# INTEGRATED WEED MANAGEMENT IN KANSAS WINTER WHEAT

by

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B.S., Iowa State University, 2001
M.S., Iowa State University, 2003

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
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# Abstract

Integrated weed management (IWM) is an ecological approach to weed control that reduces dependence on herbicides through understanding of weed biology and involves using multiple weed control measures including cultural, chemical, mechanical and biological methods.  The critical period of weed control is the duration of the crop life cycle in which it must be kept weed-free to prevent yield loss from weed interference.  Eight experiments were conducted throughout Kansas between October 2010 and June 2012 to identify this period in winter wheat grown under dryland and irrigated conditions.  Impact of henbit and downy brome density on winter wheat yields were evaluated on four farmer's fields with natural populations and on a research station with overseeded populations.  Henbit density up to 156 plants $m^{-2}$ did not affect winter wheat yield, while downy brome at a density of 40 plants $m^{-2}$ reduced yield by 33 and 13% in 2011 and 2012, respectively.  In the presence of downy brome, winter wheat should be kept weed-free approximately 30 to 45 days after planting to prevent yield loss; otherwise, weeds need to be removed immediately following release from winter dormancy to prevent yield loss due to existing weed populations.

Flumioxazin and pyroxasulfone are herbicides registered for use in winter wheat, soybean and corn for control of broadleaf and grass weeds.  Flumioxazin and pyroxasulfone were evaluated for plant response to localized herbicide exposure to roots, shoots, or both roots and shoots utilizing a novel technique.  Two weed species, ivyleaf morningglory and shattercane, as well as two crops, wheat and soybean, were evaluated for injury after localized exposures.  The location and expression of symptoms from the flumioxazin and pyroxasulfone herbicides were determined to be the shoot of seedling plants.  The utilization of preemergence herbicides in winter wheat is not a common practice, although application may protect winter wheat from early season yield losses as determined by the critical weed-free period.  Kansas wheat growers should evaluate the presence and density of weed species to determine which weed management strategy is most advantageous to preserving winter wheat yield.

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# Chapter 1- Literature Review

Winter wheat in the Central Great Plains has historically been classified as a low input and low risk crop. Over 5.6 million hectares of wheat were planted in the late 1970's to early 1980's with yields averaging around 1 metric ton per hectare (National Agricultural Statistics Service 2012). The number of hectares planted has steadily decreased across Kansas, however, recent advancements in genetics and higher commodity process are slowly changing that trend as more farmers are increasing economic inputs into managing wheat such as purchasing new seed, applying seed treatments, fall fertilizer applications, and applications of additional pesticides thus increasing productivity. In 2012, 3.8 million hectares of wheat were planted, with total production of 25.7 billion kg $ha^{-1}$ averaging to 2825 kg $ha^{-1}$ across Kansas (National Agricultural Statistics Service 2012).

Winter wheat yields are highly variable. Kansas researchers summarized over 50 years of wheat yields and determined year-to-year variability accounted for 23% of the variation in irrigated yields, and 47% of dryland yield (Holman et al. 2011). Year-to-year variability in Kansas can be attributed to erratic rainfall, warm fall temperatures, and spring freezes such that predicting yield is nearly impossible until prior to harvest. Management such as weed control and fertilizer application has also resulted in variable winter wheat yields (Jasieniuk et al. 1999; Slaton et al. 2005). Storkey and Cussans (2007) utilized a model to predict yield loss in wheat, but found it to be inaccurate due to the dominating factor of weather on wheat yield. This is consistent with previous research that has demonstrated that controlling broadleaf weeds in winter wheat was not economically feasible as wheat yields did not increase (Scott and Peeper 1994).

Within the realm of herbicides for weed management in winter wheat, application timing of herbicides has been highly studied as each new compound to the market is evaluated for optimum application timing (Carter et al. 2007; Frihauf et al. 2010; Geier and Stahlman 1996). Imazamox herbicide was evaluated with and without nitrogen fertilizer, and was found to be most effective for weed control and highest wheat yield potential with a fall application (Geier and Stahlman 2009). In additional evaluations of imazamox for weed control, yields were much higher from fall applications than from spring (Geier et al. 2004). However, a wheat grower's primary objective may be to have a clean field at harvest, not necessarily to control weeds to prevent yield loss (Scott and Peeper 1994).

The common prevalent conditions of low annual precipitation and alkaline soils should not go unnoticed in the development of chemical weed control in wheat (Peeper 1984). Development of new herbicide chemistry has diminished as herbicide-resistant crops have become the cornerstone of weed management. The first imidazolinone-tolerant crop was corn, and has since expanded to canola, rice, sunflowers, rice, and wheat (Shaner et al. 1996; White et al. 2006). Research has shown that imazamox herbicide is very effective at controlling a broad spectrum of weeds in imidazolinone-tolerant wheat (Claassen and Peterson 2002; Geier and Stahlman 2009; Kniss et al. 2008; Pester et al. 2001; Stahlman et al. 2001) and is safe to apply without adversely affecting yield (Frihauf et al. 2005; Geier et al. 2004).

Integrated weed management (IWM) is an ecological approach to weed control that reduces dependence on herbicides (Swanton and Weise 1991). IWM involves the use of several weed control measures including but not limited to cultural, chemical, mechanical and biological methods. Among these utilized in Kansas include controlled grazing, burning, cultural practices, sanitation, and herbicides. Cultural and mechanical practices included crop rotation, mowing, plowing, planting weed-free seed, delayed planting, fertilization, and planting competitive varieties (Wicks 1984; Zerner et al. 2008). Increasing wheat population has also been used for managing weeds, which has been documented for jointed goatgrass (*Aegilops cylindrica*), cheat (*Bromus secalinus*), and rye (*Secale cereal*) (Anderson 2009; Kappler et al. 1997; 2002; Koscelny et al. 1990). To successfully incorporate IWM methods, an increased understanding of the biological characteristics of weed populations is needed, particularly in the areas of crop-weed interactions and weed population dynamics.

The critical period of weed control (CPWC) is defined as the time within the life cycle of a crop that it must be kept weed-free to prevent a more than acceptable yield loss (Van Acker et al. 1993; Weaver and Tan 1987). The critical period is derived of two parts; 1) the length of time that weed control must be maintained to prevent crop yield loss, and 2) the length of time that a crop can tolerate weed interference before reducing crop yield (Weaver and Tan 1987). Assessments are based on the maximum allowable yield loss, typically ranging from 2.5 to 10% yield loss, and can subsequently be influenced by weather, moisture, soil type, weed species present, weed density, and the crop competitiveness. Numerous CPWC experiments have been conducted and have found various ranges in the CPWC for crops based on the aforementioned factors as results vary by location (Hall et al. 1992; Martin et al. 2001). The methodology for determining the CPWC has assumptions, which in turn affect weed control recommendations derived from the model (Knezevic et al. 2002). These assumptions are generally associated with 1) the dynamics of weed species present and the relative time of emergence, and 2) the way in which the CPWC is determined from two separately measured components. The CPWC in corn, for example, varied from initiating at V3 to V14; however, consistently ended at or near V14 depending on location (Hall et al. 1992). The critical weed-free period in organic winter wheat grown in England was found to be the time immediately after sowing (Welsh et al. 1999). This prevented all yield loss with the suggestion that wheat has a zero tolerance for weed competition; a 5% yield loss occurred between 506°C (November) and 1023°C (February) growing degree days after sowing. In spring wheat, the weed-free period is recognized as the one to three leaf stage of wheat (de Rocquigny 2012). The timing and duration of this period can play a large role in selecting the weed management tactic that bests fits, such as preemergence or postemergence herbicides, or the inclusion of burndown or residual herbicides. In addition, decisions on weed control in winter wheat should be a trade-off between economic impact of weeds and control cost (Benjamin et al. 2010).

Numerous weeds have been cited to reduce wheat yield, with a focus on weed density and/or their time of emergence relative to that of the wheat (Berti et al. 2008; Douglas et al. 1992; Jasieniuk et al. 1999). A 22% reduction in wheat yield was associated with Tartary buckwheat (*Fagopyrum tataricum*) that emerged with the wheat at a density of 30 plants $m^{-2}$ (De St. Remy et al. 1985). Chickweed (*Cerastium* spp.) that emerged before or at the same time as wheat had a greater effect on yield than chickweed that emerged after wheat (Inderjit 1998). Jointed goatgrass generally emerges at a similar time as wheat; nonetheless, removing jointed goatgrass by early March prevented wheat yield loss (Anderson 1993). Rye density at 4 to 5 plants $m^{-2}$ reduced wheat yield beyond its economic threshold thus requiring control (Pester et al. 2000; 2001). Italian ryegrass reduced yields by 33% in southern Illinois when allowed to compete season long (Hoskins et al. 2005). Cheat, feral rye, Italian ryegrass, jointed goatgrass, and wild oat (*Avena fatua*) emerging with the crop reduced Oklahoma wheat yields by 19, 55, 20, 21, and 28%, respectively (Fast et al. 2009). Wild oat competition with winter wheat was most affected by the time of wild oat emergence relative to that of the wheat, suggesting any delay in the onset of weed competition will aid in protecting wheat yield (O'Donovan et al. 1985). Delays in weed emergence may lessen the impact that the weeds have on crop yield, but late-emerging plants may interfere with harvest, reduce crop quality, and contribute to the weed seed bank.

Timing and application method of fertilizer inputs have been shown to significantly affect weed competition with wheat (Cralle et al. 2003; Forcella 1986; Zanin et al. 1993). Application of fertilizer inputs in the fall are a means of economic commitment to the wheat crop (Slaton et al. 2005), as it is common occurrence to divide fertilizer applications between the fall and spring, or to delay application until after overwintering (Weisz et al. 2001). The application of nitrogen also affects weed presence and density (Johri et al. 1992). Downy brome (*Bromus tectorum*), wild oat, flixweed (*Descurainia sophia*), and field pennycress (*Thlaspi arvense*) all had increased density in association with the application of a broadcast fertilizer application as opposed to subsurface banded or point-injected nitrogen (Blackshaw 2004). Blackshaw (2004) suggests that application methods of nitrogen need to be more beneficial to the crop rather than the weeds. The same conclusion was derived from a study examining wheat, rye, and phosphorus (Cralle et al. 2003), suggesting that phosphorus be placed in or near the seeded wheat row; not on the surface. Canada thistle (*Cirsium arvense*) reduced wheat yield more due to competition for nitrogen rather than Canada thistle density (Mamolos and Kalburtji 2001).

## Downy Brome

Downy brome is the predominant winter annual grass weed of winter wheat (Morrow and Stahlman 1984) that can be found throughout North America (Upadhyaya et al. 1986). The lifecycle of downy brome closely resembles that of winter wheat and likely is why it remains difficult to manage (Wicks 1997). Germination begins in the fall and can continue through early spring; however, germination in summer is also possible with adequate moisture in areas other than Kansas (Thill 1979). As with most winter annuals, downy brome seeds exhibit a short duration of dormancy and lack longevity in the seed bank (Rydrych 1974; Wicks et al. 1971). Downy brome germinates relatively close to the soil surface in the fall, overwinters as a seedling with tillers, then proceeds with stem elongation, jointing, and at that point development of seed heads in spring (Hulbert 1955). Thus, wheat and downy brome compete for the same resources of light, water, and nutrients in the soil if emerging in the relatively same time period (Dao 1987;

Massee 1976; Upadhyaya et al. 1986). Downy brome will reach physiological maturity before winter wheat with seed heads positioned at or slightly below that of wheat, providing a mechanism of dispersal at the time of wheat harvest. Downy brome occupies a range of habitats but tends to flourish in ditches, waterways, field borders, pasture, fallow land, and wheat fields. Densities of downy brome in winter wheat have been cited to reach up to 600 plants $m^{-2}$ (Rydrych and Muzik 1968; Rydrych 1974; Stougaard et al. 2004).

Many IWM strategies exist to aid in controlling downy brome infestations. Since weeds that emerge before or at the same time as the crop are most detrimental to yield, it is important to create asynchrony between crops and weeds (Blackshaw 1993). Downy brome that emerges prior to planting of winter wheat can be controlled via tillage or a burndown herbicide application to create a clean seedbed. In addition, utilizing a preemergence herbicide to delay the onset of downy brome creates an advantaged for the winter wheat (Stahlman and El-Hamid 1994). Cultural methods for improving winter wheat competitiveness may be utilization of cultivars that are tall, have broad leaves, and high seed vigor (Ghersa et al. 1994; Huel and Hucl 1996; White et al. 2004; Zerner et al. 2008). Studies in Nebraska examined different wheat cultivars and found downy brome reduced winter wheat grain yields of all cultivars by 9 to 21% at Lincoln, with yield reduction ranging from 20 to 41% at North Platte (Challaiah et al. 1986). 'Turkey' was the most competitive wheat cultivar to downy brome but it had the lowest grain yield, while 'Centurk 78', 'Centura' and 'SD 75284' had higher yields and were not quite as competitive to downy brome. Winter wheat tiller number and plant height were negatively correlated with downy brome yield, but changes in these growth parameters did not always translate into grain yield. Still, heavy reliance remains on fall or spring-applied postemergence herbicides as the primary effective tool to manage downy brome in wheat (Geier et al. 1998; 2002).

Downy brome has been recorded to reduce wheat yield up to 92% in the Pacific Northwest depending on moisture, weed density, and time of emergence (Rydych and Muzik 1968). The timing of emergence relative to the crop is more critical than density (Blackshaw 1993; Chikoye et al. 1995) and is supported by yield loss prediction models (Jasieniuk et al. 2001). Downy brome reduced wheat yields by 40% at a density of 132 plants $m^{-2}$ when emerging with the crop (Rydych 1974), whereas, downy brome densities needed to exceed 200 plants $m^{-2}$ to cause wheat yield loss from later emerging cohorts (Blackshaw 1993). Fall-applied herbicides are very effective in managing downy brome if they are applied early and provide residual activity preventing further competition and re-infestation (Stougaard et al. 2004).

Downy brome did not affect wheat yield when it emerged 21 days after wheat in the Central Plains (Stahlman and Miller 1990). Interference of downy brome with winter wheat has been shown to be influenced by nitrogen fertilizer (Anderson 1991; Ball et al. 1999). In the presence of nitrogen fertilizer, downy brome increases shoot biomass and seed production (Hulbert 1955; Thill et al. 1984). Thus, broadcast applications of nitrogen favored downy brome and resulted in reduced winter wheat response to nitrogen fertilizers when downy brome was present (Anderson 1991; Blackshaw et al. 2005; Blackshaw 2004). Densities of weeds in most wheat trials were assessed at springtime (Ball et al. 1996; Stahlman and Miller 1990), not from initial point of competition with the crop. Therefore, in many cases, the duration of weed competition and weed density remains unknown.

Management strategies for downy brome extend beyond the winter wheat cropping season. Crop rotation can be utilized if the subsequent crop life cycles (summer annual, perennial) are dissimilar to that of wheat/brome (winter annual). Utilizing these other crops are effective at reducing the weed seed bank going into the wheat crop (Rainbolt et al. 2004; White et al. 2004; Young et al. 2000).

## Henbit

Henbit (*Lamium amplexicaule*) is a winter annual broadleaf weed that is found in cultivated fields, gardens, roadsides, pastures, and waste areas and throughout most of the United States (DeFelice 2005). A single henbit plant may produce from 200 to 2,000 seed per plant (Allan 1978; Holm et al. 1997; Wilson et al. 1988). Germination occurs in the fall or early spring, with no germination occurring during summer months (Baskin et al. 1986; Blackshaw et al. 2002). Upon germination, henbit will grow prostrate, overwinter, and then mature in the understory of the wheat crop. At the time of harvest, henbit is no longer visible. However, seeds from henbit may remain viable for over 25 years, suggesting that management of this weed should focus on preventing seed production (Roberts and Boddrell 1983; Thompson et al. 1997). Henbit is cited as one of the 10 most common weeds in wheat production in the southern United States (Webster 2000), and has continued to increase in presence on no-till wheat acres (Steckel 2013). Mueller (2012) identified henbit as being present in 100% of all no-till fields sampled in

Kansas at an average density of 165 plants $m^{-2}$. Large-scale field trials in Oklahoma failed to demonstrate yield loss associated with controlling henbit (Scott et al. 1995). In addition, a single henbit plant $m^{-2}$ reduced yields less than 1% when allowed to compete with winter wheat (Streibig et al. 1989), noting it to be one of the least competitive weeds in wheat and control may not be beneficial (Mertens and Jansen 2002; Webb and Johnson 1987). Contrary to those results, Farahbakish et al. (1987) and Northam et al. (1993) both noted wheat yield losses associated with henbit or chickweed that ranged from 0.3 to 48% based on weed density. Conley and Bradley (2005) suggested that henbit in addition to crop stand loss may significantly reduce wheat yield, but not henbit alone. Therefore, there has been mixed results in regards to demonstrating an effect of henbit competition with winter wheat.

## Flumioxazin

Flumioxazin, formerly known as S-53482 and V-53482, is a *N*-phenyl phthalimide herbicide that was discovered and developed by Sumitomo Chemical Company in 1987 and is currently registered and labeled for preemergence weed control in corn, soybean, wheat and numerous other crops (Altom et al. 2000; Cranmer et al. 2000; Nagano et al. 2001; Sakaki et al. 1991; Theodoridis 2007; Yoshida et al. 1991). The mechanism of action for flumioxazin is through the inhibition of the protoporphyrinogen oxidase (PPO) enzyme of chlorophyll biosynthesis pathway in the presence of light and oxygen (Che et al. 1993; Kim et al. 1993; Konomi et al. 1993; Nagano et al. 2001; Sato et al. 1987; Yoshida et al. 1991). This inhibition induces the accumulation of protoporphyrinogen IX because of uncontrolled auto-oxidation of the substrate (Dayan et al. 1997; Duke et al. 1991). As protoporphyrinogen IX accumulates and is photoenergized by light, toxic radicals are created, which leads to degradation of the plasmalemma and tonoplast membrane lipids causing irreversible damage (Duke et al. 1990; Jacobs et al. 1991; Shibata et al. 1992). The herbicide has numerous environmental benefits including low use rate, rapid soil dissipation, and it is active in controlling numerous weed biotypes resistant to other herbicide modes of action (Alister et al. 2008; California Department of Pesticide Regulation Public Report 2003-6; Ferrell and Vencill 2003; Ferrell et al. 2005; Lu et al. 2000).

