HORSEWEED GROWTH TYPES AND INTEGRATING FALL-PLANTED CEREAL COVER CROPS FOR MANAGEMENT

By

John Allen Schramski

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ABSTRACT

HORSEWEED GROWTH TYPES AND INTEGRATING FALL-PLANTED CEREAL COVER CROPS FOR MANAGEMENT

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Recent shifts in glyphosate-resistant horseweed (*Erigeron canadensis* L.) emergence patterns and growth types at the field level have generated new management questions. Field experiments investigated the effects of cereal rye and winter wheat, seeded at 67 or 135 kg ha-1, in combination with burndown herbicide strategies or terminated at different times for managing horseweed in no-tillage soybean. In absence of effective herbicides, fall-planted cereal cover crops reduced horseweed biomass up to 70 and 33% at cover termination and five weeks after soybean planting, respectively. Integrating effective herbicide strategies improved horseweed suppression and soybean yield. Delaying termination by Planting Green improved horseweed suppression through the time of postemergence application. Additional field experiments evaluated the effects of termination timing and herbicide combinations for cereal rye termination. Glyphosate applied at 1,267 g ae ha-1 to cereal rye at early (Feekes 6) or late (Feekes 10.5) growth stages effectively terminated cereal rye. The addition of dicamba to glyphosate applied late, or clethodim alone provided less control. All herbicide combinations tested, with the exception of those which included metribuzin, provided similar control to glyphosate alone. In controlled environment experiments, a vernalization period following imbibition of water, but prior to germination, induced horseweed bolting at emergence. Additionally, bolted type horseweed in glyphosate-resistant populations was less sensitive to glyphosate than rosette type. This research provides growers strategies for managing horseweed and insight into the recent glyphosate-resistant horseweed emergence and growth type phenomena observed in the field.

Dedicated to my parents John and Jean Schramski

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CHAPTER I

LITERATURE REVIEW

Horseweed

Horseweed (*Erigeron canadensis* L.) is a plant species native to North America and is also commonly referred to as marestail or Canada fleabane (Weaver 2001). It is a member of the Asteraceae family, which is comprised of 483 genera and 4,890 taxa (USDA 2020). Horseweed's classification has changed over time. It was originally assigned to the *Erigeron* family and was reassigned to the *Conyza* family because it has fewer central hermaphroditic flowers, many pistillate flowers, and lacks ligules compared with other *Erigeron* species (Cronquist 1943). However, horseweed was recently reassigned to the *Erigeron* family in the Weed Science Society of America's Composite List of Weeds. *Erigeron canadensis* L. and *Conyza canadensis* (L.) Cronquist are listed as synonyms in the USDA Plant Database (USDA 2020).

Distribution. Horseweed is a cosmopolitan weed distributed worldwide. It is considered a weed in more than 40 crops; however, it is most common in the North Temperate Zone (Holm et al. 1997; Weaver 2001). Horseweed's geographic prevalence stretches from approximately latitudes of N 55 to S 45, which indicates it has few distinct climatic requirements (Weaver 2001). Horseweed is native to every state in the contiguous United States and has also been introduced to Alaska and Hawaii (USDA 2020). In Canada, horseweed is native to, and can be found in, every providence south of latitude N 55 except Newfoundland; however, seed production and invasive potential are limited at N 52 and beyond (Archibold 1981; USDA 2020; Weaver 2001).

Non-native to Europe, it is believed that horseweed was introduced to Europe within the last 350 years and is now considered to be the continent's most completely naturalized plant of

American origin (Frankton and Mulligan 1987; Thebaud and Abbott 1995). Horseweed's ruderal nature allows it to colonize and thrive across a wide geography, including the Mediterranean Basin, Australia, Japan, and under many different habitats (Weaver 2001). It can be found growing in orchards, vineyards, disturbed areas, recently abandoned areas, along roadsides and railways, and in agricultural fields where tillage has been reduced or eliminated (Weaver 2001).

Horseweed is considered one of the top 10 most common and troublesome weeds among all broadleaf crops in the United States and Canada (Van Wychen 2016). Along with waterhemp, (*Amaranthus tuberculatus* (Moq.) J. D. Sauer) horseweed is the most common and troublesome weed in soybean (*Glycine max* (L.) Merr.) (Van Wychen 2016). The widespread presence of horseweed can be attributed to three factors: "lack of diversity in crop rotation, reduced tillage, and herbicide resistance" (Loux et al. 2006). Many characteristics of horseweed's biology aid in its identification as well as success as a troublesome weed.

Identification. Horseweed seedlings are often incorrectly identified as whitlowgrass (*Draba verna* L.), common chickweed (*Stellaria media* (L.) Vill.), mouseear chickweed (*Cerastium fontanum* ssp. Vulgare (Hartman) Greuter and Burdet), corn speedwell (*Veronica arvensis* L.), Persian speedwell (*Veronica persica* Poir.), shepherd's-purse (*Capsella bursa-pastoris* (L.) Medicus), or other fleabane species (Loux et al. 2006). It is especially difficult to distinguish between horseweed and hairy fleabane (*Erigeron bonariensis* L.) seedlings (Shrestha et al. 2008). The rooting structure of horseweed consists of a short, slim taproot with many large, branched laterals (Frankton and Mulligan 1987; Regehr and Bazzaz 1979;). Horseweed cotyledons are hairless, lack visible veins, and are oval shaped being 2 to 3.5 mm long and 1 to 2

mm wide (Bryson and DeFelice 2009; Royer and Dickinson 1999). The first true leaves are spatula-shaped and have hair on the upper surface and leaf margin (Royer and Dickinson 1999).

Fall-emerging horseweed often form a basal rosette of dark green, lightly haired leaves for winter survival; rosettes later deteriorate during mainstem elongation, also known as bolting (Loux et al. 2006; Weaver 2001). Spring-emerging horseweed skip the rosette stage immediately bolting upon emergence and are a lighter green color than rosettes (personal observation). Stems contain many hairs, grow erect, are unbranched at the base of the plant with many branches towards the top and are typically 45 to 180 cm tall (Loux et al. 2006). Horseweed leaves are alternate, linear, simple to oblanceolate, and crowded on the stem (Bryson and DeFelice 2009). Mature horseweed leaves are sessile, pubescent, have entirely or slightly toothed margins, and decrease in size moving up the plant; leaves are 2.5 to 10 cm long and 1 to 15 mm wide (Bryson and DeFelice 2009; Loux et al. 2006). When crushed, horseweed stems and leaves produce an odor that resembles carrot (Royer and Dickenson 1999). Inflorescences are located at the top of the plant and resemble a mare's tail (Bryson and DeFelice 2009).

Horseweed inflorescences consist of many small capitula positioned on an elongated panicle (Bryson and DeFelice 2009). Acuminate involucres nearly conceal the outer flowers, pistillate ray florets, which are colored white and are 1 to 2 mm long (Frankton and Mulligan 1987). The center of the capitula is made up of receptacle flowers that are disk florets colored yellow and become fluffy at maturity (Frankton and Mulligan 1987; Weaver 2001). Each capitulum contains 60-70 seeds, which are small achenes (Weaver 2001). Achenes of horseweed are yellowish brown, hairy and have an oblong and flattened shape; they are 1 to 2 mm long with an attached pappus that is greyish-white and 3-5 mm long (Frankton and Mulligan 1987; Royer and Dickinson 1999). The attached pappus is nearly three times as long as the seed in addition to

the tall, erect stems; these traits are believed to be adaptations for long-distance seed dispersal by wind (Weaver 2001).

Flowering time and seed production. Horseweed stem elongation occurs as early as May, flowers in July, and seed production peaks in early August through September (Weaver 2001). However, weather conditions such as winter warming spells can promote early flowering; the later a winter warming spell occurs, the less time it takes horseweed to flower (Tozzi et al. 2014b). Flowering Locus C, a MADS-box gene responsible for vernalization in winter wheat (*Triticum aestivum*) and *Arabidopsis thaliana*, is present in horseweed (He et al. 2004; Rudnoy et al. 2002). This would indicate that a cold period such as winter is required for flower production; however, horseweed that germinates in the spring is still able to flower and produce seed (Regehr and Bazzaz 1979).

Tozzi and Van Acker (2014) observed spring-emerging horseweed plants flower before fall-emerging plants and plants that emerge in early fall and early spring flower before plants that emerge in late fall and late spring. The pollination period of horseweed lasts approximately two months (Ye et al. 2016). Horseweed is self-compatible and pollen release occurs before the capitula are completely opened (Mulligan and Findlay 1970, Smisek 1995). This sequence suggests horseweed is primarily self-fertilizing; however, honeybees have been noted visiting open flowers (Smisek 1995). Smisek (1995) reported horseweed outcrossing averaged 4.3%, with a range of 1.2 to 14.5%, utilizing paraquat resistance as a marker in a field population of horseweed in Essex County, ON. Similarly, in a more controlled greenhouse experiment Davis et al. (2010a) reported outcrossing rates to be between 1.1 to 4.0%. Ye et al. (2016) found that a glyphosate-resistant horseweed biotype from Tennessee released 79% of its pollen between 9:00

A.M. and 7:00 P.M., with peak production at 1:30 P.M. The seasonal pollen-release pattern of horseweed has a multimodal distribution and averages 95,000 pollen grains shed per plant per day (Ye et al. 2016). Average horseweed pollen concentration decreased to 50% at a distance of 16 m from the source, which suggests that cross-pollination occurs at close distances (Ye et al. 2016).

Following fertilization, it takes approximately three weeks for horseweed seed to mature (Weaver 2001). Not including the pappus, each seed weighs 0.072 mg of which 15% is seed coat while the rest is embryo (Fenner 1983). Each horseweed plant produces approximately 200,000 seeds in the absence of crop competition and at low plant densities (Bhowmik and Bekech 1993). However, plant density negatively affects seed production (Bhowmik and Bekech 1993; Palmblad 1968). Regehr and Bazzaz (1979) found emergence timing also affected seed production, with fall emerging plants producing more seed than those that emerged in the spring. Horseweed seed production is positively correlated to plant height (Regehr and Bazzaz 1979). However, plant height and reproductive effort, defined as seeds per aboveground dry weight, are inversely related (Regehr and Bazzaz 1979). Thus, seed production is greater for tall plants than shorter plants, but this increase is less than might be expected if energy allocation remained constant. This may suggest that energy allocation towards plant height, as a dispersal advantage, is more important than seed production (Regehr and Bazzaz 1979).

Seed dispersal. Horseweed seed can travel through human transport and water, but primarily moves through wind dispersal (Weaver 2001). The tall stems and attached pappus of horseweed are wind dispersal adaptations allowing seed to be positioned high above the ground and travel long distances (Weaver 2001). It was proposed that horseweed had dispersal abilities similar to

dandelion (*Taraxacum officinale* L.) due to similar seed surface area to seed biomass ratios (Weaver 2001). However, Andersen (1993) reported field-collected horseweed seed to have a settlement velocity of 0.278 m sec-1, which was lower than dandelion and the lowest of the 19 *Asteraceae* species tested. A lower settlement velocity increases the length of time a seed is suspended in the air and can be affected by wind (Dauer et al. 2006). Seed from plants grown under optimal greenhouse conditions had a higher average settlement velocity of 0.323 m sec-1; due to more complete seed fill, causing an increase in weight and resulting velocity (Dauer et al. 2006).

Wind-borne seed such as horseweed is subject to varying dispersal distances due to the complexity of air movement by gusts, updrafts, and boundary-layer interactions (Dauer et al. 2006). Regehr and Bazzaz (1979) observed horseweed seed input into a maize field (*Zea mays* L.) ranged from 12,500 to 125 seeds m-2 at distances of 6 and 122 m downwind from the seed source, respectively. A seed trapping study determined that horseweed seed regularly disperses 500 m from source populations, but 99% of seed remains within 100 m of the source (Dauer et al. 2007). Viable horseweed seed has been collected by remote-piloted vehicles more than 50 m above ground, also known as the Planetary Boundary Layer (PBL) (Dauer et al. 2009; Shields et al. 2006). Seed travel of more than 500 km in the PBL is possible due to lower turbulence, higher wind speeds, and more laminar flow characteristics than the Surface Boundary Layer (Shields et al. 2006). Distance of travel in the PBL is influenced by the time of day dispersal occurs as well as wind speed in the PBL (Dauer et al. 2009; Shields et al. 2006).

Seed dormancy and longevity. Horseweed seed is non-dormant and can readily germinate once released from the mother plant (Buhler and Owen 1997; Loux et al. 2006). Tozzi et al. (2014b)

reported 84 to 93% of seed recruits within the season it is shed, suggesting horseweed maintains a small seed bank. In contrast, Regehr and Bazzaz (1979) observed emergence in the fall prior to seed being shed from mature plants, indicating seed recruitment from the seed bank. Viable seed was found in the seed bank of an abandoned pasture, although horseweed was not observed in the aboveground vegetation for 20 years (Tsuyuzaki and Kanda 1996). Similarly, Leck and Leck (1998) reported large quantities of horseweed seed in the seed bank of an abandoned agricultural field, although aboveground plants were not present for over ten years. Conversely, Hayashi (1979) found horseweed seed to only remain viable for two to three years under laboratory conditions. Bhowmik and Bekech (1993) found viable horseweed seed decreased with increasing burial depth and no viable seed was found below 6 cm in a no-tillage field. Additionally, Thebaud et al. (1996) found only 1% of horseweed seed remained viable after three years on the soil surface and Davis et al. (2007) observed drastic decline of seed bank densities between 18 and 23 months after study initiation.

Germination and emergence. Horseweed is a facultative winter annual, meaning it can emerge in the fall after declining soil temperatures or facultatively at other times of the year (Cici and Van Acker 2009). Horseweed has two peak periods of emergence, April to June and August to October, but has been observed emerging throughout the season (Bhowmik and Bekech 1993; Buhler and Owen 1997; Loux et al. 2006; Main et al. 2006). The proportion of the population emerging in the spring varies; Buhler and Owen (1997) observed only 5 to 32% of total emerged plants emerged in the spring. Similarly, Regehr and Bazzaz (1979) noted that spring germination contributed little to total seed output due to the dominance of already established fall emerged horseweed. In contrast, Davis and Johnson (2008) noted over 90% of an Indiana horseweed

population emerged in the spring. In Tennessee, horseweed emergence at three locations occurred in most months of the year; however, peak emergence was either in the fall, spring, or fall and spring depending on location (Main et al. 2006). Buhler and Owen (1997) suggested that the extent of time of horseweed emergence may be dependent on availability of viable seed and space for later germinating seedlings.

The environmental conditions suitable for horseweed germination are extensive (Nandula et al. 2006). The base temperature for horseweed germination was determined to be intermediate between summer and winter annuals at 13 C (Steinmaus et al. 2000). More recent research found that base germination temperatures differed among international horseweed populations: Ontario (8-9.5 C), Iran (9.5-11 C), Spain (12.5-14 C), and the United Kingdom (11-12.5 C) (Tozzi et al. 2014a). Optimal day/night temperatures for horseweed emergence were found to be 22/16 C (Buhler and Hoffman 1999; Buhler and Owen 1997). Similarly, Nandula et al. (2006) found horseweed germination increased with increasing temperature, peaking at day/night temperatures of 24/20 C and no germination occurred at day/night temperatures of 12/6 C. Main et al. (2006) observed horseweed germination between 10 and 25 C when adequate soil moisture was available; however, horseweed emergence did not correlate with environmental conditions.

In addition to temperature, horseweed germination is influenced by other factors, including burial depth, soil properties, and light (Nandula et al. 2006). Tremmel and Peterson (1983) found horseweed germination was reduced when seed was buried 1 cm compared with seed sown on the surface. Similarly, Nandula et al. (2006) observed maximum emergence to occur on the soil surface while no germination occurred from seeds placed at 0.5 cm or deeper. Seed burial increased overwintering seed persistence and encouraged germination the following spring, especially for seed shed later in the fall (Tozzi et al. 2014b). VanGessel (2001) observed

horseweed emerging from a depth of 0.5 cm in a commercial potting mix. This is consistent with other findings that horseweed is more common and emerges faster in well-drained coarse, stony, sandy, or fertile loam soils compared with fine textured soils (Frankton and Mulligan 1987; Nandula et al. 2006).

Higher germination in well-drained soils coincides with horseweed's ability to germinate under moderate water-stress conditions and tolerate drought (Nandula et al. 2006; Shontz and Oosting 1970; Weaver 2001). Germination rates are higher after rain events and when soil moisture is high, but horseweed has little tolerance to flooding (Regehr and Bazzaz 1979; Smith and Moss 1998; Stoecker et al. 1995). Horseweed germination is better in neutral to alkaline soils compared with acidic soils and horseweed has the ability to germinate under high salinity conditions (Nandula et al. 2006).

Horseweed seed can germinate under both light and complete darkness; however, germination under light is more favorable (Nandula et al. 2006). Shontz and Oosting (1970) reported much lower germination in the dark compared with light of surface sown seeds and no germination of buried seeds in the dark. Residue from a previous crop has shown to reduce horseweed emergence over 75% due to a reduction in light quantity and quality reaching the soil surface (Bhowmik and Bekech 1993; Main et al. 2006). Increased residue can delay spring horseweed germination by four weeks and reduce total fall germination (Bhowmik and Bekech 1993). However, Milberg et al. (1996) observed significantly more horseweed seed germination following 5 seconds of light exposure compared with complete darkness. This suggest that germination may be induced by just a flash of light. If germination requirements are not met in the fall, horseweed seed has the ability to overwinter and remain viable for spring germination (Tozzi et al. 2014b).

Rosette vs. bolting growth types. Fall emerging horseweed overwinter as a rosette, while spring emerging horseweed seldom pass through or spend a short period of time as a rosette before bolting (Loux et al. 2006; Regehr and Bazzaz 1979). Horseweed that emerges later in the season, in July and August, usually remain a rosette until the following spring, but occasionally bolt and produce flowers in the fall (Loux et al. 2006). Tozzi and Van Acker (2014) suggest this enables spring emerging horseweed to spend less time and energy before bolting and flowering while not reducing fecundity. Early emergence is beneficial for seedling survival and overall productivity of both fall and spring emerging horseweed (Tozzi and Van Acker 2014). Overwintering horseweed rosettes can photosynthesize at low air temperatures and levels of light during the winter (Regehr and Bazzaz 1976). Emergence in the fall allows horseweed rosettes to begin growth early in the spring and gain a competitive advantage over spring seeded crops and emerging weeds (Buhler and Owen 1997; Main et al. 2006).

Overwintering rosettes are subject to frost heaving, which can cause seedling mortality (Regehr and Bazzaz 1979). Winter warming spells exacerbate frost heaving and cause an acclimatization response due to rapid temperature changes; mortality increases when warming spells are closer to spring and temperature fluctuations are greater (Tozzi et al. 2014b). Frost heaving and therefore winter mortality are also influenced by the number and speed of frost events as well as soil texture, organic matter, and moisture (Regehr and Bazzaz 1979). Regehr and Bazzaz (1979) found that survival of overwintering rosettes is strongly correlated with rosette size. Late emerged, small rosettes have a less developed root system and are more prone to uprooting from frost heaving (Regehr and Bazzaz 1979). In contrast, Davis and Johnson (2008) reported higher mortality when rosettes were greater than 9 cm in diameter and frost heaving was the main cause of winter mortality.

Competition. Horseweed is generally believed to be less competitive than most summer annual weeds (Loux et al. 2006). Limited studies examining horseweed competition with crops exist, but some reports exhibit significant competitive ability and detrimental yield losses in soybean and cotton. Before resistance to glyphosate, untreated horseweed reduced no-till soybean yields up to 83% (Bruce and Kells 1990). Eubank et al. (2008) observed up to 97% soybean yield loss when glyphosate-resistant horseweed was left untreated compared with burndown herbicide programs. Similarly, Byker et al. (2013a) reported soybean yield reductions of 83 to 93% when no herbicide was applied to glyphosate-resistant horseweed. In cotton, glyphosate-resistant horseweed reduced lint yield 29 to 48% (Waggoner et al. 2011). Davis and Johnson (2008) reported horseweed plants growing under a soybean canopy survived to produce seed, but late-season escapes protruding above a soybean canopy produced 88 to 98% of total seed production.

Interspecific competition studies have shown horseweed's ability to compete with other plants as well as tolerate competition (Bartelheimer et al. 2006; Shontz and Oosting 1970). Horseweed is more affected by competition in sandy soils than heavy soils and decreased nutrient availability reduces the competitive ability of horseweed (Shontz and Oosting 1970). Levang-Brilz and Biondini (2002) reported that horseweed increases its root to shoot ratio and nitrogen productivity, defined as nitrogen uptake per total plant weight, when nitrogen availability is low. The relative growth rate of horseweed was reported to be 0.16 by Levang-Brilz and Biondini (2002). Similarly, Davis et al. (2009b) calculated the mean relative growth rate of three herbicide-resistant and one susceptible horseweed population to be 0.157. In addition to characteristics that make horseweed competitive with crops, herbicide resistance has made horseweed extremely difficult to manage.

Herbicide resistance. Horseweed has been confirmed resistant to at least one herbicide site-ofaction in 18 different countries (Heap 2019). Horseweed populations have evolved resistance to acetolactate synthase (ALS) inhibitors (Group 2); photosystem II inhibitors (Group 5); photosystem II inhibitors (Group 7); glyphosate, the 5-enolpyruvyl-shikimate-3-phosphate inhibitor (EPSP) (Group 9); and paraquat, a photosystem I electron diverter (Group 22) (Heap 2019).

The first case of herbicide-resistant horseweed was reported in 1980 when Japanese researchers described biotypes resistant to the PS I diverter (Group 22) herbicide, paraquat (Loux et al. 2006). Paraquat-resistant horseweed in North America was not reported until 1994 in Mississippi (Heap 2019). Following the report of paraquat-resistance, horseweed resistant to atrazine, photosystem II inhibitor (Group 5), was found in France in 1981 followed by many other populations resistant to photosystem II inhibitors throughout the world (Heap 2019). Some populations are resistant to multiple herbicide sites of action. The first known multiple-resistant horseweed population was identified in Israel in 1993 with resistance to atrazine (Group 5) and many ALS-inhibitors (Group 2) (Heap 2019). Multiple-resistant horseweed biotypes were confirmed in Michigan blueberry orchards in 2002 with resistance to atrazine and simazine (Group 5) and the photosystem II inhibitor (Group 7), linuron (Heap 2019; Loux et al. 2006). Several other instances of multiple-resistant horseweed populations have been reported in the United States, including Ohio and Indiana, with populations resistant to glyphosate (Group 9) and ALS-inhibitors (Group 2) chlorimuron-ethyl and cloransulam-methyl (Kruger et al. 2009; Trainer et al. 2005).

While populations of horseweed resistant to different herbicide sites of actions can be found worldwide, glyphosate resistance has become widespread and influenced management in

many cropping systems the greatest. The first confirmed case of glyphosate-resistant horseweed was identified in Delaware in 2000 (VanGessel 2001). This population was collected in a glyphosate-resistant soybean field after relying solely on glyphosate for weed control for three years. The glyphosate-resistant population exhibited 8 to 13-fold level of resistance compared with a susceptible horseweed population (VanGessel 2001). By 2019, glyphosate-resistant horseweed had been confirmed in 13 countries and was the most widespread glyphosate-resistant weed (Heap 2014; 2019). In the United States, glyphosate-resistant horseweed has spread across 25 states, including Michigan in 2007 (Heap 2019). It has also been identified in many different settings including alfalfa, corn, cotton, soybean, rice, wheat, fruit, grapes, nurseries, orchards, railways, roadsides and along fence lines (Heap 2014). This is believed to be a result of horseweed's effective seed dispersal mechanism coupled with a rapid increase in glyphosate use since the introduction of Roundup Ready® crops in 1996 (Heap 2014). Horseweed resistant to glyphosate in Ohio and Indiana is most commonly reported in situations where soybean is planted continuously, glyphosate is solely relied on for weed control, and tillage is reduced or absent (Loux et al. 2006).

