HORSEWEED MANAGEMENT WITH SHADING AND COVER CROPS, AND THE TOLERANCE OF TWO HORSEWEED GROWTH TYPES TO GLYPHOSATE

By

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ABSTRACT

HORSEWEED MANAGEMENT WITH SHADING AND COVER CROPS AND THE TOLERANCE OF TWO HORSEWEED GROWTH TYPES TO GLYPHOSATE

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Field experiments were conducted to evaluate if fall-planted cereal rye in combination with narrow row soybean improved glyphosate-resistant (GR) horseweed management. At the time of a postemergence herbicide application (POST), horseweed biomass was 71 to 90% lower when soybean was planted into cereal rye, regardless of termination time, compared with no cover across all row widths. Planting green or narrow row soybean suppressed horseweed through soybean harvest and integrating an effective POST herbicide improved control. Additional field experiments found that in the absence of an effective POST herbicide, horseweed biomass was 42 and 81% lower by planting green or applying a residual herbicide compared with no cover, respectively, at soybean harvest. Similarly, planting soybean in 19 cm rows reduced horseweed biomass compared with 38 and 76 cm rows. In the greenhouse, shade levels from 35 to 92% reduced rosette and upright horseweed biomass 31 to 99% compared with the upright-type grown under 0% shade. Greater reductions occurred under 69 and 92% shade. Differences in glyphosate sensitivity between the rosette and upright horseweed growth types were not due to absorption, translocation, or total glyphosate retention; however, glyphosate retention was 21 and 18% lower on a per weight and area basis for the upright growth type. This diluted concentration may contribute to increased glyphosate tolerance found in the upright growth type. However, other factors such as differences in EPSPS gene expression may also help explain differential sensitivity if a target-mutation is discovered. This research provides growers strategies for managing horseweed and insight into potential growth type differences.

Dedicated to my family, friends, and colleagues who extended their love and guidance along the way.

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CHAPTER I

LITERATURE REVIEW

Introduction

Soybean (*Glycine max* L.) is a valuable leguminous plant and a key source of food, protein, and oil. It is divided into two marketable components: meal and oil. Soybean meal has high protein content and 97% of soybean meal is distributed as animal feed, whereas 54.3% of soybean oil in the United States goes to the food industry (USB 2021). Alternatively, soybean meal and oil are also used to replace petroleum and other ingredients in numerous industrial and consumer products (USB 2021). Soybean is the second most produced field crop in the United States with the main producing areas in the Midwest and the lower Mississippi Valley (USDA NASS 2021). In 2020, more than 33 million hectares were planted in the United States. Michigan ranks 12^{th} with nearly 81,000 hectares planted in soybean production (USDA NASS 2021). The total value for the United States in 2020 was over $46 billion with Michigan contributing over $1 billion (USDA NASS 2021). To protect profit from soybean production, it is essential to mitigate potential yield losses. One of the main causes of yield loss in soybean is competition with weeds.

A problem weed in Michigan, particularly in no-till production systems, is herbicide-resistant horseweed (*Conyza canadensis* L.). In soybeans, yield losses of 83 to 93% were observed when horseweed was not properly managed (Bruce and Kells 1990; Byker et al. 2013). In Michigan, most populations are resistant to glyphosate and acetolactate synthase (ALS) inhibiting herbicides (Hill 2020). Thus, there is a lack of effective postemergence herbicide options, especially as resistant horseweed populations continue to evolve.

Horseweed

Horseweed is a prominent, yield reducing weed native to North America that is commonly found in reduced tillage or no-till cropping systems (Buhler and Owen 1997; Weaver 2001; Loux et al. 2006). Across the Midwest, horseweed populations recently surged due to a shift towards conservation tillage land management programs, as well as an increase of herbicide resistant biotypes (Loux et al. 2006). Additionally, in Michigan agronomic cropping systems there was a shift in emergence, from fall to primarily spring and early summer (Schramski et al. 2021a). In soybean, the extended emergence and the lack of effective POST herbicide options makes it difficult to control horseweed (Loux et al. 2016), potentially leading to yield loss.

Seed Dispersal. The widespread occurrence of horseweed is partially due to each horseweed plant producing up to 200,000 seeds. Each seed is 1-mm long with an attached pappus, enabling seed dispersal via wind into the planetary boundary layer, up to 550 km from the mother plant (Bhowmik and Bekech 1993; Shields et al. 2006). In addition, horseweed can grow up to 180 cm tall; seed is positioned high above the ground, assisting in wind dispersal (Loux et al. 2006; Weaver 2001). Germ-able horseweed seed are also dispersed by water through irrigation canals and rivers from nearby field populations (Kelley and Bruns 1975). The ability of horseweed to readily enter and successfully colonize new areas, contributes to the complications associated with managing this weed species in agricultural lands.

Seed Dormancy and Longevity. Horseweed seeds generally have less dormancy than most agricultural weeds after they reach maturity, and once the seeds are shed from the source plant, they germinate readily at day/night temperatures of 22/16 C (Buhler and Owen 1997; Buhler and

Hoffman 1999). Tozzi et al. (2014) found that 84 to 93% of horseweed seed shed germinates and enters the population within the season it was shed, suggesting horseweed maintains a small seed bank. However, Leck and Leck (1998) discovered large quantities of horseweed seed in the seed bank of a fallow agricultural field, even though horseweed was not present in the aboveground vegetation for 10 previous years. Conversely, Thebaud et al. (1996) found only 1% of horseweed seed remained viable after three years on the soil surface. In addition, Davis et al. (2007) saw a drastic decline of seed bank densities between 18 and 23 months.

Germination and Emergence. There is a weak correlation between horseweed germination and soil temperature, air temperature, or rainfall (Main et al. 2006). Schramski et al. (2021b) reported that peak horseweed emergence (>80%) in Michigan soybean fields occurred when 50 to 100 growing degree days (GDDs) (base, 10 C) accumulated in the spring with adequate soil moisture, although horseweed continued to emerge until 450-600 GDD, depending on rainfall. Regehr and Bazzaz (1976) concluded horseweed's competitiveness and variable emergence timing was a result of its ability to photosynthesize over a wide range of temperatures, and various light intensities. Overwintering plants have a high chance of survival because they maintain carbon fixation and energy storage at low temperatures (Regehr and Bazzaz 1976). Conversely, Nandula et al. (2006) reported that horseweed germination increased with rising temperatures above day/night temperatures of 12/6 C and peak emergence occurred at 24/20 C. Horseweed germinated continually at increasing salt concentrations up to 160 mM NaCl, suggesting horseweed can tolerate moderate water-stress conditions (Nandula et al. 2006). Horseweed cannot germinate if seeds are 0.5 cm or deeper in the soil profile (Nandula et al. 2006), which makes it particularly problematic in no-tillage or reduced tillage systems where seeds are not buried.

Growth Type. Horseweed is a facultative winter annual wherein germination can occur in the fall when soil temperatures decline but also throughout the next season (Cici and Van Acker 2009). Numerous researchers have reported two main timings of emergence, August to October (fall) and April to June (spring) that serve as critical management timings (Bhowmik and Bekech 1993; Buhler and Owen 1997; Loux et al. 2006; Main et al. 2006). Fall-emerging horseweed form a dark green, lightly-haired basal rosette that overwinters, while spring-emerging horseweed skip, or spend a short period of time as a rosette before bolting (Loux et al. 2006; Regehr and Bazzaz 1979). The odds of fall-emerging horseweed surviving winter are very high, up to 91 percent, in the northern region of the United States (Loux et al. 2006). However, overwintering rosettes are prone to frost heaving which can result in seedling mortality (Regehr and Bazzaz 1979). Although, Regehr and Bazzaz (1979) found that as the rosette grows larger prior to winter, the odds of survival into the following spring increase. Small rosettes are more likely to uproot from frost heaving due to a less-developed root system. Conversely, higher mortality from frost heaving was observed when rosettes were greater than 9 cm in diameter (Davis and Johnson 2008). Thus, variable weather conditions with more freeze-thaw spells, and the speed of frost events are important in overwintering survival versus rosette size (Tozzi et al. 2014). In Michigan, there has been a shift in primary emergence from mainly fall-emerging rosettes to spring-emerging upright (bolted) plants that skip the rosette growth stage (Schramski et al. 2021a). Spring-emerging plants that protrude through the soybean canopy in August have the greatest chance of survival to maturity and contribute the highest number of seeds to the seed bank (Davis and Johnson 2008). Davis and Johnson (2008) found that horseweed plants that produce flower heads above the soybean canopy in late-season can contribute upwards of 88% to total seed production.

Herbicide Resistance. Another obstacle in horseweed control is its resistance to multiple herbicide sites of action. Currently, horseweed has documented resistance to at least one site of action in 18 different countries (Heap 2021). Populations resistant to acetolactate synthase (ALS) inhibitors (WSSA Group 2), photosystem II inhibitors (WSSA Group 5), photosystem II inhibitors (WSSA Group 7), the 5-enolpyruvate-shikimate-3-phosphate inhibitor (EPSP) glyphosate (WSSA Group 9), and the photosystem I electron diverter (WSSA Group 22) paraquat. However, glyphosate resistance is the most prevalent and influential herbicide resistance in many cropping systems. In the United States, GR horseweed is present in 25 states, including Michigan, and biotypes are often resistant to more than one site of action (Heap 2021). In Michigan, glyphosate-resistant (GR) horseweed biotypes are the most widespread with numerous biotypes also resistant to the ALS inhibiting herbicides (Hill 2020).

The first confirmed case of GR horseweed was identified in Delaware in 2000 (VanGessel 2001). Resistance occurred after relying only on glyphosate for weed control for three years in a glyphosate-resistant soybean field. The population exhibited an 8- to 13-fold level of resistance compared to a susceptible population. Following the release of Roundup Ready ® crops in 1996, glyphosate use increased almost 15-fold globally (Benbrook 2016), contributing to increased selection pressure for resistant individuals. By 2021, there were 14 countries with confirmed GR horseweed found in a variety of settings including roadsides, railways, orchards, fruit, grapes, nurseries, corn, cotton, wheat, and alfalfa (Heap 2021). GR horseweed is often found in settings where glyphosate is primarily relied on for weed control, especially in no-tillage or conservation tillage systems, or where soybean is planted continuously (Loux et al. 2006).

The primary mechanism of GR in horseweed is reduced translocation of glyphosate to the target site due to rapid sequestration into the vacuole (Dinelli et al. 2006; Feng et al. 2004; Ge et al. 2010; González-Torralva et al. 2012; Koger and Reddy 2005; Moretti and Hanson 2016; Nandula et al. 2005). Ge et al. (2010) examined mature leaves from GR horseweed plants 24 h after spraying with glyphosate and found glyphosate fractional occupancy of the vacuole was 85% in GR horseweed compared to less than 15% occupancy in glyphosate-susceptible horseweed. Similarly, Koger and Reddy (2005) treated horseweed rosettes with ^{14}C-glyphosate when plants had 23 to 29 leaves and found a reduction of 28 to 48% in translocation out of the treated leaf in glyphosate-resistant biotypes compared with glyphosate-susceptible biotypes. In addition to impaired translocation, glyphosate resistance in horseweed may also be due to enhanced glyphosate metabolism to other compounds (González-Torralva et al. 2012). González-Torralva et al. (2012) found that a glyphosate-resistant biotype metabolized glyphosate faster than the glyphosate-susceptible biotype; by 96 h after treatment (HAT), the glyphosate-resistant biotype converted all glyphosate in its tissue into glyoxylate, sarcosine, and aminomethylphosphonic acid. Conversely, in the susceptible population glyphosate was still present and glyoxylate was its only non-toxic metabolite detected (González-Torralva et al. 2012). Recently, the first documented case of target-site mediated glyphosate resistance in horseweed in the United States was observed in biotypes with resistance from 20 to 40X the field rate (1X = 840 g ae ha^{-1}) from Ohio and Iowa (Beres et al. 2020). A proline to serine mutation at position 106 of EPSPS2 was detected, which is the same target site mutation identified in 21 glyphosate-resistant horseweed accessions from Canada (Page et al. 2018).

Previous studies primarily evaluated glyphosate-resistant populations that emerged in the fall as rosettes. Yet the primary growth type currently being managed in Michigan soybean production systems are the spring emerging populations exhibiting the upright growth type. Schramski et al. (2021a) determined that the two distinct growth types can occur from the same parent, whether glyphosate-resistant or glyphosate-susceptible. Variations in environmental cues such as temperature, photoperiod, competition, shading, and soil moisture resulted in all horseweed populations emerging as the rosette type. However, when a 4 wk vernalization period occurred before germination, but after imbibition, the upright type was triggered in all populations. Additionally, Schramski et al. (2021a) observed that sensitivity to glyphosate in glyphosate-resistant populations was different between the upright and rosette growth types, the upright-type was 3- to 4-fold less sensitive to glyphosate compared with the rosette-type among the resistant populations. However, glyphosate-susceptible populations did not differ in sensitivity among the rosette and upright growth types. Thus, the shift towards glyphosate-resistant upright growth types with reduced glyphosate sensitivity could result in a more robust glyphosate-resistant populations.

Horseweed Management with Herbicides

Horseweed is one of the most common and troublesome weeds to control in soybean across the United States as a result of the widespread occurrence of populations resistant to glyphosate and acetolactate synthase-inhibiting herbicides (Gibson et al. 2005; Kruger et al. 2009; Zheng et al. 2011; Heap 2021). Horseweed can tolerate a variety of environmental conditions and thrives in undisturbed areas, making management especially challenging in no-till production systems. This has led to an increased reliance on herbicides for horseweed control.

Preplant burndown applications of glyphosate were applied in soybean; however, the widespread occurrence of glyphosate-resistant horseweed greatly reduced glyphosate effectiveness. Zimmer et al. (2018) reported glyphosate alone controlled GR horseweed only 33% when applied preplant. In comparison, herbicide treatments that included halauxifen-methyl, dicamba, or saflufenacil in combination with glyphosate, controlled horseweed 87 to 96%, 89%, and 93%, respectively, 35 d after burndown application (DAB) (Zimmer et al. 2018). Simpson et al. (2017) observed that horseweed control 4 wk after a preplant burndown application was 54% for glyphosate, 97% for glyphosate + dicamba, 93% for 2,4-D choline + glyphosate, 85% for glufosinate, and 92% for 2,4-D choline + glufosinate. Preplant herbicide applications provide excellent control of horseweed; however, horseweed's extended and variable emergence pattern often warrants a postemergence application (Byker et al. 2013).

The commercialization of Enlist E3® and Roundup Ready 2 Xtend® soybeans has created more postemergence (POST) herbicide options. Byker et al. (2013) reported dicamba applied POST following a preplant application in soybean provided 91 to 100% horseweed control. Similarly, Simpson et al. (2017) found that POST applications of 2,4-D choline + glyphosate, 2,4-D choline + glufosinate or glyphosate + dicamba provided ≥95% control of horseweed following a preemergence herbicide application. Excellent control of horseweed is attainable with herbicides; however, the sole use of chemical management techniques increases the selection pressure for resistance to more herbicide sites of action.

Cover Crops

Given extended horseweed emergence patterns and an increase of herbicide resistant biotypes, other methods are needed to protect crop yields. Integrating cover crops into cropping systems is a potential solution. Across the United States, cover crop acreage increased by 50% between 2012 and 2017, totaling 6.2 million hectares (SARE 2021). This increase is partially attributed to opportunities given to farmers to incorporate cover crops into their farming system through government cost-sharing (USDA ERS 2021). Additionally, benefits such as improved soil and water quality, better nutrient cycling efficiency, and increased productivity have many growers continuing to plant cover crops (Snapp et al. 2005).

Weed Suppression. Cover crops offer two periods of weed suppression. Early as the cover crop is actively growing, and later when the cover crop residue creates a mulch layer on the soil surface (Mirsky et al. 2013). During the period of active growth, cover crops compete with weeds for resources such as light and nutrients, and some cover crops species produce secondary metabolites that inhibit weed germination (Creamer et al. 1996; Davis and Liebman 2003; Shearin et al. 2008; Teasdale and Mohler 1993, 2000; Teasdale et al. 2007) After termination, cover residues decrease light penetration to the soil surface, impeding weed seedling emergence, growth, and development (Teasdale and Mohler 2000). Previous research reported that fall-planted cover crops reduce weed density and/or biomass of both fall- and spring-emerging weed species, including horseweed, early in the season (Cholette et al. 2018; Schramski et al. 2021b; Wallace et al. 2019).

Termination Times. Cover crops can be terminated early prior to planting, or after (planting green). Planting green is the practice of planting into a growing cover and terminating it after planting (SARE 2020). Methods of termination include herbicides, or the use of a mechanical implement such as a roller or roller-crimper (SARE 2021). Delaying cover crop termination allows for greater biomass accumulation. Several studies demonstrated that increasing cover crop biomass resulted in improved weed suppression (Wallace et al. 2019; Cholette et al. 2018; Finney et al. 2016; Ryan et al. 2011; Smith et al. 2011). Reed et al. (2019) found delaying cover crop termination 4 to 30 d by planting green provided 94 to 181% greater cover-crop biomass production compared with early termination.

Soil Moisture. Cover crops have variable effects on soil moisture, depending on soil and weather conditions. The presence of early-terminated cover crop residue can reduce surface evaporation and increase soil moisture retention (Clark et al. 1997; Munawar et al. 1990; Schramski et al. 2021b). Conversely, actively growing crops may increase evapotranspiration thus reducing water availability for the main crop in areas with minimal rainfall, and in turn reduce crop yield (Blanco-Canqui et al. 2015). Rogers (2017) reported that cereal rye had no effect on soil moisture at soybean planting in one site-year; however, soil moisture 4.5% higher at 7.6 cm depth when rye was terminated early the following year. Higher soil moisture was attributed to greater precipitation and cereal rye biomass prior to soybean planting, thus there was higher soil moisture retention. Actively growing cover crops can reduce soil water content, causing lower soil moisture at planting which is more prone to occur by planting green (Price et al. 2009; Mirsky et al. 2011; Reed et al. 2019; Schramski et al. 2021b). Conversely, residue remaining from less than 2,000 kg ha^{-1} of cover crop biomass can retain soil moisture in dry conditions and increase weed emergence (Haramoto and Brainard 2017; Teasdale and Mohler 2000).

C:N Ratio. Carbon (C) and nitrogen (N) are the primary elements responsible for regulating soil biological activity and nutrient cycling, and the C:N ratio of cover crops is an important factor in achieving season-long residue cover. The C:N ratio serves as the driver of residue decomposition and nutrient release in addition to concentrations of lignin and other structural carbohydrates in cover crop tissue (Wagger et al. 1998). The optimal C:N ratio is generally considered to be 24:1, as this is the ideal diet for soil microorganisms (USDA NRCS 2011). Plant residue with a lower ratio will decompose relatively quick, whereas residue with a higher ratio will decompose slower as soil microbes immobilize N to complete decomposition. Higher C:N ratios may lead to a reduction in soil N. However, ratios below 24:1 will likely lead to a surplus of N and be readily available.

Generally, non-legume cover crops such as cereal rye have C:N ratios greater than 24:1, hence they have the potential to persist longer. However, the C:N ratio is often dependent on the growth stage reached prior to termination (USDA NRCS 2011). Schramski et al. (2021b) reported that cereal rye and winter wheat terminated early at Feekes growth stages 5 and 6 had C:N ratios below 24:1, whereas C:N ratios were at or greater than 24:1 across most site years when planting green and terminated at Feekes growth stage 10.4. Similarly, Rogers (2017) observed that C:N ratios for cereal rye were 12:1 and 31:1 when terminated at Feekes growth stage 6 and 9, respectively. A non-legume cover crop such as cereal rye also increases carbon concentration as it matures (Sullivan et al. 1991). Conversely, legume cover crops such as hairy vetch tend to have lower C:N ratios and therefore are less persistent (Clark et al. 1997).