Primary focus of research regarding the mechanistic activity of herbicides that inhibit PPO has been utilizing the foliar-applied products, not necessarily those that are soil-applied such as flumioxazin, sulfentrazone, and saflufenacil. However, Sato et al. (1991) determined that the reactions within cucumber seedlings were the same for both acifluorfen, a foliar applied herbicide, and flumioxazin. It has been demonstrated with acifluorfen, one of the many herbicides that inhibit PPO, that soybeans utilize metabolic detoxification to infer tolerance and show little to no response to the herbicides (Frear et al. 1983). Ritter and Coble (1981) demonstrated that soybean exhibited tolerance to acifluorfen due to limited absorption. However, Sherman et al. (1991) examined numerous species of plants with varying susceptibility to acifluorfen and determined there could be several mechanisms of tolerance, for example, the production of protoporphyrinogen IX in response to herbicide application. Soybean susceptibility to sulfentrazone was due to differential metabolism and differential tolerance to herbicide-induced peroxidative stress (Dayan et al. 1997). Price et al. (2004) examined sickle pod (*Senna obtusifolia*), peanut (*Arachis hypogaea*), and morningglory (*Ipomoea* spp.) seedlings after root-exposure to flumioxazin. Differences in metabolism were evident, with peanut metabolizing flumioxazin three times faster than a susceptible species, morningglory. Their results suggested a high likelihood that flumioxazin injury in peanut would be associated with rainfall at the time of emergence. Bigot et al. (2011) suggest that detoxification of the herbicide may occur in grape rootstock or possible reduced uptake due to position of root system relative to the location of the flumioxazin in the soil and soil adsorption characteristics (Saladin et al. 2003).

Soybean seedlings exposed to $C^{14}$ sulfentrazone had significantly more absorption through the roots as opposed to the cotyledon and hypocotyl (Li et al. 2000). This variance in absorption has been cited as the contributing factor in the differential response of sensitive and non-sensitive weeds and crops for sulfentrazone and other herbicides (Li et al. 2000; Mangeot et al. 1979; Vencill et al. 1990).

## Pyroxasulfone

Pyroxasulfone, developed as KIH-485, is a new preemergence herbicide that is registered in corn, soybeans, and wheat for the control of many broadleaf and grass weeds (Tanetani et al. 2009). The mechanism of action for pyroxasulfone is similar to that of compounds in the chloroacetamide family, which inhibit very long chain fatty acid (VLCFA) biosynthesis (Mueller and Steckel 2011; Tanetani et al. 2011b). However, Tanetani et al. (2009; 2011a; 2011b) identified seven sites of VLCFA inhibition with pyroxasulfone, unlike the two associated with chloroacetamide compounds. Herbicides within the family of VLCFA biosynthesis inhibitors do not inhibit seed germination, only the shoot elongation of germinated seeds (Tanetani 2012). Research on dinitroaniline herbicides has confirmed that they are readily absorbed by roots and emerging shoots, but shoot exposure is more phytotoxic (Appleby and Valverde 1989; Deal and Hess 1980). Initial exploration into the activity of VLCFA herbicides utilized an activated charcoal barrier methodology to determine the importance of root and shoot exposure on the activity of nine acetanilide herbicides on peas, of which all showed greater root than shoot activity (Jordan and Harvey 1978; 1980). More recent research and technological advancements have confirmed that primary inhibition from chloroacetamide herbicides occurs in shoots of seedlings (Appleby and Valverde 1989; Böger et al. 2000). Giant foxtail (*Setaria faberi*) and shattercane (*Sorghum bicolor*) seedlings exhibited greater mortality when pyroxasulfone was applied to the shoot than the roots; while velvetleaf (*Abutilon theophrasti*) primarily absorbed pyroxasulfone through the shoot and secondarily through the roots with decreased efficacy (Kumiai internal data).

Therefore, placement of the herbicide relative to the seed or germinating seedling may be of importance. In addition, the performance of soil-applied herbicides is influenced by many factors (Holly 1962; Ogle and Warren 1954). These factors include soil pH, cation exchange capacity, organic matter, soil moisture, nutrient availability, photodecomposition, adsorption, leaching, volatilization and the overall makeup of the soil such as sand, silt, or clay (Corbin and Upchurch 1967; Upchurch and Mason 1962). Even considering all these factors, if the herbicide is not present at the optimum location for uptake and translocation, then it be ineffective.

The objectives of this research were:

1: To determine the critical period of weed control for Kansas winter wheat in dryland and irrigation conditions as to implement timely and economically feasible management practices,

2: To determine the extent of which single and mixed species of grass and broadleaf weed populations interfere with winter wheat yield, and,

3: To develop a methodology to evaluate root and shoot exposure of soil applied herbicides to weeds and crops utilizing a novel technique.

4. To measure extent of herbicide injury and weed sensitivity to flumioxazin and pyroxasulfone based on localized herbicide exposure to roots, shoots, and to both roots and shoots.

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# Chapter 2 - Weed interference and the critical period of weed control in Kansas winter wheat


## Abstract

The critical period of weed control is the duration of the crop life cycle in which it must be kept weed-free to prevent yield loss from weed interference. Eight experiments were conducted throughout Kansas between October 2010 and June 2012 to identify the critical period of weed control in winter wheat (*Triticum aestivum*) grown under dryland and irrigated conditions. Downy brome (*Bromus tectorum*) and henbit (*Lamium amplexicaule*) populations were monitored in four farmer's fields with naturally occurring populations and in one research field with an overseeded population throughout the growing season to evaluate the effect of single and mixed weed species densities on winter wheat yield. Henbit densities up to 156 plants $m^{-2}$ did not affect winter wheat yield. Downy brome at a density of 40 plants $m^{-2}$ reduced winter wheat yield by 33 and 13% in 2011 and 2012, respectively. In the presence of downy brome, winter wheat should be kept weed-free approximately 30 to 45 days after planting to prevent significant yield loss when crop and weed emerge at similar times. Otherwise, weeds need to be removed immediately following release of winter dormancy to prevent yield loss due to existing weed pressure. Kansas wheat growers should evaluate the presence and density of weed species in determining which weed management strategy is most advantageous to preserving winter wheat yield.


## Introduction

Integrated weed management (IWM) is an ecological approach to weed control that reduces dependence on herbicides (Swanton and Weise 1991). IWM involves the use of several weed control measures including but not limited to cultural, chemical, mechanical, and biological methods. A commonly used approach to IWM is the utilization of a critical period of weed control. The critical period of weed control (CPWC) is defined as the time within the life cycle of a crop when it must be kept weed-free to prevent a specific level of yield loss (Swanton and Weise 1991, Van Acker et al. 1993; Weaver and Tan 1987). The critical period is derived of two time periods, 1) the weed-free period defined as the length of time that weed control must be maintained to prevent crop yield loss, and 2) the duration of weed interference defined as the length of time that a crop can tolerate weed presence before crop yield is reduced (Weaver and Tan 1987). In spring wheat, the CPWC is recognized as occurring between the one to three-leaf stages of wheat (de Rocquigny 2012). The timing and duration of this period can play a significant role in determining when to scout for weeds and selecting the weed management tactic that best fits to provide a weed-free environment, such as preemergence or postemergence herbicides, or the inclusion of burndown or residual herbicides. In addition, decisions on weed control in winter wheat should be a trade-off between economic impact of weeds on crop yield and cost of control (Benjamin et al. 2010).

Weed management in winter wheat has historically focused on postemergence herbicide applications in either fall or spring, with applications being made in response to the presence of weeds. Winter wheat yield response to management such as weed control and fertilizer application varies greatly (Jasieniuk et al. 1999; Slaton et al. 2005). Even though numerous broadleaf weeds occur in winter wheat, some previous research found that controlling broadleaf weeds in winter wheat was not economically feasible as wheat yields did not increase when control measures were imposed (Scott and Peeper 1994). Another factor influencing winter wheat productivity in Kansas is year-to-year variability in yield. When 50 years of Kansas winter wheat yields were summarized, 47% of the variation in dryland yields was accounted for by year-to-year variability such as erratic rainfall, warm fall temperatures, and spring freezes (Holman et al. 2011).

The common prevalent conditions of low annual precipitation and alkaline soils in winter wheat production areas should not go unnoticed in the development of chemical weed control (Peeper 1984). Development of new herbicide chemistry has diminished as herbicide-resistant crops have become the cornerstone of weed management. The first imidazolinone-tolerant crop was corn and has since expanded to include varieties of canola, rice, sunflowers, rice, and wheat (Shaner et al. 1996; White et al. 2006). Research has shown that imazamox herbicide is very effective at controlling a broad spectrum of weeds in imidazolinone-tolerant wheat (Claassen and Peterson 2002; Geier and Stahlman 2009; Kniss et al. 2008; Pester et al. 2001; Stahlman et al. 2001) and is safe to apply without adversely affecting yield (Frihauf et al. 2005; Geier et al. 2004).

Within the realm of herbicides for weed management in winter wheat, application timing of herbicides has been highly researched as each new compound to the market is evaluated for optimum application timing (Carter et al. 2007; Frihauf et al. 2010; Geier and Stahlman 1996). Evaluations of imazamox for weed control determined wheat yields were much higher from fall applications than from spring (Geier et al. 2004). Welsh et al. (1999) found weeds emerging with winter wheat in Europe had a significant negative effect on yield. However, a wheat grower's primary objective may be to have a clean field for harvest efficiency, not to control weeds to prevent yield loss (Scott and Peeper 1994).

Interference of weeds in wheat has previously focused on individual species interactions with the crop, and very few noted the time of weed emergence relative to the crop. Densities of downy brome in winter wheat have been cited to reach up to 600 plants $m^{-2}$ (Rydrych and Muzik 1968; Rydrych 1974; Stougaard et al. 2004). Blackshaw (1993) determined that the time of weed emergence relative to wheat planting was more important than the density of the weeds. At a density of 25 downy brome plants $m^{-2}$, wheat yields were reduced by 10% after season-long interference, while wheat yield was not reduced regardless of density when downy brome emerged 21 days after planting (Stahlman and Miller, 1990). Henbit is frequently found, but is one of the least competitive weeds in wheat and control may not be economically beneficial in managing wheat yield (Mertens and Jansen 2002; Webb and Johnson 1987). Henbit did not cause a reduction in Missouri wheat yields at a density of 18 plants $m^{-2}$, however yields were reduced 13 and 38% at densities of 82 and 155 plants $m^{-2}$, respectively (Conley and Bradley 2005).

The objectives of this research were two-fold, 1) to determine the critical period of weed control and 2) to determine yield loss as affected by single and mixed species of grass and broadleaf weed interference for Kansas winter wheat grown under dryland and irrigated production practices as to implement IWM practices.

## Materials and Methods

### *Critical period of weed control*

Trials were conducted at three locations planted in 2010 and five locations planted in 2011. The first site (Manhattan) was located at the Kansas State University Department of Agronomy Ashland Bottoms Research Farm near Manhattan, KS and used in both 2010 and 2011. The soil was classified as a silty loam soil comprised of 10% sand, 64% silt, and 26% clay with mean pH 5.9 and soil organic matter content of 1.9%. Individual plots at this site were

3 by 9 m. The second site (Belpre) was located near Belpre, KS and utilized in 2010 and 2011. The soil at the site was loamy sand comprised of 85% sand, 9% silt, and 6% clay, pH 5.4, and organic matter content of 0.8%. Individual plots at this site were 3 by 9 m. The third site in 2010 and 2011 [Larned (a)] was located between Larned and Belpre, KS. Soil was a sandy loam comprised of 48% sand, 33% silt, and 19% clay with pH 5.2 and organic matter content of 1.3%. Individual plots were 3 by 9 m and under irrigation. The fourth location (Hays) was located at the Kansas State University Agricultural Research Center at Hays, KS in 2011. The soil was classified as a silt loam comprised of 32% sand, 52% silt, and 16% clay with pH 8.1 and organic matter content of 1.3%. Individual plots were 2.7 by 6.7 m. The final location [Larned (b)] was located near Larned, KS in 2011. Soil was a sandy loam having a composition of 71% sand, 23% silt, and 6% clay with pH 5.9 and organic matter content of 1.2%. Individual plots were 3 by 9 m and under irrigation.

The experimental sites were kept fallow in the summer prior to seeding the crop. Winter wheat seeding rates and planting dates varied by location according to local practices (Table 2.1). Fertilizer was applied according to soil test recommendations for each site. The Manhattan locations received 100 kg ha$^{-1}$ of nitrogen and 34 kg ha$^{-1}$ of phosphorus in a blend of 28% UAN and 10-34-0 as total nitrogen (N) and available phosphate ($P_2O_5$) in both years prior to planting in October. On February 15, 2011, 60 kg ha$^{-1}$ of nitrogen was applied as 46-0-0 (urea) at the Belpre site using a coarse bulk spreader, and in 2011 the field was top-dressed at planting with 24 kg ha$^{-1}$ of nitrogen as 28% UAN. At the Hays site, 45 kg ha$^{-1}$ of nitrogen was injected on September 13 as 28% UAN. At the Larned (a) site, the field received 60 kg ha$^{-1}$ of nitrogen and 54 kg ha$^{-1}$ of phosphorus as 46-0-0 and 11-52-0 (mono-ammonium phosphate) as a percentage of total nitrogen (N), available phosphate ($P_2O_5$) one week prior to planting each year in October. At Larned (b), the field was top-dressed at planting with 24 kg ha$^{-1}$ of nitrogen as 28% UAN.

The CPWC control was determined using two sets of treatments. In the first set, the winter wheat was kept weed-free for increasing durations of time utilizing a titration of imazamox (Beyond®) rates applied at planting, for a total of four rates, with the maximum rate as 52.5 g ha$^{-1}$. This rate titration varied the length of residual control provided by imazamox allowing weeds to emerge earlier at lower application rates. In the second set of treatments, weeds were allowed to interfere with the crop from the time of emergence and for increasing durations until they were removed using imazamox applied at 52.5 g ha$^{-1}$ with 1% v/v of methylated seed oil and 470 g $ha^{-1}$ of granular ammonium sulfate according to predetermined wheat development stages (Table 2.2). The herbicide was applied using a backpack sprayer with a hand-held boom with application nozzles, volume and pressure varying slightly for each location. A total of 11 treatments, including a untreated check for weedy all season and two weed-free all season long treatments were arranged in a randomized complete block design with four blocks at each site. The variety of wheat used varied by location; however, they all were resistant to imazamox herbicide. Mixed weed species populations existed at the Manhattan and Hays locations (Table 2.3). The Manhattan site was augmented with seeds of downy brome at approximately 150 seeds $m^{-2}$ and utilized existing populations of henbit and blue mustard (*Chorispora tenella*). The Hays site also received supplemental downy brome seeds at planting to increase potential density. Naturally occurring weed populations were observed at both Larned locations and at Belpre. To monitor the weed density, time of emergence, and amount of weed growth that occurred, a 1 $m^2$ sample was cut at ground level and removed from the plot area prior to herbicide application in each of the duration of weed interference plots. Winter wheat growth stage and weed density were documented, and weed biomass was collected from a 1 $m^2$ quadrat in the weedy plot and subsequently dried to obtain shoot biomass. Winter wheat harvest was conducted utilizing a small-plot combine with harvest date varying depending on year and location. Winter wheat yield was expressed as a percentage of the weed-free control for each site by replication.

Equations describing crop yield response to weed interference were fitted to the winter wheat yield data using analysis of variance in SAS 9.2 (SAS Institute 2010) and subsequently a nonlinear regression procedure in SigmaPlot v.12 (Systat Software, San Jose, CA). Years were combined for Manhattan data because of similarities in weed species and density, however all other site-years were analyzed separately because of differences in weed species and densities among sites (Table 2.3). The Gompertz equation (Hall et al. 1992; Ratkowsky 1990) was used to describe the effect of increasing lengths of weed-free period on winter wheat yield:

$$\mathrm{Y} = A\,exp\,-B\,exp\,-K\mathrm{T} \tag{1}$$

where Y is percent winter wheat yield relative to the weed-free control, *A* is the upper asymptote, *B* and *K* are parameters that determine the shape of the curve, T is time in DAP (days after planting), and exp refers to *e* (the base of the natural logarithm) raised to the specified power. A logistic equation (Ratkowsky 1990) was used to describe the effect of increasing duration of weed infestation on the yield of winter wheat:

$$Y = \frac{C + D}{1 + exp\ -A +\ BT} \qquad [2]$$

where Y is percent winter wheat yield relative to the weed-free control, *A* and *B* are parameters that determine the shape of the curve, *C* is the lower asymptote, *D* is the difference between the upper and lower asymptotes, and T is time in DAP. Standard errors of the parameter estimates are presented; the standard error of a parameter estimate is a measure of confidence, and if it is large, the parameter is poorly estimated. Using the derived Gompertz equations, the critical length of the weed-free period for winter wheat in DAP was calculated for specific yield loss levels of 5 and 10% for each site-year. Similarly, using the derived logistic equations, the critical duration of weed infestation was calculated for specific yield loss levels of 5 and 10%.

### *Henbit and downy brome interference*

Four farmer's fields in North Central Kansas were identified in the fall of 2010 and 2011 with known infestations of downy brome and henbit. Two sites in 2010 were located near Wakefield and Flush, KS. In 2011, two sites were located near Louisville, KS. In addition, an overseeded infestation of downy brome was established in the fall of 2011 at the Kansas State University Department of Agronomy Ashland Bottoms Research Farm near Manhattan, KS. All fields were planted in early October, and upon winter wheat emergence approximately forty 1 $m^2$ quadrats were randomly located within each field. In each quadrat, weed species, density, and growth stage in addition to winter wheat density and growth stage were observed on a bi-weekly basis throughout the growing season. Plot area was subject to normal winter wheat production practices with the omission of herbicide application. Winter wheat biomass and grain were collected at harvest from each 1 $m^2$ quadrat. Data were analyzed using regression analysis in SigmaPlot V.12 to determine the effect of weed density on wheat yield. The results of the regression analyses must be viewed with some caution, as the assumption of equal variance was not met; a large number of weed-free plots were utilized to estimate weed-free yield, while fewer plots were obtained at higher weed densities. However, the subsequent analyses do demonstrate correlations among factors affecting winter wheat yield.

The non-linear model (Blackshaw 1993; Cousens 1985) was fit to winter wheat yields in relation to downy brome density as determined at the mid-March evaluation timing in 2011:

$$\mathrm{Y} = Y\mathrm{wf}\left(1 - \frac{i\mathrm{X}}{100\left(1 + \frac{i\mathrm{X}}{a}\right)}\right) \quad [3]$$

where Y is the observed winter wheat yield (kg ha$^{-1}$), *Y*wf is the yield of weed-free wheat (kg ha$^{-1}$), *i* is the initial slope as weed density (X) approaches 0, and *a* represents the asymptotic maximum yield loss (%) as weed density approaches infinity (Table 2.7). A linear model described the relationship of downy brome density as observed in March 2012 to winter wheat yield:

$$\mathrm{Y} = i\mathrm{X} + Y\mathrm{wf} \quad [4]$$

where Y is the observed winter wheat yield (kg ha$^{-1}$), *Y*wf is the yield of weed-free wheat (kg ha$^{-1}$), and *i* is the slope over increasing weed density (X).

