8-2020

Response of Glyphosate-Resistant Alfalfa to Glyphosate Application in the Intermountain West

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RESPONSE OF GLYPHOSATE-RESISTANT ALFALFA TO GLYPHOSATE

APPLICATION IN THE INTERMOUNTAIN WEST

by

Logan Chet Loveland

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

Approved:

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UTAH STATE UNIVERSITY
Logan, Utah

2020

ABSTRACT

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in the Intermountain West

by

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Utah State University, 2020

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Glyphosate-resistant (GR) alfalfa (*Medicago sativa* L.) has been widely adopted in the Intermountain West United States, where alfalfa plays an important role in agriculture. Exceptional tolerance to glyphosate application has been a reported strength of this technology; however, growers have recently reported potential crop injury under certain environmental conditions. The purpose of this study was to document and characterize the injury, identify local conditions that may have contributed to crop injury, and determine best management practices for avoiding injury to GR alfalfa in the Intermountain West. The effects of glyphosate rate and application timing were investigated at 24 sites over five years, measuring the impact on alfalfa crop height and yield. Glyphosate applications were made during various seasons. Summer glyphosate applications did not injure alfalfa. Spring applications reduced crop height at 76% of the sites and biomass yield at 62% of the sites. At responsive sites, low (869 g ha^{-1} a.e.) and high (1739 g ha^{-1} a.e.) rates reduced yield by 0.53 and 1.06 Mg ha^{-1}, respectively. Alfalfa treated with a high rate when 15-20 cm tall had mean yield reductions of 16-17% compared with untreated alfalfa. Three variables were significant predictors of glyphosate injury: soil pH, glyphosate rate, and the number of days with sub-zero temperatures post-dormancy before glyphosate application. Predicted yield reduction from a one-unit increase in soil pH was 0.60 Mg ha^{-1}. Each extra day of crop exposure to sub-zero temperatures before glyphosate application increased the odds that glyphosate injury would occur by 13%. The results of these studies suggest that high rate glyphosate applications on GR alfalfa have a high probability of reducing crop height and yield in regions with high soil pH and cold spring temperatures, such as the Intermountain West. As glyphosate rate or crop height at application increased, so did the likelihood of alfalfa height and yield reductions. To mitigate the risk of injury, we recommend that spring glyphosate applications are made using low rate of glyphosate before alfalfa is 10 cm tall. If a high glyphosate rate is necessary, then application should be made at or before alfalfa is 5 cm tall.

(81 pages)

PUBLIC ABSTRACT

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Glyphosate-resistant (GR) alfalfa (*Medicago sativa* L.) has been widely adopted in the Intermountain West United States, where alfalfa plays an important role in agriculture. Exceptional tolerance to glyphosate application has been a reported strength of this technology; however, growers have recently reported potential crop injury under certain environmental conditions. The purpose of this study was to document and characterize the injury, identify local conditions that may have contributed to crop injury, and determine best management practices for avoiding injury to GR alfalfa in the Intermountain West. The effects of glyphosate rate and application timing were investigated at 24 sites over five years, measuring the impact on alfalfa crop height and yield. Glyphosate applications were made during various seasons. Summer glyphosate applications did not injure alfalfa. Spring applications reduced crop height at 76% of the sites and biomass yield at 62% of the sites. At responsive sites, low (869 g ha^{-1} a.e.) and high (1739 g ha^{-1} a.e.) rates reduced yield by 0.53 and 1.06 Mg ha^{-1}, respectively. Alfalfa treated with a high rate when 15-20 cm tall had mean yield reductions of 16-17% compared with untreated alfalfa. Three variables were significant predictors of glyphosate injury: soil pH, glyphosate rate, and the number of days with sub-zero temperatures post-dormancy before glyphosate application. Predicted yield reduction from a one-unit increase in soil pH was 0.60 Mg ha^{-1}. Each extra day of crop exposure to sub-zero temperatures before glyphosate application increased the odds that glyphosate injury would occur by 13%. The results of these studies suggest that high rate glyphosate applications on GR alfalfa have a high probability of reducing crop height and yield in regions with high soil pH and cold spring temperatures, such as the Intermountain West. As glyphosate rate or crop height at application increased, so did the likelihood of alfalfa height and yield reductions. To mitigate the risk of injury, we recommend that spring glyphosate applications are made using low rate of glyphosate before alfalfa is 10 cm tall. If a high glyphosate rate is necessary, then application should be made at or before alfalfa is 5 cm tall.

ACKNOWLEDGMENTS

Primarily I would like to thank my wife, Lizzie, for her support as I have completed this work. I am also grateful for remarkable parents, who taught me the value of hard work and ambition. I would like to thank Dr. J. Earl Creech for providing me the opportunity to learn from him. He not only played a critical role in bringing this thesis to fruition but has also become a great mentor and friend to me.

The academic and agricultural community that supported this research is a vast group of growers, researchers, and students, and I appreciate each of their efforts. Steve Orloff was principal in the discovery and establishment of this research, and I have benefitted immensely from his exceptional work. I have been privileged to work with and learn from outstanding researchers, the coauthors of this work, and am very grateful for their individual contributions and guidance.

L. Chet Loveland

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

GLYPHOSATE-RESISTANT ALFALFA PRODUCTION IN THE INTERMOUNTAIN WEST: A REVIEW OF LITERATURE

1.1 HISTORY OF ALFALFA

Records dating back to the fourth millennium, the period when writing itself originated, mention alfalfa (*Medicago sativa* L.) as a respected nutritional source of feed for livestock (Russelle, 2019). Muller et al. (2003) claims that alfalfa is believed to have originated in the Near East/Central Asia region. From there, it was introduced to other parts of the world through trade and conquest. Most speculate that the Persians introduced *läjwärd* (alfalfa) to Europe, where it became known as *lucerne* (Russelle, 2019). *Lucerne* is still the common name used in many regions of European influence (Badora & Celińska, 2020; Smith & Moore, 2020). Villagran (1923) indicates that the Spanish were likely introduced to *alfalfez* during Moor occupation, the Arabic word for alfalfa being *al-façfaçah*. Thus, many records of *M. sativa* in the United States (US) referred to alfalfa as *lucerne* until the 1960s, as most Eastern colonists were European (Pedersen, 1961). However, production in the American West had Spanish origins.

Russelle (2019) explains that “chilean clover” was imported to California from Chile around 1850, during the California Gold Rush. As the eastern and western United States became connected and greater uniformity was needed among common names, the Spanish derivation *alfalfa* was adopted and is used today. Success in California during the first few seasons caused alfalfa to spread rapidly throughout the West. It quickly became apparent that the arid climate and alkaline soil of the West made it an ideal candidate for alfalfa production. By the year 2000, just 11 western states made up 40% of total alfalfa hay production in the Unites States (Putnam et al., 2000). Today, alfalfa continues to play a major role in Western agriculture; in 2019, these same states accounted for 48% of total alfalfa production in the United States (NASS, 2020).

Chon et al. (2002) describes a few alfalfa properties that help explain why it grows particularly well in the West. Alfalfa is well-known for its autotoxic and allelopathic properties. These properties make it fiercely competitive, not only towards other plants but also against its own seed. They also stated that alfalfa, as a perennial broadleaf, grows well in environments with dryer growing seasons because it can develop a vast root system. Alfalfa roots may grow more than two meters annually, penetrating deep into the soil as it persists from year to year (Russelle, 2019). This allows alfalfa to accesss water recources well beyond the reach of most other plants.

Fundamental practices for growing alfalfa have long been recognized. Bolton (1962) claims that practices were first recorded as early as 200 B.C. by Roman naturalists. They recommended harvesting at the beginning of flowering, planting in well-drained soils, and liming the soil to increase pH. They even noted the positive effect alfalfa had on soil health, as well as the risk it posed for bloating when feeding lifestock (Bolton, 1962). These principles still prove true today.

1.2 WEED MANAGEMENT IN ALFALFA

With modern alfalfa management, it can be challenging to control weeds and maintain a pure stand. Perhaps the most successful tactics have been cultural methods. Narrow row spacing, dense populations, fertilizer applications, and companion cropping techniques have been implemented with some success (Curran et al., 1993; Huarte & Arnold, 2003). However, physical and chemical control methods have been more difficult to implement. Alfalfa's susceptibility to herbicide injury is well documented (Swan, 1972; Harvey et al., 1976; Smith, 1991). Before the introduction of glyphosate-resistant alfalfa cultivars, many herbicides failed to adequately control weeds and no single herbicide provided control for all weeds. Furthermore, alfalfa's perennial nature and close spacing prevented the use of physical/mechanical controls during non-establishment years.

It is important to keep in mind that, for most crops, proper weed control inherently results in higher yields. However, alfalfa can be an exception to this rule. Because the entire biomass is harvested, weed control can reduce yields in alfalfa. What weed control can provide, however, is improved quality. The degree to which a grower may want to control weeds is dependent on several factors, including economic incentive, crop use, and weed population, among others.

A study in Alberta, Canada on the impacts of chemical and physical control methods in alfalfa illustrates the complexity of this issue (Moyer & Acharya, 2006). The study observed the effects of various herbicides on alfalfa yield, quality, and weed control. Observations were made from the second year through the fourth year following establishment, when cultivation is not a viable control method. The study found that while a few herbicides effectively controlled the target weeds, they also resulted in crop injury, yield reduction, and even quality reduction at some sites. Moyer & Acharya (2006) attributed this reduction in quality to a shift in population from palatable weeds to unpalatable weeds, made possible by the creation of niches from herbicide application. Put simply, even herbicides effective in weed control can have other detrimental effects on the crop as well.

1.3 HISTORY OF GLYPHOSATE

Franz et al. (1997) provides an excellent overview of the discovery of glyphosate. He explains that Dr. Henri Martin first synthesized glyphosate [N-(phosphonomethyl) glucine] in 1950 while working for a small pharmaceutical company (Cilag). However, its potential as an herbicide went undiscovered for over 20 years. During that time, another company (Aldrich) purchased the original glyphosate samples. Dr. Martin changed careers, ironically enough going to work in herbicide research for another company (Ciba-Geigy). Independently, another company (Stauffer) is assumed to have crossed paths with glyphosate, as they mention it in a 1964 patent. Franz et al. (1997)claims that despite these casual encounters with glyphosate, its value as an herbicide was not discovered until the mid-1970s when Monsanto launched glyphosate into the herbicide marketplace.

A few important qualities make glyphosate a particularly desirable herbicide. As a non-selective, post-emergence, systemic herbicide, it affects nearly all annual and perennial plants (Franz et al., 1997). When in contact with the soil, glyphosate's activity quickly decreases, which might be just as important as its effectiveness when in contact with plants. Glyphosate is comparatively safe from a toxicological perspective and lacks many of the environmental side effects of other herbicides (Holly, 1985).

Once in contact with the plant, glyphosate targets 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) (Duke & Powles, 2008). EPSPS is an enzyme that acts as a catalyst in reactions necessary for the production of amino acids. Glyphosate interrupts amino acid production by binding to the EPSPS enzyme, preventing the enzyme from being used in the shikimate pathway. This, consequently, causes the plant to starve.

Glyphosate's mode of action can provide a clear indicator of whether a plant has died due to glyphosate exposure (Singh & Shaner, 1998). As seen in Figure 1.1, shikimate is produced before EPSPS is used in the pathway. Following shikimate production, glyphosate competes with the substrate phosphoenolpyruvate to bind to EPSPS. Once glyphosate binds to EPSPS, shikimate can no longer be converted into chorismate (Pline-Srnic, 2005). Thus, shikimate acid is excessively accumulated in glyphosate-affected plants (Steinrücken & Amrhein, 1980). Singh & Shaner (1998) suggest that the same indicator may be used to identify glyphosate resistance in crops and weeds; if a plant were exposed to glyphosate and high accumulations of shikimate did not occur, this would indicate glyphosate-resistance.

1.4 GLYPHOSATE-RESISTANT CROPS

Glyphosate-resistant (GR) technology is a genetic alteration that allows crops - which are otherwise sensitive to glyphosate - to continue metabolizing after exposure to the herbicide. Commercial availability for these crops began in 1996 (Dill, 2005). Within the next decade, glyphosate-resistant soybeans (*Glycine max* L.), cotton (*Gossypium hirsutum* L.), corn (*Zea mays* L.), alfalfa (*Medicago sativa* L.), and sugar beets (*Beta* *vulgaris* L.) were all available (Gianessi, 2008). Crops that have been genetically modified to express this GR trait have proved extremely popular among growers. Based on survey data from the USDA, the percent of acres planted with GR soybeans rose from 17 percent in 1997 to 94 percent in 2014. Other crops followed similar trends, as corn and cotton each rose from less than 10 percent in 1997 to greater than 95 percent in 2014 (NASS, 2020). One reason GR systems have been so widely successful is due to economic incentive on part of the grower. For example, it was estimated that implementing a GR system in soybeans reduced grower input costs by 23% (Dill, 2005). Growers also favor GR systems over conventional systems due to apparent practical advantages and improved crop safety.