Cereal Rye. Cereal rye has been the primary cover crop used in combination with soybean because of its flexible planting window, cold tolerance, high biomass production, and consistent suppression of weed biomass (Clark 2007; Hayden et al. 2012; Sherman et al. 2020). In early May, at the time of cereal rye termination, Rogers (2017) reported cereal rye provided 80 to 88% ground cover and contributed to early season winter- and summer-annual weed reductions of 74 and 84% in 2015 and 2016, respectively. In other studies, cereal cover crops reduced horseweed density 41 to 97% at the time of termination compared with no cover (Schramski et al. 2021b; Essman et al. 2020; Wallace et al. 2019; Pittman et al. 2019). Wallace et al. (2019) observed that fall-planted cereal rye reduced horseweed density both in the fall and after termination, prior to preplant herbicide applications in soybean. In addition, cereal rye residue was linked with smaller horseweed plants and reduced variability in plant size at the time of preplant soybean herbicide application. Sherman et al. (2020) determined that a cereal rye cover crop reduced horseweed density in the fall and into the following spring.

Residue from planting green has been found to suppress horseweed through POST herbicide application in soybean. Schramski et al. (2021b) reported soybean planted green into a cereal rye or winter wheat cover crop reduced horseweed biomass 46 to 93% compared with no cover at the time of POST herbicide application, 5 wk after planting (WAP); however, results were more variable in respect to horseweed density based on site-year (Schramski et al. 2021b).

Soybean Stand Establishment and Yield. The effects of a cereal rye cover crop on soybean yield and stand establishment are variable. Schramski et al. (2021b) found soybean stand was not affected cereal rye, regardless of termination time. However, soybean yield was 30 and 108% greater by planting green compared with early terminated cereal rye when an effective and noneffective POST herbicide was applied, respectively (Schramski et al. 2021b). Reed et al. (2019) reported no stand or yield reductions from planting green compared with early cover crop termination. In contrast, Liebl et al. (1992) reported delaying cereal rye termination until planting resulted in up to a 45% reduction in soybean stand and subsequent yield losses compared with conventional management, which could be exacerbated by planting green.

Soybean Row Width

Earlier canopy closure by planting in narrower row widths (19 and/or 38-cm rows) decreases weed emergence by reducing light quantity (Nelson and Renner 1999; Harder et al. 2007). Planting soybean in 19 cm rows also leads to increased soybean yield when environmental conditions are favorable (Harder et al. 2007). Narrow row spacings (19 and/or 38 cm rows) offer more equidistant plant distribution, decreasing intraspecific competition for water, nutrients, and light, and increased light interception and biomass production (Dalley et al. 2004; Wells et al. 1993). In addition, soybean planted in 19- and 38-cm rows results in earlier canopy closure by increasing leaf area index (LAI), thus creating more points for light interception (Bertram and Pederson 2004). Earlier canopy closure can also suppress weeds that escape herbicide application or emerge late in the growing season (Mickelson and Renner 1997). Burnside and Colville (1964) reported that soybean grown in 25 cm rows had a completely closed canopy 22 days earlier than soybean grown in 76 cm rows.

Board et al. (1992) attributed increased yield potential in 50 cm rows mainly to increased light interception during the vegetative and early reproductive stages, which led to greater fertile node production and more pods per fertile node. Harder et al. (2007) observed increased yield in 19- and 38-cm narrow rows compared with 76-cm rows in the moderate and high soybean populations where weeds were not effectively controlled. In weed-free and glyphosate treatments, yield in 19-cm rows was greater compared with 76-cm rows (Harder et al. 2007). Similarly, Dalley et al. (2004) found that soybean planted in 19- and 38-cm rows yielded more than in 76-cm rows as a result of higher levels of light interception throughout the growing season.

Earlier canopy development and increased soybean biomass assist in reducing weed emergence and biomass because solar radiation that stimulates weed germination and growth is intercepted by the soybean canopy (Yelverton and Coble 1991). Harder et al. (2007) observed reduced summer annual weed biomass and density in 19- and 38-cm rows compared with 76-cm rows following glyphosate application as a result of increased LAI and earlier canopy closure in narrower rows. However, when a glyphosate application did not take place there was not a difference in weed density between the three row widths (Harder et al. 2007). Similarly, Hock et al. (2006) found canopy closure was earlier in 19-cm rows, resulting in reduced total dry matter of summer annuals compared with 76-cm rows.

Shading

Light is a fundamental component for photosynthesis and plays a vital role in plant competition (Holt 1995). Previous research demonstrated that shading reduced the amount of available photosynthetically activated radiation (PAR) thus reducing the growth of plants beneath the canopy (Steckel et al. 2003; Stoller and Myers 1989). However, many weed species are well-adapted to shaded environments (Aphalo et al. 1999; Huarte and Benech Arnold 2003; Morgan and Smith 1978). Jha et al. (2008) observed that Palmer amaranth acclimated to shade levels ≤87% by lowering its light-saturated photosynthetic rate and light compensation point by increasing leaf chlorophyll content when shaded. Palmer amaranth responded to shading by branching less and increasing specific leaf area (Jha et al. 2008). Increased shade also contributes to reduced weed growth. Bello et al. (1995) observed velvetleaf (*Abutilon theophrasti* Medik.) biomass, branch number, leaf number, and plant height was reduced when grown under a 76% shade level compared with the 0 and 30% shade level. Likewise, Steckel et al. (2003) saw increasing reductions in common waterhemp (*Amaranthus rudis* Sauer) biomass at shade treatments between 40 and 99%. Common waterhemp seed production was reduced by 51 to 99% as shade level increased (Steckel et al. 2003).

Weed Management with a Cover Crop and Narrow Soybean Row Widths

Weed management outcomes in soybean with a cover crop and narrow row widths are highly variable. Hay et al. (2019) reported row-crop cultivation or narrow row widths (19- or 38-cm) plus an early terminated winter wheat cover resulted in the greatest reductions in Palmer amaranth density compared with planting soybean in 76-cm with no cover, 3 WAP. Palmer amaranth density and biomass were similar when planting soybean with no cover across all row widths. However, at 8 WAP, no differences in Palmer amaranth biomass were observed (Hay et al. 2019). Conversely, at 3 WAP waterhemp densities were greater in the cover crop treatments compared with no cover (Hay et al. 2019). Additionally, planting soybean in 76- and 38-cm rows with early terminated winter wheat did not reduce waterhemp density (Hay et al. 2019). Differences in waterhemp biomass were not present at 3- or 8-WAP across all treatments. These contradicting differences were believed to be due to growth characteristics of the weed species as well as favorable microenvironments in which surface soil moisture may have been higher with the cover crop present and narrower soybean rows. Rogers (2017) reported that cereal rye did not reduce Palmer amaranth biomass compared with the no cover treatments at peak biomass; however, 19 cm rows reduced Palmer amaranth biomass one site year compared with 76-cm rows whereas in another site year, 76-cm rows reduced Palmer amaranth biomass. Thus, cover crop and narrower row widths can have differing effects on weed suppression depending on the weed species in addition to microenvironment conditions.

The widespread occurrence of herbicide-resistant horseweed biotypes and the shift from fall-emerged rosettes to spring/summer emergence of upright plants has growers searching for new management practices. Further research is needed to better understand the effectiveness of cereal rye and narrow soybean rows as horseweed management tools in no-tillage soybean, as well as the impact of this shift in horseweed emergence.

Questions that remain to be answered:

1. Does planting green provide more horseweed suppression compared to early terminated cereal rye?
2. Does narrow soybean rows suppress horseweed?
3. Will higher cereal rye biomass from planting green and earlier canopy closure from soybean planted in narrow rows suppress horseweed more than early terminated cereal rye or soybean planted in 76 cm rows?
4. Can planting green in narrow soybean rows suppress horseweed similar to that of a PRE residual herbicide?
5. Does shading affect the growth of rosette and upright horseweed plants?
6. What mechanisms are responsible for the differential glyphosate sensitivity between rosette and upright horseweed growth types?

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CHAPTER II

NARROW ROW SOYBEAN AND A CEREAL RYE COVER CROP SUPPRESS GLYPHOSATE-RESISTANT HORSEWEED

Abstract

Alternative strategies are needed for management of glyphosate-resistant (GR) horseweed in soybean. Integrating a cereal rye cover crop with soybean planted in narrow rows may improve control and reduce herbicide selection pressure on horseweed biotypes. Four site-years of experiments were conducted in Michigan to determine if fall-planted cereal rye terminated with glyphosate 1 wk prior to (early termination) or 1 wk after (planting green) planting in combination with narrow row soybean improved GR horseweed management. At POST herbicide application, horseweed biomass was 71 to 90% lower when soybean was planted into cereal rye, regardless of termination time, compared with no cover across all row widths. Planting green or narrow row soybean suppressed horseweed through soybean harvest. When a noneffective POST herbicide (glyphosate) was applied, horseweed biomass was 36 to 46% lower when planting green compared with early terminated cereal rye and no cover. Similarly, planting soybean in 19- and 38-cm rows reduced horseweed biomass 48 and 28%, respectively, compared with 76 cm rows. Cereal rye did not affect soybean yield; however, narrow row soybean yielded 11 to 18% higher compared with 76 cm rows pooled over 3 site-years. Soybean yield was 11% higher when an effective POST herbicide was applied. In conclusion, fall-seeded cereal rye or narrow soybean rows provided horseweed suppression compared with no cover and 76 cm rows; however, the effects of early termination did not last throughout the growing season in most cases. Delaying cover crop termination by planting green reduced horseweed biomass and density through soybean harvest, but reduced yield in 1 site-year

due to an increased incidence of white mold. These cultural practices have a positive influence on suppressing horseweed that could help with an overall horseweed management strategy; however, the use of an effective POST herbicide is still needed for complete season-long horseweed management.

Introduction

Horseweed (*Conyza canadensis* L.), a facultative winter annual, is one of the most serious weed management issues in Michigan soybean [*Glycine max* (L.) Merr.] fields. If not controlled, horseweed can reduce soybean yield 83% (Bruce and Kells 1990). In a recent Michigan grower survey 86% of participants listed horseweed as their number one weed concern (E Burns and C Sprague, Michigan State University, personal communication, 2022). This ranking follows very closely to surveys conducted by the Weed Science Society of America where researchers ranked horseweed as the second most common and troublesome weed in U.S. soybean production (Van Wychen 2019). The widespread occurrence is partially attributed to horseweed producing up to 200,000 seeds per plant. These seeds are adapted for wind dispersal through an attached pappus and can travel more than 550 km from the mother plant (Bhowmik and Bekeck 1993; Shields et al. 2006). In Michigan, horseweed emergence has shifted from fall to primarily spring and early summer emergence (Schramski et al. 2021a). Schramski et al. (2021b) reported that peak horseweed emergence (>80%) occurred when 50 to 100 growing degree days (GDDs) (base, 10 C) accumulated in the spring with adequate soil moisture, although horseweed continued to emerge throughout the summer following rainfall events. Horseweed is best managed when small in size (Loux and Johnson 2010; Mellendorf et al. 2013), but this is not always feasible with its extended and variable emergence pattern.

An obstacle in horseweed control is herbicide resistance. As of 2021, horseweed has documented resistance to at least one herbicide site of action in 18 different countries (Heap 2021). Biotypes in Michigan are resistant to acetolactate synthase (ALS) inhibitors (WSSA Group 2); photosystem II inhibitors (WSSA Group 5); photosystem II inhibitors (WSSA Group 7); glyphosate, the 5-enolpyruvate-shikimate-3-phosphate inhibitor (EPSP) (WSSA Group 9); and paraquat, a photosystem I electron diverter (WSSA Group 22), and resistance to multiple sites of action have also been reported (Heap 2021). In Michigan, most horseweed populations are resistant to the ALS inhibiting herbicides and glyphosate (Hill 2020), which limits herbicide options.

Extended horseweed emergence patterns and the increased prevalence of herbicide-resistant biotypes means that herbicides alone are not enough to protect soybean yield from horseweed competition. Integrating cover crops into the cropping system is a potential solution. Across the United States, cover crop acreage increased by 50% between 2012 and 2017, totaling nearly 6.2 million hectares in 2017. Government cost-sharing to plant cover crops, as well as the ecosystem services and weed suppression they provide are reasons for this increase (USDA ERS 2021; SARE 2021). Cover crops offer two periods of weed suppression, early on when the cover crop is actively growing, and later when the cover crop residue creates a mulch layer on the soil surface (Mirsky et al. 2013; Teasdale 1996). During the period of active growth, cover crops compete with weeds for resources such as light and nutrients, delay soil warming, and some cover crop species can produce secondary plant metabolites that inhibit weed germination (Creamer et al. 1996; Davis and Liebman 2003; Shearin et al. 2008; Teasdale and Mohler 1993, 2000; Teasdale et al. 2007) causing reductions in weed density and biomass (Haramoto 2019; Hayden et al. 2012; Werle et al. 2017). After termination, cover crop residues reduce light penetration to the soil surface, hindering weed seedling growth and development (Teasdale and Mohler 2000; Wells et al. 2013). However, cover crop residues often do not persist long enough to provide season-long weed suppression (Osipitan et al. 2018; Schramski et al. 2021b).

Cereal rye (*Secale cereale* L.) is the primary cover crop used in conjunction with soybean because of its flexible planting window, cold tolerance, high biomass production, and consistent suppression of weed biomass (Clark 2007; Hayden et al. 2012; Sherman et al. 2020). Delaying cover crop termination allows for greater biomass accumulation and improves weed suppression (Wallace et al. 2019; Cholette et al. 2018; Finney et al. 2016; Ryan et al. 2011; Smith et al. 2011). Reed et al. (2019) found delaying cover crop termination 4 to 30 d by planting green provided 94 to 181% more biomass compared with early termination. Previous research observed that fall-planted cover crops reduced spring horseweed density prior to cover crop termination in soybean (Pittman et al. 2019; Schramski et al. 2021b; Wallace et al. 2019). Though, no differences in horseweed density and biomass were observed between early termination and planting green across most site-years (Schramski et al. 2021b). However, at the time of POST herbicide application (5 wk after planting (WAP)), soybean planted green in cereal rye reduced horseweed biomass 52 to 85% more compared with early terminated cereal rye across most site-years (Schramski et al. 2021b). However, cereal rye residue, regardless of termination time, was not persistent enough to suppress horseweed through soybean harvest (Schramski et al. 2021b).

Earlier canopy closure in narrow rows can suppress weeds that escape herbicide application or that emerge late in the growing season (Mickelson and Renner 1997). Harder et al. (2007) observed reduced summer annual weed biomass and density in 19- and 38-cm rows compared with 76 cm rows following glyphosate application 3 to 5 wk after treatment (WAT).

Similarly, Hay et al. (2019) reported that planting soybean in 19- or 38-cm into an early terminated winter wheat cover reduced Palmer amaranth (*Amaranthus palmeri* S. Wats.) density 65 to 67% and biomass 83% compared with soybean planted in 76 cm rows with no cover 3 WAP. Comparable density reductions were observed 8 WAP; however, suppression was not evaluated at the end of the season. Conversely, Rogers (2017) observed no interaction between cereal rye, regardless of termination time, and soybean row width on Palmer amaranth density or biomass. However, similar research has not been conducted on the effect of narrow row soybean on horseweed suppression.

Fall-planted cereal cover crops improve early-season horseweed management, but cover residues are often not persistent enough to provide season-long horseweed suppression. Meanwhile, narrow row soybeans reduce the biomass of many weeds. Can the two practices be integrated for season-long horseweed suppression in soybean? The objectives of this research were to 1) evaluate the effects of a fall planted cereal rye terminated 1 wk before and 1 wk after soybean planting on horseweed suppression, 2) determine the contribution of soybean row width on horseweed suppression by comparing soybean planted in 19-, 38-, and 76-cm rows, and 3) compare the integrated approaches of cover crop and soybean row width with and without an effective POST herbicide application on horseweed management.

Materials and Methods

Field experiments were conducted at the Michigan State University (MSU) Agronomy Farm in Lansing, Michigan in 2020 (MSU-A = 42.6872°N, -84.4914°W) and 2021 (MSU-B = 42.6845°N, -84.4887°W; MSU-C = 42.6889°N, -84.4904°W) and at the MSU Kellogg Biological Station (KBS) near Hickory Corners, Michigan in 2021 (42.4022°N, -85.3773°W) on no-tillage fields with known populations of GR horseweed. The soil types at MSU-A and MSU-B were a Conover loam (fine-loamy, mixed, active, mesic Aquic Hapludals) with pH 6.2, 7.4 and 3.2, 2.6% organic matter, respectively, and a Colwood-Brookston loam (fine-loamy, mixed, active, mesic Typic Haplaquolls) with pH 5.9 and 2.8% organic matter at MSU-C. The soil type at KBS was a Kalamazoo loam (fine-loamy, mixed, active, mesic Typic Hapludalfs) with pH 6.8 and 1.9% organic matter.

Trials were arranged in a randomized complete block split-split plot design with four replications. Plots measured 3 m wide x 11 m long. The main plot factor was cover treatment, the subplot factor was soybean row width, and the sub-subplot factor was postemergence (POST) herbicide. The main plots consisted of three cover treatments: cereal rye terminated 1 wk prior to planting (early termination), cereal rye terminated 1 wk after soybean planting (planting green), or no cover. Cereal rye was terminated by applying glyphosate (Roundup PowerMAX; Bayer CropScience, St. Louis, MO) at 1.27 kg ae ha^{-1} plus ammonium sulfate (AMS) (Actamaster; Loveland Products, Inc., Greeley, CO) at 2% w w^{-1}. The subplots consisted of three soybean row widths: 19-, 38-, and 76-cm. The sub-subplot factors were two POST herbicide application strategies: an effective POST herbicide application for GR horseweed and other weed control, or a noneffective POST herbicide application to control other weeds, but not GR horseweed. The effective POST herbicide treatment consisted of glufosinate (Liberty; BASF Corporation, Research Triangle Park, NC) at 0.66 kg ai ha^{-1} plus 2,4-D choline (Enlist One; Corteva Agriscience, Indianapolis, IN) at 1.12 kg ae ha^{-1} plus AMS at 2% w w^{-1}. The non-effective POST herbicide application was glyphosate at 1.27 kg ae ha^{-1} plus AMS at 2% w w^{-1}.

'Wheeler' rye was drilled at 67 kg ha^{-1} in 19 cm rows using a no-till drill (John Deere, Moline, IL) the fall prior to data collection. Dates for all field operations are in Table 2.1. The following spring, cereal rye was terminated, and main plots were established one week prior to (early termination) or one week after (planting green) planting soybean. Glyphosate, glufosinate, and 2,4-D choline-resistant soybean, ‘P25T09E’ or ‘P24T35E’, was planted at 500,000-, 437,500-, 375,000-, or 500,000-, 450,000-, and 387,500- seeds ha^{-1}, respectively in 2020 and 2021, in 19-, 38-, and 76-cm rows. Higher seeding rates were used in 2021 due to dry conditions at planting. POST herbicide applications were made 4 to 6 WAP when soybean was at the V2 to V4 growth stage in the no cover. All herbicide applications were made using a tractor-mounted, compressed air sprayer calibrated to deliver 177 L ha^{-1} at 207 kPa of pressure through 11003 AIXR nozzles (TeeJet, Spraying Systems CO., Wheaton, IL 60187).