The relationship of winter wheat yield to various combinations of downy brome and henbit densities was analyzed by fitting the multivariate form of the rectangular hyperbola crop yield model described by Cousens (1985) to each year separately. Swinton et al. (1994) expanded the original equation to account for multiple weed species:

$$\mathrm{Y} = Y\mathrm{wf}\left(1 - \frac{I_h\mathrm{X_h} + I_d\mathrm{X_d}}{100\left(1 + \frac{I_h\mathrm{X_h} + I_d\mathrm{X_d}}{a}\right)}\right) \quad [5]$$

where Y is the observed wheat yield (kg ha$^{-1}$), *Y*wf, $I_h$, $I_d$, and *a* are model parameters estimated from the data, and $X_h$ and $X_d$ are densities (plants m$^{-2}$) of each weed species (*h* for henbit and *d* for downy brome). Parameter *Y*wf is weed-free yield; $I_h$ and $I_d$ are crop yield loss (%) associated with each weed density as it approaches 0; and *a* represents the asymptotic maximum yield loss (%) as weed density approaches infinity. Parameter estimates for Equation 5 for each year were obtained using regression techniques (SigmaPlot v.12).

# Results

## *Critical period of weed control*

Weed density varied greatly by location and was reflective of natural and over-seeded populations established at each site. Downy brome and henbit were the most common species, with the additional presence of blue mustard at the Manhattan and Hays locations (Table 2.3). The Belpre location did not have any weeds occurring in the winter wheat crop. Weed infestations, when present, were similar each year regardless of moisture variation with emergence occurring in the fall. Densities of weeds across years at the same locations did not vary even though temperature and moisture were dissimilar. Downy brome and henbit, when present, were at similar densities that would be found in natural field populations. The absence of weed emergence in the spring did not provide evidence that the rate titration of imazamox was effective at prolonging weed infestations.

Winter wheat yield varied by location and year, ranging from 1614 to 2623 kg $ha^{-1}$ in 2011 and from 1211 to 1883 kg $ha^{-1}$ in 2012 in the weed-free control plots, whereas average yields in Kansas are 2825 kg $ha^{-1}$ (data not shown). A previous summary of Kansas winter wheat yields found year-to-year variability accounted for 23% to 47% of yield (Holman et al. 2011). Year-to-year variability in Kansas can be attributed to erratic rainfall, warm fall temperatures, and spring freezes such that predicting yield is nearly impossible until prior to harvest. Environmental conditions throughout the two growing seasons at the eight locations were unusually dry and warmer than average. Rainfall was 10 to 19 cm less than the 30-year average in 2010 and 2011 growing season, while temperatures in the spring of 2012, March through June, were above the 30-year average by approximately 6°C. There was a significant statistical relationship between crop yields and the duration of weed-free period or duration of weed infestation for the Manhattan (combined over years), Hays in 2011, Larned (a) in 2011, and Larned (b) in 2011 locations. The lack of significance for the remaining locations, Larned (a) in 2010, and Belpre in 2010 and 2011, is likely attributed to the insufficient weed presence.

The critical period, as defined by Swanton and Weise (1991), is the time interval essential to maintain a weed-free environment to prevent yield loss. The period can be adjusted based on the acceptable levels of yield loss typically ranging from 2 to 10% (Hall et al. 1992; Martin et al. 2001; Welsh et al. 1999) and is mostly dependent upon commodity price and cost of weed control. The critical period is present if the logistic and Gompertz model curves overlap in a way to create a gap where yields fall below acceptable levels (Nieto et al. 1968). This period only existed for the Larned (b) site in 2011 and ranged from 23 to 32 DAP to protect the crop from less than a 5% yield loss (Figure 2.1). The early season yield loss at this location is likely attributed to downy brome interference with the winter wheat from the onset of planting and with increasing interference under drought conditions, as the downy brome density did not change throughout the growing season (Table 2.3).

The two components of the CPWC, that is the critical weed-free period and the critical duration of weed interference will be discussed separately for each location when there was no critical period defined. Time is represented as DAP and was chosen to fulfill the objective of the study, which was to determine the CPWC for winter wheat with a view to practical application and extension. Days after planting (DAP) provides a meaningful, practical extension reference, while crop development stage varies greatly upon environment compared to a measure of meteorological time (growing degree days) that does not have a practical extension reference. However, application of herbicides in reference to crop development stage can provide a useful practical extension reference.

***Critical duration of weed interference***

Winter wheat yield decreased as the duration of weed presence increased. Season-long weed interference caused ample yield loss variability of winter wheat at Larned (a) in 2011 with only 8% reduction from weed-free, while yields at Manhattan were reduced by 29% in 2010 + 2011, and yields at Larned (b) and Hays were reduced 37 and 50%, respectively in 2011 (Figures 2.1, 2.2, 2.3, 2.4). The logistic model demonstrates that competition between winter wheat and the weeds present did not occur until after release of winter dormancy and crop and weed growth resumed for all locations except Larned (b) (Table 2.4). The Larned (b) location as fit to the logistic model, showed yield reductions occurring shortly after planting and continued to reduce yield up to winter dormancy (Figure 2.1). In this model at Larned (b), the weeds needed to be removed by 23 DAP to prevent more than 5% yield loss and removed by 30 DAP prevent more than 10% yield loss. All other logistic models determined yield loss of 5% or less to occur between 129 and 208 DAP, corresponding to a mid-March to April application timing to control weeds (Table 2.5). The timing to prevent 10% yield loss was generally only a few more days than that of the 5% tolerance. This is consistent with results of Rydrych (1974) who found

Oregon winter wheat able to endure competition from downy brome until March, at which point yield reductions began to increase significantly. At the Larned (a) location, predicted yield loss never reached 10% (Figure 2.2). In this case, the winter wheat was capable of competing with the weeds present; in this instance, the presence of henbit had no effect on yield. In summary, the duration of the weed interference for winter wheat is likely dependent on the density and species of weeds present. For example, as the density of downy brome increased, which was present at Manhattan, Hays, and Larned (b) locations, the length of time this weed species is allowed to interfere decreased.

***Critical weed-free period***

The yield of winter wheat increased as the length of the weed-free period increased, reaching maximum yield potential prior to winter dormancy, approximately 100 DAP (Table 2.6). It is important to note that no additional weed emergence occurred between fall herbicide application and harvest of wheat, therefore only weeds present in the fall attributed to crop competition and subsequent yield reduction. This is consistent with previous research identifying the role of the critical weed-free period corresponding to the weeds species and their germination pattern (Hall et al. 1992; Van Acker et al. 1993). This is also supported by Blackshaw (1993) noting that the timing of weed emergence relative to the crop is more critical than density. The Gompertz model generally fit the data for the four selected locations. The critical weed-free period for 5% yield loss based on the models were similar among Manhattan (2010 + 2011), Hays and Larned (b) locations in 2011 and equated to approximately 35 DAP (Table 2.5). The range for 10% yield loss occurred between 20 and 27 DAP for the same locations. Wicks (1966) identified the weed-free period as two weeks from planting as the time before downy brome began to affect dryland winter wheat yields in Nebraska. Larned (a) in 2011 required a weed-free period from planting until 50 DAP to prevent 5% yield loss, while preventing 10% yield loss required no weeds to be competing with the crop from the time of planting. The lack of yield response was likely due to low weed density, the weakly competitive nature of henbit and the model based on those factors. The fields were weed-free at planting across all locations; therefore, downy brome emerged at a similar time to the crop, if it occurred. The critical weed-free period for winter wheat could therefore be adjusted based on the germination and emergence pattern of the weed species in given field. Based on these results, winter wheat kept weed-free from planting to approximately one month after planting will be able to maintain maximum yield potential without reduction from competing weeds. Application of preemergence or early postemergence herbicides with residual activity would be beneficial to maintain this weed-free period and delay the onset of weeds such as downy brome or henbit, which typically emerge with the crop.

### *Henbit and downy brome interference*

Henbit and downy brome varied in density between the two growing seasons (Figures 2.5 and 2.6) because of environmental conditions. In addition, wheat yield observed in 2012 was reduced 50% from the prior year due to adverse environmental conditions at time of grain development. Stand establishment of winter wheat did not vary within years, or by location (data not shown). Henbit density ranged from 0 to 156 plants $m^{-2}$ in March 2011 and from 0 to 48 plants $m^{-2}$ in March 2012 across locations. A majority of the henbit population emerged within 60 DAP with winterkill only affecting approximately 5 to 10% of the population in either year. Henbit density, as determined at the mid-March evaluation time, was utilized for analysis as the weed had matured and perished by winter wheat harvest. There was no correlation between the density of henbit and winter wheat yield for either year as evident in Figures 2.7 and 2.10. Downy brome densities ranged from 0 to 44 plants $m^{-2}$ in March 2011 and from 0 to 172 plants $m^{-2}$ in March 2012 across locations. Similar to henbit, the majority of downy brome plants had emerged in the fall, prior to winter dormancy, with less than 5% winter mortality (data not shown).

Parameter estimates were similar when comparing the single species model (Equation 3) to that of the multi-species model (Equation 5) in 2011, suggesting that winter wheat yield loss with the multiple species was largely influenced by the presence of downy brome (Table 2.7). The parameter estimate $I_d$ represents yield loss (%) at low densities near zero. In 2011, the parameter estimate $I_d$ was approximately 13% for both single species and multi-species models; while in 2012, $I_d$ was 0.30 to 0.34% indicating less impact at low densities. Maximum predicted yield loss ($a$) was 34% at high densities in 2011. Parameter estimation for $a$ was not possible in 2012 as downy brome density was not sufficiently high enough to estimate the parameter.

Henbit density did not affect winter wheat yields in this study. This is consistent with previous results that concluded no yield loss was associated with the presence of henbit at similar densities (Conley and Bradley 2005; Scott et al. 1995; Streibig et al. 1989). Unless henbit density is exceptionally high or moisture conditions limited, the presence of henbit in winter wheat may be more associated with that of an aesthetic pest, as it appears to be one of the least competitive weeds in wheat and control may not be economically beneficial (Mertens and Jansen 2002; Webb and Johnson 1987). Considerations should also take into account future crop rotations, as henbit may interfere with planting of summer annual crops (Mueller 2012) and has been documented as an alternative host for soybean cyst nematode (*Heterodera glycines*) (Creech et al. 2007).

Downy brome density greatly affected winter wheat yield regardless of the presence or absence of henbit (Figures 2.8 and 2.10). The studied populations of downy brome emerged very close in time relative to the winter wheat crop. Thus, the impact of this population was accentuated due to season-long competition with winter wheat. In 2011, a downy brome density of 40 plants $m^{-2}$ was predicted with both models to cause 33% yield loss (Figures 2.7 and 2.8), which is similar to that observed by Stahlman and Miller (1990). In 2012, predicted wheat yield loss from 40 downy brome plants $m^{-2}$ was 13% based on parameter estimates of both the linear and multispecies models (Figures 2.9 and 2.10). Results from 2012 were also similar to that of Rydrych (1974) who found downy brome at a density of 132 plants $m^{-2}$ reduced wheat yields by 40%. Therefore, winter wheat yields could be reduced between 13 and 33% in the presence of 40 downy brome plants $m^{-2}$. This also further emphasizes the timing of weed emergence relative to the crop as an important factor in weed management decisions.

The practical application of these results is consistent with that of current production practices. Kansas winter wheat growers typically rely on fall- or spring-applied herbicides as the primary effective tool to control weeds, specifically downy brome (Geier et al. 1998; 2002). Based on these results, applications of fall-applied herbicides should occur within 30 to 40 DAP to prevent yield loss and create a more competitive environment for the crop against the weeds if they are not removed in the spring. The application of a preemergence herbicide at planting would also be beneficial in decreasing the density and delaying the onset of downy brome competition (Stahlman and El-Hamid 1994) and could be utilized if the field has a history of downy brome. To prevent yield loss, the preemergence herbicide should provide control equal to or longer than the critical weed-free period, a minimum of four weeks as determined by these results. If an herbicide application does not occur during this initial period, winter wheat yield loss can be prevented by applying an herbicide(s) upon release of winter dormancy in the spring to prevent future yield loss associated with weed competition. This practice is utilized primarily to control weeds under the assumption that all the weeds have emerged by this time and the winter wheat stand is adequate. Growers should scout to determine the density of the weeds present in the field in the spring and highlight the urgency in making this application. ALS- and ACCase-inhibiting herbicides that are presently registered in winter wheat are effective at controlling downy brome; however, if development of downy brome biotypes with herbicide resistance occurs, a change in production practices to further emphasize the duration of the weed-free period will be warranted. It can also be concluded, based on the results that yield loss did not occur with no weeds and as a result of the imazamox application (Figures A.1, A.2, A.3, and A.4). In addition, the role of weed species germination patterns relative to the crop should always be taken into account. Biological, mechanical, and cultural practices such as utilizing wheat cultivars with early season vigor, increased tillering, and selective placement of fertilizer may be utilized to delay the onset of weed competition and provide for a more competitive crop. Later emerging weeds will likely have little effect on the yield of winter wheat; however, the weeds will likely mature and contribute to the seed bank and to future weed management problems.

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## Figures and Tables

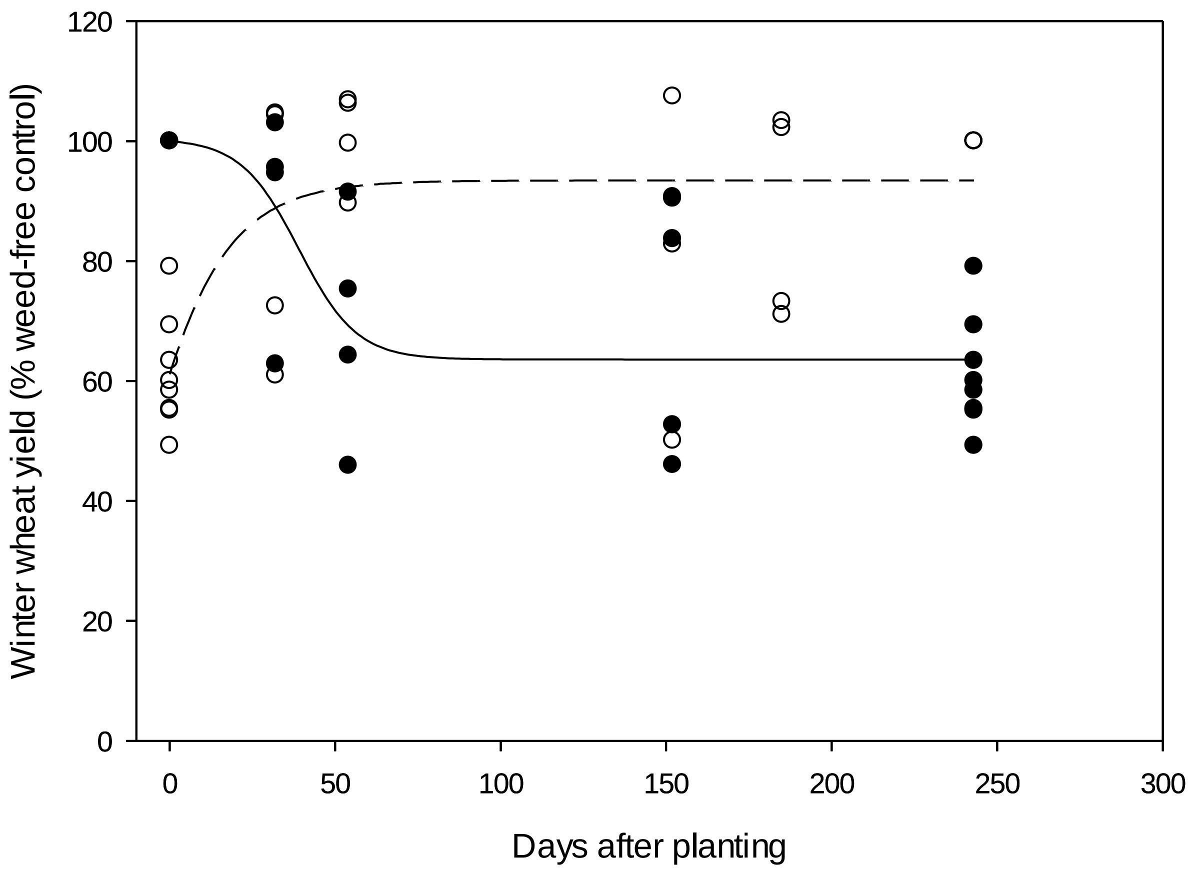


Figure 2.1 Winter wheat yield as a function of increasing duration of weed interference (●), and increasing weed-free period (○) at Larned (b), KS in 2011. Solid line was predicted from fitting the three-parameter logistic model (Equation 2) and the dashed line was predicted from the Gompertz model (Equation 1).

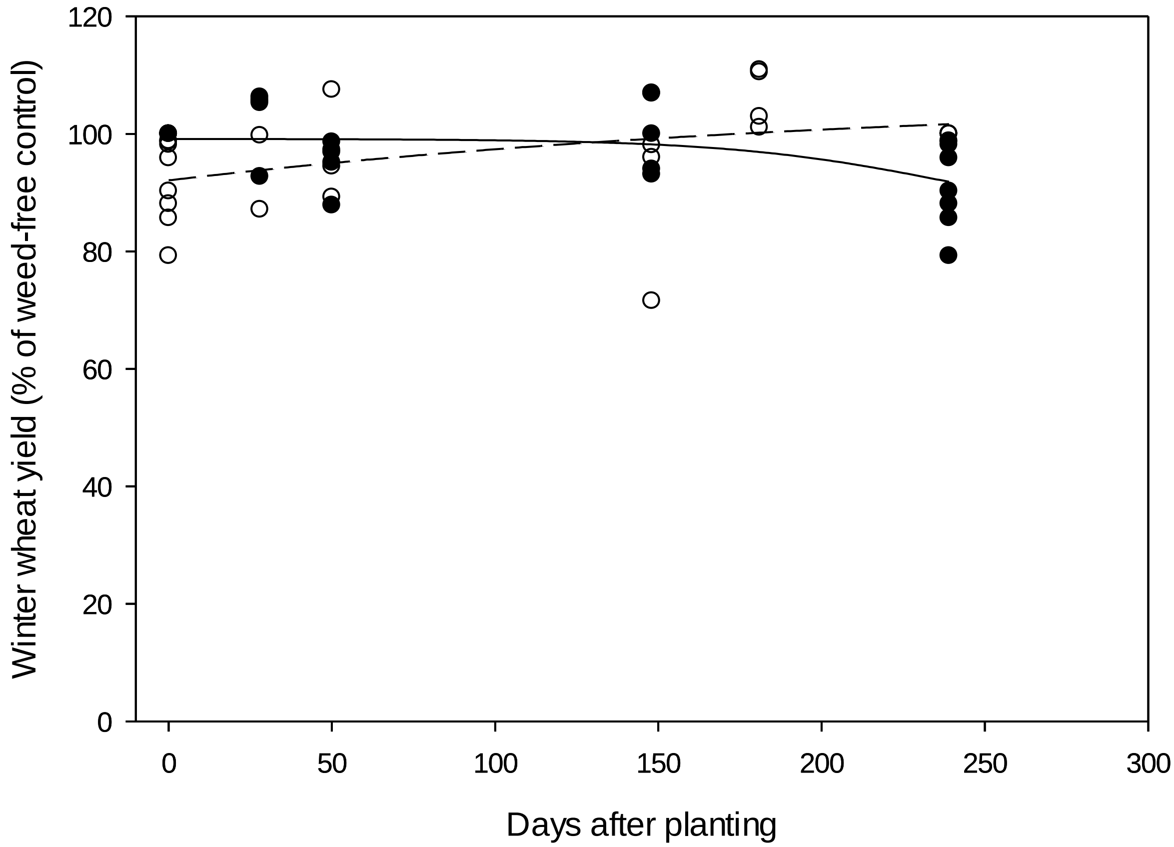


Figure 2.2 Winter wheat yield as a function of increasing duration of weed interference (●), and increasing weed-free period (○) at Larned (a), KS in 2011. Solid line was predicted from fitting the three-parameter logistic model (Equation 2) and the dashed line was predicted from the Gompertz model (Equation 1).

