	4 WAP	7 WAP	10 WAP
	g ha ⁻¹		cm	cm	cm
Growth stage ^c			V3-V4	R1-R2	R3-R4
Glyphosate	874	30 DPP	19.8	56.5	72.3
Saflufenacil +	25 +	30 DPP	21.4	64.6	82.3
glyphosate	874				
Saflufenacil +	50 +	30 DPP	19.4	62.4	78.3
glyphosate	874				
Saflufenacil +	95 +	30 DPP	20.2	65.8	82.9
imazethapyr ^d +	874				
glyphosate					
$LSD_{0.05}$			NS	NS	NS
a	a=.				
Glyphosate	874	15 DPP	21.6	62.7	79.5
Saflufenacil +	25 +	15 DPP	20.4	64.1	83.9
glyphosate	874	4.5.000	20.0	- 4	
Saflufenacil +	50 +	15 DPP	20.2	61.1	76.5
glyphosate	874	1.7. D.D.D.	10.0	<i>(</i> 0 1	01.7
Saflufenacil +	95 +	15 DPP	19.9	62.1	81.7
imazethapyr +	874				
glyphosate			MC	NG	NG
$LSD_{0.05}$			NS	NS	NS
Glyphosate	874	0 DPP	20.0	61.8	78.9
Saflufenacil +	25 +	0 DPP	20.0	61.3	77.6
glyphosate	874	0 DI I	20.1	01.5	77.0
Saflufenacil +	50 +	0 DPP	18.6	56.6	76.0
glyphosate	874	V DII	10.0	20.0	70.0
Saflufenacil +	95 +	0 DPP	20.2	62.9	77.6
imazethapyr +	874				
glyphosate					
$LSD_{0.05}$			NS	NS	NS

^a Rates are in ae for glyphosate and ai for all other products.

^b Abbreviations: DPP, days preplant; WAP, weeks after planting.

^c Growth stage was only measured in 2011.

^d Pre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

Table 3.13. Soybean yield and yield components by year for treatments applied 0 days preplant (DPP) to sensitive variety at the Central Maryland Research and Education Center (CMREC) located in Beltsville, MD.

Year	Treatment	Rate ^a	Yield	Population	Pods plant ⁻¹	Beans pod ⁻¹	Seed weight	Yield plant ⁻¹
		g ha ⁻¹	kg ha ⁻¹	Plants ha ⁻¹	no.	no.	mg seed ⁻¹	g plant ⁻¹
2010	Glyphosate	874	3284	385770	34.1	2.7	133.7	12.22
	Saflufenacil + glyphosate	25 + 874	2917	350700	34.3	2.6	162.0	14.40
	Saflufenacil + glyphosate	50 + 874	2787	333165	43.3	2.6	142.0	15.93
	Saflufenacil + imazethapyr ^b + glyphosate	95 + 874	3340	391165	36.1	2.6	142.7	13.66
	LSD _{0.05}		NS	35965	NS	NS	NS	NS
2011	Glyphosate	874	4575	274715	34.3	2.8	150.0	14.43
	Saflufenacil + glyphosate	25 + 874	4036	321475	37.0	2.8	133.3	14.02
	Saflufenacil + glyphosate	50 + 874	3888	298095	44.9	2.9	133.3	17.25
	Saflufenacil + imazethapyr + glyphosate	95 + 874	4573	289328	35.2	2.6	132.0	14.76
	$LSD_{0.05}$		NS	NS	10.0	NS	NS	NS

^aRates are in ae for glyphosate and ai for all other products.

^b Pre-packaged mix of saflufenacil at 25 g ha⁻¹ + imazethapyr at 70 g ha⁻¹ (Trade name OpTill).

Chapter 4. Conclusions

Previous research has shown that saflufenacil provides excellent control of horseweed in burndown situations (Owen et al. 2011). When tank-mixed with glyphosate, efficacy on many difficult-to-control weeds was improved over either product applied alone (Waggoner et al. 2011). Mellendorf et al. (2008) showed that combinations of saflufenacil + glyphosate were highly effective on horseweed with no signs of antagonism between the herbicides. Data from my greenhouse experiments confirmed that a tank-mix of saflufenacil + glyphosate resulted in increased efficacy on GR horseweed compared to either product alone (Table 2.1). The quick burndown offered by saflufenacil was illustrated in the GS horseweed population. Saflufenacil + glyphosate resulted in quicker control of GS horseweed compared to glyphosate applied alone (Table 2.2). Reduced efficacy at 28 DAA for all saflufenacil-containing treatments was attributed to extensive regrowth of horseweed from the crowns of the rosettes, which were larger than those usually found under field conditions (Davis and Johnson 2008). Since saflufenacil is translocated primarily in the xylem, only the foliage that was present at application was exposed to the herbicide. This broke apical dominance, which allowed regrowth. Despite the poor efficacy, a synergistic or additive response of saflufenacil + glyphosate was observed.