Since the rise of this technology, a variety of mechanisms have been used to develop glyphosate resistance crops. Pline-Srnic (2006)explains that the earliest methods focused on the progressive adaptation of cultured cells to ever-increasing glyphosate concentrations. Later work focused on achieving resistance by transforming plants with genes to break down glyphosate. Ultimately, the most effective method (which is currently used in GR crops) uses insertion of a GR form of the EPSPS enzyme into the crop. Other mechanisms behind glyphosate resistance continue to reveal themselves, primarily through the observation of mutations in GR weeds (Peterson et al., 2018; Takano et al., 2020).

1.5 GLYPHOSATE-RESISTANT ALFALFA

The commercialization of GR alfalfa was a long process, full of legal and political complexity. The first GR alfalfa plants were produced in 1997 and in-field testing began in 1999 (Jones, 2007). In 2003, Monsanto submitted a petition for nonregulated status of GR alfalfa varieties and the USDA Animal and Plant Health Inspection Service (APHIS) prepared an Environmental Assessment (EA). A large part of the assessment focused on the potential for pollen-mediated flow of genes between genetically modified and conventional varieties (Fitzpatrick et al., 2003; Jones, 2007).

APHIS issued a Finding of No Significant Impact in 2005, approving the deregulation petition. By this time, research in university and non-commercial industry settings had been performed for five years (van Deynze et al., 2004; McCaslin et al., 2006; Miller et al., 2006). Growers were now able to plant GR alfalfa and market the hay without restriction, and over 100,000 hectares were planted in the two seasons that followed (Stokstad, 2011). However, in February 2006 the Center for Food Safety sued the USDA for failing to properly investigate the impact of genetically modified seeds. Just over a year later, the United States District Court for the Northern District of California (San Francisco) ruled in favor of the plaintiff, banning genetically modified alfalfa seed nationwide (Jones, 2007).

This prevented any further plantings of GR alfalfa after 30 March 2007 and required an Environmental Impact Statement (EIS) from APHIS before GR alfalfa could once again be deregulated (Fox, 2007). After Monsanto appealed the ruling, a lengthy legal process eventually brought the trial before the United States Supreme Court in April

2010 (Dickinson, 2010). The Supreme Court confirmed the ban, stating that GR alfalfa could not be planted or sold unless the EIS was completed and concluded that it was safe for the open market (Hubbard & Hassanein, 2013). In January 2011, the EIS was completed and GR alfalfa varieties were approved without restriction for market use (Stokstad, 2011).

These proceedings were not only significant to alfalfa growers, but also to vested parties of both conventional and organic agriculture. Between 1986 and 2006, over 80 genetically engineered crops had been deregulated by the USDA; not one of them had required an EIS in the approval process before the alfalfa case (Hubbard & Hassanein, 2013). An EIS was customarily used only to further investigate crops if "significant" impacts were identified in the EA (Waltz, 2011). These events were significant not simply because they delayed the assimilation of GR alfalfa; they also set precedent in the ongoing struggle for coexistence between organic and conventional cropping systems.

Even once these legal issues were resolved, adoption of GR alfalfa varieties was much slower than with annual GR counterparts, likely due to its perennial nature requiring less frequent establishment than annual crops. Nonetheless, GR alfalfa was steadily adopted and growers began to enjoy the same apparent advantages with GR alfalfa as they did with other GR crops: simpler weed management, reduced crop injury, and improved weed control.

1.6 GLYPHOSATE INJURY TO GLYPHOSATE-RESISTANT CROPS

While it is true that the administration of GR traits allows crops to withstand glyphosate exposure, other undesirable characteristics resulting from this alteration have also been documented; these include increased fruit abortion, disease susceptibility, and sensitivity to environmental stress, among others (Pline-Srnic, 2005). More importantly, some forms of injury directly resulting from glyphosate application have been documented on various GR crops, including soybeans and cotton (Reddy et al., 2004; Pline-Srnic, 2005). For example, a Mississippi study concluded that a glyphosate metabolite may have been the source of injury in GR soybean (Reddy et al., 2004). In these cases, it was observed that temperature, water stress, growth stage, and glyphosate rate may have played a role in expressed injury. Pline-Srnic (2005) mentions that, in most cases, injury could be avoided through implementing better management practices. These included making applications at lower rates, earlier growth stages, or in periods with cooler temperatures.

GR alfalfa was heavily studied during its commercial release. Van Deynze et al. (2004), McCaslin et al. (2006), and McCordick et al. (2008) conducted studies on GR alfalfa, concluding that glyphosate application was safe for GR alfalfa. Van Deynze et al. (2004) and McCordick et al. (2008) did in fact observe slight injury following glyphosate application but deemed it irrelevant as injury was minimal, short-lived, and not evident at the time of cutting. McCaslin et al. (2006) made glyphosate application in June, July, and October and concluded that glyphosate could be applied at any stage without concern for crop safety.

Steckel et al. (2007) also observed the impact of glyphosate application on GR alfalfa and stated that it was safe for the crop. Chlorosis in GR alfalfa from glyphosate application was only observed following glyphosate application when a rate of 3476 g ha^{-1} was used (this is twice the high rate recommended by the label). They mentioned that any glyphosate injury from lower rate applications was temporary and disappeared within seven days. No reductions in yield resulted from any treatment. Given the excess rate and lack of yield reduction, it was concluded that GR alfalfa expressed good tolerance to glyphosate.

1.7 GLYPHOSATE INJURY TO GLYPHOSATE-RESISTANT ALFALFA

GR crops are no exception to the fact that local environments, including soil and climate conditions, influence the success of plant communities (Passey et al., 1982). As weather conditions become more extreme, plant responses may become more difficult to predict. For example, the degree of crop sensitivity to low temperatures is dependent on many factors, including the severity and duration of the change in temperature (Teitel et al., 1996). Whether this temperature drop actually results in a frost is further dependent on wind speed, cloud cover, topography, and other factors (Campbell & Norman, 2000; Barnhart, 2005).

Passey et al. (1982) describes the general climate of the Intermountain West as arid to semiarid, with wide seasonal fluctuations in temperature and precipitation. They also state that high elevation areas can experience frost-free periods of less than 100 days, with more than a 30-day variability in the last spring killing frost from year to year.

Extreme temperature fluctuations and frost events into late spring are typical of these areas, which may influence the occurrence of glyphosate injury in the Intermountain West.

The first recorded glyphosate injury to GR alfalfa occurred in Siskiyou County, CA in the spring of 2014. A grower, suspecting injury in his GR alfalfa, requested that his county Extension agent (Steve Orloff) examine his first crop. Upon inspection, the agent confirmed clear symptoms of crop injury, including stunting and chlorosis. Most of the field exhibited similar symptoms, apart from one strip in the middle of the field. This strip, presumably, is where the wheel line was located and was thus not sprayed when the grower made a glyphosate application earlier that spring. The crop was approximately 15-20 cm tall at application.

Several possible causes for the injury were considered. Was this the result of spray-tank contamination, or due to a bad batch of glyphosate? Were other non-herbicide related management practices at fault? Each one of these possibilities was systematically ruled out. The prospect that this was an isolated event was rejected as well when other GR alfalfa fields in the same valley displayed similar crop injury as well. This was likely more than an issue of individual farm management. After some consideration, a hypothesis was formed: Cold temperatures following an application of glyphosate may be related to the observed crop injury. From 2015-2019, 24 studies were conducted in the intermountain regions of California, Oregon, and Utah to address that hypothesis. Recently, glyphosate injury to GR alfalfa was observed in southwest Ohio in the spring of 2019, indicating that this injury may not be limited to intermountain western regions (Mark Sulc, personal communication, 3 May 2019).

1.9 SUMMARY AND OBJECTIVES

Alfalfa plays an important role in Western agriculture. In recent years, GR alfalfa varieties have reduced many of the challenges inherent in managing weeds in alfalfa. However, the true interactions between emerging GR crops and local environments may remain undetected for some time. The complicated commercial release of GR alfalfa delayed the rate at which its interactions could be observed. With less than ten consecutive years on the commercial market, there is still much to learn about its behavior.

Other GR crops have been documented to show susceptibility to injury following glyphosate applications. The injury, however, could often be mitigated by improved management practices. Observations made in the Intermountain West indicate that improved management practices for GR alfalfa may be necessary, possibly owing to the unique environmental conditions of the region. Over the past five years, 24 studies have been conducted in response to these observations. The objectives of these studies were to determine the source of injury to GR alfalfa, identify best practices to help growers mitigate risk of injury and yield loss, and assess the efficacy of using GR alfalfa in regions of the Intermountain West.

CHAPTER 2: RESPONSE OF GLYPHOSATE-RESISTANT ALFALFA TO GLYPHOSATE APPLICATION IN THE INTERMOUNTAIN WEST

2.1 INTRODUCTION

Since its introduction around 1850, alfalfa has played a principal role in agriculture in the Western United States (Putnam et al., 2000; Russelle, 2019). In 2019, just 11 western states accounted for 48% of the country's total value of alfalfa production (NASS, 2020). Alfalfa's ability to access deep water resources and withstand alkaline soils enables it to grow well in environments that may be too harsh for other crops (Chon et al., 2002). Passey et al. (1982) observed that growing conditions are particularly severe in the Intermountain West USA, where high elevations enable volatile temperature swings and frost events.

While the elements certainly provide opposition, another significant challenge for growers is weed control. A number of cultural methods have been implemented with some success, including narrow row spacing, shallow seeding, dense populations, fertilizer applications, and companion cropping techniques (Curran et al., 1993; Huarte & Arnold, 2003). However, physical and chemical control methods have been more difficult to implement. Alfalfa's susceptibility to herbicide injury is well-documented (Swan, 1972; Harvey et al., 1976; Smith, 1991). Before the advent of glyphosate-resistant alfalfa, many weeds could not be adequately controlled with herbicides, and no single herbicide provided control of all weeds. The few herbicides that controlled problematic weeds could also result in crop injury (Moyer & Acharya, 2006). For most crops, proper weed control generally results in higher yields; however, in alfalfa the entire biomass of the stand is harvested, and overall yield may be reduced as weed populations are controlled. Nevertheless, weed control is important when alfalfa is used for livestock feed as it may prevent poisonous weeds as well as improve feed quality, increasing the market value of the feed. The degree to which a grower may want to control weeds may be dependent on several factors, including economic incentive, crop use, and weed populations.

While weed control in alfalfa was once a complex issue, GR transgenics now provide a simple, effective alternative to conventional herbicides in alfalfa (van Deynze et al., 2004). Many growers have adopted this technology for apparent improvements in crop safety, quality, and herbicide application simplicity (Orloff & Putnam, 2011). However, interactions between emerging GR crops and local environments can take years to identify. The complicated commercial release of GR alfalfa delayed the rate at which these interactions could be observed (Fox, 2007; Stokstad, 2011). With less than ten consecutive years on the commercial market, it essentially remains an emerging technology.

Injury resulting from glyphosate application has been documented with other GR crops, including soybean (*Glycine max* L.) and cotton (*Gossypium hirsutum* L.) (Reddy et al., 2004; Viator et al., 2004; Pline-Srnic, 2005; Zobiole et al., 2011). Pline-Srnic (2005) observed that temperature, water stress, growth stage, and glyphosate rate may have played a role in the expression of injury in these crops. The author concluded that injury may be avoided through implementing better management practices, which included making applications at lower rates, earlier growth stages, or in periods with cooler temperatures. As with other GR crops, improved management practices in GR alfalfa may be necessary to properly benefit from this technology.