Fitness penalties have not been observed for glyphosate-resistant horseweed biotypes (Davis et al. 2009b; Zelaya et al. 2004). However, developmental differences between glyphosate-resistant and -susceptible biotypes have been conflicting. Under greenhouse conditions, Nandula et al. (2015) observed greater leaf count, rosette diameter, and root and shoot fresh weight in a glyphosate-resistant population compared with the susceptible population. In contrast, Davis et al. (2009b) reported no difference in aboveground shoot mass and seed production between glyphosate-resistant and -susceptible populations grown under field conditions. In a pot experiment, Shrestha et al. (2010) reported a glyphosate-resistant population

exhibited earlier bolting, floral bud formation, flowering, and seed set in absence of competition. However, the glyphosate-susceptible population accumulated 40% more shoot dry biomass by the onset of seed set (Shrestha et al. 2010). Under increasing levels of competition, glyphosateresistant plants were taller and had greater dry matter accumulation than susceptible plants (Shrestha et al. 2010). Koger et al. (2004) reported that the growth stage of rosette type horseweed plants did not affect glyphosate-resistance levels of three populations. However, Shrestha et al. (2007) reported tolerance to glyphosate increased when horseweed plants began to bolt compared with applications to rosette plants.

Since the confirmation of glyphosate-resistant horseweed in 2001, the mechanism of resistance has been studied extensively. Feng et al. (2004) reported glyphosate absorption was similar between resistant and susceptible biotypes collected in Delaware; however, glyphosate translocation from shoot to root was two times higher in susceptible compared with resistant biotypes. Reduced translocation was later observed in many other glyphosate-resistant horseweed populations (Dinelli et al. 2006; González-Torralva et al. 2012; Koger and Reddy 2005; Moretti and Hanson 2016; Nandula et al. 2005). These populations generally exhibit 2 to 13-fold resistance to glyphosate compared with the susceptible populations (Kruger et al. 2008; Main et al. 2004; VanGessel 2001). Ge et al. (2010) further confirmed that reduced translocation was due to rapid sequestration of glyphosate into the vacuole. Vacuolar sequestration is associated with tonoplast active transporters of the ATP-binding cassette transporters (Ge et al. 2014). Additionally, vacuolar sequestration diminished under low temperatures yielding similar glyphosate toxicity between resistant and susceptible biotypes (Ge et al. 2011).

González-Torralva et al. (2012) found vacuole sequestration along with enhanced metabolism of glyphosate to cause resistance in a horseweed population in Spain. This is the

only report of enhanced metabolism in glyphosate-resistant horseweed and studies by Dinelli et al. (2006) and Feng et al. (2004) found similar rates of metabolism between resistant and susceptible populations. In addition to reduced translocation, Dinelli et al. (2006) observed a small increase in the expression of EPSPS. Page et al. (2018) reported the first target-site resistance to glyphosate in an Ontario horseweed population which possessed the Pro-106-Ser substitution at the target site, EPSPS2. Non-target site mechanisms may also be present in this horseweed population and add to the level of resistance (Page et al. 2018). Horseweed populations in Ohio and Iowa have since been documented to possess this substitution (Beres et al. 2019). Target-site resistance to glyphosate confers much greater levels of resistance, 16 to 40fold, compared with non-target site resistance in horseweed (Beres et al. 2019; Page et al. 2018). Notwithstanding, reduced translocation is the most common mechanism of glyphosate resistance in horseweed.

Horseweed's ability to disperse seeds up to 500 km creates concern that one evolutionary event creating herbicide-resistance could result in rapid geographic spread. Dinelli et al. (2006) reported that horseweed populations collected from Delaware, Virginia, Ohio, and Arkansas shared a common non-target site resistance mechanism even though they did not share a common geographic origin. Furthermore, phylogeographic analysis showed glyphosate resistance has evolved at least four times in Delaware, Tennessee, Ohio/Indiana, and California (Yuan et al. 2010). Multiple independent origins of glyphosate resistance occurred within smaller regions such as California's Central Valley (Okada et al. 2013). Similar agronomic practices across the United States such as heavy reliance on glyphosate, no herbicide rotation, and notillage create common mechanisms through which independent evolutions of glyphosateresistance across different regions may occur (Dinelli et al. 2006). The ability of horseweed to

develop resistance to glyphosate and multiple other herbicide sites of action, along with its prolonged period of emergence make it difficult for growers to manage in glyphosate-resistant soybean.

Horseweed Management with Herbicides

Glyphosate-resistant horseweed has become a management challenge in soybean due to the limited number of effective postemergence (POST) herbicide options. Herbicides are generally more effective when horseweed plants are small or at early growth stages (Everitt and Keeling 2007; Kruger et al. 2008; Mellendorf et al. 2013; Steckel et al. 2006). However, proper timing of herbicide application is difficult due to horseweed's ability to emerge throughout the growing season. Davis et al. (2007) found that effective control of emerged horseweed before planting and utilizing a herbicide with residual activity is critical for effective management.

Extensive research on burndown herbicide applications prior to soybean planting has provided excellent control of emerged horseweed (Bruce and Kells 1990; Byker et al. 2013c; Davis et al. 2007; 2010b; Eubank et al. 2008). Prior to the evolution of glyphosate-resistant biotypes, preplant applications of glyphosate provided adequate control of horseweed (Bruce and Kells 1990). The most effective control options for glyphosate-resistant horseweed prior to soybean planting are 2,4-D (Group 4), dicamba (Group 4), glufosinate (Group 10), saflufenacil (Group 14), and paraquat (Group 22) (Byker et al. 2013c; Davis et al. 2010b; Eubank et al. 2008; Everitt and Keeling 2007; Kruger et al. 2008; 2010a; Loux et al. 2006; Loux and Johnson 2014; Mellendorf et al. 2013; Montgomery et al. 2017). However, Montgomery et al. (2017) reported that time of day when burndown treatments were applied influenced horseweed control for all of these herbicides. Control using glufosinate can vary by horseweed density and the interval

between application and planting (Eubank et al. 2008; Steckel et al. 2006). Horseweed control with paraquat and glufosinate was reduced in low air temperature or high cloud cover situations (Eubank et al. 2008; 2012; Steckel et al. 2006). Inconsistent control of horseweed was observed when burndown applications of 2,4-D were applied to plants 30 cm or taller (Kruger et al. 2010a ; Mithila et al. 2011). In contrast, Wiese et al. (1995) found horseweed was best controlled with 2,4-D when plants were 30 cm tall and actively growing. Similarly, Kruger et al. (2010a) reported size of horseweed did not influence efficacy of dicamba applications. Applying a burndown herbicide nearest to peak emergence provided the best control of horseweed (Davis et al. 2010b). To combat inconsistent control of burndown herbicide treatments and late emerging horseweed a residual herbicide may be necessary.

Davis et al. (2007) reported preemergence (PRE) herbicides with residual activity reduced horseweed densities and maximized soybean yield. They also reduced horseweed height and enhanced the activity of POST herbicides (Davis et al. 2010b). In non-ALS resistant horseweed populations, the ALS-inhibitors chlorimuron and cloransulam provided residual control of horseweed (Bruce and Kells 1990; Moseley and Hagood 1990; Davis et al. 2010b). However, due to the extent of ALS-resistance in horseweed populations, Loux and Johnson (2014) recommended avoiding ALS-inhibitors and including metribuzin (Group 5), sulfentrazone (Group 14), or flumioxazin (Group 14) as spring-applied residual herbicides for use in no-till soybean. Several studies reported reductions in horseweed density and increased soybean yield from the residual activity of metribuzin (Budd et al. 2016; Byker 2013b; Eubank et al. 2008; 2012; Kapusta 1979; Soltani et al. 2017). However, metribuzin applied at 800 to 1,600 g ha-1 to sandy loam soil when soil moisture was high caused up to 40 and 20% soybean injury at 2 and 4 weeks after emergence, respectively (Budd et al. 2016). Adding sulfentrazone to

glufosinate burndown applications provided 81 to 93% horseweed control 4 weeks after treatment (WAT) (Eubank et al. 2008). Similarly, adding flumioxazin to glufosinate provided 77% horseweed control 8 WAT (Steckel et al. 2006). Flumioxazin plus dicamba burndown applications provided 86% horseweed control 21 DAT (Owen et al. 2009). However, potential degradation of soil-applied residual herbicides along with horseweed's extended emergence pattern make it likely that postemergence herbicides will be necessary for control.

Prior to the commercialization of glyphosate-resistant soybean in 1996 and glufosinateresistant soybean in 1999, few postemergence herbicides effectively controlled horseweed (Bruce and Kells 1990; Moseley and Hagood 1990). Due to widespread resistance to glyphosate, growers must rely on glufosinate or new herbicide-resistant traits in soybean for postemergence control of horseweed. Under ideal conditions, glufosinate can effectively control emerged horseweed; however, applications made at sunrise or sunset provide significantly less control than midday applications (Montgomery et al. 2017). Roundup Ready 2 Xtend soybean were recently commercialized and provide resistance to glyphosate and dicamba (Behrens et al. 2007). Johnson et al. (2010) reported the residual activity of dicamba applied preemergence was sufficient for early-season control of horseweed, but a postemergence application improved control. Similarly, Byker et al. (2013a) reported dicamba applied preplant at 600 g ae ha-1 or sequential applications of dicamba at 300 g ae ha-1 provided greater than 90% horseweed control 8 WAT. In addition to Roundup Ready 2 Xtend soybean, the recent commercialization of Enlist E3 soybean provides resistance to 2,4-D, glyphosate, and glufosinate (Wright et al. 2010). Burndown applications of 2,4-D plus glufosinate or glyphosate provided greater than 92% horseweed control 4 WAT (Simpson et al. 2017). Additionally, applying a sequential application of these mixes provided greater than 95% control 4 WAT. However, Flessner et al. (2015) and

Kruger et al. (2010b) reported that horseweed was able to produce seed following application of dicamba and 2,4-D, respectively. Thus, the addition of cultural and mechanical control methods is necessary to slow the evolution of resistance to dicamba and 2,4-D.

Horseweed Management using Other Methods

Mechanical and cultural methods for horseweed control have been studied for their ability to help reduce the reliance on chemical control. One common method of mechanical weed control is utilizing a mower to eliminate emerged plants. However, mowing for horseweed control is not recommended because it tends to stimulate branching, which simply delays seed production and causes plants to harden off, making postemergence herbicides less effective (Shrestha et al. 2008). Horseweed is controlled using repeated applications of propane flaming, but only at the seedling growth stage (Shrestha et al. 2008).

The most effective mechanical control method studied has been tillage. Even light tillage with a disc in the fall or spring prior to planting can effectively control emerged horseweed (Brown and Whitwell 1988; Kapusta 1979). Bhowmik and Beckech (1993) reported conventional-tillage reduced and distributed seed throughout the soil profile compared with no-tillage. In a study comparing tillage intensity, horseweed was found in 61, 24, and 8% of no-till, reduced-till, and conventional-till fields, respectively (Barnes et al. 2004). Tozzi and Van Acker (2014) found that tillage did not affect horseweed emergence timing but did influence emergence levels compared with no-tillage. Emergence was similar among three seasons in tilled plots but was reduced over time in no-tillage plots, likely due to succession and competition of other weed species (Tozzi and Van Acker 2014). However, many growers in Michigan are adopting no-

tillage practices to reduce soil erosion and compaction, conserve soil moisture, and decrease fuel expenses.

Cultural weed control methods such as crop rotation, crop residue, and cover crops have been studied for their possible contributions to horseweed management. After the first two years of an experiment comparing soybean-soybean and soybean-corn crop rotations, Davis et al. (2007) found no difference in horseweed plant or seed bank densities. However, after a second cycle of the crop rotations it was reported that horseweed densities were reduced in the soybeancorn rotation (Davis et al. 2009a). The authors suggested this was likely due to herbicide rotation in corn rather than crop competition. Field surveys in Indiana found horseweed in 63% of double-crop soybean, 51% of full-season continuous soybean, and 47% of soybean following corn fields, respectively (Barnes et al. 2004). Plant residue from a previous corn crop can delay horseweed emergence and reduce total emergence up to 80% in no-tillage fields (Bhowmik and Bekech 1993). Similarly, Main et al. (2006) found residue from a corn or cotton crop reduced horseweed emergence by 77% compared with no residue. The residue cover from previous crop may also increase winter mortality of fall-emerging horseweed by delaying emergence and slowing growth (Buhler and Owen 1997). Including a cover crop to a crop rotation could provide additional residue cover to aid in management of horseweed.

Cover Crops

Cover crops and green mulches are crops employed for purposes beyond the typical food, fiber, and fuel uses of cash crops. Traditionally, cover crops were used to protect the soil from erosion and nutrient loss while covering the ground and can be thought of as short-term rotations between main crops (Reeves 1994). Cover crops are typically planted into or after a cash crop and killed prior to planting the next crop, whereas living mulches are cover crops planted before

or simultaneously with a main crop and kept living throughout the growing season (Hartwig and Ammon 2002). Brassicas, legumes, and grasses are the three major categories of cover crops and their lifecycle can be summer annual, winter annual, or perennial (Dabney et al. 2010). Management decisions and species selection of cover crops is determined by the objectives of the grower. Many cover crop options are available, and they are often mixed or combined depending on the goals of the grower, region the cover crops will be grown in, and time of year the cover crops will be established. Cover crops are being planted on a greater number of acres each year as farmers seek to resist soil erosion, build soil health, and discover new benefits of cover crops (CTIC 2017). According to the 2017 USDA Census of Agriculture, cover crops were planted on 3.88% of total cropland hectares in the U.S (USDA-NASS 2017).

Benefits/services. Cover crops provide value to cropping systems by actively growing when soil might otherwise be fallow (Dabney et al. 2001). Cover crops provide many ecosystem services that benefit crop performance, nutrient cycling, and general cropping system function (Snapp et al. 2005). They improve surface- and groundwater quality by reducing soil erosion, nitrogen leaching, and phosphorus runoff (Adeli et al. 2011; Qi and Helmers 2009). Legume cover crops can enhance growth and yield of marketable crops by their ability to fix nitrogen (Coombs et al. 2017). Nitrogen fixation from a legume or nitrogen scavenging from other cover crops can improve nitrogen retention and reduce fertilizer costs (Dabney et al. 2010). Cover crops also provide environments for pollinators and beneficial predators of insects and weed seeds (Decourtye et al. 2010; Ward et al. 2011). In the U.S., survey respondents similarly noted the key benefits of utilizing cover crops were improved soil health, yield consistency, yield increases, and controlling herbicide-resistant weeds (CTIC 2017). An increase in the utilization of cover

crops is expected as cost share programs or incentives rise as well as grower interest and awareness.

Increasing occurrences of herbicide-resistant weed populations have increased the interest of using cover crops as a mechanism for weed suppression. Cover crops compete with weeds chemically via allelopathy and physically through direct competition for resources and modification to the soil environment (Conklin et al. 2002; Creamer et al. 1996; Dyck and Liebman 1994; Snapp et al. 2005). Allelopathy covers both the chemical- inhibitory and stimulatory effects of one plant on another plant; however, most of the focus is on the interference of germination, growth, or development of another plant (Kruse et al. 2000). Allelochemicals have been identified in many cover crop species and classes such as benzoxazinoids, heliannuols, and benzoquinones offer potential utility for weed suppression (Kelton et al. 2012). Allelochemical concentration is dependent on the age and density of the allelopathic plant when terminated as well as soil- pH, organic matter content, moisture, and availability of alternative carbon sources to microorganisms (Blum et al. 1993; Blum 1996; Kruse et al. 2000). Exudation of allelochemicals are achieved via root exudation, leaching from dead or living tissue, or through volatilization of aboveground plant parts (Jabran et al. 2015). Previous research has shown that allelochemicals are more effective on broadleaf weeds than grasses (Barnes and Putnam 1986; Weston et al 1989). Allelochemical suppression of weed emergence or growth generally occurs at cover crop termination and is typically short-lived (Kruidhof et al. 2009). Until 2012, allelopathic effects were difficult to distinguish from direct competition and physical interference in the field, so a protocol to verify allelopathy of well know allelochemicals was developed for field experiments (Rice et al. 2012; Teasdale et al. 2012).

Physical suppression of weeds by cover crops can occur either in the form of living plants or through the residue remaining after cover crop termination (Teasdale et al. 2007). Numerous studies have found that cover crops can suppress weeds via resource competition if grown simultaneously (Akobundu et al. 2000; Blackshaw et al. 2001; Brennan and Smith 2005; Creamer and Baldwin 2000; Favero et al. 2001; Grimmer and Masiunas 2004; Peachey et al. 2004; Stivers-Young 1998). The ability of different cover crop species to optimally compete with weeds can vary substantially with climatic conditions and soil fertility (Brainard et al. 2011). In most cases, there is a negative correlation between cover crop and weed biomass (Akemo et al. 2000; Ross et al. 2001; Sheaffer et al. 2002). However, Brainard et al. (2011) found that even when cover crops reduced weed biomass by 90% or more, certain weed species were capable of producing large quantities of seed. Some cover crop residues also harbor seedling diseases which infect certain weed species (Conklin et al. 2002). In addition, living cover crops provide habitat for beneficial seed predators, which can help reduce the weed seed bank (Gallandt et al. 2005). Following cover crop termination, the remaining cover crop residue forms a mulch layer which impedes weed germination and emergence (Mirsky et al. 2013). Teasdale and Mohler (1993) found that light and temperature cues required for weed seed germination can be inhibited by cover crop residue on the soil surface. High levels of mulch also create a physical barrier for emerged weed seedlings, which inhibits upward movement of seedlings and downward penetration of light (Mirsky et al. 2013). However, cover crop residues do not persist long enough to provide weed suppression throughout the season in long season grain crops due to residue degradation (Osipitan et al. 2018).

C:N degradation. Plant residue degradation is influenced by the initial chemical composition of the residue as well as site dependent factors such as climatic conditions and soil organisms (Eiland et al. 2001). Carbon to nitrogen ratio (C:N) is the most common tool for predicting residue decomposition rates when other chemical characteristics are similar (Wagger et al. 1998). Soil microorganisms thrive when the C:N ratio of an added residue is near 24:1 (NRCS 2011). Cover crop residues with a lower C:N ratio are quickly broken down by soil microorganisms while residues with higher C:N ratios degrade slower as microorganisms immobilize soil nitrogen to complete decomposition (NRCS 2011). In general, non-legume cover crops such as cereal rye have C:N ratios higher than 24:1 and therefore have the potential to persist longer (Kuo and Jellum 2002). Legume cover crops such as hairy vetch have higher nitrogen concentrations and therefore lower C:N ratios, making them less persistent (Clark et al. 1997). Mixing legume cover crops with grasses leads to transfer of nitrogen to the non-legume crop and lowers the overall C:N ratio (Ranells and Wagger 1996). Previous research has shown that the C:N component of non-legume cover crops such as cereal rye increases as it matures (Sullivan et al. 1991). If weed suppression is the primary objective, biomass accumulation and residue persistence are important factors in cover crop species selection.

Winter annual cereal cover crops. Horseweed can emerge in the fall or spring, so cover crop species with similar lifecycles could potentially be utilized as a suppression tool. Winter annual cover crops are planted in late summer or fall, initiate growth before winter, and rapidly accumulate biomass during the spring prior to planting a summer crop (Teasdale 1996). These cover crops are highly effective at reducing erosion on bare soils through the winter months (Snapp et al. 2005) and can decrease nitrate leaching by up to 50% (Fraser et al. 2013). When

utilized in no-till systems, winter cover crops reduce diurnal fluctuations in soil temperature by decreasing the maximums and increasing the minimums compared with conventional tillage systems (Dabney et al. 2001). In addition to these benefits, Cholette et al. (2018) observed glyphosate-resistant horseweed suppression by winter annual cover crops from May to September in corn compared with the no cover crop control. Similarly, Wallace et al. (2019) reported horseweed density reductions of 52 to 86% with fall-planted cover crops in no-till soybean compared with the no cover crop control. Winter annual small grain cover crops overwinter in colder climates better than other species and can experience rapid growth at temperatures as low as 10 C (Clark 2007). Wallace et al. (2019) observed winter-hardiness as an important cover crop attribute to effectively reduce the number of large horseweed plants at the time of herbicide application. For these reasons, small grain cover crops that are winter-hardy, such as cereal rye (*Secale cereale* L.) and winter wheat (*Triticum aestivum* L.), need to be considered as potential suppression tools for glyphosate-resistant horseweed.

Cereal rye and winter wheat are two of the most commonly grown winter annual cover crops prior to no-till soybean in the Midwest. When utilized as a cover crop, they provide many of the same benefits, with some small differences (Clark 2007). Winter wheat is more commonly grown as a cash crop, with roughly 12,750,000 hectares planted and 10,000,000 hectares harvested in the U.S. in 2019 (USDA-NASS 2019). Additionally, wheat doubles as a forage for winter grazing in some areas, such as the southern Great Plains (Edwards et al. 2011). Cereal rye can also be used for forage, which can help alleviate forage shortages for livestock farmers (Ketterings et al. 2015; Kim et al. 2016). Among temperate region cereal cover crops, cereal rye is recognized as the easiest to establish, the most cold tolerant, the most productive, and the earliest to head (Dabney et al. 2001). Cereal rye can germinate at 1 C, exhibit vegetative growth

at 3 C, and survive temperatures as low as -30 C (Clark 2007; Fowler and Carles 1979). Winter wheat is a less costly alternative to cereal rye and is slower to mature, making it more manageable in the spring and less likely to become a weed than cereal rye (Clark 2007). Similar to cereal rye, winter wheat is extremely winter-hardy and can withstand temperatures as low as -21 C (Fowler and Carles 1979). However, winter wheat is more prone to winter damage and mortality than cereal rye (Duiker 2014; Peltonen-Sainio et al. 2011).

Previous research on winter annual cereal grains as cover crops has mainly focused on cereal rye, although winter wheat and others provide similar benefits (Meisinger et al. 1991). Cereal rye provides many ecosystem services, including improved soil physical properties and decreased wind and water erosion (Meisinger et al. 1991; Kaspar et al. 2001). Cereal rye and winter wheat are nitrogen scavengers and compared with a no cover crop control reduced nitrate leaching by 50 and 63%, respectively (Kaspar et al. 2007; Kladivko et al. 2004). When utilized in a corn-soybean rotation, Villamil et al. (2006) found that cereal rye improved water-aggregate stability and effectively trapped soil phosphorus compared with winter fallow. Additionally, Eckert (1991) reported an increase in exchangeable potassium concentrations near the soil surface. Cereal rye and winter wheat have extensive root systems and can provide low resistance paths for cash crop roots in compacted soils, while the mulch left following termination can limit evaporation and provide soil water conservation in a droughty season (Williams and Weil 2004). Lastly, numerous studies reported weed suppression from cereal grain cover crops (Misrky et al. 2013; Ryan et al. 2011a; 2011b; Teasdale and Mohler 2000). Among survey respondents in the U.S. in 2016, cereal rye was the most popular cover crop and winter wheat was the third most popular cereal grain, following cereal rye and oats (Avena sativa L.) (CTIC 2017).
Cover crop biomass. Depending on the ecosystem service in question, the degree to which cereal rye and winter wheat are beneficial is often dependent on biomass production (Finney et al. 2016). For example, Ryan et al. (2011b) found weed biomass decreased with increasing levels of cereal rye residue and weeds were completely suppressed when biomass levels were above 1,500 kg ha-1. Previous research found that among a wide range of locations, cereal rye generally produced more biomass than winter wheat (Bauer and Reeves 1999; Cornelius and Bradley 2017; Duiker 2014; Kaspar and Bakker 2015; McCormick et al. 2006; Price et al. 2006; Reeves et al. 2005). Haramoto (2019) reported cereal rye accumulated nearly three times as much biomass as winter wheat in one year of a study. However, other studies observed no differences in winter wheat and cereal rye biomass differences between cereal rye and winter wheat when seeded at 34 kg ha-1, but no difference at 112 kg ha-1 in one year of a study. Generally, larger differences in biomass of cereal rye and winter wheat were observed when temperatures were low and few growing degree days (GDD) were accumulated.

Cereal rye and winter wheat biomass accumulation prior to spring termination is influenced by weather and generally greater the farther south they are grown. For example, cereal rye cover crop produce an average biomass of 3,300 to 4,500 kg ha-1 in the Northeastern U.S. (Clark 2007) while biomass levels in the Southeastern U.S. consistently ranged from 6,000 to 11,000 kg ha-1 (Price et al. 2012; Reberg-Horton et al. 2012; Reeves et al. 2005). In Michigan, cereal rye whole plant (roots + shoots) biomass levels up to 12,777 kg ha-1 were reported when covers were planted in early September and harvested in June (Hill 2014). However, whole plant biomass levels less than 1,000 kg ha-1 were reported in the same study when planted in November and harvested in early May (Hill 2014). Other studies reported cereal rye

aboveground biomass ranged from 800 to 2,900 kg ha-1 in Michigan (Rogers 2017; Snapp et al. 2005). These cereal rye biomass production levels agree with those found in nearby Ohio and Ontario (Akemo et al. 2000; Vyn et al. 1999). Since biomass accumulation may be limited by climatic region, cultural practices are important to consider.