Throughout the growing season air temperature and precipitation data were collected from the Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, MI) stations closest to each trial (data not shown). Temperature and precipitation 30-yr averages were collected from the National Oceanic and Atmospheric Administration (https://www.noaa.gov) (data not shown).

Data Collection. At each cereal rye termination timing, aboveground cereal rye biomass, weed density, and weed biomass was collected from two randomly placed 0.25 m^2 quadrats per plot. In addition to GR horseweed, shepherd’s purse [*Capsella bursa-pastoris* (L.) Medic], common chickweed [*Stellaria media* (L.) Vill.], whitlowgrass (*Draba verna* L.), purple deadnettle (*Lamium purpureum* L.), henbit (*Lamium amplexicaule* L.), and dandelion (*Taraxacum officinale* F. H. Wigg.) were present at early termination at all sites in 2021. In 2020, only GR horseweed was present at early termination. Subsamples of cereal rye biomass were analyzed for C:N ratios by A&L Great Lakes Laboratories, Inc. (Fort Wayne, Indiana) using a TruMac CNS Macro

Analyzer (LECO Corporation, St. Joseph, MI). Percent ground cover was measured using line-transects (Laflen et al. 1981) laid diagonally across each plot at the planting green termination. The presence of cover crop, GR horseweed, other weeds, or no vegetation was recorded at every 30 cm point along a 11 m transect and converted to a percentage. When soybean reached the V2 growth stage in the no cover plots, percent ground cover was measured again with the addition of marking the presence of soybean. At the time of POST herbicide application and prior to soybean harvest, GR horseweed density and biomass were collected from two randomly placed 0.25 m^2 quadrats per plot. Height of 20 random plants per plot was also measured. Biomass samples were dried for approximately 7 d at 65 C and weighed. Soybean growth and development was evaluated bi-weekly until soybean reached R1 stage based on the hybrid method (Pedersen 2009).

Soil moisture was measured with a Field Scout TDR 300 Soil Moisture Meter (FieldScout, Spectrum Technologies, Aurora, IL) by collecting five measurements per plot at a depth of 7.6 cm at the time of soybean planting and at 4-6 WAP. Prior to soybean planting and again after soybean harvest, soil samples (30-cm depth) from each cover treatment were collected and analyzed for soil nitrate levels (Soil and Plant Nutrient Laboratory, Michigan State University). Soybean was harvested using a small-plot research combine (Massey-Ferguson 8XP, AGCO, Duluth, GA). Yields were adjusted to 13% moisture.

Statistical Analysis. Data analysis was performed using PROC GLIMMIX in SAS OnDemand (SAS Institute, 2021) at $\alpha = 0.05$. The statistical model consisted of cover treatment, soybean row width, POST herbicide application, and their interactions as fixed effects. Each year-location combination was considered an environment sampled at random from a population as suggested by Carmer et al. (1989). Environment (individual year and location), replication nested within environments, the interaction between cover treatment and replication nested within environments, and the interaction between cover treatment and soybean row width nested within environments were considered random effects. Replications were used as an error term for testing the effects of environment, and data were combined over all environments for each measurement except for soybean yield. Normality of residuals were examined using the UNIVARIATE procedure ($\alpha \leq 0.05$). Squared and absolute value residuals were examined with Levene's test to confirm homogeneity of variances ($\alpha \leq 0.05$). Data were combined over main effects when interactions were not significant. Treatment means were separated using Fisher's Protected LSD at $\alpha \leq 0.05$ when ANOVA indicated a significant main effect or interaction. Non-transformed means for horseweed density and biomass are presented because the arcsine and square root transformation did not improve the normality of the data.

Results and Discussion

Early-Season Horseweed Suppression. At the time of early termination, cereal rye reduced horseweed density and biomass by 3-fold compared with the no cover plots, where 31 plants m^{-2} and 3 g m^{-2} of horseweed biomass were present (data not shown). Additionally, in 2021 cereal rye reduced biomass of other weeds by 76% compared with no cover (42 g m^{-2}); however, other weed density was not affected by cereal rye (14 to 20 plants m^{-2}). Horseweed diameter at the time of early termination averaged 2 cm and there was not a substantial amount of horseweed biomass present. Although there can be some fall horseweed emergence, in Michigan annual cropping systems peak horseweed emergence (>80%) generally occurs when 50 to 100 GDDs (base, 10 C) have accumulated and there is adequate soil moisture (Schramski et al. 2021b).

However, horseweed can continue to emerge throughout the summer following rainfall events. In our research, GDD accumulation from the first of the year through early termination was 138-146 GDDs (base, 10 C) (Table 2.1); however, due to reduced precipitation in April (28-73 mm) and May (6-110 mm), peak emergence did not take place until later. Total precipitation across site-years in April was 10 to 50 mm less and 0 to 87 mm lower in May than the 30-yr average (data not shown).

Cereal rye at the time of early termination was at Feekes stage 8 with 1,842 kg ha^{-1} of biomass and GDD accumulation from rye planting to termination ranged between 418 to 561 GDDs (base, 4.4 C) (Table 2.1). Previous research has reported slightly lower (756-1,359 kg ha^{-1}) aboveground biomass in early terminated cereal rye in Michigan, likely due to less GDD accumulation (315-326 GDD) (base, 4.4 C) (Schramski et al. 2021b). Schramski et al. (2021b) reported horseweed biomass was reduced 59 to 70% by cereal cover crops compared with no cover, similar to what was observed in our research. Christenson (2015) reported slightly higher horseweed biomass reductions of 84 to 92% when cereal rye was terminated early compared with no cover.

At soybean planting, mean volumetric soil moisture content was 9-11%. Moisture was not affected by cereal rye, regardless of termination time because of conditions were dry (data not shown). At planting, cereal rye was terminated for 1 wk in the early terminated plots and in Feekes stage 10.5 in the planting green plots. Prior to soybean planting, precipitation was 6 to 110 mm in May across site-years (data not shown). Previous research has reported cereal rye, regardless of termination time, did not influence soil moisture at soybean planting (Rogers 2017; Schramski et al. 2021b). Cereal rye, regardless of termination time, reduced soil nitrate (NO_3-N) 8 to 14 kg N ha^{-1} compared with no cover (24 kg N ha^{-1}) at soybean planting. Similarly, Hill et al. (2016) found that a cereal rye cover crop reduced soil inorganic N up to 13 kg N ha^{-1} at the time of dry bean planting. By reducing plant available N, cereal rye may be more competitive and suppress weeds, an asset in legume crops such as soybean that fix their own N and grow well in low soil N conditions (Wells et al. 2013).

Delaying termination 15 to 20 d by planting green resulted in an additional 230-252 GDDs compared with early termination (base, 4.4 C). Cereal rye was at Feekes growth stage 10.5.1 (Table 2.1), which increased cover biomass by 132% to 4,280 kg ha^{-1} and provided 12% more ground cover compared with early termination (Table 2.2). Similarly, Reed et al. (2019) reported delaying cover crop termination 4 to 30 d by planting green produced up to 181% greater cover-crop biomass production compared with early termination. During this timeframe, delaying termination by planting green increased the C:N ratio of cereal rye to 42:1 compared with 27:1 for the early termination timing (Table 2.2). In contrast, Schramski et al. (2021b) reported C:N ratios below 24:1 when cereal rye was terminated early at Feekes growth stage 5 to 6, and C:N ratios of 16:1 and 30:1 when terminated a week after planting at Feekes 10.4 to 10.5. We likely observed higher C:N ratios due to slightly further development of cereal rye at the time of cover termination. The optimum C:N ratio is 24:1 as this is the ideal diet for soil microorganisms (USDA NRCS 2011). Plant residue below this ratio will decompose relatively quick compared with C:N ratios larger than 24:1 (Jahanzad et al. 2016; Odhiambo and Bomke 2001; USDA NRCS 2011), providing a potentially longer period of horseweed suppression by planting green. Greater cover biomass production and higher C:N ratios likely contributed to N immobilization in the cereal rye treatments at soybean planting.

Although we observed a significant increase in cover biomass by planting green, we did not see an increase in horseweed suppression at the time of planting green termination compared with early termination. This was likely due to delayed horseweed emergence as a result of the dry conditions mentioned earlier. At planting green termination, cereal rye, regardless of termination time, reduced horseweed density and biomass. Cereal rye reduced horseweed density 57 to 65% compared with no cover (Table 2.2). Likewise, previous studies found cover crops reduced horseweed density 41 to 97% at the time of termination compared with no cover (Schramski et al. 2021b; Essman et al. 2020; Wallace et al. 2019; Pittman et al. 2019). Horseweed biomass was reduced 71% by planting into cereal rye, regardless of termination time, compared with no cover (Table 2.2). Similarly, Schramski et al. (2021b) reported that a cereal cover, regardless of termination time, reduced horseweed biomass at the time of termination. Less variable plant sizes and smaller horseweed plants at the time of preplant soybean herbicide application were linked with residue from a cereal rye cover crop (Wallace et al. 2019). Additionally, horseweed size at the time of herbicide application impacts selection intensity for glyphosate resistance within resistant populations (Wallace et al. 2019). The ability of cereal rye to reduce horseweed size could reduce the selection pressure for resistant individuals and provide growers greater horseweed control at the time of burndown application.

Mid-Season Horseweed Suppression. In late June, approximately 4 WAP, when soybean was at the V2 growth stage in the no cover treatment, cereal rye ground cover was 12% higher by planting green (51%) compared with early termination (data not shown). Horseweed ground cover was similar between early terminated cereal rye and planting green (7-9%) but was significantly lower in both compared with no cover (17%) (data not shown). Additionally, soybean planted in narrow rows reduced horseweed ground cover 5% compared with 76 cm rows (14%) (data not shown). At this time, soybean was at VC in the planting green treatments, 1 to 2 growth stages behind the early terminated and no cover treatments. This delay lasted until R1 soybean. Delays in soybean growth were likely due to N deprivation or shading from planting green cover crop residue. Cereal rye did not affect soil moisture which ranged from 13-15%, 4 to 6 WAP (data not shown). Wells et al. (2013) reported that cereal rye created an exceedingly low N environment that resulted in N deprivation in pigweed and in soybean before nodulation; however, soybean plants recovered from N deficit once nodulation became active, whereas pigweed continued to be negatively affected and reductions in pigweed density were observed.

At the time of POST herbicide application, 4 to 6 WAP, there was an interaction between cover treatment and soybean row width on horseweed density and biomass. All treatment combinations reduced horseweed density compared to soybean planted in 76 cm rows with no cover. However, horseweed density was reduced most (67-80%) when soybean was planted in narrow rows into cereal rye, regardless of termination time (Table 2.3). Similarly, Hay et al. (2019) reported soybean planted in 19- and 38-cm rows into an early terminated winter wheat cover crop reduced Palmer amaranth density 49 to 55% compared with soybean planted in 76 cm rows with no cover, 8 WAP. Earlier soybean canopy closure in narrower rows likely suppressed horseweed compared with 76 cm rows. In contrast, Schramski et al. (2021b) and Wallace et al. (2019) reported no reduction in horseweed density at the time of POST herbicide application when a cereal rye cover crop was present.

Horseweed biomass was 71 to 90% lower when soybean was planted into cereal rye, across all row widths, compared with no cover (Table 2.3). The greatest biomass reductions were observed by planting green in all row widths compared with early terminated cereal rye in 76 cm rows and no cover for all row widths. Similarly, Schramski et al. (2021b) reported soybean planted green into a cereal rye or winter wheat cover crop reduced horseweed biomass 46 to 93%

compared with no cover at the time of POST herbicide application. Averaged over soybean row width, horseweed height was 8- and 10-cm shorter in early terminated cereal rye and planting green plots compared with no cover, respectively (Table 2.3). Peak emergence was estimated to have taken place between planting green termination and POST herbicide application (data not shown). Horseweed density was similar between early terminated cereal rye and planting green, but the planting green cover was more competitive and able to reduce horseweed growth more. Reductions in horseweed size at the time of POST herbicide application may also improve herbicide effectiveness when managing GR horseweed.

Late-Season Horseweed Suppression. Prior to soybean harvest, horseweed density was 42% lower in the planting green treatments compared with no cover, regardless of soybean row width or POST herbicide treatment (Table 2.4). In contrast, Schramski et al. (2021b) reported that cereal cover crop residue did not persist long enough to provide horseweed suppression through soybean harvest. This was likely due to lower C:N ratios (<30:1) and less biomass production in that study. While there were no interactions with cover treatment, there was an interaction between soybean row width and POST herbicide treatment on horseweed density. Soybean planted in 19- and 38-cm rows reduced horseweed density 59 and 32%, respectively, compared with 76 cm rows when a noneffective POST herbicide was applied (Table 2.4). The addition of an effective POST herbicide resulted in greater reductions in horseweed density in 19 cm rows than in 76 cm rows. Additionally, there was an interaction between cover treatment and POST herbicide, as well as soybean row width and POST herbicide on horseweed biomass. When a noneffective POST herbicide was applied, horseweed biomass was 36 to 46% lower by planting green compared with early terminated cereal rye and no cover (Table 2.4). Greater reductions

(69%) in horseweed biomass were observed when planting into cereal rye, regardless of termination time, compared with no cover (39 g m^{-2}) when an effective POST herbicide application was made. In contrast, Schramski et al. (2021b) did not observe an effect of cover crop or termination time on horseweed biomass at soybean harvest when an effective POST herbicide was applied. Planting soybean in 19- and 38-cm rows reduced horseweed biomass 48 and 26% compared with 76 cm rows, respectively, when a noneffective POST was applied (Table 2.4). Planting soybean in 19 cm rows reduced horseweed biomass more compared with 76 cm rows when an effective POST herbicide treatment was applied. In contrast, previous research found narrower rows (19- and 38-cm) did not reduce summer annual weed biomass prior to soybean harvest (Harder et al. 2007). Similarly, Rogers (2017) reported no difference in Palmer amaranth control between 19- and 76-cm rows at soybean harvest.

Horseweed height was 13 to 18 cm shorter in soybean planted in narrower rows compared with 76 cm rows (66 cm) prior to soybean harvest (Table 2.4). Moreover, there was an interaction between cover and POST herbicide treatments. Planting green reduced horseweed height 37-43% compared with early termination and no cover when a noneffective POST herbicide was applied. Regardless of termination time, cereal rye reduced horseweed height 58 to 64% compared with no cover when an effective POST herbicide was applied (36 cm) (Table 2.4). Horseweed biomass reductions were also observed with a cereal rye cover compared with no cover with an effective POST herbicide application. In this study, horseweed plants with flower heads were often above the soybean canopy in treatments with a noneffective POST herbicide application or when soybean was planted in 76 cm rows (personal observation). Horseweed plants that produce flower heads above the soybean canopy in August to October can contribute upwards of 88% to total seed production (Davis and Johnson 2008). Shorter horseweed plants at the end of the growing season may result in less seed production and reduce the seed bank.

Soybean Yield. Due to a high incidence of white mold (*Sclerotinia sclerotiorum* (Lib.) de Bary), MSU-C was separated from the remaining site-years. Combined over MSU-A, MSU-B and KBS, cereal rye, regardless of termination time, did not affect soybean yield which ranged from 4,077 to 4,362 kg ha^{-1} (Table 2.5). Likewise, others reported no effect on soybean yield from a cereal rye cover crop (Pittman et al. 2019; Schramski et al. 2021b). In addition, cereal rye did not affect NO_3-N concentrations (Table 2.5), supporting what Hill et al. (2016) reported in dry beans that were planted into early terminated cereal rye. We also observed that soybean planted in narrower rows yielded 11 to 18% higher compared with soybean in 76 cm rows (Table 2.5). Harder et al. (2007) reported soybean yielded greater in 19 cm rows compared with 38 and 76 cm rows. Furthermore, by applying an effective POST herbicide application yield was 11% higher compared with a noneffective POST herbicide application (Table 2.5).

There was a cover treatment and POST herbicide application interaction at MSU-C. When a noneffective POST was applied, yield was similar among cover treatments (3,643-3,934 kg ha^{-1}) likely due to greater horseweed competition. However, when an effective POST herbicide was applied, early terminated cereal rye yielded 10% more than planting green (3,842 kg ha^{-1}) (Table 2.5). This was likely due to a high incidence of white mold within the planting green treatments. There was also an interaction between soybean row width and POST herbicide application at MSU-C. Soybean yield was lower when soybean was planted in 76 cm rows with a noneffective POST herbicide application compared with all other treatments (Table 2.5). Whereas, yield was similar in narrower rows, regardless of POST herbicide application (3,911-

4,162 kg ha^{-1}). At MSU-C, there was a high incidence of white mold in the planting green and narrow row soybean treatments. This was likely due to above average rainfall in June, July, and August that totaled 356 mm compared with the 30-yr average of 259 mm (data not shown). As a result, planting green and narrow soybean rows created an environment beneath the closed canopy favorable for sclerotia germination. This site was also surrounded by corn and a woodlot that may have limited air flow creating a greater risk for infection. When planting soybean in narrow rows, there was a higher incidence of white mold compared with 76 cm rows, which likely diminished the yield advantage of narrow rows. Previous research has reported higher white mold disease severity in narrow row soybean causing significant yield loss (Grau and Radke 1984).

Overall, planting soybean in narrow rows into a cereal rye cover crop is a promising horseweed management tool; however, growers should be cautious of favorable environmental conditions for white mold development created by planting green or narrow soybean rows. The addition of a cereal rye cover crop reduced horseweed emergence and density at cover crop termination. By the time of POST herbicide application, narrow row soybean planted into cereal rye, regardless of termination time, reduced horseweed density 67 to 80% compared with 76 cm rows with no cover, whereas soybean planted green in narrow rows reduced horseweed size 90% compared with 76 cm rows with no cover. Reductions in horseweed size at the time of burndown and POST herbicide applications may improve herbicide effectiveness and potentially reduce the selection pressure for further development of herbicide-resistant populations. In contrast to previous research, planting green suppressed horseweed through soybean harvest, likely due to higher biomass (4,280 kg ha^{-1}) and later growth stages at termination, thus resulting in a more persistent residue due to a higher C:N ratio. At soybean harvest, horseweed biomass was reduced

91% or more by planting into cereal rye or by planting in 19 cm rows when an effective POST herbicide application was made. Narrow row soybean or soybean planted green with an effective herbicide program can be implemented as an additional horseweed management strategy for early- and late-season horseweed suppression.

APPENDIX

APPENDIX
Tables

Table 2.1. Cereal rye seeding and termination dates, GDDs[a,b,c] until cereal rye termination, soybean planting, POST herbicide application, and soybean harvest dates for the four experimental locations.

	Site			
Operation	MSU-A	MSU-B	MSU-C	KBS
Cereal rye seeding	October 4, 2019	October 16, 2020	November 9, 2020	October 12, 2020
Early termination	May 24, 2020	May 13, 2021	May 13, 2021	May 14, 2021
GDDs (base, 4.4C)	561	521	418	560
GDDs (base, 10 C)	138	142	142	146
Soybean planting	June 1, 2020	May 25, 2021	May 25, 2021	May 24, 2021
Planting green termination	June 8, 2020	June 2, 2021	June 2, 2021	June 3, 2021
GDDs (base, 4.4 C)	791	764	661	812
GDDs (base, 10 C)	287	289	289	300
POST application	June 29, 2020	June 24, 2021	July 7, 2021	July 1, 2021
Soybean harvest	October 31, 2020	October 18, 2021	October 18, 2021	October 19, 2021

[a]Abbreviation: GDDs, growing degree days; MSU, Michigan State University; KBS, Kellogg Biological Station.
[b]GDDs (base, 4.4 C) accumulated from the time of cereal rye planting in the fall until termination.
[c]GDDs (base, 10 C) accumulated from January 1 until cover termination for horseweed emergence.