Saflufenacil applications to soybean grown on coarse-textured soils with less than 2% OM are currently restricted to 30 DPP for a rate of 25 g ha⁻¹, and 44 DPP for a rate of 37.5 g ha⁻¹ (Anonymous 2012). Reduced plant population for saflufenacil at 50 g ha⁻¹ applied 0 DPP and reduced yield plant⁻¹ for saflufenacil at 50 g ha⁻¹ applied 15 DPP seem to confirm the current label (Tables 3.8 and 3.13). These data indicate poor crop safety in cases of application overlap. Trends in the data also showed that saflufenacil at 25 g ha⁻¹ had higher levels of crop injury and reduced yields when compared to the pre-packaged mix of saflufenacil at 25 g ha⁻¹ +

imazethapyr at 70 g ha⁻¹ (Tables 3.8 and 3.13). This suggests that the prepackaged mix of saflufenacil + imazethapyr [packaged as a water-dispersible granule (WG)] may increase crop safety when compared to the liquid emulsifiable concentrate (EC) formulation of saflufenacil. Previous work by Frihauf et al (2010a) found that the EC formulation of saflufenacil provided better weed control and higher rates of injury to wheat than a WG formulation of saflufenacil. Data from this experiment concur with that of Frihauf et al. (2010a) indicating that saflufenacil shows more herbicidal activity in the EC formulation than the WG formulation.

Regardless of application timing, soil texture, saflufenacil formulation, or product rate, the tolerant soybean varieties never showed any injury symptoms or reductions in yield (Tables 3.5, 3.7, 3.9, and 3.11). Grossman et al. (2011) showed that corn was inherently tolerant to saflufenacil based on metabolism of the herbicide. Weed species examined in that study could not metabolize saflufenacil and thus experienced high rates of injury and total plant death. Given wide variations in genetics between soybean varieties, it is possible that varieties categorized as tolerant to saflufenacil can readily metabolize the herbicide whereas sensitive varieties cannot metabolize the chemical at a rapid enough rate to avoid crop injury and yield loss. It seems appropriate for BASF to keep their current saflufenacil label that restricts application timings on coarse-textured soils.

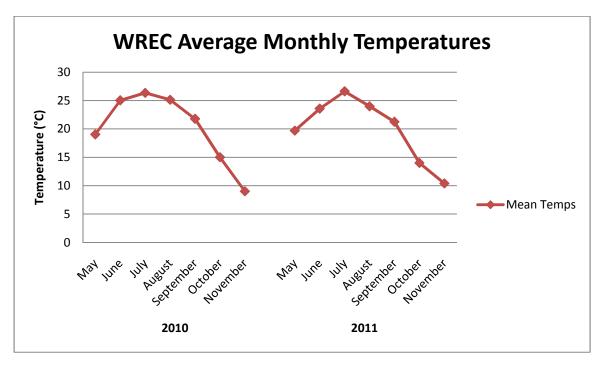
Saflufenacil's utility for controlling GR horseweed on the Delmarva Peninsula may be limited. Data from WREC suggest that farmers on the peninsula with medium-textured soils can apply saflufenacil up to 50 g ha⁻¹ at 0 DPP with no yield loss (Tables 3.5 and 3.6). This would allow control of horseweed after the majority of plants have emerged. However, with many fields containing coarse-textured soils with low OM, saflufenacil applications will be restricted to 30 DPP for much of the region. While these applications show promise in controlling fall-

germinated horseweed populations, as well as those that germinate throughout May, there is still some probability of populations avoiding the more effective burndown treatment and germinating once residual activity of saflufenacil starts to fail. Previous research shows this could be as early as 21 DAA (Owen et al. 2011).

In conclusion, while saflufenacil appears to increase efficacy of glyphosate on both GR and GS horseweed populations, its utility in Maryland and the Delmarva Peninsula is limited based on preplant application restrictions on the label. If a variety screening program were maintained to identify saflufenacil-tolerant varieties of soybean, the herbicide would prove to be a valuable tool in controlling GS and GR horseweed populations in Maryland.