Manipulating cultural practices such as planting and termination date, seeding rate, and nitrogen application can potentially effect biomass production. Previous research has found that cereal rye biomass production is greater at earlier planting dates compared with later planting dates (Duiker and Curran 2005; Farsad et al. 2011; Price et al. 2012; Webster et al. 2016). For example, Mirsky et al. (2011) reported roughly 1,000 to 3,000 kg ha-1 more biomass production from cereal rye planted in late August to mid-September compared with October plantings in Pennsylvania. Feyereisen et al. (2006) created a mechanistic model confirming the importance of fall GDD accumulation and found that, until the first week of May, cereal rye planted in mid-September produced twice as much biomass as rye planted in October. Similarly, Blue et al. (1990) determined 400 GDD accumulation is necessary for optimal winter wheat yields in Nebraska. In addition, studies on winter wheat for grain yield have found delayed planting reduced tillering and subsequent yield (Blue et al. 1990; Dahlke et al. 1993; Sander and Eghball 1999). Fisher et al. (2011) did not collect biomass but reported greater nitrogen uptake in earlier planted winter wheat, which likely resulted in higher biomass. In Michigan, Rogers (2017) observed cereal rye biomass production of 2,180 and 1,250 kg ha-1 at GDD accumulations of 640 and 400 (base 4.4 C), respectively.

Delaying spring termination of cereal rye and winter wheat can also be an effective practice to increase biomass production. A study comparing biomass accumulation following 10day incremental delays in spring termination found cereal rye biomass increased 37% with each

delay (Mirsky et al. 2011). In Michigan, Hill (2014) similarly reported that cereal rye planted in mid-September and terminated in early-June had the greatest amount of biomass while cereal rye planted in early-November and terminated in mid-May resulted in the lowest. In a study comparing termination timings, Ashford and Reeves (2003) found that winter wheat reached maximum biomass at a later growth stage than cereal rye, resulting in less residue for soil cover and lower soil water content when terminated early.

To compensate for delayed establishment by factors such as weather and late harvest, researchers have investigated seeding rate and nitrogen fertility on biomass productivity. The recommended seeding rate for cereal rye and winter wheat when drilled is 67-135 kg seed ha-1 (Haramoto 2019). A study conducted in Ohio comparing cereal rye seeding rates and planting dates found that seeding rate may have more of an effect on spring biomass accumulation than planting date (Lamb 2018). However, many previous studies report seeding rate to have no effect on cereal rye biomass (Brennan et al. 2009; Masiunas et al. 1995; Ryan et al. 2011a; Webster et al. 2016). For example, a study comparing 'Wheeler' cereal rye seeded at 56, 110, and 170 kg ha-1 in Indiana, Illinois, and Kentucky observed no differences in biomass production (Masiunas et al. (1995). Boyd et al. (2009) observed increased aboveground biomass with increasing seeding rates of cereal rye (90 to 270 kg ha-1) when sampled 10 weeks after planting but found no differences in biomass when sampled prior to termination. The lack of seeding rate effect on biomass production has been attributed to cereal rye's ability to tiller (Masiunas et al. 1995; Ryan et al. 2011a). In winter wheat grown for grain yield, researchers have observed increased yield at higher seeding rates, particularly when planting is delayed (Blue et al. 1990; Dahlke et al. 1993; Sander and Eghball 1999).

Though cereal rye and winter wheat scavenge nitrogen from the soil, research has shown supplemental nitrogen (N) applications can increase biomass compared with the control (Ryan et al. 2011a; Webster et al. 2016). For example, Mirsky et al. (2017) determined a fall application of 72.4 kg N ha-1 to cereal rye was required to reach maximum biomass averaged across three termination growth stages. However, increases in biomass may be dependent on existing soil N content. Webster et al. (2016) observed no significant increase in biomass from spring applications of 17 kg ha-1 N when 34 kg ha-1 N was applied at planting. Extensive research has indicated that N applications for winter wheat are critical for grain yield, but management as a cover crop differs. To ensure adequate tillering and biomass production, N is important in early winter wheat growth stages and supplemental N should be considered if low soil fertility exists (Clark 2007).

The N content in cereal grains generally increases as termination is delayed, but the N concentration relative to carbon (C) decreases and leaves a higher C:N ratio (Clark et al. 1997). A study in Maryland found the C:N ratio of cereal rye increased from 23:1 in late March to 42:1 in late April (Clark et al. 1997). In a study comparing cereal rye and winter wheat, the growth stage by which C:N ratio increased the most varied by year and location, but cereal rye consistently had a higher C:N ratio than winter wheat (Ashford and Reeves 2003). When C:N ratios of cover residues are greater than 25:1, soil N can be immobilized and potentially affect the subsequent crop (Pantoja et al. 2016; Schomberg et al. 2007). Previous studies have reported N immobilization by cereal rye, especially when terminated during reproductive growth stages and when biomass is high (Clark 2007; Ruffo et al. 2004; Wyland et al. 1995). Persistent residue due to a high C:N ratio can increase the presence of both damaging and beneficial insects. Seed corn maggot (*Delia platura* Meigen), a pest of soybean, reduced soybean stands when planted

into cereal rye residue (Hammond 1990). Untung (1978) observed increased armyworm (*Psudaletia unipuncta*) damage in corn no-tilled into cereal rye compared with corn grown conventionally. However, Laub and Luna (1991) found that terminating cereal rye by mowing reduced armyworm densities compared with spray treatments by physically destroying the larvae.

Cover crop termination. Cover crop termination can be achieved via winterkill, mechanically, or by herbicide application (Legleiter et al. 2012). Cereal rye and winter wheat are winter annuals that do not winterkill in Michigan. Tillage, a mechanical termination method, incorporates cover crop residue into the soil prior to planting, but can negate the benefits of a cover crop and is not suitable in no-till systems (Legleiter et al. 2012). Another mechanical method of termination for no-till systems is by mowing with a flail mower or stalk chopper. However, it is suggested to mow cereal grains at head emergence for maximal biomass and minimal regrowth (Wilkins and Bellinder 1996). Flail mowing is recommended for mowing cereal grains to achieve even distribution of residue on the soil surface; however, this method finely chops the residue, which decomposes faster than a thick mulch (Creamer and Dabney 2002). Wilkins and Bellinder (1996) reported soil absorbed between 55 and 70% photosynthetically active radiation (PAR) 8 weeks after mowing when cereal rye and winter wheat were mowed at first node, while plants mowed at kernel-filling absorbed less than 5%.

An additional mechanical method for even residue distribution when terminating cereal rye and winter wheat is the use of a roller-crimper, which lays the cover crop flat while damaging the vascular tissue by crimping (Ashford and Reeves 2003). Similar to mowing, termination is more consistent at maturity and is best achieved at anthesis or later (Ashford and

Reeves 2003; Mirsky et al. 2009; Wayman et al. 2014). In addition to mechanical termination methods, cereal rye and winter wheat are successfully terminated with a herbicide application alone (Devore et al. 2013), or with a combination of herbicide and mowing (Moore et al. 1994) or roller-crimper (Ashford and Reeves 2003). For example, Price et al. (2009) observed greater than 97% cereal rye termination with rolling followed by a glyphosate application and 85% with rolling or glyphosate alone. Similarly, Ashford and Reeves (2003) observed 94% kill of cereal rye and winter wheat with full labeled rates of glyphosate (1.68 kg ai ha-1) or paraquat (0.69 kg ai ha-1), and with roller-crimper and half rates of glyphosate (0.84 kg ai ha-1) or paraquat (0.35 kg ai ha-1).

The mechanism of termination can influence the distribution of cover crop residue on the soil surface and therefore affect subsequent crop and weed emergence. Herbicide-killed covers are often left partially standing, whereas mowed or rolled covers rest on the soil surface following termination (Mirsky et al. 2011). Putnam and Defrank (1983) determined that in general, large seeded plants grow normally or benefit from cover residue while small-seeded plants experience severe injury. Comparing roller-crimper and herbicide termination methods in cereal rye, Davis (2010) found no difference in soybean yield. However, soybean stand reductions ranging from 10 to 35% have been observed in cereal rye residue compared with no cover crop (Moore et al. 1994; Reddy 2001). A 14% stand reduction was observed by Reddy (2001) when soybean were no-tilled into winter wheat. Timing of termination in relationship to crop planting can also affect establishment of crop stand and subsequent yield. For example, Liebl et al. (1992) observed significant soybean stand reduction and yield loss when soybean were planted into cereal rye terminated at planting compared with cereal rye terminated 2 weeks prior to planting and conventional treatments. Previous research observed varying effects on

soybean yield when utilizing a cereal rye cover crop compared with no cover crop. Results range from decreased yield (Reddy 2001), no effect on yield (Koger et al. 2002; Liebl et al. 1992), increased yield but reduced profitability (De Bruin et al. 2005; Reddy 2003), to increased yield and profitability (Ateh and Doll 1996).

Planting Green. No-tilled systems typically experience cooler and wetter soil conditions at planting compared to tilled systems (Blevins et al. 1971; Carter 1994; Imholte and Carter 1987; Mock and Erbach 1977). This problem can be intensified by the presence of cover crops (Teasdale and Mohler 1993; Unger and Vigil 1988). Since these scenarios often delay main crop establishment, growers have begun delaying cover crop termination until main crop planting or shortly after, instead of terminating cover crops 1-2 weeks before planting; this process is referred to as "Planting Green" (Reed et al. 2019). Soil moisture at planting can be reduced if cover crops are allowed more time to grow and accumulate biomass (Mirsky et al. 2011). Consequently, Planting Green potentially improves soil conditions at planting and provides a better seed bed for main crop establishment. Increased cover crop biomass resulting from delayed termination may also improve many of the ecosystem services previously mentioned. Additionally, cover crop residues help conserve summer soil moisture in droughty conditions and could benefit a main crop (Blanco-Canqui et al. 2015; Clark et al. 1997; Unger and Vigil 1998; Vincent-Caboud et al. 2017). Of survey respondents in the U.S., 39% of participants have tried Planting Green (CTIC 2017). Furthermore, the most important reasons survey respondents tried Planting Green were to "get more out of their cover crops", suppress weeds, attain more surface biomass, manage soil moisture, and simplify crop establishment (CTIC 2017).

Reed et al. (2019) observed 94 to 181% greater cover crop biomass production in soybean from Planting Green treatments compared to early terminated treatments. In the same study, Planting Green provided 7 to 24% drier and 0.7 to 2.4 C cooler soil conditions at soybean planting, and increased soil moisture during dry periods following planting (Reed et al. 2019). Similarly, 62% of survey respondents reported improved soil moisture management when Planting Green (CTIC 2017). Liebl et al. (1992) observed 32 to 45% soybean stand reduction in cereal rye terminated at planting compared with conventional management, which resulted in lower soybean yield. In contrast, Reed et al. (2019) reported no stand reductions or effect on yield from Planting Green compared with early terminated cover crops. Of survey respondents who planted green in the U.S., 61% reported improved weed control compared with their typical practices. Similarly, Liebl et al. (1992) found cereal rye terminated at planting to provide equal to or better control of all weed species compared with other tillage treatments. Further research is needed to understand the effectiveness of Planting Green in no-till soybean and its use to manage herbicide-resistant horseweed.

Weed suppression by cover crops. Cereal rye and winter wheat suppress weeds through a combination of chemical and physical mechanisms (Kruse et al. 2000). Several studies showed the chemical effects, through allelopathy, of cereal rye and winter wheat residues (Barnes and Putnam 1983; 1986; Przepiorkowski and Gorski 1994; Putnam 1988; Putnam and DeFrank 1983; 1990; Shilling et al. 1985). Cereal rye is one of the most allelopathic crops and 16 allelochemicals have been identified (Jabran et al. 2015; Schulz et al. 2013). Winter wheat also has some of the same phenolic acid allelochemicals as cereal rye (Weston 1996). Additionally, cultivar and growth stage can affect the allelochemical and concentration present in a species, as

they can vary greatly in cereal rye (Schulz et al. 2013). Several cereal rye and winter wheat cultivars which possess allelopathic abilities have been identified (Jabran et al. 2015; Schulz et al. 2013). For example, Mahmood et al. (2013) screened 35 wheat cultivars for allelopathic potential against wild oat (*Avena fatua* L.) and found 11 cultivars provided 42 to 83% suppression. Similarly, the cereal rye cultivar 'Wheeler' was determined to have the highest concentration of DIBOA (2,4-dihrdroxy-1,4-(2H) benzoxazine-3-one) in a study analyzing allelopathic control of redroot pigweed (*Amaranthus retroflexus* L.) and goose grass (*Eleusine indica* L. Gaertn.) (Reberg-Horton et al. 2005). However, allelopathic suppression is short lived, as Chou and Patrick (1976) determined the maximum allelochemical concentration in cereal rye occurs within 20 days after decomposition.

Cereal rye and winter wheat residue physically suppress weed germination and establishment by providing a physical barrier and by modifying the microenvironment of the soil surface (Teasdale 1996). In absence of herbicides, cereal rye and winter wheat reduced weed biomass 68% and 21%, respectively (Norsworthy et al. 2004). In addition, Malik et al. (2008) reported cereal rye reduced weed density by 50% 4 WAP compared with a no cover crop control; however, reduction in densities were short lived and similar to no cover crop control 8 WAP. As with other ecosystem services, several studies have found correlation between increased cereal grain biomass and greater weed suppression (Finney et al. 2016; Ryan et al. 2011b, Smith et al. 2011). Heavy cover crop residues, such as cereal rye, provided weed suppression up to 6 weeks after desiccation (Dabney et al. 2001). The rapid growth of cereal rye and winter wheat prior to desiccation make them effective at controlling winter annual and early-summer annual weeds. Numerous studies reported winter annual weed suppression from a cereal rye cover crop (Hayden et al. 2012; Teasdale et al. 2007; Teasdale and Mohler 1993). For example, Cornelius

and Bradley (2017) reported 70% and 50% reduction in winter annual weed emergence from cereal rye and winter wheat, respectively. In addition, numerous other studies observed suppression of early-season summer annual weeds when utilizing a cereal rye or winter wheat cover crop (Mirsky et al. 2011; Price et al. 2006; 2012; Saini et al. 2006). Cornelius and Bradley (2017) observed 42% late-season summer annual suppression from cereal rye; however, this was not as effective as using a PRE residual herbicide program. The ability of cereal rye and winter wheat to suppress winter annual and early-summer annual weeds could make them effective tools for managing herbicide-resistant horseweed.

Horseweed suppression by cover crops. Recent studies reported that cereal rye and winter wheat aided in suppression of herbicide-resistant, small-seeded broadleaf weeds, particularly *Amaranthus* spp. (Loux et al. 2017; Montgomery et al. 2018; Wiggins et al. 2015). However, studies demonstrating the ability of these cover crops to suppress horseweed are limited. One study found that both cereal rye root and shoot residue have allelopathic activity on horseweed, inhibiting germination up to 50% (Przepiorkowski and Gorski 1994). The available research on horseweed control mainly focuses on physical suppression in no-till corn or soybean systems. Wallace et al. (2019) observed a negative correlation between fall and spring cereal rye biomass production and horseweed density at the time of burndown. Similarly, Pittman et al. (2019) reported horseweed density at cover termination was reduced 88 to 97% in cereal rye and cereal rye containing mixtures compared with no cover. Cereal rye terminated prior to planting no-till corn provided glyphosate-resistant horseweed control of 82% and 48% at corn planting and in September, respectively (Cholette et al. 2018). In no-till soybean, inclusion of cereal rye provided reductions in horseweed density compared with no cover crop control (Lamb 2018;

Wallace et al. 2019). In a corn-soybean rotation, Davis et al. (2007) observed similar reductions in spring-emerged horseweed density from a winter wheat cover crop and spring-applied residual herbicide one month after planting the main cash crop. Additionally, incorporating a winter wheat cover crop into a corn-soybean rotation reduced horseweed seed bank density (Davis et al. 2007). Wallace et al. (2019) reported cereal rye cover crop can reduce the proportion of large horseweed plants at time of burndown application.

As growers continue to adopt reduced tillage practices they will be challenged by the spread of herbicide-resistant horseweed. Additionally, a recent shift from a winter annual to a primarily summer annual lifecycle, with emergence throughout the growing season, has required new management practices in Michigan. Further research is needed to better understand the significance of this lifecycle shift and the effectiveness of cereal rye and winter wheat as horseweed management tools in no-tillage soybean.

Questions that remain to be answered:

- 1. Do fall-planted cereal cover crops provide horseweed control?
- 2. How does cover crop termination time effect horseweed management?
- 3. Are herbicides needed in addition to cover crops to manage horseweed in no-till soybean?
- 4. What effects do cereal rye growth stage and tank-mix combinations have on cereal rye termination?
- 5. What factors affect horseweed growth type and how does growth type influence sensitivity to glyphosate?

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CHAPTER II

INTEGRATING FALL-PLANTED CEREAL COVER CROPS AND BURNDOWN HERBICIDES FOR GLYPHOSATE-RESISTANT HORSEWEED MANAGEMENT Abstract

The increased occurrence of glyphosate-resistant (GR) horseweed (Erigeron canadensis L.) escapes in no-tillage crop production fields warrants a need for improved and integrated weed management tactics. Field experiments were conducted in three site-years in Michigan to investigate the effects of cereal rye and winter wheat, seeded at 67 or 135 kg ha-1, and three burndown herbicide strategies for GR horseweed suppression in no-tillage soybean. The burndown strategies were: control (glyphosate only), effective burndown, and effective burndown plus residual. Cereal rye produced more biomass in two site-years and provided more ground cover than winter wheat. GR horseweed densities at cover termination were 47 to 96% lower with cover crops compared with no cover at these locations. Cereal rye and winter wheat reduced GR horseweed biomass 59 to 70% compared with no cover at this time. At the time of postemergence herbicide application, approximately five weeks after planting, cover crops reduced GR horseweed biomass greater than 33% in absence of an effective burndown at two site-years. Additional suppression from cover crops was minimal in the presence of an effective burndown and adding residual herbicides reduced horseweed biomass 99% at this time. Cover crops did not affect late-season GR horseweed suppression or soybean yield. Soybean yield was highest in residual treatments followed by effective burndown and control at two site-years. Utilizing cereal rye and winter wheat for early-season GR horseweed management as a supplement to effective herbicides could provide growers effective GR horseweed suppression while reducing selection pressure for resistance to more herbicide sites of action.

Introduction

Horseweed (*Erigeron canadensis* L.) is a facultative winter annual plant native to North America that grows in many environments, including roadsides, railways, and reduced or notillage crop production fields (Weaver 2001). Each plant can produce up to 200,000 seeds, that are approximately 1 mm in length with an attached pappus which facilitates wind dispersal into the planetary boundary layer for travel of over 500 km (Bhowmik and Bekech 1993; Shields et al. 2006; Weaver 2001). Peak emergence occurs in May and in late August to early September in the North Central Region of the United States; however, emergence has been observed throughout the growing season (Buhler and Owen 1997; Tozzi and Van Acker 2014). Predicting horseweed emergence can be difficult, as emergence is not correlated to soil temperature, air temperature, or rainfall pattern (Main et al. 2006). Horseweed seeds are non-dormant and readily germinate on the soil surface, making horseweed especially difficult to manage in no-tillage situations. (Buhler and Owen 1997).

A lack of effective postemergence (POST) herbicide options makes horseweed management in no-tillage soybean challenging (Bruce and Kells 1990; Moseley and Hagood 1990). In Michigan, soybean yield losses of 83% were reported when horseweed was not controlled (Bruce and Kells 1990). Control is further exacerbated with herbicide-resistant populations. Currently, horseweed is resistant to at least one herbicide site-of-action in 18 countries (Heap 2020). In the United States, horseweed resistant to glyphosate (WSSA group 9) was first confirmed in Delaware in 2001 (VanGessel 2001) and has since been identified in 25 states, over a large number of hectares (Heap 2020). In Michigan, horseweed is resistant to acetolactate synthase inhibitors (WSSA group 2), triazine herbicides (WSSA group 5), diuron (WSSA group 7), and paraquat (WSSA group 22) (Heap 2020). In many cases, horseweed

populations are resistant to multiple herbicide sites of action (Heap 2020). Therefore, utilizing herbicides with effective sites of action is necessary for horseweed management.

Effective control of emerged horseweed prior to planting, and a residual herbicide to control later emerging plants, is necessary in no-tillage soybeans (Loux et al. 2006). Previous research found preplant applications of the auxinic herbicides 2,4-D or dicamba (WSSA group 4) provided control of emerged glyphosate-resistant (GR) horseweed (Byker et al. 2013; Eubank et al. 2008; Keeling et al. 1989; McCauley et al. 2018). However, auxinic herbicide effectiveness on larger plants has been inconsistent, and tools to reduce horseweed size at burndown are needed (Keeling et al. 1989; Kruger et al. 2010; Wiese et al. 1995). In addition, utilizing residual herbicides at burndown, such as metribuzin (WSSA group 5), flumioxazin, or sulfentrazone (WSSA group 14) controlled horseweed up to 8 weeks after application (Eubank et al. 2008; Steckel et al. 2006). However, horseweed's continued emergence throughout the growing season necessitates the need for additional management strategies.

Recent on-farm adoption of cover crops for various ecosystem services has piqued interest in their utility as an additional weed suppression tool. Cover crops suppress weeds via resource competition while living and by creating a mulch layer on the soil surface following termination (Teasdale et al. 2007). The mulch left following termination suppresses weeds by modifying light quantity and quality, reducing soil surface temperature, and creating a physical barrier to seedling emergence (Teasdale and Mohler 1993). Fall-planted cover crops compete with weeds in the fall and following spring (Teasdale 1996). Cereal rye (*Secale cereale* L.) and winter wheat (*Triticum aestivum* L.) are two of the commonly grown cover crops prior to no-till soybeans in the Midwest due to their winter hardiness and biomass production (CTIC 2017). Cereal rye produces similar or greater biomass compared with winter wheat, depending on

seeding rate (Haramoto 2019). Weed biomass decreases with increasing levels of cover crop biomass (Ryan et al. 2011).

Recent studies found that fall-planted cover crops reduced horseweed density prior to cover crop termination in the spring (Pittman et al. 2018; Wallace et al. 2019). Similarly, Cholette et al. (2018) observed 76 to 95% visual suppression of GR horseweed in mid-May by fall-planted cover crops in a subsequent corn crop. Horseweed suppression by cereal rye and winter wheat cover crops later in the growing season varies. Davis et al. (2007) observed similar horseweed density between a winter wheat cover crop and spring-applied residual herbicides one month after burndown in one of a four-year study. In contrast, Wallace et al. (2019) reported no density reduction at the time of a POST herbicide application by a cereal rye cover crop. Lateseason horseweed suppression is likely dependent on cover crop biomass accumulation prior to burndown and the persistence of the residue.

Prolonged emergence of GR horseweed has reduced effectiveness of chemical control for no-tillage soybean growers. Integrated weed management strategies that provide effective suppression need to be determined and implemented. As growers continue to adopt cover crops for their numerous benefits, questions about their utility for GR horseweed management remain. The objective of this research was to evaluate cereal rye and winter for GR horseweed suppression. Our studies examined the impact of different seeding rates of cover crops, as well as combinations of cover crops and different burndown herbicide strategies, on GR horseweed control and soybean yield.

Materials and Methods

Field experiments were conducted in commercial fields in Isabella County, Michigan in 2018 (43.6128°N, -84.8777°W) and 2019 (43.6255°N, -84.9812°W) and at the Michigan State University (MSU) Agronomy Farm in East Lansing, Michigan in 2019 (42.6876°N, -84.4907°W). Sites were selected based on GR horseweed escapes the previous season. The soil types in Isabella County were a Selfridge sand (loamy, mixed, active, mesic Aquic Arenic Hapludalfs) with pH 6.4 and 2.2% organic matter in 2018 and a Wasepi loamy sand (coarse-loamy, mixed, semiactive, mesic Aquollic Hapludalfs) with pH 5.2 and 2.2% organic matter in 2019. The soil type at MSU was a Conover loam (fine-loamy, mixed, active, mesic Aquic Hapludalfs) with pH 5.7 and 3.0% organic matter.