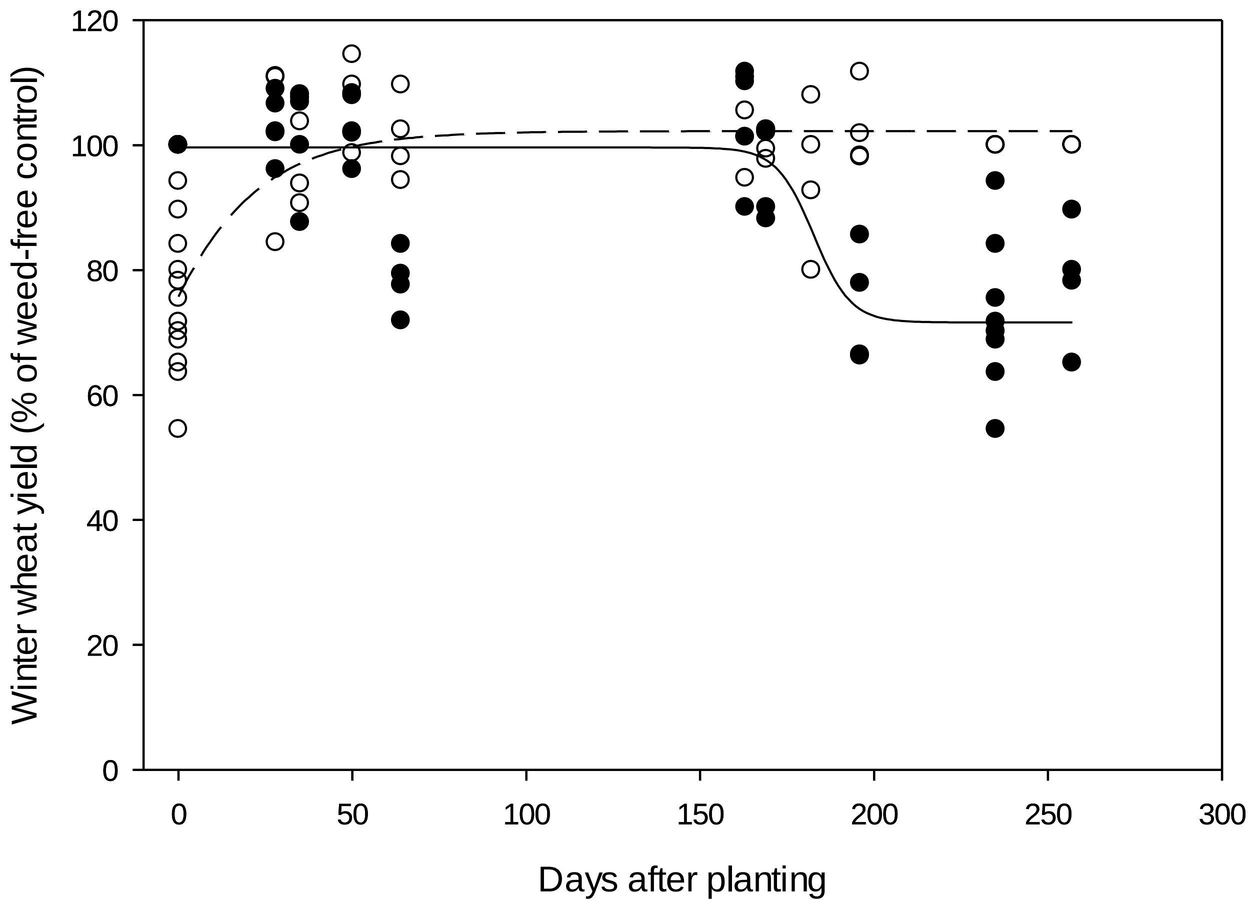


Figure 2.3 Winter wheat yield as a function of increasing duration of weed interference (●), and increasing weed-free period (○) at the Kansas State University Department of Agronomy Ashland Bottoms Research Farm near Manhattan, KS in 2011. Solid line was predicted from fitting the three-parameter logistic model (Equation 2) and the dashed line was predicted from the Gompertz model (Equation 1).

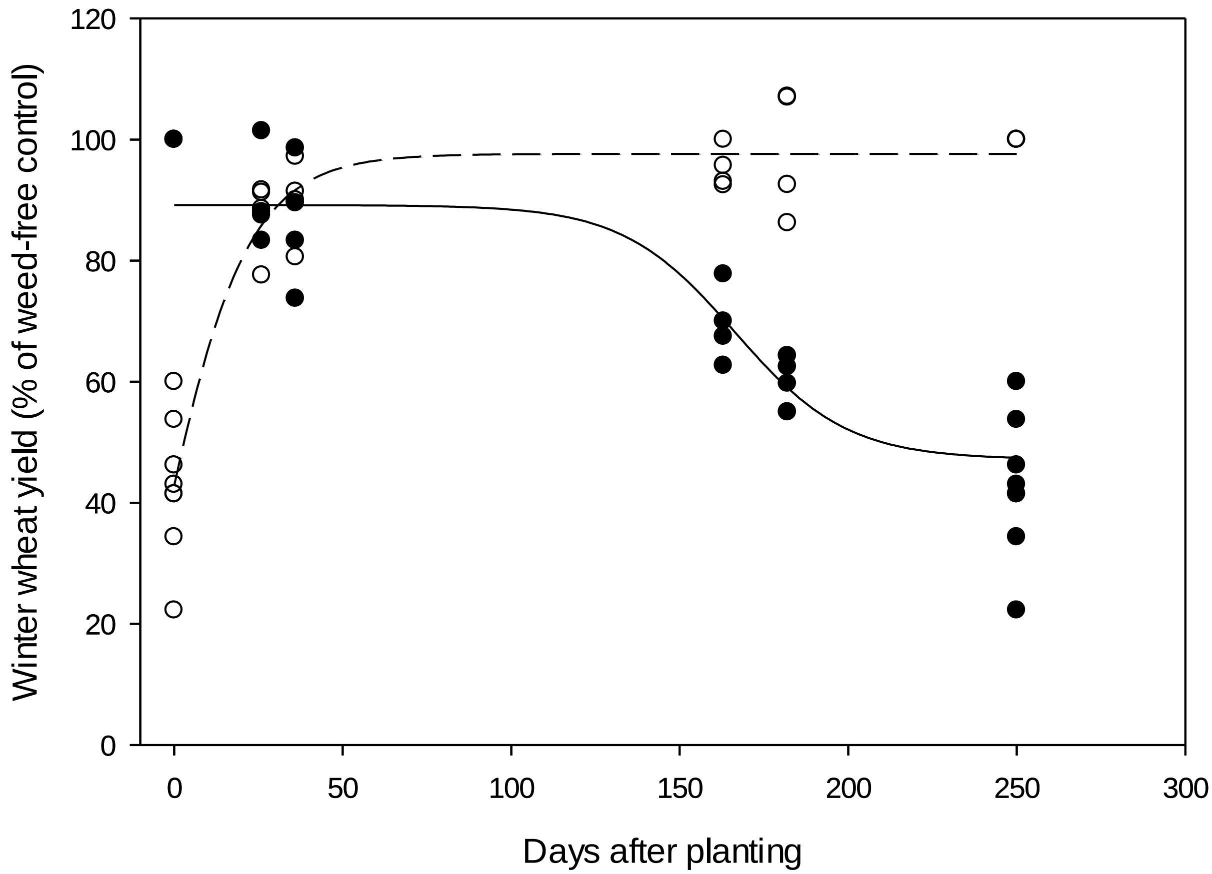


Figure 2.4 Winter wheat yield as a function of increasing duration of weed interference (●), and increasing weed-free period (○) at the Kansas State University Agricultural Research Center at Hays, KS in 2011. Solid line was predicted from fitting the three-parameter logistic model (Equation 2) and the dashed line was predicted from the Gompertz model (Equation 1).

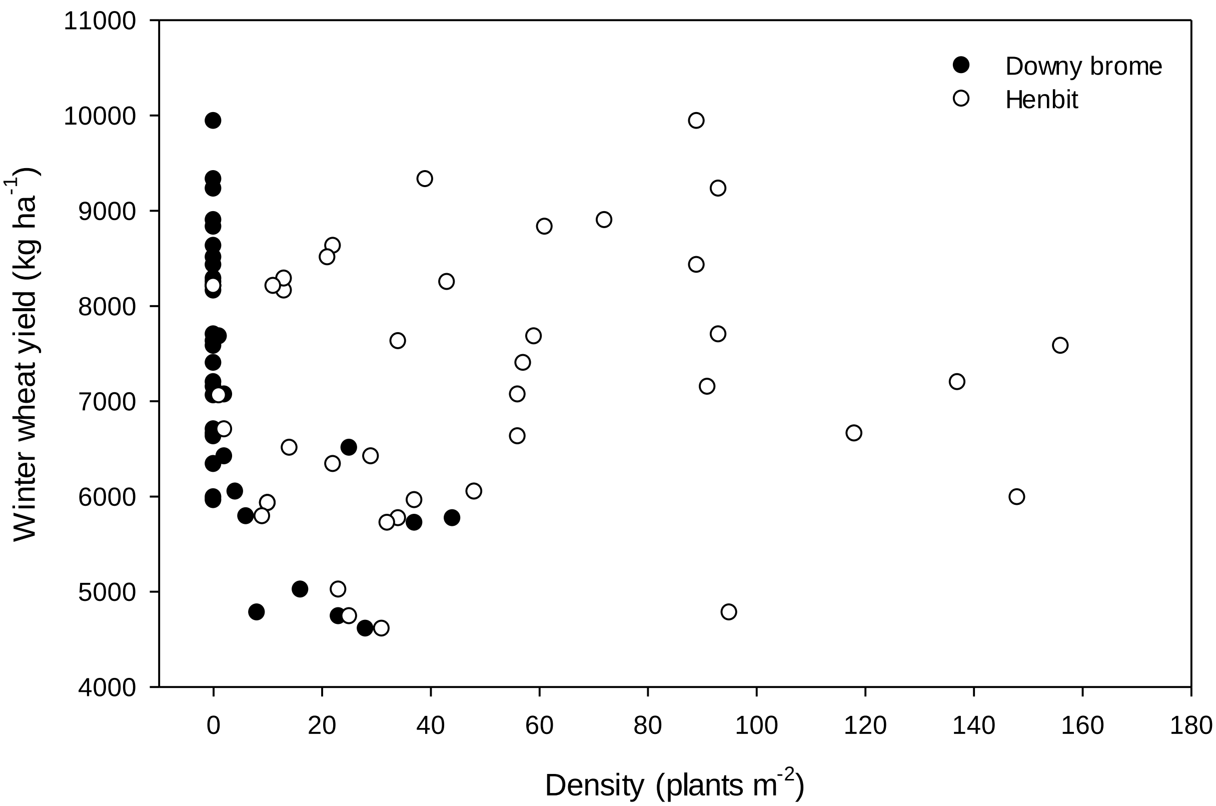


Figure 2.5 Winter wheat yields at two locations in north central Kansas as affected by the density of downy brome and henbit in 2011.

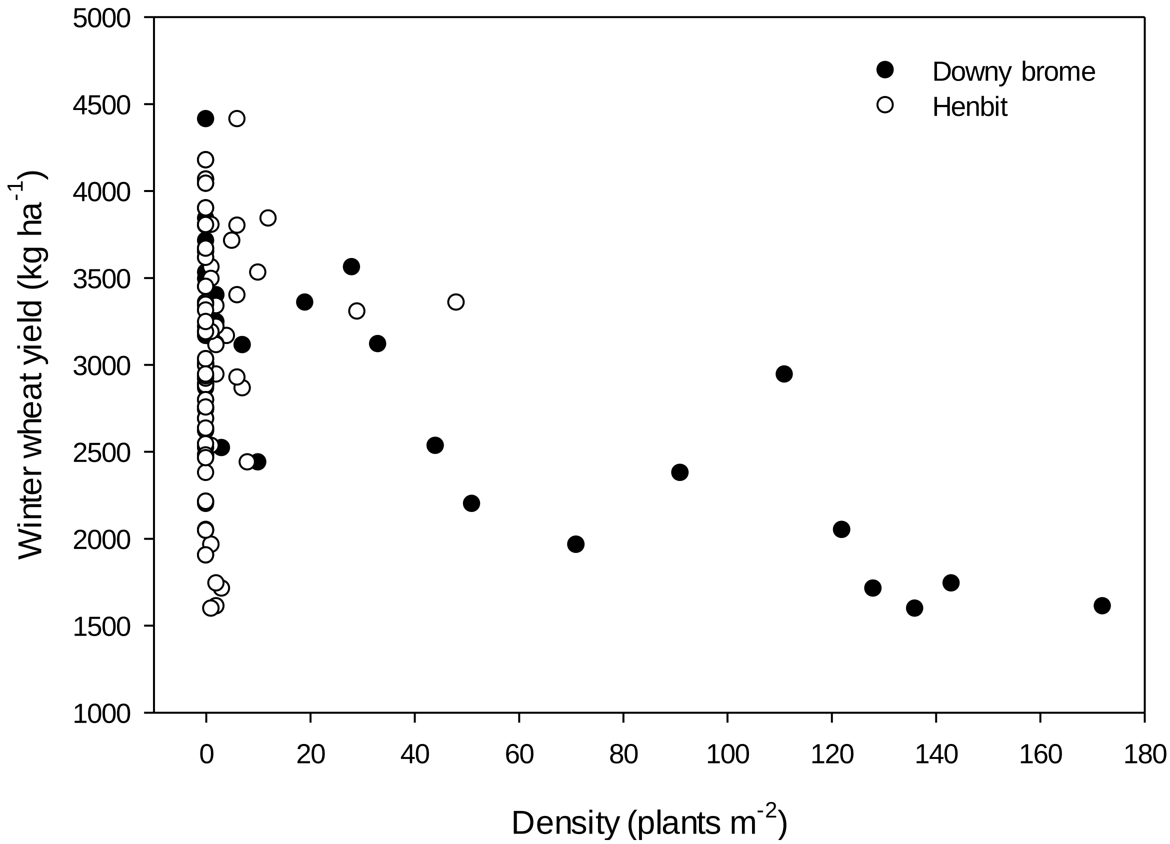


Figure 2.6 Winter wheat yields at three locations in north central Kansas as affected by the density of downy brome and henbit in 2012.

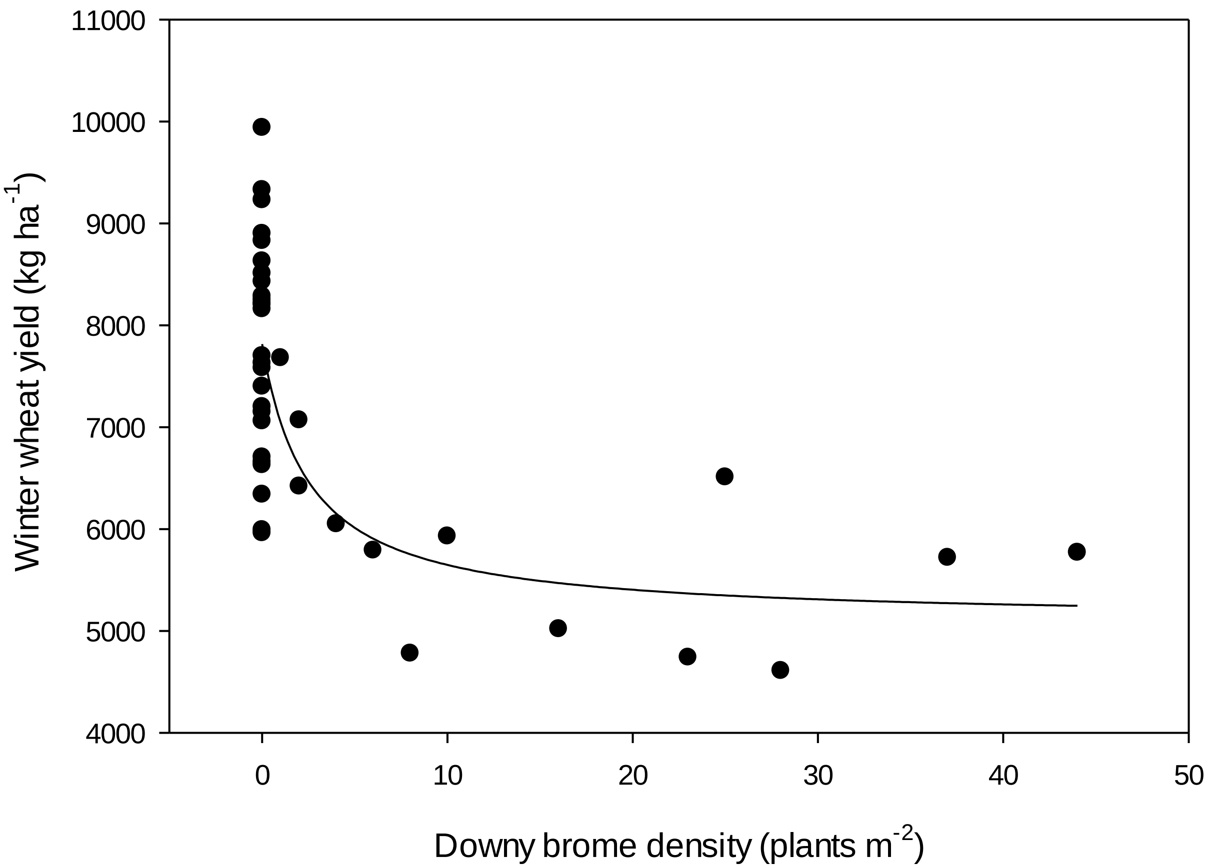


Figure 2.7 Winter wheat yield as affected by downy brome density in 2011 as fitted to Equation 3, where $Y$ = 7813*(1-(13.75*x)/(100*(1+13.75*x/34.78)))) with a $R^2$ of 0.52.