GR alfalfa was heavily studied during its commercial release. Van Deynze et al. (2004), McCaslin et al. (2006), and McCordick et al. (2008) conducted studies on GR alfalfa, concluding that glyphosate application was safe for GR alfalfa. Van Deynze et al. (2004) and McCordick et al. (2008) did in fact observe slight glyphosate injury but deemed it irrelevant as injury was short-lived and not evident at the time of cutting. McCaslin et al. (2006) made glyphosate applications in June, July, and October and concluded that glyphosate could be applied without concern for crop safety. Steckel et al. (2007) also observed the impact of glyphosate application on GR alfalfa and stated that it was safe for the crop. Chlorosis in GR alfalfa from glyphosate application was only observed following glyphosate application when a rate of 3476 g a.e. ha^{-1} was used (this is twice the high rate recommended by the label). He mentioned that any glyphosate injury from lower rate applications was temporary and disappeared within seven days. No reductions in yield resulted from any treatment. Given the excess rate and lack of yield reduction, it was concluded that GR alfalfa expressed good tolerance to glyphosate.

The first documented glyphosate injury to GR alfalfa that we are aware of occurred in Siskiyou County, CA in the spring of 2014. A grower, suspecting injury in his GR alfalfa, requested that his farm advisor (Steve Orloff, University of California Agriculture and Natural Resources) examine his first crop. Upon inspection, the advisor confirmed clear symptoms of crop injury, including stunting and chlorosis. Most of the field exhibited similar symptoms, apart from one strip in the middle of the field. The strip without symptoms was where the wheel line was located and was left untreated when the grower made a glyphosate application earlier that spring when the crop was approximately 15-20 cm tall.

Several possible causes for the injury were considered. Was this the result of spray-tank contamination, or due to a bad batch of glyphosate? Were other non-herbicide related management practices at fault? Each one of these possibilities was systematically ruled out. The prospect that this was an isolated event was rejected when other GR alfalfa fields in the same valley displayed similar crop injury as well. After some consideration, a hypothesis was formed: Cold temperatures following an application of glyphosate may be related to the observed crop injury. From 2015-2019, experiments were conducted at 24 sites in the intermountain regions of California, Oregon, and Utah to address that hypothesis. The objectives of the study were to document and characterize the injury and determine best management practices for avoiding injury to GR alfalfa in the Intermountain West.

2.2 MATERIALS AND METHODS

2.2.1 Experimental design

Experiments were established from 2015 to 2019 at 24 sites in the Intermountain region of California, Oregon, and Utah. Trials were conducted in producers' fields in existing stands of GR alfalfa. All management practices, with the exception of herbicide application and harvest, were conducted by the grower using commercial, field-scale equipment. All sites were irrigated and located in regions that generally produce three to four alfalfa cuttings per growing season. These field sites spanned from 40°20'2" to

43°20’10” N lat and 122°51’20” to 111°57’16” W long. Four soil orders were represented, with soil pH ranging from 5.8 to 8.3. Sites ranged in elevation from 733 to 1378 m (Soil survey staff, n.d.). Alfalfa stand age varied from two to eight years old. Details of the study locations are summarized in Table 2.1.

The experimental design at each site was a randomized complete block design with four replications and 14 treatments (Table 2.2). Two references were used: an untreated check and a conventional herbicide control. The conventional control was a mix of metribuzin (Sencor 75 DF, Bayer CropScience, Research Triangle Park, NC) and paraquat (Gramoxone SL 2.0, Syngenta Crop Protection, Greensborough, NC) at 750 and 562 g ha^{-1} a.i., respectively. The other 12 treatments received applications of glyphosate (Roundup PowerMax, Bayer CropScience, Research Triangle Park, NC) at various crop heights and glyphosate rates. There were six crop heights at application: 5, 10, 15, 20, 30, and 40 cm. Each of these application heights received one of two glyphosate rates: low (869 g a.e. ha^{-1}) or high (1739 g a.e. ha^{-1}). Treatments have been abbreviated for simplicity. These abbreviations sequentially indicate crop height at application, chemical, and rate. To illustrate, 5GL would indicate alfalfa treated at 5 cm tall with glyphosate at a low rate. Not all treatments were implemented at every location (Table 2.3).

2.2.2 Herbicide application

Treatments were applied using a CO_2-pressurized backpack sprayer, with a carrier volume of 140 L ha^{-1} in UT (Sites 19, 20, and 24) and 187 L ha^{-1} at all other sites. Plot size at Site 1 and all UT sites was 28 m^2; at all other sites plot size was 18 m^2.

Application of the conventional control was made in the early spring while the alfalfa was dormant. At the 21 sites with spring applications of glyphosate, applications were made at different application heights before the first harvest. The remaining three sites received glyphosate application during second crop regrowth in July (Sites 13 and 14) and third crop regrowth in August (Site 18).

2.2.3 Data collection

The three measured response variables were first cut yield, first cut height, and second cut yield. Alfalfa crop height was measured immediately preceding the first harvest. Ten plant heights per plot were taken by measuring the tallest plant within a random 0.2 m^2 area to the end of the stem. First and second cut yields were taken at bud to early flowering stage, as is recommended to maximize nutrient concentration when used for animal feed (Sheaffer et al., 2000). At all sites in CA and OR, a Carter forage harvester (Carter Manufacturing Company, Inc., Brookston, IN) was used to harvest the center of the plot and record wet weight. At the UT sites the center of the plots were harvested and wet weight measured with a Hege 212 forage harvester (Wintersteiger AG, Ried im Innkreis, Austria). The harvested area at Site 1 was 7.04 m^2; Sites 19, 20, and 24 had a harvested area of 11.74 m^2. The harvested area at all other sites was 4.51 m^2. After wet weight measurements were recorded, a 700 g sub-sample was collected for moisture analysis. Each sub-sample was weighed and subsequently dried at 60°C in a forced air oven for seven days. Once dry, sub-samples were weighed again to determine percent moisture and calculate dry matter yield for each plot.

Weather data were collected from two sources: local weather stations and on-site temperature loggers (HOBO TidbiT v2 Temperature Data Logger, Onset Computer Corporation, Bourne, MA). On-site temperature measurement devices were placed at the top of the alfalfa canopy and raised to canopy height as subsequent glyphosate applications were made. Weather station data were accessed online through public networks provided by the states of CA and UT (California Department of Natural Resources, 2019; University of California, 2019; Utah State University, 2019).

2.2.4 Data analysis

Experiments were conducted at 24 sites over five years. Each experiment was a randomized complete block design in which whole plots were grouped into four blocks and randomly assigned to treatments. Data were first analyzed across sites using the MIXED procedure of SAS (SAS Institute, 2004) at $P \leq 0.05$. Site and treatment (all individual glyphosate rate and application height treatments), and their interaction were considered fixed effects, while block and interactions involving block were considered random. This analysis indicated a significant interaction between site and treatment for both harvest height ($P < 0.001$) and yield ($P = 0.003$). Thus, all subsequent data were analyzed by site because some sites showed injury while others did not. Six separate analyses were conducted to investigate various datasets. The first analysis (A1) used treatment as the fixed effect and harvest height as the dependent variable. The second analysis (A2) exclusively compared glyphosate treatments applied when the crop was 5-20 cm tall to one another. It observed application height, glyphosate rate, and their interaction as fixed effects with harvest height measured as a percent of the untreated check as the dependent variable.

Yield of the first alfalfa cutting was analyzed as the dependent variable in the third analysis (A3) with treatment as the fixed effect. In the fourth analysis (A4), first crop yield as a percent of the untreated check was analyzed as the dependent variable with rate, application height, and their interaction as fixed effects. Data exclusively from glyphosate treatments applied between 5-20 cm tall was used in this analysis. The fifth analysis (A5) observed the impact of application height, glyphosate rate, and their interaction on yield as a percent of the untreated check in studies from 2019 (Sites 21-24), observing all treatments. This was done to evaluate whether there was a threshold within crop height at application where glyphosate application was once again safe for the crop. Yield of the second alfalfa cutting from Sites 1-3 was treated as the dependent variable in the sixth analysis (A6), using treatment as the fixed effect. Due to differences in overall alfalfa harvest height and yield between locations and years, pooled data were analyzed and discussed as a percent of the untreated check at the respective site.

Multiple linear regression was then used to predict the effect of application height on harvest height and biomass yield. Data were analyzed by site using the REG procedure of SAS at sites where crop height at application influenced harvest height or yield ($P < 0.05$). These sites were selected based on results from A2 and A4. The dependent variables were harvest height and biomass yield. The independent variable was crop height at application.

2.3 RESULTS AND DISCUSSION

Weather conditions varied among the locations and years. An evaluation of how local weather and soil conditions may have contributed to the occurrence of glyphosate injury can be found in chapter three.

2.3.1 Harvest height response to glyphosate application

Data observing the response of crop height at first harvest was collected at 17 sites, as data were not available at all sites (Table 2.3). Alfalfa crop height at harvest was influenced by treatment at 13 sites (76%). The four nonresponsive sites were studies from 2016 in CA. At the 12 responsive sites where no rate × height interaction occurred, mean harvest height across all treatments ranged from 28.6 to 87.4 cm. Four treatments produced the greatest height reductions from the untreated check: 10GH, 15GH, 20GL, and 20GH. Treatment 20GH was the only glyphosate treatment imposed at every site, reducing crop height from the untreated check 85% of the time with a mean height reduction of 6.7 cm. Only 15GH resulted in greater mean height reduction from the untreated check, at 7.1 cm. 15GH reduced height 89% of the time. A high rate treatment was usually responsive (72% of the time) unless applied when the crop was 5 cm tall (Figure 2.1).

Crop height at harvest was influenced by rate at five sites; however, rate was only marginally nonresponsive at Site 6 (P = 0.053) (Table 2.3). At responsive sites, the low and high rate of glyphosate reduced harvest height by 3 cm and 6 cm respectively compared to the untreated check. The greatest height reduction from glyphosate occurred at site 20, with the high rate reducing height by 24% and the low rate reducing height by 14%.

Crop height at glyphosate application resulted in a response at eight sites (Table 2.3). At these sites, mean harvest height reduction from a 5cm application height was minimal, just 0.9 cm from the untreated check. However, applications made when the crop was 10, 15, and 20 cm tall reduced mean harvest height from the untreated check by 4.1, 5.9, and 6.9 cm, respectively. In linear regression models, all regression coefficients were significant at $b(\beta) < 0.01$ (R^2 0.18-0.50). In these models, mean predicted harvest height was reduced by 0.61% for each 1 cm increase in crop height at application. Harvest height reduction was even greater (0.72% cm^{-1}) in linear regression models where $b(\beta) \leq 0.001$ (R^2 0.28-0.50). These models suggest that harvest height is reduced as application height increases; however, the R^2 value indicates that they may need to be regarded with some caution. A significant interaction between rate × height occurred at Site 7. At this site, treatment 20GH reduced harvest height from the untreated check by 21%, while the conventional control reduced height by 18%. The three other treatments, 10GL, 10GH, and 20GL, reduced height from the untreated check by 10-12%.

While a few early studies observed the response of GR alfalfa to glyphosate application, none of them measured crop height response to glyphosate application (van

Deynze et al., 2004; McCaslin et al., 2006; Steckel et al., 2007; McCordick et al., 2008). These studies observed other important variables, including crop injury, yield, and forage quality. In these studies, stunting was generally considered an element of crop injury; thus, documentation isolating the impact of glyphosate application on crop height at harvest is not available.

2.3.2 Alfalfa biomass yield response to glyphosate application

Glyphosate reduced yield compared to the untreated check at 13 of the 21 studies conducted in the spring prior to first cutting (Table 2.4). All sites except Site 18 were included in these analyses. Across responsive sites, treatments 15GH and 20GH reduced biomass yield compared to the untreated check by 0.99 and 0.86 Mg ha^{-1}, respectively. These two treatments were responsible for the greatest yield reductions at all responsive sites except at Site 4, where 10GH reduced yield from the untreated check by 0.90 Mg ha^{-1} (Figure 2.2). Treatment 15GH resulted in biomass yield reductions at all eight sites where it was used. Treatment 20GH decreased yield 77% of the time. Three sites responded to glyphosate rate. At these sites, mean yield reductions at low and high rates were 0.53 (9%) and 1.06 Mg ha^{-1} (18%) from the untreated check, respectively.

Of the sites that had reduction in biomass yield, all but two showed yield reductions due to crop height at application (Table 2.4). In total, application height influenced yield at 10 sites. Similar to the response observed with harvest height, application at 5 cm had little impact on crop yield, with a mean yield reduction of 0.7%. Application at 10 cm caused a 5% yield reduction. However, the 15 (13%) and 20 cm

(14%) application heights resulted in significant yield reductions. In linear regression models, all regression coefficients were significant at $b(\beta) < 0.01$ (R^2 0.10-0.50) (Table 2.5). In these models, mean predicted biomass yield was reduced by 0.91% for each 1 cm increase in application height. Mean predicted yield reduction was 1% cm^{-1} in linear regression models where $b(\beta) \leq 0.001$ (R^2 0.28-0.50). Site 12 had a significant rate × height interaction. Here, yield as a percent of the untreated check ranged from 83-106%. Three treatments produced greater mean yield reductions than the conventional control (6%); treatments 10GL, 15GH, and 20GH reduced yield from the untreated check by an average of 10, 12, and 7%, respectively.

