Factors Influencing Soybean Tolerance to Diflufenican

by

Andrew Stephen Laplante

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ABSTRACT

FACTORS INFLUENCING SOYBEAN TOLERANCE TO DIFLUFENICAN

Andrew Stephen Laplante
University of Guelph, 2022

Advisor:
François J. Tardif

Diflufenican (DFF) is a selective, contact, residual herbicide under development by Bayer Crop Science for use in corn and soybean. It is a novel active ingredient and site of action for field crop production in North America and is highly effective on *Amaranthus* species. Differential soybean tolerance to DFF was observed at several field locations with cultivar selection, cultural practices, and environmental conditions all thought to influence tolerance. Field and growth room studies indicate no differences in tolerance exists among soybean cultivars and that crop response is instead related to rainfall timing and intensity. Rain splash from intense rainfall was shown to be a key mechanism for increased DFF exposure on soybean seedlings. Preplant applications were successful at reducing soybean phytotoxicity to DFF. This may permit the use of increased field rates, achieving improved residual weed control while maintaining crop safety.

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1. Literature Review

1.1 Introduction

The use of herbicides is the most reliable and cost-efficient method of controlling weeds in crop production. While their effective and repeated use has been a major contributor to the substantial increases in yield over the last 70 years, this practice has increased the prevalence of herbicide-resistant weeds (Heap 2014a). Over the years, herbicide use has shifted with the occurrence of widespread herbicide resistance and the development of new herbicide modes of action (Heap 2014a). Today however, as the same small subset of effective herbicides are used as the primary form of weed control on increasingly large areas, herbicide-resistant weeds have begun to appear at a much greater rate (Heap 2014b). Powerful and complex resistance mechanisms now threaten crop production with multiple and cross resistant weeds, and fewer effective herbicides to control them.

Several highly competitive weed species have evolved herbicide resistance in the soybean (*Glycine max* (L.) Merr.) growing regions of Canada (OMAFRA 2017). Among these, are a number of key *Amaranthus* species, which are often cited as some of the most troublesome herbicide-resistant weeds (Heap 2014a). The spread of localized multiple resistant waterhemp (*A. tuberculatus* (Moq.) Sauer) and the northward range expansion of Palmer amaranth (*A. palmeri* S. Wats.)) are of particular concern for Canadian soybean production (Kistner and Hatfield 2018, Schryver et al. 2017b). New weed management tools are needed to combat these weed species and preserve the value of soybean production to the Canadian economy.

Current efforts to slow the evolution of herbicide-resistant weeds focus on the diversification of herbicide regimes. It is now common to use a residual herbicide (preplant or preemergence) followed by an in-crop post-emergence herbicide in a two-pass program (Craigmyle et al. 2013, Kumar et al. 2021). This has shown to not only improve weed control, but also reduce selection pressure for herbicide-resistant weeds (Craigmyle et al. 2013, Underwood et al. 2017). Efforts have also been made to incorporate new herbicide-resistant technologies into crops in order to diversify in-crop applications (Schryver et al. 2017a). An additional strategy is to expand the use of certain modes of action into new cropping systems (Duke 2012). While the introduction of novel modes of action is the ideal approach, there is still value in extending older herbicides into new crops or growing regions. This can provide farmers with additional effective herbicides for their rotation and give them a chance against herbicide-resistant weeds while also reducing selection pressure.

Diflufenican (DFF) is a selective, residual herbicide under development by Bayer Crop Science for use in corn (*Zea mays* L.) and soybean. From a resistance management perspective, DFF has a mode of action (Group 12) that is underutilized and is a novel active ingredient for field crop production in North America. Additionally, DFF is highly selective on key broadleaf species like *A. tuberculatus*, *A. palmeri,* and other *Amaranthus* species. Pending approval in Canada and the U.S., the registration of DFF is intended to target these species and be an important tool to slow the evolution and impact of herbicide-resistant weeds.

Throughout the initial field testing of DFF, many studies reported differences in soybean tolerance to the herbicide. In trials across North America, varying degrees of bleaching were observed in early season soybean growth at several field rates. The proposed DFF registration in soybean would include a wide range of doses. There is interest in using the maximum dose to achieve longer residual control of all key *Amaranthus* species, however, as this might negatively impact the crop, a further understanding of the factors influencing soybean tolerance is required. Since DFF is yet to become commercially available, investigations into the sources of this variable tolerance are needed to better position DFF in the market and advise growers more effectively on the use of this product in soybean.

1.2 Diflufenican

Diflufenican is a pyridinecarboxamide herbicide that inhibits the phytoene desaturase enzyme within the carotenoid biosynthesis pathway. It was originally discovered in 1979 and brought to market in the 1980s by May & Baker Limited (now part of Bayer Crop Science) as a preemergence and early post-emergence herbicide in cereals (Cramp et al. 1987, Haynes and Kirkwood 1992). The increased adoption of winter cereals paired with the occurrence of weeds resistant to substitute-urea herbicides created the need for a persistent, broad-spectrum herbicide to control winter-germinating species into the spring (Cramp et al. 1987). Today, the use pattern has expanded to include applications in lentil, field pea, oilseed poppy, lupin, and legume cover crops. Mainly within the European and Australian markets, DFF is commonly found in co-formulations and co-packs with several other active ingredients and is sold under various trade names and suppliers. At doses of 50 to 250 g ai ha^{-1}, DFF is effective against a wide range of broadleaf and grass species (Haynes and Kirkwood 1992).

1.2.1 Activity

DFF is a residual, contact herbicide with activity as a preemergence or early post-emergence application (Cramp et al. 1987). Once applied to the soil, the low solubility (0.05 mg/L in water at 20˚C) and adsorptive capacity (K_{ow} = 4.2 at pH 7, mean K_{foc} = 1622-7431 ml/g) allow DFF to form a stable herbicide layer on the soil surface (Catchpole and Plumbe 1993, EFSA 2008). Germinating weeds absorb DFF through their shoots as they emerge through the top 1-2 cm of soil (Catchpole and Plumbe 1993). Although shoot uptake is the main route for absorption, germinating weeds in the herbicide layer can absorb DFF through their roots (Catchpole and Plumbe 1993). This activity is thought to grant deeper germinating species a degree of depth protection from the herbicide (Haynes and Kirkwood 1992). Seedlings emerge bleached or develop bleached tissues in early growth stages and are susceptible to photooxidation and plant death. DFF provides residual control of broadleaf weeds and can be used as part of a season-long weed management program (Kyndt et al. 1985). Its persistence in the soil (DT_{50} = 44.3- 248.5 days, lab, 20˚C) allows for control of weeds with extended emergence periods (Catchpole and Plumbe 1993, EFSA 2008). When DFF is applied to larger, emerged weeds, limited mobility can result in minimal adverse effects or localized bleaching and chlorosis, stressing the need for applications to target the apex of the plant (Knight and Kirkwood 1991, Sharma et al. 1996). The activity of DFF is dependent upon reaching these apical meristems or other meristematic tissues within target plants (Catchpole and Plumbe 1993, Knight and Kirkwood 1991).

Previous research has documented the activity of DFF applied to both plant and soil material. Haynes and Kirkwood (1992) applied ^{14}C-labelled DFF to various application sites on numerous weed and crop species to determine factors influencing selectivity. From this methodology, it was concluded that differential rates of herbicide uptake was the main mechanism responsible for differences between tolerant and susceptible species. When applied to the leaves of tolerant crops like wheat and barley, over 90% of the radiolabelled DFF was recovered from a leaf wash (Haynes and Kirkwood 1992). Only 0.3% to 1% was recovered in the leaf tissue, and just 0.1% to 0.2% had been translocated and recovered from the rest of the plant (Haynes and Kirkwood 1992). In contrast, 6% to 9% of the applied DFF was recovered from the leaves of susceptible weed species (Haynes and Kirkwood 1992). Similar trends were observed in this study with DFF applications to the soil. Morphological and physiological characteristics were thought to be key factors contributing to the variation in herbicide uptake (Haynes and Kirkwood 1992). Overall, these results suggest that the lipophilic nature of DFF (K_{ow} = 4.2) allow the herbicide to readily adsorb to cuticular waxes and endogenic material. Poor uptake and translocation were also identified as limiting factors in other studies examining DFF activity and properties (Ahmad 1991, Knight and Kirkwood 1991, Sharma et al. 1996). Applications to young plant tissues, such as the hypocotyl or coleoptile demonstrated the highest rates of uptake and injury, and it is thought that the thinner cuticular waxes in young plant material increases the amount of herbicide reaching meristematic tissues (Ahmad 1991, Haynes and Kirkwood 1992). Foliar applications to more developed plant material have demonstrated strong sorption to cuticular waxes and negligible phloem mobility, resulting in minimal and localized damage (Knight and Kirkwood 1991, Sharma et al. 1996). The limited absorption and translocation of leaf applied DFF are responsible for this herbicide being primarily soil applied.

The adsorptive capacity of DFF also influences its activity when applied to the soil. Soil organic matter content and soil clay content have both shown to be inversely related to DFF availability (Benoit et al. 2008, Tejada 2011). DFF was strongly adsorbed in soils with increased levels of organic material, however when organic matter content is low, soil texture is the determinant factor for herbicide adsorption (Benoit et al. 2008, Tejada 2011). Little desorption occurs once the herbicide is bound to these fractions within the soil (Benoit et al. 2008, Willkommen et al. 2019). Again, this is credited to the lipophilicity ($K_{ow} = 4.2$) and low solubility (0.05 mg/L) of the molecule (Rouchaud et al. 1991). While most studies examining the behaviour of DFF in soil focus on environmental fate and persistence, the inverse relationship between soil adsorption and phytotoxicity can still be considered (Peter and Weber 1985, Weber et al. 1974). The interaction between DFF and soil properties may influence efficacy and phytotoxicity, similar to what has been observed with other soybean herbicides including metribuzin, sulfentrazone, and pendimethalin (Coble and Schrader 1973, Grey et al. 1997, Szmigielski et al. 2009, Urach Ferreira et al. 2020). In these studies, decreased phytotoxicity and weed control were observed with higher levels of organic matter and clay.

1.2.2 Mode of Action

The bleaching action of DFF indicates inhibitory activity in the biosynthesis of photosynthetic pigments. While there are several modes of action with inhibitory action along the carotenogenic pathway, phytoene desaturase has been identified as the primary target site (Bramley 1993, Sandmann et al. 1984). Phytoene desaturase is the first enzyme involved in the desaturation sequence that converts phytoene into coloured carotenoids (BögerBöger 1996). This membrane bound enzyme catalyzes the dehydrogenation reactions transforming phytoene to zeta-carotene, a vital precursor reaction in the biosynthesis of other coloured carotenoids like beta-carotenes (BögerBöger 1996, Sandmann et al. 1991). In early studies on the inhibitory action of similar herbicides, a reduction in carotenoids and chlorophyll, with a simultaneous accumulation in phytoene was the first indicator of this target site (Bartels and Watson 1978). More recent research confirmed this target site and demonstrated non-competitive and reversible binding by herbicide compounds to phytoene desaturase (Sandmann et al. 1984, 1989). Consequently, this interference with the substrate site results in a decrease in carotenoids, xanthophyll, and chlorophyll within susceptible plants (BögerBöger 1996, Sandmann et al. 1991).

The accumulation of reactive oxygen species (ROS) caused by the lack of carotenoids is the ultimate cause of death following applications of DFF. Carotenoids are essential to the photosynthetic pathway due to their role in harvesting light and protecting chlorophyll from photooxidation (Anderson and Robertson 1960). Under high light conditions, singlet oxygen is formed when triplet state chlorophyll transfer their excess energy to molecular oxygen (Böger 1996). These ROS are highly volatile and can cause damage to the photosynthetic unit and cell membrane destruction (Böger 1996). The presence of carotenoids, which are essentially "antioxidants", allows singlet oxygen to be quenched. Carotenoid molecules have a high number of double bonds (nine or more) and use these to quench singlet oxygen and dissipate the excess energy as heat (Bartels and Watson 1978). Susceptible plants treated with DFF are no longer be able to accumulate these protective pigments and are vulnerable to photooxidation. Since phytoene desaturase inhibiting herbicides stop the formation of coloured carotenoids, these herbicides cause bleaching in new plant tissues (Böger 1996). Existing green plant tissues will not be affected. The bleaching symptomology is typically followed by chlorosis and eventual necrosis in affected tissues (Bartels and Watson 1978). This results in rapid plant death in newly emerged and young rapidly growing weeds, but also generates the potential for unsightly crop phytotoxicity.

1.3 Soybean

1.3.1 History and Botany

Cultivated soybean (*Glycine max* [L.] Merr.) is an herbaceous annual legume of the Fabaceae family that is widely grown globally (Hartman et al. 2011, Singh 2017). Soybean was first domesticated as early as 2500 BCE and were commonly used for components in food and medicine in China, Japan, and Korea (Hymowitz 1970, Kumudini 2010). By the 16th century, soybean as cultivated in much of Asia and later was grown in Europe and America as an ornamental plant and forage crop (Hymowitz 1970, Kumudini 2010). Advancements in soybean processing at the start of the 20th century lead to rapid growth in soybean production in America, and major production has expanded to South America and Asia as processing capabilities and demand continue to grow (Ali 2010, Hartman et al. 2011).

This versatile crop has a multitude of uses. Global demand for soybean production is driven by its use in protein meal and vegetable oil (Ali 2010; Hartman et al. 2011). Increasing demand paired with the wide range of geographic adaptability have allowed soybean production to increase more than any other major crop since 1970 (Ali 2010; Hartman et al. 2011). As a result, an estimated 6% (75.5 million ha) of the world's arable land is used for growing soybean (Hartman et al. 2011). Soybean has become one of the top traded commodities in the world and production is expected to increase further through expanded production systems and improvements to yields (Ali 2010; Hartman et al. 2011).

1.3.2 Morphology

Soybean is an annual plant with an erect and bushy growth pattern. The vegetative growth of soybean is classified by the development and number of leaf types: the cotyledons (seed leaves), the unifoliates (primary simple leaves), and the trifoliates (pinnately trifoliate leaves) (Singh 2017). The cotyledons and unifoliate leaves occur on the first two nodes of the plant and are oppositely arranged, while all further nodes are alternately arranged and bear trifoliate leaves (Singh 2017). Soybean is pubescent and can have trichomes on its leaves, stems, sepals, and pods (Kumudini 2010). Below ground, development is characterized by initial taproot growth then the subsequent development of a network of secondary roots (Singh 2017). Like other legumes, this root system establishes a symbiotic relationship with nitrogen fixing bacteria called rhizobia that induce nodules on legume roots (Kumudini 2010). The reproductive stage is indicated by the development of self-pollinating purplish flowers and subsequent pod establishment (Singh 2017). Pods can have one to four seeds and mature in about 50-80 days after fertilization (Singh 2017).

1.3.3 Canadian Soybean Production

In Canada, the expanded production of soybean has resulted in increased importance to the Canadian economy. Soybean occupies the fourth largest seeded area among principal field crops in Canada, generating CDN$3.26 billion in farm cash receipts in 2020 (Statistic Canada 2021). With production ranging from 6 to 7 million metric tons (MMT) over the last 5 years, Canada has consistently been ranked seventh in the world for soybean production (Statistics Canada 2021). A 40% increase in production from 2011 to 2021 has allowed Canada to become a significant exporter of high-quality soybean, with approximately two thirds of production being exported (Soy Canada 2021; Statistics Canada 2021). Historically, southern Ontario has been responsible for the majority of soybean production in Canada, however advancements in soybean breeding have expanded the land base for potential soybean production (Statistics Canada 2021; Voldeng et al. 1997). Early maturing soybean cultivars have allowed Quebec and Manitoba to significantly contribute to overall production in Canada (Statistics Canada 2021). The high economic value generated by soybean warrant active protection against a variety of pests.

Among challenges affecting soybean production, weeds are very concerning. Weed interference is often cited as one of the greatest threats to soybean production (Oerke 2006, Soltani et al. 2017). This occurs through competition for essential resources such as light, moisture and nutrients (Soltani et al. 2017). Globally, it was estimated that about 37% of attainable soybean production could be lost to weed competition (Oerke 2006). In North American production systems, potential losses in soybean yield could reach 52.1% if weeds are left unchecked (Soltani et al. 2017). Using this figure with average soybean prices from 2007-2013, corresponding losses in value would approximate US$16 billion in the United States and US$ 400 million in Canada (Soltani et al. 2017). Current loss estimates would likely be significantly higher given the increases in production since the date of this study.

Herbicides are the primary form of weed control in Canada but overreliance on this form of control may lead to reductions in efficacy. The repeated use of a select few herbicides has increased the prevalence of herbicide-resistant weeds (Heap 2014b). With Canada among the nations with the greatest reported number of herbicide-resistant weeds (51 unique resistant weeds), this represents an increasing threat to soybean production (Heap 2022). Weeds of the *Amaranthus* genus are known as some of the most troublesome herbicide-resistant weeds (Heap 2014a). High genetic diversity within weed populations, prolific seed production, and a record of developing resistance to numerous herbicides indicates a high threat of future resistance and negative economic impacts (Heap 2014a). Of particular concern are *A. palmeri* and *A. tuberculatus.* The dioecious nature of these species allows them to rapidly produce resistant biotypes to many herbicide molecules, which has lead to large yield losses in soybean across the United States (Bensch et al. 2003, Davis et al. 2015). When present at soybean emergence at densities of 8 plants/m^2, *A. palmeri* and *A. tuberculatus* reduced soybean yield by 78% and 56% respectively (Bensch et al. 2003). Models have shown that the range of these species will expand into the key soybean growing regions of Canada, stressing the need for an increased arsenal of effective herbicides (Kistner and Hatfield 2018). The high activity of DFF on these key *Amaranthus* spp. highlights its potential importance to field crop production in Canada.

1.4 Factors Influencing Soybean Tolerance to Herbicide

1.4.1 Differential Cultivar Tolerance

Differential tolerance among soybean cultivars has been documented with numerous herbicides, including chlorimuron (Newsom and Shaw 1995), imazethapyr (Wixson and Shaw 1991), metribuzin (Barrentine et al. 1976, Coble and Schrader 1973, Mangeot et al. 1979), bentazon (Connelly et al. 1988, Hayes and Wax 1975, Wills 1976), sulfentrazone (Dayan et al. 1997, Hulting et al. 2001, Swantek et al. 1998), flumioxazin (Taylor-Lovell et al. 2001), saflufenacil (Miller et al. 2012), dimethenamid, and metolachlor (Osborne et al. 1995). Of the aforementioned herbicides, extensive research has been performed on metribuzin, bentazon, and the protoporphyrinogen oxidase (PPO) inhibiting herbicides.