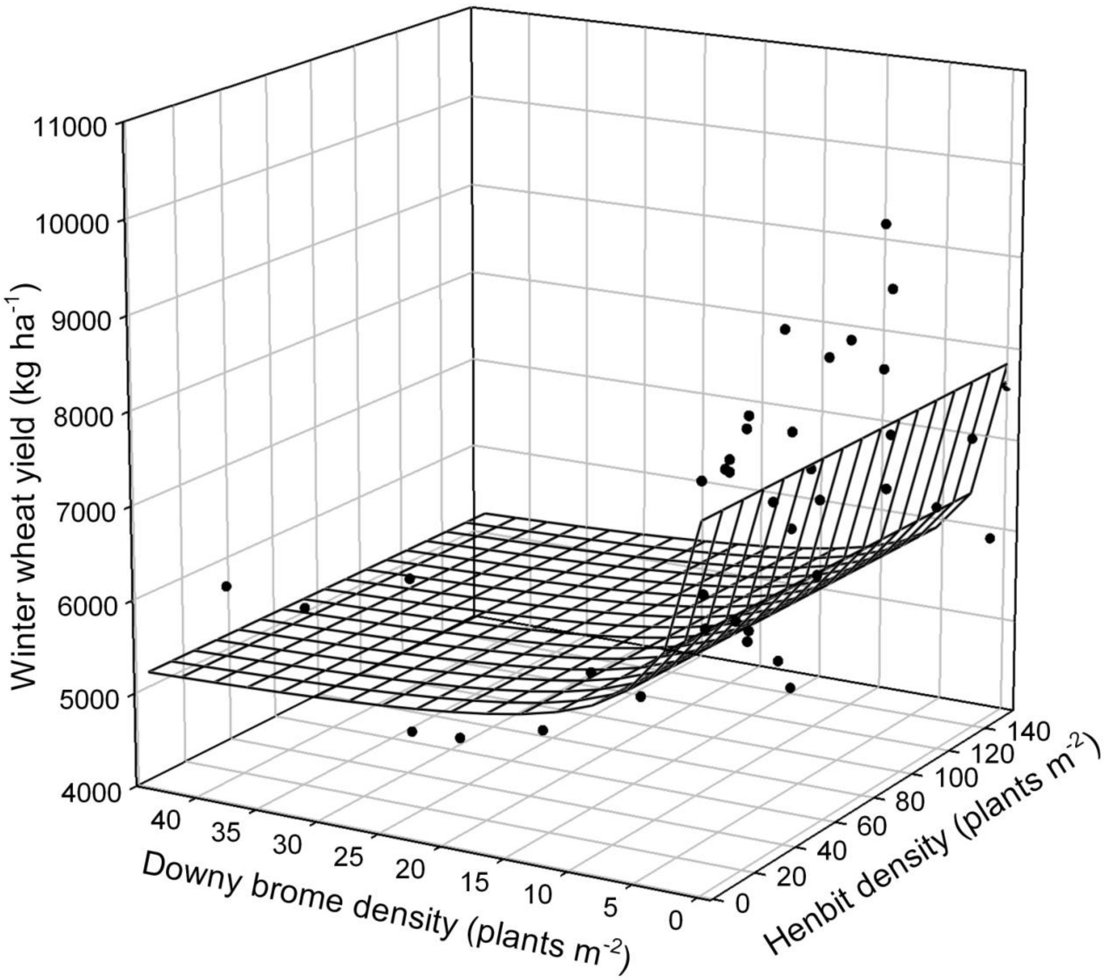


Figure 2.8 Predicted winter wheat yield as affected by downy brome and henbit density in 2011 as fitted to Equation 5 where $Y_{WF}$ = 7812, i$h$ = -0.0008, i$d$ = 13.09, and $a$ = 34.87 with a $R^2$ of 0.50.

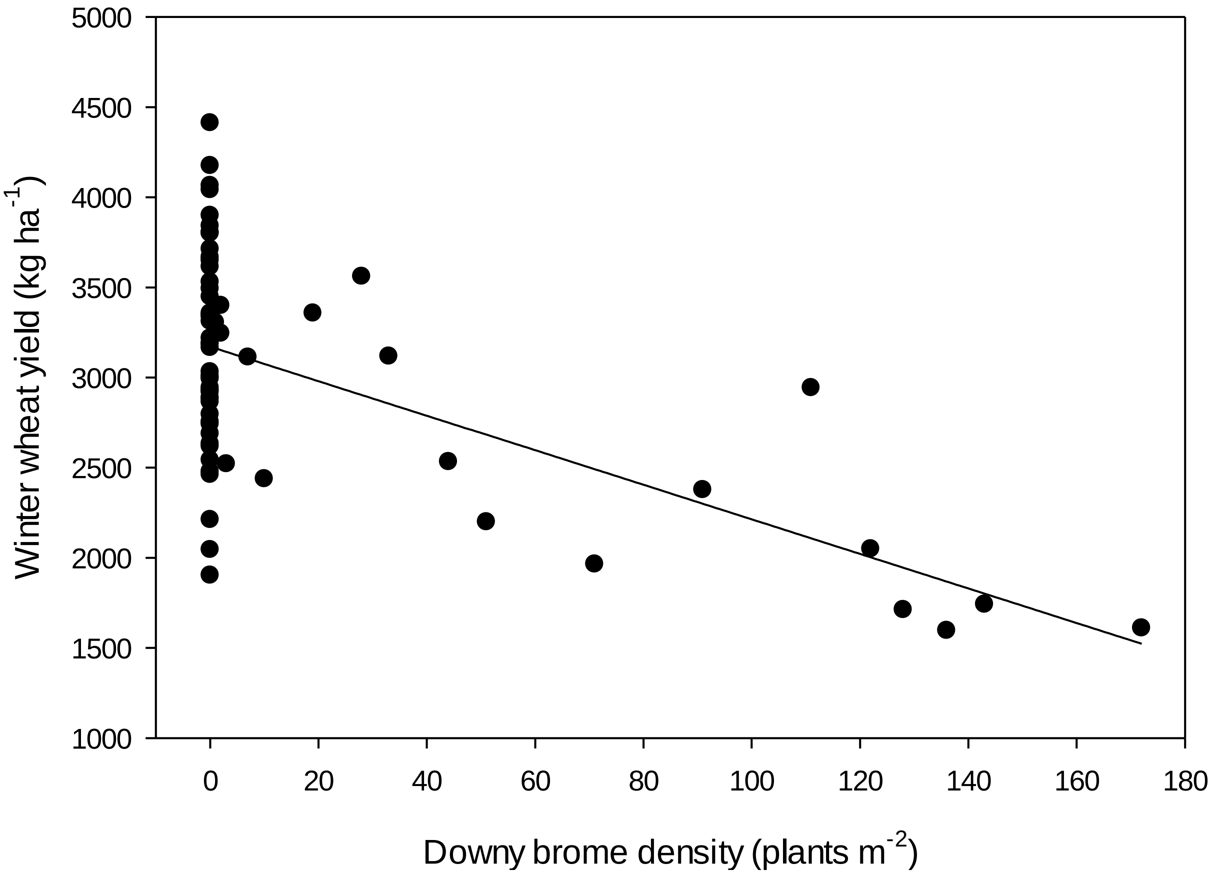


Figure 2.9 Winter wheat yield as affected by downy brome density in 2012. Relationship between winter wheat yield and downy brome density as fit to Equation 4, where $Y = -9.58x + 3172$, with a $R^2 = 0.35$.

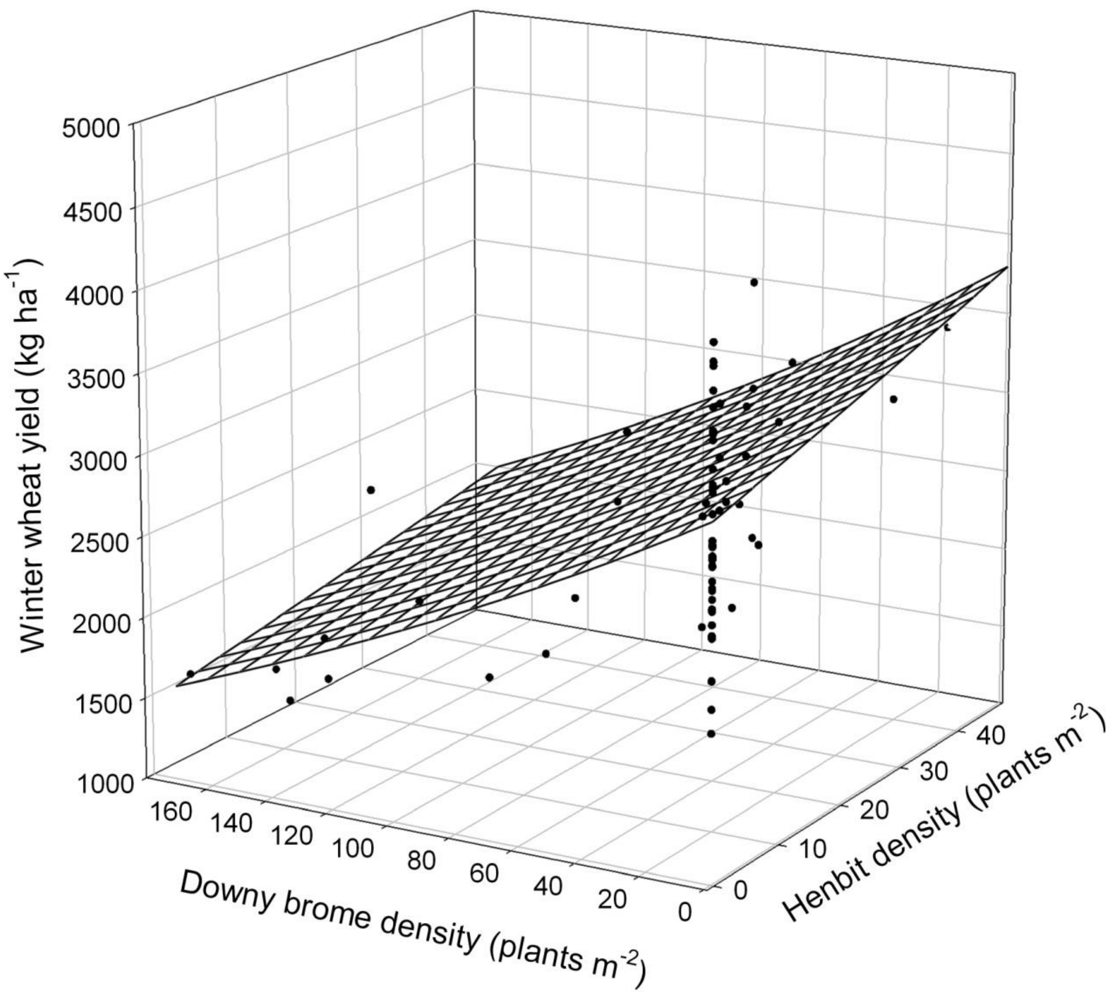


Figure 2.10 Predicted winter wheat yield as affected by downy brome and henbit density in 2011 as fitted to Equation 5 where $Y_{WF}$ = 3142, i$h$ = -0.40, i$d$ = 0.35, and $a$ = 261 with a $R^2$ of 0.36.

Table 2.1 Planting, herbicide application and harvest information for winter wheat established in 2010 and 2011 across Kansas.

| | | | | | | Herbicide application dates | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Year initiated | Moisture | Planting date | Variety | Seeding rate (kg $ha^{-1}$) | TRT A | TRT B | TRT C | TRT D | TRT E | Harvest date |
| Manhattan | 2010 | Dryland | 6 Oct | AP503 | 83.8 | 6 Oct | 10 Nov | 9 Dec | 24 Mar | 20 Apr | 20 Jun |
| Belpre | 2010 | Dryland | 30 Sep | Infinity CL | 94.9 | 18 Oct | 10 Nov | 9 Dec | 4 Mar | NA† | 30 Jun |
| Larned (a) | 2010 | Irrigated | 18 Oct | AP503 | 106.1 | 23 Oct | 10 Nov | 9 Dec | 4 Mar | NA | 30 Jun |
| Manhattan | 2011 | Dryland | 17 Oct | AP503 | 83.8 | 17 Oct | 14 Nov | 6 Dec | 28 Mar | NA | 8 Jun |
| Belpre | 2011 | Dryland | 20 Oct | AP503 | 78.2 | 21 Oct | 14 Nov | 6 Dec | 14 Mar | NA | 13 Jun |
| Hays | 2011 | Dryland | 5 Oct | Clara CL | 68.1 | 6 Oct | 31 Oct | 10 Nov | 16 Mar | 4 Apr | 11 Jun |
| Larned (a) | 2011 | Irrigated | 18 Oct | AP503 | 106.0 | 20 Oct | 15 Nov | 7 Dec | 14 Mar | NA | 12 Jun |
| Larned (b) | 2011 | Irrigated | 14 Oct | Infinity CL | 100.5 | 20 Oct | 15 Nov | 7 Dec | 14 Mar | NA | 12 Jun |

† Not applied.

Table 2.2 Winter wheat growth stages and description of timing of herbicide application for each treatment code to establish treatments for increasing weed-free period and duration of weed interference.

| | Treatment | Application code | Imazamox rate (g $ha^{-1}$) | Corresponding growth stage and timing | Feekes stage | Zadoks stage |
|---|---|---|---|---|---|---|
| Weed-free period | 1 | TRT A | 52.5 f/b 35.0 | Weed-free all season. Preemergence to weed and crop, second application prior to winter dormancy, | 0 | 0 |
| | 2 | TRT A | 52.5 | Residual control of weeds to 2 to 3 leaf wheat, fall application | | |
| | 3 | TRT A | 35.0 | Residual control of weeds to tillering wheat, prior to winter dormancy | | |
| | 4 | TRT A | 17.5 | Residual control of weeds to tillering wheat, resumption of growth in early spring | | |
| | 5 | TRT A | 8.8 | Residual control of weeds to stem elongation of wheat, prior to jointing in spring | | |
| Duration of weed interference | 6 | TRT B | 52.5 | 2 to 3 leaf wheat, fall application | 2 | 21 |
| | 7 | TRT C | 52.5 | Tillering wheat, prior to winter dormancy | 3 | 26 |
| | 8 | TRT D | 52.5 | Tillering wheat, resumption of growth in early spring | 4 | 30 |
| | 9 | TRT E | 52.5 | Stem elongation of wheat, prior to jointing in spring | 5 to 6 | 30 to 31 |
| | 10 | | | Weedy all season | | |

Table 2.3 Species spectrum, density, and mean shoot biomass in weed interference control treatments in November and March at eight site-years in Kansas.

| Location | Year | Time of observation | Weed species | Density | Total density | Total shoot biomass |
|---|---|---|---|---|---|---|
| | | | | Plants $m^{-2}$ | | g $m^{-2}$ |
| Manhattan | 2010 | November | Downy brome | 52 | 104 | 0.56 |
| | | | Henbit | 48 | | |
| | | | Blue mustard | 4 | | |
| | | March | Downy brome | 72 | 114 | 31.53 |
| | | | Henbit | 40 | | |
| | | | Blue mustard | 2 | | |
| Belpre | 2010 | November | None | | | |
| | | March | None | | | |
| Larned (a) | 2010 | November | Henbit | 8 | 8 | 0.01 |
| | | March | Henbit | 4 | 4 | 0.41 |
| Manhattan | 2011 | November | Downy brome | 76 | 76 | 0.51 |
| | | March | Downy brome | 22 | 24 | 6.20 |
| | | | Henbit | 2 | | |
| Belpre | 2011 | November | None | | | |
| | | March | None | | | |
| Hays | 2011 | November | Downy brome | 100 | 120 | NR† |
| | | | Blue mustard | 20 | | |
| | | March | Downy brome | 12 | 22 | NR |
| | | | Blue mustard | 10 | | |
| Larned (a) | 2011 | November | Henbit | 11 | 11 | 0.01 |
| | | March | Henbit | 6 | 6 | 0.56 |
| Larned (b) | 2011 | November | Downy brome | 78 | 78 | 0.44 |
| | | March | Downy brome | 42 | 42 | 13.55 |

† NR = Not recorded.

Table 2.4 Winter wheat yield response (expressed as a percentage of the weed-free control) to increasing duration of weed infestation in days after planting of the crop at several sites in Kansas in 2010 and 2011. Logistic equation parameter estimates are followed by standard errors in parentheses. Refer to Materials and Methods for a description of the logistic model fitted.

| | | Parameter estimates† | | | | |
|---|---|---|---|---|---|---|
| Location | Year | *A* | *B* | *C* | *D* | $R^2$ |
| Manhattan | 2010 + 2011 | 34.00 (24.59) | 0.18 (0.13) | 71 (1.69) | 28 (2.05) | 0.52 |
| Hays | 2011 | 10.00 (6.44) | 0.06 (0.03) | 47 (2.20) | 42 (2.86) | 0.82 |
| Larned (a) | 2011 | 6.86 (10.66) | 0.029 (0.04) | 84 (1.95) | 15 (1.97) | 0.17 |
| Larned (b) | 2011 | 4.45 (3.70) | 0.11 (0.09) | 63 (4.22) | 36 (9.30) | 0.46 |

† Parameter estimates *A* and *B* are parameters that determine the shape of the curve, *C* is the lower asymptote, *D* is the difference between the upper and lower asymptotes, and T is time in DAP (days after planting).

Table 2.5 The length of the weed free period and the duration of weed interference for winter wheat in days after planting (DAP) as calculated from fits of the Gompertz and logistic equations (Equations 1 and 2) for each site-year at 5 and 10% yield loss levels.

| | | Weed-free period | | Duration of weed interference | |
|---|---|---|---|---|---|
| | | 5% | 10% | 5% | 10% |
| Location | Year | DAP | | | |
| Manhattan | 2010 + 2011 | 34 | 20 | 174 | 178 |
| Hays | 2011 | 37 | 27 | 129 | 143 |
| Larned (a) | 2011 | 50 | 0 | 208 | ∞† |
| Larned (b) | 2011 | 32 | 20 | 23 | 30 |

†Using the fitted equation, predicted yield loss was <10% even when weed remained to crop harvest.