In 2018, the experiment was established as a split-plot randomized complete block design with three replications. In 2019, the experiment was established as a split-split plot randomized complete block design with three replications. Each plot measured 3 m wide by 9 m long. The main plot factor was cover crop, the subplot factor was burndown herbicide strategy, and the sub-subplot factor in 2019 was postemergence (POST) herbicide. The main plots consisted of five cover crop factors: 1) winter wheat seeded at a low rate of 67 kg ha-1 (WWL), 2) winter wheat seeded at a high rate of 135 kg ha-1 (WWH), 3) cereal rye seeded at a low rate of 67 kg ha-1 (CRL), 4) cereal rye seeded at a high rate of 135 kg ha-1 (CRH), and 5) no cover crop control (NC). The subplots consisted of three burndown herbicide strategies: 1) control, 2) effective burndown, and 3) effective burndown plus residual (Table 2.1). The sub-subplot factors in 2019 were two POST herbicide application strategies: 1) an effective POST herbicide application or 2) a non-effective POST herbicide application only to control other weeds, but not GR horseweed (Table 2.1).
Main plots of 'Wheeler' cereal rye and 'Sunburst' winter wheat were sown in 19 cm rows using a no-till drill (Great Plains, Salina, KS) the fall prior to data collection. Dates for all field operations can be found in Table 2.2. Cover crops were terminated, and burndown strategy subplots were established one week prior to soybean planting the following spring. Glyphosate and dicamba-resistant soybean 'AG 26X8' (Roundup Ready 2 Xtend, Bayer CropScience, St. Louis, MO) was planted in 76 cm rows at a seeding rate of 383,000 seeds ha-1. POST herbicide applications were made approximately five weeks after planting (WAP) when emerged GR horseweed was approximately 10 cm tall. In 2018, a POST herbicide application of glyphosate + dicamba was applied to individual plots if needed. This treatment was applied to all of the control (glyphosate-only) and effective burndown plots. In 2019, POST herbicide application was a sub-subplot factor and was established at this time. 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 TTI nozzles (TeeJet Technologies, Spraying Systems Co., Wheaton, IL).

Data collection. Prior to cover crop termination, percent ground cover was measured using linetransects (Laflen et al. 1981) laid diagonally across each main cover crop and no cover crop plot. Presence of cover crop, GR horseweed, other weed, or no vegetation was recorded at every 30 cm point along a 9 m transect and converted to a percentage. Aboveground cover crop and weed density and biomass were collected at this time from two randomly placed 0.25 m² subsamples in each plot. In addition to GR horseweed, annual bluegrass (*Poa annua* L.), common chickweed (*Stellaria media* (L.) Vill.), shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.), and dandelion (*Taraxacum officinale* F. H. Wigg.) were present during the time of burndown subplot establishment for all site-years. Spring whitlowgrass (*Draba verna* L.) and white campion

(*Silene latifolia* Poir.) were also present at the 2019 locations. Subsamples of cover crop 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). GR horseweed density and biomass were collected again from two randomly placed 0.25 m² subsamples in all plots at the time of POST herbicide application and prior to soybean harvest. At soybean harvest, fall-emerged horseweed rosettes were segregated from fully mature horseweed plants. Biomass samples were dried for approximately 7 d at 65 C and weighed.

Soil moisture was measured at the time of soybean planting 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. When soybean reached the VE growth stage, percent ground cover of terminated vegetation was reassessed using the line-transect method described above. Soybean populations were also assessed in all plots at this time. Soybean was harvested for yield using a small-plot research combine (Massey-Ferguson 8XP, AGCO, Duluth, GA) with a 1.5 m header. Yields were adjusted to 13% moisture.

Precipitation and temperature data were obtained throughout the growing season from the Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, MI) stations located in Mecosta, Mount Pleasant, and East Lansing for Isabella 2018, Isabella 2019, and MSU 2019, respectively (Table 2.3).

Statistical analysis. Data analysis was conducted using lmer in R v. 3.6.2 (R Development Core Team 2019). The statistical model consisted of site-year (individual year and location), cover crop treatment, and burndown herbicide strategy as fixed effects and replication nested in site-year and the interaction between cover crop treatment and replication nested within site-year as

the random effects. Replications were used as an error term for testing the effect of site-year, and data were combined over site-year when the interaction of site-year and cover crop treatment or burndown herbicide was not significant. The cover crop treatment by replication interaction was used as an error term to test the effect of cover treatment. Additionally, preplanned contrasts were performed to compare cover species pooled over cover rate and cover rates pooled over cover species. Data for horseweed density and biomass at harvest and soybean yield were analyzed separately by POST herbicide treatment. 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 the Levene's test for unequal variances. In cases of marked deviations from normality, the data were log-transformed and further analyses were performed using the transformed data. For all experiments, treatment means were separated using Fisher's LSD at $\alpha < 0.05$.

Results and Discussion

Cover crop response variables. Site-year had a significant effect on (P = <0.05) cover crop biomass, ground cover at termination, and soil moisture at soybean planting; therefore, these data were analyzed separately by site-year. Averaged over all treatments, cover crop biomass at termination was highest at Isabella 2018 (1,205 kg ha-1) followed by MSU 2019 (999 kg ha-1) and Isabella 2019 (709 kg ha-1). Differences in cover crop biomass were likely a function of accumulated precipitation and growing degree days (GDD) between cover crop planting and termination, as well as soil type at the three locations. The Isabella sites were planted on sandy and loamy sand soils in 2018 and 2019, respectively. However, cover crops were sown earlier (~3 weeks) and received greater amounts of precipitation between planting and termination, at

Isabella in 2018 compared with 2019 (Tables 2.2 and 2.3). Mirsky et al. (2011) reported increases of cereal rye biomass of 1,000 to 3,000 kg ha-1 when planted in late August to mid-September compared with October plantings. Furthermore, cereal rye and winter wheat were terminated at Feekes stage 6 at Isabella in 2018 compared to Feekes stage 5 at the 2019 sites. GDD accumulations between cover crop planting and termination were 541 and 315 (base 4.4 C) for the Isabella 2018 and 2019 sites, respectively (Table 2.4). Similarly, MSU 2019 was sown later, received less precipitation, and accumulated less GDD (326) compared with Isabella 2018. However, soil type at MSU 2019 was a loam, which was believed to have greater nitrogen availability due to higher clay and silt content as well as higher soil organic matter (Hassink 1994).

The greatest amount of biomass was produced by cereal rye at the high seeding rate at Isabella 2018 and MSU 2019 (Table 2.4). Combined over seeding rates, cereal rye biomass was 1,550 kg ha-1 at Isabella 2018 and MSU 2019. These levels are similar to previous studies in Michigan where aboveground biomass of fall-planted cereal rye ranged from 800 to 2,900 kg ha-1 (Rogers 2017; Snapp et al. 2005). At Isabella 2018 and MSU 2019, cereal rye biomass was also significantly greater than winter wheat. This is consistent with previous findings that cereal rye produces more biomass than winter wheat (Cornelius and Bradley 2017; Haramoto 2019). At Isabella in 2019, biomass production among CRH, CRL, and WWH treatments was similar, and all were greater than WWL (Table 2.4). Combined over cover species, the high seeding rate only increased cover crop biomass at MSU 2019 (Table 2.4). This is consistent with previous research, where cereal cover crops often compensate for lower seeding rates by tillering (Masiunas et al. 1995).

Combined over site-years and seeding rates, the carbon to nitrogen ratio (C:N) of cereal rye (18:1) was greater than winter wheat (15.1) at termination (Table 2.4). Similar differences in C:N ratios between cereal rye and winter wheat were reported by Ashford and Reeves (2003). However, the ideal C:N ratio for a microbial diet is 24:1 (NRCS 2011). Cover residues with C:N ratios higher than 24:1 decompose more slowly compared with residues with C:N ratios lower than 24:1 (Odhiambo and Bomke 2001). Therefore, the persistence of cereal rye and winter wheat residues in this study were predicted to be similar, and not last the entire growing season.

Variability in cover crop biomass resulted in variability of ground cover at termination. Cereal rye provided more ground cover at the time of termination compared with winter wheat at all sites (Table 2.5). Combined over seeding rate, cereal rye provided 65, 51, and 56% ground cover at Isabella 2018, 2019, and MSU 2019, respectively. There was no difference in ground cover for high and low seeding rates of cereal rye at any site. In contrast, WWH provided 3 and 7% more ground cover compared with WWL at Isabella 2018 and MSU 2019, respectively (Table 2.5).

Cereal rye maintained more ground cover than winter wheat three weeks after cover crop termination when soybeans were at the VE growth stage (Table 2.5). Cereal rye and winter wheat provided 38 and 25% ground cover, respectively, combined over site-years and seeding rates. Similar to what was observed at cover crop termination, there were no differences in ground cover between seeding rates of cereal rye. However, WWH provided as much ground cover as CRL and 9% more than WWL.

Soil moisture at soybean planting was influenced by cover crops, but results were not consistent. At Isabella 2018, soil moisture was higher in the cereal rye plots, while at Isabella 2019 soil moisture was higher in the winter wheat plots compared with the no cover controls

(Table 2.6). At MSU 2019, soil moisture was higher in all cover crop treatments compared with no cover, and cereal rye plots held more moisture compared with winter wheat plots (Table 2.6). The effects of cover treatments on soil moisture between the sites is believed to be a function of cover crop biomass, soil texture, and precipitation prior to planting. Precipitation in the four weeks prior to soybean planting was 73, 104, and 106 mm for Isabella 2018, Isabella 2019, and MSU 2019, respectively (Table 2.3). Greater cereal rye biomass at Isabella 2018 and MSU 2019 resulted in higher soil moisture retention in the cereal rye cover treatments. Although winter wheat biomass was relatively low, extensive precipitation and a finer soil texture at MSU 2019 resulted in winter wheat also retaining soil moisture compared with the no cover plots.

Horseweed suppression at cover crop termination. Initial horseweed emergence occurred between April 25th and May 14th in all site-years and all horseweed plants exhibited a summer annual lifecycle. Horseweed density at the time of cover crop termination varied significantly by site-years; densities of no cover plots were 1,916, 714, and 21 plants m-2 at Isabella 2018, 2019, and MSU 2019, respectively (Table 2.7). Horseweed density was reduced in all cover treatments at Isabella 2018 and all cover treatments, except WWH at MSU 2019. Horseweed densities were reduced 47 to 68% and 57 to 96% by cover crops compared with no cover at Isabella 2018 and MSU 2019, respectively. Wallace et al. (2019) reported greater than 80% horseweed density reductions at the time of burndown by cereal rye monocultures, which were the highest and most consistent horseweed density reductions of all cover crop monocultures studied. Similarly, Pittman et al. (2018) reported 88 to 97% horseweed density reduction by cover crops compared to the no cover control; however, no differences were observed between cereal rye-mixtures and legume-mixtures or monocultures. In our study, there was no difference in density reduction

between cereal rye and winter wheat or by seeding rate. At Isabella 2019, cover crops did not reduce horseweed density at the time of cover termination compared with no cover. We attribute this to the dense horseweed stand and low cover crop biomass accumulated at the time of termination.

Applying a burndown herbicide close to peak emergence provides optimal control of horseweed (Davis et al. 2010). We evaluated horseweed biomass at cover termination as a measure of cereal cover crops ability to potentially improve burndown applications. In our study, horseweed emerged approximately one to two weeks prior to cover termination and completed a summer annual lifecycle. Combined over site-year, cover crops reduced horseweed biomass 59 to 70% compared with no cover at the time of cover termination (Table 2.7). Hayden et al. (2012) reported that cereal rye reduced winter annual weed biomass 95 to 97% in a study located in Michigan. However, cereal rye biomass in this study ranged from 3,300 to 5,870 kg ha-1 and weed densities were 139 plants m-2. Total weed biomass is a useful measurement for horseweed suppression; however, biomass can be misleading, as it is a result of both weed density and the size of individual plants. Wallace et al. (2019) evaluated size of individual horseweed plants at the time of burndown and found cereal rye, alone and in mixtures, reduced horseweed size and improved size uniformity. In our study, horseweed biomass was reduced by all cover treatments, regardless of whether or not horseweed density was reduced. No differences were detected between cereal rye and winter wheat, or by seeding rate (Table 2.7). We attribute the earlyseason horseweed suppression to the presence of a cover crop competing for nutrients and light.

Horseweed suppression following herbicide establishment. Horseweed density at the time of POST herbicide application, five to six weeks after burndown herbicide applications, in the

control plots combined over cover treatments were 491, 202, and 37 plants m-2 for Isabella 2018, Isabella 2019, and MSU 2019, respectively (Table 2.8). Burndown herbicide treatment had a greater impact on horseweed density than cover treatment at this time. Applying a residual herbicide at cover crop termination reduced horseweed density greater than 99% at POST herbicide application compared with the control in all site-years (Table 2.9). These findings are similar to Davis et al. (2007) who reported soil-applied residual herbicides reduced horseweed density 98% one month after burndown. Without the residual herbicide, the effective burndown treatment reduced horseweed density 60 and 51% compared with the control at Isabella 2019 and MSU 2019, respectively. At Isabella 2018, the effective burndown and control plots had similar horseweed densities. This is a result of significant horseweed emergence following the burndown herbicide treatment. Davis et al. (2007) reported similar horseweed densities one month after burndown treatment when using a winter wheat cover crop compared with a spring-applied residual herbicide in one of a four-year study. However, winter wheat was less effective than residual herbicides in the other years. Cover crops did not reduce horseweed density compared with no cover in any of the burndown treatments in our study. These findings are similar to Wallace et al. (2019) who reported no reduction in horseweed density at the time of POST by a cereal rye cover crop.

Both cover crops and burndown treatments had a significant effect on horseweed biomass at the time of POST herbicide application. Although cover crops did not reduce horseweed density, horseweed biomass was reduced by cover treatments in absence of an effective burndown or residual herbicide. Within the control plots, all cover crops reduced horseweed biomass compared with no cover, with exception of the low seeding rates of both winter wheat and cereal rye at Isabella 2019 (Table 2.9). Cover crop treatments reduced horseweed biomass by

at least 33 and 36% compared with the no cover control at Isabella 2018 and MSU 2019, respectively. At Isabella 2019, WWH and CRH reduced horseweed biomass 37 and 58%, respectively. Horseweed biomass was reduced in no cover plots that received an effective burndown 49, 79, and 83% compared with the no cover control at Isabella 2018, 2019, and MSU 2019, respectively. Within effective burndown treatments, only WWL and WWH at Isabella 2018 reduced horseweed biomass compared with no cover. Applying a residual herbicide reduced horseweed biomass 99% compared with the no cover control (Table 2.9). Any effect cover crops had within residual herbicide plots was overwhelmed by this level of suppression. Overall, cover crops effectively reduced horseweed biomass, but the magnitude was less evident as burndown treatment effective POST herbicide was only applied to control and effective burndown herbicide treatments at Isabella 2018.

Prior to soybean harvest, sampled horseweed was separated by recently emerged rosettes and inflorescent plants expected to produce viable seed. Horseweed emerging in July and August typically overwinter as rosettes and do not contribute to the seed bank that growing season (Loux et al. 2006). For this reason, only inflorescent horseweed density and biomass data are presented in Table 2.10. At Isabella 2018, only newly emerged rosette horseweed plants were present in the control and effective burndown treatments, due to the effectiveness of the POST dicamba application. Cover crop treatment had no effect on density or biomass in the residual herbicide plots where inflorescent horseweed was present (Table 2.10). Similarly, horseweed density and biomass were greater in 2019 in plots that received a non-effective POST herbicide application. Pooled over the 2019 sites, burndown and residual treatments reduced horseweed density 84 and 38% compared with the control when a non-effective POST herbicide application was made

(Table 2.10). However, neither cover crop treatment nor burndown treatment had an effect on horseweed biomass at Isabella 2019. At MSU 2019, residual and effective burndown treatments reduced horseweed biomass 95 and 31% compared with the control, respectively.

When an effective POST herbicide application was made in 2019, neither cover crop treatment nor burndown treatment influenced horseweed density or biomass at soybean harvest (Table 2.10). The effects of cover treatment and burndown herbicide had little impact on lateemerging horseweed rosettes (data not shown). These results support previous research that cover crop residues do not persist long enough to provide weed suppression throughout the growing season (Osipitan et al. 2018). However, low horseweed densities at the 2019 sites following an effective POST herbicide application indicate that late-emerging horseweed plants did not significantly contribute to seed production. The ability of cereal rye and winter wheat to provide horseweed suppression at planting through the time of POST herbicide application provides an additional integrated weed management tool.

Soybean establishment and yield. Pooled over site-year and burndown treatment, cover crops did not affect soybean stand, with exception of CRH (Table 2.11). Soybean stand was 4% lower in the CRH treatment compared with no cover. Soybean stand has been reported to be reduced 10 to 35% when planted in a cereal rye cover crop (Moore et al. 1994; Reddy 2001). However, cover crops did not affect soybean yield at any site-year, when averaged over burndown treatment and in presence or absence of an effective POST herbicide application (Table 2.11). Burndown treatment also had no effect on soybean yield, regardless of POST herbicide application at MSU 2019 where soybean yields were relatively high and horseweed pressure was low. However, soybean yields at Isabella 2018 for the residual and effective burndown

treatments were 52 and 19% higher, respectively, compared with the control. When dicamba was not applied POST at Isabella 2019, soybean yields were 145 and 75% higher for the residual and effective burndown treatments, respectively, compared with control. Yield losses ranging from 83 to 93% were reported in soybean when horseweed was not effectively controlled (Bruce and Kells 1990; Byker et al. 2013).

We conclude that cereal rye and winter wheat cover crops effectively suppressed GR horseweed through the time of POST herbicide application. Cereal rye produced more biomass than winter wheat; however, horseweed suppression throughout the growing season was similar for both cover crops. Seeding rate had little impact on horseweed suppression. Fall planted cereal cover crops reduced horseweed density and biomass at the time of burndown application and can be used as an additional tool to reduce reliance on herbicides early in the season. At the time of POST herbicide application, approximately five weeks after burndown, cover crops did not impact horseweed density but did reduced horseweed biomass. This could improve the effectiveness of POST herbicide applications of the new herbicide-resistant soybean technologies. More research is needed to understand how cover crops aid horseweed suppression under the various soybean systems. Though cover crops suppressed horseweed through the time of POST herbicide application, an effective burndown with residual herbicides provided the greatest horseweed control and improved soybean yield. Utilizing cereal rye and winter wheat for early-season horseweed management as a supplement to effective herbicides could provide growers effective suppression while reducing evolution of resistance to more herbicide sites of action.

APPENDIX

APPENDIX Tables

Table 2.1. Herbicide application timings, active ingredients, and product information for three different herbicide strategies (sub-plots) and two different POST herbicide strategies (sub-sub plots) used for management of glyphosate-resistant horseweed.

Herbicide strategiesa	Active ingredientsb Trade names		Rates
Burndown subplots			g ai or ae ha-1
Control	glyphosate	Roundup PowerMAX _c	1,267
Effective burndown	glyphosate + 2,4-D ester	Roundup PowerMAXc + 2,4-D LV4d	1,267 + 560
Effective burndown + residual	glyphosate + 2,4-D ester + flumioxazin + metribuzin	Roundup PowerMAX _c + 2,4-D LV4 _d + Valor _e + Metribuzin 75 _f	1,267 + 560 + 717 + 420
POST sub-sub plots			
Non-effective POST	glyphosate	Roundup PowerMAX _c	1,267
Effective POST	glyphosate + dicamba	Roundup PowerMAXc + XtendiMaxc	1,267 + 560

^aAll burndown herbicide applications were made approximately 7 d prior to planting and POST applications were made approximately 5 weeks after planting.

bAll herbicide treatments with the exception of glyphosate + dicamba were applied with 2% w w-1 of ammonium sulfate. A drift-reduction agent at 0.5% w w-1 was included with the Effective POST treatment.

Bayer CropScience, St. Louis, MO

dLoveland Products, Inc., Greeley, CO

eValent U.S.A. Corporation, Walnut Creek, CA

fWinfield Solutions, St. Paul, MN

Operation	Isabella 2018	Isabella 2019	MSU 2019
Cover crop seeding	September 27, 2017	October 18, 2018	October 17, 2018
Termination	May 14, 2018	May 21, 2019	May 14, 2019
Soybean planting	May 21, 2018	May 29, 2019	May 27, 2019
POST application	June 29, 2018	July 3, 2019	July 3, 2019
Soybean harvest	October 16, 2018	October 23, 2019	October 9, 2019

Table 2.2. Cover crop seeding and termination, soybean planting, POST herbicide application, and soybean harvest dates for the three experimental locations.

		Isabella		MS	U
Month	2018	2019	5-yr ave.	2019	5-yr ave.
		mm		mr	n
Fall prior	249	51	164	54	154
April	43	27	75	72	72
May	103 (46)b (68)c	96 (61) (92)	91	85 (45) (76)	86
June	57	89	90	115	89
July	68	22	62	58	62
August	197	86	99	18	91
September	64	129	81	92	86
October	19d	131	110	31	109
Total					
Cover crope	338	139	-	171	-
Soybeanf	437	461	533	323	523

Table 2.3. Monthly and 5-yr average precipitation at Isabella County in 2018 and 2019 and Michigan State University in 2019.a

^aMichigan Automated Weather Network, <u>http://www.agweather.geo.msu.edu/mawn/</u>, Michigan State University, East Lansing, MI. ^bPrecipitation up to cover crop termination.

cPrecipitation up to soybean planting.

dThe harvest month does not include rainfall after harvest.

eTotal precipitation is a total of rainfall from cover crop planting until termination, not including precipitation in December, January, February, and March.

fTotal precipitation is a total of rainfall from soybean planting until harvest.

		C:N ratio		
Cover crop treatments _a	Isabella 2018	Isabella 2019	MSU 2019	Combined sites
		kg ha-1		
Winter wheat – low (WWL)	713 db	561 b	301 d	15:1 b
Winter wheat – high (WWH)	1,015 c	756 a	605 c	15:1 b
Cereal rye – low (CRL)	1,347 b	756 a	1,359 b	18:1 a
Cereal rye – high (CRH)	1,747 a	762 a	1,731 a	18:1 a
Contrastsc				
Winter wheat vs. cereal ryed	**	NS	**	*
High vs. low seeding ratee	NS	NS	**	NS
GDD at terminationf	541	315	326	

Table 2.4. Cover crop dry biomass, C:N ratios, and growing degree days (GDD) accumulated at the time of cover crop termination.

bMeans followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

cSignificance is designated as: * = P < 0.05; ** = P < 0.001; NS denoted $P \ge 0.05$.

dContrasts comparing cover crop species pooled over seeding rate.

eContrasts comparing seeding rates pooled over cover crop species.

fGrowing degree days (GDD) (base 4.4 C) accumulated from the timing of planting until termination.

	Ground cover					
		At VE soybean				
Cover crop treatments _a	Isabella 2018	Isabella 2019	MSU 2019	Combined sites		
			%			
Winter wheat – low (WWL)	48 сь	42 b	42 b	20 c		
Winter wheat – high (WWH)	58 b	49 a	43 b	29 b		
Cereal rye – low (CRL)	65 a	49 a	54 a	36 ab		
Cereal rye – high (CRH)	65 a	52 a	58 a	40 a		
Contrastsc						
Winter wheat vs. cereal rye	**	*	**	**		
High vs. low seeding rate	*	NS	NS	NS		

Table 2.5. Cover crop ground cover at cover termination and soybean growth stage VE.

bMeans followed by the same letter within a column are not statistically different at $\alpha \le 0.05$.

cSignificance is designated as: * = P < 0.05; ** = P < 0.001; NS denoted $P \ge 0.05$.

dContrasts comparing cover crop species pooled over seeding rate.

eContrasts comparing seeding rates pooled over cover crop species.