Table 2.2. Cereal rye biomass and C:N ratios at each termination time, and cereal rye ground cover and the effect of cereal rye on horseweed density and biomass at planting green termination.

Cover treatment	Cereal rye			Horseweed	
	Biomass	C:N ratio	Groundcover	Density	Biomass
	—— kg ha^{-1} ——		—— % ——	—— plants m^{-2} ——	—— g m^{-2} ——
No cover	NA[a]	NA	NA	49 a	7 a
Early termination	1842 b[b]	27:1 b	46 b	17 b	2 b
Planting green	4280 a	42:1 a	58 a	21 b	2 b
Effects (P-values)					
Cover treatment	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a]Abbreviations: NA, not applicable.

[b]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

Table 2.3. Interaction between cover treatment and soybean row width on horseweed density, biomass, and height at the time of POST herbicide application, 4-6 wk after planting (WAP).

Cover treatment	Row width (cm)	Density	Biomass	Height[b]
		—— plants m^{-2} ——	—— g m^{-2} ——	—— cm ——
No cover	19	51 bc[a]	43 b	19
	38	51 bc	44 b	19
	76	116 a	63 a	19
Early termination	19	32 cd	13 cde	11
	38	38 cd	14 cd	11
	76	60 b	18 c	11
Planting green	19	23 d	6 f	8
	38	34 cd	8 ef	9
	76	43 bc	9 def	10
Effects (P-values)				
Cover treatment		<0.0001	<0.0001	<0.0001
Row width		0.0018	0.0173	0.8843
Cover treatment x row width		0.0137	0.0386	0.8651

[a]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.
[b]The main effect of cover treatment was significant for horseweed height. Horseweed height was reduced 8 and 10 cm by terminating cereal rye early (11 cm) and planting green (9 cm), respectively.

Table 2.4. Interactions between cover treatment and POST herbicide application, and soybean row width and POST herbicide treatment on horseweed density, biomass, and height at soybean harvest.

Cover treatment	POST treatment	Density[a]	Biomass	Height[b]
		—— plants m^{-2} ——	—— g m^{-2} ——	—— cm ——
No cover	Noneffective	18	153 a	108 a
Early termination		16	129 a	99 a
Planting green		11	82 b	62 b
No cover	Effective	6	39 c	36 c
Early termination		3	12 d	13 d
Planting green		2	12 d	15 d
Row width (cm)				
19	Noneffective	9 c[c]	83 c	81
38		15 b	119 b	91
76		22 a	161 a	98
19	Effective	2 e	10 e	14
38		3 de	15 de	15
76		6 cd	37 d	35
Effects (P-values)				
Cover treatment		0.0202	0.0001	<0.0001
Row width		0.0064	0.0033	0.0315
POST		<0.0001	<0.0001	<0.0001
Cover treatment x row width		0.6906	0.7995	0.7447
Cover treatment x POST		0.3782	0.0148	0.0002
Row width x POST		0.0148	0.0158	0.4184
Cover treatment x row width x POST		0.8387	0.4891	0.3798

[a]The main effect of cover treatment was significant for horseweed density. Density was reduced 42% by planting green (7 plants m^{-2}) compared with no cover (12 plants m^{-2}).

[b]The main effect of row width was significant for horseweed height. Height was reduced 13 to 18 cm by planting in narrower rows (48-53 cm).

[c]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$

Table 2.5. Main effects of cover treatment, soybean row width, and POST herbicide treatment on soybean yield and soil nitrate at harvest.

	Soil nitrate	Soybean yield	
Main effects	Combined sites	3 site-years[a]	MSU-C[c,d,e]
Cover treatment	—— kg N ha^{-1} ——	——— kg ha^{-1} ———	
No cover	32	4098	3930
Early termination	30	4362	4010
Planting green	27	4077	3888
Row width (cm)			
19	NA[a]	4493 a[b]	4095
38	NA	4237 a	4036
76	NA	3807 b	3697
POST			
Noneffective	NA	3960 b	3793
Effective	NA	4399 a	4092
Effects (P-values)			
Cover treatment	0.1189	0.1315	0.6274
Row width	NA	0.0099	0.2307
POST	NA	0.0004	0.0080
Cover treatment x row width	NA	0.7871	0.1171
Cover treatment x POST	NA	0.9215	0.0353
Row width x POST	NA	0.7487	0.0166
Cover treatment x row width x POST	NA	0.9707	0.2305

[a]Abbreviations: NA, not applicable, 3 site-years = MSU-A, -B, and KBS.

[b]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

[c]There was a high incidence of white mold in the planting green and narrow row treatments at MSU-C, therefore it was separated from the remaining site-years.

[d]There was an interaction between cover treatment and POST. When a noneffective post was applied, yield was similar between cover treatments (3,643-3,934 kg ha^{-1}). However, when an effective POST was applied, early terminated cereal rye (4,217 kg ha^{-1}) yielded 10% higher compared with planting green (3,842 kg ha^{-1})

Table 2.5 (cont'd).

[e]There was an interaction between row width and POST. Yield was similar in narrow rows, regardless of POST herbicide application (3,911-4,162 kg ha^{-1}); however, yield was 21% higher in 76 cm rows with an effective POST (4,055 kg ha^{-1}) compared with 76 cm rows with a noneffective POST application (3,339 kg ha^{-1}).

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CHAPTER III

CONTRIBUTIONS OF SHADING, SOYBEAN ROW-WIDTH, AND PLANTING GREEN ON HORSEWEED MANAGEMENT COMPARED WITH SOIL-APPLIED RESIDUAL HERBICIDES

Abstract

Glyphosate-resistant (GR) horseweed is a problematic weed for Michigan soybean growers. Additionally, rosette- and upright- horseweed growth types have been observed co-emerging during mid- to late-summer in several Michigan fields. In the greenhouse, shade levels from 35 to 92% reduced rosette and upright horseweed biomass 31 to 99% compared with the upright growth type grown under 0% shade. Greater reductions in biomass occurred under 69 and 92% shade. Thus, increased shading by planting in narrow rows and/or planting green into cereal rye may improve horseweed suppression. A field experiment conducted over three site-years compared the effect of fall-planted cereal rye terminated with glyphosate 1 wk after planting (planting green) with a preemergence (PRE) residual herbicide program (glyphosate + 2,4-D choline + flumioxazin + metribuzin) on horseweed control in soybean planted in three row widths (19-, 38-, and 76-cm). Planting green or applying a residual herbicide program across all row widths reduced horseweed biomass 86 to 91% and 95 to 99%, respectively, compared with soybean planted with no cover in 76 cm rows, 4 to 6 wk after planting (WAP). At soybean harvest, when a noneffective postemergence (POST) herbicide (glyphosate) was applied horseweed biomass was 42 and 81% lower by planting green or applying a residual herbicide program compared with no cover, respectively. Similarly, planting soybean in 19 cm rows reduced horseweed biomass compared with 38- and 76-cm rows. When an effective POST program was applied, similar horseweed biomass reductions were observed by planting green or

applying a residual herbicide across all row widths. Additionally, soybean yield and economic returns were similar between planting green and applying a residual herbicide in 1 of 2 site-years. Integrating planting green and an effective POST herbicide program offers an alternative horseweed management strategy to applying a residual preemergence herbicide program.

Introduction

Horseweed (*Conyza canadensis* L) is one of the most problematic weeds in Michigan and is ranked as the second most common and troublesome weed in U.S. soybean [*Glycine max* (L.) Merr.] production (E Burns and C Sprague, Michigan State University, personal communication, 2021; Van Wychen 2019). Horseweed can tolerate a variety of environmental conditions and thrives in undisturbed areas, such as reduced-tillage or no-tillage production systems, making management more challenging (Weaver 2001). Each plant can produce up to 200,000 seeds that have an attached pappus adapted for wind dispersal for up to 550 km from the source plant (Bhowmik and Bekeck 1993; Shields et al. 2006). Considered a facultative winter annual, horseweed emergence is observed throughout the growing season (Buhler and Owen 1997; Tozzi and Van Acker 2014; Weaver 2001). In Michigan, Schramski et al. (2021b) reported horseweed plants exhibited a summer annual life cycle with initial emergence occurring between late-April and mid-May and peak emergence (>80%) occurred when 50 to 100 growing degree days (GDDs) (base, 10 C) accumulated with adequate soil moisture. Additional emergence occurred through the spring and early summer and late emergence into July followed rainfall events. Both rosette- and upright- horseweed growth types have been observed co-emerging in mid- to late summer in Michigan (Schramski et al. 2021a). Horseweed is best managed when small (Loux and Johnson 2010; Mellendorf et al. 2013), however because of its extended and variable emergence pattern, this has complicated management efforts due to variability in size.

Horseweed germination is not affected by light intensity or quality (Gorski et al. 1977; Nandual et al. 2006); however, decreasing light intensity from 100 to 25% of full sunlight has reduced biomass of rosette horseweed (Bekech 1988). In the same study horseweed rosettes eventually bolted, and the average plant height decreased from 192 to 92 cm as shade levels increased. Weed biomass reductions in other weed species are also observed as irradiance levels are reduced. Steckel et al. (2003) reported each additional increase in shade (0, 40, 68, and 99%) reduced common waterhemp (*Amaranthus rudis* Sauer) biomass by 24, 49, and >99% for an early emerging cohort (May) and by 37, 51, and 99% for a later emerging cohort (June). However, common waterhemp height was similar among shade treatments, except with the 99% shade level, regardless of emergence cohort (Steckel et al. 2003). Similarly, Bello et al. (1995) reported velvetleaf (*Abutilon theophrasti* Medik.) biomass, branch number, leaf number, and plant height were consistently lower when grown under 76% shade compared with 0 and 30% shade.

One way to increase shade levels and lower light interception under a canopy is to plant soybean in narrow rows. Narrow row soybean produces more biomass and provides earlier canopy development, reducing weed emergence and growth. This is a result of the soybean canopy intercepting the solar radiation that is needed to stimulate weed seed germination and weed growth (Yelverton and Coble 1991). Summer annual weed biomass and density were lower when soybean was planted in 19- and 38-cm rows compared with 76-cm rows, 3 to 5 wk after glyphosate application, as a result of earlier canopy closure that increased leaf area index (LAI) (Harder et al. 2007). Similarly, Rich and Renner (2007) reported eastern black nightshade

(*Solanum ptycanthum* Dun.) biomass was lower in soybean planted in 19- compared with 76-cm rows; however, density was more variable and was often not affected.

Horseweed is resistant to at least one herbicide site of action in 18 countries (Heap 2021). However, in Michigan glyphosate-resistant (GR) (WSSA Group 9) horseweed biotypes are the most widespread and there are numerous biotypes that are also resistant to the acetolactate synthase (ALS) inhibitors (WSSA Group 2) (Hill 2020). Horseweed management requires effective control of plants prior to planting and a residual soil-applied herbicide to control later emerging plants (Loux et al. 2006). Previously, preplant (PP) and PRE glyphosate applications were used to control horseweed in soybean. However, the widespread occurrence of GR and multiple-resistant horseweed has greatly reduced glyphosate effectiveness and limited options for control with PRE and postemergence (POST) herbicides without the use of newer herbicide-resistant soybean traits. For example, Simpson et al. (2017) reported that the addition of dicamba (WSSA Group 4) or 2,4-D choline (WSSA Group 4) to glyphosate improved horseweed control to 93 and 85%, respectively, compared with glyphosate alone (54%). Additionally, glufosinate (WSSA group 10) alone or in combination with 2,4-D choline resulted in 85 and 92% control, respectively, 4 wk after a preplant application. These herbicides could not be used in soybean without the development of herbicide-resistant traits. The use of PRE residual herbicides such as metribuzin (WSSA group 5), flumioxazin, or sulfentrazone (WSSA group 14) provide horseweed control for up to 8 wk after application (Davis et al. 2007, 2009; Eubank et al. 2008; Steckel et al. 2006). However, residual herbicides often have lengthy rotation intervals due to their persistence in the soil. In a diverse agricultural state, such as Michigan, rotation restrictions limit grower options for season-long horseweed control. Therefore, additional management strategies are needed.

Recently, research has been focused on integrating cover crops as a weed management tool. Cover crops suppress weeds by competing for light and nutrients, and specific cover crop species generate secondary metabolites that inhibit weed germination causing reductions in weed density and biomass (Creamer et al. 1996; Davis and Liebman 2003; Haramoto et al. 2019; Hayden et al. 2012; Shearin et al. 2008; Teasdale and Mohler 1993, 2000; Teasdale et al. 2007; Werle et al. 2017). The primary cover crop used in soybean is cereal rye [*Secale cereale* (L.)] because of its flexible planting window, cold tolerance, vast amounts of biomass production, and consistent suppression of weeds (Clark 2007; Hayden et al. 2012; Sherman et al. 2020). In several studies, fall-planted cereal rye reduced horseweed density and/or biomass compared with no cover prior to cover crop termination in the spring (Pittman et al. 2019; Schramski et al. 2021b; Wallace et al. 2019). Other studies compared cereal rye terminated prior to soybean planting to preplant residual herbicides on horseweed management. Schramski et al. (2021c) reported that a preplant application of flumioxazin + metribuzin provided greater horseweed suppression than early terminated cereal rye, 5 wk after planting (WAP). Similarly, Essman et al. (2020) observed greater reductions in horseweed density in June when a preplant residual herbicide was applied compared with early terminated cereal rye with a PRE herbicide application of flumioxazin. However, there can be lengthy rotation intervals for residual herbicides that may impact a farmer's crop rotation.

Previous research found that weed suppression by cereal cover crops improves with increasing cover crop biomass (Finney et al. 2016; Ryan et al. 2011b; Smith et al. 2011). One way to increase cover crop biomass is by planting green. Planting green is the agronomic practice of planting into a growing cover crop, allowing it to accumulate more cover biomass, and terminating it after planting (SARE 2021). Mirsky et al. (2011) reported that cereal rye biomass increased 37% with each 10-d delay. Additionally, Schramski et al. (2021b) reported soybean planted green into cereal rye reduced horseweed biomass 52 to 85% compared with early terminated cereal rye. However, cereal rye residue did not persist long enough, regardless of termination time, to provide season-long horseweed suppression. Currently, there is a lack of research on horseweed management comparing the practice of planting green with preplant residual herbicides.

The extended and variable emergence of horseweed and prevalence of herbicide-resistant biotypes has created many challenges in the management of horseweed. Preplant residual herbicide applications provide excellent control of horseweed; however, this increases the selection pressure for horseweed that is resistant to more sites of action and there are often lengthy rotation intervals that may limit grower options. Fall-planted cereal cover crops improve early-season horseweed management; however, cover residue is often not persistent enough to provide season-long horseweed suppression. Additionally, it has been reported that narrow soybean rows contribute to reductions in summer annual weed density and biomass. Many studies have investigated the effects of fall-seeded cover crops on horseweed management, but research is absent on integrating a cereal rye cover crop and narrow soybean rows. Therefore, the objectives of this research were to 1) determine the effect of shade on the growth of rosette and upright horseweed growth types, and 2) examine horseweed suppression when planting green in combination with narrow soybean rows compared with a PRE residual herbicide.

Materials and Methods

Greenhouse Experiment. Horseweed seed collected from Lansing, MI (42.6845°N, -84.4887°W) was used to generate two growth types, rosette and upright. Horseweed seeds were planted on the surface of 30 x 30 cm flats filled with potting media (Suremix Perlite, Michigan Grower Products, Inc., Galesburg, MI) and watered. Flats were placed in a vernalization chamber set at 4 C with 8 h photoperiod for 4 wk to stimulate the upright growth type. After 4 wk, vernalized flats and flats planted with seed from the same parent plant (to generate the rosette siblings) were placed in the greenhouse set at 25 ± 5 C, with a midday light intensity of 1,000 μmol m^{-2} s^{-1}, and a 16 h photoperiod. Upright and rosette flats were subjected to four shade treatments: 0, 30, 60, and 90%. Shade environments were created by covering structures with forest green colored woven shade cloth (Agriculture Solutions, Strong, ME) for the 30 and 60% shade treatments, and with a black shade cloth (Shatex Corporation, Delta, BC) rated for 90% shade. Three wk after emergence, seedlings were transplanted into 10 x 10 x 12 cm pots filled with potting media, one horseweed plant pot^{-1}. Photosynthetically active radiation (PAR) was measured at plant height using a MultispeQ (PhotoSynQ, East Lansing, MI). PAR was converted to a percent of the nonshaded control to determine the estimated shade % from each cloth. Based on weekly PAR measurements, actual percent shade for the 30%, 60%, and 90% cloth was 35%, 69%, and 92%, respectively. Plants were watered and fertilized as needed to promote optimum plant growth. Aboveground biomass was harvested at 6, 7, 8, and 9 wk after planting. Biomass was dried for 7 d at 60 C and weighed. Dry weights were converted to a percent of the final weight of the no shade (0%) upright growth type. Horseweed height and diameter were collected at 9 WAP. All treatments were replicated five times and repeated in time.

Field Experiment. Field experiments were conducted at the Michigan State University (MSU) Agronomy Farm in Lansing, Michigan in 2020 (MSU-A = 42.6872°N, -84.4914°W) and 2021 (MSU-B = 42.6845°N, -84.4887°W; MSU-C = 42.6889°N, -84.4904°W) in no-tillage fields with known populations of GR horseweed. The soil types at MSU-A and MSU-B were a Conover loam (fine-loamy, mixed, active, mesic Aquic Hapludals) with pH 6.2, 7.4 and 3.2, 2.6% organic matter, respectively, and a Colwood-Brookston loam (fine-loamy, mixed, active, mesic Typic Haplaquolls) with pH 5.9 and 2.8% organic matter at MSU-C.

In 2020, the experiment was arranged in a randomized complete block split plot design with four replications. In 2021, the experiment was arranged in a randomized complete block split-split plot design with four replications. Plots measured 3 m wide by 11 m long. The main plot factor was early-season management strategy consisting of 1) cereal rye terminated one wk after soybean planting with glyphosate (Roundup PowerMAX; Bayer CropScience, St. Louis, MO) at 1.27 kg ae ha^{-1} + ammonium sulfate at 2% w w^{-1} (AMS) (Actamaster; Loveland Products, Inc., Greeley, CO) (planting green), 2) a no cover plus PRE residual herbicide program that included glyphosate at 1.27 kg ae ha^{-1} + 2,4-D choline (Enlist One; Corteva Agriscience, Indianapolis, IN) at 1.12 kg ae ha^{-1} + flumioxazin (Valor; Valent U.S.A. Corporation, Walnut Creek, CA) at 0.07 kg ai ha^{-1} + metribuzin (Metribuzin 75; Winfield Solutions, St. Paul, MN) at 0.31 kg ha^{-1} + AMS at 2% w w^{-1}, and 3) a no cover control that was treated with glyphosate at 1.27 kg ae ha^{-1} + AMS at 2% w w^{-1} PRE. The subplot factor was soybean row width; 19-, 38-, and 76-cm. The sub-subplot factor in 2021 was POST herbicide program consisting of an effective POST program of glufosinate (Liberty; BASF Corporation, Research Triangle Park, NC) at 0.66 kg ai ha^{-1} + 2,4-D choline at 1.12 kg ae ha^{-1} + AMS at 2% w w^{-1}, or a noneffective POST program of glyphosate at 1.27 kg ae ha^{-1} + AMS at 2% w w^{-1} to only control other weeds, but not GR horseweed. In 2020 at MSU-A, only glyphosate at 1.27 kg ae ha^{-1} + AMS at 2% w w^{-1} was applied POST all plots.