Appendix A

Figure 1. Average temperatures and precipitation during the growing season for 2010 and 2011 at the Wye Research and Education Center (WREC) located in Queenstown, MD.



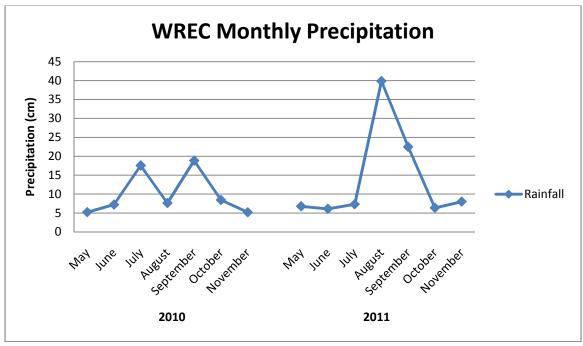
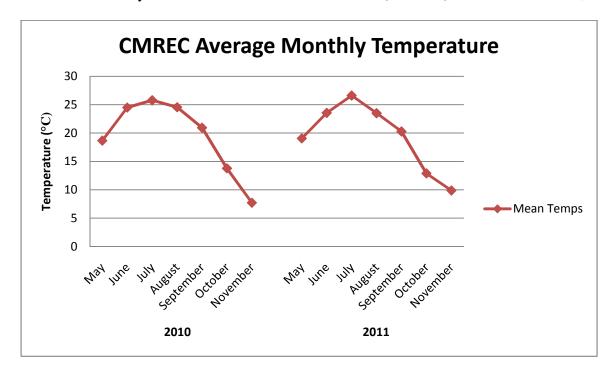
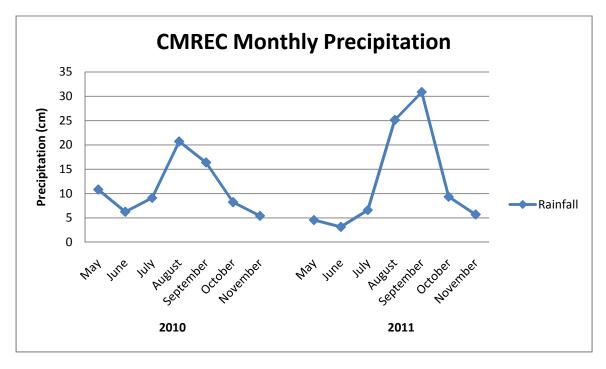


Figure 2. Average temperatures and precipitation during the growing season for 2010 and 2011 at the Central Maryland Research and Education Center (CMREC) located in Beltsville, MD.





Appendix B

Table 1. Weather conditions at time of spray applications at the Wye Research and Education Center (WREC) located in Queenstown, MD.

WREC Application Conditions								
	2010				2011			
Timing	30 DPP ^a	15 DPP	0 DPP	POST	30 DPP	15 DPP	0 DPP	POST
Date	4 May	20 May	8 June	1 July	9 May	24 May	6 June	22 June
Air temp (°C)	24.4	25.6	27.8	27.8	22.2	26.7	30.6	32.2
% humidity	30	35	40	35	25	60	45	60
% cloud cover	0	0	0	0	0	66	0	66
Soil temp (°C at 10.15 cm depth)	21.1	23.9	27.8	26.7	21.1	26.7	31.1	32.2

^a Abbreviations: DPP, days preplant; POST, postemergence.

Table 2. Weather conditions at time of spray applications at the Central Maryland Research and Education Center (CMREC) located in Beltsville, MD.

CMREC Application Conditions								
		2010				2011		
Timing	30 DPP ^a	15 DPP	0 DPP	POST	30 DPP	15 DPP	0 DPP	POST
Date	5	19	1	21	2	16	31	24
	May	May	June	June	May	May	May	June
Air temp (°C)	22.2	16.7	25.6	32.8	21.1	23.9	32.2	28.9
% humidity	40	30	70	25	40	65	65	40
% cloud cover	0	66	33	10	66	66	10	66
Soil temp (°C at 10.15 cm depth)	21.1	15.6	25.6	32.2	19.4	23.9	31.1	28.9

^a Abbreviations: DPP, days preplant; POST, postemergence.

Table 3. Weather conditions at the time of greenhouse application.

Greenhouse Spray Conditions							
	Study 1 Study 2						
Date	2 June 2011	2 December 2011					
Air temp (°C)	32.2	14.3					
% humidity	65	65					
% cloud cover	0	0					

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