Cover crop treatments _a	Isabella 2018	Isabella 2019	MSU 2019
		% moistureb	
No cover	18.6 ac	18.7 a	20.2 a
Winter wheat – low (WWL)	19.1 a	20.5 b	22.5 b
Winter wheat – high (WWH)	19.7 a	20.7 b	23.4 bc
Cereal rye – low (CRL)	22.0 b	19.0 a	23.9 с
Cereal rye – high (CRH)	22.4 b	19.6 ab	25.9 d
Contrastsd			
Winter wheat vs. cereal ryee	**	*	**
High vs. low seeding ratef	NS	NS	*

Table 2.6. Soil moisture at 7.6 cm depth measured at the time of soybean planting in the cover crop and no cover plots.

bSoil moisture reported as volumetric water content and measured with a TDR 300 Soil Moisture Meter (FieldScout, Spectrum Technologies, Aurora, IL).

cMeans followed by the same letter within a column are not statistically different at $\alpha \le 0.05$.

dSignificance is designated as: * = P < 0.05; ** = P < 0.001; NS denoted $P \ge 0.05$.

eContrasts comparing cover crop species pooled over seeding rate.

rContrasts comparing seeding rates pooled over cover crop species.

		Biomass		
Cover crop treatmenta	Isabella 2018	Isabella 2018 Isabella 2019		Combined sites
		_plants m-2		g m-2
No cover	1,916 bb	714	21 c	6.4 b
Winter wheat – low (WWL)	614 a	603	4 ab	2.4 a
Winter wheat – high (WWH)	934 a	516	12 bc	2.6 a
Cereal rye – low (CRL)	936 a	589	1 a	1.9 a
Cereal rye – high (CRH)	1,022 a	489	9 ab	1.9 a
Contrastsc				
Winter wheat vs. cereal ryed	NS	NS	NS	NS
High vs. low seeding ratee	NS	NS	NS	NS

Table 2.7. Horseweed density and biomass at the time of cover crop termination.

bMeans followed by the same letter within a column are not statistically different at $\alpha \le 0.05$.

cSignificance is designated as: * = P < 0.05; ** = P < 0.001; NS denoted P \ge 0.05.

dContrasts comparing cover crop species pooled over seeding rate.

eContrasts comparing seeding rate pooled over cover crop species.

	Horseweed density					
	Isabella 2018	Isabella 2019	MSU 2019			
Cover crop treatment _a (Main effect)		plants m-2				
No cover	76	32 аь	8			
Winter wheat – low (WWL)	61	30 a	9			
Winter wheat – high (WWH)	104	31 a	9			
Cereal rye – low (CRL)	98	55 b	11			
Cereal rye – high (CRH)	154	36 ab	12			
Burndownc (Main effect)						
Control	491 b	202 c	37 c			
Effective burndown	363 b	87 b	18 b			
Effective burndown + residual	3 a	1 a	0.1 a			
Effects (p-values)		p-value				
Cover crop treatment	0.1941	0.0254	0.2342			
Burndown	< 0.0001	< 0.0001	< 0.0001			
Cover treatment x burndown	0.3588	0.4333	0.1561			

Table 2.8. Main effects of cover crop and burndown herbicide treatments and P-values for horseweed density at the time of POST herbicide application.

^aWinter wheat and cereal rye were seeded at 67 and 135 kg ha-1 for the low and high seeding rates, respectively.

bMeans followed by the same letter within a column are not statistically different at $\alpha \le 0.05$. cBurndown herbicide information is presented in Table 2.1.

	Horseweed biomass				
Burndowna	Cover crop treatmentb	Isabella 2018	Isabella 2019	MSU 2019	
			g m-2		
Control	No cover	121.0 fc	99.8 fg	36.9 f	
	Winter wheat – low (WWL)	77.2 de	79.0 ef	23.7 e	
	Winter wheat – high (WWH)	72.4 de	62.9 de	15.5 cde	
	Cereal rye – low (CRL)	78.0 de	115.9 g	23.5 e	
	Cereal rye – high (CRH)	80.0 e	42.4 cd	17.1 de	
Effective	No cover	61.5 cd	20.7 bc	6.4 ab	
burndown	Winter wheat – low (WWL)	37.4 b	16.8 abc	3.8 ab	
	Winter wheat – high (WWH)	41.4 b	28.3 c	7.5 abc	
	Cereal rye – low (CRL)	52.4 bc	71.9 e	12.1 bcd	
	Cereal rye – high (CRH)	46.2 bc	28.3 c	11.9 bcd	
Effective	No cover	0.1 a	0.0 a	0.0 a	
burndown +	Winter wheat – low (WWL)	0.0 a	0.1 ab	0.0 a	
residual	Winter wheat – high (WWH)	0.2 a	0.1 ab	0.0 a	
	Cereal rye – low (CRL)	0.2 a	0.3 ab	0.0 a	
	Cereal rye – high (CRH)	0.1 a	0.2 ab	0.4 a	
Effects			p-value		
Cover crop tro	eatment	0.0734	0.0002	0.2897	
Burndown		< 0.0001	0.0028	0.0017	
Cover treatme	ent*burndown	0.0036	< 0.0001	0.0001	

Table 2.9. Effect of cover crop treatment and burndown interaction on horseweed biomass at the time of POST herbicide application.

^aBurndown information is presented in Table 2.1.

^bWinter wheat and cereal rye were seeded at 67 and 135 kg ha-1 for the low and high seeding rates, respectively.

cMeans followed by the same letter within a column are not statistically different at $\alpha \le 0.05$.

Table 2.10. Main effects of cover treatment and burndown herbicide and P-values for inflorescent horseweed density and biomass at the time of soybean harvest.

	Horseweed density			Horseweed biomass			
		No POSTa	POST		No POST		POST
	ISB 2018bc	2019 sites	2019 sites	ISB 2018b	ISB 2019	MSU 2019	2019 sites
Cover treatmenta (Main effect)		plants m-2			g	m-2	
No cover	2	29	1	2.9	76.3	99.5	0.9
Winter wheat – low (WWL)	0.1	28	0	0.2	76.8	137.6	0.0
Winter wheat – high (WWH)	2	22	0.4	4.8	68.6	114.5	0.1
Cereal rye – low (CRL)	1	42	0	4.7	84.7	147.6	0.0
Cereal rye – high (CRH)	2	27	0.3	3.7	33.7	177.1	0.3
Burndowne (Main effect)							
Control	0 af	50 c	1	0.0 a	150.7g	233.0 c	0.7
Effective burndown	0 a	31 b	0.1	0.0 a	53.4	162.0 b	0.1
Effective burndown + residual	4 b	8 a	0	9.8 b	0.0	11.0 a	0.0
Effects (p-values)							
Cover crop treatment	1.0000	0.9582	0.8985	1.0000	0.6765	0.5284	0.7122
Burndown	0.0011	0.0383	0.8881	0.0043	0.1747	< 0.0001	0.8998
Cover treatment x burndown	0.2703	0.8863	0.9832	0.1946	0.2922	0.3647	0.9788

^aAbbreviations: No POST, non-effective POST herbicide; POST, effective POST herbicide.

bSite abbreviations: ISB 2018, Isabella 2018; ISB 2019 Isabella 2019; 2019 sites, Isabella 2019 and MSU 2019 combined. cEffective POST herbicide (glyphosate + dicamba) was applied to control and effective burndown herbicide treatments at Isabella 2018.

dWinter wheat and cereal rye were seeded at 67 and 135 kg ha-1 for the low and high seeding rates, respectively.

eBurndown information is presented in Table 2.1.

fMeans followed by the same letter within a column are not statistically different at $\alpha \le 0.05$.

gTreatment differences were not detected due to the variability of horseweed biomass in the control and effective burndown treatments.

	Soybean stand	Soybean Yield					
			No Po	OSTa	PO	POST	
	Combined sites	Isabella 2018b	Isabella 2019	MSU 2019	Isabella 2019	MSU 2019	
Cover treatment: (Main effect)	plants 3 m row-1			kg ha-1			
No cover	55 ad	1,733	1,600	3,091	1,961	3,432	
Winter wheat – low (WWL)	57 a	1,852	1,698	3,039	1,969	3,453	
Winter wheat – high (WWH)	55 ab	2,010	1,658	3,144	2,084	3,336	
Cereal rye – low (CRL)	55 a	1,867	1,683	2,862	2,064	3,315	
Cereal rye – high (CRH)	53 b	1,915	1,661	2,839	2,355	3,456	
Burndowne (Main effect)							
Control	54	1,514 c	958 c	2,655	2,003	3,479	
Effective burndown	54	1,809 b	1,678 b	3,157	2,078	3,244	
Effective burndown + residual	54	2,302 a	2,344 a	3,173	2,179	3,472	
Effects (p-values)							
Cover treatment	0.0242	0.8444	0.8662	0.8476	0.9371	0.6685	
Burndown	0.4300	0.0043	0.0003	0.1381	0.7261	0.6464	
Cover treatment x burndown	0.7501	0.7532	0.7034	0.6681	0.7927	0.7069	

Table 2.11. Main effects of cover treatment and burndown herbicide and P-values for soybean stand and yield.

aAbbreviations: No POST, non-effective POST herbicide; POST, effective POST herbicide.

bEffective POST herbicide was applied to control and effective burndown herbicide treatments at Isabella 2018.

eWinter wheat and cereal rye were seeded at 67 and 135 kg ha-1 for the low and high seeding rates, respectively.

dMeans followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

eBurndown information is presented in Table 2.1.

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CHAPTER III

EFFECTS OF FALL-PLANTED CEREAL COVER CROP TERMINATION TIME ON GLYPHOSATE-RESISTANT HORSEWEED SUPPRESSION

Abstract

Integrated strategies for management of glyphosate-resistant (GR) horseweed (Erigeron canadensis L.), such as cover crops, are needed to reduce reliance on herbicides. Planting a cover crop after corn or soybean harvest in the upper Midwest may reduce horseweed establishment and growth. Field experiments were conducted in three site-years in Michigan to determine if cereal rye and winter wheat, seeded at 67 or 135 kg ha-1, and terminated with glyphosate at 1,267 g ae ha-1 one week prior to (early termination) or one week after (Planting Green) soybean planting would suppress establishment and growth of GR horseweed. Delaying termination by Planting Green provided 212 to 272% more cover crop biomass compared with early termination. At the time of termination, cover crops reduced GR horseweed biomass 41 to 89% compared with no cover. Delaying termination by Planting Green increased the C:N ratio of cover crop residue, which improved residue persistence and GR horseweed suppression at the time of POST herbicide application, approximately five weeks after planting. Planting Green cover crops reduced GR horseweed biomass 46 to 93% compared with no cover at the time of POST herbicide application, while early terminated treatments provided less consistent suppression. Cover crops alone did not suppress GR horseweed through soybean harvest. Soybean yield was 30 to 108% greater in Planting Green compared with early terminated cover crops at two site-years. Cereal rye and winter wheat, seeded at 67 or 135 kg ha-1, provided earlyseason GR horseweed suppression. Delaying termination by Planting Green may improve GR horseweed suppression through the time of POST herbicide application.

Introduction

Horseweed (*Erigeron canadensis* L.) is a facultative winter annual weed species with a ruderal nature, that thrives across a wide geography and many different habitats. It is native to North America where it grows along roadsides, railways, and in crop production fields with reduced or no-tillage (Weaver 2001). Horseweed plants can produce up to 200,000 seeds, which are capable of wind dispersal into the planetary boundary layer and travel over 500 km due to an attached pappus (Bhowmik and Bekech 1993; Shields et al. 2006; Weaver 2001). Horseweed emergence can be difficult to predict as it is not correlated with soil temperature, air temperature, or rainfall (Main et al. 2006). Emergence has been observed throughout the growing season; however, peak emergence occurs in May and in late August to early September in the North Central Region (Buhler and Owen 1997; Tozzi and Van Acker 2014). Horseweed seeds are non-dormant and readily germinate on the soil surface, making horseweed especially difficult to manage in no-tillage situations. (Buhler and Owen 1997).

Horseweed is considered one of the top 10 most common and troublesome weeds in broadleaf crops in the United States and Canada (Van Wychen 2016). Soybean yields were reduced 83% when horseweed was left uncontrolled (Bruce and Kells 1990). Yield loss has intensified due to the widespread occurrence of herbicide-resistant populations and lack of effective management options. Currently, 18 countries have confirmed horseweed populations resistant to at least one herbicide site-of-action (Heap 2020). Horseweed was the first weed with confirmed resistance to glyphosate (WSSA group 9) in the United States (VanGessel 2001). Since this discovery, glyphosate-resistant (GR) horseweed populations were confirmed in 25 states and populations are often resistant to more than one herbicide site-of-action (Heap 2020). In Michigan, horseweed with resistance to acetolactate synthase inhibitors (WSSA group 2),

triazine herbicides (WSSA group 5), diuron (WSSA group 7), and paraquat (WSSA group 22) have also been confirmed (Heap 2020). The spread of herbicide-resistant horseweed populations, in addition to potential resistances to other herbicide sites of action, makes utilizing additional management strategies necessary.

Cover crops are being rapidly adopted for the ecosystem services they provide to the soil and as a weed suppression tool. Cover crops suppress weeds via resource competition and space capture while living, and by creating a mulch layer on the soil surface following termination (Mirsky et al. 2013; Teasdale et al. 2007). Additionally, cover crops reduce weed emergence by inhibiting seed germination through the release of allelochemicals (Kelton et al. 2012). However, allelopathic suppression is short lived and generally occurs within 20 days after cover crop decomposition (Chou and Patrick 1976). The mulch left following termination suppresses weeds by modifying light quantity and quality, changing soil surface temperature, and by creating a physical barrier to seedling emergence (Teasdale and Mohler 1993). In addition, high levels of mulch also create a physical barrier for emerging weed seedlings, which inhibits upward movement of seedlings and downward penetration of light (Mirsky et al. 2013). However, cover crop residues often do not persist long enough to provide season-long weed suppression in longseason grain crops due to residue degradation (Osipitan et al. 2018).

Fall-planted cover crops suppress winter annual weeds in the fall as well as the following spring (Teasdale 1996). This is critical for GR horseweed suppression since it emerges in the fall or early spring. Among fall-planted cover crops, cereal rye (*Secale cereale* L.) and winter wheat (*Triticum aestivum* L.) are two of the most commonly grown cover crops prior to soybeans (CTIC 2017). Cornelius and Bradley (2017) reported 70 and 50% reduction in winter annual weed emergence from cereal rye and winter wheat, respectively. Recent studies reported that

fall-planted cover crops reduced horseweed density prior to cover crop termination in the spring (Pittman et al. 2019; Wallace et al. 2019). Wallace et al. (2019) observed that cereal rye, alone or in mixtures, reduced horseweed size and improved horseweed size uniformity at burndown. Late-season horseweed suppression by fall-planted cover crops is less consistent. Davis et al. (2007) observed similar reductions in horseweed density by a winter wheat cover crop compared with spring-applied residual herbicides one month after burndown in one of four years. In contrast, Wallace et al. (2019) reported cereal rye terminated 10 days before soybean planting did not reduce horseweed density at the time of POST herbicide application.

Several studies found weed suppression by cereal cover crops improves with increasing cover crop biomass (Finney et al. 2016; Ryan et al. 2011b; Smith et al. 2011). Cereal rye generally produces more biomass than winter wheat (Bauer and Reeves 1999; Cornelius and Bradley 2017; Duiker 2014; Kaspar and Bakker 2015; McCormick et al. 2006; Price et al. 2006; Reeves et al. 2005). Adjusting seeding rate is a method to potentially increase cereal cover crop biomass accumulation. However, many previous studies reported adjusting seeding rate to have little effect on biomass accumulation of cereals due to tillering as a means of compensation (Brennan et al. 2009; Masiunas et al. 1995; Ryan et al. 2011a; Webster et al. 2016). Delaying cover crop termination is another method to increase biomass accumulation. A study comparing incremental delays in spring termination found cereal rye biomass increased 37% with each 10-day delay (Mirsky et al. 2011).

To offset delayed planting in no-till systems due to cooler and wetter soil conditions, many growers have begun "Planting Green," where cover crop termination is delayed until shortly after planting of the main crop (CTIC 2017). Delaying cover crop termination 4 to 30 days compared with early termination, Planting Green provided 94 to 181% greater cover crop

biomass production in Pennsylvania (Reed et al. 2019). Soil moisture at planting may be reduced if cover crops are allowed more time to grow and accumulate more biomass (Mirsky et al. 2011). Reed et al. (2019) reported Planting Green provided 7 to 24% drier and 0.7 to 2.4 C cooler soil conditions at soybean planting, and increased soil moisture during dry periods following planting in Pennsylvania. However, Liebl et al. (1992) found delaying cereal rye termination until planting in Illinois resulted in up to 45% soybean stand reduction and subsequent yield loss compared with conventional management. In contrast, Reed et al. (2019) reported no stand reductions or effect on yield from Planting Green compared with early terminated cover crops. Additional information on the effectiveness of Planting Green as a weed suppression tool is needed.

Challenges surrounding GR horseweed management require additional strategies. Fallplanted cereal cover crops may improve horseweed management; however, little is known about Planting Green for weed suppression. The objective of this research was to evaluate the effects of fall-planted cereal cover crops for management of GR horseweed. We compared winter wheat and cereal rye seeded at two recommended seeding rates for their ability to suppress GR horseweed. Additionally, we examined how termination time affected cover crop biomass, residue persistence, and, ultimately, GR horseweed suppression.

Materials and Methods

Field experiments were conducted in commercial fields in Isabella County, Michigan in 2018 (43.6128°N, -84.8777°W) and 2019 (43.6255°N, -84.9812°W) and at the Michigan State University (MSU) Agronomy Farm in East Lansing, Michigan in 2019 (42.6876°N, -84.4907°W). Sites were selected based on GR horseweed escapes the previous season. The soil types in Isabella County were a Selfridge sand (loamy, mixed, active, mesic Aquic Arenic Hapludalfs) with pH 6.4 and 2.2% organic matter in 2018 and a Wasepi loamy sand (coarse-loamy, mixed, semiactive, mesic Aquollic Hapludalfs) with pH 5.2 and 2.2% organic matter in 2019. Soils at MSU were Conover loam (fine-loamy, mixed, active, mesic Aquic Hapludalfs) with pH 5.7 and 3.0% organic matter.

In 2018, the experiment was established as a split-plot randomized complete block design with three replications. In 2019, the experiment was established as a split-split plot randomized complete block design with three replications. Each plot measured 3 m wide by 9 m long. The main plot factor was cover crop, the subplot factor was cover crop termination timing, and the sub-subplot factor in 2019 was postemergence (POST) herbicide. The main plots consisted of five cover crop factors: 1) winter wheat seeded at a low rate of 67 kg ha-1 (WWL), 2) winter wheat seeded at a high rate of 135 kg ha-1 (WWH), 3) cereal rye seeded at a low rate of 67 kg ha-1 (CRL), 4) cereal rye seeded at a high rate of 135 kg ha-1 (CRH), and 5) no cover crop control (NC). The subplots consisted of two cover crop termination timings: 1) one week prior to soybean planting (early termination) or 2) one week after soybean planting (Planting Green). Cover crops were terminated by applying glyphosate (Roundup PowerMAX; Bayer CropScience, St. Louis, MO) at 1,267 g ae ha-1 plus ammonium sulfate (Actamaster; Loveland Products, Inc., Greeley, CO) at 2% w w-1. The sub-subplot factors in 2019 were: 1) an effective POST herbicide application, or 2) a non-effective POST herbicide application. The effective POST herbicide application consisted of glyphosate at 1,267 g ae ha-1 plus dicamba (XtendiMax; Bayer CropScience, St. Louis, MO) at 560 g ae ha-1 with a drift-reduction agent (Intact, Bayer CropScience, St. Louis, MO) at 0.5% w w-1. The non-effective POST herbicide application consisted of glyphosate at 1,267 g ae ha-1 plus ammonium sulfate at 2% w w-1.

Main plots of 'Wheeler' cereal rye and 'Sunburst' winter wheat were sown in 19 cm rows using a no-till drill (Great Plains, Salina, KS) the fall prior to data collection. Dates for all field operations can be found in Table 3.1. Cover crops were terminated and subplots were established one week prior to (early termination) or one week after (Planting Green) planting soybean the following spring. Glyphosate and dicamba-resistant soybean 'AG 26X8' (Bayer CropScience, St. Louis, MO) was planted in 76 cm rows at a seeding rate of 383,000 seeds ha-1. POST herbicide applications were made approximately five weeks after soybean planting (WAP) when emerged horseweed was approximately 10 cm tall. In 2018, the effective POST herbicide application was applied to all plots. In 2019, POST herbicide application was a sub-subplot factor and was established at this time. 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 TTI nozzles (TeeJet Technologies, Spraying Systems Co., Wheaton, IL).

Data collection. Prior to early termination, aboveground cover crop biomass and weed density and biomass were collected from two randomly placed 0.25 m2 subsamples in each early termination plot. Measurements were taken using the same method in each Planting Green plot at the time of Planting Green termination, approximately two weeks later. In addition to horseweed, annual bluegrass (*Poa annua* L.), common chickweed (*Stellaria media* (L.) Vill.), shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.), and dandelion (*Taraxacum officinale* F. H. Wigg.) were present at the time of early termination subplot establishment in all site-years. Spring whitlowgrass (*Draba verna* L.) and white campion (*Silene latifolia* Poir.) were also present at the 2019 locations. Common lambsquarters (*Chenopodium album* L.) had emerged by the time of Planting Green termination in all site-years. Cover crop subsamples of 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 was collected from two random 0.25 m² subsamples in all plots at the time of POST herbicide application and again prior to soybean harvest. At soybean harvest, fall-emerged horseweed rosettes were segregated from fully mature horseweed plants. Biomass samples were dried for approximately 7 d at 65 C and weighed.

Soil moisture was measured at the time of soybean planting 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. When soybean reached the VE growth stage, percent ground cover was measured using line-transects (Laflen et al. 1981) laid diagonally across each plot. The presence of cover crop residue, horseweed, other weed, or no vegetation was recorded at every 30 cm point along a 9 m transect. Soybean populations were also assessed in all plots at this time. Soybean was harvested for yield using a small-plot research combine (Massey-Ferguson 8XP, AGCO, Duluth, GA) with a 1.5 m header. Yields were adjusted to 13% moisture.

Two permanent 0.25 m² quadrats were established in each no cover plot to measure horseweed emergence throughout the growing season. Each week, newly emerged horseweed seedlings were counted and removed from these quadrats.

Precipitation and temperature data were obtained throughout the growing season from the Michigan Automated Weather Network (http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, MI) stations located in Mecosta, Mount Pleasant, and East Lansing for Isabella 2018, Isabella 2019, and MSU 2019, respectively (Table 3.2). Daily soil temperature was collected using HOBO Pendant® Temperature/Alarm Data Loggers (Onset Computer Corporation, Bourne, MA) placed in the soil at a depth of 2 cm.
Statistical analysis. Data analysis was conducted using lmer in R v. 3.6.2 (R Development Core Team 2019). The statistical model consisted of site-year (individual year and location), cover treatment, and termination time as fixed effects and replication nested in site-year and the interaction between cover treatment and replication nested within site-year as the random effects. Replications were used as an error term for testing the effect of site-year, and data were combined over site-year when the interaction of site-year and cover treatment or termination time was not significant. The cover treatment by replication interaction was used as an error term to test the effect of cover treatment. Additionally, preplanned contrasts were performed to compare cover crop species pooled over cover crop seeding rates and cover crop seeding rates pooled over cover crop species. Data for horseweed density and biomass at harvest and soybean yield were analyzed separately by POST herbicide treatments. 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 the Levene's test for unequal variances. In cases of marked deviations from normality, the data were log-transformed and further analyses were performed using the transformed data. For all experiments, treatment means were separated using Fisher's Protected LSD at $\alpha \leq 0.05$.

Results and Discussion

Horseweed emergence. Peak emergence occurred the week prior to April 25th at MSU 2019 and May 14th at Isabella 2018 and 2019 (Figure 3.1). Greater than 80% of total emergence occurred over the period of a week at each of these locations. Horseweed continued to emerge at each location throughout the spring and early summer. Horseweed emergence reached 100% the week

prior to June 25th, July 2nd, and July 17th at Isabella 2018, MSU 2019, and Isabella 2019, respectively. Emergence occurred in September and October at each site following seed shed (data not shown). This data was omitted because emerging plants were believed to originate from newly shed seed. Predicting peak horseweed emergence would allow growers to adjust their management plans for effective control. From our data collection it is clear that peak emergence dates vary by location and year.