Table 2.6 Parameter estimates (and standard errors) for Gompertz equation (Equation 1) for winter wheat yield response (expressed as a percentage of the weed-free control) to increasing weed free period in days after planting the crop at several sites in Kansas in 2010 and 2011. Refer to Materials and Methods for a description of the Gompertz model.

| | | Parameter estimates† | | | |
|---|---|---|---|---|---|
| Location | Year | *A* | *B* | *K* | $R^2$ |
| Manhattan | 2010 + 2011 | 102 (1.46) | 0.29 (0.03) | 0.05 (0.05) | 0.61 |
| Hays | 2011 | 97 (4.53) | 0.82 (0.42) | 0.07 (0.06) | 0.89 |
| Larned (a) | 2011 | 106 (32.70) | 0.14 (0.30) | 0.005 (0.19) | 0.09 |
| Larned (b) | 2011 | 93 (4.53) | 0.42 (0.10) | 0.06 (0.06) | 0.40 |

† Parameter estimates are *A* is the upper asymptote, *B* and *K* are parameters that determine the shape of the curve, T is time in DAP (days after planting).

Table 2.7 Parameter estimates (and standard errors) from Equations 3, 4, and 5 for downy brome and combination of downy brome and henbit interference with winter wheat. Refer to Results for a description of the fitted models.

| | | Parameter estimates† | | | | |
|---|---|---|---|---|---|---|
| Year | Weed species | $Y_{WF}$ | $I_h$ | $I_d$ | $a$ | $R^2$ |
| | | kg ha$^{-1}$ | ---------- % ---------- | | | |
| 2010-2011 | Downy brome | 7817 (186) | | 13.75 (10.85) | 34.78 (6.99) | 0.52 |
| | Downy brome + henbit | 7812 (217) | -0.0008 (0.02) | 13.08 (17.16) | 34.86 (7.18) | 0.50 |
| 2011-2012 | Downy brome | 3172 (70) | | 0.30 (0.04) | | 0.35 |
| | Downy brome + henbit | 3142 (75) | -0.40 (0.27 | 0.35 (0.23) | 260.62 (975.14) | 0.36 |

† Parameter estimates $Y_{WF}$ is weed-free yield; $I_h$ and $I_d$ are crop yield loss (%) as density of each species approaches zero; and $a$ represents the maximum yield loss (%) as weed density approaches infinity.

# Chapter 3 - Differential exposure of four plant species to flumioxazin and pyroxasulfone

## Abstract

Flumioxazin and pyroxasulfone are herbicides currently registered and labeled for preemergence application in corn, soybean and wheat for the control of many broadleaf and grass weeds. The goals of this research were to 1) develop a methodology that could evaluate root and shoot exposure of soil-applied herbicides to weeds and crops, and 2) measure crop and weed response to root and shoot exposure to flumioxazin and pyroxasulfone. The specific objective of the study was to determine the extent of injury based on localized herbicide exposure to roots, shoots, and to both roots and shoots utilizing a novel technique. Herbicides evaluated included flumioxazin, pyroxasulfone, and the combination of flumioxazin + pyroxasulfone. The 1x field use rates utilized were flumioxazin at 71.5 g ai ha$^{-1}$ and pyroxasulfone at 89 g ai ha$^{-1}$. Two weed species, ivyleaf morningglory (*Ipomoea hederacea*) and shattercane (*Sorghum bicolor*), as well as two crops wheat and soybean, were evaluated for injury after root, shoot or root + shoot exposures. Weeds were exposed to 0.25, 0.5 and 1x field use rates, whereas crops were exposed to 1, 2, and 4x field use rates. Pre-germinated seedlings were transferred into specially designed two-petri dish combination to physically limit seedling exposure to herbicides, such that the hypocotyl or coleoptile was on one side (shoot-exposure dish) and roots on the other side (root-exposure dish). The location and expression of symptoms from the flumioxazin and pyroxasulfone herbicides were determined to be the shoot of germinating seedlings.

## Introduction

Preemergence herbicides are the foundation for chemical weed management in soybean (*Glycine max)*; however, the application of a preemergence herbicide is not common practice within winter wheat (*Triticum aestivum*) production. Flumioxazin, sold as Valor® herbicide, is used currently throughout the United States as a preemergence soybean herbicide, and recently was registered for use in winter wheat (EPA registration #59639-99). Pyroxasulfone, sold as Zidua® herbicide, was recently registered for preemergence use in soybeans, with pending registration for application in winter wheat (EPA registration #7969-338). These two herbicides are characterized as having low use rates, flexibility in crop rotation, and provide residual control of broadleaf and grass weeds. Ivyleaf morningglory has become a prominent weed throughout the Midwest in the past decade due to its inherent tolerance to glyphosate and delayed emergence relative to other summer annual weeds (Baucom and Mauricio 2004). Shattercane is a large-seeded summer annual grass that is not as prevalent, but can also be found throughout the Midwest in row crop production (USDA 2013). Both flumioxazin and pyroxasulfone can be applied to effectively control either weed species; however, in many instances the grower may run the risk of crop injury if herbicides are not applied in a timely matter or heavy rainfall occurs at the time of crop emergence.

Flumioxazin, formerly known as S-53482 and V-53482, is a *N*-phenyl phthalimide herbicide that was discovered and developed by Sumitomo Chemical Company in 1987 and is currently registered and labeled for preemergence weed control in corn, soybean, wheat and numerous other crops (Cranmer et al. 2000; Theodoridis 2007; Yoshida et al. 1991). The mechanism of action for flumioxazin is the inhibition of protoporphyrinogen oxidase (PPO) of chlorophyll biosynthesis pathway in the presence of light and oxygen (Nagano et al. 2001; Sato et al. 1987; Yoshida et al. 1991). This inhibition induces the accumulation of protoporphyrinogen IX because of uncontrolled autooxidation of the substrate (Dayan et al. 1997a; Duke et al. 1991). As protoporphyrinogen IX accumulates and is photoenergized by light, toxic radicals are generated, which leads to degradation of the plasmalemma and tonoplast membrane lipids causing irreversible damage (Duke et al. 1990; Jacobs et al. 1991; Shibata et al. 1992). The herbicide has numerous environmental benefits including low use rate of 70 to 105 g $ha^{-1}$, rapid soil dissipation, and is active in controlling biotypes of weeds resistant to different herbicide modes of action (Alister et al. 2008; California Department of Pesticide Regulation Public Report 2003-6; Ferrell and Vencill 2003; Ferrell et al. 2005; Lu et al. 2000).

To understand the mechanistic activity of herbicides that inhibit PPO, researchers have focused on activity of foliar-applied products, such as acifluorfen and lactofen and not necessarily those that are soil-applied such as flumioxazin, sulfentrazone, and saflufenacil. However, Sato et al. (1991) determined that the reactions within cucumber (*Cucumis sativis* L. cv Sachikaze) seedlings were the same for both acifluorfen and flumioxazin. It was demonstrated with acifluorfen, one of the many herbicides that inhibit PPO, that soybeans utilize metabolic detoxification to infer tolerance and show little to no response to the herbicide (Frear et al. 1983). Sherman et al. (1991) examined numerous species of plants with varying susceptibility to acifluorfen and determined there could be several mechanisms of tolerance, such as the production of protoporphyrin IX, in response to herbicide application. Soybean susceptibility to sulfentrazone was due to differential metabolism and differential tolerance to herbicide-induced peroxidative stress (Dayan et al. 1997a). Price et al. (2004) examined sickle pod (*Senna obtusifolia*), peanut (*Arachis hypogaea*), and ivyleaf morningglory (*Ipomoea hederacea*) seedlings after root-exposure to flumioxazin. Differences were evident, with peanut metabolizing flumioxazin three times faster than the susceptible morningglory species. Soybean seedlings exposed to radio-labeled $C^{14}$ sulfentrazone had significantly more absorption through the roots as opposed to absorption through the cotyledon or hypocotyl (Li et al. 2000). This variance in absorption has been cited as the contributing factor to the differential response of sensitive and non-sensitive weeds and crops for sulfentrazone, another PPO-inhibiting herbicide and metribuzin, a PS II-inhibiting herbicide (Li et al. 2000; Mangeot et al. 1979).

Pyroxasulfone, developed as KIH-485, is a new preemergence herbicide that is registered in corn, soybeans, and wheat for the control of many broadleaf and grass weeds at use rates of 125 to 250 g $ha^{-1}$ (Steele et al. 2005; Tanetani et al. 2009). The mechanism of action for pyroxasulfone is similar to that of compounds in the chloroacetamide family, such as alachlor, acetochlor and metolachlor, which inhibit very long chain fatty acid (VLCFA) biosynthesis (Tanetani et al. 2009; Tanetani et al. 2011). Tanetani et al. (2009; 2011) identified seven sites of VLCFA inhibition with pyroxasulfone, unlike the two associated with other chloroacetamide compounds. Herbicides within the family of VLCFA biosynthesis do not inhibit seed germination but inhibit shoot elongation of germinated seeds (Tanetani 2012). More recent research and technological advancements have confirmed the target of chloroacetamide herbicides as causing primary inhibition in emerging shoots of seedlings (Böger et al. 2000). Shoots of giant foxtail (*Setaria faberi*) and shattercane seedlings exposed to pyroxasulfone were controlled more than those with roots exposed to pyroxasulfone; velvetleaf (*Abutilon theophrasti*) control was primarily attributed to absorption of pyroxasulfone through the shoot and secondarily through the roots (Kumiai internal data).

Placement of soil-applied herbicides relative to seed germination and subsequent plant emergence is of great importance. This would suggest for flumioxazin and other soil-applied PPO herbicides that after germination, inhibition does not occur until light reaches the seedling, suggesting that selectivity is based on placement of seed relative to location of herbicide in the soil. In a greenhouse study, Vencill (2002) reported that peanut injury from flumioxazin was related to planting depth. Previous research examining controlled exposure has utilized plastic barriers as means of physical restraint or layers of activated charcoal, which inactivates the herbicide (Appleby and Furtick 1965; Donald 1988; Jordan and Harvey 1980). The efficiency of deactivation depends on soil organic matter, physical condition of the soil medium, herbicide activity, and crop sensitivity (Ahrens 1964; Fishel 2011; Toth et al. 1987; Warren 1973). Limitations to the methodology may be associated with the solubility of the herbicide that is to be deactivated in addition to physical placement of layers of activated charcoal in containers, which may permit herbicide to seep along the outside walls.

The performance of soil-applied herbicides is influenced by many factors (Holly 1962; Ogle and Warren 1954). These factors include soil pH, cation exchange capacity, organic matter, soil moisture, nutrient availability, photodecomposition, adsorption, leaching, volatilization and the overall texture of the soil such as sand, silt, or clay (Corbin and Upchurch 1967; Upchurch and Mason 1962). Even considering all these factors, if the herbicide is not present at the optimum location for uptake and translocation into the germinating weed seedling, then it will not be effective.

The goals of this research are to 1) develop a methodology that could evaluate root and shoot exposure of soil-applied herbicides to weeds and crops and 2) measure crop and weed response to root and shoot exposure to flumioxazin and pyroxasulfone. The hypothesis is that flumioxazin will affect the roots of plants, while pyroxasulfone would affect the shoots; a combination product (flumioxazin + pyroxasulfone) would have different physical sites of contact and absorption that would affect both the root and shoot of germinating seeds or seedling plants. The specific objective of the study was to determine the extent of injury based on localized herbicide exposure to roots, shoots, and to both roots and shoots utilizing a novel technique.

## Materials and Methods

This procedure was modified from Parker (1966), who utilized square petri dishes in an inverted fashion to examine separate exposure of roots and shoots of seedlings to EPTC.

Herbicides evaluated included flumioxazin, pyroxasulfone, and the combination of flumioxazin + pyroxasulfone. The 1x field use rates utilized were flumioxazin at 71.5 g ai $ha^{-1}$ and pyroxasulfone at 89 g ai $ha^{-1}$. Two weed species, ivyleaf morningglory and shattercane, as well as wheat and soybean were evaluated for injury after root, shoot, or root + shoot exposures. Weeds were exposed to 0.25, 0.5 and 1x field use rates, whereas crops were exposed to 1, 2, and 4x field use rates. The herbicide solution utilized in this methodology was equivalent to that of a spray solution for herbicide application, thus more concentrated than an actual field spray application or what would be found in the soil solution.

The bottoms of two square petri dishes were fused together utilizing a soldering iron by making five indentations between the two dishes. The melted plastic fused the dishes together and also created grooves for seedling placement (Figure 3.1). The grooves were cleaned of excess plastic residue. The lids of the two dishes were attached to each other using clear duct tape to allow hinge-like flexibility. One dish was designated as the shoot-exposure dish and the other dish designated as the root-exposure dish. Individual petri dishes were then filled with 125 g of dry silica sand.

Weed and crop seed were germinated in moist silica sand in the greenhouse to approximately 2 cm shoot growth for grasses and 5 cm shoot growth for broadleaves. Three to five pre-germinated seedlings were then transferred into each specially designed two-petri dish combination to physically limit plant exposure, such that the hypocotyl or coleoptile was on one side (shoot-exposure dish) and roots on the other side (root-exposure dish). The seed of grasses, if still attached, was placed in the groove itself as to physically limit the exposure to root or shoot cells. Upon placement of seedlings into the two-petri dish combination, they were then transferred to a growth chamber where conditions were 24°C with a 24 hour photoperiod. Three replicates were placed in a growth chamber at one time. The dishes were laid flat and no effort was made to avoid phototropism; however, none was observed.

Each half of the two-petri dish combination was then filled with appropriate herbicide treatment and covered. The herbicide treatments included none (30 ml distilled water), 30 ml herbicide solution for shoot exposure only, 30 ml herbicide solution for root exposure only, or 30 ml herbicide solution in each half of dish for both root + shoot exposure. Each herbicide by species combination was replicated at least six times. Flumioxazin treatments were evaluated after 48 hours, whereas the pyroxasulfone and flumioxazin + pyroxasulfone treatments were evaluated after 72 hours. The variation in evaluation timing was due to the differences between the rate of symptom development of each herbicide. Each two-petri dish combination was examined to determine the number of plants injured or dead. The numbers were then converted to a percentage of affected plants per treatment relative to the untreated water control:

$$\% \text{ of plants affected} = \frac{\#\text{ in control} - \#\text{ survived treatment}}{\#\text{ in control}} \quad 100 \qquad [6]$$

Data were analyzed for each plant species individually utilizing the MIXED procedure in SAS 9.2 (SAS Institute 2010) with block (growth chamber) and replication within block set as random. Data were then separated using the LSMEANS procedure at alpha = 0.05 significance.

## Results and Discussion

The methodology utilized in this experiment proved to be effective in distinguishing plant survival based on zone of exposure (root and/or shoot). This analysis can therefore be used to evaluate plant susceptibility and selectivity to herbicides. Results were generated quickly and efficiently such that this methodology could be utilized for screening crop and weed selectivity to new compounds prior to greenhouse or field trials. In addition, this methodology can be used to demonstrate herbicide mode of action and symptomology for herbicide physiology courses or weed science training. Considerations should be made to account for the utilization of non-inert media, a high concentration of herbicide, in addition to the maximum light intensity as it specifically pertains to photosynthetic reactions. Unfortunately, only large-seeded weeds and crops were evaluated due to methodology constraints and therefore should be taken into consideration for future utility.

### *Soybean and ivyleaf morningglory*

There was a significant interaction for herbicide and area of exposure as it affected soybean and ivyleaf morningglory seedlings (Table 3.1). Soybean seedlings were affected by root + shoot exposure and shoot exposure to flumioxazin and all areas of exposure to pyroxasulfone. Root exposure to flumioxazin and exposure to the combination of flumioxazin + pyroxasulfone did not result in significant injury (Table 3.2). Ivyleaf morningglory seedlings were affected by shoot and root + shoot exposure to pyroxasulfone and flumioxazin +

pyroxasulfone. Flumioxazin had a similar affect regardless of area of exposure. Ivyleaf morningglory seedlings will emerge from depths of 15 cm, however most emergence occurs when seed are less than 2.5 cm from the soil surface (Cole 1976). Broadleaf weed seedlings that emerge from within this depth of the soil profile are positioned for exposure to herbicides and would subsequently be affected. There were no significant differences between shoot exposure and the combination of root + shoot exposure for any herbicide (Figure 3.2). These results indicate that any reaction of soybean when roots and shoots are in contact with flumioxazin in excess of the labeled rate is likely associated with the hypocotyl and apical exposure. The parallel response in sensitivity is likely a function of similarities in broadleaf plant physiology and development between the crop and weed. The relatively high injury associated with root exposure of soybean to pyroxasulfone is similar to the results of Jordan and Harvey (1978; 1980). They found VLCFA herbicides have greater root than shoot activity on processing peas (*Pisum sativum*), which have a very similar growth and development pattern to that of soybean. Varietal sensitivity of soybeans to preemergence herbicides due to variation in metabolism rates has been cited in previous research (Dayan et al. 1997b; Hutchinson et al. 2005; Taylor-Lovell et al. 2001) and may aid in explaining the level of response we observed in the study.

The percentage of soybean plants affected by herbicide exposure increased as rate increased (Table 3.3). Flumioxazin, pyroxasulfone, and the combination herbicide are currently registered for use on soybean at the 1x field use rate examined in this experiment. The results confirm a level of crop safety, as only 60% of soybean seedlings were injured at the 2x field use rate, but also demonstrate that injury can be significant if misapplication occurs resulting in application rate greater than two-fold field use rate. The utility of this methodology may help with analysis of varietal sensitivity or aid in explaining herbicide injury to soybean seedlings.

Ivyleaf morningglory did not differ in response when exposed to herbicides at the 0.25x or 0.5x field use rates of herbicides as greater than 76% of the seedlings were injured (Table 3.4), while the greatest response occurred at the 1x field use rate with 88.8% of ivyleaf morningglory affected. This is consistent with previous field trial research of flumioxazin, pyroxasulfone, and the combination of two herbicides that are characterized as only providing suppression of *Ipomoea* spp. at this 1x rate.