The fall prior to data collection 'Wheeler' cereal rye was drilled at 67 kg ha^{-1} in 19 cm rows using a no-till drill (John Deere, Moline, IL). Dates for all field operations can be found in Table 3.1. The next spring, glyphosate, glufosinate, and 2, 4-D choline-resistant soybean 'P25T09E' or 'P24T35E' was planted at 500,000-, 437,500-, 375,000-, or 500,000-, 450,000-, and 387,500- seeds ha^{-1} in 2020 and 2021, respectively, in 19-, 38-, and 76-cm rows. Higher seeding rates were used in 2021 due to dry conditions. The burndown plus residual treatments were established 3 days after soybean planting (DAP) or one week prior to soybean planting in 2020 and 2021, respectively. Cereal rye was terminated one wk after soybean planting the following spring. POST herbicide applications were made 4 to 6 WAP when horseweed was 10 cm tall in the control. At one site, POST herbicides applications were delayed due to weather and average horseweed height was 20 cm tall. All herbicide applications were made using a tractor-mounted, compressed air sprayer calibrated to deliver 177 L ha^{-1} at 207 kPa of pressure through 11003 AIXR nozzles (TeeJet, Spraying Systems CO., Wheaton, IL 60187).

Throughout the growing season temperature and precipitation data was collected from the Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, MI) stations located in East Lansing (data not shown). Temperature and precipitation 30-yr averages were collected from the National Oceanic and Atmospheric Administration (https://www.noaa.gov) (data not shown).

Data Collection. At planting green termination, aboveground cereal rye biomass and weed density and biomass were collected from two randomly placed 0.25 m^2 subsamples per plot. Subsamples of cereal rye biomass were analyzed for C:N ratios by A&L Great Lakes Laboratories, Inc. (Fort Wayne, Indiana) using a TruMac CNS Macro Analyzer (LECO

Corporation, St. Joseph, MI). Horseweed density and biomass were also collected at the time of POST herbicide application and prior to soybean harvest. Biomass samples were dried for approximately 7 d at 65 C and weighed.

Canopy closure was measured in the PRE residual treatments 6, 7, 8, 10, and 11 WAP using the mobile device application Canopeo (Oklahoma State University, Stillwater, OK). Three images were taken randomly per plot using the Canopeo application on a smartphone (iPhone X, Apple®) held 5 ft above the soybean canopy. Images were then analyzed for percent green cover based on selection of pixels according to ratios of R/G, B/G (Paruelo et al. 2000; Liang et al. 2012), and the excess green index (Richardson et al. 2007; Chen et al. 2010) with a threshold setting of 0.95. Green cover ranged from 0 (no green cover) to 1 (100% green cover). Soybean was harvested for yield using a small-plot research combine (Massey-Ferguson 8XP, AGCO, Duluth, GA). Yields were adjusted to 13% moisture.

Economic Analysis. The net economic returns in response to each treatment was calculated by subtracting estimated treatment cost from gross income. Gross income was calculated in USD ($) ha^{-1} by multiplying soybean yield by soybean prices of $0.37 kg^{-1} ($10.00 bu^{-1}) and $0.55 kg^{-1} ($15.00 bu^{-1}). The cost of each treatment was calculated by using the average soybean seed, cereal rye seed, herbicide, and adjuvant prices from June 2021 and January 2022 price sheets provided by major agricultural retailers in the Midwest. Soybean seed cost for 140,000 seeds was estimated at $60.00 and cereal rye seed cost plus custom planting was estimated at $58.52 for 67 kg ha^{-1}. A custom application fee of $22.23 ha^{-1} was included for each herbicide application timing in the program.

Statistical Analysis. Upright and rosette horseweed biomass response and canopy closure data were analyzed using the drc package in R v. 4.0.2 (R Development Core Team 2020). Three-parameter log logistic models (Equation 1) were fitted for each shade level by growth type combination and soybean row width as selected by the drc modelFit function using the lack of fit test. The effective time to reach 25% (T_{25}) biomass compared with the 0% shade upright growth type was determined using the ED function for the rosette- and upright-type within each shade level. For canopy closure, the effective time to reach 75 (T_{75}) and 90% (T_{90}) canopy closure was determined using the ED function for each row width. Time is the d after planting (DAP) for the shade and canopy closure data, respectively

$$y = c + \frac{d-c}{1+exp[(b(log(x)-log(e))]} \qquad \text{[Eq. 1]}$$

For this equation, y is the biomass response (percent of the 0% shade upright-type) or the % canopy closure; *x* is the time (DAP), *c* and *d* are the lower and upper limits, respectively, *b* is the relative slope around *e*, and e is the T_{25} (Streibig 1988). Shade level by growth type differences in T_{25} values and row width differences in T_{75} and T_{90} values (based on a t-statistic with $\alpha \leq 0.05$) were compared using the EDcomp function.

Final biomass, upright height, and rosette diameter were analyzed using analysis of variance (ANOVA) in the lmer function of R v. 3.6.0 (R Development Core Team 2020). Fixed factors were shade level and growth type, and their respective interaction. Random factors included replication and shade level by replication. Normality assumption was checked by examining histogram and normal probability plots of the residuals. Unequal variance assumption was assessed by visual inspection of the side-by-side box plots of the residuals followed by

Levene's test for unequal variances. Treatment means were separated using Fisher's Protected LSD at $\alpha \leq 0.05$.

Field experiment data analysis was performed using PROC GLIMMIX in SAS OnDemand (SAS Institute, 2021) at $\alpha = 0.05$. The statistical model consisted of early-season management strategy, soybean row width, POST herbicide application, and their interactions as fixed effects. Each year-location combination was considered an environment sampled at random from a population as suggested by Carmer et al. (1989). Environment (individual year and location), replication nested within environments, the interaction between early-season strategy and replication nested within environments, and the interaction between early-season strategy and soybean row width nested within environments were considered random effects. Replications were used as an error term for testing the effects of environment, and data were combined over all environments for each measurement except for soybean yield and economic return. Data for horseweed density and biomass at harvest, soybean yield, and economic return were analyzed separately by POST herbicide treatment. Normality of residuals were examined using the UNIVARIATE procedure. Squared and absolute value residuals were examined with Levene's test to confirm homogeneity of variances. Data were combined over main effects when interactions were not significant. Treatment means were separated using Fisher's Protected LSD at $\alpha \leq 0.05$. Nontransformed means for horseweed density and biomass are presented because the arcsine and square root transformation did not improve the normality of the data.

Results and Discussion

Horseweed Response to Shade. Shade had a significant effect on the growth of rosette and upright horseweed plants. As shade level increased the rate of biomass accumulation for the upright growth type decreased (Figure 3.1) with 1.66- and 2.42-times slower biomass accumulation under 35 and 69% shade, respectively (Table 3.2). These increased shade levels also led to an additional 2 and 19 d to reach 25% (T_{25}) biomass accumulation in relation to the upright growth type under 0% shade. The rate of biomass accumulation for the rosette type grown under 0% shade was 2.27-times slower compared with the upright growth type under 0% shade (Table 3.2). There were no differences in rates for the rosette growth type the 0, 35, and 69% shade treatments, but the higher shade levels caused significant delays in the time to reach 25% biomass accumulation and these delays were generally longer for the rosette compared with the upright growth type. At the highest shade level (92%), neither growth type reached 25% biomass accumulation by the end of the experiment. High shade levels (>40%) were reported to slow the growth rate of other weeds, such as common waterhemp (Steckel et al. 2003).

Final horseweed height and biomass was reduced for the upright growth type with increasing shade level (Table 3.2). Biomass was 30 and 77% lower when the upright growth type was grown under 35 and 69% shade, respectively. For rosettes, final diameter was not different between the 0 and 35% shade; although, biomass was 33% less. At 69% shade, there was no difference in horseweed biomass between the upright and rosette growth types. Horseweed, regardless of growth type, grown under 92% shade produced very little biomass and was >99% lower than the 0% shade treatments. Previous research showed that decreasing light intensity from 100 to 25% of full sunlight reduced biomass of rosette horseweed (Bekech 1988). Similarly, Steckel et al. (2003) reported less common waterhemp biomass with increased shading; however, there was no difference in final height.

Overall, increased shading up to 69% slowed the rate of horseweed growth in the upright, but not the rosette, growth type. However, higher shade levels delayed the time to reach 25%

biomass accumulation within each growth type and the time required was longer for the rosette compared with the upright growth type. Higher shade levels also reduced final horseweed height and biomass.

Horseweed Suppression at Planting Green. Cereal rye suppressed horseweed similar to the PRE residual herbicide treatment at the time of planting green termination (1 WAP). At this time, cereal rye was at Feekes stage 10.5.1 with a dry biomass of 4,384 kg ha^{-1} and a C:N ratio of 41:1 (data not shown). Horseweed density was 54 and 80% lower by planting green or applying a residual herbicide, compared with the no cover control (Table 3.3). Likewise, horseweed biomass was not different between the planting green and residual herbicide treatments (10-20 g m^{-2}). At termination, biomass was extremely low due to the relatively small size of horseweed plants (<2.5 cm average diameter) and horseweed biomass for the planting green treatment was not different than the no cover control. However, the residual herbicide treatment reduced horseweed biomass by 76% compared with no cover control (Table 3.3). Previous studies reported horseweed densities reductions of 41 to 97% from fall planted cover crops at the time of early termination compared with no cover (Schramski et al. 2021b; Essman et al. 2020; Wallace et al. 2019; Pittman et al. 2019). Similarly, Owen et al. (2009) reported >86% horseweed control 21 d after application (DAA) of various preplant residual herbicide programs. Pittman et al. (2019) reported greater horseweed density reductions from fall-planted cover crops compared with fall-applied metribuzin + chlorimuron-ethyl.

Horseweed Suppression at POST Application. Horseweed continued to emerge after cereal rye termination. Horseweed density increased 3-fold between cereal rye termination and the

POST herbicide application in the no cover control with soybean planted in 76 cm rows (Tables 3.3, 3.4). Schramski et al. (2021b) reported prolonged horseweed emergence until 450-600 GDD (base, 10 C), depending on rainfall. In our research, GDD accumulation at the time of planting green termination was 287 to 289 (base, 10 C) (Table 2) and at the time of POST herbicide application, 500 to 703 GDDs (base, 10 C) had accumulated (data not shown). Prior to planting green termination in June, rainfall was 3-20 mm; however, later rainfall events totaling 71 to 157 mm occurred throughout the rest of June likely stimulating horseweed emergence (data not shown).

At the time of POST herbicide application, each early-season strategy x soybean row width combination reduced horseweed density and biomass compared with soybean planted in 76 cm rows in the no cover control (Table 3.4). Soybean planted in narrow rows (19- or 38-cm) reduced horseweed density and biomass by over 2- and 1.7-fold, respectively, compared with 76 cm rows when no early-season horseweed management strategy was in place. Rich and Renner (2007) found that planting soybean in 19 cm rows reduced eastern black nightshade biomass compared with 76 cm rows. The PRE residual treatment of metribuzin + flumioxazin provided the greatest horseweed suppression for all three soybean row widths. Horseweed density was lower in 19- than 76-cm rows; however, reductions in horseweed biomass were not different among soybean row widths (96-99%). Schramski et al. (2021c) observed similar reductions in horseweed density and biomass at the time of POST herbicide application in soybean when a residual herbicide was applied. Across all row widths, planting green reduced horseweed density and biomass 65 to 83% and 86 to 91%, respectively, compared with soybean planted in 76 cm rows with no cover (Table 3.4). Planting soybean in 19 cm rows reduced horseweed density 2-fold more than 76 cm rows in the planting green treatments; however, there were no differences in horseweed biomass among row widths. Similar horseweed biomass reductions were observed between the combination of planting green in 19 cm rows and the residual herbicide treatment with soybean planted in 38- and 76-cm rows. Only the 19 cm row by PRE residual herbicide combination suppressed horseweed biomass more. Similar weed density reductions were reported in narrow row by cover crop combinations. Hay et al. (2019) reported soybean planted in 19- and 38-cm rows into an early terminated winter wheat cover crop reduced Palmer amaranth (*Amaranthus palmeri* S. Wats.) density 49-55% compared with soybean planted in 76 cm rows with no cover.

The advantage of soybean planted in narrow rows for horseweed suppression was likely due to quicker canopy development. Soybean planted in 38- and 19-cm rows reached 75% (T_{75}) canopy closure 1- and 2.5-wk ahead of 76 cm rows, respectively (Figure 3.2). The soybean canopy reached 90% closure 7.5 WAP for 19 cm rows. It took an additional 1.25 and 2.5 wk for the 38- and 76-cm rows to reach this point, respectively. Greater horseweed suppression from earlier canopy closure in narrow row soybean was supported by our greenhouse research. At 69% shade, which would have occurred prior to 6 WAP in 19 cm in our field study, biomass of both rosette and upright horseweed growth types was reduced by greater than 75% (Table 3.2). Any horseweed emerging after 90% canopy closure would likely not produce much biomass. Earlier canopy closure by planting in narrower rows likely contributed to greater reductions in horseweed density and biomass at the time of POST herbicide application. Additionally, planting green cover residue was persistent enough to suppress horseweed until the time of POST herbicide application, but the magnitude of suppression was less evident compared with applying a PRE residual herbicide.

Horseweed Suppression at Soybean Harvest. At soybean harvest, early-season strategy and soybean row width continued to have significant effect on horseweed density and biomass when a noneffective postemergence herbicide. Planting soybean in 19- and 38-cm rows suppressed horseweed density 2.7- and 2-times more than soybean planted 76 cm rows, respectively, when no early-season horseweed management strategy was in place (Table 3.5). The effect of row width on horseweed density was also important for soybean planted green. The 19-cm row width was the only spacing that reduced horseweed density within the planting green treatments, although the 38-cm row width planted green had lower horseweed numbers than soybean planted in 76 cm rows with no cover. Soybean planted green in 19-cm rows also had similar horseweed numbers to the PRE residual treatments for all three soybean row widths which provided the greatest horseweed suppression. Unlike horseweed density, only main effects were significant for early-season strategy and soybean row width on horseweed biomass. Horseweed biomass was reduced most with the PRE residual herbicide treatment (81%) and planting green reduced horseweed biomass 67% compared with no cover across all three row widths (Table 3.5). Across all early-season strategies, horseweed biomass was only reduced when planting soybean in 19 cm rows. Soybean planted in 19 cm rows reduced horseweed biomass 38 and 50% compared with 38- and 76-cm rows, respectively. Similarly, Schramski et al. (2021c) reported an 84% reduction in horseweed density when a PRE herbicide with residuals and a noneffective POST herbicide application took place compared with no cover. However, he observed no effect of cereal rye terminated early on horseweed density or biomass prior to soybean harvest. In our study, the cereal rye C:N ratio was relatively high at 42:1 when planting green, whereas Schramski et al. (2021c) had cereal rye C:N ratios of <24:1. Therefore, the residue in our study was likely more persistent through soybean harvest, resulting in a longer horseweed suppression period.

While it is important to know what effects early-season strategies will have on horseweed control throughout the season, growers are likely going to need an integrated approach that includes an effective POST herbicide application for season-long horseweed management. Therefore, each early-season strategy by soybean row width combination was also treated with an effective POST herbicide of glufosinate + 2,4-D choline. For these treatments, there was an interaction between early-season strategy and soybean row width on horseweed density and biomass (Table 3.5). Planting soybean in 19- and 38-cm rows when no early-season horseweed management strategy was in place suppressed horseweed density and biomass 2.7- to 5.5-, and 2.4- to 4.2-fold, respectively, compared with 76 cm rows when an effective POST herbicide was applied. Across all row widths, horseweed density and biomass were reduced most when a PRE residual herbicide was applied or when soybean was planted green. Similar horseweed density reductions were observed among the combination of planting soybean in 19 cm rows with no cover and applying a PRE herbicide with residuals and planting green across all row widths. Our results show that when an effective POST herbicide is integrated, horseweed control is similar between planting green and applying a PRE residual herbicide.

Soybean Yield and Economic Return. Due to a high incidence of white mold (*Sclerotinia sclerotiorum* (Lib.) de Bary), MSU-C was separated from MSU-A and MSU-B. Combined over MSU-A and MSU-B, there was a main effect of early-season strategy and soybean row width on soybean yield when a noneffective POST herbicide was applied. By applying a PRE residual herbicide, soybean yield was 14 to 21% higher compared with the no cover control and planting green (Table 3.6). Yield was also 19 and 12% higher when soybean was planted in 19- and 38-cm rows compared with 76 cm rows, respectively. When an effective POST herbicide was applied, horseweed control was higher in the planting green treatments. Thus, soybean yield was similar for planting green and applying a PRE herbicide with residuals. Planting soybean in 19 cm rows yielded 9 to 10% higher than soybean planted in 38- and 76-cm rows (Table 3.6). These findings support Schramski et al. (2021c) who reported soybean yield was 52 to 145% higher when a preplant residual herbicide treatment was applied with a noneffective POST compared with a no cover control; however, they observed no effect on soybean yield by planting into an early terminated cereal rye cover. Additionally, Harder et al. (2007) reported soybean planted in 19 cm rows yielded higher than soybean planted in 38 and 76 cm rows.

At MSU-C, there was a high incidence of white mold in the planting green and narrow row soybean treatments. This was likely due to above average rainfall in June, July, and August in 2021 that totaled 356 mm compared with the 30-yr average of 259 mm (data not shown). As a result, the cover residue by planting green and narrow soybean rows created a moist soil surface beneath the closed canopy favorable for sclerotia germination. In addition, this site was bordered by corn and a woodlot that may have reduced air flow creating a larger risk for infection. When a noneffective POST herbicide was applied, there was a main effect of early-season strategy on soybean yield. Similar to MSU-A and MSU-B, by applying the PRE residual herbicide treatment soybean yield was 17 and 25% higher compared with planting green and no cover, respectively (Table 3.6). In contrast to MSU-A and MSU-B, there was no effect of soybean row width on yield, regardless of POST herbicide application. There was a higher incidence of white mold when planting in 19- and 38-cm rows compared with 76 cm rows, which likely diminished the yield advantage of narrower rows. Grau and Radke (1984) reported there was greater disease severity when in narrow row soybean, resulting in significant yield loss. At MSU-C, soybean yield was similar between the no cover control and the PRE residual herbicide treatment when an effective POST herbicide was applied; however, yield was 13% lower by planting green compared with applying a residual herbicide, likely due to the high incidence of white mold.

Program costs based on June 2021 pricing ranged from \$244.67 to 358.77 kg ha^{-1} for those that included a noneffective POST herbicide treatment and were \$286.03 to 400.13 kg ha^{-1} for those that involved an effective POST herbicide treatment (Table 3.7). Economic returns generally followed the same trend as soybean yield. There were no significant differences in economic returns among treatments whether soybean was marketed at \$0.37 kg^{-1} (\$10.00 bu^{-1}) or \$0.55 kg^{-1} (\$15.00 bu^{-1}); therefore, economic return is based on a market price of \$0.37 kg^{-1}. Additionally, we examined the impact of increased herbicide costs for the 2022 growing season due to glyphosate and glufosinate shortages; however, this did not change the differences between treatments compared with 2021 herbicide costs (data not shown).