The conventional control reduced crop height at harvest compared with the untreated check at 60% of the sites. At sites where yield was influenced by treatment, mean yield reduction from the conventional control was 0.20 Mg ha^{-1}. Treatments 5GL and 5GH were the only treatments with less mean yield reduction (0.02 and 0.05 Mg ha^{-1}, respectively). While the standard for yield in these studies is the untreated check, it is important to keep in mind that the conventional control represents the realistic alternative to GR alfalfa for many growers. Yield reduction from the conventional control (0.20 Mg ha^{-1}) was comparative to the reduction from 10GL (0.21 Mg ha^{-1}). Yield reductions resulting from remaining treatments (10GH, 15GL, 15GH, 20GL, and 20GH) ranged from 0.44 to 0.99 Mg ha^{-1}.

These data suggest that, when managing GR alfalfa, less crop injury may result from the use of glyphosate than with conventional herbicides as long as glyphosate is applied at or before 5 cm of growth. An application at a taller crop height may result in injury equivalent to or greater than what would occur in a conventional system. It is important to note that these findings do not negate the value of GR technology. Conventional methods never outperformed treatments where glyphosate was applied at 5 cm tall, regardless of rate. In fact, at the six responsive sites where all three treatments were present, 5GL and 5GH increased yield compared with the conventional control 33% of the time.

These results may appear to conflict with earlier conclusions (van Deynze et al., 2004; McCaslin et al., 2006; Steckel et al., 2007; McCordick et al., 2008); however, when reviewing the methods of earlier studies none of them were conducted under similar conditions, particularly regarding site location, environmental conditions, and stand age. Steckel et al. (2007) stated chlorosis was only observed in GR alfalfa following glyphosate application when a rate of 3476 g ha^{-1} was used prior to the fourth cutting in the third and fourth years of a five-year study. This is twice the maximum labelled rate. For all other applications, including applications made in the spring, any glyphosate injury observed was temporary and disappeared within seven days. No reductions in yield resulted from any treatment. Given the excessive application rate and the absence of an effect on yield, it was concluded that GR alfalfa expressed good glyphosate tolerance. This trial was located in Jackson, TN (just 125 m in elevation) and experienced warmer spring temperatures than the locations used in our study, which may be why no injury was observed.

Van Deynze et al. (2004), McCaslin et al. (2006), and McCordick et al. (2008) studied the effect of various herbicides (including glyphosate) and glyphosate application timing on GR alfalfa. McCordick et al. (2008) and van Deynze et al. (2004) observed slight glyphosate injury but deemed it irrelevant as injury was minimal (6%), short-lived, and not evident at the time of cutting. These studies were conducted in the establishment year and subsequent spring following establishment. While the mechanism behind glyphosate injury remains unknown, stand age may influence crop susceptibility to injury.

One example of the effect of stand age occurred at Sites 20 and 24 in UT in 2018 and 2019, which were in their sixth and second years of production, respectively. High and low rates of glyphosate applied at 15 and 20 cm decreased yield compared with the untreated check at both sites; however, mean yield reduction from the untreated check from these treatments was 11% greater in the sixth-year stand than in the second-year stand. Treatment 20GH reduced mean biomass yield of the six-year-old stand by 1.9 Mg ha^{-1}, while the same treatment in a second-year stand caused a 0.4 Mg ha^{-1} reduction. This and other practical observations suggest that young alfalfa stands may be less susceptible to glyphosate injury than older stands. However, variable weather and soil conditions between locations and years makes it difficult to test such notions, and missing stand age data prevented us from performing a satisfactory analysis (Table 2.1). A proper investigation would require simultaneous establishment of trials at various stand ages in the same location and year.

McCaslin et al. (2006) made glyphosate application in June, July, and October and concluded that glyphosate could be applied at any stage without concern for crop safety. In our present study, all sites where treatments were made in the summer also indicated a lack of influence on harvest height and biomass yield (Table 2.4). This would suggest that cool spring temperatures may have contributed to the occurrence of glyphosate injury. Thus, these early assessments of GR alfalfa, given the young stand ages and herbicide applications during warm summer months, is not altogether contrary to what we might expect based on observations from our present study.

2.3.3 Injury observations

2.3.3.1 Injury in subsequent cuttings

In early studies during 2015 and 2016, many observations were made to better understand the behavior of glyphosate injury. One of these observations included harvesting the second cutting at Sites 1, 2, and 3. First cutting yield data indicated a significant yield response at Sites 1 ($P = 0.001$) and 2 ($P = 0.004$); however, second cut data at both sites failed to produce a yield response to treatment ($P = 0.300$ and 0.879, respectively). Site 3 did not experience a yield response to treatment in either cutting. This suggests that the impacts of glyphosate injury may be limited to the cutting in which it occurs. As yield response occurred in first cutting at sites in the following years, we continued to observe the behavior of the second cutting at these sites. In these instances, it seemed that no residual effect of glyphosate injury lingered in subsequent cuttings.

2.3.3.2 Tall crop glyphosate applications

Another important set of observations were made in 2019 trials. After observing that alfalfa was particularly susceptible to injury at 15-20 cm tall, we wanted to know if there was a threshold within application height where it once again became safe to apply glyphosate to the crop. Studies from 2019 included glyphosate treatments applied at 30 and 40 cm tall with low and high rates. Data from this analysis (A5) indicated that crop height at application had a significant impact on alfalfa yield at all four sites ($P < 0.033$). Neither rate ($P > 0.065$) nor rate × height interaction ($P > 0.440$) influenced yield at any site. At Site 21, application height had no influence on yield when the data were analyzed without the 30 and 40 cm tall applications (P=0.089). However, once these treatments were included application height proved responsive (P=0.033). This was due to significant yield reductions from the 30 cm application height (Figure 2.4).

Application at 20 cm tall produced the lowest yield of all treatments at Sites 22 and 23. At sites 21-23, yield from a 40 cm application height was the same as the untreated check; yield also increased compared with the lowest yielding application height at their respective site. These data suggest that alfalfa may not be as susceptible to injury at a 40 cm application height as it is at 20 or 30 cm. However, when making management decisions a grower should also consider the potentially undermined impacts of a later application. Passing over the crop to make the herbicide application could reduce yield. Weeds at this stage have had more time to possibly set seed before herbicide application and some weeds show greater tolerance to glyphosate when they are large (Jordan et al., 1997). Even if effective weed control was achieved, the dead weeds would likely reduce forage quality. Thus, the best time to apply glyphosate is likely when the weeds - and crop - are short.

2.3.3.3 Determining injury occurrence

Treatments resulting in reduced harvest height were not always accompanied by a biomass yield reduction. Sites expressed a response to both harvest height and yield 53% of the time (Figure 2.5). Treatment influenced yield alone 18% of the time; conversely, crop height at harvest was exclusively impacted 23% of the time. At the 17 sites where both harvest height and yield data were taken, only Site 3 lacked either a harvest height or yield response. Often, it was difficult to observe visual injury symptoms before first cut; nevertheless, significant differences in yield would be measured at harvest. Conversely, apparent stunting was measured at times without a significant yield reduction to reflect it. Notably, this suggests that differences in crop height at harvest alone may not be the best indicator of glyphosate injury. An assessment of many indicators is likely necessary to properly assess whether glyphosate injury has occurred, including crop stunting, biomass yield loss, and the expression and behavior of crop injury symptoms.

2.4 CONCLUSIONS

Our conclusion is that GR alfalfa in the Intermountain West was often impacted by glyphosate application. The degree to which a crop expresses injury may be influenced by several factors. GR alfalfa was not impacted by summer applications of glyphosate, indicating that cool spring temperatures may be a necessary condition for glyphosate injury to occur. This also suggests that glyphosate applications made before the first cutting require more consideration than applications made later in the season. Sites that expressed first cutting yield reductions did not express reductions in the second cutting, indicating that the effects of glyphosate on alfalfa did not persist through the season.

Application of metribuzin + paraquat at dormancy (standard herbicide treatment used in non-GR alfalfa), did not out yield glyphosate applied at 5 cm tall, regardless of the glyphosate rate. However, alfalfa growers applying glyphosate to GR alfalfa may observe injury similar to that of dormant treatments in non-GR alfalfa if glyphosate is applied improperly. Injury symptoms sometimes worsened as glyphosate rate or crop height at application increased, prior to 20 cm of alfalfa growth. However, as crop height at application increased from 20 to 40 cm, the crop usually became less susceptible to injury (75% of the time). Crop stunting did not always reduce biomass yield; as such, growers should be thoughtful when assessing the occurrence of glyphosate injury.

To mitigate the risk of injury during spring application of glyphosate, growers should spray early using the lowest recommended rate. When using a low rate, application should be made at or before the crop is 10 cm tall. When using a high rate, application should be made at or before 5 cm. Of reasonable concern for some growers is that early applications of glyphosate may not control late-emerging weeds. In such cases, a tank mix of glyphosate with a soil residual herbicide would be recommended to provide adequate weed control.

TABLE 2.1 Site properties for 24 trials in California, Oregon, and Utah from 2015 to 2019 including year, location, elevation, stand age (including establishment year), and soil texture.

Site	Year	Location[a]	Elevation	Stand Age[b]	Soil Texture
			m		
1	2015	Tulelake	1229	5	Silty clay
2	2016	Scott Valley	829	-	Sandy loam
3		Scott Valley	832	-	Sandy loam
4		Scott Valley	859	3	Silty clay loam
5		Christmas Valley	1320	-	Loamy sand
6		Susanville	1270	-	Sandy loam
7		Scott Valley	840	3	Sandy loam
8		Scott Valley	841	-	Sandy loam
9		Susanville	1245	-	Silty clay
10		Scott Valley	841	-	Sandy loam
11		Macdole	1366	-	Silty clay loam
12		Tulelake	1230	4	Silt loam
13	2017	Scott Valley	840	4	Sandy loam
14		Scott Valley	840	4	Sandy loam
15		Scott Valley	840	4	Sandy loam
16		Susanville	1218	-	Loamy sand
17		Montague	733	-	Clay
18		Scott Valley	840	4	Sandy loam
19		Cornish	1378	5	Loamy sand
20	2018	Cornish	1378	6	Loamy sand
21	2019	Scott Valley	840	6	Sandy loam
22		Tulelake	1229	8	Silty clay
23		Susanville	1218	6	Loamy sand
24		Cornish	1378	2	Sandy loam

[a]Christmas Valley is located in Oregon, Cornish is located in Utah, and the remaining locations are in California.

[b]Establishment year included in stand age.

TABLE 2.2 Herbicide active ingredient, rate, and crop height at application. Treatment name is an abbreviation of application height, chemical, and rate.

Treatment ID	**Application Height**	**Active Ingredient**	**Acid Equivalent**	**Treatment Name**
	cm		g ha^{-1}	
T1				Untreated check
T2	Dormancy	Metribuzin + Paraquat	750[a] + 562[a]	Conventional control
T3	5	Glyphosate	869	5GL
T4	5	Glyphosate	1739	5GH
T5	10	Glyphosate	869	10GL
T6	10	Glyphosate	1739	10GH
T7	15	Glyphosate	869	15GL
T8	15	Glyphosate	1739	15GH
T9	20	Glyphosate	869	20GL
T10	20	Glyphosate	1739	20GH
T11	30	Glyphosate	869	30GL
T12	30	Glyphosate	1739	30GH
T13	40	Glyphosate	869	40GL
T14	40	Glyphosate	1739	40GH

[a]Expressed as active ingredient.

TABLE 2.3 Model significance observing the effect of treatment and the impact of rate, crop height at application, and their interaction on alfalfa first crop height at harvest. Preliminary analysis indicated a site × treatment interaction; as such, data was analyzed by site. Harvest height data were not available for all sites.