### *Winter wheat*

The interaction between herbicide and herbicide rate significantly affected winter wheat survival (Table 3.5) and the area of exposure averaged across herbicides significantly affected winter wheat survival (Table 3.6). Greater than 64% of winter wheat seedlings exposed to the combination of flumioxazin + pyroxasulfone were injured regardless of rate. Flumioxazin and pyroxasulfone affected 36 and 20% of the winter wheat, respectively when applied alone at the 1x field use rate. The percentage of injured winter wheat seedlings increased two-fold as the rate increased from 1x to 4x from either herbicide alone. Surprisingly, there was not 100% survival of wheat seedlings when exposed to flumioxazin or pyroxasulfone at the 1x field use rate. This result was unexpected due to the current registration of pyroxasulfone (Sakura® APVMA #: 63998/75259) as a preemergence herbicide in winter wheat in Australia. The wheat variety utilized in this study was a localized variety, 'Fuller'; therefore, differences in sensitivity may be varietal or morphological differences in coleoptile development and length for different wheat varieties (Klepper et al. 1984; Liatukas and Ruzgas 2011; McMaster 1997; Pereira et al. 2002). Area of exposure was significant as greater than 67% of winter wheat seedlings were injured with shoot exposure, while only 28% were injured with root exposure (Table 3.6). Therefore, winter wheat injury in response to root + shoot exposure could be attributed primarily to shoot exposure.

### *Shattercane*

The interaction of herbicide and area of exposure was significant in regards to shattercane susceptibility (Table 3.7). Shoot and root + shoot exposure resulted in greater than 87% of shattercane seedlings being affected regardless of herbicide, while less than 30% of shattercane seedlings were affected by only root exposure to pyroxasulfone or flumioxazin + pyroxasulfone (Figure 3.3). Position of shattercane seed and subsequent germination within the soil profile will greatly affect the control obtained from these herbicides, as seed that germinates within the upper 2 cm of the soil surface will have a significantly less chance of survival due to shoot exposure to the herbicides.

Seedling morphology and its role in crop and weed sensitivity must continue to be evaluated. Specifically, there is a need to focus on the coleoptile, as our results indicated that grass seedling survival was most evident when only roots were exposed, therefore protection from or reduced absorption of herbicides via the coleoptile may describe variance in grass species response to these herbicides when they germinate from below the zone of herbicide activity in the soil. Simply the type of grass seedling emergence can influence the location of the coleoptile and growing point of the seedling (Peterson 1990) which is important for herbicide activity as reflected in the results.

## Conclusion

Overall, flumioxazin appeared to affect shoots and roots of broadleaf seedlings, and shoots more so than roots of grass seedlings. This is contrary to the hypothesis, which stated that predominantly root exposure was limiting seedling survival in response to flumioxazin. These results do confirm previous work that determined both root and shoot activity of VLCFA inhibiting herbicides, such as pyroxasulfone, to have primarily shoot responsiveness (Appleby and Valverde 1989; Böger et al. 2000; Kumiai internal data). Therefore, application of the combination product, flumioxazin + pyroxasulfone, would result in generally greater shoot than root activity affecting the germinating seedling survival and determining potential for injury.

Resistance to foliar-applied PPO-inhibiting herbicides has been documented for common waterhemp (*Amaranthus rudis*) and common ragweed (*Ambrosia artemisiifolia*) (Patzoldt et al. 2002; Rousonelos et al. 2012). However, weed resistance to either pyroxasulfone or flumioxazin, both soil-applied herbicides, has not been reported (Heap 2013). Busi et al. (2012) determined that a recurrent application of pyroxasulfone at low doses has the potential for rapid development of herbicide resistance in Italian ryegrass (*Lolium rigidum)*. The utility of two modes of action, as present in the combination of flumioxazin and pyroxasulfone, will be beneficial in mitigating the development of herbicide resistance in susceptible weeds to either compound.

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## Figures and Tables


Figure 3.1 Ivyleaf morningglory seedlings placed into fused two-petri dish combination demonstrating the physical barrier of root or shoot exposure to herbicide treatment. Treatments from left to right in photo are shoot, root, and root + shoot exposures.

| 1x Rate | | | 0.5x Rate | | | 0.25x Rate | | | No herbicide |
|---|---|---|---|---|---|---|---|---|---|
| Shoot | Root | Root + shoot | Shoot | Root | Root + Shoot | Shoot | Root | Root + Shoot | |

Figure 3.2 Ivyleaf morningglory as result of selective exposure to three rates of flumioxazin + pyroxasulfone in growth chamber after 72 hours.

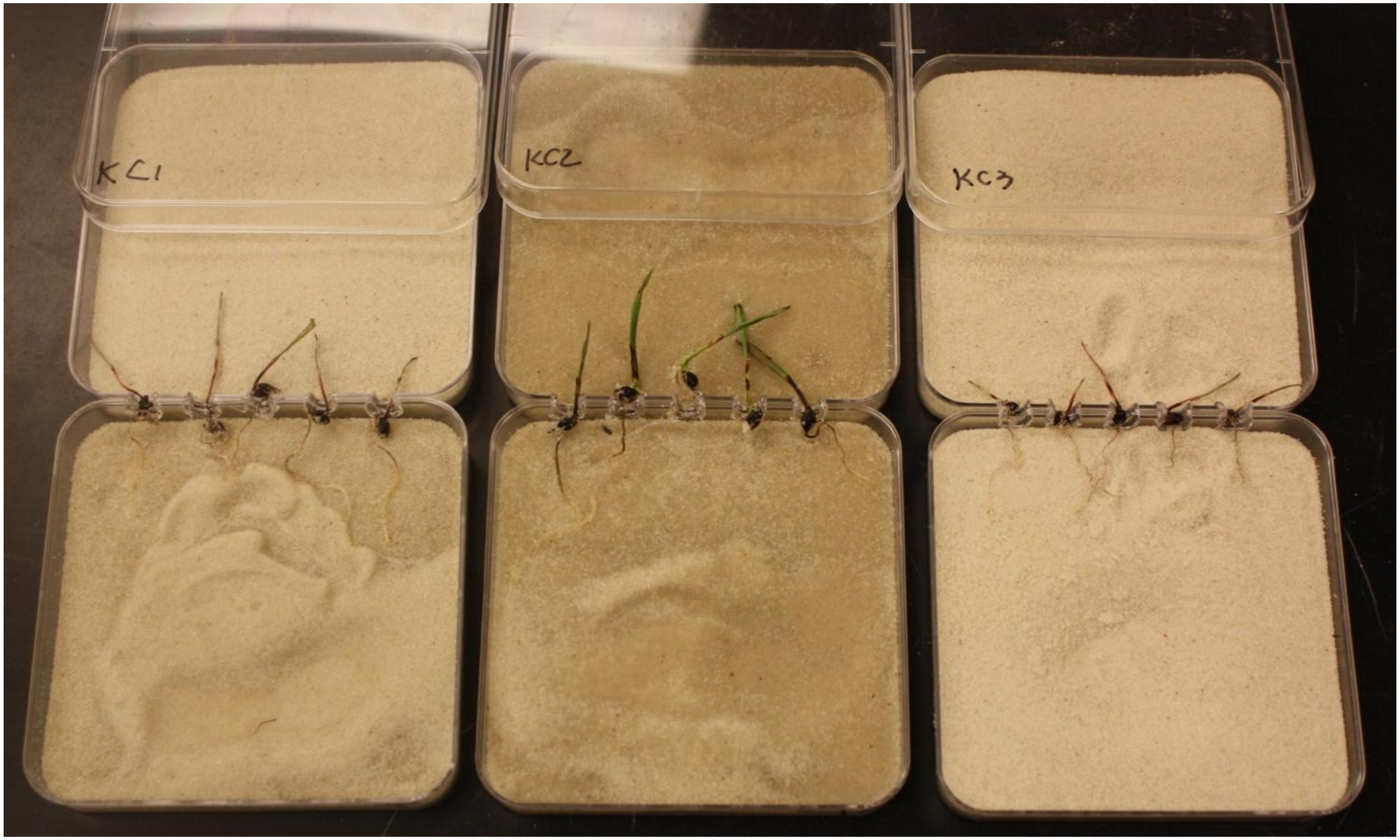


Figure 3.3 Shattercane seedlings as affected by area of herbicide exposure. Treatments from left to right are shoot, root, and root + shoot exposures.

Table 3.1 F values and their significance for the fixed effects of herbicide, area of exposure, herbicide rate, and their interactions for % affected plants relative to control for each species. Blocks and replication within blocks were considered random effects for analysis.

| Main Effect | Soybean | Winter wheat | Ivyleaf morningglory | Shattercane |
|---|---|---|---|---|
| Herbicide | 37.94****† | 28.22*** | 10.12*** | 13.53*** |
| Exposure | 6.27** | 43.71*** | 46.18*** | 106.70*** |
| Rate | 17.64**** | 22.37*** | 6.13** | 1.01 |
| Herbicide x exposure | 6.21**** | 1.51 | 9.82*** | 3.28* |
| Herbicide x rate | 0.25 | 4.45*** | 0.26 | 0.45 |
| Exposure x rate | 2.04 | 1.69 | 2.15 | 2.02 |
| Herbicide x rate x exposure | 1.22 | 0.89 | 1.45 | 0.74 |

† F values for the main effect are statistically significant *P = 0.05, **P = 0.01, ***P = 0.001, ****P ≤ 0.0001.

Table 3.2 Percentage of soybean and ivyleaf morningglory seedlings affected by interaction of herbicide and area of exposure averaged across herbicide rates.

| Species | Herbicide | Area of exposure | | |
|---|---|---|---|---|
| | | Root | Shoot | Root + shoot |
| | | -------------------- % ------------------- | | |
| Soybean | Flumioxazin | 47 ef† | 85 ab | 70 bcd |
| | Pyroxasulfone | 98 a | 83 abc | 72 bcd |
| | Flumioxazin + pyroxasulfone | 22 g | 51 e | 42 ef |
| Ivyleaf morningglory | Flumioxazin | 83 bc | 91 ab | 96 a |
| | Pyroxasulfone | 54 d | 74 c | 90 ab |
| | Flumioxazin + pyroxasulfone | 31 e | 100 a | 99 a |

†Numbers followed by different letters within each species are statistically different at the 0.05 probability level.

Table 3.3  The percentage of soybean plants as affected by herbicide rate, averaged across herbicides and area of exposure.

| Herbicide rate | Affected plants (%) |
|---|---|
| 4x field use | 76 a† |
| 2x field use | 60 b |
| 1x field use | 46 c |

†Numbers followed by different letters are statistically different at the 0.05 probability level.

Table 3.4 Percentage of ivyleaf morningglory plants affected by herbicide rate, averaged across herbicides and area of exposure.

| Herbicide rate | Affected plants (%) | |
|---|---|---|
| 1.0x field use | 89 | a† |
| 0.5x field use | 77 | b |
| 0.25x field use | 76 | b |

†Numbers followed by different letters are statistically different at the 0.05 probability level.

Table 3.5  Percentage of winter wheat plants affected by herbicide and herbicide rate averaged across area of exposure.

| Herbicide | Field use rate | | |
|---|---|---|---|
| | 1x | 2x | 4x |
| | % | | |
| Flumioxazin | 36 c† | 53 b | 70 a |
| Pyroxasulfone | 20 d | 23 cd | 63 ab |
| Flumioxazin + pyroxasulfone | 64 ab | 74 a | 74 a |

†Numbers followed by different letters are statistically different at the 0.05 probability level.

Table 3.6  The percentage of winter wheat plants affected by area of herbicide exposure averaged across herbicide and herbicide rate.

| Area of exposure | Affected plants (%) |
|---|---|
| Root | 29 c† |
| Shoot | 67 a |
| Root + shoot | 58 b |

† Numbers followed by different letters are statistically different at the 0.05 probability level.

Table 3.7 Percentage of shattercane seedlings affected by herbicide and the area of herbicide exposure averaged across herbicide rate.

| Herbicide | Area of exposure | | |
| --- | --- | --- | --- |
| | Root | Shoot | Root + shoot |
| | -------------------- % ------------------- | | |
| Flumioxazin | 66 b† | 99 a | 100 a |
| Pyroxasulfone | 28 c | 87 a | 92 a |
| Flumioxazin + pyroxasulfone | 30 c | 90 a | 91 a |

† Numbers followed by different letters are statistically different at the 0.05 probability level.

# Appendix A - Figures and Tables

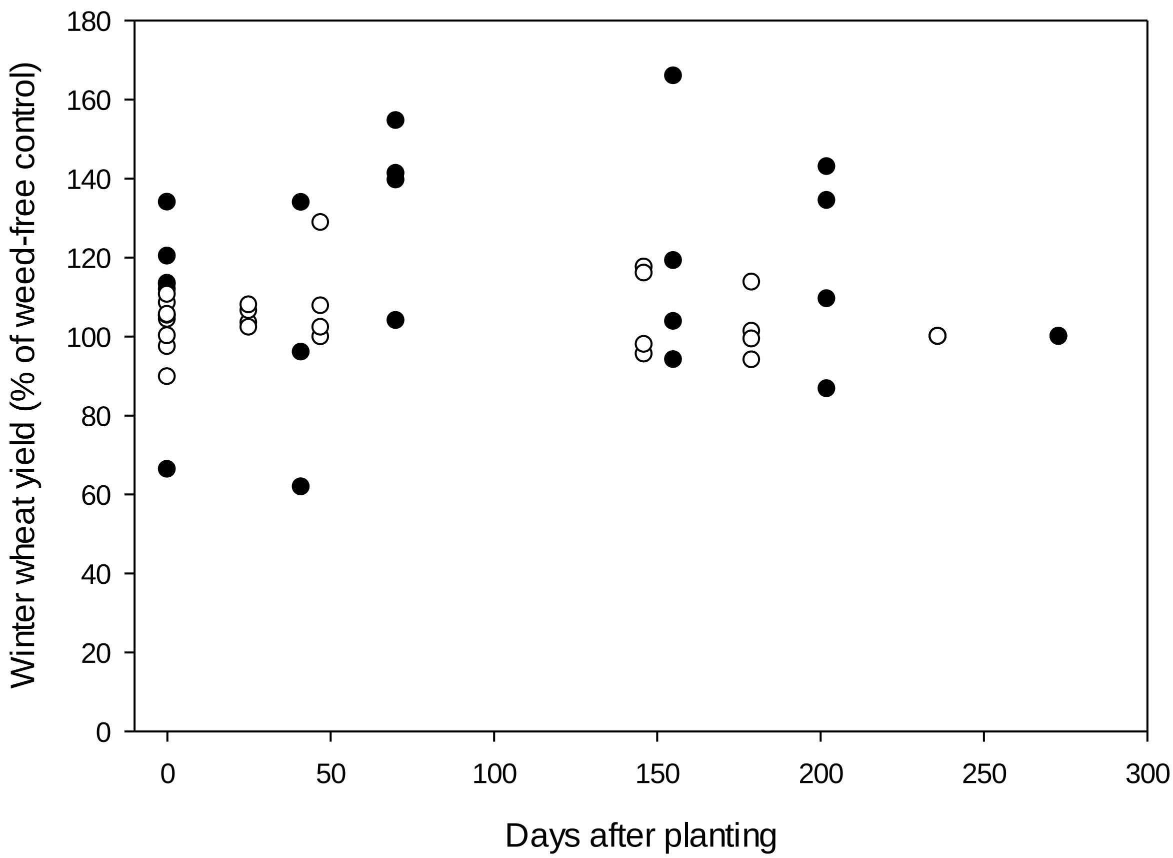


Figure A.1 Winter wheat yield as a function of increasing duration of weed-free period at Belpre, KS in 2010 (●) and 2011 (○).

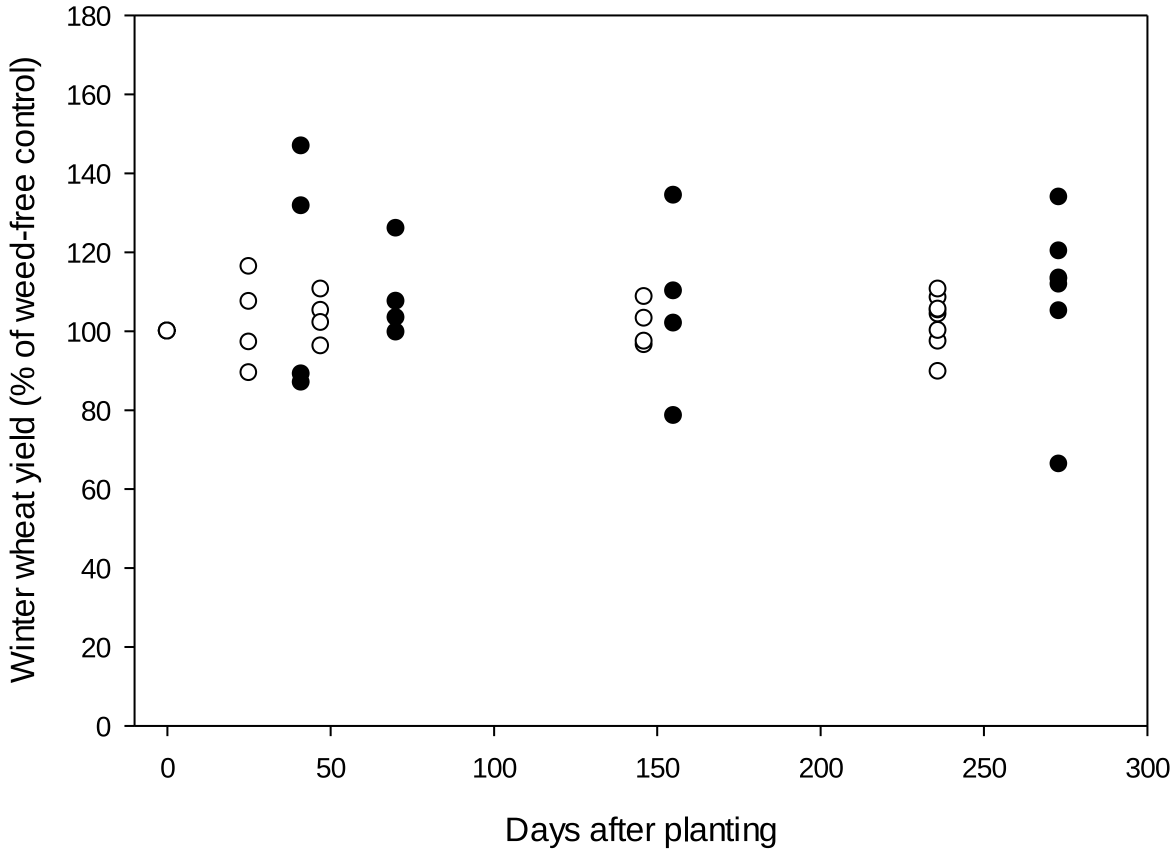


Figure A.2 Winter wheat yield as a function of increasing duration of weed interference at Belpre, KS in 2010 (●) and 2011 (○).