Metribuzin is a Group 5 herbicide, that is applied preemergence in soybean. Following its initial registration in soybean, various amounts of damage were widely observed in the crop. Coble and Shrader (1973) reported that herbicide rate, soil organic matter, and rainfall can influence phytotoxicity, but hydroponic testing confirmed cultivar selection also played a significant role in susceptibility to metribuzin (Barrentine et al. 1976). The physiological mechanism responsible for differences in tolerance was found to be herbicide metabolism (Mangeot et al. 1979).

Similar conclusions were found with the Group 6 herbicide bentazon. A 100-fold difference in tolerance was observed among soybean cultivars from various growing regions (Wax et al. 1974). The translocation of radiolabeled bentazon was four times greater in susceptible cultivars and was thought to be the physiological basis for cultivar sensitivity (Wills 1976). However, Hayes and Wax (1975) found that the residual bentazon within the plant and the production of metabolites differed between tolerant and susceptible cultivars. The presence of specific enzymes responsible for conjugation resulted in 80-90% metabolism of bentazon in tolerant cultivars as opposed to 10-15% in susceptible cultivars (Connelly et al. 1988). It was then concluded that the ability to metabolize bentazon at a higher rate was the main mechanism causing differential tolerance (Connelly et al. 1988, Hayes and Wax 1975).

Preemergence applications of PPO inhibiting herbicides can cause varying degrees of injury among soybean cultivars. Notable differences in tolerance to sulfentrazone led to the classification of cultivars as having a low, medium, or high tolerance to the herbicide (Hulting et al. 2001). It was originally hypothesized that differential tolerance to sulfentrazone is related to a cultivar's ability to metabolize the molecule and tolerate the peroxidative stress brought on by PPO inhibiting herbicides (Dayan et al. 1997), but more recent work has demonstrated that differing rates of root uptake immediately following seed germination can also cause differences in tolerance (Li et al. 2000). Differential cultivar tolerance to flumioxazin and saflufenacil also exists, but these molecules have not been studied as extensively and tolerance mechanisms have not been confirmed (Miller et al. 2012, Taylor-Lovell et al. 2001).

Despite the potential risk of injury associated with the use of these herbicides, they remain in use across Canadian soybean production. Many seed companies now release tolerance ratings for cultivars and an understanding of how soil, weather, and farming practices influence soybean injury is well established. The extensive research performed to screen cultivars and the additional examination of other management strategies has minimized the risks associated with these effective herbicides.

1.4.2 Cultural Factors

1.4.2.1 Planting Date

Planting date is one of the most important agronomic factors influencing soybean yield potential. In the soybean growing regions of Canada, studies have shown that planting earlier (late April to early May) can maximize seed quality and yield (Kandel et al. 2016, OMAFRA 2017). This can have implications on the response of soybean to herbicides. While farmers may choose early planting to maximize yield potential, cold, wet weather conditions in early spring can increase the risk of soybean injury from soil applied herbicides (OMAFRA 2017, Poston et al. 2008). Frequent rainfall events in this period increase herbicide availability within the soil and intense rainfall shortly after emergence can lead to rain splash damage (Poston et al. 2008, Yoshida et al. 1991). These factors in combination with reduced herbicide metabolism in cold temperatures can lead to high-risk scenarios when soybean is planted early (Poston et al. 2008). This effect has been observed when metribuzin and PPO-inhibitors are applied to soybean in such conditions (Hulting et al. 2001, Miller et al. 2012, Poston et al. 2008). Considering the activity of DFF, cold temperatures could also decrease the growth rate of soybean seedlings and prolong their contact with the herbicidally active layer prior to emergence. In southern Ontario, where average temperatures in April and May differ by ~6.5 ˚C, there is value in investigating how the conditions associated with various planting dates influence soybean phytotoxicity.

1.4.2.2 Planting Depth

Soybean in Canada can be planted as shallow as 2.5 cm in ideal moisture conditions and up to 7.25 cm deep when planting to sufficient moisture (OMAFRA 2017). While planting depth can interact with a number of factors to influence emergence and stand quality, this range of depths can also have implications on soybean response to herbicide (Fehr et al. 1973, Grabe and Metzer 1969, Johnson and Wax 1979). Research has shown that soybean tolerance to metribuzin is influenced by planting depth in certain field scenarios, with greater planting depths offering a degree of depth protection from herbicide injury (Coble and Schrader 1973). It is also commonly stated in many residual herbicides labels that planting at least 3.75 cm deep can reduce the risk of crop injury.

The preemergence activity of DFF results in effective control of species with extended germination (Catchpole and Plumbe 1993). Prolonged shoot contact in the herbicidally active layer can allow for greater uptake to occur (Catchpole and Plumbe 1993). This interaction could also be expressed with differences in planting depth. Similar to cases where low temperatures may prolong soybean emergence, it is possible the slower rates of growth and emergence associated with deeper planting depths could also increase soybean seedling exposure to DFF and result in greater phytotoxicity.

1.4.2.3 Application Timing

Increasing the time interval between residual herbicide application and crop emergence can decrease the severity of injury in soybean (Hulting et al. 2001, Moshier and Russ 1981, Priess et al. 2020). This is best achieved using preplant applications (Moshier and Russ 1981, Priess et al. 2020). Preplant applications lengthen the period for herbicide activation and increase the probability of adequate herbicide dilution, movement, and degradation throughout the soil (Priess et al. 2020, Yoshida et al. 1991). This decreases the availability of herbicide in the soybean germination zone and prevents concentrated herbicide from remaining at the soil surface (Hulting et al. 2001, Priess et al. 2020, Yoshida et al. 1991). With less concentrated herbicide at the soil surface, emerging seedlings will be less susceptible when breaking through the soil, and the risk of splash damage from further rainfall events will be reduced (Yoshida et al. 1991).

Altering application timing has been explored with other herbicides with narrow safety margins in soybean. High rates of metribuzin applied three weeks prior to planting caused minor adverse effects, while metribuzin applied at planting resulted in stand, height, and yield reductions (Moshier and Russ 1981). Sulfentrazone demonstrated similar trends with no injury when applied preplant (Dirks et al. 2000), and 4-61% injury among various cultivars after a preemergence application (Taylor-Lovell et al. 2001). This was shown more recently in a study that investigated the performance and phytotoxicity of numerous herbicide mixtures in soybean applied both at planting and 12-16 days prior to planting (Priess et al. 2020). It was concluded that crop injury from herbicides with narrow safety margins in soybeancan be reduced by applying herbicide 12-16 days before planting, however residual activity against key weeds must be considered (Priess et al. 2020). Manipulating the timing between application and emergence can be an efficient way of reducing crop injury.

1.4.3 Environmental Conditions

1.4.3.1 Moisture

The activity and uptake of soil applied herbicides is directly linked to environmental conditions. Many herbicides require adequate soil moisture for activation, bringing the herbicide into solution and distributing it through the surface layers of the soil (Walker 1971). However, excessive moisture during germination and emergence can increase the availability of herbicide and increase the risk of crop injury (Walker 1971). Herbicides may leach from the soil surface to the depth of emerging seedlings or to the root zone of the crop, resulting in phytotoxicity. Such an effect has been observed with PPO inhibiting herbicides in soybean or isoxaflutole in corn (Hixson 2008; Nelson and Penner 2007). The timing and intensity of rainfall can also influence activity and introduce new avenues of exposure. Concentrated residual herbicide on the soil surface can be splashed onto emerging shoots during rainfall events and cause severe injury (Hartzler 2004; OMAFRA 2017; Wise et al. 2015). Splash damage in soybean has been documented with the use of metribuzin and PPO inhibiting herbicides, with some cases severe enough to warrant replanting (Hartzler 2004). Given that DFF uptake has demonstrated similar patterns of activity to some of these herbicides, it is possible that soybean seedlings may be similarly vulnerable in these adverse conditions. The stability of DFF on the soil surface decreases the likelihood of leaching to lower levels in the soil (Rouchaud et al. 1991). Consequently, this may leave a relatively high concentration on the soil surface for potential splashing damage.

1.4.3.2 Soil

The activity and performance of soil-applied herbicides are also influenced by soil properties (Peter and Weber 1985, Weber et al. 1974). An increase in soil organic matter content and clay content are both inversely related to herbicide activity and are often cited as the predominant factors for inactivating soil applied herbicides (Peter and Weber 1985, Weber et al. 1974). This has implications on both weed control and crop safety, necessitating the need for soil specific rate recommendations within a number of herbicide labels (Coble and Schrader 1973, Grey et al. 1997, Peter and Weber 1985, Szmigielski et al. 2009). While DFF availability is also influenced by soil properties such as organic matter and texture, research directly linking these characteristics to efficacy or phytotoxicity is limited. DFF persisted longer in soils with higher organic matter and clay content due to adsorption, however this herbicide would be unavailable for plant uptake (Benoit et al. 2008, Tejada 2011). Higher herbicidal activity is typically observed on soils with lower organic matter and clay content, and this aligns with observations made by Bayer during the initial field testing of DFF.

In general, DFF is highly persistent and degrades slowly within the soil (DT_{50} = 44.3-248.5 days). Numerous studies have shown that despite differences in soil parameters (C, N, pH, and dehydrogenase activity), there is little variation in rates of DFF degradation (Bending et al. 2006, Norgaard et al. 2015). The molecule is mainly broken down by microbial activity with rates of metabolism following zero order kinetics (Bending et al. 2006, Rouchaud et al. 1991). Locations with a repeated DFF use do not demonstrate enhanced rates of biodegradation, however locations with warm and wet climates did experience increased degradation rates (Bending et al. 2006, Rouchaud et al. 1991). While climatic conditions influenced degradation, heavy rainfall did not result in leaching, with DFF and its metabolites only found in the top 0-10 cm of soil (Rouchaud et al. 1991).

1.5 Study Objectives and Hypotheses

1.5.1 Objectives

The overall goal of this research was to better understand the factors that influence soybean tolerance to DFF, prior to its registration in the U.S. and Canada. Diflufenican has potential to be an important tool for growers across North America due to its ability to control key *Amaranthus* species including herbicide-resistant biotypes. Understanding the factors that influence soybean tolerance to DFF and identifying strategies that increase soybean tolerance will permit the effective use of this herbicide in soybean. Observations in preliminary research performed by Bayer Crop Science Canada indicated that tolerance may be influenced by cultural practices, cultivar selection, and environmental conditions, therefore, the objectives of this research were:

1. Determine the effect of several cultural factors on soybean tolerance to DFF and identify best management practices with the use of DFF in soybean.
2. Design a simple and consistent screening procedure to test soybean cultivar tolerance to DFF and determine if differential tolerance to DFF occurs among soybean cultivars.
3. Determine the influence of rain splash on soybean phytotoxicity to DFF.

1.5.2 Hypotheses

1. The cultural factors (planting date, planting depth, cultivar selection, application timing and DFF rate) used in this field study and the interactions between them will influence soybean tolerance to DFF.
2. Given the large differences in soybean cultivar tolerance observed at the field-scale, it is hypothesized that 10 soybean cultivars will demonstrate differential tolerance to DFF in the growth room screening procedure.
3. Soybean will demonstrate increased phytotoxicity to DFF when rainfall occurs at or just following soybean emergence.

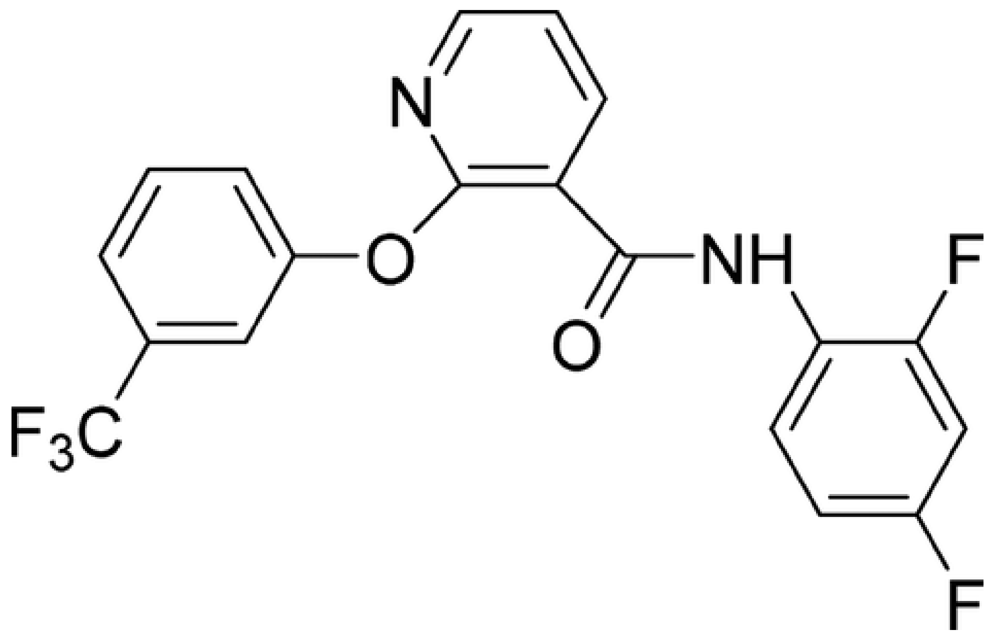


Figure 1.1 The structure of diflufenican (Song et al. 2018).

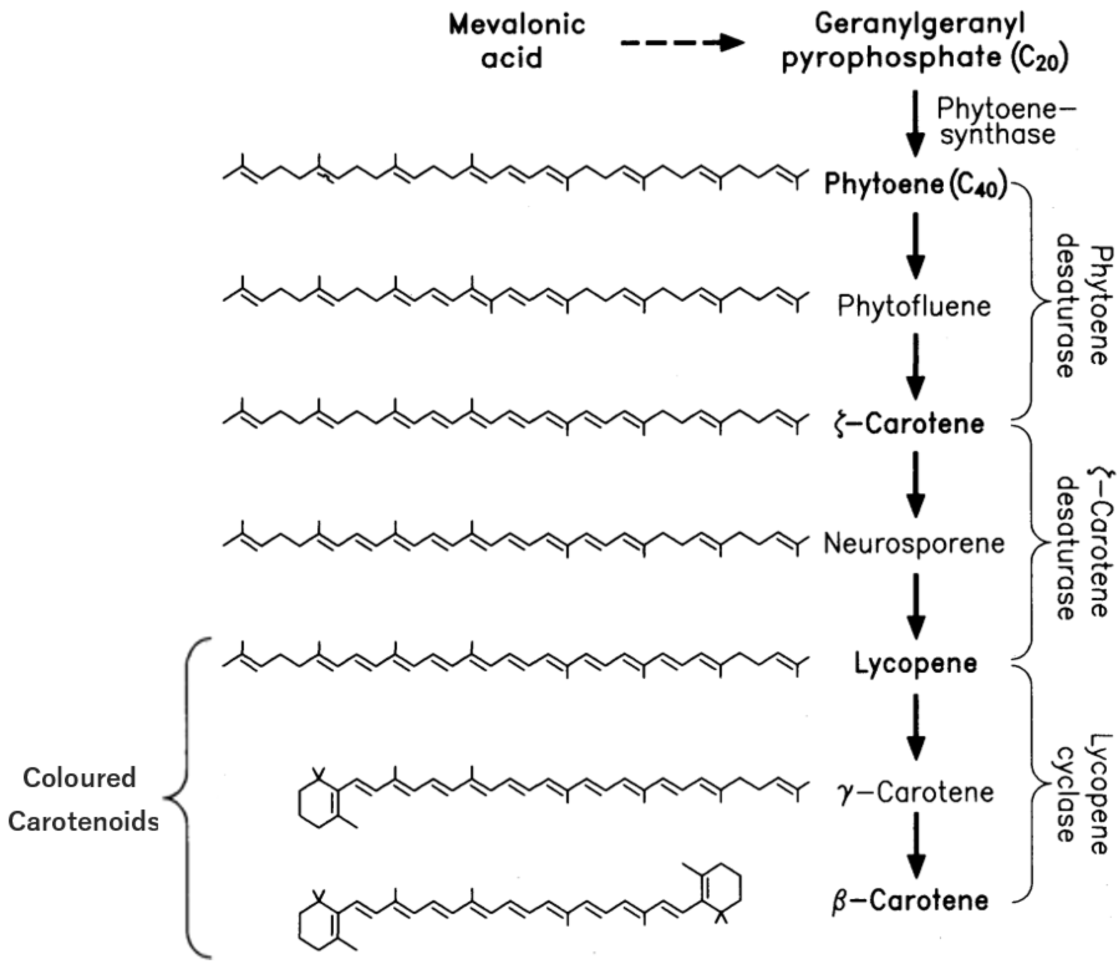


Figure 1.2 Carotenoid biosynthesis pathway from mevalonic acid to beta-carotene. The brace brackets on the right show the membrane bound enzymes responsible for the stepwise introduction of double bonds into the carotenes. The carotenes printed in bold are the end products of the enzyme activity from phytoene desaturase, zeta-carotene desaturase, and lycopene cyclase (Böger 1996).

2. The Influence of Cultural Factors on Soybean Tolerance to Diflufenican

2.1 Abstract

Examining the influence of cultural practices on soybean phytotoxicity can be used to improve tolerance, identify high-risk scenarios, and contribute to the best management practices associated with herbicides. This is necessary with the new herbicide diflufenican (DFF; Group 12), since soybean injury has been variable within preliminary field trials. DFF applied preemergence provides excellent control of key *Amaranthus* spp., however, to enable higher field rates, a greater understanding of the factors that influence soybean tolerance is required. A factorial split-plot field trial examining the effects of planting date, planting depth, cultivar, application timing, and application rate was used to determine the influence of cultural factors on soybean tolerance to DFF. Preplant (PP) applications one week prior to planting reduced soybean phytotoxicity in both 2020 and 2021 regardless of other cultural practices used. This demonstrates the potential to increase the recommended rate and achieve more consistent residual control of hard to control weeds. Environmental conditions associated with three planting dates (~1-week apart) demonstrated the importance of rainfall timing and intensity on the activity of DFF in soybean. The timing of significant rainfall events in relation to soybean emergence directly influenced the degree of injury observed, as evidence suggests soybean have a relatively narrow susceptible growth stage and are susceptible to splash damage. While early season phytotoxicity was high (up to 50%), soybean recovered with minimal reductions in yield. Soybean biomass and yield were never reduced from PP applications of DFF.