When a noneffective POST herbicide was applied at MSU-A and MSU-B, economic return was highest by applying a PRE residual herbicide (\$1,165 ha^{-1}) (Table 3.8). Regardless of POST herbicide application, higher economic return was observed when soybean was planted in 19 cm rows compared with 76 cm rows. The application of an effective POST herbicide improved soybean yield in the planting green treatments; therefore, economic return was similar between planting green and applying a PRE residual herbicide (\$1,092-1,167 ha^{-1}).

Similar to MSU-A and MSU-B, applying a PRE residual herbicide resulted in the highest economic return when a noneffective POST herbicide was applied at MSU-C. Due to white mold, the yield advantage of narrow rows were diminished, resulting in no effect of row width on soybean yield, regardless of POST herbicide application. Additionally, applying an effective

POST did not improve soybean yield in the planting green treatments and economic return was \$187 ha^{-1} lower than the no cover and \$215 ha^{-1} lower than PRE residual herbicide treatments (Table 3.8). Overall, planting green resulted in similar soybean yields and economic return to applying a PRE residual herbicide treatment when integrated with an effective POST herbicide program in one site-year. To diminish the risks of white mold development, variety resistance, soil type, field history, and the environment surrounding the field should be assessed.

In conclusion, planting green suppressed horseweed season-long. However, suppression was not to the magnitude of applying a PRE herbicide with residuals unless soybean was planted green in 19 cm rows. When a residual herbicide was applied across all row widths, there was 96 to 99% horseweed suppression at the time of POST herbicide application. In comparison, horseweed density was only reduced 65 to 83% by planting green; however, horseweed biomass was 86 to 91% lower which likely improved POST herbicide efficacy. Planting soybean in narrow rows contributed to greater reductions in horseweed density and biomass at POST herbicide application and soybean harvest due to earlier canopy closure. However, this effect was diminished when a residual herbicide was applied. Greenhouse experiments demonstrated that rosette- and upright-type horseweed were greatly affected by shade and as shade levels increased, greater reductions in biomass were observed. Thus, earlier canopy closure can play a substantial role in reducing horseweed growth as well as suppressing late season emergence. Soybean yield and economic return was similar when planting green or applying a PRE herbicide with residuals in one site-year when integrated with an effective POST herbicide program. Conversely, reduced soybean and economic return occurred in one site-year when planting green or in narrow row soybean due to a high incidence of white mold. Thus, planting green is a practical alternative horseweed management strategy for growers, especially for those whose crop rotation limits residual herbicide options. Although, growers should take field history, field environment, and soybean variety resistance into consideration to diminish the risks of white mold development.

APPENDIX

APPENDIX
Tables and Figures

Table 3.1. Cereal rye seeding and termination dates, GDDs[a,b,c] until planting green termination, PRE herbicide application, soybean planting, POST herbicide application, and soybean harvest dates for the three experimental locations.

	Site		
Operation	MSU-A	MSU-B	MSU-C
Cereal rye seeding	October 4, 2019	October 16, 2020	November 9, 2020
PRE application	June 4, 2020	May 13, 2021	May 13, 2021
Soybean planting	June 1, 2020	May 25, 2021	May 25, 2021
Planting green termination	June 6, 2020	June 2, 2021	June 2, 2021
GDDs (base, 4.4 C)	791	764	661
GDDs (base, 10 C)	287	289	289
POST application	June 24, 2020	June 24, 2021	July 7, 2021
Soybean harvest	October 31, 2020	October 18, 2021	October 18, 2021

[a]Abbreviation: GDDs, growing degree days; MSU, Michigan State University

[b]GDDs (base, 4.4 C) accumulated from the time of cereal rye planting until termination.

[c]GDDs (base, 10 C) accumulated from January 1 until cover termination for horseweed emergence.

Table 3.2. Rate of biomass accumulation, biomass accumulation T_{25} (±SE)[a], and final height, diameter, and biomass for the upright and rosette growth types of horseweed in a greenhouse study.

Growth type	Shade level	Rate[b]	Biomass T_{25}[c]	Height	Diameter	Final biomass
	—— % ——	—— % d^{-1} ——	—— d ——	—— cm ——	—— cm ——	— g $plant^{-1}$ —
Upright	0	11.34 (±0.97)	45 (±0.72)	33 a[e]	-	2.52 a
	35	6.84 (±0.58)	47 (±0.96)	29 b	-	1.74 b
	69	4.69 (±1.19)	64 (±2.07)	15 c	-	0.58 cd
	92	0 (-)[d]	>63	2 d	-	0.0087 e
Rosette	0	4.99 (±0.48)	46 (±1.21)	-	16 a	1.44 b
	35	4.16 (±0.61)	53 (±1.63)	-	17 a	0.96 c
	69	3.88 (±1.91)	>63	-	12 b	0.30 de
	92	0 (-)	>63	-	2 c	0.0038 e
Effects (P-values)						
Shade		-	-	<0.0001	<0.0001	<0.0001
Growth type		-	-	-	-	<0.0001
Shade level * growth type		-	-	-	-	<0.0001

[a]Abbreviations: SE, standard error.
[b]Rate is the % biomass accumulation per day (d).
[c]T_{25} is the time required to reach 25 biomass accumulation relative to upright growth type under 0% shade.
[d]SE could not be calculated for 92% shade because no biomass was accumulated.
[e]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

Table 3.3. Main effect of early-season strategy on horseweed density and biomass at the time of planting green termination, 1 wk after planting (WAP).

Early-season management strategy[a]	Horseweed density	Horseweed biomass
	—— plants m^{-2} ——	—— g m^{-2} ——
No cover	56 a[b]	42 a
No cover + PRE residual	11 b	10 b
Planting green (cereal rye)	26 b	20 ab
Effects (P-value)		
Early-season management strategy	<0.0001	0.0153

[a]Abbreviations: No cover control = glyphosate only; No cover + PRE residual = glyphosate + 2,4-D choline + flumioxazin + metribuzin

[b]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

Table 3.4. Interaction between early-season strategy and soybean row width on horseweed density and biomass at the time of POST herbicide application (4 to 6 WAP).

Early-season management strategy	Row width	Horseweed Density	Horseweed Biomass
	—— cm ——	– plants m^{-2} –	—— g m^{-2} ——
No cover	19	71 b[b]	41 b
	38	75 b	44 b
	76	171 a	76 a
No cover + PRE residual	19	2 e	1 e
	38	6 de	3 de
	76	7 d	4 de
Planting green (cereal rye)	19	29 c	7 cd
	38	45 bc	10 c
	76	60 b	11 c
Effects (P-values)			
Early-season management strategy		<0.0001	<0.0001
Row width		0.0038	0.0052
Early-season management strategy x row width		<0.0001	0.0002

[a]Abbreviations: No cover control, glyphosate only; No cover + PRE herbicide with residuals, glyphosate + 2,4-D + flumioxazin + metribuzin

[b]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

Table 3.5. Interaction between early-season strategy and soybean row width on horseweed density and biomass at the time of soybean harvest for plots treated with and without an effective POST[a] application of glufosinate and 2,4-D.

		Noneffective		Effective	
Early-season management strategy	Row width	Density	Biomass[b]	Density	Biomass
	— cm —	— plants m^{-2} —	— g m^{-2} —	— plants m^{-2} —	— g m^{-2} —
No cover	19	13 cd[c]	133	4 bc	33 bc
	38	17 bc	169	8 b	58 b
	76	35 a	184	22 a	141 a
No cover + PRE residual	19	1 e	6	0 c	0 d
	38	3 e	31	1 c	7 cd
	76	4 de	55	1 c	1 cd
Planting green (cereal rye)	19	6 de	47	0 c	0 d
	38	18 bc	100	4 bc	25 cd
	76	26 ab	135	5 bc	28 bcd
Effects (P-values)					
Early-season management strategy		<0.0001	<0.0001	<0.0001	<0.0001
Row width		0.0045	0.0026	0.0309	0.0492
Early-season management strategy x row width		0.0229	0.8834	<0.0001	<0.0001

[a]Abbreviations: Noneffective, glyphosate; Effective, 2,4-D + glufosinate; No cover control, glyphosate only; No cover + PRE herbicide with residuals, glyphosate + 2,4-D + flumioxazin + metribuzin

[b]The main effects of early-season strategy and row width were significant for horseweed biomass when a noneffective POST was applied. Horseweed biomass was reduced 42 and 81% by planting green (94 g m^{-2}) or applying a residual herbicide (31 g m^{-2}) compared with no cover control (162 g m^{-2}), respectively. Horseweed biomass was reduced 38 to 50% by planting soybean in 19 cm rows (62 g m^{-2}) compared with 38- and 76-cm rows (100-125 g m^{-2}).

[c]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

Table 3.6. Main effects of early-season strategy and soybean row width on soybean yield for plots treated with either a noneffective or effective POST[a] herbicide application for horseweed control.

	Soybean yield			
	MSU-A and MSU-B	MSU-B[b]	MSU-C[c]	
Main effects	Noneffective	Effective	Noneffective	Effective
Early-season management strategy	kg ha^{-1}			
No cover	3373 b[d]	3319 b	3643 b	4200 ab
No cover + PRE residual	4078 a	4199 a	4536 a	4452 a
Planting green (cereal rye)	3568 b	3980 a	3862 b	3851 b
Row width (cm)				
19	3955 a	4070 a	4264	4109
38	3738 a	3727 b	4200	4193
76	3327 b	3702 b	3578	4202
Effects (P-values)				
Early-season management strategy	0.0012	0.0012	0.0010	0.0168
Row width	0.0009	0.0039	0.0762	0.7834
Early-season management strategy x row width	0.8538	0.5781	0.3184	0.1090

[a]Abbreviations: Noneffective, glyphosate; Effective, 2,4-D + glufosinate; No cover control, glyphosate only; No cover + PRE herbicide with residuals, glyphosate + 2,4-D + flumioxazin + metribuzin

[b]Effective POST herbicide only applied at MSU-B and -C.

[c]There was a high incidence of white mold in the planting green and narrow row soybean treatments at MSU-C, therefore, it was separated from the remaining site-years.

[d]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

Table 3.7. Treatment costs[a] (June 2021) for horseweed management programs for plots treated with either a noneffective or effective POST[b] herbicide application for horseweed control.

Early-season management strategy	Row width	POST Noneffective	POST Effective
	—cm—	—USD $ ha^{-1}—	
No cover	19	294.97	336.33
	38	271.15	312.50
	76	244.67	286.02
No cover + PRE residual	19	358.77	400.13
	38	334.94	376.30
	76	308.47	349.83
Planting green (cereal rye)	19	353.49	394.85
	38	329.67	371.02
	76	303.19	344.55

[a]Total treatment costs = soybean seed costs + cereal rye seed and planting costs + herbicide costs + adjuvant costs + application costs. Average price of seed, herbicide and adjuvants were calculated from multiple price lists. Herbicide application cost = $22.31 ha^{-1}.

[b]Abbreviations: Noneffective, glyphosate; Effective, 2,4-D + glufosinate; No cover control, glyphosate only; No cover + PRE herbicide with residuals, glyphosate + 2,4-D + flumioxazin + metribuzin

Table 3.8. Economic return[a,b,c] for horseweed management programs for soybean marketed at \$0.37 kg^{-1} (\$10.00 bu^{-1}) using price lists from June 2021 for plots treated with and without an effective POST herbicide application[d].

	Economic return			
	MSU-A and MSU-B	MSU-B[e]	MSU-C[f]	
Main effects	Noneffective	Effective	Noneffective	Effective
Early-season strategy	USD \$ ha^{-1}			
No cover control	970 b[g]	908 b	1068 b	1232 a
No cover + PRE residual	1165 a	1167 a	1333 a	1260 a
Planting green (cereal rye)	982 b	1092 a	1117 b	1045 b
Row width (cm)				
19	1117 a	1118 a	1257	1133
38	1062 ab	1016 b	1231	1187
76	938 b	1033 b	1029	1218
Effects (P-values)				
Early-season strategy	0.0089	0.0082	0.0059	0.0125
Row width	0.0028	0.0161	0.0921	0.3183
Early-season strategy x row width	0.8593	0.5781	0.4947	0.1090

[a]Net return = (yield x price) – treatment costs. Crop selling price = \$10.00 bu^{-1}.
[b]No differences in mean separation when crop selling price = \$15.00 bu^{-1}.
[c]No differences in mean separation using January 2022 price lists.
[d]Abbreviations: Noneffective, glyphosate; Effective, 2,4-D + glufosinate; No cover control, glyphosate only; No cover + PRE herbicide with residuals, glyphosate + 2,4-D + flumioxazin + metribuzin
[e]Effective POST herbicide only applied at MSU-B and -C.
[f]There was a high incidence of white mold in the planting green and narrow row soybean treatments at MSU-C, therefore, it was separated from the remaining site-years.
[g]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

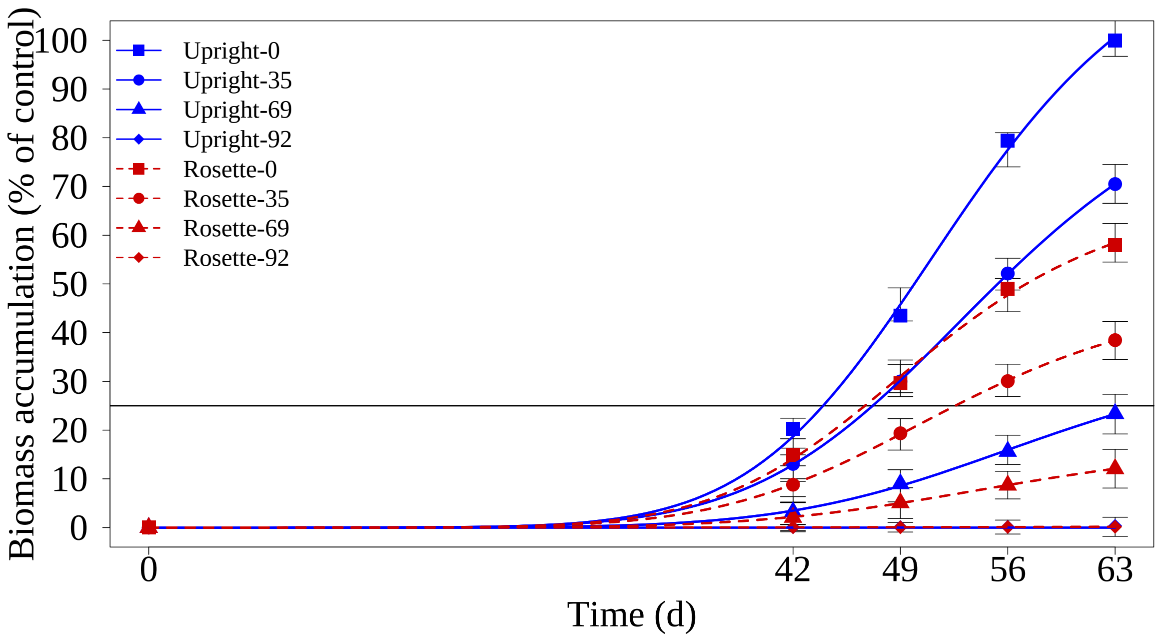

Figure 3.1. Time to reach 25% biomass accumulation of rosette and upright horseweed plants grown under 0, 35, 69, and 92% shade. Biomass is presented as a percent of the biomass of the upright growth type grown under 0% shade at 63 d.

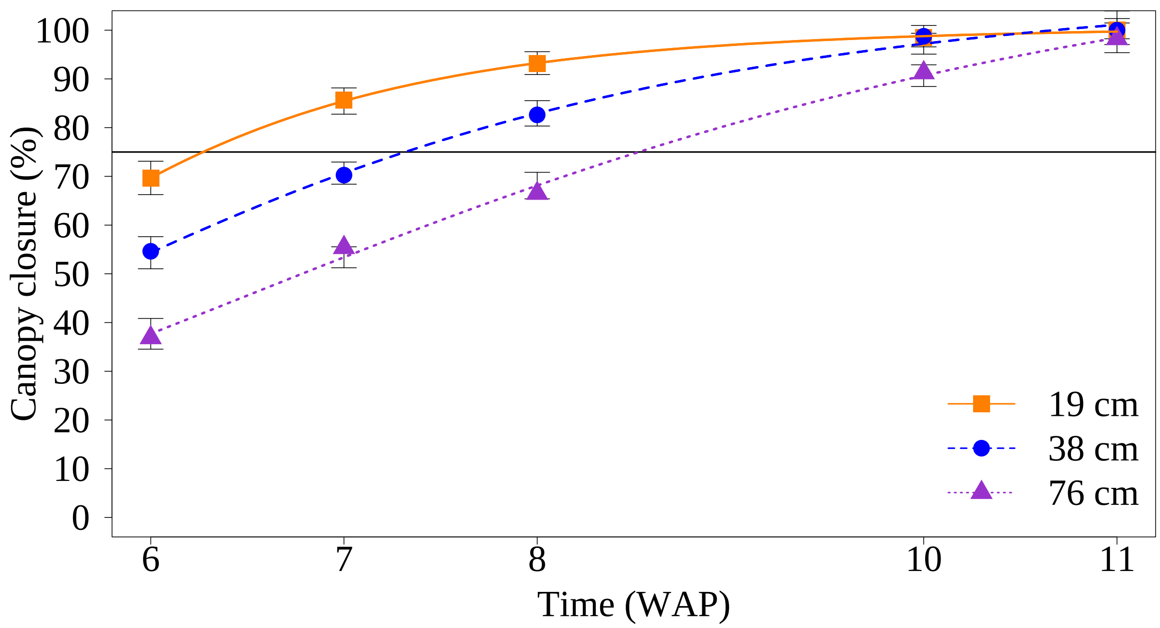

Figure 3.2. Canopy closure (%) from 6 to 11 wk after planting (WAP) for soybean planted in 19-, 38, and 76-cm rows combined over three site-years.

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CHAPTER IV

INVESTIGATIONS INTO DIFFERENTIAL GLYPHOSATE SENSITIVITY BETWEEN TWO HORSEWEED GROWTH TYPES

Abstract

Phenotypic differences, “rosette” and “upright” growth types, of newly emerged horseweed have been observed co-occurring in Michigan fields. Previous research found that “upright” plants from two glyphosate-resistant populations were 3- and 4-fold less sensitive to glyphosate than their rosette siblings. However, differences in sensitivity between the growth types in the susceptible population were not observed. Further experiments were conducted to investigate whether differential glyphosate sensitivity of the growth types was due to glyphosate retention, absorption, or translocation. The total amount of glyphosate retained on horseweed’s leaf surface was similar for both growth types; however, on a per weight and area basis the upright growth type retained 21 and 18% less glyphosate, respectively. Glyphosate absorption was up to 85%, 168 HAT, and was not different between the rosette and upright growth types or between the susceptible (S) and resistant (R) biotypes. Additionally, there was no difference in translocation between the two growth types within each biotype at any time point. Interestingly, at 168 HAT ^{14}C-glyphosate translocation was higher in the S rosette compared with the two growth types from the R biotype; however, the S upright-type was similar to R across both growth types. Thus, glyphosate resistance in the R biotype may be due to target-site resistance instead of impaired translocation, which has been cited as the primary mechanism of glyphosate resistance in horseweed. These results suggest that reduced glyphosate interception and retention on a per weight and area basis of the upright growth type may contribute to increased glyphosate tolerance due to a diluted concentration of glyphosate. However, there is likely another factor

related to the mechanism of resistance within the R biotype that is contributing to a 3-fold difference in glyphosate sensitivity between the two growth types, such as alterations in EPSPS gene expression or other genetic variations.