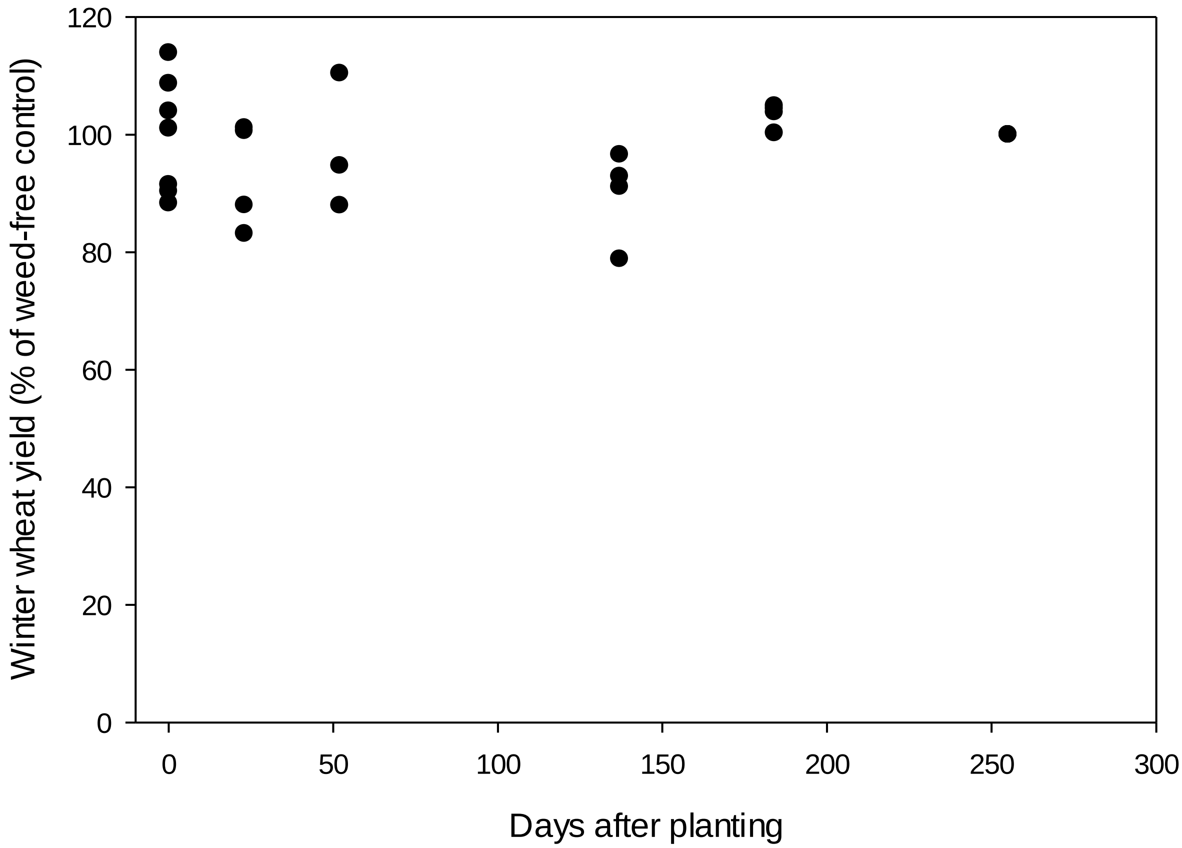


Figure A.3 Winter wheat yield as a function of increasing weed-free period for Larned (a), KS in 2010.

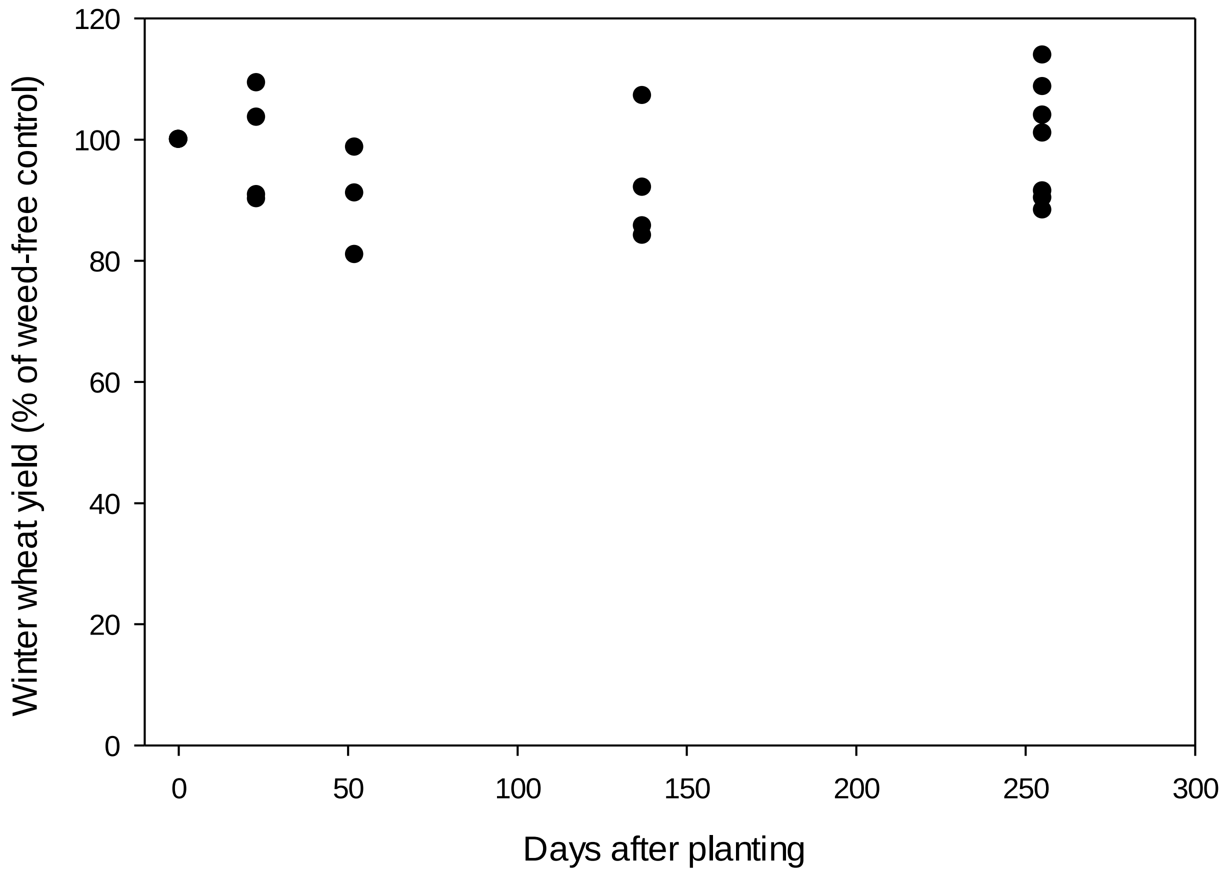


Figure A.4  Winter wheat yield as a function of increasing duration of weed interference at Larned (a), KS in 2010.

Table A.1 High and low monthly temperatures, 30-yr average high and low temperature, total monthly precipitation and 30-yr average monthly precipitation for Kansas State University Department of Agronomy Ashland Bottoms Research Farm near Manhattan, KS from 2010 through 2012.

| | | Average Air Temperature (C) | | 30-YR Average Air Temperature (C) | | Rainfall | 30-YR Ave |
|---|---|---|---|---|---|---|---|
| Month | Year | High | Low | High | Low | Total (cm) | Rainfall (cm) |
| OCT | 2010 | 23.9 | 5.9 | 20.9 | 6.2 | 3.4 | 6.6 |
| NOV | 2010 | 13.8 | 0.1 | 13.0 | -0.7 | 4.3 | 3.8 |
| DEC | 2010 | 5.2 | -6.6 | 5.8 | -6.7 | 0.4 | 3.5 |
| JAN | 2011 | 1.4 | -9.9 | 4.8 | -8.1 | 2.3 | 2.3 |
| FEB | 2011 | 4.9 | -8.7 | 8.1 | -5.9 | 2.4 | 3.5 |
| MAR | 2011 | 12.3 | -0.1 | 13.9 | -0.9 | 4.3 | 6.7 |
| APR | 2011 | 19.7 | 5.7 | 19.6 | 5.4 | 7.8 | 7.9 |
| MAY | 2011 | 23.7 | 10.9 | 24.8 | 12.0 | 11.7 | 11.1 |
| JUN | 2011 | 31.2 | 18.0 | 30.1 | 17.2 | 10.6 | 12.1 |
| Average | | 15.1 | 1.7 | 15.7 | 2.1 | | |
| Total | | | | | | 47.1 | 57.6 |
| OCT | 2011 | 22.4 | 6.7 | 20.9 | 6.2 | 5.3 | 6.6 |
| NOV | 2011 | 13.0 | 0.1 | 13.0 | -0.7 | 10.4 | 3.8 |
| DEC | 2011 | 6.9 | -4.2 | 5.8 | -6.7 | 9.9 | 3.5 |
| JAN | 2012 | 8.9 | -5.4 | 4.8 | -8.1 | 0.3 | 2.3 |
| FEB | 2012 | 9.0 | -3.7 | 8.1 | -5.9 | 5.7 | 3.5 |
| MAR | 2012 | 21.2 | 7.0 | 13.9 | -0.9 | 6.7 | 6.7 |
| APR | 2012 | 22.4 | 8.2 | 19.6 | 5.4 | 5.5 | 7.9 |
| MAY | 2012 | 28.3 | 14.5 | 24.8 | 12.0 | 3.0 | 11.1 |
| JUN | 2012 | 31.7 | 18.4 | 30.1 | 17.2 | 11.5 | 12.1 |
| Average | | 18.2 | 4.6 | 15.7 | 2.1 | | |
| Total | | | | | | 58.4 | 57.6 |

Table A.2 High and low monthly temperatures, 30-yr average high and low temperature, total monthly precipitation and 30-yr average monthly precipitation for Belpre, KS from 2010 through 2012.

| | | Average Air Temperature (C) | | 30-YR Average Air Temperature (C) | | Rainfall Total | 30-YR Ave |
|---|---|---|---|---|---|---|---|
| Month | Year | High | Low | High | Low | (cm) | Rainfall (cm) |
| | | 29.9 | 14.4 | 27.8 | 13.6 | 2.0 | 4.3 |
| OCT | 2010 | 23.8 | 5.9 | 21.3 | 6.6 | 0.2 | 3.7 |
| NOV | 2010 | 12.7 | -1.7 | 12.5 | -1.0 | 7.6 | 2.6 |
| DEC | 2010 | 6.5 | -6.7 | 6.9 | -5.7 | 0 | 2.0 |
| JAN | 2011 | 5.2 | -9.7 | 5.2 | -7.4 | 0.2 | 1.6 |
| FEB | 2011 | 7.1 | -8.8 | 9.1 | -4.7 | 0.1 | 1.7 |
| MAR | 2011 | 13.1 | 0.1 | 14.1 | -0.4 | 2.8 | 4.7 |
| APR | 2011 | 21.2 | 5.1 | 19.5 | 4.8 | 2.7 | 5.7 |
| MAY | 2011 | 25.9 | 10.4 | 24.4 | 10.9 | 8.0 | 7.6 |
| JUN | 2011 | 34.9 | 18.3 | 30.5 | 16.4 | 3.8 | 8.0 |
| Average | | 18.0 | 2.7 | 17.1 | 3.3 | | |
| Total | | | | | | 27.2 | 41.8 |
| | | | | | | | |
| OCT | 2011 | 21.3 | 8.7 | 21.3 | 6.6 | 0.9 | 3.7 |
| NOV | 2011 | 12.3 | 1.0 | 12.5 | -1.0 | 3.3 | 2.6 |
| DEC | 2011 | 4.4 | -3.7 | 6.9 | -5.7 | 2.0 | 2.0 |
| JAN | 2012 | 9.3 | -3.8 | 5.2 | -7.4 | 0.2 | 1.6 |
| FEB | 2012 | 8.8 | -3.1 | 9.1 | -4.7 | 2.4 | 1.7 |
| MAR | 2012 | 20.2 | 5.4 | 14.1 | -0.4 | 6.1 | 4.7 |
| APR | 2012 | 21.3 | 8.7 | 19.5 | 4.8 | 6.0 | 5.7 |
| MAY | 2012 | 29.1 | 13.4 | 24.4 | 10.9 | 0.2 | 7.6 |
| JUN | 2012 | 31.7 | 16.4 | 30.5 | 16.4 | 3.1 | 8.0 |
| Average | | 17.6 | 4.8 | 15.9 | 2.2 | | |
| Total | | | | | | 24.2 | 37.5 |

Table A.3 High and low monthly temperatures, 30-yr average high and low temperature, total monthly precipitation and 30-yr average monthly precipitation for Larned (a), KS from 2010 through 2012.

| | | Average Air Temperature (C) | | 30-YR Average Air Temperature (C) | | | | 30-YR Average Rainfall |
|---|---|---|---|---|---|---|---|---|
| Month | Year | High | Low | High | Low | Rainfall (cm) | Irrigation (cm) | (cm) |
| OCT | 2010 | 24.5 | 6.1 | 21.7 | 6.3 | 0.5 | 5.1 | 4.2 |
| NOV | 2010 | 13.2 | -1.2 | 12.7 | -1.1 | 5.4 | 1.3 | 3.0 |
| DEC | 2010 | 6.8 | -7.0 | 6.8 | -6.1 | 0 | 0 | 2.1 |
| JAN | 2011 | 5.3 | -9.8 | 5.1 | -7.9 | 0.2 | 0 | 1.6 |
| FEB | 2011 | 7.3 | -9.1 | 8.9 | -5.4 | 0.1 | 0 | 2.1 |
| MAR | 2011 | 13.3 | 0.1 | 14.1 | -0.8 | 3.1 | 1.9 | 5.1 |
| APR | 2011 | 20.8 | 4.4 | 19.8 | 4.7 | 2.3 | 13.0 | 5.4 |
| MAY | 2011 | 23.9 | 10.5 | 24.7 | 10.9 | 4.8 | 7.6 | 8.4 |
| JUN | 2011 | 33.9 | 17.8 | 30.7 | 16.4 | 4.7 | 2.5 | 9.0 |
| Average | | 16.6 | 1.3 | 16.0 | 1.9 | | | |
| Total | | | | | | 21.0 | 31.4 | 40.8 |
| | | | | | | | | |
| OCT | 2011 | 22.7 | 5.8 | 21.7 | 6.3 | 3.6 | 2.3 | 4.2 |
| NOV | 2011 | 13.0 | -1.1 | 12.7 | -1.1 | 4.5 | 0.0 | 3.0 |
| DEC | 2011 | 5.1 | -5.2 | 6.8 | -6.1 | 5.6 | 0.0 | 2.1 |
| JAN | 2012 | 9.3 | -5.4 | 5.1 | -7.9 | 0.2 | 0.0 | 1.6 |
| FEB | 2012 | 8.7 | -4.7 | 8.9 | -5.4 | 1.9 | 0.0 | 2.1 |
| MAR | 2012 | 20.1 | 4.4 | 14.1 | -0.8 | 6.1 | 1.4 | 5.1 |
| APR | 2012 | 21.7 | 7.2 | 19.8 | 4.7 | 5.8 | 1.3 | 5.4 |
| MAY | 2012 | 31.4 | 11.6 | 24.7 | 10.9 | 1.7 | 0.0 | 8.4 |
| JUN | 2012 | 32.2 | 15.9 | 30.7 | 16.4 | 5.7 | 0.0 | 9.0 |
| Average | | 18.2 | 3.2 | 16.0 | 1.9 | | | |
| Total | | | | | | 35.1 | 4.9 | 40.8 |

Table A.4 High and low monthly temperatures, 30-yr average high and low temperature, total monthly precipitation and 30-yr average monthly precipitation for the Kansas State University Research Center Hays, KS in 2011.

| | | Average Air Temperature (C) | | 30-YR Average Air Temperature (C) | | Rainfall | 30-YR Ave |
|---|---|---|---|---|---|---|---|
| Month | Year | High | Low | High | Low | Total (cm) | Rainfall (cm) |
| OCT | 2011 | 22.3 | 5.3 | 20.3 | 5.6 | 4.0 | 4.1 |
| NOV | 2011 | 13.3 | -2.1 | 12.6 | -1.9 | 3.1 | 2.4 |
| DEC | 2011 | 4.4 | -7.1 | 5.8 | -7.4 | 5.1 | 1.8 |
| JAN | 2012 | 9.5 | -6.5 | 5.7 | -8.4 | 0.1 | 1.3 |
| FEB | 2012 | 8.8 | -5.6 | 7.9 | -6.4 | 3.2 | 1.8 |
| MAR | 2012 | 21.2 | 3.3 | 13.6 | -1.3 | 3.6 | 4.6 |
| APR | 2012 | 21.3 | 8.1 | 19.3 | 4.6 | 7.3 | 5.4 |
| MAY | 2012 | 29.1 | 12.5 | 24.3 | 10.8 | 4.0 | 8.3 |
| JUN | 2012 | 35.1 | 17.7 | 30.2 | 16.3 | 2.2 | 7.2 |
| Average | | 18.3 | 2.9 | 15.5 | 1.3 | | |
| Total | | | | | | 32.5 | 36.8 |

Table A.5 High and low monthly temperatures, 30-yr average high and low temperature, total monthly precipitation and 30-yr average monthly precipitation for Larned (b), KS in 2011 and 2012.

| | | Average Air Temperature (C) | | 30-YR Average Air Temperature (C) | | | | 30-YR Average Rainfall |
|---|---|---|---|---|---|---|---|---|
| Month | Year | High | Low | High | Low | Rainfall (cm) | Irrigation (cm) | (cm) |
| OCT | 2011 | 22.8 | 6.8 | 21.7 | 6.3 | 3.9 | 0 | 4.2 |
| NOV | 2011 | 13.5 | -0.8 | 12.7 | -1.1 | 4.8 | 0 | 3.0 |
| DEC | 2011 | 5.2 | -5.0 | 6.8 | -6.1 | 5.6 | 0 | 2.1 |
| JAN | 2012 | 9.4 | -4.9 | 5.1 | -7.9 | 0.2 | 0 | 1.6 |
| FEB | 2012 | 8.8 | -3.8 | 8.9 | -5.4 | 2.1 | 0 | 2.1 |
| MAR | 2012 | 20.6 | 5.3 | 14.1 | -0.8 | 6.4 | 5.1 | 5.1 |
| APR | 2012 | 21.9 | 8.6 | 19.8 | 4.7 | 5.3 | 5.4 | 5.4 |
| MAY | 2012 | 30.6 | 13.1 | 24.7 | 10.9 | 1.6 | 8.3 | 8.4 |
| JUN | 2012 | 32.2 | 16.9 | 30.7 | 16.4 | 5.8 | 0 | 9.0 |
| Average | | 18.4 | 4.0 | 16.0 | 1.9 | | | |
| Total | | | | | | 35.5 | 18.8 | 40.8 |