			A1 Model Significance[c]	A2 Model Significance[d]		
Year	**Site ID[a]**	**Treatment ID[b]**	**Treatment**	**Rate**	**Height**	**Rate × Height**
			P > F			
2015	1	T1, T9, T10	0.044*	0.025*	n/a	n/a
2016	2	T1, T2, T5, T6, T9, T10	0.117	0.512	0.494	0.969
	3	T1, T2, T5, T6, T9, T10	0.065	0.805	0.807	0.191
	4	T1, T2, T5, T6, T9, T10	0.126	0.260	0.848	0.401
	6	T1, T2, T5, T6, T9, T10	0.018*	0.053	0.561	0.444
	7	T1, T2, T5, T6, T9, T10	<0.001***	0.008*	0.036*	0.043*
	8	T1, T2, T5, T6, T9, T10	<0.001***	0.976	0.120	0.182
	9	T1-T10	0.007**	0.839	0.372	0.221
	10	T1, T8, T10	<0.001***	n/a	0.177	n/a
	11	T1, T2, T5-T10	<0.001***	0.035*	0.005**	0.264
	12	T1-T10	0.056	0.068	0.736	0.904
2017	19	T1-T10	<0.001***	0.031*	<0.001***	0.100
2018	20	T1-T10	<0.001***	0.037*	<0.001***	0.359
2019	21	T1-T10	0.048*	0.806	0.016*	0.345
	22	T1-T10	<0.001***	0.363	<0.001***	0.937
	23	T1-T10	<0.001***	0.110	0.008**	0.605
	24	T1-T10	0.015*	0.482	0.009**	0.545

[a]See Table 2.1.
[b]See Table 2.2.
[c]Analysis A1 used treatment as the fixed effect and harvest height as the dependent variable.
[d]Analysis A2 compared glyphosate treatments to one another, using crop height at application, glyphosate rate, and their interaction as fixed effects with crop height at harvest measured as a percent of the untreated check as the dependent variable.
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

TABLE 2.4 Model significance observing the effect of treatment and the impact of glyphosate rate, crop height at application, and their interaction on alfalfa first crop biomass yield. Preliminary analysis indicated a significant site × treatment interaction; as such, data were analyzed by site with 23 sites evaluated.

			A3 Model Significance[c]	A4 Model Significance[d]		
Year	**Site ID[a]**	**Treatment ID[b]**	**Treatment**	**Rate**	**Height**	**Rate × Height**
			——— $P > F$ ———			
2015	1	T1, T9, T10	0.001**	0.189	n/a	n/a
2016	2	T1, T2, T5, T6, T9, T10	0.004**	0.273	0.018*	0.965
	3	T1, T2, T5, T6, T9, T10	0.842	0.866	0.869	0.768
	4	T1, T2, T5, T6, T9, T10	0.042*	0.103	0.143	0.845
	5	T1, T5, T6, T9, T10	0.730	0.420	0.881	0.652
	6	T1, T2, T5, T6, T9, T10	0.048*	0.017*	0.017*	0.312
	7	T1, T2, T5, T6, T9, T10	0.605	0.665	0.429	0.589
	8	T1, T2, T5, T6, T9, T10	0.003**	0.549	0.005**	0.417
	9	T1-T10	0.197	0.888	0.154	0.567
	10	T1, T8, T10	0.005**	n/a	0.192	n/a
	11	T1, T2, T5-T10	0.584	0.399	0.784	0.685
	12	T1-T10	0.003**	0.557	0.029*	0.018*
2017	13	T1, T5, T6	0.421	0.412	n/a	n/a
	14	T1, T5, T6, T9, 10	0.570	0.609	0.486	0.737
	15	T1, T5, T6, T9, T10	0.568	0.277	0.502	0.652
	16	T1-T10	<0.001***	0.002**	<0.001***	0.064
	17	T1-T10	0.395	0.348	0.601	0.471
	19	T1-T10	0.008**	0.117	0.001***	0.152
2018	20	T1-T10	<0.001***	0.003**	<0.001***	0.303
2019	21	T1-T10	0.135	0.133	0.089	0.339
	22	T1-T10	0.008**	0.290	0.008**	0.798
	23	T1-T10	<0.001***	0.158	0.023*	0.491
	24	T1-T10	0.010**	0.957	0.002**	0.872

[a]See Table 2.1.
[b]See Table 2.2.
[c]Analysis A1 used treatment as the fixed effect and first crop yield as the dependent variable.
[d]Analysis A2 compared glyphosate treatments to one another, using crop height at application, glyphosate rate, and their interaction as fixed effects with first crop yield measured as a percent of the untreated check as the dependent variable.
*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. ***Significant at the 0.001 probability level.

TABLE 2.5 Linear regression equations, adjusted R^2 values, model significance relating the effect of alfalfa crop height at application on alfalfa harvest height and alfalfa yield by site. Sites not listed showed no response to application height.

Site	Effect of crop height at application		
	Equation[a]	Adjusted R^2	Model significance
	Alfalfa harvest height		
19	$y = 107.0 - 0.788x$	0.323	0.001
20	$y = 103.3 - 0.852x$	0.497	<0.001
21	$y = 104.2 - 0.626x$	0.282	0.001
22	$y = 95.7 - 0.449x$	0.229	0.003
23	$y = 94.9 - 0.620x$	0.281	0.001
24	$y = 102.7 - 0.307x$	0.182	0.007
	Alfalfa biomass yield		
12	$y = 100.9 - 0.399x$	0.102	0.045
16	$y = 96.6 - 1.297x$	0.503	<0.001
19	$y = 110.8 - 0.987x$	0.194	0.007
20	$y = 104.5 - 1.282x$	0.349	<0.001
22	$y = 105.2 - 0.932x$	0.319	<0.001
23	$y = 99.1 - 0.805x$	0.211	0.005
24	$y = 104.9 - 0.635x$	0.283	0.001

[a]Where *x* is crop height at application (cm) and *y* is crop harvest height/biomass yield as a percent of the untreated check.

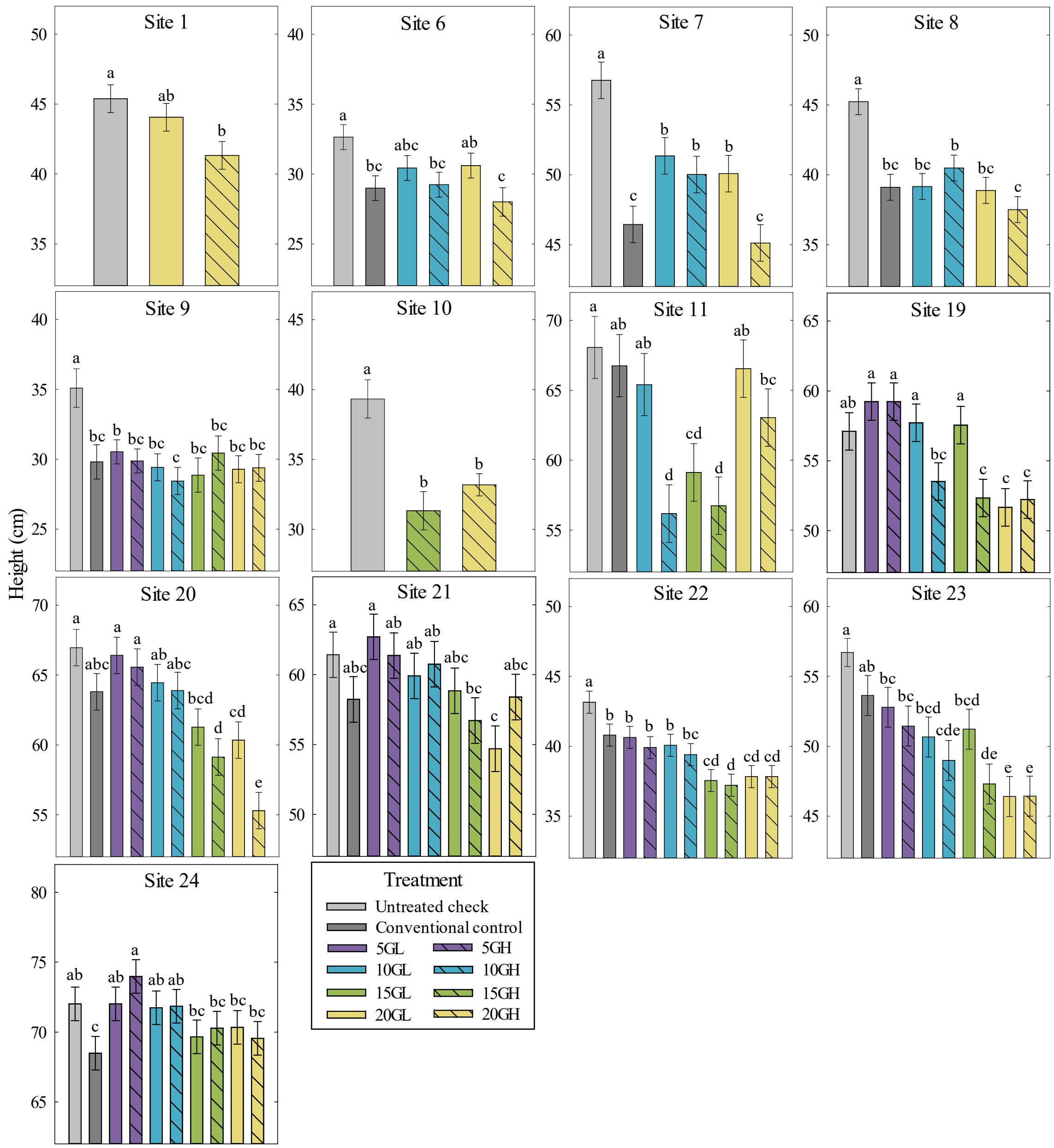

FIGURE 2.1 Relationship between application heights at low and high glyphosate rates on alfalfa crop height (cm) at harvest. Data were analyzed by site (A1). The conventional control was a mix of metribuzin + paraquat. Abbreviations for glyphosate treatments indicate the application height, chemical, and rate used. For example, treatment 5GL was a 5 cm application of glyphosate at a low rate.

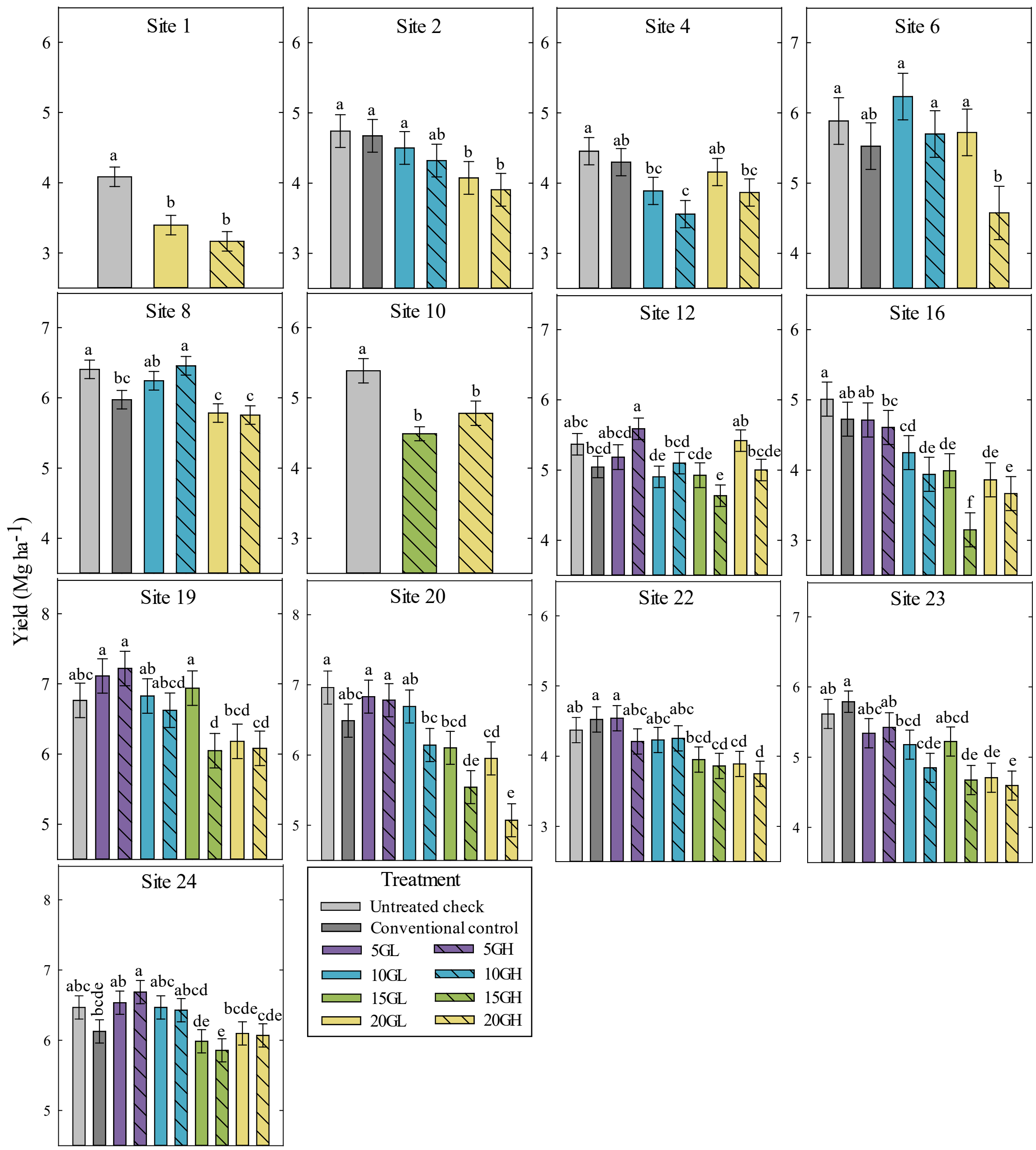

FIGURE 2.2 Relationship between application heights at low and high glyphosate rates on alfalfa biomass yield (Mg ha^{-1}). Data were analyzed by site (A3). The conventional control was a mix of metribuzin + paraquat. Abbreviations for glyphosate treatments indicate the application height, chemical, and rate used. For example, treatment 5GL was a 5 cm application of glyphosate at a low rate.