The horseweed emergence data was also examined by accumulated growing degree days (GDD) and precipitation. Peak emergence occurred when 50 to 100 GDD's (base 10 C) were accumulated at each site-year (Figure 3.2). Tracking GDD's and emergence across different sites and years is needed to support this conclusion. However, growers could use a GDD model to determine when to make burndown herbicide applications. Late emergence in June at Isabella 2018 occurred in the weeks following heavy rainfall (Figure 3.3). Similarly, late emergence occurred at Isabella 2019 and MSU 2019 during plateaus in precipitation following rain events (Figures 3.4 and 3.5). Our data suggest that initial periods of peak emergence depend on accumulated GDD's and late emergence follows rainfall. Therefore, a GDD model could be developed to determine burndown herbicide application timing and we recommend scouting for newly emerged horseweed seedlings after rain events to appropriately time POST herbicide applications.

Cover crop response traits. Differences among accumulated precipitation and GDD's prior to early termination resulted in varying amounts of initial cover biomass. Cover crops were sown approximately three weeks earlier at the Isabella 2018 site, leading to more GDD and precipitation between cover crop planting and early termination (Table 3.1). Accumulated

GDD's between cover crop planting and early termination were 541, 315, and 326 (base 4.4 C) for Isabella 2018, 2019, and MSU 2019, respectively. In addition, precipitation prior to early termination at Isabella 2018 was two times greater than at Isabella 2019 and MSU 2019 (Table 3.2). Cover crop biomass, averaged over cover treatment, at the early termination was 1,240, 560, and 1,000 kg ha-1 for Isabella 2018, 2019, and MSU 2019, respectively (Table 3.3). Soil type differences between the 2019 sites may explain the differences in cover crop biomass. At Isabella 2019, the sandy soil type didn't allow cover crops to compensate for lack of GDD's and precipitation between planting and termination. In contrast, the MSU 2019 soil type was a loam, which generally has greater nitrogen availability due to the higher clay and silt content as well as higher soil organic matter (Hassink 1994). This allowed MSU 2019 cover crops to accumulate more biomass even though planting was relatively late. Previous studies in Michigan have found similar ranges (800 to 2,900 kg ha-1) of aboveground cereal rye biomass (Rogers 2017; Snapp et al. 2005; Hill 2014).

Delaying termination 15 to 20 days by Planting Green allowed cover crops to accumulate more biomass at each site-year. Average cover biomass at the time of Planting Green termination was 3,870, 1,970, and 3,720 kg ha-1 at Isabella 2018, 2019, and MSU 2019, respectively (Table 3.3). Mirsky et al. (2011) reported 10-day incremental delays in termination resulted in 37% higher cereal rye biomass with each delay. Between early termination and Planting Green, cover crops accumulated an additional 227, 171, and 222 GDD at Isabella 2018, 2019, and MSU 2019, respectively. Lower initial biomass and colder temperatures between terminations resulted in a smaller increase in cover biomass at Isabella 2019 compared with the other site-years. The Planting Green biomass produced at Isabella 2018 and MSU 2019 was similar to that reported by Hayden et al. (2012) who found cereal rye produced 3,300 to 5,870 kg ha-1 when planted in early September and terminated in mid to late May in Michigan. At MSU 2019, there was an interaction between cover crop treatment and termination time. In general, cover crop biomass was higher for Planting Green treatments. However, CRH terminated early produced similar biomass to WWL with the Planting Green termination. Combined over termination time and seeding rate, cereal rye produced 31 and 53% more biomass than winter wheat at Isabella 2018 and MSU 2019, respectively (Table 3.3). This supports previous research that found cereal rye produced more biomass than winter wheat (Cornelius and Bradley 2017; Haramoto 2019). However, winter wheat benefited from delayed termination more than cereal rye at MSU 2019, increasing biomass 452 and 216%, respectively. Seeding rate had no effect on cover crop biomass, which is likely due to cereal cover crops compensating for lower seeding rates by tillering (Masiunas et al. 1995).

When terminated early, cereal rye and winter wheat were at Feekes stage 6 at Isabella 2018 and Feekes stage 5 at the 2019 sites. Cover crops at the Planting Green termination time reached Feekes stage 10.4 at Isabella 2019, and Feekes stage 10.5 at Isabella 2018 and MSU 2019. At Isabella 2018, cover crop treatment and termination time did not affect the C:N ratio of the harvested cover crop biomass (Table 3.3). This is in contrast to previous research which found that the C:N ratio of cereal cover crops increased as they matured (Sullivan et al. 1991). At the 2019 sites, there was an interaction between cover crop treatment and termination time. Pooled over cover crop treatment, C:N ratio was 16:1 and 29:1 for cover crops harvested at the early and Planting Green termination times, respectively (Table 3.3). The ideal C:N ratio for a microbial diet is 24:1; cover residues with C:N ratios above 24:1 (NRCS 2011; Odhiambo and Bomke 2001). All Planting Green covers had a C:N ratio at or above 24:1, while early terminated

covers were below 24:1 at the 2019 sites. Additionally, cereal rye generally had a higher C:N ratio compared with winter wheat, which is similar to previous findings (Ashford and Reeves 2003).

Cover crops had no effect on soil moisture at soybean planting, regardless of termination time, at the Isabella sites. In contrast, soil moisture at MSU 2019 followed a trend of increasing soil moisture with increased cover crop biomass. Pooled over termination time, soil moisture was 2.6, 2.9, and 3.7% higher in WWH, CRL, and CRH plots compared with no cover (Table 3.4). However, soil moisture was 1.8% lower in Planting Green cover crops compared with early terminated covers. Soil moisture at planting can be reduced if cover crops are allowed more time to grow and accumulate biomass (Mirsky et al. 2011). At the Isabella sites, low precipitation between terminations and coarse soil texture allowed water movement through the soil regardless of cover residue. The MSU 2019 site received more rainfall and was on a loam soil type. The presence of early terminated cover crop residue likely led to less surface evaporation and greater soil moisture retention by the loam soil. In contrast, evapotranspiration by Planting Green cover crops resulted in drier soils at MSU 2019. Similarly, Reed et al. (2019) reported drier soils when Planting Green compared with terminating cover crops before planting soybeans. Cover crop treatment had no effect on daily soil temperature fluctuations. At Isabella 2018, soil temperature fluctuated less in Planting Green treatments compared with early termination treatments five days around the time of soybean planting (data not shown). In general, soil temperatures within termination times were similar and differences never exceeded 2 C.

To identify how cover treatment and termination time affected cover residue left to suppress GR horseweed, ground cover was measured when soybean reached the VE growth stage (~ 3 WAP). The hypothesis was that higher cover crop biomass at termination and a higher

C:N ratio would result in more persistent ground cover and therefore GR horseweed suppression later in the season. Pooled over cover treatment, early terminated and Planting Green cover crops provided 31 and 56% ground cover, respectively (Table 3.4). This was a function of Planting Green having higher cover biomass at termination and a more persistent residue compared with early termination. Averaged over termination time, cereal rye and winter wheat provided 47 to 50 and 34 to 43% ground cover, respectively. Additionally, cereal rye often lodged creating more ground cover compared with winter wheat which remained upright (personal observation). The mulch layer formed by cover residue provides weed suppression (Mirsky et al. 2013), so greater GR horseweed suppression is expected when cover crop ground cover is high.

Horseweed suppression at cover crop termination. Initial horseweed emergence occurred between April 25th and May 14th in all three site-years and all horseweed plants exhibited a summer annual lifecycle. Horseweed density at the early termination timing varied greatly between site-years; densities in the no cover plots were 1,845, 715, and 82 plants m-2 at Isabella 2018, 2019, and MSU 2019, respectively. Due to inter- and intra-specific competition between terminations, horseweed densities in no cover plots were lower at the Planting Green termination time with 748 and 251 plants m-2 at Isabella 2018 and 2019, respectively. In contrast, relatively low horseweed and other weed presence resulted in drastically higher horseweed density by the time of the Planting Green termination at MSU 2019. At Isabella 2018, all cover crop treatments, with exception of WWH, reduced horseweed density 46 to 56% compared with no cover (Table 3.5). Similarly, previous studies have found cover crops reduced horseweed density 80% at the time of termination compared with no cover (Wallace et al. 2019; Pittman et al. 2018). At Isabella 2019, horseweed densities in Planting Green treatments were 66% lower compared with the early termination treatments (Table 3.5). However, cover crops did not reduce horseweed density compared with the no cover control at this site. We attributed the horseweed density reduction between termination timings to intraspecies competition. At MSU 2019, neither cover treatment nor termination time had an effect on horseweed density.

Similar to density, horseweed biomass at cover crop termination varied among site-years and termination times. Pooled over termination time, cover crops reduced horseweed biomass 41 to 89% compared with the no cover control at Isabella 2019 (Table 3.5). These results are similar to Hayden et al. (2012) who reported cereal rye reduced winter annual weed biomass 95 to 97% in Michigan. There was an interaction between cover treatment and termination time at MSU 2019 (P = 0.0033). With the exception of WWH terminated early, cover crops reduced horseweed biomass 81 to 88% compared with the no cover control terminated at the same respective time (Table 3.5). However, horseweed biomass in winter wheat terminated Planting Green was similar to no cover terminated early. Horseweed experienced rapid growth between terminations at this site-year and biomass increased 173% in the no cover control plots. As a result, the main effect of cover treatment was masked at MSU 2019.

Horseweed suppression varied by termination time at Isabella 2018. Combined over termination time, only WWL provided horseweed suppression compared with no cover at Isabella 2018 (Table 3.5). When horseweed biomass data were analyzed separately by termination time, the early termination cover crop treatments reduced horseweed biomass by greater than 59% compared with the no cover control (data not shown). Applications of glyphosate were made to terminate cover crops and control other weeds. Thus, delaying termination by Planting Green allowed a longer period of growth for cover crops as well as other weed species. In no cover plots, dandelion and common chickweed biomass increased by greater

than 200% between early and Planting Green terminations at Isabella 2018 (data not shown). Horseweed biomass in no cover plots at Planting Green termination was 82% lower compared with early termination at this site-year. Thus, dandelion and chickweed provided horseweed competition when cover crops were absent and termination was delayed. Additionally, Planting Green cover crops reduced other weed biomass 88% compared with the no cover plots at Isabella 2018 (data not shown). These findings suggest weed diversity was reduced at this site-year when cereal rye and winter wheat were left to compete with weeds. More diverse weed communities are less competitive and less prone to dominance by herbicide-resistant species, such as horseweed (Storkey and Never 2018). Therefore, cover crop presence may increase the overall competitiveness of the weed community in certain scenarios.

With the exception of Planting Green at Isabella 2018, we observed horseweed biomass reduction at the time of termination from cereal cover crops. Similarly, Wallace et al. (2019) reported cereal rye alone or in mixtures reduced horseweed size and improved size uniformity at burndown. The effectiveness of the auxinic herbicides 2,4-D and dicamba is less consistent on large horseweed plants (Keeling et al. 1989; Kruger et al. 2010; Wiese et al. 1995). The ability of cereal rye and winter wheat to reduce biomass at termination could provide growers greater GR horseweed control at the time of burndown herbicide application.

Horseweed suppression following termination. POST herbicide applications were made approximately five weeks after planting. At this time, cover treatment and termination time had no effect on horseweed density (Table 3.6). Similarly, Wallace et al. (2019) reported no reduction in horseweed density at the time of POST herbicide application when using a cereal rye cover crop. Mirsky et al. (2011) observed greater suppression of weed density 8 WAP from high biomass producing cereal rye varieties, earlier planting dates, and later termination dates. We observed a similar trend in our study, but only for horseweed biomass. Planting Green cover crops reduced horseweed biomass at the time of POST herbicide application 46 to 93% compared with no cover (Table 3.6). Early terminated cover crops provided less horseweed suppression at each site-year. The high seeding rates, with exception of CRH at Isabella 2018, reduced horseweed biomass 18 to 58% compared with no cover. Cover crops seeded at the low rate and terminated early did not provide horseweed suppression at the time of POST herbicide application. Norsworthy et al. (2004) reported cereal rye and winter wheat reduced weed biomass 68 and 21% three weeks after corn emergence, respectively. The reduced horseweed biomass in the Planting Green treatments five weeks after planting is worth noting. Reducing horseweed size at the time of POST herbicide application may provide improved herbicide effectiveness when managing GR horseweed.

Horseweed density and biomass was collected and separated by rosettes and inflorescent plants expected to produce viable seed prior to soybean harvest. Horseweed emerging in July and August typically overwinter as rosettes and do not contribute to the seed bank that growing season (Loux et al. 2006). For this reason, only inflorescent horseweed density and biomass data are presented. At Isabella 2018, an effective POST herbicide application was made to all plots and no horseweed were present at the time of soybean harvest (Table 3.7). Neither cover crop treatment nor termination time affected horseweed density at Isabella 2019 when an effective POST herbicide application was made. Similarly, cover crops did not reduce horseweed density compared with the no cover control at MSU 2019. Combined over the 2019 sites, neither cover crop treatment nor termination time affected horseweed biomass at soybean harvest when an effective POST herbicide application was made.

In 2019, sub-sub plots were created to measure the ability of cereal rye and winter wheat to suppress horseweed in the absence of an effective POST herbicide application. Consequently, horseweed density and biomass were higher in these plots. When a non-effective POST herbicide was applied at Isabella 2019 or MSU 2019, cover crops did not reduce horseweed density compared with the no cover control (Table 3.8). However, horseweed biomass was 69% lower in the Planting Green treatments compared with early termination timing at MSU 2019. Similarly, biomass was reduced 86% in Planting Green treatments compared with early termination timing at MSU 2019. Similarly, biomass was reduced 86% in Planting Green treatments compared with early termination treatments at Isabella 2019. However, due to variability in the biomass collected in Planting Green treatments at Isabella 2019 a significant difference was not detected between termination times. A meta-analysis found that cover crop residues often do not persist long enough to provided weed suppression throughout the season in long-season crops (Osipitan et al. 2018). Our data in two of three site-years generally supported this.

Soybean establishment and yield. Pooled over site-year and termination time, cover crops had no effect on soybean stand establishment compared with the no cover control (Table 3.9). However, soybean stands were higher in WWL compared with other cover crops. This is in contrast to previous studies who reported 10 to 35% soybean stand reduction in a cereal rye cover crop (Moore et al. 1994; Reddy 2001). Additionally, soybean stand was similar between early terminated and Planting Green cover crops (Table 3.9). This is in contrast to what Liebl et al. (1992) found, where delaying cereal rye termination until planting reduced soybean stand up to 45% compared with conventional management and resulted in subsequent yield loss. Reed et al. (2019) observed no stand reductions from planting soybean into green cover crops. Cover crop treatment had no effect on soybean yield at any site-year (Table 3.9). Termination time

affected soybean yield under two circumstances. At Isabella 2018, all plots received an effective POST herbicide application and soybean yields in Planting Green covers were 30% higher than early terminated covers. When a non-effective POST herbicide was applied at Isabella 2019, soybeans in the Planting Green covers yielded 108% higher than early terminated covers (Table 3.9). We believe reduced early-season weed competition through the time of when a POST herbicide application would have been applied resulted in higher yields at these locations. Termination time differences were not detected at Isabella 2019 with a non-effective POST herbicide or at MSU 2019, regardless of POST herbicide. We believe the effective POST herbicide application at Isabella 2019 made up for horseweed competition differences early in the season. Soybean yields were higher at MSU 2019 and horseweed competition was relatively low, resulting in no differences among cover crop treatments or termination times. Similarly, Reed et al. (2019) reported no soybean yield difference between termination times. Our findings suggest utilizing cereal rye and winter wheat terminated at either time results in similar or higher soybean yield compared with no cover.

In conclusion, cereal rye and winter wheat effectively suppressed GR horseweed early in the season in a no-till soybean system. At the times of cover crop termination and POST herbicide application, cover crops suppressed horseweed by reducing biomass through resource competition rather than affecting horseweed emergence. Cereal rye produced more biomass and provided more ground cover than winter wheat. However, horseweed suppression was similar between cover species and cover seeding rates throughout the season. Delaying cover crop termination by Planting Green increased cover crop biomass, ground cover, and residue persistence. This ultimately led to greater horseweed suppression through the time of POST herbicide application. However, cereal cover crops alone were not effective at controlling

horseweed until soybean harvest. More research is needed to explore how effective herbicides can be integrated with Planting Green cover crops. Planting Green covers reduced soil moisture at planting in one site-year and did not negatively affect soybean stand. Soybean yields were higher with Planting Green covers compared with early terminated covers at two site-years, likely due to greater early-season horseweed suppression. Cereal rye and winter wheat cover crops provide growers an additional strategy for GR horseweed management. Delaying cover crop termination by Planting Green provides additional horseweed suppression through the time of a POST herbicide application.

APPENDIX

APPENDIX Tables and Figures

and soybean narvest dates for the three experimental locations.							
Operation	Isabella 2018	Isabella 2019	MSU 2019				
Cover crop seeding	September 27, 2017	October 18, 2018	October 17, 2018				
Early termination	May 14, 2018	May 21, 2019	May 14, 2019				
Soybean planting	May 21, 2018	May 29, 2019	May 27, 2019				
Planting Green termination	May 30, 2018	June 5, 2019	June 3, 2019				
POST application	June 29, 2018	July 3, 2019	July 3, 2019				
Soybean harvest	October 16, 2018	October 23, 2019	October 9, 2019				

Table 3.1. Cover crop seeding and termination, soybean planting, POST herbicide application, and soybean harvest dates for the three experimental locations.

	Isabella			MS	ЛSU
Month	2018	2019	5-yr ave.	2019	5-yr ave.
		mm		mr	n
Fall prior	249	51	164	54	154
April	43	27	75	72	72
May	103 (46)b (68)c (73)d	96 (61) (92)	91	85 (45) (76)	86
June	57	89 (5)	90	115 (22)	89
July	68	22	62	58	62
August	197	86	99	18	91
September	64	129	81	92	86
October	19e	131	110	31	109
Total precipitation f					
Cover – Early termination	338	139	-	171	-
Cover – Planting Green	365	179	-	233	-
Soybean	437	461	533	323	523

Table 3.2. Monthly and 5-yr average precipitation at Isabella County in 2018 and 2019 and Michigan State University in 2019.a

^aMichigan Automated Weather Network, <u>http://www.agweather.geo.msu.edu/mawn/</u>, Michigan State University, East Lansing, MI. ^bPrecipitation up to early cover crop termination.

^cPrecipitation up to soybean planting.

dPrecipitation up to Planting Green cover crop termination.

eThe harvest month does not include rainfall after harvest.

fTotal precipitation is a total of rainfall from planting until cover crop termination, not including precipitation in December, January, February, and March and the total rainfall from soybean planting until harvest.

¥ ¥		Cover crop biomass			C:N 1	atio
Termination time	Cover crop treatmenta	Isabella 2018	Isabella 2019	MSU 2019	Isabella 2018	2019 sites
			kg ha-1			
Early	Winter wheat - Low	746	561	321 еь	20:1	13:1 f
	Winter wheat – High	1,048	756	605 e	17:1	14:1 ef
	Cereal rye – Low	1,381	756	1,359 d	18:1	18:1 de
	Cereal rye – High	1,777	762	1,731 cd	17:1	18:1 d
Planting Green	Winter wheat – Low	3,003	1,715	2,291 bc	17:1	24:1 c
-	Winter wheat – High	3,506	2,268	2,819 b	17:1	25:1 c
	Cereal rye – Low	4,217	1,917	4,791 a	16:1	30:1 b
	Cereal rye – High	4,748	1,980	4,973 a	15:1	36:1 a
Effects (p-values)						
Cover treatment		0.2726	0.7913	< 0.0001	0.2469	0.0213
Termination timec		< 0.0001	< 0.0001	< 0.0001	0.0685	< 0.0001
Cover treatment x tim	e	0.7169	0.6775	0.0026	0.3079	0.0363
Contrastsd						
Winter wheat vs. cerea	al ryee	**	NS	**	NS	*
High vs. low seeding	ratef	NS	NS	NS	NS	NS
GDDg						
Early termination		541	315	326		
Planting Green		768	486	548		

Table 3.3. Interaction effect of termination time and cover crop treatment, P-values, and contrasts on cover crop dry biomass, C:N ratios, and growing degree days (GDD) accumulated at the time of cover crop termination.

bMeans followed by the same letter within a column are not statistically different at $\alpha \le 0.05$.

^cThe main effect of termination time was significant for cover crop biomass at Isabella 2018 and 2019; Planting Green cover crop biomass was greater than early termination.

dSignificance is designated as: * = P < 0.05; ** = P < 0.001; NS denoted $P \ge 0.05$.

eContrasts comparing cover crop species pooled over seeding rate and termination time.

fContrasts comparing seeding rates pooled over cover crop species and termination time.

gGrowing degree days (GDD) (base 4.4 C) accumulated from the timing of planting until termination.

			Ground cover	
	Isabella 2018	Isabella 2019	MSU 2019	Combined sites
Cover treatmenta (Main effect)		_% moistureb_		%
No cover	19.1	17.7	20.1 ac	
Winter wheat – low (WWL)	18.6	18.9	22.0 ab	34 c
Winter wheat – high (WWH)	19.5	20.0	22.7 b	43 b
Cereal rye – low (CRL)	22.4	18.5	23.0 b	47 ab
Cereal rye – high (CRH)	22.3	19.0	23.8 b	50 a
Termination time (Main effect)				
Early	20.4	19.7	23.2 b	31 b
Planting Green	20.4	17.9	21.4 a	56 a
Effects (p-values)				
Cover treatment	0.0768	0.6165	0.0035	< 0.0001
Termination time	0.8574	0.4712	0.0063	< 0.0001
Cover treatment x time	0.9080	0.8798	0.3559	0.6118

Table 3.4. Soil moisture at 7.6 cm depth measured at the time of soybean planting and cover crop ground cover at soybean growth stage VE.

bSoil moisture reported as volumetric water content and measured with a TDR 300 Soil Moisture Meter (FieldScout, Spectrum Technologies, Aurora, IL).

cMeans followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

	Density			Biomass			
	Isabella 2018	Isabella 2019	MSU 2019	Isabella 2018	Isabella 2019	MSU 2019	
Cover treatment _a (Main effect)		plants m-2			g m-2		
No cover	1,296 bb	483	144	6.6 b	10.8 c	13.6	
Winter wheat – low (WWL)	573 a	433	32	3.1 a	6.4 b	1.4	
Winter wheat – high (WWH)	772 ab	366	114	4.5 ab	3.9 ab	2.4	
Cereal rye – low (CRL)	686 a	377	9	4.1 ab	2.1 ab	0.3	
Cereal rye – high (CRH)	694 a	292	27	4.2 ab	1.3 a	0.5	
Termination time (Main effect)							
Early	1013	582 b	35	6.2	5.5	2.0	
Planting Green	595	198 a	95	2.9c	4.3	5.3	
Effects (p-values)							
Cover treatment	0.0193	0.4056	0.6886	0.0162	0.0065	0.1027	
Termination time	0.1917	0.0119	0.7977	0.1762	0.5981	0.8498	
Cover treatment x time	0.2791	0.9279	0.1730	0.0800	0.9439	0.0033d	

Table 3.5. Main effects of cover treatment and termination time and P-values for horseweed density and biomass at the time of cover crop termination.

bMeans followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

cCompetition from other weeds between termination times is believed to have reduced biomass between terminations.

^dThe interaction effect for cover treatment and time was significant. In general, cover crops reduced horseweed biomass compared with no cover at each respective termination time. Increase in horseweed biomass effected the main effect of cover treatment.

		Density		Biomass	
Termination time	Cover treatmenta	Combined sites	Isabella 2018	Isabella 2019	MSU 2019
		plants m-2		g m-2	
Early	No cover	263	111.3 еь	99.8 ef	3.3 e
	Winter wheat - Low	262	73.2 cde	79.0 de	3.1 de
	Winter wheat – High	317	62.7 cd	62.9 cd	2.4 bc
	Cereal rye – Low	346	66.8 cde	115.9 f	3.1 de
	Cereal rye – High	321	77.8 de	42.4 bc	2.7 cd
Planting Green	No cover	273	44.5 c	77.9 de	3.5 e
	Winter wheat - Low	221	16.4 b	15.4 ab	1.9 ab
	Winter wheat – High	259	9.0 a	5.8 ab	1.8 a
	Cereal rye – Low	249	9.8 ab	19.2 ab	1.5 a
	Cereal rye – High	281	6.9 a	7.2 ab	1.5 a
Effects (p-values)					
Cover treatment		0.1952	0.1952	0.0009	0.0054
Termination time		0.8623	< 0.0001	0.0552	< 0.0001
Cover treatment x time		0.9725	0.0096	0.0376	0.0001

Table 3.6. Interaction effect of termination time and cover treatment interaction and P-values for horseweed density and biomass at the time of POST herbicide application.