2.2 Introduction

Diflufenican (DFF) is a selective, contact, residual herbicide under development by Bayer Crop Science for control of dicot weeds in corn and soybean (Cramp et al. 1987; Haynes and Kirkwood 1992). Originally brought to market in the 1980s, DFF has mainly been utilized in cereal crops in Europe and Australia but is a novel active ingredient for field crop production in North America (Bayer 2021; Cramp et al. 1987)). DFF can be applied preemergence (PRE) or early postemergence (POST) to target weeds and is highly effective on important broadleaf weeds such as waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer) Palmer amaranth (*Amaranthus palmeri* S. Watson), and other *Amaranthus* species (Bayer, unpublished data). Pending approval in Canada and the U.S., the registration of DFF is intended to target these species and be an important tool to slow the evolution and impact of herbicide-resistant weeds (Bayer 2021).

DFF belongs to the pyridinecarboxamide chemical group and has inhibitory action within the carotenoid biosynthesis pathway (Sandmann et al. 1991). The primary target site is inhibition of phytoene desaturase (PDS), which is a membrane bound enzyme responsible for the production of coloured carotenoids (Böger 1996; Sandmann et al. 1991). Carotenoids are antioxidants that protect against photooxidation by quenching singlet oxygen within the photosynthetic unit (Böger 1996). Within susceptible plants, the inhibition of PDS creates a shortage of these protective pigments resulting in photooxidation (Böger 1996). This is expressed as bleaching in newly generated plant tissues, followed by chlorosis and eventual necrosis (Böger 1996; Sandmann et al. 1991). Weeds typically emerge bleached then quickly succumb to photooxidation (Catchpole and Plumbe 1993).

Applied pre-emergence (PRE), the stability of DFF on the soil surface provides residual weed control, with susceptible weeds absorbing the herbicide through their shoots as they emerge through the herbicide layer in the soil (Catchpole and Plumbe 1993). Although emerging shoots are the main route for absorption, additional uptake can occur when shallow germinating weeds have roots present in the herbicide zone (Catchpole and Plumbe 1993). DFF uptake is highest in young plant material and is limited by the lipophilicity of the molecule (Ahmad 1993; Haynes and Kirkwood 1992; Knight and Kirkwood 1991). Little translocation occurs, so uptake in the apex of the plant is necessary for high activity (Ahmad 1993; Knight and Kirkwood 1991).

Throughout initial field testing, many studies reported differences in soybean injury from DFF. In trials across North America, varying degrees of bleaching were observed in early season soybean growth at several field rates (Bayer Crop Science Inc, unpublished data). The DFF submission to the regulatory agencies in Canada and the US included a rate range, with a desired higher rate for improved residual control of *Amaranthus* species. However, a further understanding of the factors that influence soybean tolerance is required to enable the use of the higher field rates. It was thought that cultivar selection, cultural practices, and environmental conditions may all influence soybean tolerance to DFF.

Manipulating cultural factors to improve soybean tolerance allows growers to better manage the risks associated with residual herbicide applications. Numerous studies have explored these practices with herbicides like metribuzin and sulfentrazone and have aided in generating best management strategies for their use in soybean (Coble and Schrader 1973; Moshier and Russ 1981; Priess et al. 2020; Young et al. 2003). As a result, it is understood that planting date, planting depth, application timing, and application rate can all influence soybean tolerance. Considering these cultural factors, along with cultivar tolerance and environmental conditions has allowed for the continued effective use of these potentially injurious soybean herbicides.

In the soybean growing regions of Canada, studies have shown that planting earlier (late April to early May) can maximize seed quality and yield, however, this can have implications on the response of soybean to herbicides (Kandel et al. 2016; OMAFRA 2017). Unfavourable environmental conditions after planting can delay germination and emergence, reduce early plant growth, and reduce herbicide metabolism while also increasing herbicide availability and uptake, creating high-risk scenarios for soybean injury (Moomaw and Martin 1978; Poston et al. 2008; Swantek et al. 1998). This effect has resulted in heightened injury with both metribuzin and sulfentrazone in soybean (Coble and Schrader 1973; Hulting et al. 2001; Swantek et al. 1998). Conversely, late planting dates can also be damaging by reducing the recovery interval between exposure and physiological maturity, leading to greater yield loss relative to early applications (Young et al. 2003). Understanding how soybean tolerance to DFF is influenced by the range of conditions observed from late April to early June is important to manage early season phytotoxicity. The inclusion of multiple planting dates in this study was used to examine how various conditions during germination and emergence impact soybean phytotoxicity from DFF.

Similar to cold and wet conditions, deeper planting depths may also prolong the soybean germination and emergence period as well as contact with the herbicide zone within the soil, leading to reduced seedling vigour and higher rates of herbicide exposure (Wyse et al. 1976). Considering that DFF is highly active on weed species with prolonged germination, the potential for increased injury exists in soybean as well (Catchpole and Plumbe 1993). Alternatively, Haynes and Kirkwood (1992) suggest that there may be a degree of depth protection associated with DFF due to the potential for root uptake. This is consistent with the activity of metribuzin which is less phytotoxic on deeper planted soybean (Coble and Schrader 1973).

Another effective strategy used to improve crop tolerance is increasing the interval between herbicide application and crop emergence. While the longevity of weed control must be considered, this is best achieved using preplant (PP) applications (Priess et al. 2020). Applying herbicides PP increases the likelihood of adequate rainfall for herbicide activation and dilution of herbicide throughout the soil profile prior to crop emergence (Priess et al. 2020; Yoshida et al. 1991). This moves concentrated herbicide away from the soybean germination zone and soil surface, reducing herbicide availability and the risk of herbicide splashing onto emerged seedlings from rainfall after soybean emergence (Priess et al. 2020; Yoshida et al. 1991). High rates of metribuzin applied 3-weeks prior to planting caused minor adverse effects, while the same rate applied at planting resulted in stand, height, and yield reductions (Moshier and Russ 1981). Applications of sulfentrazone alone, as well as tank mixes of sulfentrazone + cloransulam-methyl and saflufenacil + dimethenamid-P + pyroxasulfone + metribuzin also reduced soybean phytotoxicity when applied two weeks before planting compared to PRE applications (Priess et al. 2020).

The influence of planting date, planting depth, and application timing on soybean tolerance to DFF was examined using a range of DFF field rates and two soybean cultivars thought to differ in tolerance. It was hypothesized that these cultural factors and the interactions between them would influence soybean tolerance to DFF. More specifically, through manipulating these cultural factors, it was hypothesized that: 1) the environmental conditions associated with the various planting dates (ie. temperature and moisture) will generate differences in the response of soybean to DFF, 2) the deeper planting depth (5 cm) will be more injurious than the shallow planting depth (2.5 cm), and 3) PP applications will result in less soybean injury from DFF compared to PRE applications. The objective of this research was to gain an understanding of how planting date, planting depth, application timing, application rate, and cultivar selection affect soybean tolerance to DFF. This research will provide the foundation of information needed to generate best management practices for the use of this product in soybean.

2.3 Materials and Methods

The field trials for this study were conducted at the Elora Research Station (near Elora, Ontario, Canada) in 2020 and 2021.. In 2020 the soil was a Guelph loam (brunisolic gray brown luvisol, 51% sand, 36% silt, 13% clay, pH 7.2, 3.2% OM). The previous year had canola and soybean. In 2021, soil was a London loam (gleyed brunisolic gray brown luvisol, 38% sand, 47% silt, 15% clay, pH 7.2, 4.5% organic matter). Soil analyses were performed by SGS Canada Inc., Guelph, ON, Canada. The previous crop was alfalfa. In both years, fields were prepared using shallow tillage and a cultipacker to create an even seedbed.

To examine the five cultural factors, the experiment was designed as a factorial split-plot design arranged in a randomized complete block design with four replications. The main plot factors were planting date, planting depth, and cultivar. Three planting dates, 7 to 10 days apart were used to capture differences in environmental conditions during germination and emergence. Planting depths of 2.5 cm and 5 cm were used to represent “shallow” and “deep” planting. The two cultivars used were DKB 008-81 and P09A53X. DKB 008-81 was thought to be a known susceptible cultivar used in previous Bayer Crop Science field trials. Difficulties in initial screening of cultivars meant that the susceptibility of the second cultivar was unknown prior to use in the trial.

Each main plot contained 10 subplots, which were combinations of application timing and application rate. Three application timings were used. A PP application one week before planting, a PRE application at planting (E-PRE), and a PRE application three to five days after planting (L-PRE). At each application timing, DFF was applied at 90, 180, and 360 g ai ha^{-1} to the subplots. DFF was supplied by the manufacturer (500 g ai L^{-1} suspension concentrate; Bayer Crop Science Inc., Calgary, AB, Canada). One untreated subplot was used as a check for all application timing and rate combinations within each main plot. These factors and levels resulted in 12 main plots (3 Dates x 2 Depths x 2 Cultivars = 12) each containing 10 subplots (3 Timings x 3 Rates + 1 UTC = 10) resulting in 120 total treatments that were randomly assigned (Figure 1). All experimental plots were 4 rows wide and 6 m long, with 76 cm between rows.

DFF was applied with a compressed air small plot sprayer equipped with six nozzles (TeeJet AI1102-VS, Spraying System, Wheaton IL, USA) delivering a volume of 200 L ha^{-1} at a pressure of 200 kPa and a speed of 4.0 km h^{-1}. Nozzles were positioned 50 cm above target. The entire experimental area was maintained weed free with applications of glyphosate (Roundup WeatherMax, 560 g ae L^{-1}, Bayer Crop Science Inc., Calgary, AB, Canada) at 900 g ae ha^{-1} to remove the confounding effects of weed interference. The remaining weeds were hand weeded as necessary.

Estimates of visible soybean injury from DFF including the combined effect of bleaching, chlorosis, stunting, and malformation were determined at 7, 14, 28, and 56 DAE on a scale of 0% to 100%, with 0% being no crop injury and 100% being crop death. At the same assessment interval, 1 m of row was photographed for use in the photo processing software Canopeo. This rapid image analysis tool can be used to quantify Fractional Green Canopy Cover (FGCC) (Patrignani and Ochsner 2015). Analysis of images is based on colour ratios of red to green (R/G) and blue to green (B/G) and an excess green index of (2G-R-B). Canopeo is typically used to estimate canopy development, light interception, and evapotranspiration partitioning, but the bleaching and stunting symptomology of DFF allows the software to be an objective injury assessment tool to pair with subjective visible injury ratings. An above ground biomass harvest occurred at 28 DAE. Soybean plants were counted and cut from a 2 m segment in an outside row then dried and weighed. Final yield was taken at harvest maturity from the two middle rows of each plot.

Statistical Methods

Data was analyzed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Treatment by year interactions were significant, therefore the data from each year was analyzed and presented separately. Soybean canopy cover via Canopeo, biomass, and yield data were standardized into percent reduction from the control plots sharing the same combination of treatment factors. Yield was adjusted to 13% moisture content prior to analysis. Visible injury data was transformed into the BETA distribution for analysis. The fixed effects included in the statistical model were the main effects of cultivar, planting depth, planting date, application timing, application rate, and all possible two-, three-, four-, and five-way combinations of these factors. The random effects were replication and replication by the main plot factors of planting date, planting depth, and cultivar. An analysis of variance was calculated using PROC GLIMMIX using an alpha value set to ≤0.05

to determine the difference among treatment groups. Tukey's multiple comparison test was utilized to determine differences among the treatment means. The assumptions for each analysis were met for all response variables except for the visible injury assessment at 7 DAE in 2021 and the visible injury assessments at 56 DAE in both years. These data sets had an inflated number of zero values and could not be analyzed. The Spearman correlation analysis through the PROC CORR procedure was used to determine the level of agreement between visible injury ratings and Canopeo canopy cover measurements. This procedure was also used to calculate the correlation coefficients among response variables, mainly to identify if early season phytotoxicity can be used as a predictor for losses in biomass and yield.

2.4 Results and Discussion

Significant effects for soybean injury from DFF were observed in 2020 and 2021 at 7, 14, and 28 DAE (Table 2.1). Most main effects were significant at 7 and 14 DAE, but the impact of application timing and DFF rate persisted to 28 DAE in both years. Multiple 2-way interactions were significant at 7 and 14 DAE and the majority involved planting date, application timing, and DFF rate (Table 2.1). Most 3-way and 4-way interactions that were significant occurred at 14 DAE and among these, the most common factors were planting date and DFF rate. Visible injury was most pronounced with E-PRE and L-PRE applications and was reduced when DFF was applied PP (Table 2.2). Regardless of application time, soybean injury was increased as the rate of DFF increased. In 2020, the level of soybean injury 7 DAE ranged between 0.2% and 50% (Table 2.2). Soybean phytotoxicity levels in 2020 remained similar 14 DAE, then decreased to acceptable levels by 28 DAE excluding the 360 g ai ha^{-1} rate applied E-PRE and L-PRE. (Table 2.2). The amplitude of soybean injury was less in 2021, with the highest level of injury observed at 14 DAE from the 360 g ai ha^{-1} rate applied L-PRE (Table 2.2). Similar to 2020, soybean injury decreased by 28 DAE in 2021, and ranged from 0% to 10% (Table 2.2). By 56 DAE minimal soybean injury was observed in both trial years, resulting in no significant differences among the treatments (data not shown).

The significant effect of planting date was seen in the severity of soybean injury across the three planting dates, which appeared to be related to the environmental conditions shortly following emergence. In 2020, significant rainfall events 1 to 3 DAE of the soybean planted on May 20th and May 31st caused soybean injury ranging from 3% to 42% at 7 DAE (Table 2.3). Conversely, when minor rainfall was received following the emergence of the soybean planted June 9th, a maximum injury of only 12.1% was observed (Table 2.3). A 10.4 mm rainfall event on June 23, 2020 (6 DAE), increased the injury associated with the June 9th planting date at 14 DAE, but phytotoxicity levels still remained below the other planting dates (Table 2.3). Rainfall timing also influenced the phytotoxicity associated with the three planting dates in 2021, with the latest planting date (June 4th) receiving just 2.9 mm of rain in the first 9 DAE resulting in relatively lower levels of soybean injury.

Similarly, significant effects for reduction in soybean canopy cover were observed both years (Table 2.4). In 2020, all main effects were significant at 7 DAE, and with the exception of planting depth, remained significant at 14 and 28 DAE (Table 2.4). In 2021, there was less consistency among assessment dates: only planting depth and date were significant at 7 DAE while at 14 DAE, all main effects except cultivar were significant. Only the effects related to DFF applications (application timing and rate) were significant at 28 DAE (Table 2.4). The two-way interactions were more consistent in both years and were similar to the effects observed with the soybean injury data. Thus, the majority of significant interactions occurred between planting date, application timing, and DFF rate at 7 and 14 DAE (Table 2.4). These same factors were also most often involved in significant three- and four-way interactions 7 and 14 DAE (Table 2.4).

Increased soybean tolerance to DFF when applied PP was also observed in soybean canopy cover assessments (Table 2.5). In 2020, reductions in soybean canopy cover 7 DAE had a similar range as the soybean injury ratings, spanning from non-detectable to 51% (Table 2.5). Greater reductions in soybean canopy cover were observed at 14 DAE, with the 360 g ai ha^{-1} rate applied E-PRE and L-PRE reducing canopy cover by 49% and 64% (Table 2.5). These levels of reduction persisted to 28 DAE. Reductions in canopy cover were less severe in 2021, only reaching 27% at 14 DAE for the highest rate and L-PRE application timing (Table 2.5). Once again, the level of canopy cover reduction remained relatively unchanged until 28 DAE (Table 2.5).

Substantial rainfall events shortly after emergence resulted in significant differences among planting dates in the response of soybean canopy cover. In both trial years soybean appeared most sensitive when significant rainfall events occurred 1 to 6 DAE. In 2020, 31.8 mm 2 DAE resulted in a significant canopy cover reductions up to 56%, while in 2021, a 26.1 mm rainfall event 4 DAE resulted reductions up to 35% (Table 2.6). Conversely, the latest planting dates in both trial years received only minor rainfall within the first 1 to 6 DAE, which resulted in canopy cover reductions ranging from 0% to 11.3% (Table 2.6).

Early season soybean injury resulted in significant reductions in biomass at 28 DAE. All main effects except planting depth were significant in 2020 and just application timing and DFF rate were significant in 2021 (Table 2.7). Fewer significant high-level interactions existed with the response of soybean biomass, however, interactions between planting date, application timing, and DFF rate were again the most prominent in both 2020 and 2021 (Table 2.7). In 2020, the highest-level significant interactions were three-way interactions between planting date, application timing, and DFF rate, and between cultivar, planting depth, and application timing (Table 2.7). A significant four-way interaction involving cultivar, planting date, planting depth, and DFF rate was observed in 2021 (Table 2.7). The increased soybean injury observed with PRE applications compared to PP produced significant differences in biomass among the various application timings (Table 2.8). In 2020, PP applications reduced biomass by 0% to 5%, while E-PRE applications reduced biomass by 4% to 24%, and L-PRE by 5% to 43% (Table 2.8). In 2021, most application timing and DFF rate combinations resulted in minor reductions in biomass with the exception of the 360 g ai ha^{-1} rate applied E-PRE and L-PRE (Table 2.8).

Fewer significant main effects and interactions were observed with the response of soybean yield. The main effects of timing and rate were significant, with later application timings and higher DFF rates resulting in lower yields in 2020 (Table 2.7 and 2.8). The effect of cultivar was also significant as DKB-008-81 had lower yield (data not shown). Only two interactions were significant in 2020, a two-way interaction between date and application timing and a four-way interaction between cultivar, planting depth, application timing, and DFF rate (Table 2.9). In 2021, all single effects except planting date had a significant effect on yield (Table 2.7). Dissimilar to 2020, P09A53X had lower yield compared to DKB-0081, while the deeper planting also resulted in a greater yield loss (data not shown). The only high-level interaction (3 or more factor interaction) occurred between cultivar, application timing, and DFF rate (Table 2.7). Consistent with the 56 DAE soybean injury ratings, many of the treatments recovered to show minimal adverse effects on yield. This is consistent with other soybean tolerance studies, which demonstrated the plasticity of soybean and the crop's ability to recover from severe early season injury (Arsenijevic et al. 2021; bra and Habetz 1989; Taylor-Lovell et al. 2001). The largest impacts on yield were observed with PRE applications and the higher DFF rates, with significant reductions in yield mainly occurring in treatments including PRE application timings and the 360 g ai ha^{-1} rate (Table 2.8).