Introduction

Horseweed (*Conyza canadensis* L.) is considered a facultative winter annual; germination can occur in the fall when soil temperatures decline or facultatively during other times of the year (Cici and Van Acker 2009). However, there are two main horseweed emergence timings, April to June (spring) and August to October (fall) that serve as critical horseweed control periods (Bhowmik and Bekech 1993; Buhler and Owen 1997; Loux et al. 2006; Main et al. 2006). Fall-emerging horseweed typically form dark green, lightly haired, basal rosettes that overwinter, while spring emerging horseweed skip or spend a short period of time as a rosette before bolting, forming an upright growth type (Loux et al. 2006; Regehr and Bazzaz 1979). In Michigan field cropping systems, primary horseweed emergence has shifted from fall to spring/summer, and therefore from a rosette to an upright growth type. In addition, the rosette and upright growth types have been observed co-emerging during the summer with visual differences in glyphosate tolerance (Schramski et al. 2021). Schramski et al. (2021) found that horseweed growth type was not strictly genetically controlled but instead could emerge from the same parent plant. The upright growth type seems to be environmentally triggered by a vernalization period of 4 wk following water imbibition, but before germination. Horseweed is primarily a self-pollinating species with ≤10% cross pollination, thus many horseweed biotypes have been evolving independently, and it is likely that agronomic factors such as recurring herbicide applications, lack of herbicide rotation, and no-tillage have selected for similar traits on various genetic pools of horseweed convergently (Dinelli et al. 2006).

Horseweed has documented resistance to at least one site of action in 18 different countries, including biotypes resistant to acetolactate synthase (ALS) inhibitors (WSSA Group 2); photosystem II inhibitors (WSSA Group 5); photosystem II inhibitors (WSSA Group 7); glyphosate, the 5-enolpyruvate-shikimate-3-phosphate inhibitor (EPSP) (WSSA Group 9); and paraquat, a photosystem I electron diverter (WSSA Group 22) (Heap 2021). However, glyphosate resistant biotypes are the most prevalent. The first confirmed case of glyphosate-resistant horseweed was identified in Delaware in 2000 (VanGessel 2001). This occurred after relying only on glyphosate for weed control for three years in a glyphosate-resistant soybean field. Following the release of Roundup Ready ® crops in 1996, glyphosate use increased almost 15-fold (Benbrook 2016), contributing to increased selection pressure for resistant individuals. By 2021, there were 14 countries with confirmed glyphosate-resistant horseweed (Heap 2021). Within the United States, glyphosate-resistant horseweed is present in 25 states, including Michigan.

Common mechanisms of herbicide resistance in weeds include an altered target site, reduced absorption, reduced translocation to the target site, or rapid metabolic detoxification. The main mechanism of glyphosate resistance in horseweed was reported to be rapid glyphosate sequestration into the vacuole which results in reduced translocation to the target tissue (i.e. the meristem) (Dinelli et al. 2006; Feng et al. 2004; Ge et al. 2010; González-Torralva et al. 2012; Koger and Reddy 2005; Moretti and Hanson 2016; Nandula et al. 2005). However, much of this was performed on rosette horseweed plants. Recently, the first documented case of target-site mediated glyphosate resistance in horseweed in the United States was observed in biotypes with resistance from 20- to 40-times the field use rate (1X = 840 g ae ha^{-1}) from Ohio and Iowa (Beres et al. 2020). A proline to serine mutation at position 106 of EPSPS2 was detected, the same target site mutation identified in 21 glyphosate-resistant horseweed accessions from Canada (Page et al. 2018).

Schramski et al. (2021) reported that the upright type from two glyphosate-resistant biotypes were 3- and 4-fold less sensitive to glyphosate than their rosette siblings; however, these differences were not observed in the susceptible biotype (Schramski et al. 2021). The level of resistance in the rosette and upright growth types were 84 to 386X and 26 to 97X, respectively. Similarly, Shrestha et al. (2007) reported increased levels of glyphosate resistance with increasing growth stage, determined by the number of leaves per plant, within the susceptible and resistant horseweed populations. Additionally, glyphosate tolerance increased when plants began to grow upright in the resistant and susceptible populations. In contrast, Koger et al. (2004) found no differences in glyphosate tolerance among growth stages in the rosette growth types. Based on these findings, our main objective was to determine if differential glyphosate sensitivity between the rosette- and upright-horseweed plants with known glyphosate resistance were due to higher glyphosate interception and retention, absorption, and/or translocation.

Materials and Methods

Growth Parameters. Seed from the same parent plants of the glyphosate-resistant (MSU-18 or R) and -susceptible (S-117 or S), horseweed biotypes studied in Schramski et al. (2021) were used for this experiment. To generate the upright growth type, ~0.6 g of seed from each biotype was surface planted in 30 x 30 cm flats filled with potting media (Suremix Perlite, Michigan

Grower Products, Inc., Galesburg, MI) and imbibed with water. These flats were placed in a vernalization room set to 4 C with an 8-h photoperiod for 4 wk then moved to a greenhouse. At that time, flats with seed to produce rosette siblings were planted using the same method described above, without a vernalization period. Flats were placed in the greenhouse at 25 ± 5 C and a total midday light intensity of 1,000 μmol m^{-2} s^{-1} photosynthetic photon flux with 16-h days. After 3 wk, seedlings were transplanted, one plant pot^{-1}, to 10 x 10 x 12 cm pots filled with potting media. Plants were watered and fertilized as needed to promote optimum plant growth. Individual plants were grown to an average rosette size of 10 cm wide and an upright size of 7 cm tall (approximately 42-d old).

Retention. Glyphosate interception and retention was examined by applying 1.27 kg ae ha^{-1} of glyphosate (Roundup PowerMAX; Bayer CropScience, St. Louis, MO) plus ammonium sulfate (AMS) (Actamaster; Loveland Products, Inc., Greeley, CO) at 2% w w^{-1} with Chicago Sky Blue dye (2.5 g L^{-1}) (Chem-Impex International, Inc., Wood Dale, IL) to both rosette- and upright-growth types at plant sizes as previously described. The method used was modified from the technique described by Boldt and Putnam (1980). Herbicide applications were made with a single-track sprayer (Generation 4, DeVries Manufacturing, Inc., Hollandale, MN) equipped with an 8001E TeeJet flat-fan nozzle (TeeJet Technologies, Wheaton, IL) calibrated to deliver 187 L ha^{-1} at 193 kPa of pressure.

Immediately after application, plants were excised at the soil surface and the retained dye was collected by a 30-s agitated rinse of the plant in 10 ml of a nonionic surfactant at 0.25% v v^{-1} with water solution. An additional 5 ml of the nonionic surfactant-water solution was used to collect the remaining retained dye. A 1-ml aliquot of the rinsate was used to measure absorbance with a spectrophotometer at 625 nm. Absorbance values were compared with those of a standard curve prepared for the Chicago Sky Blue dye. The technique was similar to that used by Sprague et al. (1999). Horseweed plants were dried at 60 C for 7 d and weighed to determine aboveground biomass.

Prior to spray application, exposed leaf area (cm^2) from above was measured. All plants were photographed using an iPhone X® (Apple) with a white background and a ruler as a size reference, and the photos were processed to obtain the leaf area using ImageJ software (National Institutes of Health, Bethesda, MD; University of Wisconsin Laboratory for Optical and Computation Instrumentation, Madison, WI). The average distance between the camera and plant was 30 cm. There were 20 replications of rosette and upright plants, and the study was repeated in time.

Absorption and Translocation. The uppermost fully developed leaf of the R and S rosette and upright growth types at 10 cm wide and 7 cm tall, respectively, was targeted for radiolabeled ^{14}C-glyphosate application. These leaves were covered with aluminum foil and the remainder of the plant was sprayed with unlabeled glyphosate at 1.27 kg ae ha^{-1} plus AMS at 2% w w^{-1}. Spray applications were made as previously described in the retention study. The aluminum foil was removed immediately after spray application. Each plant was treated with 1.67 kBq of ^{14}C-glyphosate (50 mCi $mmol^{-1}$ specific activity, 99% purity). The spotting solution contained the appropriate amounts of ^{14}C-glyphosate, unlabeled glyphosate, AMS, and water to give the same concentration as in the 1.27 kg ae ha^{-1} application of glyphosate. Each treated leaf was spotted on the adaxial leaf surface with 10 1-μl droplets and placed in a growth chamber maintained at 25/20 C day/night temperature with a 16-h photoperiod (1,000 $\mu mol\ m^{-2}\ s^{-1}$).

Plants were harvested at 0, 12, 24, 72, and 168 h after treatment (HAT). At harvest, each plant was divided into treated leaf, above treated leaf, below treated leaf, and roots. Unabsorbed ^{14}C-glyphosate was removed by placing the treated leaf in a 20-ml scintillation vial containing 3 ml of a methanol:water (1:9 v v^{-1}) solution and agitating it for 30 sec followed by a 1 ml rinse with the methanol:water (1:9 v v^{-1}) solution as the treated leaf was removed from the scintillation vial. The samples for each plant were immediately placed in the freezer and stored at -30 C until further analysis. Each plant part was combusted in a biological sample oxidizer. The $^{14}CO_2$ released from the biological oxidizer was trapped in 20 ml of scintillation fluid (PerkinElmer, Groningen, The Netherlands) (Carbo-Sorb® E:Permafluor® E^+, 1:1 [v/v]) and the radioactivity was quantified using a liquid scintillation counter (PerkinElmer, Boston, MA) (Tricarb 4910TR Liquid Scintillation Analyzer). Radioactivity in the 4 ml leaf wash solution was quantified with the addition of 16 ml of Ultima Gold™ scintillation fluid (PerkinElmer, Groningen, The Netherlands). The technique was similar to that used by Sprague et al. (1999). Each study had five replications and was repeated in time.

Glyphosate absorption was calculated as the sum of the total ^{14}C in the plant parts divided by the total ^{14}C recovered, including the treated leaf wash. The amount of ^{14}C present in the leaf wash and the plant sections was considered as total ^{14}C recovered, which averaged 90% of applied ^{14}C-glyphosate. ^{14}C translocation out of the treated leaf was calculated by taking the amount of ^{14}C recovered in the plant parts, excluding the treated leaf, divided by the total ^{14}C recovered in the plant.

Statistical Analysis. Retention and translocation data were analyzed using PROC GLIMMIX in SAS OnDemand (SAS Institute, 2021) at $\alpha = 0.05$. The statistical model included the main effect of growth type and growth type within biotype for the retention and translocation experiments, respectively. Data were combined over repetition in time, and replication was treated as a random effect. Normality of residuals were examined using the UNIVARIATE procedure ($\alpha \leq 0.05$). Squared and absolute value residuals were examined with Levene's test to confirm homogeneity of variances ($\alpha \leq 0.05$). Treatment means were separated using Fisher's Protected LSD at $\alpha \leq 0.05$ when ANOVA indicated a significant main effect.

Absorption and translocation over time was analyzed using the drc package in R v. 4.0.2 (R Development Core Team 2020). Three-parameter log logistic models (Equation 1) were fitted for the rosette and upright growth types within each biotype as selected by the drc modelFit function using the lack of fit test. The effective dose to reach 50% absorption was determined using the ED function for each biotype and growth type.

$$y = c + \frac{d-c}{1+exp[(b(log(x)-log(e))]} \qquad \text{[Eq. 1]}$$

For the equation above, *y* is the percent absorption, *x* is the time (HAT), *c* and *d* are the lower and upper limits, respectively, *b* is the relative slope around *e*, and e is the ED_{50} (Streibig 1988). Relative growth type within each biotype differences in ED_{50} values (based on a t-statistic with $\alpha \leq 0.05$) were compared using the EDcomp function.

Results and Discussion

Interception and Retention. At 42-d after planting, the average height of the upright plants was 7 cm and the average diameter of the rosette plants was 10 cm (data not shown). The upright growth type accumulated 30% more biomass (358 mg plant^{-1}) and had 20% more leaf area was exposed (89 cm^2 plant^{-1}) compared to the rosette growth type (Table 4.1, Figures 4.1A and B). Total glyphosate interception and retention was not different among the rosette and upright growth types which ranged from 0.77 to 0.78 g ae of glyphosate per plant (Table 4.2). However, the upright growth type retained 21% less glyphosate on a per weight (0.0022 g ae mg^{-2}) and 18% less on a per area basis (0.0088 g ae cm^2) than the rosette type (Table 2). In theory, the upright growth type should have intercepted more glyphosate per plant since leaf area was higher; however, differences in leaf arrangement likely altered spray interception. Previous research has not found differences in total glyphosate retention between glyphosate-susceptible and -resistant biotypes (Feng et al. 2004; González-Torralva et al. 2012). Based on these results, reduced glyphosate retention on a per weight and area basis in the upright growth type may result in a more diluted concentration of glyphosate inside the plant compared with the rosette growth type. This may contribute to differences in sensitivity between the rosette- and upright growth type with known glyphosate resistance, similar to what we know about the poor effectiveness of glyphosate when it is sprayed on larger plants of any species. However, we believe this relatively small change is unlikely to be the only or primary mechanism responsible for the 3- to 4-fold difference in sensitivity we see between rosette and upright resistant plants.

Absorption and Translocation. There were no differences in glyphosate absorption among the rosette and upright growth types across both biotypes. Each growth type x biotype combination reached 50% of its total absorption (ED_{50}) 11 and 15 HAT (Figure 4.2). Similarly, past research did not find reduced glyphosate absorption to be a mechanism of resistance in horseweed (Dinelli et al. 2006; Feng et al. 2004; González-Torralva et al. 2012; Koger and Reddy 2005). Maximum glyphosate absorption ranged between 75 and 85%, plateauing around 72 HAT (Figure 4.2). González-Torralva et al. (2012) reported no significant differences in absorption between resistant and susceptible horseweed biotypes with peak absorption occurring at 96 HAT when 71 and 62% of glyphosate was absorbed, respectively. Similarly, Feng et al. (2004) observed no differences in glyphosate absorption among 11 biotypes of susceptible and resistant horseweed at 4-5 DAT. These results suggest that glyphosate absorption does not contribute to differences in glyphosate sensitivity between the upright and rosette growth types, or between resistant and susceptible biotypes.

There was no difference in translocation among the rosette and upright plants within each biotype at any time point (Figure 4.3). Additionally, translocation was similar between all growth type x biotype combinations at 12 and 24 HAT. However, by 72 to 168 HAT, differences in translocation were observed between the S upright-type and both R growth types (Figure 4.3). Radioactivity was distributed throughout the plant with a majority remaining in the treated leaf at all time points, regardless of growth type within biotype. At 168 HAT, 71 to 75% of the applied ^{14}C-glyphosate remained in the treated leaf in both R growth types, and the S upright-type, whereas only 59% remained in the treated leaf for the S rosette-type (Table 4.3). The amount of ^{14}C-glyphosate translocated out of the treated leaf in the rosette and upright growth types at 168 HAT was 19 and 11% in the S biotype, respectively. For the R biotype, ^{14}C-glyphosate translocation out of the treated leaf in the rosette and upright plants at 168 HAT was 9 and 7%, respectively. Interestingly, at 168 HAT ^{14}C-glyphosate translocation was higher in the susceptible rosette compared with the upright and rosette growth types from the resistant biotype; however, the S upright growth type was similar to the R biotype across both growth types. Translocation was greater to the above and/or below treated leaves compared with the roots. However, there were no clear differences that would help to explain differential sensitivity between the rosette and upright growth types with known glyphosate resistance (Table 4.3). There was minimal translocation to the roots (0.75 to 2.7%), but the S upright-type translocated the least to the roots compared with all other growth type x biotype combinations. Previous research found lower glyphosate levels in the treated leaf of susceptible biotypes compared with the resistant biotypes (Feng et al. 2004; Koger and Reddy 2005). However, we only observed this when examining the S rosette-type compared with the R rosette-type. No differences were observed between the upright-type from the R and S biotypes. In addition, prior studies have observed reduced ^{14}C-glyphosate translocation to the crown and other leaves in resistant biotypes (Feng et al. 2004; Koger and Reddy 2005); however, by comparing growth types we observed that this was not always the case. Conversely, González-Torralva et al. (2012) reported no differences in translocation to the leaves when grouped together between resistant and susceptible horseweed biotypes. Dinelli et al. (2006) reported that more ^{14}C-glyphosate was translocated to the leaves compared with the roots in rosette horseweed, supporting what we found across all growth type biotype combinations. In contrast, prior research has found roots to be the strongest sink when applying ^{14}C-glyphosate to rosette horseweed (Feng et al. 2004; González-Torralva et al. 2012; Koger and Reddy 2005). This may have been due to different growth conditions prior to and after ^{14}C-glyphosate application.

Generally, non-target site resistance mechanisms such as impaired translocation due to rapid vacuolar sequestration have been identified as the most common mechanism of glyphosate resistance in horseweed (Dinelli et al. 2006; Feng et al. 2004; Ge et al. 2010; González-Torralva et al. 2012; Koger and Reddy 2005; Moretti and Hanson 2016). This is likely due to prior research primarily investigating non-target site resistance mechanisms and only in rosette growth types. Our research supports these findings since translocation was impeded in the R rosette-type compared with the S rosette-type 168 HAT. Interestingly, our research differs when considering upright plants. Translocation differences were not evident at 168 HAT between the S upright growth type and the R upright and rosette growth types despite resistant upright plants still being very glyphosate resistant; therefore, it is likely that a yet to be discovered resistance mechanism is at least partially responsible for glyphosate resistance in the MSU-18 biotype.

Recently, the first documented case of target-site mediated glyphosate resistance in horseweed in the United States was observed in highly resistant biotypes, 20 to 40X the field rate (1X = 840 g ae ha^{-1}), from Ohio and Iowa (Beres et al. 2020). A proline to serine mutation at position 106 of *EPSPS2* was detected, which is the same target site mutation that was identified in 21 glyphosate-resistant horseweed accessions from Canada (Page et al. 2018). Based on the recent discovery of a target site mutation in glyphosate-resistant horseweed biotypes coupled with what we found regarding translocation, the primary mechanism of resistance in earlier documented glyphosate-resistant horseweed biotypes may not be due to reduced translocation since each study only examined the rosette growth type. Thus, these studies (Dinelli et al. 2006; Feng et al. 2004; Ge et al. 2010; González-Torralva et al. 2012; Koger and Reddy 2005; Moretti and Hanson 2016) should re-examine translocation with the inclusion of the upright growth type to confirm if reduced translocation is present in both growth types. It is also possible that that these biotypes, including MSU-18, possess a target site mutation that works synergistically with non-target or other unknown mechanisms. Stacked target-site and non-target site resistance mechanisms have been observed in waterhemp [*Amaranthus tuberculatus* (Moq.) J.D. Sauer], rigid ryegrass (*Lolium rigidum* Gaud.), and annual bluegrass (*Poa annua* L.) (Bostamam et al. 2012; Kaundun et al. 2011; Laforest et al. 2021; Nandula et al. 2013).

These results suggest that differences in glyphosate sensitivity among the rosette and upright growth types with known glyphosate resistance were not due to higher glyphosate absorption, translocation, nor the total amount of glyphosate intercepted and retained on horseweed's leaf surface. As expected, we did not observe these differences in the glyphosate-susceptible biotype either. However, the upright growth type intercepted and retained 21 and 18% less glyphosate on a per weight and per area basis, thus the concentration of glyphosate may be diluted resulting in slightly higher glyphosate tolerance in the upright growth type in the R biotype. Though, this difference is not likely fully responsible for the 3- to 4-fold difference in glyphosate sensitivity between the rosette and upright growth type in the glyphosate-resistant biotype.