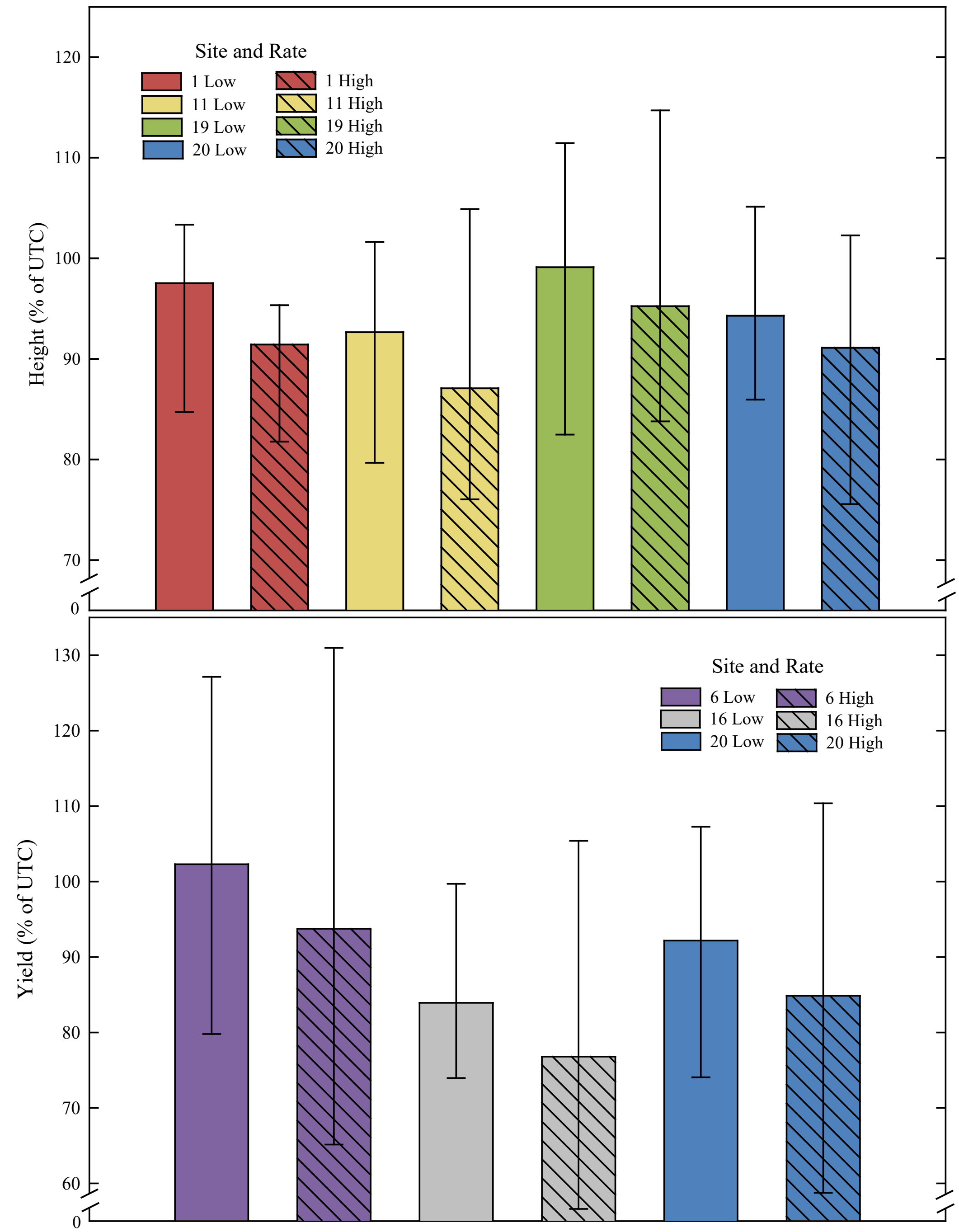

FIGURE 2.3 Relationship between low and high rates of glyphosate on alfalfa harvest height and yield as a percentage of the untreated check (UTC). Sites not shown indicated no significant harvest height or yield response to rate.

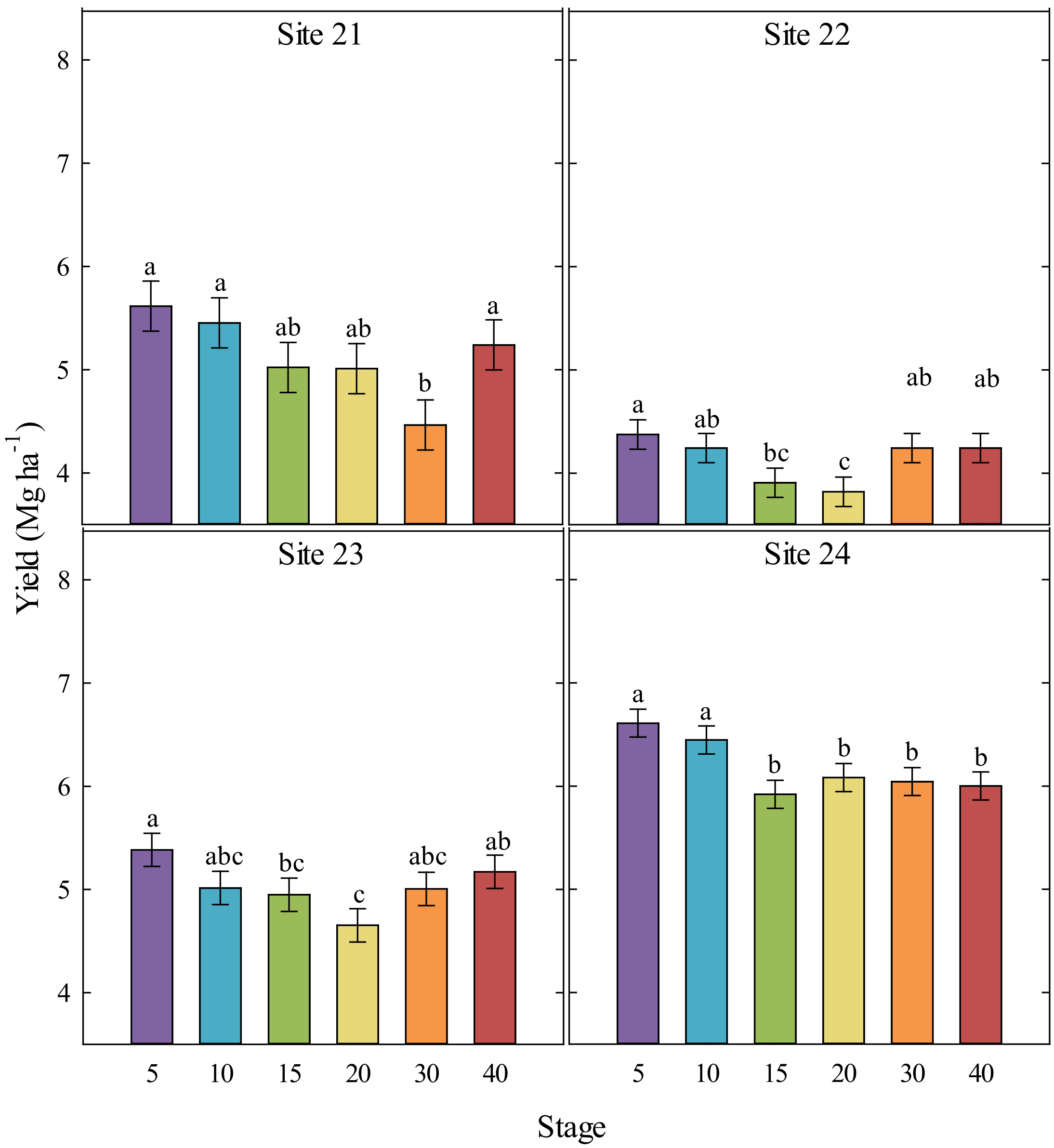

FIGURE 2.4 Alfalfa yield response to glyphosate applied at different alfalfa heights at four sites in CA and UT in 2019.

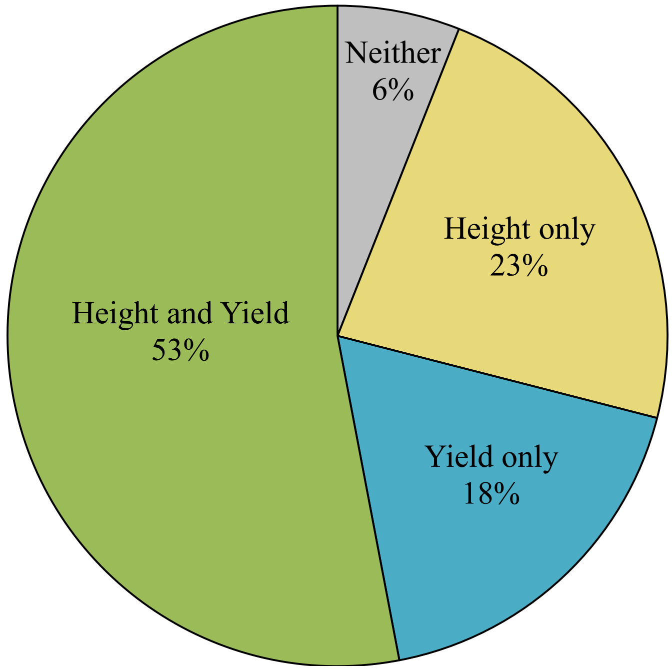

FIGURE 2.5 The percentage of sites (17) where spring applied glyphosate reduced alfalfa first cutting yield and height at harvest.

CHAPTER 3

THE IMPACT OF LOCAL CONDITIONS ON GLYPHOSATE INJURY IN GLYPHOSATE-RESISTANT ALFALFA

3.1 INTRODUCTION

Alfalfa production is an integral part of agriculture in the western United States, in part due to plant attributes that afford success in the region (Putnam et al., 2000). Russelle (2019) observed that alfalfa roots may grow more than 2 meters annually which enables access to water resources deep beneath the soil surface, essential in arid western climates. Other characteristics, including allelopathic properties and alkaline tolerance, have enabled alfalfa to successfully establish itself as a staple crop for many western growers (Chon et al., 2002; An et al., 2016). In terms of production value, 11 western states comprised 48% of annual alfalfa production in the United States in 2019 (NASS, 2020).

With modern alfalfa management, it can be challenging to control weeds and maintain a pure stand. Some cultural methods have been successfully implemented, including narrow row spacing, dense populations, fertilizer application, and companion cropping (Curran et al., 1993; Huarte & Arnold, 2003). However, alfalfa's susceptibility to herbicides make chemical control more difficult to implement (Swan, 1972; Harvey et al., 1976). Before the advent of GR alfalfa, no single herbicide provided control for all weeds in alfalfa. The few herbicides that controlled problematic weeds could also result in crop injury (Moyer & Acharya, 2006).

While weed control in alfalfa was once a complex issue, GR transgenics now provide a simple, effective alternative to conventional herbicides in alfalfa (van Deynze et al., 2004). Initial studies indicated that GR alfalfa provided improved crop safety compared to conventional herbicides (McCaslin et al., 2006; Steckel et al., 2007; McCordick et al., 2008). Van Deynze et al. (2004) found that these systems provide growers with effective, broad-spectrum weed control as well. McCaslin et al. (2006) noted that another advantage of GR alfalfa was improved flexibility when timing herbicide applications.