In 2020, correlations between soybean injury and Canopeo ratings at the same assessment interval were acceptable, with correlation coefficients ranging from -0.67 to -0.74 (Table 2.9). This was likely aided by the severity of injury observed in 2020, which created clear treatment differences. Soybean injury ratings throughout the season remained strongly correlated from 7 DAE to 28 DAE (r = 0.71 to 0.90), and Canopeo ratings over this period had a slightly weaker correlation (r = 0.64 to 0.73)(Table 2.9). The Canopeo canopy cover values were more strongly correlated to mid-season biomass and final yield than soybean injury estimates, however the overall correlation strength between early season variables and final yield was low. Soybean biomass at 28 DAE was the best predictor of impacts on final yield (r = 0.62) (Table 2.9).

The correlation strength between all response variables was weaker in 2021 (Table 2.10). There was little agreement between soybean injury ratings and the Canopeo values (r = 0.12 to 0.27) and no response variables were an acceptable predictor of final yield. The weak relationship between the response variables was likely due to the lower overall soybean injury in this trial year. As a result, the differences between treatments were not as drastic and harder to distinguish for both soybean injury ratings and the Canopeo software. In addition, the low levels of soybean injury led to an inflated number of zero values for soybean injury, which can inhibit the utility of the data in various statistical analyses.

The prevalence and consistency of the five cultural factors within the significant main and interaction effects can be used to further analyze their role in determining the response of soybean to DFF. Results from both 2020 and 2021 indicate that DFF rate, application timing, and planting date were the most prevalent significant factors observed among the several response variables, however they differed in the consistency of their impact on soybean phytotoxicity. The effects of DFF rate and application timing were consistent in both years and across all treatment combinations. Predictably, the phytotoxic effects of DFF were greater as rates increased. In addition, the PP application consistently resulted in lower soybean phytotoxicity, regardless of cultivar, planting date, and planting depth. This is in agreement with the original hypothesis and consistent with previous studies examining soybean response to soil-applied herbicides (Moshier and Russ 1981; Priess et al. 2020). Phytotoxicity from the 180 g ai ha^{-1} DFF rate was reduced by up to 25% using PP applications. This is comparable to a study conducted by Priess et al. (2020), where a 20% to 61% reduction in injury was achieved by applying sulfentrazone two weeks prior to planting and the 25% to 30% reduction in injury was observed when a tank mixture of saflufenacil + dimethenamid-P + pyroxasulfone + metribuzin was also applied two weeks before planting (Priess et al. 2020). Even the negative impacts of increased metribuzin rates applied on sandy soils with high pH and low organic matter could be mitigated by utilizing a preplant interval of three weeks (Moshier and Russ 1981).

The one week PP interval used in this study was just as successful at reducing soybean phytotoxicity from DFF. These results indicate the potential to increase rates, which would help growers obtain more consistent broadleaf weed control. The 180 g ai ha^{-1} rate generally resulted in minimal adverse effects across all response variables when applied PP. These effects were equivalent or less phytotoxic than the 90 g ai ha^{-1} rate applied E-PRE or L-PRE. Increasing the recommended rate to 180 g ai ha^{-1} using PP applications would have major implications on the level of control observed with key *Amaranthus* species. The improved consistency and length of residual weed control would enhance early season weed control of species like waterhemp and Palmer amaranth and delay or reduce the need for subsequent POST applications.

It is suggested that the buffering capacity of PP applications against soybean phytotoxicity is due to the increased likelihood of proper activation prior to soybean emergence (Priess et al. 2020). Preplant applications lengthen the interval between herbicide application and soybean emergence, producing more opportunities for an initial activating rain event. Rainfall can move concentrated herbicide away from the soybean germination zone and reduce the risk of injury (Priess et al. 2020; Yoshida 1991). While PP applications generally reduced phytotoxicity in this study, inconsistencies in the level of protection did not always align with rainfall events recorded at the site. This suggests there may be a high threshold of rainfall required to fully activate this relatively insoluble molecule. Soil disturbance from planting equipment may also be responsible for the reduced phytotoxicity associated with PP applications. In theory, this could break up the stable DFF layer on the soil surface and lead to less herbicidal activity. This is similar to tillage following herbicide applications reducing residual weed control (Chauhan et al.

2006). While the coulters on the planter used in this trial did disrupt the soil surface, this may be more applicable to no-till situations where more aggressive trash whippers are employed.

Planting date was involved in several significant main and interaction effects within the study, however the direction of the effect was not as consistent and appeared to be related to the weather conditions shortly following soybean emergence. This allowed for a degree of predictability in the response of soybean to DFF, however subtle differences in rainfall intensity or soybean staging relative to the timing of rainfall still generated a wide range of soybean response to DFF. This aligns with the findings from growth room studies using DFF, which identified rain splash as a main avenue for DFF exposure to soybean seedlings, and a relatively narrow window of high susceptibility during early soybean development (Chapter 3 and 4). Thus, small differences in rainfall timing and soybean growth stage could result in large differences in the resulting DFF injury.

This is a potential explanation for the inconsistent effect of planting depth and cultivar selection within the study. Both the shallow and deep planting depths demonstrated a higher relative susceptibility across the different planting dates included in the study. For example, in 2020, at the first planting date, the shallow planting depth resulted in an average canopy cover reduction of 19%, while the deep planting depth resulted in just 6%. However, at the latest planting date in 2020, the shallow planted soybean appeared more tolerant with a 9% reduction in canopy cover when compared to the 26% reduction observed with the deep planting depth (Table 2.11). The two planting depths used in this study generated small differences in the timing of soybean emergence and early seedling development. Linking these observations with the rainfall data for the study site, it is thought that the differences in growth stage just after emergence and the timing and intensity of rainfall in this period contributed to the resulting variability in soybean injury. For instance, rainfall events 1 to 3 DAE would be more injurious to the faster emerging shallow planted soybean. However, by 4 to 6 DAE, the deeper planted soybean would advance to the more susceptible growth stage, and rainfall events in this period would be more phytotoxic to the soybean. This scenario occurred in 2020, and the timeline of emergence and rainfall can be seen in Figures 2.2a and 2.2b. Since the uptake and activity of DFF in soybean appears to occur after emergence, this negates the potential for depth protection that has been observed with metribuzin, or the potential for increased exposure from prolonged contact in the soil during soybean germination (Catchpole and Plumbe 1993; Coble and Schrader 1973). Ultimately, this suggests that manipulating planting depth cannot be used to increase soybean tolerance to DFF.

Similarly, physiological differences in soybean emergence and growth rate could also create differences in soybean growth stage and result in variability among cultivars planted on the same date. This likely aided in the development of the initial hypothesis that differential cultivar tolerance to DFF existed among soybean cultivars. However, these observations indicate that a cultivar could easily be falsely identified as a highly susceptible or tolerant cultivar at the field-scale simply based on when it was exposed to a significant rainfall event. Since growth room screening of cultivars did not demonstrate differential tolerance, physiological differences among cultivars in relation to the timing and intensity of rainfall events is a more likely explanation for the large differences in cultivar tolerance observed at the field-scale.

Conclusions

DFF toxicity to soybeans can be reduced if the appropriate cultural and herbicide application practices are employed. Preplant applications one week prior to planting did not cause as much injury when compared to E-PRE and L-PRE applications. The relative safety of PP applications suggests there is potential to make use of the higher DFF field rates in soybean and improve the residual control of *Amaranthus* species. However, as suggested by Priess et al. (2020), it is possible that applying DFF PP could reduce the longevity of weed control, and this warrants further examination under field conditions.

The importance of rainfall for DFF activity and phytotoxicity was also identified through the inclusion of numerous planting dates across the two years of this study. Rainfall timing and intensity are of particular importance due to the rain splash potential of DFF and the narrow window of susceptibility exhibited by soybean during the development of a cultivar screening procedure (Chapter 3). In addition to this, planting soybean shallow or deep did not afford any additional protection from DFF, as injury showed to be more dependent on rainfall after emergence.

Canopeo was used in this study as a tool to obtain an objective injury rating to pair with subjective soybean injury ratings. Its utility appeared promising in 2020, however its correlation to soybean injury ratings was lower in 2021. This indicates that the effectiveness of this tool can be limited when treatment differences are small and can be confounded by natural variation in crop growth and morphology. Canopeo remains a useful tool for quick and easy canopy cover measurements, all possible with just a smartphone. The simplicity of the technology was the attraction and it was also shown to be successfully incorporated into other studies examining soybean phytotoxicity (Arsenijevic et al. 2021). As with any measurement tool, proper operation is essential to produce accurate data. Some of the operational limitations outlined by Patrignani and Ochsner (2015) could be considered in this study such as consistency of photo height and adequate photo height. A smartphone mounted on a tripod was used in this study, which could easily be impacted by the uneven surface present in the fields. This could slightly confound the data by altering the photo frame from plot to plot. Additionally, increasing the picture height to include the whole plot is thought to be better practice than photographing a portion of each plot, however this would likely require special equipment or drone technology (Patrignani and Ochsner 2015). This should all be considered when incorporating this software into a study.

Table 2.1 Significance level for the effects of cultivar, planting depth, planting date, application timing and DFF rate and their interactions for soybean visible injury assessed 7, 14, and, 28 DAE in 2020 and 2021.[a]

Source of variation	df	Visible Injury 7 DAE 2020	Visible Injury 7 DAE 2021[c]	Visible Injury 14 DAE 2020	Visible Injury 14 DAE 2021	Visible Injury 28 DAE 2020	Visible Injury 28 DAE 2021
Cultivar (C)	1	*	N/A	*	NS	NS	NS
Depth (D)	1	NS[b]	N/A	NS	*	NS	NS
Date (A)	2	*	N/A	*	*	NS	*
Timing (T)	2	*	N/A	*	*	*	*
Rate (R)	2	*	N/A	*	*	*	*
C*D	1	NS	N/A	NS	NS	NS	NS
C*A	2	NS	N/A	NS	NS	NS	NS
C*T	2	NS	N/A	NS	NS	NS	NS
C*R	2	*	N/A	*	NS	NS	NS
D*A	2	*	N/A	*	*	NS	NS
D*T	2	NS	N/A	NS	*	NS	NS
D*R	2	NS	N/A	NS	*	NS	NS
A*T	4	*	N/A	*	*	*	*
A*R	4	*	N/A	*	*	NS	*
T*R	4	*	N/A	*	*	NS	NS
C*D*A	2	NS	N/A	NS	NS	NS	NS
C*D*T	2	NS	N/A	NS	NS	NS	NS
C*D*R	2	NS	N/A	NS	NS	NS	NS
C*A*T	4	NS	N/A	NS	*	NS	NS
C*A*R	4	NS	N/A	NS	*	NS	NS
C*T*R	4	NS	N/A	*	NS	NS	NS
D*A*T	4	NS	N/A	NS	*	NS	NS
D*A*R	4	NS	N/A	*	*	NS	NS
D*T*R	4	NS	N/A	NS	NS	NS	NS
A*T*R	8	NS	N/A	*	*	NS	NS
C*D*A*T	4	NS	N/A	NS	NS	NS	NS
C*D*A*R	4	NS	N/A	NS	*	NS	NS
C*D*T*R	4	NS	N/A	NS	NS	NS	NS
C*A*T*R	8	NS	N/A	NS	*	NS	NS
D*A*T*R	8	*	N/A	*	NS	NS	NS
C*D*A*T*R	8	NS	N/A	NS	NS	NS	NS

[a] Visible injury was determined using the combined effects of bleaching, chlorosis, stunting, and malformation on a 0% to 100% scale where 0 indicates no injury and 100 denotes complete plant death

[b] NS = non-significant

[c] Zero inflated data: analysis did not meet assumptions

[*] Significant differences at $a \leq 0.05$

Table 2.2 Effect of DFF rate and application timing (PP, E-PRE, and L-PRE) on soybean visible injury at 7, 14, and 28 DAE in 2020 and 2021. Data are the mean of two cultivars, two planting depths, three planting dates, and four replications. [a]

		Visible injury (%)				
		2020			2021	
Timing	DFF rate	7 DAE[b]	14 DAE	28 DAE	14 DAE	28 DAE
PP	90	0.2 a	0.3 a	0.0 a	0.2 a	0.0 a
	180	2.5 a	3.4 a	0.5 ab	1.3 ab	0.2 a
	360	10.6 b	12.4 b	5.5 ab	4.6 c	3.3 c
E-PRE	90	2.3 a	3.5 a	0.1 ab	1.0 ab	0.1 a
	180	11.8 b	10.5 b	3.7 ab	4.3 c	1.9 b
	360	28.9 c	33.3 d	20.3 c	14.2 e	7.5 d
L-PRE	90	11.6 b	10.4 b	0.8 ab	2.0 b	0.2 a
	180	27.8 c	24.1 c	8.2 b	7.9 d	2.5 bc
	360	49.9 d	50.1 e	46.3 d	20.1 f	10.4 e

[a] Visible injury was determined using the combined effects of bleaching, chlorosis, stunting, and malformation on a 0% to 100% scale where 0 indicates no injury and 100 denotes complete plant death
[b] Means within a column with the same letter are not significantly different according to the Tukey HSD test at $a \leq 0.05$

Table 2.3 Effect of planting date and DFF rate on soybean visible injury at 7, 14, and 28 DAE in 2020 and 2021. Data are the mean of two cultivars, two planting depths, three application timings, and four replications. [a]

		Visible injury (%)				
		2020			2021	
Planting Date	DFF rate	7 DAE[b]	14 DAE	28 DAE	14 DAE	28 DAE
Planting 1[c] May 20, 2020 May 21, 2021	90	2.5 a	2.5 a	0.0 a	0.8 a	0.2 ab
	180	14.8 b	9.7 ab	1.1 ab	4.6 c	0.7 bc
	360	42.0 d	27.9 c	12.8 b	10.7 d	4.8 d
Planting 2 May 31, 2020 May 27, 2021	90	5.2 a	6.1 a	0.2 a	1.7 ab	0.02 a
	180	13.6 b	12.5 b	3.2 ab	6.2 c	0.6 abc
	360	32.3 c	39.1 d	25.3 c	18.5 e	6.3 de
Planting 3 June 9, 2020 June 4, 2021	90	0.4 a	0.9 a	0.3 ab	0.7 a	0.2 ab
	180	4.6 a	7.9 a	4.9 ab	2.6 b	2.4 c
	360	12.1 b	22.3 c	20.4 c	9.8 d	8.6 e

[a] Visible injury was determined using the combined effects of bleaching, chlorosis, stunting, and malformation on a 0% to 100% scale where 0 indicates no injury and 100 denotes complete plant death
[b] Means within a column with the same letter are not significantly different according to the Tukey HSD test at $a \leq 0.05$
[c] Calendar planting dates for 2020 and 2021 are included below each numbered planting date

Table 2.4 Significance level for the effects of cultivar, planting depth, planting date, application timing and DFF rate and their interactions for soybean canopy cover reduction assessed 7, 14 and 28 DAE in 2020 and 2021. [a]

Source of variation	df	Canopy cover 7 DAE		Canopy cover 14 DAE		Canopy cover 28 DAE	
		2020	2021	2020	2021	2020	2021
Cultivar (C)	1	*	NS	*	NS	*	NS
Depth (D)	1	*	*	NS	*	NS	NS
Date (A)	2	*	*	*	*	*	NS
Timing (T)	2	*	NS	*	*	*	*
Rate (R)	2	*	NS	*	*	*	*
C*D	1	NS[b]	NS	*	NS	NS	NS
C*A	2	NS	*	NS	NS	NS	NS
C*T	2	NS	NS	NS	NS	NS	NS
C*R	2	NS	NS	NS	NS	NS	NS
D*A	2	*	NS	*	NS	NS	NS
D*T	2	NS	NS	NS	NS	NS	NS
D*R	2	*	NS	*	NS	NS	NS
A*T	4	*	NS	*	*	*	NS
A*R	4	*	NS	*	*	*	*
T*R	4	*	NS	*	*	*	*
C*D*A	2	NS	NS	*	NS	*	NS
C*D*T	2	NS	NS	NS	NS	NS	NS
C*D*R	2	NS	NS	NS	*	NS	NS
C*A*T	4	*	*	NS	NS	NS	NS
C*A*R	4	NS	*	NS	NS	*	NS
C*T*R	4	NS	NS	NS	*	NS	NS
D*A*T	4	NS	NS	NS	*	NS	NS
D*A*R	4	NS	*	NS	NS	NS	NS
D*T*R	4	*	NS	NS	NS	NS	NS
A*T*R	8	*	NS	*	*	NS	NS
C*D*A*T	4	NS	*	NS	NS	NS	NS
C*D*A*R	4	NS	*	NS	NS	NS	NS
C*D*T*R	4	NS	NS	*	NS	*	NS
C*A*T*R	8	NS	NS	NS	NS	NS	NS
D*A*T*R	8	*	NS	NS	NS	NS	NS
C*D*A*T*R	8	NS	NS	NS	NS	*	NS

[a] Canopy cover measured using Canopeo photo processing software (fractional green canopy cover (%))
[b] NS = non-significant
[*] Significant differences at $a \leq 0.05$

Table 2.5 Effect of DFF rate and application timing (PP, E-PRE, and L-PRE) on soybean canopy cover reduction at 7, 14, and 28 DAE in 2020 and 2021. Data are the mean of two cultivars, two planting depths, three planting dates, and four replications. [a]

		Canopy cover reduction (%)					
		2020			2021		
Timing	DFF rate	7 DAE[b]	14 DAE	28 DAE	7 DAE	14 DAE	28 DAE
PP	90	1.4 a	1.5 a	0.4 a	0.3 ab	0.0 a	0.8 a
	180	0.0 a	2.4 a	5.1 ab	1.4 ab	0.0 a	2.7 a
	360	10.8 bc	16.6 bc	16.4 cd	0.0 a	2.2 bc	6.7 a
E-PRE	90	6.5 ab	9.9 ab	7.7 abc	5.1 ab	0.4 ab	0.6 a
	180	13.3 bc	19.8 c	17.4 d	0.0a	4.3 bc	4.0 a
	360	37.1 d	48.7 d	47.4 f	5.6 ab	18.9 d	20.0 b
L-PRE	90	15.4 c	15.8 bc	13.4 bcd	1.8 ab	0.0 a	1.8 a
	180	32.7 d	41.6 d	30.6 e	0.0a	7.3 c	6.3 a
	360	50.7 e	64.5 e	68.1 g	6.7 b	27.1 e	26.2 b