^aWinter wheat and cereal rye were seeded at 67 and 135 kg ha-1 for the low and high seeding rates, respectively.

bMeans followed by the same letter within a column are not statistically different at $\alpha \le 0.05$.

		Biomassa		
	Isabella 2018	Isabella 2019	MSU 2019	2019 sites
Cover treatment _b (Main effect)		plants m-2		g m-2
No cover	0	3	0 ac	0
Winter wheat – low (WWL)	0	0.3	0 a	0
Winter wheat – high (WWH)	0	0.7	0.7 b	0.2
Cereal rye – low (CRL)	0	0	0 a	0
Cereal rye – high (CRH)	0	0.7	0.3 ab	0.2
Termination time (Main effect)				
Early	0	2	0.4	0.2
Planting Green	0	0.2	0	0
Effects (p-values)				
Cover treatment	1.000	0.1428	0.0131	0.9877
Time	1.000	0.6233	0.1413	0.7342
Cover treatment x time	1.000	0.2759	0.1498	0.9968

Table 3.7. Main effects of cover treatment and termination time and P-values for inflorescent horseweed biomass and density at the time of soybean harvest when an effective POST herbicide (glyphosate plus dicamba) was applied.

aInflorescent horseweed were not present at Isabella 2018; therefore, only horseweed biomass data from 2019 is presented.

bWinter wheat and cereal rye were seeded at 67 and 135 kg ha-1 for the low and high seeding rates, respectively.

cMeans followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

	Den	sity	Bion	nass
	Isabella 2019	MSU 2019	Isabella 2019	MSU 2019
Cover treatmenta (Main effect)	plants	s m-2	g n	1-2
No cover	52 abь	39	122.7	144.1
Winter wheat – low (WWL)	27 ab	37	106.8	142.7
Winter wheat – high (WWH)	17 a	35	74	124.0
Cereal rye – low (CRL)	62 b	38	87.7	148.3
Cereal rye – high (CRH)	21 ab	37	39.9	203.1
Termination time (Main effect)				
Early	59	34	150.7	232.8 b
Planting Green	13	41	21.9	71.4 a
Effects (p-values)				
Cover treatment	0.0325	0.7469	0.5326	0.1247
Time	0.3319	0.3875	0.3623	0.0004
Cover treatment x time	0.2070	0.4597	0.7680	0.1650

Table 3.8. Main effects of cover treatment and termination time and P-values for inflorescent horseweed biomass and density at the time of soybean harvest when a non-effective POST herbicide (glyphosate only) was applied.

bMeans followed by the same letter within a column are not statistically different at $\alpha \le 0.05$.

	Soybean stand			Soybean yield₁			
		No POST		· · ·	POST		
	Combined sites	Isabella 2019	MSU 2019	Isabella 2018	Isabella 2019	MSU 2019	
Cover treatmentb (Main effect)	plants m row-1			kg ha-1			
No cover	55 abc	1,170	2,565	1,312	1,455	3,272	
Winter wheat – Low	57 a	1,492	2,932	1,873	1,758	3,366	
Winter wheat – High	54 b	1,394	2,949	1,922	1,947	3,373	
Cereal rye – Low	54 b	1,690	2,693	1,896	1,918	3,411	
Cereal rye – High	53 b	1,526	2,496	2,018	2,139	3,380	
Time (Main effect)							
Early	54	943 b	2,655	1,570 b	1,975	3,479	
Planting Green	55	1,966 a	2,799	2,038 a	1,712	3,242	
Effects (p-values)							
Cover treatment	0.0004	0.4133	0.5002	0.2918	0.1370	0.9689	
Termination time	0.3123	0.0030	0.7866	0.0167	0.2990	0.2607	
Cover treatment x time	0.3936	0.7756	0.4528	0.6895	0.7650	0.7328	

Table 3.9. Main effects of cover treatment and termination time and P-values for soybean stand and yield for plots treated with and without a POST application of dicamba.

aIsabella 2019 and MSU 2019 received POST herbicide applications of No POST (glyphosate only) or POST (glyphosate + dicamba; Isabella 2018 received a POST (glyphosate + dicamba) on all plots.

^bWinter wheat and cereal rye were seeded at 67 and 135 kg ha-1 for the low and high seeding rates, respectively.

cMeans followed by the same letter within a column are not statistically different at $\alpha \le 0.05$.



Figure 3.1. Cumulative horseweed emergence as a percent of the seasonal total by date at the Isabella 2018, Isabella 2019, and MSU 2019 sites.



Figure 3.2 Cumulative horseweed emergence as a percent of the seasonal total by growing degree day (base 10 C) at the Isabella 2018, Isabella 2019, and MSU 2019 sites.



Figure 3.3. Weekly horseweed emergence and precipitation at the Isabella 2018 site.



Figure 3.4. Weekly horseweed emergence and precipitation at the Isabella 2019 site.



Figure 3.5. Weekly horseweed emergence and precipitation at the MSU 2019 site.

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LITERATURE CITED

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CHAPTER IV

ENVIRONMENTAL CUES AFFECTING HORSEWEED GROWTH TYPE AND THEIR SENSITIVITY TO GLYPHOSATE

Abstract

Horseweed (*Erigeron canadensis* L.) is a facultative winter annual weed, that can emerge at any time of the year. Fall emerging horseweed overwinters as a rosette, while spring emerging horseweed skips the rosette stage and immediately bolts upon emergence. In Michigan, primary emergence recently shifted from winter-annual plants emerging as rosettes to a summer-annual lifecycle emerging as bolted type. Additionally, rosette and bolted type plants emerge simultaneously in mid-summer. Growth chamber experiments were conducted to determine whether both horseweed growth types could originate from a single parent and how environmental cues influence growth type. The effects of temperature and photoperiod, competition, shading, and soil moisture only resulted in the rosette growth type in four horseweed populations. However, a vernalization period of four weeks following imbibition of water, but prior to germination, resulted in the bolted growth type. Dose-response experiments were conducted to determine whether glyphosate sensitivity differed between horseweed growth types from the same parent. Bolted type horseweed were seven and three-fold less sensitive to glyphosate than rosette type in the glyphosate-resistant ISB-18 and MSU-18 populations, respectively. Glyphosate sensitivity was not different between growth types of the susceptible population. Future research is needed to understand what influences seed to germinate in the fall versus spring, and how this coincides with the mechanism of resistance to decrease sensitivity to glyphosate. These results suggest that while horseweed populations shift from winter to summer annual lifecycles, concurrent increases in glyphosate resistance could occur.

Introduction

Horseweed (*Erigeron canadensis* L.) is a weed species native to North America where it can complete its lifecycle as a winter or summer annual (Weaver 2001). It has a ruderal nature and thrives across a wide geography, particularly in undisturbed environments such as no-till crop production fields (Weaver 2001). Horseweed is a primarily self-pollinating species that can produce up to 200,000 seeds per plant (Weaver 2001). Each seed is 1 mm long with an attached pappus, facilitating seed dispersal over 500 km via wind into the planetary boundary layer (Bhowmik and Bekech 1993; Shields et al. 2006). The first report of glyphosate-resistant horseweed was confirmed in Delaware in 2001 from seed collected in a field following three consecutive years of using glyphosate exclusively (VanGessel 2001). Despite the ability to disperse by wind, many glyphosate-resistant horseweed populations have evolved independently and share a common resistance mechanism (Dinelli et al. 2006; Okada et al. 2013).

Predicting timely herbicide applications to manage horseweed can be difficult because emergence is not correlated with soil temperature, air temperature, or rainfall (Main et al. 2006). Seeds are non-dormant and readily germinate once shed from the parent plant (Buhler and Owen 1997). Peak horseweed emergence occurs in May and in late August to early September, but emergence has been observed throughout the growing season (Buhler and Owen 1997; Tozzi and Van Acker 2014). Base temperatures for germination vary by population and germination can occur if adequate soil moisture is available (Main et al. 2006; Tozzi et al. 2014). Although horseweed can complete its lifecycle as a winter annual, a vernalization period has not been confirmed as a requirement for flowering. However, Flowering Locus C (FLC), a MADS-box gene responsible for vernalization in winter wheat (*Triticum aestivum*) and *Arabidopsis thaliana*, is present in horseweed (He et al. 2004; Rudnoy et al. 2002). In these other species, flowering is

repressed by high levels of FLC expression until a cold period attenuates expression through vernalization (He et al. 2004). This indicates that a vernalization period such as winter is required for flower production; however, horseweed that germinates in the spring is still able to flower and produce seed (Regehr and Bazzaz 1979).

Fall emerging horseweed overwinters as a rosette while spring emerging horseweed seldom passes through, or only spends a short period of time in, the rosette stage before bolting (Loux et al. 2006; Regehr and Bazzaz 1979). Horseweed's ability to skip the rosette stage and immediately bolt to set seed in the same season was observed in many field populations (Buhler and Owen 1997; Regehr and Bazzaz 1979; Tozzi and Van Acker 2014). Similarly, Koger et al. (2004) reported a Mississippi population bolted 15 days after planting under greenhouse conditions. In 2018 and 2019, Michigan horseweed emergence primarily occurred in the spring and all seedlings skipped the rosette stage to immediately bolt. Additionally, simultaneous emergence of rosette and bolted type horseweed in the same field during mid to late summer has been observed in Michigan.

Reduced translocation of glyphosate to the target site caused glyphosate-resistance in many horseweed populations (Dinelli et al. 2006; Feng et al. 2004, González-Torralva et al. 2012; Koger and Reddy 2005; Moretti and Hanson 2016; Nandula et al. 2005). Furthermore, reduced translocation was caused by rapid sequestration of glyphosate into the vacuole and glyphosate resistance was reversed under low temperatures (Ge et al. 2010; 2011). Springemerging, bolted type horseweed seedlings in a confirmed glyphosate-resistant population exhibited leaf chlorosis and plant death when exposed to glyphosate in the early spring in Michigan (personal observation). The mechanism of glyphosate resistance was not characterized in this horseweed population; however, the level of visual injury observed in this glyphosate-
resistant horseweed population generated questions of how growth type influenced sensitivity to glyphosate.

Information is lacking on the environmental cues which determine growth type in spring emerging horseweed. The high level of spring emergence in Michigan may influence management practices and resistance dynamics. Can rosette and bolted growth type horseweed seedlings occur from a single parent plant? Does growth type influence the level of glyphosate sensitivity? There is evidence that skipping the rosette stage and immediately bolting at emergence results in less time and energy spent prior to flowering (Tozzi and Van Acker 2014). Lysenko (1928) discovered slight imbibition of water prior to germination made winter wheat seed susceptible to vernalization. Do horseweed seeds imbibe water, survive the winter months without germinating, and skip the rosette stage to conserve energy?

This research was conducted to understand more about the dynamics of rosette and bolted type horseweed. The first objective was to determine whether rosette and bolted type horseweed plants were produced from a single parent plant, and which environmental cues were involved? The second objective was to determine if growth type influences sensitivity to glyphosate.

Materials and Methods

Seed Collection. Seed heads were collected in fall 2018 from naturally senescing horseweed plants that emerged in the spring as the bolted type. Individual seed heads were kept separate and assigned a lot number. Each seed head lot was threshed, and stored in labeled manila envelopes in the dark at 4 C. Glyphosate-resistant horseweed seed samples were obtained from two commercial fields in Isabella County near Mount Pleasant, Michigan (ISB-18) (43.6128°N, - 84.8777°W) and (ISB-19) (43.6255°N, -84.9812°W) and two fields at the Michigan State

University (MSU) Agronomy Farm in East Lansing, Michigan (MSU-18) (42.6876°N, -84.4907°W) and (MSU-19) (42.6854°N, -84.4886°W). Seed for a known susceptible horseweed population (S-117) was collected from a commercial field near Saint Johns, Michigan (43.0966°N, -84.5830°W).

Growth Type Experiment. Threshed horseweed seeds from one lot of the ISB-18, ISB-19, MSU-18, and MSU-19 populations were weighed and divided into 5 mg allotments. An allotment of seed was planted on the surface of a 5 x 5 cm pot filled with potting media (Suremix Perlite, Michigan Grower Products, Inc., Galesburg, MI) and watered. Pots were replicated 8 times, placed in potting trays in growth chambers, and subjected to various biotic and abiotic stresses. Daytime light intensity was set to 240 µmol m-2 s-1 photosynthetic photo flux at plant height in a 15 h day, unless otherwise stated. Plants were watered as needed to maximize growth, with exception of the soil moisture experiment. After emergence, pots were thinned to one horseweed plant pot-1, with exception of the competition experiment. Experiments were terminated when horseweed seedlings were large enough to determine the growth type. Horseweed plants typically emerge as the rosette growth type under greenhouse settings (personal observation). Thus, we used the occurrence of bolted type emergence as a reference to determine differences between treatments.

Temperature and Photoperiod. Horseweed growth type was determined in growth chambers based on daily average fluctuating temperatures and photoperiods typical of what is observed in May and July in Michigan. May and July were chosen because horseweed emerging in May are typically the bolted growth type while in July simultaneous emergence of both rosette and bolted growth types occurs (Figure 4.1). May and July daily average fluctuating temperatures and

photoperiod in East Lansing, MI are 16/4 C with 10 h photoperiod and 27/16 C with 15 h photoperiod, respectively (http://www.agweather.geo.msu.edu/mawn/, Michigan State University, East Lansing, MI). Pots of horseweed seeds were placed in growth chambers set to the four combinations of May and July daily fluctuating temperatures and photoperiod (16/4 C, 10 h; 16/4 C, 15 h; 27/16 C, 10 h; 27/16 C, 15 h).

Competition. The effects of intraspecies competition on horseweed growth type was determined using the July daily average fluctuating temperatures and photoperiod. Pots of horseweed seed were placed in growth chambers set to 27/16 C with 15 h photoperiod and subject to either intraspecies competition (five plants pot-1) or no competition (one plant pot-1).

Shading. The effects of shading on horseweed growth type was also determined using the July daily average fluctuating temperatures and photoperiod. Pots of horseweed seed were placed in growth chambers set to 27/16 C with 15 h photoperiod and subjected to shading treatments of 0, 30, 60, and 80%. Woven shade cloth colored forest green (Agriculture Solutions, Strong, ME) was placed over individual pots to create shading treatments.

Soil Moisture. Soil moisture effects on horseweed growth type were determined by placing pots of horseweed seed in growth chambers set to 27/16 C with 15 h photoperiod. Soil moisture treatments consisted of 50, 75, and 100% field capacity. Field capacity of potting media was determined by saturating a pot with a known weight of oven-dried potting media for 24 h. After 24 h, potting media was considered to be at 100% field capacity and pots were weighed to determine the amount of water being held. The amount of water needed in each pot for 50 and 75% field capacity treatments was determined by taking 50 and 75% of the water weight needed in 100% field capacity. Pots were weighed daily and the appropriate amount of water was added to maintain soil moisture throughout the experiment.

Vernalization. The effects of vernalization prior to germination on horseweed growth type was determined for each population. Horseweed seeds were surface-planted in pots filled with potting media and watered as described above. Prior to germination, pots were placed in the Michigan State University Wheat Breeding and Genetics Program's vernalization room set to 4 C with 8 h photoperiod. Horseweed seeds were subject to vernalization period treatments of 2, 4, or 6 wk based off the vernalization requirement of *Arabidopsis thaliana* (Nordborg and Bergelson 1999). Following a vernalization period, pots were moved into growth chambers set to 27/16 C daily average temperatures with 15 h photoperiod.

Dose-Response Experiment. Dose-response experiments were conducted to determine if glyphosate sensitivity was affected by growth types from the same parent plant. The populations used were two known glyphosate-resistant, ISB-18 and MSU-18, and a glyphosate-susceptible, S-117. To obtain the bolted growth type, 0.5 g of seed from each population was surface-planted in 28 x 55 cm trays filled with potting media (Suremix Perlite, Michigan Grower Products, Inc., Galesburg, MI) and watered. Bolted type trays were placed in the Michigan State University Wheat Breeding and Genetics Program's vernalization room set to 4 C with 8 h photoperiod for 4 wk. At the end of the 4 wk period, rosette type trays were established by planting seeds in trays as described above with no vernalization period. Rosette and bolted growth type trays were simultaneously placed in the greenhouse at 25 ± 5 C and sunlight was supplemented to provide a total midday light intensity of 1,000 µmol m-2 s-1 photosynthetic photon flux at plant height in a 16 h day. After emergence, seedlings were transplanted to 10 x 10 cm pots filled with potting media, one horseweed plant pot-1. Plants were watered and fertilized as needed to promote optimum plant growth. Herbicide applications of glyphosate (Roundup PowerMAX, Bayer Crop

Science, St. Louis, MO) were applied to horseweed plants approximately 5 wk after emergence with a single nozzle (8001E, TeeJet Technologies, Wheaton, IL) track sprayer calibrated to deliver 187 L ha-1 at 193 kPa of pressure. Rosette plants were approximately 12 cm wide and bolted plants were approximately 18 cm tall at the time of application. The glyphosate rate 1.27 kg ae ha-1 represented a 1X field use rate. Application rates ranged from 1/32 to 8X for the S-117 population and 1/8 to 32X for the ISB-18 and MSU-18 populations. All treatments contained spray grade ammonium sulfate (AMS) (Actamaster, Loveland Products, Inc., Loveland, CO) at 2% w w-1 and nonionic surfactant (NIS) (Activator 90, Loveland Products, Inc., Loveland, CO) at 0.5% v v-1. Non-treated controls for each horseweed population (S-117, ISB-18, and MSU-18) were also included in the experiment. Pots were arranged in the greenhouse in a randomized complete block design. Treatments consisted of growth type and glyphosate rate combinations. Each treatment was replicated five times and the experiment was repeated in time. Horseweed control was evaluated 7 and 14 DAT on a scale of 0 to 100. Aboveground biomass was harvested 14 DAT and dried at 60 C for 7 d and weighed.

Statistical analysis. The growth type experiments were terminated when horseweed seedlings were large enough to determine the growth type. Rosette and bolted type plants were typically distinguishable 4 wk after emergence. Rosette plants formed a basal rosette, that was dark green in color (Figure 4.2). Bolted plants skipped the rosette stage, bolted upright, and were light green in color (Figure 4.3). Plants were counted and the total number of plants was calculated as (*# bolted plants* + *# rosette plants*). The proportion of bolted plants (*% bolted*) was calculated using Equation 1. Since simultaneous emergence of rosette and bolted type plants was observed in the field, we used 50% bolted type plants as our baseline to distinguish treatment differences.

Growth type experiments were repeated in time if the proportion of bolted plants was greater than 50%.

$$\% bolted = \frac{\# bolted \ plants}{\# \ total \ plants} (100\%)$$
 [Eq. 1]

Dose-response data were analyzed using the drc package in R (R version 3.6.2, R Development Core Team 2019). In order to keep results objective, horseweed control ratings were not used in data analysis. Dry weights from each experiment were converted to a percent of the non-treated control for each population (S-117, ISB-18, MSU-18). The appropriate model for each population was determined using the mselect function. Four-parameter log logistic models (for S-117 and ISB-18; Equation 2) and a three-parameter log logistic model (MSU-18; Equation 3) were fitted to the data as selected by the drc modelFit function using the lack-of-fit test. The effective dose to reduce biomass 50% compared with the non-treated control (ED₅₀) was determined using the ED function for each population and growth type.

$$y = 0 + \frac{d-0}{1 + exp[(b(log(x) - log(e))]}$$
 [Eq. 2]

$$y = c + \frac{d-c}{1 + exp[(b(log(x) - log(e))]}$$
 [Eq. 3]

For both equations, *y* is the biomass response (percent of non-treated control), *x* is the dose, *c* and *d* are the lower and upper limits, respectively, *b* is the relative slope around *e*, and *e* is the ED₅₀ (Streibig, 1988). Relative population differences in ED₅₀ values (based on a t-statistic with $P \le 0.05$) were compared using the EDcomp function and selectivity indices (R/S ratio; Knezevic et al. 2007), which are the ratios between two ED₅₀ values from dose-response curves. In addition, relative differences between bolted and rosette ED₅₀ values were compared (Bt/Ro ratio) for each population using the same method.

Results and Discussion

Growth Type Experiment. Regardless of daily average fluctuating temperature or photoperiod, 100% of emerged horseweed plants formed the rosette type (Table 4.1). Horseweed that emerge in May in Michigan typically skip the rosette stage and immediately bolt. For this reason, we hypothesized that May daily average fluctuating temperatures (27/16 C) and photoperiod (15 h) would result in a high proportion of bolted type horseweed. Simultaneous emergence of rosette and bolted type horseweed generally occurs in July (Figure 4.1); thus, we hypothesized mimicking July temperatures (27/16 C) and photoperiod (15 h) would result in a split population between growth types. However, regardless of temperature and photoperiod combination, no plants bolted. Thus, horseweed seed shed from a bolted plant can emerge as a rosette type and simultaneous emergence of both growth types observed in the field can occur from one horseweed biotype.

Regardless of the stress tested, 100% of emerged horseweed formed the rosette type (Table 4.1). Extensive research has been conducted on horseweed germination factors such as temperature, intraspecies competition, light, and soil moisture (Buhler and Hoffman 1999; Buhler and Owen 1997; Main et al. 2006; Nandula et al. 2006; Palmblad 1968; Steinmaus et al. 2000; Tozzi et al. 2014). However, the influence of these factors on growth type was undetermined. Additionally, numerous studies on herbicide-resistant horseweed populations in the rosette stage have been conducted under controlled conditions in a greenhouse or growth chamber (Davis et al. 2009; Dinelli et al. 2006; Feng et al. 2004; Koger and Reddy 2005; Koger et al. 2004; Main et al. 2004; Page et al. 2018; Shrestha et al. 2010; Tani et al. 2015; VanGessel 2001; Zelaya et al. 2004). In regard to what are standard controlled growing environments, our findings of only rosette type plants is consistent with previous research.

Exposing horseweed seeds to a vernalization period of 2, 4, or 6 weeks resulted in at least 62% bolted type seedlings in all populations (Table 4.1). The susceptible population (S-117) was included and this experiment was repeated two additional times with vernalization for 4 weeks. Each additional run resulted in 100% bolted type horseweed seedlings for all populations (data not shown). Horseweed seed is non-dormant and readily germinates once released from the mother plant (Buhler and Owen 1997; Loux et al. 2006). A vernalization requirement has not been confirmed for horseweed. However, Flowering Locus C (FLC), a MADS-box gene responsible for vernalization in winter wheat and Arabidopsis thaliana, is present in horseweed (He et al. 2004; Rudnoy et al. 2002). In these other species, flowering is repressed by high levels of FLC expression until a cold period attenuates expression through vernalization (He et al. 2004). Lysenko (1928) discovered slight imbibition of water prior to germination made wheat seed susceptible to vernalization. In our study, horseweed seeds were sown and watered prior to a vernalization period, so seeds would have imbibed water prior to vernalization. Skipping the rosette stage and immediately bolting at emergence results in less time and energy spent prior to flowering (Tozzi and Van Acker 2014). Future exploration of FLC expression in horseweed is needed to understand whether this species has a vernalization requirement, and how it can be met. In addition, more research is needed on the factors that influence horseweed to germinate in the fall immediately after being shed versus the following spring.

Dose-Response Experiment. The dose-response analysis confirmed that the S-117 horseweed population was more sensitive to glyphosate compared with the ISB-18 and MSU-18 populations. The ED₅₀ values for S-117 rosette and bolted growth types were 0.02 and 0.06 kg ae ha-1, respectively (Figure 4.4). The ED₅₀ values for ISB-18 rosette and bolted types were 0.34

and 2.34 kg ae ha-1, respectively (Figure 4.5). The respective R/S ratios were 37 and 18X for ISB-18 bolted and rosette plants (Table 4.2). The ED₅₀ values for MSU-18 rosette and bolted types were 7.73 and 23.29 kg ae ha-1, respectively (Figure 4.6). The respective R/S ratios were 370 and 407X for MSU-18 bolted and rosette plants (Table 4.2). The ED₅₀ and R/S ratio in ISB-18 and MSU-18 are higher than those previously reported, 2 to 13-fold resistant, in populations with non-target site resistance (Krueger et al. 2008; Main et al. 2004; VanGessel 2001). However, R/S ratios of 20X have been reported in horseweed populations in Ontario, Ohio, and Indiana (Beres et al. 2015; Page et al. 2018). Page et al. (2018) characterized a Pro-106-Ser substitution in EPSPS2 of an Ontario horseweed population which significantly increased levels of glyphosate-resistance. In our research, a significant (>50%) reduction in aboveground biomass relative to the untreated control was obtained for all populations. In ISB-18, visible injury ratings indicate that complete control was obtained in the doses used. In contrast, MSU-18 was not controlled at our highest dose, 32X the label rate. Both ISB-18 and MSU-18 displayed higher resistance ratios than an accession from Michigan with known non-target site resistance examined by Page et al. (2018). Nonetheless, the mechanisms of resistance present in ISB-18 and MSU-18 have not been characterized and it is unclear how high a dose would be required to completely control MSU-18.