However, the true cumulative effect of GR trait administration to a crop can be difficult to project. Undesirable characteristics in GR cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* L.) were documented years after their commercial release (Reddy et al., 2004; Viator et al., 2004; Pline-Srnic, 2005; Zobiole et al., 2011). Among these disadvantages were greater sensitivity to environmental stress and susceptibility to glyphosate application. Pline-Srnic (2005) stated that temperature, water stress, growth stage at glyphosate application, and glyphosate rate may have played a role in expressed injury.

Local environments, including soil and climate conditions, influence the success of plant communities (Passey et al., 1982). For example, high soil pH levels (>7.4) have been known to reduce the availability of several nutrients such as P, Zn, and Fe (Fernandez & Hoeft, 2020). Weather conditions, such as growing degree days or temperature stress, influence factors essential to crop development (Hollinger & Angel, 2020). Alfalfa, specifically, has been known to show susceptibility to spring frost events

(Andresen et al., 2001; Barnhart, 2005). These influences and others play a particularly influential role in areas like the Intermountain West where extreme conditions exist. Passey et al. (1982) describes the general climate of the Intermountain West as arid to semiarid, with wide seasonal fluctuations in temperature and precipitation. Furthermore, high elevation areas can experience frost-free periods of less than 100 days, with more than 30-day variability in the last spring killing frost from year to year. Extreme temperature fluctuations and frost events into late spring are typical of these areas.

The degree of crop sensitivity to low temperatures is dependent on many factors, including the severity and duration of the change in temperature (Teitel et al., 1996). Whether this temperature drop results in an actual frost is further dependent on other variables such as wind speed, cloud cover, and topography (Campbell & Norman, 2000; Barnhart, 2005). For this reason, frost remains difficult to measure and project, despite recent advances in measuring and forecasting these other independent variables (Childs, 2003; Jung & Broadwater, 2014). Various types of frost may even impact crops differently. Critchfield (1966) observed that some plants killed by advection frost may only be damaged by radiation frost.

Environmental conditions of the Intermountain West may influence the occurrence of glyphosate injury to GR alfalfa. The first recorded glyphosate injury to GR alfalfa occurred in Siskiyou County, CA in the spring of 2014. A grower, suspecting injury in his GR alfalfa, requested that his county Extension agent (Steve Orloff) examine his first crop. Upon inspection, the agent confirmed clear symptoms of crop injury, including stunting and chlorosis. Most of the field exhibited similar symptoms, apart from one strip in the middle of the field. The strip was where the wheel line was located and was thus not sprayed when the grower made a glyphosate application earlier that spring. The crop was approximately 15-20 cm tall at application.

The prospect that this was an isolated event was rejected when other GR alfalfa fields in the same valley displayed similar crop injury as well. After some consideration, a hypothesis was formed: Cold temperatures following an application of glyphosate may be related to the observed crop injury. Experiments were conducted in 2016, 2017, and 2019 at 19 sites in the intermountain regions of California, Oregon, and Utah to address that hypothesis. The purpose of this study was to characterize the injury and identify local environmental conditions that may have contributed to the occurrence and intensity of crop injury in GR alfalfa in the Intermountain West. Observing the local environments where these studies took place may lead to an improved understanding of how glyphosate injury may respond to local soil and weather conditions. Using this information to improve management practices for GR alfalfa could benefit growers, particularly in regions where crop stunting and yield loss have been documented.

3.2 MATERIALS AND METHODS

3.2.1 Experimental design

Experiments investigating the effects of glyphosate injury to GR alfalfa were conducted at a total of 24 sites during 2015-2019. However, some of those sites were omitted from the present study. Three sites were removed because applications of glyphosate were made during the summer months instead of the spring. Two more sites were omitted because they lacked on-site air temperature measurements. Considering these omissions, experiments were conducted at 19 sites in 2016, 2017, and 2019 in the Intermountain region of California, Oregon, and Utah. Trials were conducted in producers' fields on existing stands of GR alfalfa. All management practices, with the exception of herbicide application and harvest, were conducted by the grower using commercial, field-scale equipment. All sites were irrigated and located in regions that generally produce three to four alfalfa cuttings per growing season. These field sites spanned from 40°20'2" to 43°20'10" N lat and 122°51'20" to 111°57'16" W long. Four soil orders were represented, with soil pH ranging from 5.8 to 8.3 and site elevation ranging from 733 to 1378 m (Soil survey staff, n.d.). Alfalfa age varied from two- to eight-year-old stands. Details of the study locations are summarized in Table 3.1.

The experimental design was a randomized complete block in which whole plots were grouped into four blocks and randomly assigned to one of nine treatments. Treatment refers to any single combination of glyphosate rate (low or high) and crop height at application (5, 10, 15, or 20 cm). An untreated check was included as a reference. The remaining eight treatments consisted of glyphosate (Roundup PowerMax, Bayer CropScience, Research Triangle Park, NC) at various glyphosate rates and application heights. There were four application heights: 5, 10, 15, and 20 cm. Each of these application heights received one of two glyphosate rates: low (869 g a.e. ha^{-1}) or high (1739 g a.e. ha^{-1}). Not all glyphosate treatments were included at every location. Treatments were applied using a CO_2-pressurized backpack sprayer, with a carrier volume of 140 L ha^{-1} in UT (Sites 15 and 19) and 187 L ha^{-1} at all other sites. Plot size was 3.0 × 9.1 m at UT sites and 3.0 × 6.1 m at all others. Spring applications of glyphosate were made to GR alfalfa following the end of dormancy but before the first cut.

3.2.2 Data collection

The measured response variable was first cut biomass yield. Yield was measured at bud to early flowering stage, as is recommended to maximize nutrient concentration when used for animal feed (Sheaffer et al., 2000). At all sites in CA and OR, a Carter forage harvester (Carter Manufacturing Company, Inc., Brookston, IN) was used to harvest the center 4.51 m^2 of the plot. At the UT sites the same was done with a Hege 212 forage harvester (Wintersteiger AG, Ried im Innkreis, Austria) with a harvested area of 11.74 m^2. After wet weight measurements were recorded, a 700 g sub-sample was collected for moisture analysis. Each sub-sample was weighed and subsequently dried at 60°C in a forced air oven for seven days. Once dry, sub-samples were weighed again to determine percent moisture and calculate dry matter yield for each plot.

Data regarding local soil and weather conditions at each site were collected with the intent of determining which factors, if any, may have influenced the occurrence and intensity of glyphosate injury (Table 3.2). Soil data were collected online using the web soil survey provided by the USDA-NRCS (Soil survey staff, n.d.). The estimated soil pH, soil texture, and elevation for the dominant soil series at each site were collected from these surveys. Weather data were collected from two sources: local weather stations and on-site temperature loggers (HOBO TidbiT v2 Temperature Data Logger, Onset Computer Corporation, Bourne, MA). Daily air temperature data from the nearest NOAA weather station to each site were collected from the Utah State University Climate Center Database, University of California Statewide Integrated Pest Management Program, and the California Irrigation Management Information System (California Department of Natural Resources, 2019; University of California, 2019; Utah State University, 2019).

Various metrics were calculated using weather station data, including the number of days with temperature events ≤0°C following end of dormancy before glyphosate application, the number of days with temperature events ≤0°C following glyphosate application before the first cut, the number of days with temperature events ≤0°C from post dormancy to the first cut, the minimum temperature on the day of glyphosate application, the minimum temperature in the three days surrounding glyphosate application, and the minimum temperature in the seven days surrounding glyphosate application.

These same metrics were calculated using on-site temperature measurements. In addition, the following were calculated using on-site measurements: the hours of exposure to temperatures ≤0°C on the day of herbicide application, the hours of exposure to temperatures ≤0°C in the three days surrounding herbicide application, and the hours of exposure to temperatures ≤0°C in the seven days surrounding glyphosate application.

3.2.3 Data analysis

Data were analyzed in three steps. Data were first analyzed using the MIXED procedure of SAS (SAS Institute, 2004) at $P \leq 0.05$. Site and treatment (all individual glyphosate rate and application height treatments), and their interaction were considered fixed effects, while block and interactions involving block were considered random. This preliminary analysis indicated a significant interaction between site and treatment for biomass yield (P = 0.003). Thus, all subsequent data were analyzed by site because some sites had glyphosate injury and others did not. First crop yield was then analyzed as the dependent variable using treatment as the fixed effect. This analysis was reported in Chapter 2 and was used to determine which sites had significant injury to glyphosate applications at various crop application heights.

The second step was to conduct multiple logistic regression to determine which combination of management and environmental conditions influenced the occurrence (yes vs. no) of glyphosate injury. In logistic regression, an odds ratios (in lieu of a probability) is used to predict the likelihood of an event occurrence (Allison, 2012). The odds ratio states the number of times an event will occur (numerator) over the number of times the event will not occur (denominator). Thus, for the present study, an odds ratio larger than one indicates an increased likelihood that glyphosate injury will occur when that variable is present. A number smaller than one indicates the opposite. A multiple logistic regression model was developed using the LOGISTIC procedure of SAS. The dependent variable for this model was output from the MIXED procedure described previously in the first step: a dichotomous variable indicating which treatments resulted in a yield response. Multiple logistic regression models are based on the equation $y_i = \{1 + \exp[-(\beta_0 + \beta_i x_i]\}^{-1}$. In this study, y_i was the predicted odds ratio for alfalfa yield response to glyphosate application, β_0 was the intercept, β_i were the linear coefficients, and x_i were the independent variables.

The third step of the analysis was to conduct multiple linear regression utilizing the REG procedure of SAS to predict the level of glyphosate injury to alfalfa yield. The dependent variable was yield as a difference from the untreated check. The same independent variables were considered for both the logistic and linear regression models. Variables included rate (low or high), crop height at application (5, 10, 15, or 20 cm), soil texture (sand, silt, or clay), site elevation (low or high), soil pH, and the 15 various temperature metrics calculated from weather station and on-site measurements. The stepwise selection method was used for both model types with $P \leq 0.10$ for entry levels and $P \leq 0.15$ for stay levels (Kutner et al., 2004).

The logistic and linear regression models were analyzed both with and without the 15 various temperature metrics to determine whether the addition of these metrics improved predictions. Autocorrelation between metrics was considered when various models were analyzed. Including metrics from data measured at weather stations did not improve fit statistics [i.e., percent of concordant pairs, Somers' D Statistics, for logistic models (Allison, 2012) or correlation coefficients for linear models]; thus, they were removed from the models. Measures of hours of exposure to sub-zero temperatures as well as minimum temperatures surrounding glyphosate application also failed to improve the models. Several iterations in various combinations of these six variables in the model slightly altered the prediction of glyphosate injury occurrence, and sometimes these variables were highly correlated. For example, including these variables in the models presented in this study reduced percent concordance in the logistic regression (Table 3.3) by 0.2% and decreased R^2 in the linear regression model (Table 3.4) by 0.006%. Since omitting them only slightly changed the model, we chose to exclude them.

3.3 RESULTS AND DISCUSSION

3.3.1 Predicting glyphosate injury occurrence

Three predictors were significant in the logistic regression model forecasting the occurrence of glyphosate injury following glyphosate application: Soil pH, glyphosate rate, and the number of days experiencing temperatures ≤0°C post-dormancy before glyphosate application (Table 3.3). This model was 84% accurate at identifying the response or non-response of alfalfa yield to glyphosate treatments. The regression indicated that soil pH increased the odds of a yield reduction occurrence more than any other variable. As soil pH increased, so did the odds of alfalfa crop yield reduction. This variable had the largest 95% confidence interval of the three variables, indicating that it also contained the most variation.

Interpreting the mechanisms between soil pH and glyphosate injury is difficult because soil pH is linked to many factors related to plant growth. Soil pH has been shown to influence microbial populations, cation exchange capacity, and nutrient behavior within the soil (Thomas, 1967; Matschonat & Vogt, 1997; Xing et al., 2019; Shen et al., 2019). These, in turn, impact crop nutrient availability, which is generally reduced as soil pH becomes more extremely acidic or alkaline (Alam et al., 1999; Fernandez & Hoeft, 2020). In the present study, the mean estimated soil pH was 7.4 with a range of 5.8 - 8.3; thus, GR alfalfa observed in this study was primarily exposed to alkaline soils.