[a] Canopy cover measured using Canopeo photo processing software (fractional green canopy cover (%))
[b] Means within a column with the same letter are not significantly different according to the Tukey HSD test at $a \leq 0.05$

Table 2.6 Effect of planting date and DFF rate on soybean canopy cover reduction at 7, 14, and 28 DAE in 2020 and 2021. Data are the mean of two cultivars, two planting depths, three application timings and four replications. [a]

		Canopy cover reduction (%)					
		2020			2021		
Planting Date	DFF rate	7 DAE[b]	14 DAE	28 DAE	7 DAE	14 DAE	28 DAE
Planting 1[c] May 20, 2020 May 21, 2021	90	2.3 a	0.0 a	5.3 a	4.7 ab	0.0 a	3.7 a
	180	10.2 ab	9.2 b	17.7 bcd	4.5 ab	2.0 a	2.8 a
	360	31.2 c	32.6 de	49.7 e	7.4 ab	9.8 b	12.4 ab
Planting 2 May 31, 2020 May 27, 2021	90	13.5 b	22.2 cd	9.2 ab	0.0 a	0.0 a	0.0 a
	180	27.5 c	41.4 e	22.5 cd	0.0 a	8.8 ab	0.0 a
	360	56.0 d	67.4 f	52.0 e	0.0 a	35.9 c	19.9 b
Planting 3 June 9, 2020 June 4, 2021	90	7.4 ab	9.2 b	7.0 ab	8.9 b	0.0 a	3.7 a
	180	6.2 ab	13.3 bc	12.8 abc	8.3 b	0.0 a	10.7 ab
	360	11.3 ab	29.8 d	30.3 d	11.3 b	2.5 a	20.6 b

[a] Canopy cover measured using Canopeo photo processing software (fractional green canopy cover (%))
[b] Means within a column with the same letter are not significantly different according to the Tukey HSD test at $a \leq 0.05$
[c] Calendar planting dates for 2020 and 2021 are included below each numbered planting date

Table 2.7 Significance level for the effects of cultivar, planting depth, planting date, application timing and DFF rate and their interactions for soybean biomass assessed at 28 DAE and final yield in 2020 and 2021.[a]

		Biomass		Yield	
Source of variation	df	2020	2021	2020	2021
Cultivar (C)	1	*	NS	*	*
Depth (D)	1	NS[b]	NS	NS	*
Date (A)	2	*	NS	NS	NS
Timing (T)	2	*	*	*	*
Rate (R)	2	*	*	*	*
C*D	1	NS	NS	NS	NS
C*A	2	NS	NS	NS	NS
C*T	2	NS	NS	NS	NS
C*R	2	NS	NS	NS	NS
D*A	2	NS	*	NS	NS
D*T	2	NS	NS	NS	NS
D*R	2	*	*	NS	NS
A*T	4	*	NS	*	NS
A*R	4	*	NS	NS	NS
T*R	4	*	*	NS	NS
C*D*A	2	NS	NS	NS	NS
C*D*T	2	*	NS	NS	NS
C*D*R	2	NS	NS	NS	NS
C*A*T	4	NS	NS	NS	NS
C*A*R	4	NS	NS	NS	NS
C*T*R	4	NS	NS	NS	*
D*A*T	4	NS	NS	NS	NS
D*A*R	4	NS	NS	NS	NS
D*T*R	4	NS	NS	NS	NS
A*T*R	8	*	NS	NS	NS
C*D*A*T	4	NS	NS	NS	NS
C*D*A*R	4	NS	*	NS	NS
C*D*T*R	4	NS	NS	*	NS
C*A*T*R	8	NS	NS	NS	NS
D*A*T*R	8	NS	NS	NS	NS
C*D*A*T*R	8	NS	NS	NS	NS

[a] Biomass represents above ground dry mass clipped at the soil surface 28 DAE and final yield was taken at maturity from the middle two rows of each plot then adjusted to 13% moisture

[b] NS = Non-significant

[*] Significant at $a \leq 0.05$

Table 2.8 Effect of DFF rate and application timing (PP, E-PRE and L-PRE) on soybean biomass at 28 DAE and final yield in 2020 and 2021. Data are the mean of two cultivars, two planting depths, three planting dates and four replications. [a]

		Biomass reduction (%)[b]		Yield reduction (%)	
Timing	DFF rate	2020	2021	2020	2021
PP	90	0.0 a	0.0 a	0.0 a	0.0 a
	180	0.7 a	0.0 a	0.0 a	0.7 abc
	360	5.3 a	2.0 ab	0.0 a	0.0 a
E-PRE	90	4.4 a	0.0 a	8.7 ab	0.0 a
	180	5.2 a	0.4 a	0.0 a	0.0 a
	360	24.5 c	9.8 bc	13.1 ab	3.9 bc
L-PRE	90	4.6 a	1.2 a	2.3 a	0.0 a
	180	14.9 b	4.4 ab	7.3 ab	0.8 abc
	360	42.8 d	15.7 c	27.0 b	4.5 c

[a] Biomass represents above ground dry mass clipped at the soil surface 28 DAE and final yield was taken at maturity from the middle two rows of each plot then adjusted to 13% moisture

[b] Means within a column with the same letter are not significantly different according to the Tukey HSD test at $a \leq 0.05$

Table 2.9 Correlation of soybean injury and canopy cover reduction assessed at 7, 14, and 28 DAE, biomass assessed at 28 DAE, and final yield in 2020.

	C7[a]	C14	C28	V7[b]	V14	V28	Biomass	Yield
C7	1.00	0.73	0.64	-0.70	-0.69	-0.59	0.56	0.35
C14		1.00	0.70	-0.66	-0.74	-0.67	0.62	0.38
C28			1.00	-0.65	-0.67	-0.67	0.76	0.58
V7				1.00	0.90	0.71	-0.52	-0.19
V14					1.00	0.82	-0.54	-0.20
V28						1.00	-0.54	-0.26
Biomass							1.00	0.62
Yield								1.00

[a] C7, C14, C28 represent Canopeo canopy cover assessments at 7, 14, and 28 DAE

[b] V7, V14, V28 represent soybean injury assessments at 7, 14, and 28 DAE

Table 2.10 Correlation of soybean injury and canopy cover reduction assessed at 7, 14, and 28 DAE, biomass assessed at 28 DAE, and final yield in 2021.

	C7[a]	C14	C28	V7[a]	V14	V28	Biomass	Yield
C7	1.00	0.17	0.27	0.12	0.08	0.08	0.11	-0.09
C14		1.00	0.59	0.12	0.10	0.05	0.50	-0.09
C28			1.00	0.09	0.03	0.01	0.47	-0.04
V7				1.00	0.73	0.53	0.19	-0.11
V14					1.00	0.81	0.12	-0.21
V28						1.00	0.06	-0.24
Biomass							1.00	0.03
Yield								1.00

Table 2.11 Effect of planting date and planting depth on soybean canopy cover reduction at 14 DAE in 2020. Data are the mean of two cultivars, three application timings, three application rates, and four replications. [a]

Planting Date (Precipitation)	Planting Depth (cm)	Reduction in canopy cover (%)[b]
Planting Date 1	2.5	18.7 bc
(6.3 mm 1 DAE)[c]		
(9.1 mm 2 DAE)	5	6.4 a
Planting Date 2	2.5	42.1 d
(31.8 mm 2 DAE)		
	5	45.2 d
Planting Date 3	2.5	9 ab
(2.8 mm 2 DAE)		
(10.4 mm 6 DAE)	5	25.8 c

[a] Canopy cover measured using Canopeo photo processing software (fractional green canopy cover (%))

[b] Means within a column with the same letter are not significantly different according to the Tukey HSD test at $a \leq 0.05$

[c] Precipitation data from 1 to 6 DAE is included under each planting date. [a]

Figure 2.1. Example of a replicated block used in the factorial split-plot design. The main plots consist of three factors including planting date (Early, Middle, Late), planting depth (Shallow, Deep), and cultivar (1 = DKB 008-81, 2 = P09A53X). Combinations of the main plot factors are listed along the bottom of the block and one of the 12 main plots is outlined in light green. Within each main plot there are 10 subplots, which are combinations of application timing (PP, ePRE, lPRE) and DFF rate (90, 180, 360 g ai ha^{-1}), plus one untreated check for all combinations of application timing and rate. An example of a subplot is outlined in dark green within the figure.

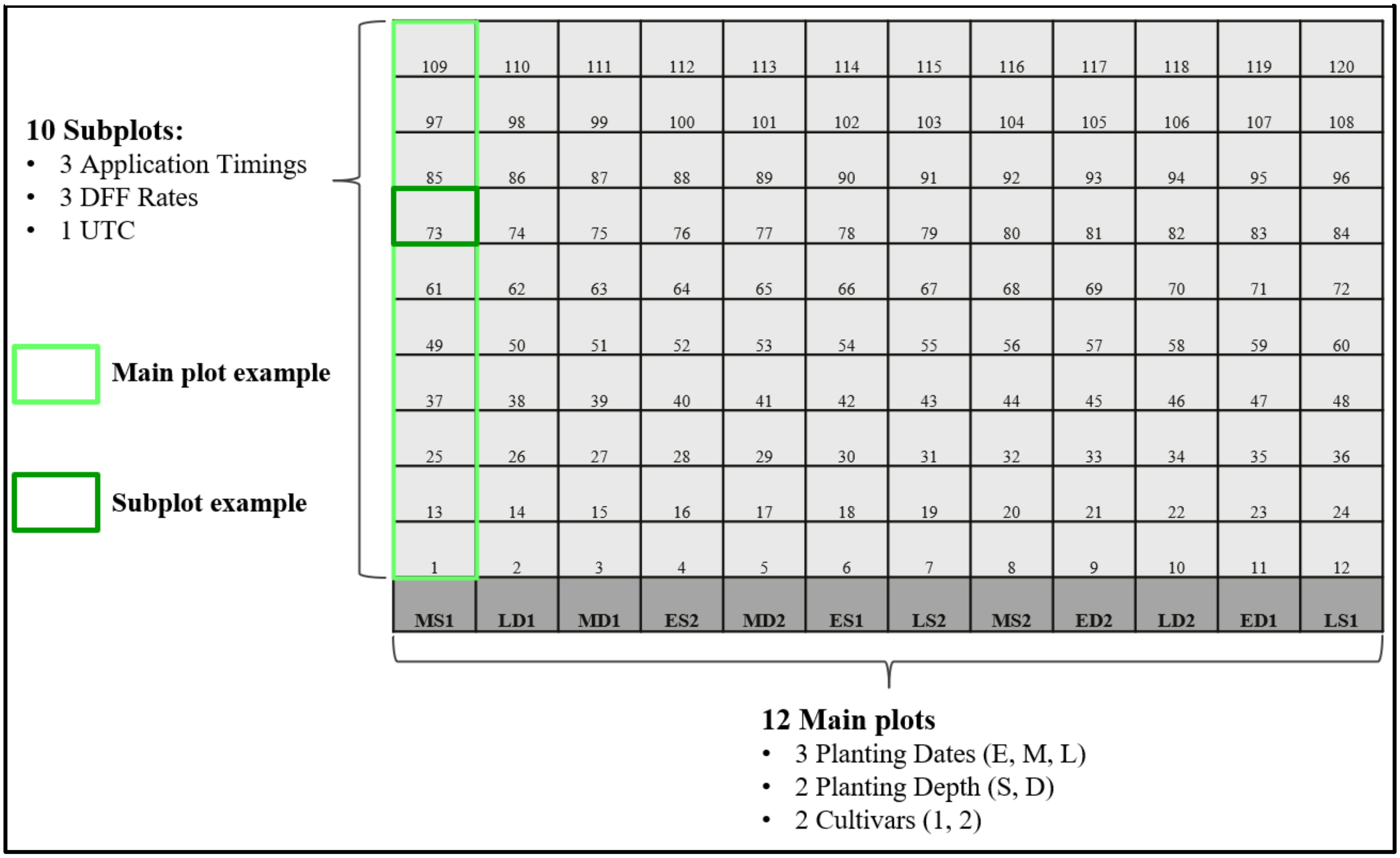


Figure 2.2a. Timeline indicating the approximate growth stage of soybean planted 2.5 cm and 5 cm deep and the timing of significant rainfall events that occurred for the early planting date in 2020 at Elora Research Station (Emergence: May 27, 2020). The narrow susceptible growth stage and strong influence of rain splash exposure generates differences in soybean phytotoxicity between the two planting depths. In this scenario significant rainfall 1 to 3 DAE (days after emergence) resulted in the shallow planted soybean to exhibit more phytotoxicity.

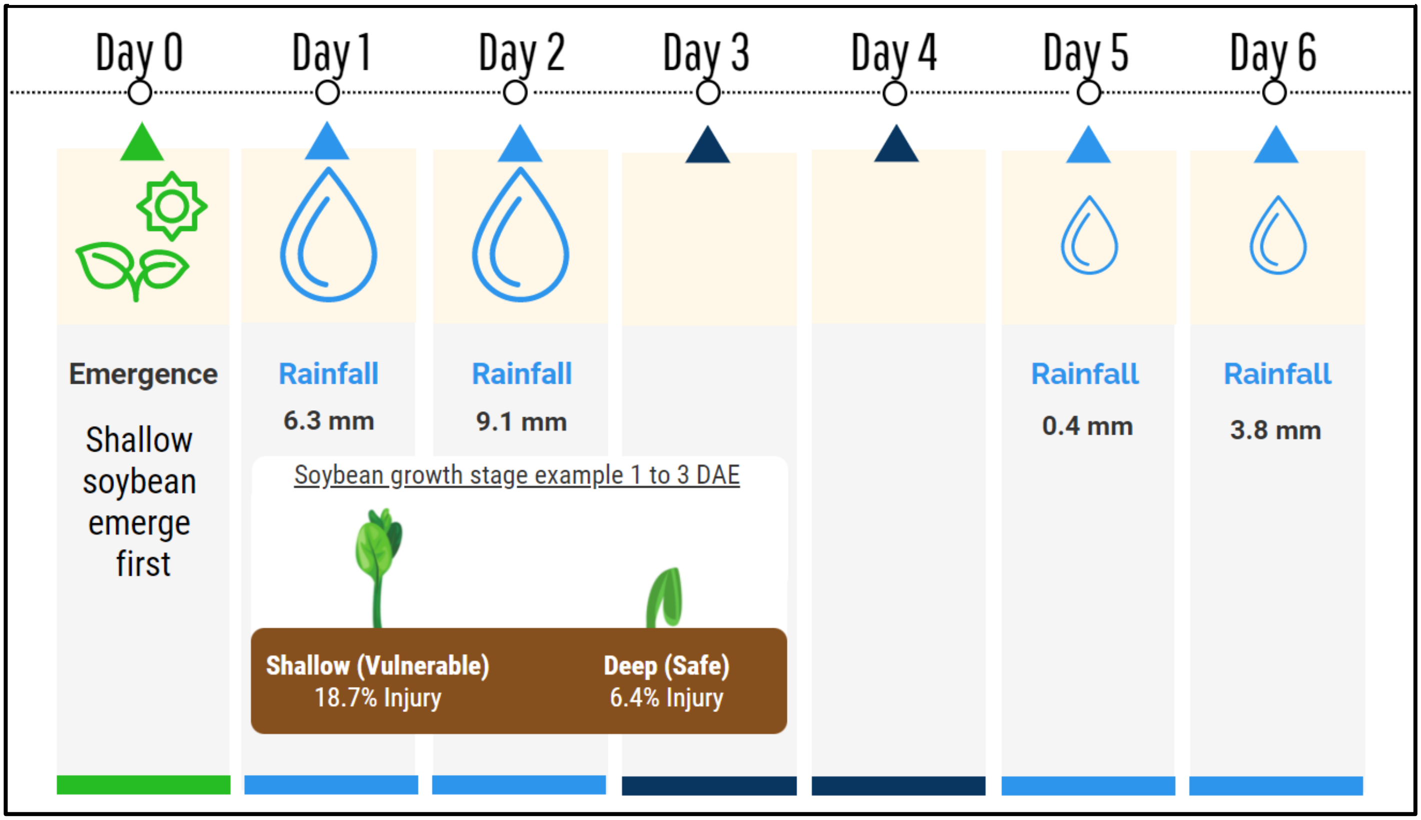
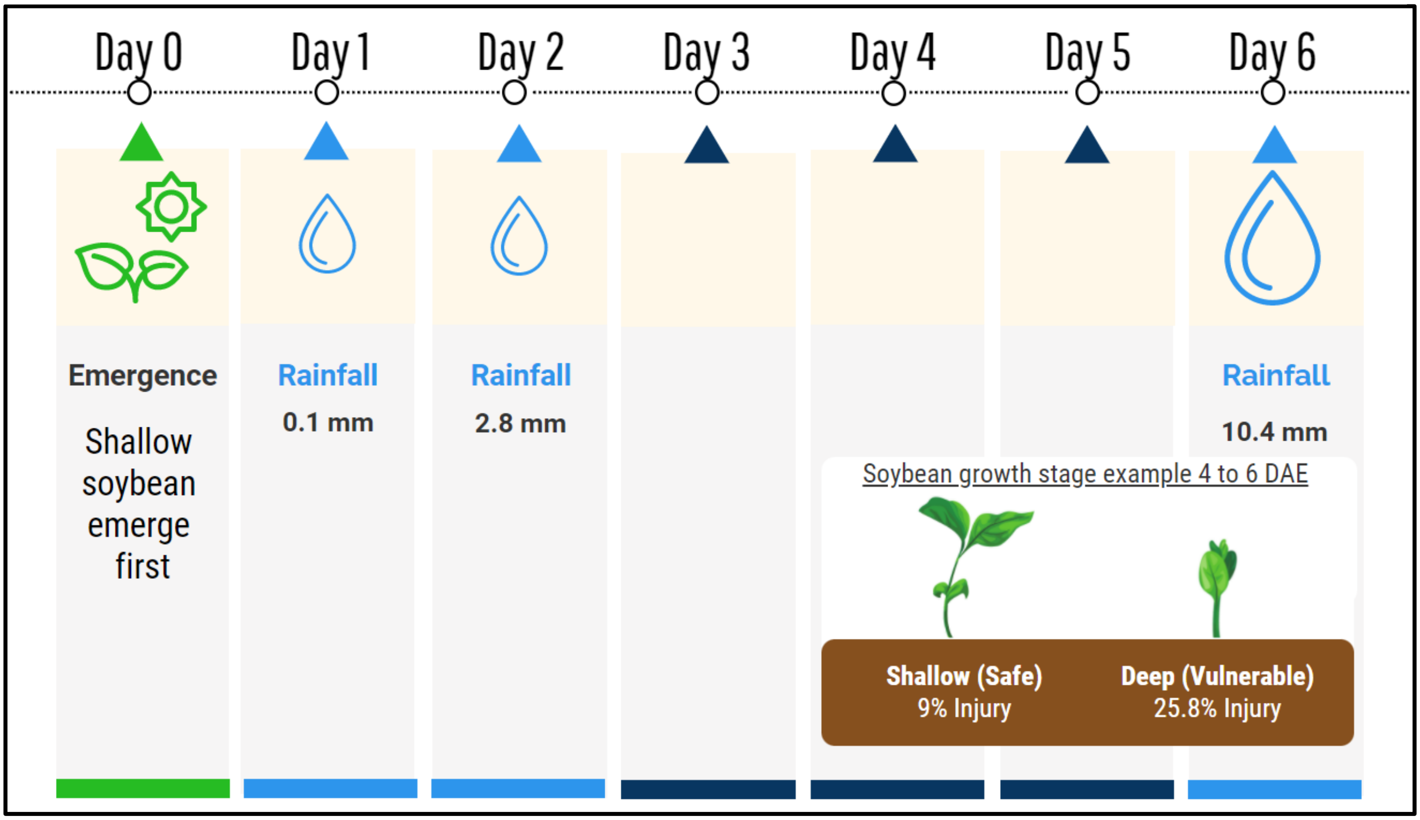