Recently, Laforest et al. (2020) reported the first chromosome-scale genome sequence of horseweed, which revealed at least 4 EPSPS-like genes (three of which seem to be pseudogenized). Because of this, care should be taken when amplifying and sequencing EPSPS from horseweed so as not to accidently sequence one of these non-functional copies of EPSPS. This genome will greatly assist a genome-wide association study (GWAS) to look for genetic variations amongst S and R biotypes to identify the mechanism of glyphosate resistance. Once, the mechanism of resistance has been established, additional studies should examine if there are differences within the resistance mechanism between the rosette- and upright-type with known glyphosate resistance. There may be other contributing factors to the differential sensitivity among the rosette and upright growth types, such as differences in EPSPS gene expression especially if a target-mutation is discovered such has been found in recent horseweed biotypes.

APPENDICES

APPENDIX A: Chapter IV Results
Tables and Figures

Table 4.1. Horseweed biomass and exposed leaf area (±SE)[a] for rosette and upright horseweed growth types at herbicide application[b] for interception and retention of glyphosate with Chicago Sky Blue dye.

Growth type	Biomass	Leaf area
	—— mg $plant^{-1}$ ——	—— cm^2 $plant^{-1}$ ——
Rosette	277 (±9.95) b[c]	74 (±2.16) b
Upright	385 (±9.95) a	89 (±2.12) a
Effects (P-value)		
Growth type	<0.0001	<0.0001

[a]Abbreviations: SE, standard error.
[b]Glyphosate plus Chicago Sky Blue dye applications were made approximately 42 d after planting.
[c]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

Table 4.2. Glyphosate retention (±SE)[a] on a per weight, area, and plant basis by rosette and upright horseweed growth types at 42 d after planting.

Growth type	Biomass	Leaf area	Total
	—— g ae mg^{-2} ——	—— g ae cm^{-2} ——	—— g ae $plant^{-1}$ ——
Rosette	0.0028 (±0.0001) a[b]	0.0107 (±0.0004) a	0.7808 (±0.0239)
Upright	0.0022 (±0.0001) b	0.0088 (±0.0004) b	0.7664 (±0.0233)
Effects (P-value)			
Growth type	<0.0001	<0.0001	0.6866

[a]Abbreviations: SE, standard error.

[b]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

Table 4.3. ^{14}C-glyphosate translocation and distribution (±SE)[a] in rosette and upright glyphosate-resistant and glyphosate-susceptible horseweed biotypes at 168 h after treatment[b].

Biotype	Translocation[c]	Glyphosate distribution[d]			
		Above treated leaf	Below treated leaf	Roots	Treated leaf
		% of applied			
MSU-18:Rosette	9.0 (±2.61) b[e]	0.72 (±0.03) b	6.0 (±1.16) ab	2.2 (±0.41) a	70.8 (±3.96) a
MSU-18:Upright	7.4 (±2.61) b	1.7 (±0.58) b	3.4 (±0.22) b	2.4 (±0.41) a	73.8 (±3.96) a
S-117:Rosette	19.0 (±2.82) a	6.5 (±3.04) ab	9.8 (±2.35) a	2.7 (±0.42) a	58.7 (±4.21) b
S-117:Upright	11.2 (±2.82) ab	9.3 (±2.22) a	1.2 (±0.22) c	0.75 (±0.42) b	75.0 (±4.21) a
Effects (P-value)					
Biotype	0.0388	0.0085	0.0009	0.0017	0.0225

[a]Abbreviations: SE, standard error; MSU-18, glyphosate-resistant; S-117, glyphosate-susceptible.
[b]Plants were grown in the greenhouse before ^{14}C-glyphosate application at 25 ± 5 C with a 16 h photoperiod. After application, plants were maintained in a growth chamber at 25/20 C day/night temperature with a 16-h photoperiod
[c] ^{14}C-glyphosate outside of treated leaf (above treated leaf, below treated leaf, and roots) is considered translocation.
[d] ^{14}C-glyphosate distribution throughout the plant is based on percent of ^{14}C-glyphosate applied.
[e]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

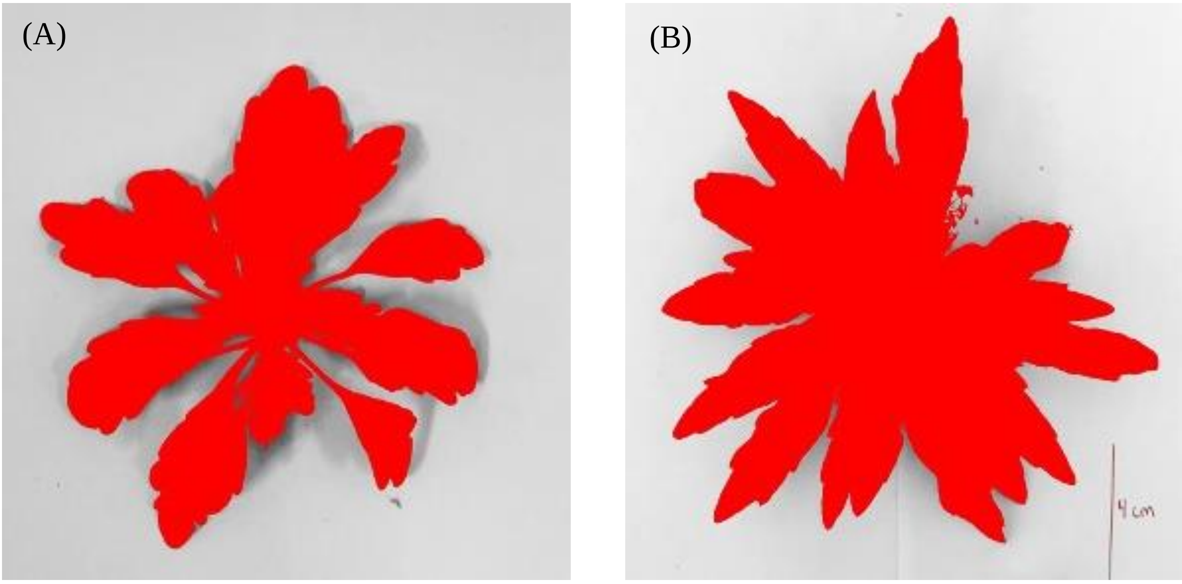

Figure 4.1. Exposed leaf area from above at the time of glyphosate application for (A) the rosette (74 cm^2), and (B) upright (89 cm^2) horseweed growth types.

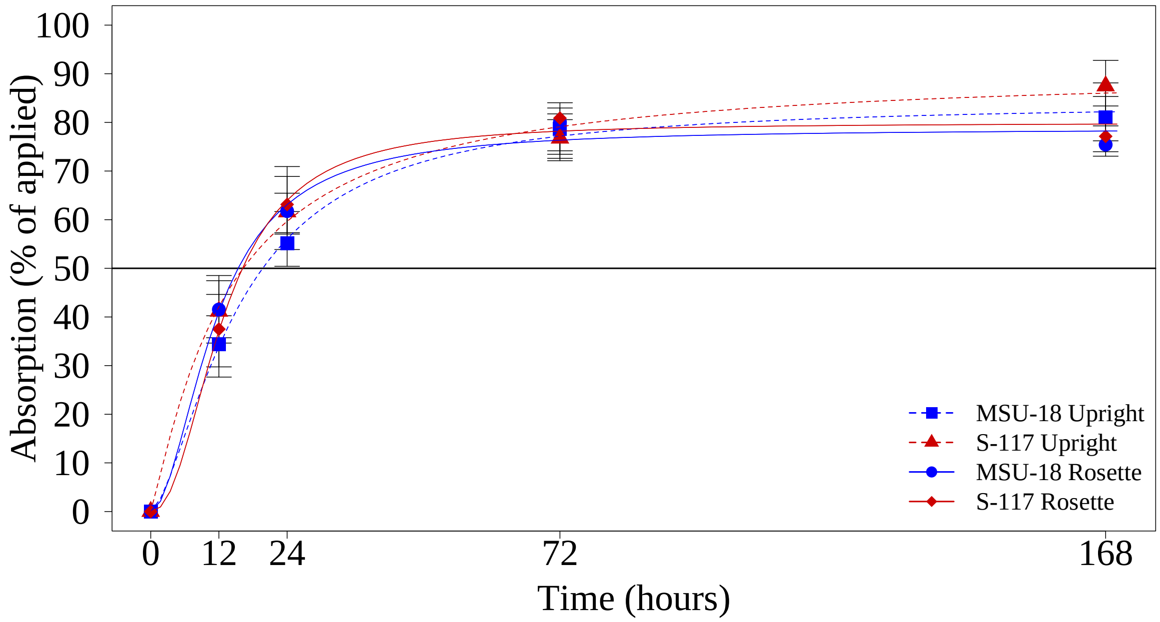

Figure 4.2. ^{14}C-glyphosate absorption over time in rosette and upright plants from glyphosate-resistant (MSU-18) and glyphosate-susceptible (S-117) horseweed biotypes.

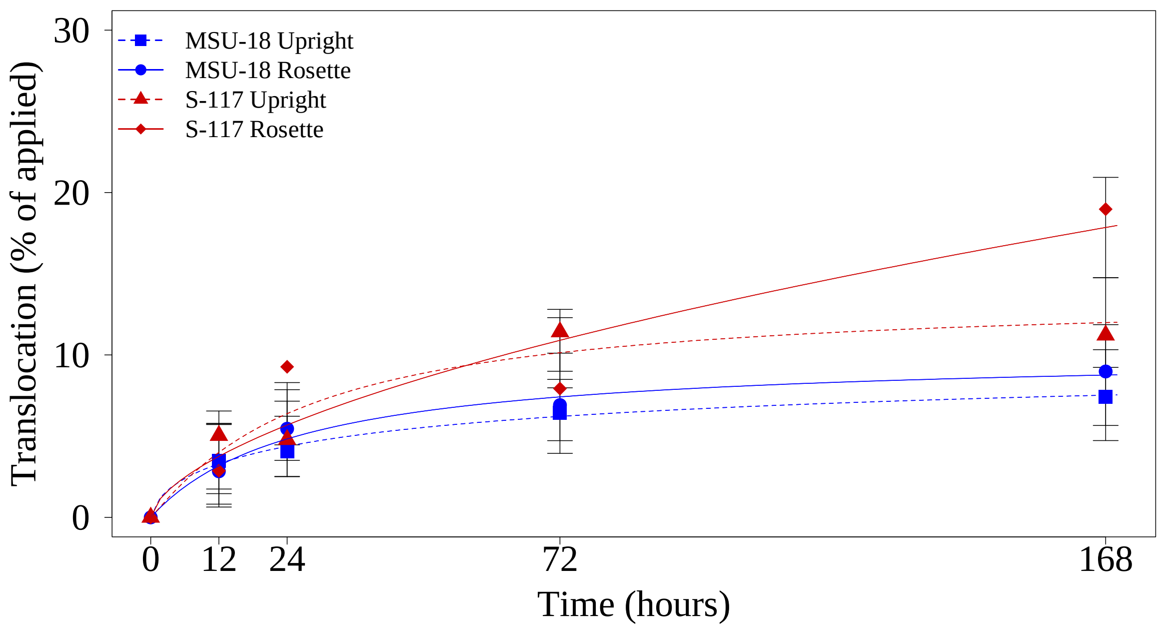

Figure 4.3. ^{14}C-glyphosate translocation over time out of the treated leaf in rosette and upright glyphosate-resistant (MSU-18) and glyphosate-susceptible (S-117) horseweed biotypes.

APPENDIX B: Supplementary information, integrating fall-planted winter wheat and soybean row widths to manage horseweed in no-tillage soybean

In the fall of 2019, a field experiment was established to evaluate the effect of a fall-planted winter wheat cover crop terminated one week before soybean planting and one week after soybean planting for the suppression of glyphosate-resistant horseweed in no-till soybean. In addition, we wanted to determine the contribution of soybean row width on horseweed suppression by comparing soybean planted in 19-, 38-, and 76-cm rows, as well as compare the integrated approaches of a winter wheat cover crop and soybean row width with and without an effective POST herbicide application on horseweed management. Winter wheat was drilled as a cover crop at 728,434 seeds ha^{-1} in 19 cm rows using a no-till drill (John Deere, Moline, IL) the fall prior to data collection. Dates for all field operations can be found in Table 1. Winter wheat was terminated, and main plots were established one week prior to (early termination) or one week after (planting green) planting soybean the following spring. Glyphosate, glufosinate, and 2,4-D choline-resistant soybean, 'P25T09E', was planted at 500,000-, 437,500-, 375,000 seeds ha^{-1}. POST herbicide applications were made 4 WAP when soybean was at the V2 growth stage in the no cover. All herbicide applications were made using a tractor-mounted, compressed air sprayer calibrated to deliver 177 L ha^{-1} at 207 kPa of pressure through 11003 AIXR nozzles (TeeJet, Spraying Systems CO., Wheaton, IL 60187). This study was only conducted in one site-year and the following tables include data pertaining to this experiment.

Table 4.4. Winter wheat seeding and termination dates, GDDs[a,b,c] until winter wheat termination, soybean planting, POST herbicide application, and soybean harvest date.

Operation	MSU
Winter wheat seeding	October 15, 2019
Early termination	May 24, 2020
GDDs (base, 4.4C)	489
GDDs (base, 10 C)	165
Soybean planting	June 1, 2020
Planting green termination	June 8, 2020
GDDs (base, 4.4 C)	718
GDDs (base, 10 C)	313
POST application	June 29, 2020
Soybean harvest	October 31, 2020

[a]Abbreviation: GDDs, growing degree days; MSU, Michigan State University.
[b]GDDs (base, 4.4 C) accumulated from the time of cereal rye planting until termination.
[c]GDDs (base, 10 C) accumulated from January 1 until cover termination for horseweed emergence.

Table 4.5. Winter wheat biomass and C:N ratios at each termination time, and winter wheat ground cover and the effect of winter wheat on horseweed density and biomass at planting green termination.

	Winter wheat			Horseweed	
Cover treatment	Biomass	C:N ratio	Groundcover	Density	Biomass
	—— kg ha^{-1} ——		—— % ——	— plants m^{-2} —	—— g m^{-2} ——
No cover	NA[a]	NA	NA	32 a	1 a
Early termination	3,188 b[b]	17:1 b	89 a	0 b	0 b
Planting green	9,211 a	30:1 a	90 a	0 b	0 b
Effects (P-values)					
Cover treatment	<0.0001	<0.0001	0.8922	<0.0001	0.0233

[a]Abbreviations: NA, not applicable.

[b]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

Table 4.6. Main effects of cover treatment and soybean row width on horseweed density, biomass, and height at the time of POST herbicide application, 4 wk after planting (WAP).

Main effects	Density	Biomass[b]	Height
Cover treatment	— plants m^{-2} —	— g m^{-2} —	— cm —
No cover	81 a[a]	36 a	14 a
Early termination	39 b	2 b	4 b
Planting green	1 c	0.05 b	1 c
Row width (cm)			
19	28	8 b	6
38	43	13 ab	6
76	51	17 a	6
Effects (P-values)			
Cover treatment	<0.0001	0.0468	<0.0001
Row width	0.2824	<0.0001	0.5113
Cover treatment x row width	0.2520	0.0052	0.8360

[a]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$

[b]There was an interaction between cover treatment and row width on horseweed biomass. Biomass was reduced 94 to 100% by planting into cereal rye (0-3 g m^{-2}) across all row widths compared with planting soybean in 76 cm rows with no cover (49 g m^{-2}).

Table 4.7. Interactions between cover treatment and POST herbicide application, and soybean row width and POST herbicide treatment on horseweed density, biomass, and height at soybean harvest.

Cover treatment	POST treatment	Horseweed density	Horseweed biomass	Horseweed height[b]
		—— plants m^{-2} ——	—— g m^{-2} ——	—— cm ——
No cover	Noneffective	78 a[a]	295 a	73 a
Early termination		58 a	38 b	35 b
Planting green		12 b	9 c	13 c
No cover	Effective	4 b	5 c	8 c
Early termination		1 b	0 d	0 d
Planting green		1 b	0 d	0 d
Row width (cm)	POST treatment			
19	Noneffective	45	90 b	33
38		49	78 b	35
76		54	173 a	53
19	Effective	0	0 c	0
38		1	0.01 c	0.25
76		4	5 c	8
Effects (P-values)				
Cover treatment		0.0013	<0.0001	<0.0001
Row width		0.7550	0.1325	0.0200
POST		<0.0001	<0.0001	<0.0001
Cover treatment x row width		0.9210	0.0887	0.0182
Cover treatment x POST		0.0005	<0.0001	<0.0001
Row width x POST		0.9512	0.0180	0.1089
Cover treatment x row width x POST		0.9713	0.0115	0.7449

[a]Means followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$

[b]The main effect of row width was significant for horseweed height. Height was reduced 14 cm by planting in narrower rows (17 cm) compared with 76 cm rows (31 cm).

Table 4.8. Main effects of cover treatment, soybean row width, and POST herbicide treatment on soil nitrate and soybean yield at harvest.

Main effects	Soil nitrate	Soybean yield[b]
Cover treatment	—— $kg\ N\ ha^{-1}$ ——	—— $kg\ ha^{-1}$ ——
No cover	29	3,169
Early termination	32	3,143
Planting green	30	3,016
Row width (cm)		
19	NA[a]	3109
38	NA	3021
76	NA	3198
POST		
Noneffective	NA	3059
Effective	NA	3160
Effects (P-values)		
Cover treatment	0.1189	0.6312
Row width	NA	0.7971
POST	NA	0.4707
Cover treatment x row width	NA	0.4097
Cover treatment x POST	NA	0.2413
Row width x POST	NA	0.0313
Cover treatment x row width x POST	NA	0.4674

[a]Abbreviations: NA, not applicable.

[b]There was an interaction between row width and POST on soybean yield. Yield was similar when planted in narrow rows, regardless of POST herbicide application (2827-3216 $kg\ ha^{-1}$). However, when an effective POST was applied, planting soybean in 76 cm rows (3,477 $kg\ ha^{-1}$) yielded 19% higher compared with 76 cm rows with a noneffective POST application (2,920 $kg\ ha^{-1}$).

APPENDIX B: Supplementary information, precipitation data

Table 4.9. Monthly and 30-yr average precipitation at Michigan State University (MSU) in 2020 and 2021 and at Kellogg Biological Station (KBS) in 2021[a,b].

	MSU			KBS	
Month	2020	2021	30-yr ave.	2021	30-yr ave.
	mm			mm	
Fall prior	158	105	143	142	159
April	73	36	83	28	78
May	110 (101)[c] (9)[d]	6 (4) (1)	93	22 (8) (3)	94
June	74 (3)[e]	177 (20)	96	233 (10)	81
July	42	83	75	114	85
August	69	96	88	122	93
September	109	74	71	88	83
October	70[f]	97	79	145	90
Total					
Cover crop	332[g]	145	-	178	-
Soybean	437[h]	674	728	894	486

[a]Michigan Automated Weather Network, http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, MI.
[b]National Oceanic and Atmospheric Administration, https://www.noaa.gov, U.S. Department of Commerce
[c]Precipitation data up to early termination
[d]Precipitation data up to soybean planting.
[e]Precipitation data up to planting green termination
[f]The harvest month includes rainfall after harvest.
[g]Total precipitation is a total of rainfall from planting until termination, not including precipitation in December, January, February, and March.
[h]Total precipitation is a total of rainfall from planting until end of October.

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