Discussing the potential influence that nutrient availability might have on glyphosate injury illustrates the complexity of soil pH estimate interpretation. In alfalfa, P and K are important macronutrients for plant growth (James et al., 1995; Jungers et al., 2019). Fernandez & Hoeft (2020) found that soil pH greater than 7.4 can reduce the availability of K. Optimum soil pH for P availability occurs around 6.5 (Lindsay, 1979). Penn & Camberato (2019) observed that phosphorous fixation by calcium occurs as soil pH increases above this threshold. Thus, GR alfalfa in our study may have exhibited greater yield reduction from glyphosate at sites with high soil pH due to reduced availability of these nutrients. If glyphosate were to inhibit plant growth in an environment already deficient in nutrients, nutrient deficiencies may be magnified through glyphosate application.

The influence of micronutrient availability is not limited to immediate effects on the crop alone. Alam et al. (1999) observed that when Fe, Mn, Zn, and Cu availability were reduced in calcareous soils, adding P to the medium antagonized these deficiencies more under high pH conditions. This would suggest that, at a high pH site, measured yield loss may have occurred from a micronutrient deficiency exacerbated by an application of P fertilizer. These are just a few possible ways that high soil pH may have influenced the occurrence of glyphosate injury. More research is needed to identify why high soil pH may increase the likelihood that glyphosate injury will occur. Furthermore, the influence of glyphosate on alfalfa macro- and micronutrient uptake needs to be examined.

The second significant variable in selected models was one that growers can control: glyphosate rate. A high rate glyphosate application, as opposed to a low rate, increased the odds of glyphosate injury. In our study, the high rate (1739 g a.e. ha^{-1}) was twice the low rate (869 g a.e. ha^{-1}). Logistic regression indicated that doubling the rate increased the odds of injury by 285% (Table 3.3). According to the model, using a high rate would produce the same effect as increasing the soil pH by 0.44 or exposing the crop to 22 extra days of sub-zero temperatures.

The number of days post-dormancy before application with temperatures ≤0°C was the third variable significant in the logistic regression model. The range for this variable was 0-53 days, with a mean of 15. Each extra day with sub-zero temperatures before application increased the odds of injury by 13%. As days with sub-zero temperature increased from 0-53, the predicted frequency of alfalfa yield reduction increased 689%.

The intent of selecting 0°C as the critical value in analyzing this variable was to evaluate the possible effect that a frost event might have on glyphosate injury in GR alfalfa. Alfalfa's susceptibility to frost events may impact its response to glyphosate application. However, measurements of ambient air temperature are insufficient to properly identify when frost events have occurred (Campbell & Norman, 2000; Barnhart, 2005). As subzero temperatures have been identified as a significant factor in injury occurrence, perhaps the predictive power of the model would increase through implementing more accurate measures of frost events, such as infrared thermography (Wisniewski et al., 1997; Gómez Muñoz et al., 2016). Conversely, creating a model based on a simple measure of ambient air temperature provides growers with a practical method for implementing improved management practices in the field, without the need to invest significant labor and capital in more advanced instrumentation.

The number of days with sub-zero temperatures preceding treatment application is inherently linked to the application timing established by our treatments. An application at a 20 cm crop height will have more total days post-dormancy before application than a treatment applied at 5 cm, thus increasing the total number of possible days for sub-zero temperatures to occur. Depending on weather conditions at each location and year, added days may also be warmer, reducing the impact that extra days have on this metric.

3.3.2 Predicting glyphosate injury intensity

The same variables significant in predicting glyphosate injury occurrence were also key indicators for predicting the level of glyphosate injury (Table 3.4). The linear regression model was significant at $P < 0.001$, and indicated that soil pH, glyphosate rate, and the number of days experiencing temperatures ≤0°C following dormancy before glyphosate application were significant in predicting glyphosate injury intensity. Again, soil pH was the most influential variable on crop yield reduction. Differences in yield reduction were particularly stark at the four sites with soil pH levels ≥ 8.0, with a mean yield reduction of 0.59 Mg ha^{-1}. Mean yield reduction at sites with soil pH < 8.0 was 0.25 Mg ha^{-1}. Predicted yield reduction for each one-unit increase in soil pH was 0.60 Mg ha^{-1}.

When predicting injury intensity, the number of days with sub-zero temperatures before application became more influential than glyphosate rate. Each extra day with sub-zero temperatures was predicted to reduce alfalfa yield by 0.06 Mg ha^{-1}. This variable ranged from 0-53 days, which on the latter end would result in a 3.18 Mg ha^{-1} yield reduction. Thus, alfalfa exposed to more days of sub-zero temperatures before glyphosate application generally exhibited greater yield reduction. Treatments applied on the lower half of that range (0-26 days) had a mean yield reduction of 0.36 Mg ha^{-1}. It has been observed that air temperature can impact not only the translocation and efficacy of glyphosate but also the response of other GR crops to glyphosate application (Schultz & Burnside, 1980; Pline et al., 1999). In glyphosate-sensitive species, translocation has been shown to increase at higher temperatures (Schultz & Burnside, 1980). Pline et al. (1999) observed that GR soybean grown at 35°C has significantly lower chlorophyll content in the newest trifoliate that GR soybean grown at 15 or 25°C when treated with glyphosate at 1500 g a.e. ha^{-1}. As a perennial crop, GR alfalfa differs from annual GR crops in that it can receive glyphosate application early in the season during periods of sustained cold temperatures and frost events. This could suggest an undescribed interaction of glyphosate with alfalfa plant physiology under these unique conditions.

Linear regression predicted that yield reduction from applying a high rate of glyphosate instead of a low rate was 0.02 Mg ha^{-1}. However, this estimate is likely understated for treatments applied at 15 and 20 cm crop heights. As shown in the previous chapter, there was little difference in biomass yield between rates for treatments applied at a 5 cm crop height. However, greater yield reduction generally resulted from a high glyphosate rate when applications were made at 10, 15, and 20 cm tall. Across all application heights, a low glyphosate rate resulted in mean yield reduction of 0.61 Mg ha^{-}

^{1}, while mean yield reduction resulting from high rate treatments was 1.01 Mg ha^{-1}. In GR soybean, Pline et al. (1999) found that a glyphosate rate of 2000 g a.e. ha^{-1} could cause chlorophyll reduction in the newest developing trifoliates. The maximum recommended rate for GR soybean is 3360 g a.e. ha^{-1}.

3.3.3 Other possible influences

While soil texture, site elevation, crop height at application, and various weather metrics were not significant in our final models, this does not imply that they do not influence glyphosate injury. Rather, they may be less influential than significant variables. Furthermore, there are many other factors that may influence GR alfalfa tolerance to alfalfa that were not addressed. For example, the influence of stand age could not be evaluated since this information was only available at 47% of the sites. While the mechanism behind glyphosate injury remains unknown, practical observations made in these studies suggest that an analysis of stand age could possibly improve the ability of predicting crop susceptibility to glyphosate injury. Variable weather and soil conditions among locations and years makes it difficult to test such notions, and missing stand age data prevented us from performing a satisfactory analysis (Table 3.1). A proper investigation would require simultaneous establishment of trials at various stand ages in the same location and year.

3.4 CONCLUSIONS

The occurrence and intensity of glyphosate injury in GR alfalfa was influenced by local conditions in the Intermountain West. Crop yield reduction due to glyphosate injury was both more likely to occur and more intense at locations with higher soil pH levels. This predictor influenced glyphosate injury more than any other variable and might be accounting for other factors that influence plant development, such as crop nutrient availability. Yield reduction also occurred and increased in intensity as the crop experienced more days of sub-zero temperatures post-dormancy before glyphosate application. Each extra day with sub-zero temperatures before application increased the odds of injury occurrence by 13%.

Although growers have little control over soil pH and ambient air temperature, our analysis identified management practices that may help reduce the risk of injury. First, making applications earlier in the spring will likely reduce the number of days the crop is exposed to sub-zero temperatures before glyphosate application. Second, applying a low rate of glyphosate will reduce both the likelihood that injury will occur as well as the level of injury when it does. The odds of glyphosate injury occurrence were 285% greater when a high rate of glyphosate was applied as opposed to a low rate. In regions with high soil pH and cold spring temperatures, such as the Intermountain West, implementing these management practices should help mitigate glyphosate injury to GR alfalfa in these areas.

TABLE 3.1 Site properties for 19 sites in CA, OR, and UT in 2016, 2017, and 2019 including year, location, elevation, stand age, soil texture, and soil pH.

Site	Year	Location[a]	Elevation	Stand Age[b]	Soil Texture	pH
			m			
1	2016	Scott Valley	829	-	Sand	7.4
2		Scott Valley	832	-	Sand	6.8
3		Scott Valley	859	3	Clay	7.5
4		Christmas Valley	1320	-	Sand	8.3
5		Susanville	1270	-	Sand	7.0
6		Scott Valley	840	3	Sand	7.6
7		Scott Valley	841	-	Sand	7.4
8		Susanville	1245	-	Clay	8.2
9		Scott Valley	841	-	Sand	7.4
10		Macdole	1366	-	Silt	5.8
11		Tulelake	1230	4	Silt	7.0
12	2017	Scott Valley	840	4	Sand	7.6
13		Susanville	1218	-	Sand	8.1
14		Montague	733	-	Clay	6.7
15		Cornish	1378	5	Sand	7.6
16	2019	Scott Valley	840	6	Sand	7.6
17		Tulelake	1229	8	Clay	7.5
18		Susanville	1218	6	Sand	8.1
19		Cornish	1378	2	Sand	7.9

[a]Christmas Valley is located in Oregon, Cornish is located in Utah, and the remaining locations are in California.

[b]Establishment year included in stand age.

TABLE 3.2 Variables considered as potential predictors of glyphosate injury. Soil texture, site elevation, application height, and application rate were treated as categorical. All other variables were considered continuous.

Parameter Type	Category	Units/critical values
Soil textural class	Sand	
	Silt[a]	
	Clay	
Soil properties	Soil pH	
Site elevation	Low	<914 m
	Moderate[a]	914-1280 m
	High	>914 m
Application height	5[a]	cm
	10	cm
	15	cm
	20	cm
Application rate	869[a]	g a.e. ha-1
	1739	g a.e. ha-1
On-site measurement	Days before treatment	Temperature <0°C
	Days after treatment	Temperature <0°C
	Days during first crop	Temperature <0°C
	Day of treatment	Minimum temperature (°C)
	Three days surrounding treatment	Minimum temperature (°C)
	Seven days surrounding treatment	Minimum temperature (°C)
	Day of treatment	Hours of exposure to temperature <0°C
	Three days surrounding treatment	Hours of exposure to temperature <0°C
	Seven days surrounding treatment	Hours of exposure to temperature <0°C
Weather station measurement	Days before glyphosate treatment	Temperature <0°C
	Days after glyphosate treatment	Temperature <0°C
	Days during first crop	Temperature <0°C
	Day of treatment	Minimum temperature (°C)
	Three days surrounding treatment	Minimum temperature (°C)
	Seven days surrounding treatment	Minimum temperature (°C)

[a]Used as the reference in class variable comparisons. Variables within a parameter type without a reference category were analyzed as independent continuous variables.

TABLE 3.3 Parameter estimates of odds ratios with 95% Walk confidence intervals (CI) and Somers' D statistic observing the effect of soil texture, soil pH, site elevation, glyphosate application rate, glyphosate application height, and ambient temperature on alfalfa crop yield response.

Parameter Type	**Category**	**Units/critical values**	**Odds Ratio**	**Estimate**	**95% CI**	**Max Rescale R2**	**Concordance**	**Somers' D**
							%	
	Model					0.3878	83.7	0.674
Soil properties	Soil pH		7.512	2.0165	2.01-28.04			
Application rate	869[a]	g a.e. ha^{-1}						
	1739	g a.e. ha^{-1}	3.849	0.6739	1.34-11.09			
On-site measurement	Days before treatment	Temperature <0°C	1.13	0.1222	1.06-1.21			

[a]Used as the reference in class variable comparisons.

TABLE 3.4 Parameter estimates for linear regression observing the effect of soil texture, soil pH, site elevation, glyphosate application rate, glyphosate application height, and ambient temperature on alfalfa crop yield response.

Parameter Type	Category	Units/critical values	Estimate	*P* > *F*	Adj R^2
			Mg ha^{-1}		
	Intercept		5.112	<0.001	0.418
Soil properties	Soil pH		-0.599	<0.001	
Application rate	869[a]	g a.e. ha^{-1}			
	1739	g a.e. ha^{-1}	-0.020	<0.001	
On-site temperature	Days before glyphosate application	Temperature <0°C	-0.056	<0.001	

[a]Used as the reference in categorical variable comparisons

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