Figure 2.2b. Timeline indicating the approximate growth stage of soybean planted 2.5 cm and 5 cm deep and the timing of significant rainfall events that occurred for the late planting date in 2020 at Elora Research Station (Emergence: June 17, 2020). The narrow susceptible growth stage and strong influence of rain splash exposure generates differences in soybean phytotoxicity between the two planting depths. In this scenario significant rainfall 4 to 6 DAE (days after emergence) resulted in the deep planted soybean to exhibit more phytotoxicity



3. Soybean Cultivar Tolerance to Diflufenican

3.1 Abstract

The new soybean herbicide diflufenican (DFF; Group 12) demonstrates excellent preemergence (PRE) control of key *Amaranthus* spp., however, variable soybean tolerance has occurred under field conditions and is thought to be influenced by differential cultivar sensitivity. A growth room screening procedure was designed to evaluate the tolerance of 10 soybean cultivars to DFF. The herbicide was applied to various plant structures at several growth stages on the soybean seedlings to identify a highly susceptible site and growth stage for screening. Although DFF is applied PRE in soybean, postemergence (POST) applications at the cotyledon stage generated the most consistent response in soybean. Cultivars were treated with POST applications of DFF at rates ranging from 0.1 to 10,000 g ai ha^{-1} and were evaluated for visible injury 7 days after application (DAA). Despite large differences in tolerance at the field-scale, no notable differences in tolerance to DFF were observed under growth room conditions. The results of this study indicate that variability among cultivars is not the main explanation behind the variable responses observed in the field. Instead, it suggests other factors occurring at the field-scale influence soybean injury caused by DFF.

3.2 Introduction

Diflufenican (DFF) is a selective, contact, residual herbicide that has mainly been used in cereals and legumes in Europe since the 1980s (Cramp et al. 1987, Haynes and Kirkwood 1992). In North America, it is a novel active ingredient under development by Bayer Crop Science for use in corn and soybean (Bayer 2021). DFF can be applied preemergence (PRE) or early postemergence (POST) to target weeds and is highly effective on important broadleaf weeds such as waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer), Palmer amaranth (*Amaranthus palmeri* S. Watson), and other *Amaranthus* species (Bayer Crop Science, unpublished data). Applied PRE, the stability of DFF on the soil surface provides residual activity, with susceptible weeds absorbing the herbicide through their shoots as they emerge through the herbicide treated layer (Catchpole and Plumbe 1993).

DFF belongs to the pyridinecarboxamide chemical group and has inhibitory action within the carotenoid biosynthesis pathway (Sandmann et al. 1991). The primary target site is phytoene desaturase (PDS), an enzyme involved in a precursor reaction in the production of coloured carotenoids (Böger 1996; Sandmann et al. 1991). Carotenoids are essentially antioxidants that protect against photooxidation by quenching singlet oxygen within the photosynthetic unit (Böger 1996). Within susceptible plants, the inhibition of PDS creates a shortage of these protective pigments resulting in photooxidation (Böger 1996). This is expressed as bleaching in newly generated plant tissues, followed by chlorosis and eventual necrosis (Böger 1996; Sandmann et al. 1991). Weeds typically emerge bleached then quickly succumb to photooxidation (Catchpole and Plumbe 1993).

Anecdotal evidence from research across North America suggested differences in soybean tolerance to the DFF could possibly be linked to genetic differences among cultivars. Historically, soybean cultivars have demonstrated variable tolerance to several herbicides, including metribuzin and various protoporphyrinogen oxidase (PPO) inhibiting herbicides (Barrentine et al. 1976; Coble and Schrader 1973; Dayan et al. 1997; Hulting et al. 2001; Taylor-Lovell et al. 2001). In such cases, significant differences in herbicide tolerance among soybean cultivars were observed and cultivar screening allowed classification based on tolerance. Differential tolerance is often associated with varying rates of herbicide metabolism among cultivars and the resulting differences in relative tolerance has allowed for studies to classify cultivars as having low, medium, or high herbicide tolerance (Barrentine et al. 1976; Dayan et al. 1997; Hulting et al. 2001; Mangeot et al. 1979). This practice has been adopted by several seed companies, which indicate similar tolerance ratings within seed guides (Anonymous 2019). Further studies investigated the influence of cultural and environmental factors on soybean tolerance to these herbicides (Coble and Schrader 1973; Johnson and Wax 1979; Moshier and Russ 1981; Priess et al. 2020). This established best management practices and identified high-risk scenarios with the use of these herbicides, and ultimately allowed for the continued effective use of these products in soybean.

Similar studies are required to develop a better understanding of soybean tolerance to DFF prior to its registration in Canada and the US. This research will identify the existence of differential tolerance among soybean cultivars to DFF and could provide a foundation for more widespread screening and tolerance studies. Since the use of DFF in soybean is novel, no previous screening has been performed to confirm potential differences in cultivar tolerance. Thus, the objectives of this research were to design a simple and consistent screening procedure to test cultivar tolerance to DFF and to determine if differential tolerance to DFF occurs among soybean cultivars. Select cultivars that had exhibited divergent tolerance response to DFF in field trials, potentially indicating genetic based differences, were used to determine if their relative tolerance was consistent in a controlled environment. Cultivars with known high tolerance to metribuzin and PPO inhibiting herbicides were also included to determine if there was a link with DFF tolerance. Given the large difference in DFF tolerance observed at the field-scale, it was hypothesized that a set of 10 soybean cultivars would demonstrate differential tolerance within the screening procedure.

3.3 Materials and Methods

Seed Material

Ten soybean cultivars were selected based on current market availability, adaption to Ontario conditions and response to herbicides (Table 3.1). There are indications that cultivar DKB 008-81 has increased susceptibility to DFF based on previous field trials and it was included as a relatively susceptible cultivar. Cultivars P11A10 and P04A60R were included based on their known moderate or high susceptibility to metribuzin and PPO inhibitors (Anonymous 2019).

Growth Conditions and Experiment Design

Screening took place in a growth room at the University of Guelph, Guelph, Ontario in 2021. Growth room conditions were maintained using a 16-hour photoperiod, a day/night temperature regime of 26˚/21˚ C, and a relative humidity of 65%. Light was provided by LED tubes and bulbs at a level of 400 μmol m^{-2} s^{-1}.

Plastic pots (17.8 cm by 12.7 cm by 5 cm – LxWxD) were filled with a peat moss based potting mix (Sunshine No. 4, Sun Gro Horticulture Canada Ltd., Agawam, MA, USA) and placed in plastic flats (55.9 cm by 30.5 cm by 5 cm) for sub irrigation. The pots were watered to saturation prior to sowing. Each pot contained eight seeds planted 2 cm deep and was randomly allocated within the growth room. At the cotyledon stage, after initial development of the unifoliate leaves between the cotyledons, each pot was thinned to the four most uniform soybean seedlings and randomly assigned a treatment level (Figure 3.1). Despite DFF's usual application timing as a PRE herbicide in soybean, bleaching activity under our conditions was only observed with applications to young aboveground plant structures. Preliminary tests demonstrated that soybean seedlings are highly susceptible in the cotyledon stage as the unifoliate leaves are initially developing in the growth point and applications to the cotyledons at this stage generated the most consistent and uniform response to DFF (Figure 3.1).

After thinning, herbicide treatments were applied POST using a single nozzle track spray chamber. The sprayer was equipped with a TeeJet 8002E nozzle tip calibrated to deliver 210 L ha^{-1} at 276 kPa (Spraying Systems, Wheaton IL, USA). DFF formulated as a 500 g L^{-1} suspension concentrate was supplied by Bayer Crop Science (Bayer Crop Science Inc., Calgary, AB, Canada). The herbicide was sprayed at 0, 0.1, 1, 10, 100, 1,000, and 10,000 g ai ha^{-1}. Following application, pots were returned to the growth room, grown out for one week following application, and sub irrigated as needed. Sub irrigation was required to avoid movement of the herbicide from splashing. Visible soybean injury ratings included the combined effect of bleaching, chlorosis, stunting, and malformation of the unifoliate were completed one week after treatment.

Experimental design and statistical analysis

The pots were placed in the growth room according to a completely randomized design (CRD) with seven treatment levels (herbicide doses) and six replications. The experiment was repeated twice over time. Initial Generalized Linear Mixed Model (GLMM) analysis of visible soybean injury (%) using PROC GLIMMIX (SAS 9.4, SAS Institute Inc., Cary, NC, USA) with the beta distribution revealed no significant interactions between herbicide dose and replications over time. Consequently, data from both replications over time were pooled. Data was further analyzed with PROC GLIMMIX as a two-factor factorial CRD with cultivar and herbicide dose as the two treatment factors. Visible soybean injury (%) was transformed into a BETA distribution then analyzed using the PROC GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Alpha value for variance analysis was set to ≤0.05 to determine the effect of the two treatment factors and their interaction. Tukey's multiple comparison test was utilized to determine differences among the treatment means. The assumptions for the statistical analysis were met.

3.4 Results and Discussion

There was no interaction between DFF dose and soybean cultivar so the main effects are presented (Table 3.2). There was no difference among the soybean cultivars evaluated so the effect of DFF dose is presented (Table 3.3). DFF phytotoxicity was first observed in all soybean cultivars at 10 g ai ha^{-1} (1%) and increased as the rate of DFF applied increased (Table 2). The variability within and between units also increased as rates increased.

The maximum observed level of injury was 23% at the highest DFF dose. This was surprising considering this dose of 10,000 g ai ha^{-1} drastically exceeded the normally recommended doses of 60 to 180 g ai ha^{-1}. Moreover, the severity of injury observed when doses of 90 to 360 g ai ha^{-1} of DFF were applied PRE in field trials was much higher (Chapter 2). This disparity in phytotoxicity may be due to a number of factors related to the unique activity of DFF in soybean. DFF activity in soybean can be influenced by significant rainfall events that splash concentrated herbicide onto emerged seedlings (Chapter 4). This could generate an increased level of exposure and the continued rewetting could extend the period of uptake with this relatively insoluble and immobile molecule. This effect was accounted for and removed from the screening procedure by sub irrigating the experimental units, which may have resulted in comparably less DFF uptake and activity than observed under field conditions despite POST applications being used.

Previous studies examining soybean cultivars tolerance to herbicides found a high level of correlation between tolerance in the field and tolerance under laboratory or greenhouse conditions (Barrentine et al. 1976; Hulting et al. 2001; Taylor-Lovell et al. 2001). This permitted a reliable classification of cultivars and further studies examining the mechanisms of tolerance (Barrentine et al. 1976; Hulting et al. 2001). The DFF screening procedure used in this study was not able to confirm that the variability in response to DFF observed in field trials is due to genetic variation among cultivars. This suggests that other factors under field conditions may explain the causes of soybean injury from DFF.

Differential herbicide metabolism is usually the basis for soybean cultivar sensitivity to herbicides (Connelly et al. 1988; Dayan et al. 1997; Mangeot et al. 1979). This is similar to differences observed between tolerant crops and susceptible weeds species (De Carvalho et al. 2009). Previous research has shown that this may not be the case with DFF (Haynes and Kirkwood 1992). Instead, it is thought that differences in uptake and translocation are the predominant factors influencing tolerance to DFF (Haynes and Kirkwood 1992). Only 0.3% to 1% of radiolabelled DFF was absorbed by the leaves of tolerant crops like wheat and barley compared to 6% to 9% uptake in susceptible weed species (Haynes and Kirkwood 1992). This aligns with other studies reporting that DFF activity in certain weed species is limited by poor uptake and mobility, with physiological and morphological differences between species thought to generate the differences in uptake (Ahmad 1991; Haynes and Kirkwood 1992; Knight and Kirkwood 1991). It is possible that physiological differences among soybean cultivars may be involved in the variable tolerance observed at the field-scale. For example, differences in seedling growth rate and emergence could influence the level of exposure to DFF or the period of susceptibility when DFF can be absorbed through young plant tissue, especially considering the highly susceptible growth stage identified in the screening procedure was relatively narrow.

Soybean cultivar selection does not appear to influence tolerance to DFF. The 10 cultivars screened for tolerance in this study all demonstrated a similar response to POST DFF applications ranging from 0.1 to 10,000 g ai ha^{-1}. This information highlights the fact that other factors may influence soybean tolerance to DFF at the field level. Physiological differences among soybean cultivars may play a role in the observed difference in tolerance in DFF field trials, however the resulting phytotoxicity is likely related to the environmental and cultural conditions within given field scenarios. Further studies should be performed to examine the dynamic relationship between these factors and how they ultimately influence tolerance.

Table 3.1 Soybean cultivar name, company, maturity, and trait for ten cultivars used in this study.

Cultivar	Company	CHU	HTR trait	Notes
DKB 008-81	Dekalb	2500	RR2X	Possible susceptibility to DFF
DKB 04-41	Dekalb	2650	RR2X	
DKB 12-16	Dekalb	2875	RR2X	
B011DE	Brevant	2550	E3	
P05A35X	Pioneer	2675	RR2X	
P06A51X	Pioneer	2675	RR2X	
P06A13R	Pioneer	2675	RR	
P09A53X	Pioneer	2750	RR2X	
P11A10	Pioneer	2800	NGMO	Susceptible to PPO inhibitors; Reduced tolerance to metribuzin
P04A60R	Pioneer	2625	RR	Tolerant to PPO inhibitors Reduced tolerance to metribuzin

RR: tolerance to glyphosate (Roundup Ready trait)
RR2X: tolerance to glyphosate and dicamba (Roundup Ready 2 Xtend trait)
E3: tolerance to 2,4-D, glyphosate and Glufosinate (Enlist E3 trait)

Table 3.2 Results of generalized mixed model variance analysis (ANOVA) for the effects of cultivar, herbicide dose, and their interaction on soybean injury (%) from diflufenican applied POST to ten soybean cultivars.

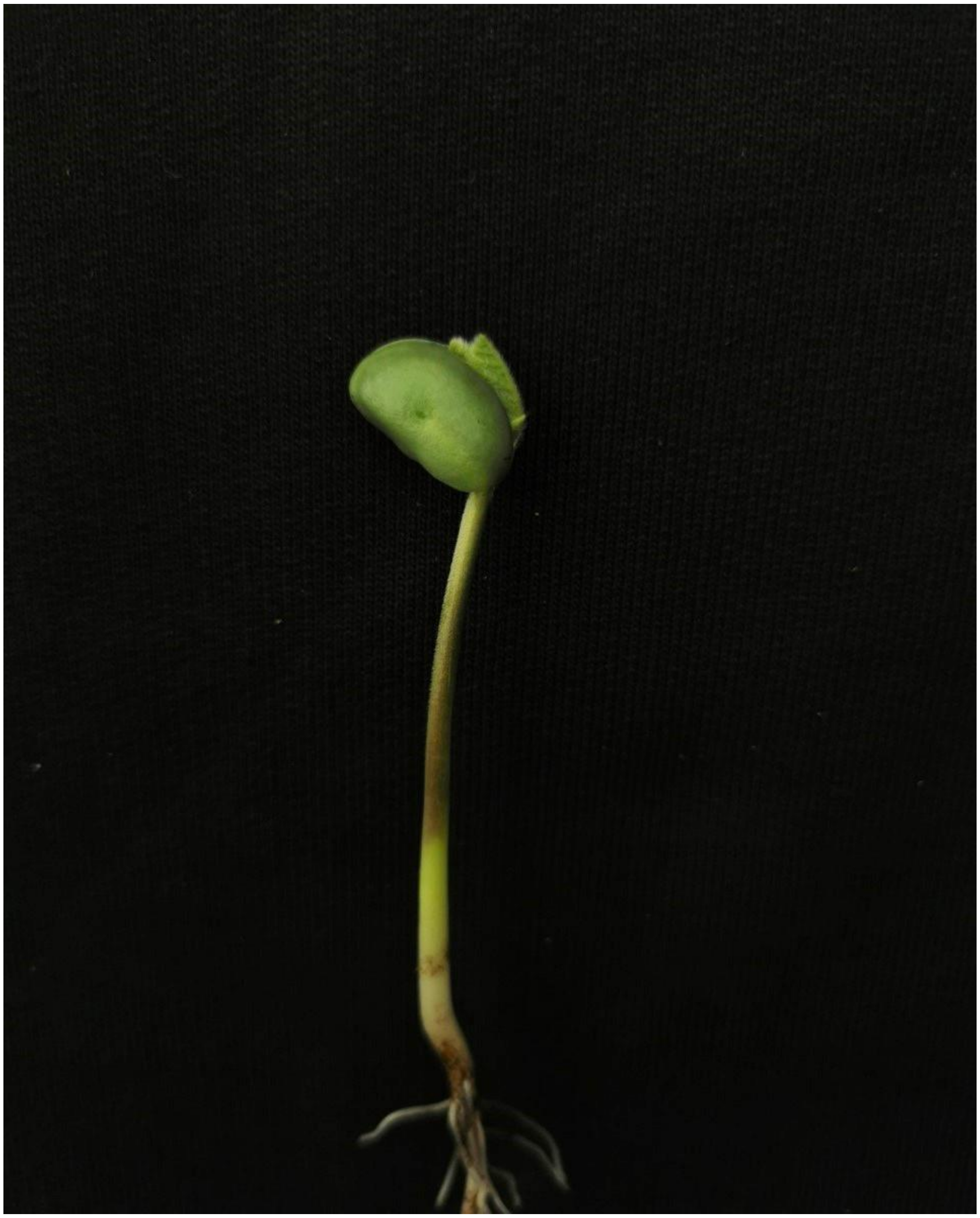
Fixed effect	Num df	Den df	F Value	Pr > F
Cultivar	9	770	0.72	0.6868
Dose	6	770	1570.39	<.0001
Cultivar*Dose	54	770	1.31	0.0724

Table 3.3 Soybean injury dose response to POST application of diflufenican averaged over ten cultivars

Dose (g ai ha^{-1})	Visible Injury (%) [a]	Standard Error
0	0 a	0
0.1	0 a	0
1	0 a	0
10	1 b	0.06
100	5 c	0.1
1,000	14 d	0.2
10,000	23 e	0.3

[a] Means followed by the same letter in a column are not significantly different according to Tukey's multiple comparison test at $\alpha<0.05$

Figure 3.1 Soybean growth stage at time of POST DFF application. DFF applied to the cotyledon as the unifoliate leaves are initially developing between the cotyledons was identified as a highly susceptible location and growth stage for DFF activity in soybean.