Sensitivity to glyphosate was different among bolted and rosette growth types in the ISB-18 and MSU-18 populations, but not in S-117. The Bt/Ro ratio was 7 and 3X for ISB-18 and MSU-18, respectively (Table 4.2). Koger et al. (2004) found that growth stage of rosette type horseweed plants did not affect glyphosate-resistance levels of three populations. However, Shrestha et al. (2007) reported tolerance to glyphosate increased when horseweed plants began to bolt. In our study, rosette and bolted growth types were placed in the greenhouse, emerged, and

treated at the same time. Bolted growth types grew faster and accumulated more biomass by the time of treatment compared with rosette plants of the same parent (personal observation). Thus, our results could be due to differences in total leaf surface, leaf morphology, or spray coverage. However, no differences in glyphosate sensitivity were observed between S-117 bolted and rosette type plants. This indicates that the mechanism of resistance present in ISB-18 and MSU-18 in combination with growth type influences glyphosate sensitivity.

Variations in temperature and photoperiod, competition, shading, and soil moisture only resulted in the rosette growth type of horseweed. However, a vernalization period prior to germination resulted in the bolted growth type for all populations. In previous research, a Mississippi horseweed population bolted 21 DAP (Koger et al. 2004). However, our study is the first report of intentionally triggering horseweed to skip the rosette growth stage and immediately bolt in a controlled setting. Additionally, we confirmed that both rosette and bolted growth types form from seed from the same parent plant. We determined that bolted plants in two glyphosate-resistant populations were less sensitive to glyphosate than rosette plants from the same parent. Future research to characterize the mechanisms of resistance in these populations could provide insight into the differences among growth types. The most common mechanism of resistance identified in previous research has been non-target site resistance mechanisms such as vacuolar sequestration and impaired translocation (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). If non-target site resistance is present in these populations, how does growth type influence this mechanism? Does horseweed growth type affect resistance to other herbicide sites of action? Our results show that the recent shift from winter-annual plants emerging as rosettes

to a primarily summer-annual lifecycle emerging as bolted type plants could result in new management challenges.

APPENDIX

APPENDIX

Tables and Figures

		Proportion of emerged bolted plantsa			
Condition		ISB-18	ISB-19	MSU-18	MSU-19
<i>Temperature (C)</i>	Photoperiod (h)		%		
16/4b	10	0	0	0	0
16/4	15	0	0	0	0
27/16	10	0	0	0	0
27/16	15	0	0	0	0
<i>Competition</i> c					
No competition		0	0	0	0
Intraspecies competition		0	0	0	0
Shading (%)					
0		0	0	0	0
30		0	0	0	0
60		0	0	0	0
80					
Soil moisture (% field capacity)					
50		0	0	0	0
75		0	0	0	0
100		0	0	0	0
Vernalizationa time (weeks)					
2		62	100	84	100
4e		100	100	100	100
6		100	100	100	100

Table 4.1. Effects of growth conditions on the determination of horseweed growth type (rosette or bolted) from seed collected from individual parent plants.

^aProportion of emerged bolted plants was calculated using Equation 1.

bAverage of daily/nightly fluctuating temperatures.

cDaily/nightly fluctuating temperatures and photoperiod for competition, shading, soil moisture, and vernalization experiments were 27/16 C and 15 h, respectively.

dVernalization time consisted of exposure to 4 C with 8 h photoperiod following imbibition of water.

eVernalization for 4 wks was repeated in time with the addition of the S-117 population. All horseweed plants from all populations formed the bolted growth type.

		ED50a	\pm S.E.	R/S_b	Bt/Roc
Туре	Population				
		kg ae	ha-1		
Bolted (Bt)	ISB-18	2.34	0.43	37X	7X
	MSU-18	23.29	3.50	370X	3X
	S-117	0.06	0.02		\mathbf{NS}_{d}
Rosette (Ro)	ISB-18	0.34	0.01	18X	
	MSU-18	7.73	1.04	407X	
	S-117	0.02	0.01		

Table 4.2. ED₅₀ values, standard errors (\pm S.E.), and ED₅₀ ratios of populations (R/S) and growth types (Bt/Ro) following glyphosate application.

^aED₅₀ is the required dose to reduce horseweed biomass 50%.

 $_{\rm b}$ R/S is the ED₅₀ ratio of a resistant population and the susceptible of the same growth type.

cBt/Ro is the ED50 ratio of bolted and rosette type plants within a population.

dED₅₀ values between susceptible bolted and rosette type plants were not significantly different at $\alpha \le 0.05$.



Figure 4.1. Rosette (left) and bolted (right) type horseweed plants emerging simultaneously in a field in mid-summer.



Figure 4.2. Rosette type horseweed seedling identified in the growth type experiment as forming a rosette, dark green in color.



Figure 4.3. Bolted type horseweed seedling identified in the growth type experiment as bolting upright, light green in color.



Figure 4.4. Biomass of bolted and rosette horseweed plants of a susceptible population (S-117) in response to applications of glyphosate. Lines were fitted using Equation 2.



Figure 4.5. Biomass of bolted and rosette horseweed plants of the ISB-18 population in response to applications of glyphosate. Lines were fitted using Equation 2.



Figure 4.6. Biomass of bolted and rosette horseweed plants of the MSU-18 population in response to applications of glyphosate. Lines were fitted using Equation 3.

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CHAPTER V

EFFECTS OF GROWTH STAGE AND HERBICIDE TANK MIXTURES ON CEREAL RYE TERMINATION

Abstract

Termination of cover crops, such as cereal rye (Secale cereale L.), and weed control are often achieved simultaneously through applications of herbicides. Factors such as growth stage and additional herbicides present in tank-mixtures may impact cereal rye termination and cause undesired competition and subsequent crop yield loss. Thus, separate field experiments were conducted in 2018 and 2019 in Michigan to investigate the effects of timing and herbicide combinations on cereal rye termination. In the termination timing experiment, treatments which included glyphosate at 1,267 g ae ha-1, with the exception of glyphosate plus dicamba applied late, applied at early (Feekes 6) and late (Feekes 10.5) growth stages provided 100% cereal rye control 14 DAT. Clethodim alone provided less than 67% control 28 DAT. Additionally, cereal rye control with clethodim was greater when applied at the early compared with late termination time 14 DAT, but control was similar between times 28 DAT. In the herbicide combination experiment, the addition of saflufenacil, 2,4-D, or dicamba to glyphosate provided similar cereal rye control as glyphosate alone. The inclusion of the residual herbicides flumioxazin or sulfentrazone to any of these treatments also provided similar control to glyphosate alone. However, tank-mixtures which included metribuzin provided less than 55% cereal rye control 7 DAT and metribuzin in combination with glyphosate plus dicamba provided the lowest control of all combinations tested. Glyphosate applied at 1,267 g ae ha-1 or greater is essential for terminating cereal rye. Growers should be conscious of potential antagonistic effects when tankmixing metribuzin or dicamba with glyphosate for additional weed control.

Introduction

Across the United States, implementation of cover crops continues to increase annually as growers intend to improve agricultural sustainability while capitalizing on federal conservation payments (CTIC 2017). Cover crops provide many ecosystem services that benefit crop performance, nutrient cycling, and general cropping system function (Snapp et al. 2005). Cover crops are also used as an integrated weed management tool. Cover crops compete with weeds for resources while living and the remaining residue following termination provides additional physical suppression (Mirsky et al. 2013; Teasdale et al. 2007). Winter annual cover crops compete with emerged weeds in the fall as well as early emerging weeds the following spring (Teasdale 1996). Cereal rye (*Secale cereale* L.) is one of the most commonly grown winter annual cover crops due to its winter hardiness and ability to produce biomass (CTIC 2017). Despite numerous known advantages, growers are reluctant to utilize winter cereals as cover crops because of additional costs and management requirements.

A primary concern of growers is the ability to effectively terminate winter cereal cover crops before soybean planting (Cornelius and Bradley 2017). If not effectively terminated, cover crops can become weeds and compete with the subsequent cash crop (Nascente et al. 2013; White and Worsham 1990). Thelen et al. (2004) reported competition for soil moisture from interseeded cereal rye reduced soybean yield 17 to 22%. Cover crop termination in no-till production systems is most commonly achieved by herbicides. Previous research found glyphosate-based (WSSA group 9) termination programs provided the best control of cereal rye (Cornelius and Bradley 2017; Palhano et al. 2018; Whalen et al. 2020). Glyphosate is commonly used as a burndown before soybean planting due to its broad-spectrum weed control. However, the widespread occurrence of glyphosate-resistant weeds and availability of new herbicide-

resistant soybean technologies may shift reliance away from glyphosate alone at pre-plant. Glufosinate (WSSA group 10) applied alone provided limited activity on cereal rye (Palhano et al. 2018; Whalen et al. 2020). The addition of clethodim (WSSA group 1) to glyphosate provided similar or improved control of winter cereals compared with glyphosate alone (Cornelius and Bradley 2017; Whalen et al. 2020). However, the effectiveness of clethodim applied alone was not determined in these studies. Additionally, cereal rye growth stage may vary due to environmental conditions and planting date. More information on effective cereal rye termination programs at different growth stages is needed.

In addition to using cover crops as an integrated weed management approach, producers are encouraged to add soil-applied residual herbicides with effective burndown treatments prior to or at planting. Integrating multiple herbicide sites of action reduces selection for herbicideresistance and improves control of agronomically important herbicide-resistant weeds such as waterhemp (Amaranthus tuberculatus (Moq.) Sauer), Palmer amaranth (Amaranthus palmeri S. Wats.), and horseweed (Erigeron canadensis L.) (Beckie 2006). The utility of these herbicide combinations to terminate a cereal rye cover crop needs to be considered. Common burndown herbicides such as 2,4-D (WSSA group 4), dicamba (WSSA group 4), or saflufenacil (WSSA group 14) provide control of glyphosate-resistant horseweed (Budd et al. 2016; Byker et al. 2013; Eubank et al. 2008; Keeling et al. 1989; McCauley et al. 2018). These burndown herbicides tank-mixed with glyphosate provide similar cereal rye control compared with glyphosate alone (Palhano et al. 2018; Whalen et al. 2020). However, the addition of soil-applied residual herbicides could antagonize cereal rye control. Antagonism was observed when sulfentrazone (WSSA group 14) was added to glyphosate for barnyardgrass (Echinochloa crusgalli L.) control (Starke and Oliver 1998). Similarly, Selleck and Baird (1981) reported an

antagonistic effect on quackgrass (*Agropyron repens* L.) when metribuzin (WSSA group 5) was added to glyphosate. However, adding metribuzin to glyphosate plus 2,4-D provided excellent control of cereal rye (Whalen et al. 2020). Common herbicide tank-mixtures before soybean planting need to be evaluated for their utility for cereal rye termination.

With the increased adoption of cereal rye as a cover crop across the United States, it is critical to understand the most effective strategies for termination. Additionally, the effectiveness of herbicides for termination needs to be examined at different growth stages and in the various tank-mixtures recommended to control herbicide-resistant weeds. Therefore, the objectives of this research were to determine: 1) the effect of cereal rye growth stage on herbicides used for termination, and 2) the effect of common herbicide tank-mixtures applied prior to soybean planting on cereal rye termination.

Materials and Methods

Field experiments were conducted in 2018 and 2019 at the Michigan State University (MSU) Agronomy Farm in East Lansing, Michigan (42.6876°N, -84.4907°W). The soil types were a Conover loam (coarse-loamy, mixed, semiactive, mesic Aquic Hapludalfs) with pH 6.9 and 2.7% organic matter in 2018, and pH 5.7 and 3.0% organic matter in 2019. 'Wheeler' cereal rye was sown at 67 kg ha-1 in 19 cm rows using a no-till drill (Great Plains, Salina, KS) the fall prior to data collection. Sowing dates were November 28, 2017 and November 8, 2018.

Separate field studies were established for the termination timing and herbicide combination experiments. Each plot measured 3 m wide by 9 m long. Plots were established in the spring when producers would typically terminate a cereal rye cover crop. 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 TTI nozzles (TeeJet Technologies, Spraying Systems Co., Wheaton, IL).

Termination timing experiment. Treatments were arranged in a two-factor factorial randomized complete block design with four replications. The factors were herbicide treatment and termination timing. The treatment combinations were glyphosate alone at two different rates, glyphosate in combination with dicamba or clethodim, and clethodim alone applied to cereal rye at Feekes stages 6 (early) or 10.5 (late). Herbicide information and rates are presented in Table 5.1.

Herbicide combination experiment. Treatments were arranged in a two-factor factorial randomized complete block design with four replications. The factors were burndown herbicide and soil-applied residual herbicide. The burndown herbicides were glyphosate alone and in combination with saflufenacil, 2,4-D ester, or dicamba. Burndown herbicides were tank-mixed with soil-applied residual herbicide treatments of none, flumioxazin, sulfentrazone, or metribuzin to create treatment combinations. Herbicide applications were made when cereal rye was at Feekes stage 6 and 9 in 2018 and 2019, respectively. Herbicide information and rates are provided in Table 5.1.

Data collection. Cereal rye termination was evaluated 14 and 28 d after treatment (DAT) and 7 and 14 DAT for the termination timing and herbicide combination experiments, respectively. Evaluations were based on a scale of 0 to 100% with 0 representing no control and 100 indicating complete cereal rye termination.

Statistical analysis. All data analysis was conducted using lmer in R v. 3.6.2 (R Development Core Team 2019). The statistical models used consisted of year, factor 1 (termination time or burndown herbicide), and factor 2 (herbicide or residual herbicide) as fixed effects and replication nested in year, the interactions between factor 1 and replication nested within year, and factor 2 and replication nested within year as random effects. Replications were used as an error term for testing the effect of site-year, and data were combined over site-year when the interaction of site-year and main effects was not significant. 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 the Levene's test for unequal variances. For all experiments, treatment means were separated using Fisher's Protected LSD at $\alpha \leq 0.05$.

Results and Discussion

Termination timing experiment. Application of early termination treatments was delayed in 2019 due to heavy spring rainfall. As a result, cereal rye at the early termination timing was 40 cm tall and at Feekes stage 6 and 65 cm tall and at Feekes stage 9 in 2018 and 2019, respectively. Cereal rye was 130 cm tall and at Feekes stage 10.5 when late termination treatments were applied in both years; however, the interval between early and late applications was 14 d longer in 2018 compared with 2019. Termination treatments containing glyphosate, with the exception of glyphosate plus dicamba applied late, provided 100% cereal rye control 14 DAT, regardless of cereal rye growth stage at application (Table 5.2). Previous research has shown glyphosate alone provides excellent cereal rye control (Cornelius and Bradley 2017; Palhano et al. 2018; Whalen et al. 2020). The addition of dicamba to glyphosate applied late resulted in 88% cereal rye

control at this time, which may indicate potential antagonistic effects from dicamba when tankmixed with glyphosate and applied to mature cereal rye. Clethodim was less effective at terminating cereal rye. At 14 DAT, clethodim provided 38 and 19% cereal rye control when applied at the early and late growth stages, respectively. Since clethodim needs to be translocated to the growing point of the plant, our results indicate that translocation and subsequent control may be less for more mature cereal rye.

Control of cereal rye with glyphosate plus dicamba applied late only progressed to 89% by 28 DAT (Table 5.2). Complete termination of cereal rye was not achieved until 42 DAT with this treatment (data not shown). With this application becoming more common due to the commercial introduction of dicamba-resistant soybean technology, further research on grass weed and cover crop species control is needed. Control of cereal rye 28 DAT was similar for clethodim applied early or late, with 56 and 67% control, respectively (Table 5.2). However, cereal rye control at 28 DAT was significantly less with clethodim compared with glyphosate. Young et al. (2016) observed similar results when clethodim and glyphosate were applied alone to cereal rye at tillering. In our study, 80% control was not achieved from clethodim until 42 and 35 DAT in 2018 and 2019, respectively (data not shown).

Herbicide combination experiment. Herbicide combination treatments were delayed in 2019 due to excessive rain at the time of termination. At the time of application, cereal rye was 40 cm tall and at Feekes stage 6 and 65 cm tall and at Feekes stage 9 in 2018 and 2019, respectively. However, cereal rye control was similar and thus combined over years. At 7 DAT, glyphosate alone provided 76% cereal rye control (Table 5.3). The addition of saflufenacil, 2,4-D, or dicamba to glyphosate without a residual herbicide provided similar or better cereal rye control

at this time compared with glyphosate alone. Previous research has found glyphosate alone or in combination with clethodim, 2,4-D, dicamba, or saflufenacil effectively controls cereal rye (Cornelius and Bradley 2017; Palhano et al. 2018; Whalen et al. 2020). A potential issue when adding burndown and/or soil-applied residual herbicides to glyphosate could be antagonism on grass control, specifically cereal rye termination. For example, the addition of sulfentrazone to glyphosate resulted in antagonistic effects on quackgrass (Selleck and Baird 1981). In our study, herbicide combinations which included metribuzin provided less than 55% control, with metribuzin in combination with glyphosate plus dicamba only providing 31% control 7 DAT. Additionally, cereal rye control with treatments containing flumioxazin, sulfentrazone, or no residual herbicide provided similar or greater cereal rye control than glyphosate alone at this time. This indicates that only tank-mixes which included metribuzin induced antagonism 7 DAT.

At 14 DAT, we did not detect any differences between treatments. However, all treatments provided greater than 98% cereal rye control, with the exception of metribuzin in combination with glyphosate plus dicamba (Table 5.3). Previous research has found dicamba (Flint and Barrett 1989) and metribuzin (Starke and Oliver 1988) antagonize glyphosate control of weedy grass species. We suspect metribuzin and dicamba antagonistic effects are additive when tank-mixed, resulting in less cereal rye control 14 DAT. The recent commercial introduction of dicamba-resistant soybean and subsequent increase in applications which include dicamba and glyphosate has created a focus on grass control. Metribuzin is commonly applied at soybean planting because it provides cost-effective horseweed (Eubank 2008) and waterhemp control (Hausman et al. 2017). Growers should be cautious when selecting herbicide combinations for weed control while simultaneously terminating cereal rye.

In conclusion, termination treatments which included glyphosate, with the exception of glyphosate plus dicamba applied late, effectively controlled cereal rye at early (Feekes 6) and late (Feekes 10.5) growth stages. Applications of clethodim alone provided less control and may take up to 42 DAT to completely terminate cereal rye. Additionally, at 14 DAT clethodim was less effective at controlling cereal rye at the later growth stage compared with the earlier growth stage. For this reason, growers should include glyphosate when terminating a cereal rye cover crop. Adding 2,4-D, saflufenacil, or dicamba burndown herbicides to glyphosate provided similar cereal rye control to glyphosate alone. The addition of flumioxazin or sulfentrazone as a residual herbicide also provided similar control to glyphosate alone. In contrast, tank-mixtures which included metribuzin provided less than 55% cereal rye control 7 DAT and may delay complete termination. Additionally, the combination of metribuzin with glyphosate plus dicamba provided the lowest cereal rye control 14 DAT. In our study, we did not tank mix clethodim with dicamba; however, growers looking to avoid glyphosate may consider this application for grass and broadleaf control. This combination has been reported to be antagonistic for volunteer corn control (Underwood et al. 2016). For this reason, growers aiming to terminate a cereal rye cover crop while using dicamba or metribuzin for weed control should consider making split applications. We recommend applying glyphosate alone to terminate cereal rye first and allowing a 7 day period before making metribuzin or dicamba applications.

APPENDIX

APPENDIX Tables

Table 5.1. Herbicide information for all products used in the termination timing and herbicide combination experiments.					
Herbicide	Rates	Trade name	Manufacturer	Location	
	g ai or ae ha-1				
2,4-D ester	560	2,4-D LV4	Loveland Products, Inc.	Greeley, CO	
Dicamba	560	XtendiMax	Bayer CropScience	St. Louis, MO	
Clethodim	102	Select Max	Valent Co.	Walnut Creek, CA	
Flumioxazin	90	Valor	Valent Co.	Walnut Creek, CA	
Glyphosate	1,267, 1,681	Roundup PowerMAX	Bayer CropScience	St. Louis, MO	
Metribuzin	420	Metribuzin 75	Winfield Solutions	St. Paul, MN	
Saflufenacil	25	Sharpen	BASF Corporation	Research Triangle Park, NC	
Sulfentrazone	280	Spartan	FMC Corporation	Philadelphia, PA	

			Cereal rye control	
Time	Herbicidea	Rate	14 DAT	28 DAT
		g ai or ae ha-1	%	
Early (Feekes 6)b	Glyphosate	1,267	100 af	100 a
	Glyphosate high	1,681	100 a	100 a
	Glyphosate + dicambac	1,267 + 560	100 a	100 a
	Glyphosate + clethodimd	1,267 + 102	100 a	100 a
	Clethodime	102	38 c	55 c
Late (Feekes 10.5)	Glyphosate	1,267	100 a	100 a
	Glyphosate high	1,681	100 a	100 a
	Glyphosate + dicamba	1,267 + 560	88 b	89 b
	Glyphosate + clethodim	1,267 + 102	100 a	100 a
	Clethodim	102	18 d	66 c
Effects (p-values)				
Time			1.0000	1.0000
Herbicide			< 0.0001	0.0002
Time x herbicide			0.0356	0.0436

Table 5.2. The effects of termination time and herbicide and their interaction on cereal rye control 14 and 28 days after treatment.

aAll herbicide treatments except for glyphosate + dicamba were applied with 2% w w-1 of ammonium sulfate.

bEarly termination was delayed in 2019 due to heavy rainfall, resulting in early application at Feekes 9.

cA drift-reduction agent was added to all treatments containing dicamba at 0.5% w w-1. dNonionic surfactant was added to glyphosate + clethodim at 0.25% w w-1.

eCrop oil concentrate was added to clethodim at 1% w w-1.

fMeans followed by the same letter within a column are not statistically different at $\alpha \le 0.05$.
		Cereal rye	Cereal rye controla	
Residual	Burndownb	7 DAT	14 DAT	
		%	%	
None	Glyphosate	76 fc	100	
	Glyphosate + saflufenacil	81 cd	100	
	Glyphosate + 2,4-D	76 f	100	
	Glyphosate + dicamba	75 f	100	
Flumioxazin	Glyphosate	81 cd	100	
	Glyphosate + saflufenacil	85 a	100	
	Glyphosate + 2,4-D	82 bc	100	
	Glyphosate + dicamba	79 cde	100	
Sulfentrazone	Glyphosate	76 ef	100	
	Glyphosate + saflufenacil	84 ab	100	
	Glyphosate + 2,4-D	81 c	100	
	Glyphosate + dicamba	77 def	100	
Metribuzin	Glyphosate	37 i	99	
	Glyphosate + saflufenacil	54 g	98	
	Glyphosate + 2,4-D	44 h	98	
	Glyphosate + dicamba	31 j	90	
Effects (p-values)				
Residual		< 0.0001	1.000	
Burndown		< 0.0001	1.000	
Residual x burndown		< 0.0001	1.000	

Table 5.3. The effects of soil applied residual and burndown herbicide and their interaction on cereal rye control 7 and 14 days after treatment.

^aCereal rye was at Feekes stages 6 and 9 at the time of application in 2018 and 2019, respectively.

bAll herbicide treatments except for those containing dicamba were applied with 2% w w-1 of ammonium sulfate. Methylated seed oil was added to all treatments containing saflufenacil at 1% w w-1. A drift reduction agent was added to all treatments containing dicamba at 0.5% w w-1. cMeans followed by the same letter within a column are not statistically different at $\alpha \leq 0.05$.

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