4. The Influence of Rain Splash on Phytotoxic Response of Soybean Treated with Diflufenican and Flumioxazin

4.1 Abstract

Rainfall events at or just after soybean emergence can cause crop phytotoxicity from soil-applied herbicides. Concentrated herbicide on the soil surface can splash onto susceptible plant tissues on the hypocotyl, cotyledon, and growing points, which can result in severe crop injury, crop loss, or yield loss. Despite this, little formal research has been conducted to understand how rain splash contributes to phytotoxicity in soybean. The unique activity of the soil-applied herbicide diflufenican (DFF; Group 12) prompted an investigation into its splash damage potential in soybean. In preliminary trials, soybean demonstrated variable tolerance to DFF, with several factors thought to influence soybean injury. While rainfall was thought to be a factor, rain splash was not considered until splash damage was observed during the development of a cultivar screening procedure. A simple irrigation method by herbicide treatment factorial experiment was designed to determine the influence of rain splash on soybean injury from DFF. To generate rain splash, simulated rainfall was applied from 50 cm above the surface of the pots, while a drip irrigation system was used as the other irrigation method. Flumioxazin, a herbicide with reported cases of splash damage was also used in the same methodology. Results indicated that soybean phytotoxicity was directly related to irrigation method, with significant injury from both herbicides only observed in units treated with herbicide and simulated rainfall. The deposition of water and soil material on the soybean seedlings demonstrate the increased level of exposure that can occur when rainfall events occur at or shortly after soybean emergence. Relating these findings to other DFF field trials, rainfall timing and intensity appears to be a prominent factor influencing soybean tolerance to the herbicide. In addition, this information can

be linked to other factors thought to influence soybean tolerance and used to better manage the early season injury risks associated with these herbicides.

4.2 Introduction

Unfavourable environmental conditions during soybean germination and emergence can increase the risk of phytotoxicity from soil-applied herbicides (Moomaw and Martin 1978; Poston et al. 2008; Swantek et al. 1998). Cold, wet soils can reduce plant growth and metabolism while also increasing herbicide availability and uptake, leading to high-risk scenarios (Hulting et al. 2001; Poston et al. 2008). Excessive moisture from early season rainfall moves herbicide material from the soil surface to the depth of emerging seedlings or to the root zone of the crop (Hartzler 2004; Hixson 2008). Here, young susceptible plant structures more readily absorb the herbicide. Less soluble herbicides can remain at the soil surface and be more damaging after seedlings have broken through the soil (Wauchope 1978; Hartzler 2005). This can also occur when the first activating rainfall takes place as the crop emerges (Hartzler 2005; Yoshida et al. 1991). In these scenarios, rainfall timing and intensity must also be considered, as concentrated herbicide in solution and on soil material can be splashed from the soil surface onto the emerged seedlings.

The occurrence of splash damage from herbicides is generally understood by both farmers and weed scientists, however, reports on the topic remain mostly anecdotal. Limited peer-reviewed literature exists examining the splashing potential of various herbicides or the influence it has on soybean phytotoxicity. Rain splash is also rarely referenced in herbicide labels, yet in conditions conducive to rain splash, necrotic lesions on seedlings and speckle chlorosis and necrosis on the lower leaves can be commonly observed on emerged soybean (Hartzler 2004).

Some information on rain splash can be found in the extension literature. These articles all report heavy rainfall events at or just after soybean emergence and significant injury in trials treated with protoporphyrinogen oxidase (PPO) inhibiting herbicides (Hartzler 2004, 2005; Wise et al. 2015). Herbicide was splashed and washed onto soybean hypocotyls, cotyledons, and growing points, leaving necrotic lesions on the contacted tissues (Hartzler 2004, 2005; Wise et al. 2015). This type of exposure has also been documented with metribuzin (OMAFRA 2017). In severe cases, the hypocotyls can become girdled, or the growing point can be completely killed, resulting in crop loss (Hartzler 2004). It is thought that the degree of injury depends on several factors including herbicide selection, herbicide rate, soil type, rainfall amount and intensity, growth stage of the soybean, and cultivar sensitivity (Hartzler 2005).

Diflufenican (DFF) represents an under-utilized mode of action (Group 12) and is a novel active ingredient for field crop production in North America. The primary target site of DFF is phytoene desaturase, an enzyme involved in a precursor reaction in the production of coloured carotenoids (Böger 1996). Similar to other bleaching herbicides, the resulting decrease in these protective pigments leaves susceptible plants bleached and vulnerable to photooxidation (Böger 1996). The low solubility of DFF allows it to form a stable herbicide layer on the soil surface, offering excellent residual activity on dicot weeds (Catchpole and Plumbe 1993, Haynes and Kirkwood 1992). It is particularly effective on important species like waterhemp (*Amaranthus tuberculatus* (Moq.) Sauer), Palmer amaranth (*Amaranthus palmeri* S. Watson), and other *Amaranthus* species (Bayer, unpublished data). Pending approval in Canada and the U.S., the registration of DFF is intended to target these species and be an important tool to slow the evolution and impact of herbicide-resistant weeds (Bayer 2021).

Throughout the initial field testing of DFF, many studies reported differences in soybean tolerance to the herbicide. Thus, studies investigating the factors influencing soybean tolerance to DFF were required. It was thought that cultivar selection, cultural practices, and environmental conditions may all influence soybean tolerance. Initial hypotheses on the splash damage potential of DFF were formed through observations from the development of a cultivar screening procedure. DFF activity was shown to be dependent on contact with foliage shortly after emergence. Severe bleaching comparable to field scenarios was observed with applications to the cotyledon just prior to unifoliate development, however, the question remained how the soil-applied herbicide was transferred to this location on the plant for uptake. As the screening methodology progressed using foliar applications at the cotyledon stage, the overhead water technique used in the growth room demonstrated how herbicide material may be transferred to additional plant material at this susceptible growth stage. Untreated units neighbouring those with high rates displayed bleaching symptomology, indicating the potency and splash damage potential of DFF in soybean.

Identifying potential avenues of soybean exposure to DFF is essential for reducing the risk of early season injury. Additionally, linking rainfall timing and intensity to other factors in field scenarios can help build a more complete understanding of the variable tolerance observed when DFF is applied in soybean. This study aimed to determine the influence of rain splash on soybean tolerance to DFF. An experiment was designed to test the hypothesis that rain splash from simulated rainfall would cause measurable soybean phytotoxicity and lead to heightened bleaching symptomology. To confirm the accuracy of the methodology and further understand the role of rain splash on herbicide injury in soybean, the experiment was also conducted with flumioxazin, a PPO inhibiting herbicide known to cause splash damage in soybean (Hartzler 2004, 2005; Wise et al. 2015).

4.3 Materials and Methods

The experiments were performed in a controlled growth room environment with a 16-hour photoperiod, a day/night temperature regime of 26/21C, and a relative humidity of 65%. Light was provided by LED tubes and bulbs at a level of 400 μmol m^{-2} s^{-1}.

Four soybean seeds were planted 2.5 cm deep in 12.7 X 12.7 cm square pots filled with 100% field soil medium. The soybean cultivar DKB 008-81 was provided by Bayer Crop Science for the experiment. The soil used for this study was collected from the Elora Research Station (Elora, ON, Canada) and is a London loam series soil (50% silt, 31% sand, 19% clay, pH 7.4, 4.2% organic matter) (soil test performed by A&L Laboratories Inc., London, ON Canada). Prior to sowing, experimental units were prepared by installing an irrigation dripper to the center of each pot and applying 100 ml of water. Additionally, the soil surface was dampened for better sowing conditions. After planting, the irrigation system was set to deliver 70 ml of water per day until soybean emergence.

Two herbicides were examined separately in experiments designed according to a two-factor factorial randomized complete block design (RCBD) with six replications. The factors were irrigation type and herbicide rate with each factor having two levels, resulting in four total treatments. The irrigation treatments included a drip irrigation treatment using one dripper per pot, and a simulated rainfall treatment applied from 50 cm above the pot surface using a 1 L plastic (PET) bottle (Figure 4.1). Two holes (1.5875 mm; 1/16 inch) were drilled in the bottle cap and a hole (2.38125 mm; 3/32 inch) was drilled near the center, on the side of the bottle to allow for airflow. Both irrigation types received 70 ml of deionized water per day, with the simulated rainfall treatment applied in two 35 ml applications to avoid excess pooling on the pot surface; the second application was done one minute after the first. Experimental units receiving the simulated rainfall treatment were temporarily removed from the study area during treatment to avoid splashing onto neighbouring units. Irrigation treatments began at soybean emergence so that the herbicide on the soil surface was not disturbed and were continued for the duration of the experiment. The two herbicide treatment levels were 0 and 360 g ai ha^{-1} for DFF (500 g L^{-1} suspension concentrate formulation; Bayer Crop Science Inc. Calgary, AB, Canada) and 0 and 210 g ai ha^{-1} for flumioxazin (51.1% water dispersible granule formulation; Nufarm Canada, Calgary, AB, Canada). Herbicide treatments were applied pre-emergence three days after planting using a single nozzle track spray chamber. The sprayer was equipped with a TeeJet 8002E nozzle tip calibrated to deliver 210 L ha^{-1} at 276 kPa (Spraying Systems, Wheaton IL, USA). Following the herbicide application, the pots were randomized within the growth room bench space and grown out for one week. Visible soybean injury ratings were recorded on a scale of 0 to 100%, where 0% means no visible injury and 100% means complete plant necrosis and death; ratings included the combined effect of bleaching, chlorosis, stunting, and malformation.

Statistical Methods

The factorial RCBD experiment with four treatments and six replications was performed twice with each herbicide. The soybean injury data was pooled for each herbicide and transformed into the BETA distribution for analysis. The herbicide and irrigation method treatments were used as fixed effects and replication as the random effect within the factorial RCBD design. Soybean injury (%) was analyzed using the PROC GLIMMIX procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). An analysis of variance (ANOVA) with an alpha value set to ≤0.05 was used to determine differences among treatment groups. Tukey's multiple comparison test was utilized to determine differences among the treatment means. The assumptions for the statistical analysis were met.

4.4 Results and Discussion

The level of DFF injury observed in soybean was directly related to the irrigation treatment, with bleaching and stunting occurring in all units subject to 360 g ai ha^{-1} DFF and the simulated rainfall and little to no injury occurring in units with the same herbicide rate and the drip irrigation (Table 4.1). Soybean sprayed with flumioxazin demonstrated a similar trend, with necrotic lesions and severe stunting only occurring in units treated with the simulated rainfall (Table 4.2). The simulated rainfall treatment generated considerable deposition of both water and soil material on the soybean seedlings (Figure 4.2). This demonstrated the increased level of exposure that can occur when preemergence herbicides are applied. Herbicide in solution and on soil material reached more plant material overall, and the young plant tissues readily absorbed both herbicides. This interaction between the two factors was significant at seven days after treatment when plants were evaluated for visible injury (Tables 4.1 and 4.2).

The severity of injury differed between the two herbicides when splashed onto the soybean seedlings. Average visible injury for DFF was 6%, while the flumioxazin treatment generated visible injury of 27% on average (Tables 4.3 and 4.4). This is likely related to the mode of action of each herbicide. Flumioxazin splashed on to soybean seedlings caused injury wherever it landed, producing necrotic lesions on a significant proportion of the impacted seedlings. As reported in the field, this completely killed the growing point of the plant, halting growth at the cotyledon stage in some units (Hartzler 2004). Conversely, DFF splashed on to the soybean seedlings does not generate bleaching where the herbicide makes contact with the plant. While DFF is generally considered a contact herbicide, its activity within the carotenogenic pathway can only express the bleaching symptomology in new plant tissues. Therefore, seedlings were able to advance to the unifoliate stage, where varying degrees of bleaching were observed in the unifoliate leaves. Some individual soybean seedlings within the experimental units treated with DFF demonstrated injury as high as 30% and it is thought that minor differences in soybean growth stage during the early rainfall applications created variability within units, as DFF activity has shown to be related to application location and growth stage in soybean (Chapter 3). A single unit subject to DFF and the drip irrigation treatment showed minor bleaching symptoms while the rest showed no adverse effects. These results indicate that rainfall is directly related to the degree of DFF phytotoxicity observed in soybean. Rainfall timing and intensity has potential to increase the level of DFF exposure and activity and could be contributing factors to the variable tolerance observed when DFF is applied in soybean.

This is the first conclusive demonstration that soybean injury from DFF is dependent on rain splash and reveals the importance of both rainfall intensity and timing to the resulting soybean phytotoxicity. These discoveries were aided by examining the activity of DFF during the development of a cultivar screening procedure. DFF only demonstrated phytotoxicity when applied to the foliage, and applications to the cotyledon as the unifoliates are first developing in the growing point was identified as a highly susceptible location and growth stage for DFF activity (Chapter 3). Consequently, this necessitates that DFF applied to the soil be transferred 1 to 3 inches off the soil surface to the cotyledons on the soybean seedlings. The use of simulated rainfall in this experiment demonstrated the transfer of herbicide from the soil surface to the soybean seedlings via the splashing of herbicide in solution or on soil material. This emphasizes the role of intense rainfall events to facilitate this transfer of herbicide material and increase DFF exposure and uptake in soybean. Additionally, rainfall timing is also key for DFF activity in soybean, since the highly susceptible growth stage identified in soybean is relatively narrow (Chapter 3). This may be due to the lipophilicity of the molecule (K_{ow} = 4.2) and the relatively limited uptake and mobility of DFF in plant tissues (Haynes and Kirkwood 1992; Knight and Kirkwood 1991). Previous studies have shown that applications to young plant tissues, such as the hypocotyl or coleoptile demonstrated the highest rates of uptake and injury, and it is thought that the thinner cuticular waxes at this stage increase the amount of herbicide reaching meristematic tissues (Ahmad 1991; Haynes and Kirkwood 1992). This is somewhat consistent with the susceptible cotyledon stage identified in soybean (Chapter 3). It is possible that comparable rates of DFF uptake could occur through the hypocotyl and cotyledons as the soybean are initially breaking through the soil surface, however, at this earlier growth stage there is little new tissue generated that can exhibit the bleaching symptomology. A more formal investigation of the uptake and translocation patterns of DFF in soybean would confirm these suggestions.

The identification of a highly susceptible growth stage and the mechanism of exposure in soybean can be used to pinpoint high-risk scenarios with applications of DFF. Therefore, rainfall timing and intensity must be considered in future field trials examining DFF in soybean. In addition, this information could be used to revisit data from previous trials that did not consider this relationship to make connections between rainfall events and observed phytotoxicity. For example, significant rainfall events (~25 to 30 mm) two days after soybean emergence appeared to align with the susceptible growth stage and lead to the most severe phytotoxicity in various DFF field trials in 2020 and 2021 (data not shown).

Understanding how rainfall timing and intensity influence tolerance at the field-scale could also reveal how these events interact with other factors thought to influence soybean tolerance. This was considered in a concurrent trial examining how cultural factors can influence soybean tolerance to DFF (Chapter 2). For example, two planting depths were used to determine whether shallow or deep planting can be used to mitigate against DFF injury. However, at some planting dates the shallow planting depth appeared more tolerant, while at others the deep planting depth appeared to be more tolerant. Upon review of the rainfall data for the site, it is hypothesized that differences in soybean staging between the two planting depths relative to the timing of rainfall generated the variability in the observed level of tolerance (Figure 4.3). This same effect may also be observed among soybean cultivars, where physiological differences in growth rate and emergence can result in cultivars planted on the same date differing in their growth stage. Since the highly susceptible growth stage occurs in a narrow window, these minor differences in staging could create large differences in tolerance, leading to false assumptions about the role of relative cultivar tolerance or planting depth.

While farmers have little control over rainfall patterns, they can still reduce the risk of splash damage from soil-applied herbicides. One strategy is to expand the time interval between herbicide application and soybean emergence. This increases the likelihood of adequate activation and dilution within the soil and reduces the concentration of herbicide on the soil surface that could potentially cause splash injury (Hulting et al. 2001; Swantek et al. 1998). This has been demonstrated with PPO inhibiting herbicides and the labels for sulfentrazone and flumioxazin try to account for this by suggesting applications no later than three days after planting (Anonymous 2017; Anonymous n.d.). Some studies recommend these herbicides be applied even earlier, using preplant applications to reduce the risk of injury even further (Priess et al. 2020). This was observed with DFF in 2020 and 2021 field trials, with preplant applications consistently reducing DFF phytotoxicity across a variety of environmental conditions (Chapter 2). More research is required to examine the potential for preplant applications to reduce phytotoxicity while maintaining residual control on key weed species.

Management tactics related to soil type could also be examined. PPO inhibiting herbicides already have soil type specific field rates related to texture and organic matter, and DFF has shown to be similarly influenced by organic and clay fractions within the soil (Anonymous 2017; Anonymous n.d.; Tejada 2011). While herbicide activity is typically greater on coarse textured soils, it must be considered that poorly drained soils can promote pooling and splashing potentially increasing herbicide exposure (Hartzler 2005).

Rainfall timing and intensity must be considered when examining soybean tolerance to the new soybean herbicide DFF. Identifying this alternative avenue of exposure is important for understanding variable tolerance in soybean and must be examined in various field scenarios to generate the foundational knowledge required for proper management. Rainfall events have shown to interact with other factors influencing soybean tolerance and more research is needed to examine these relationships.

This simple methodology was effective at demonstrating the splash damage potential of two soil-applied herbicides in soybean. While small droplets from a height of 50 cm may not fully capture the same magnitude of splashing that can occur from rainfall events at the field scale, these methods were still effective at moving water and soil material from the soil surface to the aboveground portion of the plants. Substituting various crops and herbicides into this design would be straightforward and could further expand the understanding of how splashing contributes to phytotoxicity in various crop-herbicide scenarios.

Table 4.1 Results of generalized mixed model variance analysis (ANOVA) for the effects of irrigation type, herbicide rate, and their interation on visual injury (%) from diflufenican in soybean 1-week after treatment. [a]

Fixed effect	Num df	Den df	F Value	Pr > F
Herbicide	1	44	57.24	<.0001
Irrigation	1	44	52.89	<.0001
Herbicide*Irrigation	1	44	52.89	<.0001

[a] Estimate for random block effect was zero and was removed from the analysis

Table 4.2 Results of generalized mixed model variance analysis (ANOVA) for the effects of irrigation type, herbicide rate, and their interaction on visual injury (%) from flumioxazin in soybean 1-week after treatment. [a]

Fixed effect	Num df	Den df	F Value	Pr > F
Herbicide	1	44	64.36	<.0001
Irrigation	1	44	46.77	<.0001
Herbicide*Irrigation	1	44	46.77	<.0001

[a] Estimate for random block effect was zero and was removed from the analysis

Table 4.3 Response of soybean phytotoxicity to diflufenican and irrigation method.

Treatment	Visual Injury (%) [a]	Standard Error
Drip Irrigation – 0 g ai ha^{-1}	0 a	0.036
Simulated Rainfall – 0 g ai ha^{-1}	0 a	0.036
Drip Irrigation – 360 g ai ha^{-1}	0.09 a	0.039
Simulated Rainfall – 360 g ai ha^{-1}	6 b	0.65

[a] Means with the same lowercase letter in a column are not significantly different at $\alpha<0.05$ using Tukey's multiple comparison test

Table 4.4 Response of soybean phytotoxicity to flumioxazin and irrigation method.

Treatment	Visual Injury (%) [a]	Standard Error
Drip Irrigation – 0 g ai ha^{-1}	0 a	0.19
Simulated Rainfall – 0 g ai ha^{-1}	0 a	0.19
Drip Irrigation – 210 g ai ha^{-1}	0.67 a	0.27
Simulated Rainfall – 210 g ai ha^{-1}	27 b	3

[a] Means with the same lowercase letter in a column are not significantly different at $\alpha<0.05$ using Tukey's multiple comparison test

Figure 4.1 Demonstration of simulated rainfall treatment. A 1 L plastic bottle with two 1/16" holes drilled in the bottle cap was used to deliver water to the units from ~50 cm above the surface.

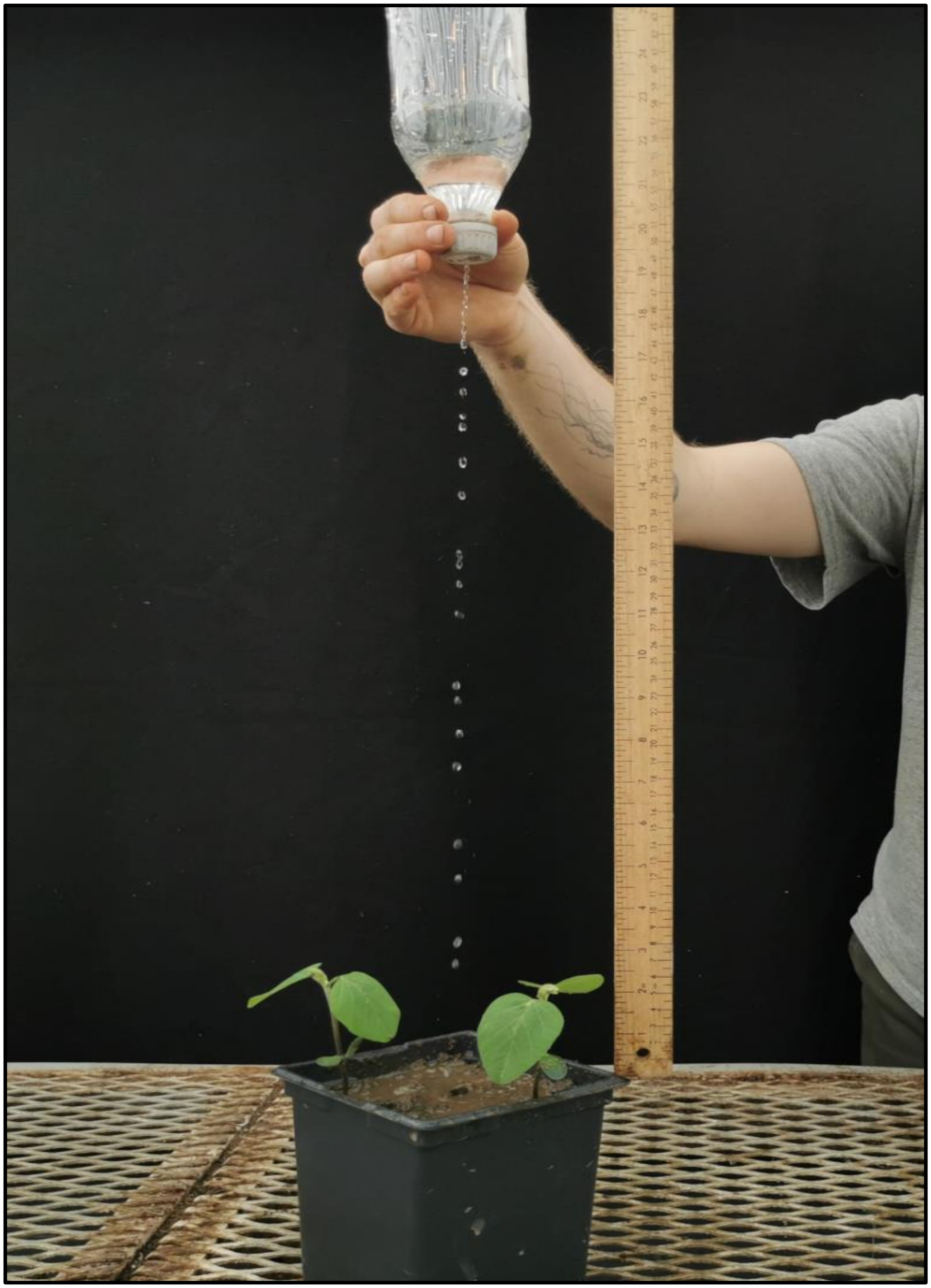
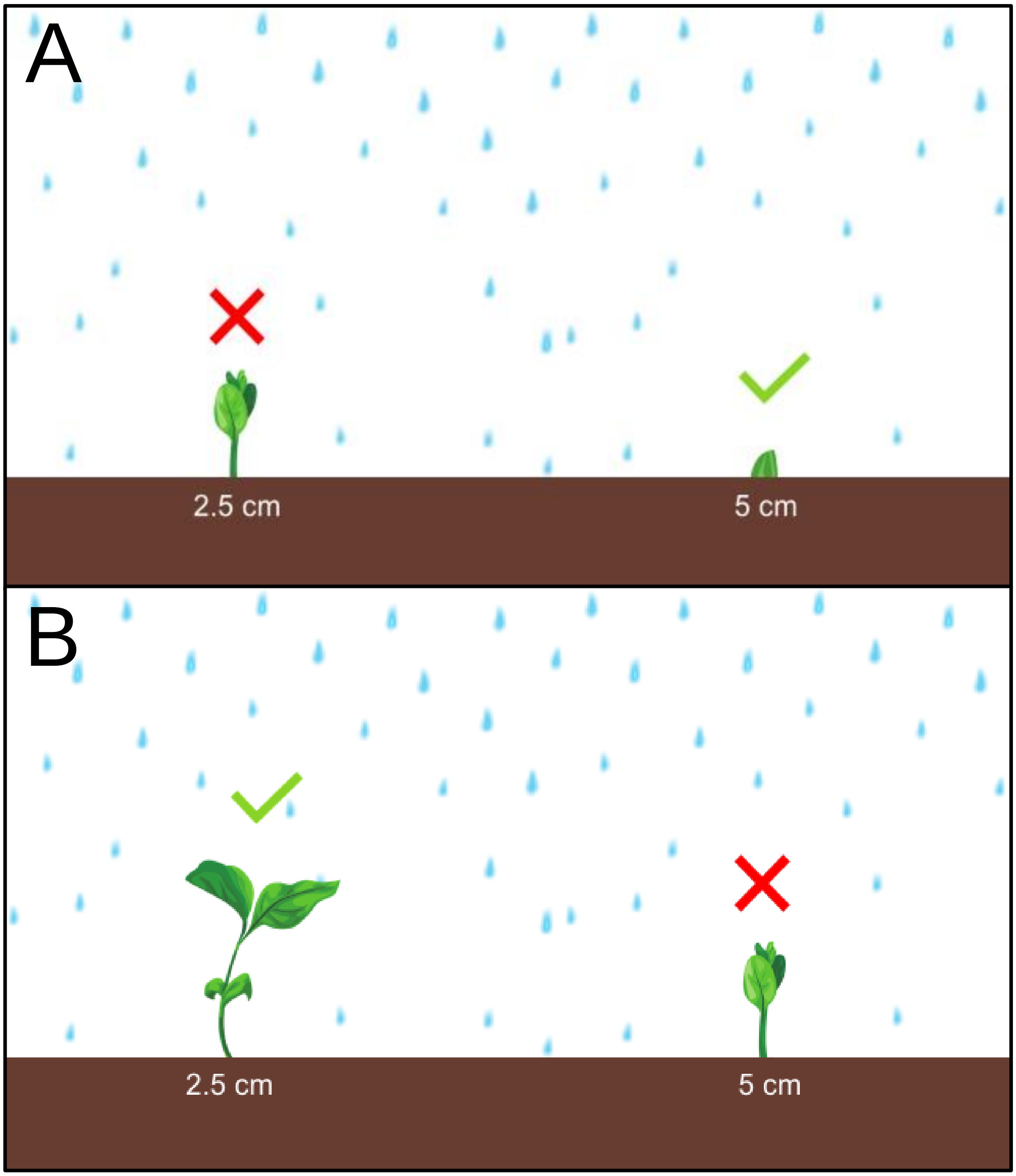


Figure 4.2 Deposition of water and soil material on soybean seedlings after simulated rainfall treatment. Deposition can also be observed on plastic ID stake.

Figure 4.3 Example of soybean growth stage differences between soybean planted 2.5 cm and 5 cm deep. Differences in growth stage at the time of rainfall created significant differences in DFF phytotoxicity, with both depths demonstrating increased phytotoxicity depending on rainfall timing during emergence. Soybean seedlings with the red "x" represent seedlings at the susceptible growth stage, resulting in severe bleaching. Panel A represents a scenario where rainfall occurred 1 to 3 DAE and the shallow planted soybean in the susceptible growth stage. Panel B represents a scenario where rainfall occurred 3 to 6 DAE and the later emerging deep planted soybean in the susceptible growth stage.



5. General Discussion

5.1 Contributions

The registration of DFF for weed management in corn and soybean in North America is intended to target troublesome *Amaranthus* spp. found in these cropping systems. This will provide a new mode of action for herbicide-resistant weeds within these cropping systems and the effective use of DFF in two-pass herbicide programs will help slow the evolution and impact of herbicide-resistant weeds.

Understanding the factors influencing soybean tolerance to DFF is essential to enable the use of increased field rates. This will allow for improved residual weed control of waterhemp and Palmer amaranth, while also maintaining an acceptable level of crop safety. Historically, soybean tolerance has shown to be influenced by cultivar selection, cultural practices, and environmental conditions, so proper management of these factors can permit the effective use of DFF in soybean. Examining how these factors influence soybean tolerance to DFF prior to its registration will allow Bayer Crop Science to make better recommendations upon its registration and provide growers with an excellent residual herbicide option to control broadleaf weeds.

The use of DFF in soybean is novel, therefore this research was necessary to provide the foundational information required to generate best management practices for the use of DFF in soybean. This research contributes valuable knowledge about the sources of variability in the response of soybean to DFF while also providing consistent and replicable methodologies to test soybean cultivar tolerance to DFF and the influence of splash damage from herbicides.

Results from the growth room screening procedure indicate that cultivar selection is not responsible for the large differences in soybean tolerance to DFF observed at the field-scale. Instead, evidence from the other aspects of the study suggests that physiological differences among soybean cultivars relative to rainfall timing may be a more likely cause of differences in soybean injury from DFF. Eliminating differential cultivar sensitivity from the potential sources of variability reduces the complexity of the issue for Bayer Crop Science and growers. Resources no longer need to be devoted to more widespread screening of soybean cultivars for sensitivity to DFF and growers will not have to consider cultivar selection when using DFF for weed management in soybean.

Rainfall timing and intensity influence soybean injury from DFF. This was confirmed during the controlled rain splash experiment performed in the growth room. After this experiment, the influence of rain splash was considered with the weather data from various field trials and used to draw further conclusions regarding soybean tolerance to DFF. The simple design of this growth room experiment and its effectiveness with an additional herbicide demonstrates its potential for use in other herbicide-crop combinations where rain splash is thought to influence crop phytotoxicity. This can be used to further understand the influence of rain splash on crop phytotoxicity overall, as there is little peer reviewed literature on this topic.

The result from the cultural factors field trial builds on the understanding of how cultural factors influence crop phytotoxicity from soil-applied herbicides. Dynamic interactions can occur between planting date, planting depth, cultivar selection, herbicide rate, and application timing that influence crop phytotoxicity. The multiple planting dates and their corresponding environmental conditions aligned with the results observed in the rain splash experiment and previous soybean tolerance research. It is common for excess precipitation and cold temperatures to increase the risk of phytotoxicity, and this is also the case with DFF. The greatest potential for soybean injury from DFF is if the first rainfall event occurs 1 to 3 DAE, and DFF applications should be managed based on predicted rainfall events. PP applications increase the likelihood of adequate rainfall to move the herbicide into the top 1.0 cm of soil prior to soybean emergence, and this may be the reason for reduced phytotoxicity observed when DFF was applied 1-week before planting in this study. Reduced soybean phytotoxicity through the use of PP applications demonstrates the potential to increase the recommended field rate while maintaining an acceptable level of crop safety. This has major implications for the effectiveness of DFF and overall weed management, as higher rates of DFF will provide more consistent residual control of weed species that are considered to be some of the most troublesome in North American field crop production. DFF is an important new tool for farmers to combat the evolution and impact of herbicide-resistant weeds, and this research provides valuable information for growers on how to optimize its use in soybean.

5.2 Limitations

The growth room screening procedure was limited by the inability to obtain additional data points to measure the response of soybean to DFF. Several potential response variables were evaluated for their utility in measuring soybean phytotoxicity from DFF, however none produced consistent results. This contributed to delays in developing a final screening procedure and reduced the total number of cultivars screened. If the experiment could be performed again, the inclusion of more soybean cultivars would strengthen conclusion regarding their relative sensitivity to DFF.

The field trial aspect of this research was limited by the number of times the experiment was replicated. The scale and complexity of this trial permitted only two replications, both at the same research station in 2020 and 2021. It is understood that DFF activity varies depending on soil texture and organic matter content, therefore it cannot be assumed that soybean would respond the same way to similar cultural practices in other field scenarios. In addition, more replications would permit stronger conclusions regarding the interactions between the various cultural factors. Finally, the potassium deficiency observed in 2020 was a major hinderance to final interpretation of this study. Given that injury symptoms were more severe in 2020, it would have been valuable to examine the true final impact on yield resulting from such high levels of injury. This limits the overall strength of conclusions relating to the impact of soybean phytotoxicity from DFF and yield.

5.3 Future Research

Further research on DFF should explore the weed control efficacy and soybean phytotoxicity from PP applications. While this study demonstrated that PP applications can reduce soybean phytotoxicity, more replications of this research are necessary to move forward with potential changes in rate or application timing on the herbicide label. The length of residual weed control and soybean tolerance from PP applications must be examined across a wider range of soil and environmental conditions to obtain the optimum PP interval for DFF in soybean. There could also be value in evaluating the performance of PP applications in no-till situations with increased crop residues. Exploring no-till scenarios would also provide the opportunity to investigate if the soil disturbance from aggressive trash whippers can reduce soybean injury from DFF.

This study solely examined the phytotoxic effects of DFF in soybean, however, this active ingredient will likely be used in combination with other soil-applied herbicides to increase the spectrum of weed control. Therefore, future studies should use these potential tank-mixes to determine if the result of this study remain consistent when DFF is paired with other herbicides.

All future trials should closely record soybean emergence and early development in relation to significant rainfall events. Increased replications will expose DFF applications in soybean to a wider range of environmental conditions, and this can be used to further pinpoint the window of high susceptibility in early season soybean growth. This can also be used to track how much rainfall may be required to adequately safen DFF applications in soybean.

If consistent differences in cultivar tolerance are observed once DFF is brought to market and used on a wider scale, the screening procedure should be revisited. The methodology used in this study remains the only effective procedure to test soybean cultivar tolerance to DFF. Careful consideration must be given to reports of poor cultivar tolerance, as unfortunate timing of significant rainfall and soybean growth stage can lead to false assumptions about a cultivar's sensitivity to DFF.

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